STATE LEVEL WORKSHOP ON TRAINING OF TRAINERS (ToT)

SANCTIONED by DBT, GOI
UNDER SKILL VIGYAN PROGRAMME

सत्यमेव जयते

EMPOWERING RESEARCHERS OF TOMORROW

Organised by
TELANGANA STATE COUNCIL OF SCIENCE & TECHNOLOGY (TSCOST)

ENVIRONMENT, FORESTS AND SCIENCE & TECHNOLOGY DEPARTMENT
GOVERNMENT OF TELANGANA
4TH FLOOR, ARANYA BHAVAN, SAIFABAD, HYDERABAD

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Foreword

It is with great pleasure and a sense of accomplishment that I extend my greetings to the esteemed readers of this Training of Trainers (ToT) Book, documenting the comprehensive proceedings of the ToT program held as a part of DBT - Skill Vigyan Program during November 1st – 4th, 2023.

I am honored to serve as the Program In charge for this momentous event, facilitated under the aegis of the Telangana State Council of Science & Technology (TSCOST). The DBT - Skill Vigyan Program, an initiative of paramount significance, aims to bolster the foundations of the biotechnology sector by fostering skill development, innovation, and knowledge transfer.

This ToT Book stands as a testament to the dedication and collaborative efforts of all those involved in making this program a resounding success. Throughout the four days of intensive training, we were privileged to host 10 distinguished Guest Lecturers, each one of them are experts in their respective fields. Their invaluable insights and expertise have been meticulously captured in the lecture notes presented within this compilation, ensuring a lasting reference for future endeavors in biotechnology education and training.

A highlight of the program was the Industrial Training Sessions conducted on the 3rd of November 2023, where participants had the unique opportunity to engage with and learn from the practical nuances of two leading pharmaceutical companies – Sai Life Science & Curia Pharmaceuticals Ltd. This hands-on experience is integral to bridging the gap between theoretical knowledge and real-world application, a cornerstone of effective training in the dynamic field of biotechnology.

As we delve into the contents of this ToT Book, I am confident that the wealth of knowledge encapsulated within its pages will serve as a guiding light for all those committed to advancing biotechnology education and training. The collaborative spirit demonstrated during this event, coupled with the dedication of the participants and the expertise of our esteemed Guest Lecturers, has undoubtedly set a new standard for the Skill Vigyan Program.

I extend my sincere gratitude to all the Guest Lecturers, the participants and the Organizing Team for their unwavering commitment to the success of this Training of Trainers program. I sincerely hope this ToT Book serves as a useful reference for educators and trainers, fostering a continuous cycle of learning and growth in the field of biotechnology.

Dr. Ahmed Kamal
Consultant - TSCOST
Program In charge, DBT - Skill Vigyan Program

Foreword

It is with immense pride and satisfaction that I extend my warmest greetings to the distinguished readers of this Training of Trainers (ToT) Book, commemorating the successful execution of the DBT - Skill Vigyan Program ToT held from the 1st to the 4th of November 2023.

It is my distinct honor to have led our dedicated team in orchestrating this significant event. The DBT - Skill Vigyan Program represents a pivotal initiative aimed at enhancing the competencies of professionals in the field of biotechnology & Intellectual Property, and this ToT Book encapsulates the collective efforts of the entire TSCOST team in ensuring its success.

The core of this program lies in the exchange of knowledge, and I am pleased to note that we had the privilege of hosting 10 eminent Guest Lecturers during this ToT. Their profound insights and expertise, meticulously recorded in the lecture notes within this compilation, are intended to serve as an enduring resource for academicians, students, and all stakeholders invested in advancing biotechnology education and training.

The DBT - Skill Vigyan Program encompasses three integral components: the Faculty Training Program (FTP), the Student Training Program (STP), and the Entrepreneurship Development Program (EDP). The FTP is tailored to empower educators with the latest advancements in biotechnology, equipping them to impart cutting-edge knowledge to their students. The STP, designed for students, provides a platform for skill development and hands-on learning, fostering a new generation of adept biotechnologists. Simultaneously, the EDP nurtures the entrepreneurial spirit, offering aspiring innovators the guidance and resources necessary to transform their ideas into viable ventures.

Furthermore, TSCOST serves as a pivotal focal point for academic institutions and universities, acting as a conduit for communication with key scientific government departments such as the Department of Science and Technology (DST) and the Department of Biotechnology (DBT). This strategic role underscores our commitment to facilitating seamless collaboration and communication, ensuring that academic institutions remain abreast of the latest developments in the scientific landscape.

May this ToT Book stand as a testament to our collective dedication to advancing biotechnology education and training, fostering a culture of innovation and excellence in the state of Telangana and beyond.

Sri Marupaka Nagesh
Member Secretary,
TSCOST, Hyderabad

Hyderabad
20/11/2023

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I. GENESIS, FUNCTIONS & APEX BODIES:

Recognizing the importance of Science& Technology for the overall socio-economic development throughout the country, the Department of Science & Technology (DST), Government of India played catalytic role in establishing State S&T Councils in all the States and Union Territories in association with the respective Governments. Accordingly, Government of Telangana constituted the Telangana State Council of Science & Technology (TSCOST) to function under the aegis of the Environment, Forests, Science & Technology Department, Govt. of Telangana.

Article 51(A)(H) of the Indian Constitution emphasizes the possession of scientific temper by all the citizens.

Telangana State Council of Science & Technology (TSCOST) acts as the focal point for formulation, planning, coordination, and promotion of S&T activities and helps in preparing State S&T plans, facilitating technological interventions, disseminating S&T information, and fostering the spirit of scientific temper among various sections of society.

Government of Telangana formed the General Council and Executive Committee with eminent personalities from administrative, scientific, and academic circles to govern and guide TSCOST.

State Science & Technology Program (SSTP) Division of DST, GoI provide the necessary guidelines and programmatic support from time to time.

Government of Telangana and Department of Science & Technology (DST), Govt. of India provide the program and manpower budgetary support.

II. THRUST AREAS:

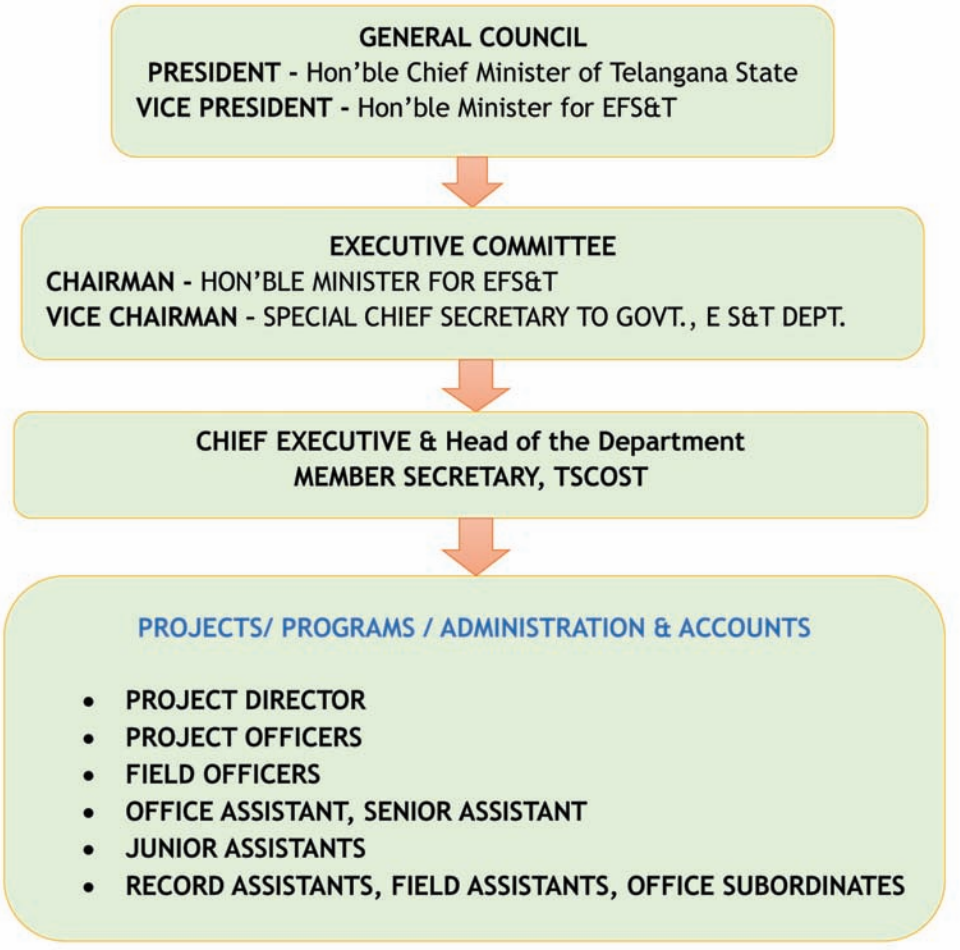
A. Facilitation for Registration of Patents, Copy Rights & Geographical Indicators (GIs) generated in the State for protection of Intellectual Property Rights (IPR)

B. Transfer of Technologies from Central R&D Institutions to the State Institutions for societal applications

C. Coordination with Universities to assess the research work and to provide Central / State funding tie-ups such as Young Scientist Fellowship and Research Grants etc

D. Popularization of Science, Skill Development Activities for the benefit of Students / unemployed youth

III. STRUCTURE OF THE COUNCIL:



IV. MAJOR ACTIVITIES:

TSCOST is implementing the following Unique Schemes & Programs with the support from Government of Telangana, Department of Science & Technology (DST), Govt. of India and other GoI Departments/ Research & Development Institutions.

i. Established **SC & ST Cell** at Warangal with support of DST, GoI with a budget of Rs. 8.08 Lakhs for a period of 3 years. Main functions of SC & ST Cell include GIS based Scientific Survey in Telangana State for SC & ST Communities, identifying & implementing of relevant Science, Technology & Innovation interventions for the development of SC and ST Communities, etc.

ii. Established **Patent Information Centre (PIC)** in Telangana State with support of DST, GoI with an annual budget of **Rs. 25.01 Lakhs**. Awareness on Intellectual Property Rights (IPRs) such as Patents, Copyrights, Trademarks, Industrial Designs etc. to the Researchers, Innovators, Contributors, Designers etc. and Coordination for facilitation of IPRs are the main functions of the PIC. TSCOST signed an MoU with RGUKT-IIIT, Basara and works in association with TIFAC, TSIC, T-Hub, We-Hub, Academic and Research Universities etc.

iii. Implementing the **Skill Vigyan Initiative Project** sponsored by Department of Biotechnology (DBT), Govt. of India with a budget of **Rs. 319.70 Lakhs** for a period of 3 years. TSCOST coordinates the implementation of Skill Development Programs in Life Sciences, Health Care and Agriculture sectors in association with the R&D and Academic Institutions, Departments, Training Institutions for the benefit of Students, Faculty and Prospective Entrepreneurs.

iv. Implementing **Research Projects under the State Science & Technology Programme (SSTP)** Scheme of Department of Science & Technology (DST), Govt. of India for introducing S&T Interventions for solving Location Specific Problems. DST sanctioned a budget grant of Rs. 100.00 Lakhs for this purpose. 26 Research Projects are in progress in various fields like Biodiversity, Artificial Intelligence (AI), Health & Medical, etc

v. TSCOST is operating and maintaining the **Regional Science Centre (RSC), Warangal** with the support of Govt. of Telangana. Programs and activities of the RSC include Demonstration of Scientific Principles through Interactive Exhibits, Workshops/Face to Face/ Interactive sessions on various aspects of Science & Technology.

vi. TSCOST is establishing **Innovation Hub at RSC, Warangal** with the support of National Council of Science Museums (NCSM), Ministry of Culture, Govt. of India. The **Budget of Rs. 160.00 Lakhs** is to be shared by TSCOST and NCSM, over a period of 3 years. Innovation Hub will act as springboard for new ideas and motivates youth toward providing solutions to the problems through innovation.

vii. TSCOST is implementing **Science Popularization and Communication Activities** such as National Children's Science Congress, National Science Day Celebrations, National Mathematics Day Celebrations, etc. to pursue the objectives of spreading scientific temper, scientific literacy and strengthening scientific capabilities among a wide cross section of society i.e., child scientists, teachers, innovators, research scholars, etc.

Collaborations:

i. Executed Memorandum of Agreement (MoA) with Department of Biotechnology (DBT), GoI, Life Sciences Sector Skill Development Council (LSSSDC), Agriculture Skill Council of India (ASCI), Professor Jayashanker Telangana State Agricultural University, Osmania University, Jawaharlal Nehru Technological University, Hyderabad, etc., and with the Research Institutions like NIPER, FCRI, CCMB, etc., in respect of Skill Vigyan Initiative.

ii. Executed Memorandum of Understanding (MoU) with National Council of Science Museums (NCSM), Ministry of Culture, GoI for establishment of Innovation Hub at Regional Science Centre, Warangal & with Rajiv Gandhi University of Knowledge and Technologies (RGUKT) for implementation of Science, Technology & Innovation activities.

iii. Associated with TSCHE, SCERT, Dept. of School Education, CESS, TISS, JNAFAU, and certain other Academic & Research Institutions in respect of specific S&T Programs.

The Department of Biotechnology (DBT), operating under the Government of India, has been at the forefront of promoting and regulating biotechnology research, development, and innovation in the country. DBT plays a pivotal role in fostering scientific excellence, innovation, and skill development in the biotechnology sector. Through its Skill Vigyan Programme, DBT supports three essential development programs: the Student Development Program, Entrepreneur Development Program, and Faculty Development Program.

SKILL VIGYAN PROGRAMME:

The Skill Vigyan Programme is a flagship initiative by DBT that encompasses the Student, Entrepreneur, and Faculty Development Programs. It is part of DBT's overarching strategy to promote skill development in biotechnology and align the workforce with industry needs. Through this comprehensive program, DBT plays a pivotal role in nurturing talent, fostering innovation, and bridging the gap between academia and industry.

ENTREPRENEUR DEVELOPMENT PROGRAM:

To promote innovation and entrepreneurship in biotechnology, DBT's Entrepreneur Development Program supports aspiring biotech entrepreneurs. This program offers funding, mentorship, and resources to individuals and startups with innovative biotechnology ideas. DBT's goal is to foster a culture of innovation and entrepreneurship, ensuring that promising biotech ventures have access to the necessary resources to transform their ideas into successful businesses. The Entrepreneur Development Program is instrumental in helping biotech startups bridge the gap between research and commercialization.

FACULTY DEVELOPMENT PROGRAM:

DBT recognizes the crucial role played by educators and researchers in advancing biotechnology. The Faculty Development Program is aimed at enhancing the quality of biotechnology education by providing support to faculty members. This includes grants for research projects, opportunities for professional development, and access to state-of-the-art infrastructure. By investing in faculty development, DBT ensures that educational institutions can offer world-class biotechnology programs and keep pace with the rapidly evolving field.

STUDENT DEVELOPMENT PROGRAM:

DBT's Student Development Program is designed to nurture the next generation of biotechnologists. This initiative provides financial support, scholarships, and training to undergraduate and postgraduate students pursuing biotechnology-related courses. The program aims to develop a skilled workforce, enabling students to acquire hands-on experience and knowledge that is aligned with the evolving needs of the biotechnology sector. By offering grants and scholarships, DBT encourages students to pursue careers in biotechnology and contribute to the growth of this field.

ROLE OF TSCOST IN PROGRAM IMPLEMENTATION IN TELANGANA:

In the state of Telangana, the implementation of these development programs under the Skill Vigyan Program is facilitated by the Telangana State Council of Science & Technology (TSCOST). TSCOST acts as a vital intermediary, ensuring that the benefits of DBT's initiatives reach the eligible individuals and institutions in the state. This partnership between TSCOST and DBT serves as a vital bridge connecting the central government's initiatives with the grassroots of Telangana's biotechnology sector. It plays a critical role in managing the application process, disbursing grants and scholarships, and monitoring the progress of the programs at the state level. TSCOST's involvement at the state level further ensures that the benefits of these programs are effectively delivered and utilized in Telangana, contributing to the growth of the biotechnology sector in the region.

TSCOST's involvement at the state level extends beyond mere administrative tasks. It goes to the heart of fostering a conducive ecosystem for biotechnology growth in Telangana. By acting as a liaison between the central government and the local stakeholders, TSCOST serves as a catalyst for the effective delivery and utilization of these programs. This symbiotic relationship contributes to the overall growth and development of the biotechnology sector in Telangana.

Telangana State Council of Science & Technology (TSCOST)

DBT – Skill Vigyan Program

Training of Trainers (ToT) Program

Telangana State Council of Science & Technology (TSCOST) initiated the 2nd year program of Skill Vigyan initiative supported by Department of Biotechnology (DBT), GoI, by conducting the Training of Trainers (TOT) Program to all the Coordinators/ Principal Investigators of Grantee Institutes (NIPER, CCMB, PJTSAU, FCRI, CPMB, OU, JNTUH). In this connection, the TOT program was initiated at TSCOST, Aranya Bhavan, Hyderabad and it was 04 (Four) days Program arranged in offline mode, including the Industrial visits.

The objective of the program is to train the faculty in the area of life sciences and biotechnology. Faculty are trained in so that these trainers can train the other faculty and students in their respective institutions.

It is a 4-day program conducted during November 1st to 4th, 2023. All the Principal Investigators of TSCOST-DBT skill vigyan Initiative were invited to attend the program along with their team of trainers. The methodology followed for conducting the program is through lecture sessions by experts in various fields of science and industry.

As earlier mentioned, Skill Vigyan initiative is a 3-years project supported by Department of Biotechnology (DBT), GoI, sanctioned to TSCOST. The Partner Institutes of this project are:

1. National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad
2. Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad
3. CSIR–Centre for Cellular and Molecular Biology (CCMB), Hyderabad
4. Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad
5. Centre for Biotechnology, Institute of Science & Technology, Jawaharlal Nehru Technological University (JNTU), Hyderabad
6. Forest College and Research Institute (FCRI), Hyderabad

The consolidated list of participants for Training of Trainers (ToT) Program is as follow:

SL. NO.	NAME OF THE PARTICIPANT	ORGANIZATION	CONTACT NO.
	FACULTY TRAINING PROGRAM (FTP)		
1	Dr. Ramakrishna Kancha	CPMB, OU	7075047600
2	Dr. Lavanya Tayi	CPMB, OU	8706092737
3	Ms. Sai Charitha Mullaguri	CPMB, OU	9032550505
4	Dr. Anuradha PJTSAU	9866661350	
5	Dr. Lakshmi Prasanna	PJTSAU	9573316477
6	Dr. SNCVL Pushpavally	PJTSAU	9052748970
8	Dr. Archana B. SivaCCMB	9441233939	
9	Dr. Saloni Kakkar	CCMB	7837160549
10	Dr. Leela Kumari	CCMB	9394824180
11	Dr. A. Uma	JNTU	9848120819
12	Dr. Ch. Kalyani	JNTU	9949234486
13	Dr. Venkanna	JNTU	9505459857
14	Dr. Ranjit	JNTU	9985289499
15	Dr. Venkateshwara Reddy	JNTU	8977375801
16	Dr. K. Venkat Rao	NIPER	9779183370
17	Mr. Arbaz Sujait Shaik	NIPER	7507869848
18	Ms. Vemula DivyaNIPER	6305359094	
19	Dr. Santhosh Kumar Guru	NIPER	8114747477
20	Mr. Biswajit Dey	NIPER	8981918034
21	Ms. Essha ChatterjeeNIPER	8016114001	
22	Ms. Bhagyashree Patra	NIPER	9496196698
23	Ms. Swathi LakshmiNIPER	9496196698	
	STUDENT TRAINING PROGRAM (STP)		
24	Dr. S. GnanadhamuNIPER	9866906386	
25	Ms. Nehal Bhatt	NIPER	7227838310
26	Ms. Pilli Pushpa	NIPER	9963748875
27	Mrs. Vijaya Madhyanapu Golla	NIPER	7508076504
28	Mr. H M Chandra Mouli	NIPER	9182095430
	ENTREPRENEURSHIP DEVELOPMENT PROGRAM (EDP)		
29	Prof. M. Mamatha FCRI	9885226957	
30	Mr. P. Rajasekhar FCRI	8466871929	
31	Mr. Bochu Jeevan FCRI	7032223597	
32	Dr. Mohammed Abdul Waseem	FCRI	7876531614
33	Dr. B. Lakshmi NIPER	9885154574	
34	Ms. Dharipally Harini	NIPER	8247692649

DAY 01

DATE: 01st November 2023

VENUE: 06th Floor, Aranya Bhavan, Hyderabad

No. of Participants: 35

Detailed Agenda	
Day 1 **1st November, 2023**	**Particulars**
10:00 - 10:30 AM	Registration of Participants
10:30 - 11:00 AM	Inaugural Session - Welcome Speech by Dr. Ahmed Kamal, Consultant, TSCOST - Opening Remarks & Address by Member Secretary, TSCOST - Introduction & Keynote Address by Chief Guest - Prof. M. Vijjulatha, Vice Chancellor, Telangana Mahila Viswa Vidyalayam, Hyderabad
11:00 - 11:45 AM	Session 1 - Quality Control in Pharma Industry - Speaker: Dr. M. V. Narendra Kumar Talluri - Affiliation: Daicel Chiral Technologies (India) Pvt Ltd, IKP Knowledge Park, Shamirpet, Hyderabad. - Presentation and Q&A
11:45 - 12:00 PM	Tea Break
12:00 - 1:00 PM	Session 2 - Workshop on Animal Cell Culture - Topic: Apoptosis Cancer Cell Signalling - Molecular Tools and Methods - Speaker: Dr. Raghu Gogada - Affiliation: Chief Scientific Officer, Scigene Bio Pvt Ltd, Hyderabad. - Presentation and Q&A
1:00 - 2:00 PM	Lunch Break
2:00 - 3:00 PM	Session 3 - Entrepreneurship Development Program - Topic: IP Strategy for Entrepreneurs - Speaker: Swapna Vanamali - Affiliation: NiMSME Yousaufguda - Presentation and Q&A
3:00 - 4:00 PM	Session 4 - Hands-on Skill Development Training
	- Topic: Advanced Area of Life Science and Biotechnology - Recombinant Molecular Expression Technologies - Speaker: Dr. Anil Pasupulati, Assistant Professor, Hyderabad University - Q&A Session
4:00 PM	Day-1 Session Concluding Remarks - Recap of the day's highlights - Announcements for Day 2 - Acknowledgment of Speakers, Sponsors, and Participants - Closing Remarks and Thank You

REGISTRATION OF PARTICIPANTS:

Institution	Sl. No.	Name of the Participants
National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad	01.	Dr. S. Gnanadhamu
	02.	Dr. Santhosh Kumar Guru
	03.	Dr. K. Venkat Rao
	04.	Dr. B. Lakshmi
	05.	Ms. Nehal Bhatt
	06.	Ms. Pilli Pushpa
	07.	Mrs. Vijaya Madhyanapu Golla
	08.	Mr. H M Chandra Mouli
	09.	Mr. Biswajit Dey
	10.	Ms. Esha Chatterjee
	11.	Ms. Bhagyashree Patra
	12.	Ms. Swathi Lakshmi
	13.	Mr. Arbaz Sujait Shaik
	14.	Ms. Divya
	15.	Ms. Dharipally Harini
Jawaharlal Nehru Technological University (JNTHU), Hyderabad	16.	Dr. A. Uma
	17.	Dr. L. Saida
	18.	Dr. Ch. Kalyani
	19.	Dr. Venkanna
	20.	Dr. Ranjit
	21.	Dr. Venkateshwara Reddy
Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad	22.	Dr. C. V. Sameer Kumar
	23.	Dr. Anuradha
	24.	Dr. Lakshmi Prasanna
	25.	Dr. SNCVL Pushpavally
Forest College and Research Institute (FCRI), Hyderabad	26.	Prof. M. Mamatha
	27.	Dr. Mohammed Abdul Waseem
	28.	Mr. Bochu Jeevan
	29.	Mr. Pathapelly Rajashekhar
Centre for Cellular & Molecular Biology (CCMB), Hyderabad	30.	Dr. Archana B. Siva
	31.	Dr. Saloni Kakkar
	32.	Dr. Leela Kumari
Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad	33.	Dr. Rama Krishna
	34.	Dr. Lavyana Tayi
	35.	Ms. Sai Charitha Mullaguri

WELCOME SPEECH BY DR. AHMED KAMAL, CONSULTANT, TSCOST:

Dr. Ahmed Kamal, the astute and dedicated program in-charge, stepped forward to extend a warm and gracious welcome to the assemblage of trainers and educators representing various prestigious universities such as NIPER, JNTUH, OU - CPMB, PJTSAU, and FCRI. His welcome speech was both an affirmation of the collective passion for knowledge sharing and an acknowledgment of the tireless efforts that had gone into the meticulous organization of the Training of Trainers (ToT) Program under the Skill Vigyan Initiative.

In his welcoming address, Dr. Kamal encapsulated the essence of the event by recognizing the significance of bridging the knowledge gap in the field of biotechnology. He emphasized the vital role that educators from renowned institutions played in moulding the future generation of scientists & innovators. By hosting participants from diverse academic backgrounds, the program aimed to create a collaborative space for the exchange of ideas, experiences, and expertise.

Dr. Kamal went on to express his heartfelt appreciation for the dedication and hard work put into orchestrating this grand event. The effort invested by the organizing committee, the participating universities, and the support of the Skill Vigyan Initiative were fundamental in ensuring the program's success.

Dr. Kamal outlined the core objectives and the comprehensive agenda for the next four days. He elucidated how the Training of Trainers (ToT) Program was designed to provide participants with a holistic understanding of biotechnology, intellectual property, and their interconnectedness. The participants were encouraged to actively engage in the workshops and sessions, foster collaboration, and build a knowledge repository that would empower them to shape the educational landscape in their respective institutions.

As Dr. Kamal concluded his welcome speech, he expressed his hopes that the participants would find the program not only informative but also enriching. He underlined that the success of the Skill Vigyan Initiative hinged on the eagerness of educators and trainers to absorb knowledge, impart it to their students, and drive scientific innovation.

In his closing remarks, Dr. Kamal encapsulated the ethos of Skill Vigyan Initiative, mentioning the integral components like Students Training Programs (STP), Faculty Training Programs (FTP), and Entrepreneurship Development Programs (EDP). These programs, he emphasized, were structured to cater to different facets of biotechnology and equipped the participants with a versatile skill set. He encouraged the trainers and educators to explore these offerings, highlighting the wealth of resources at their disposal.

OPENING REMARKS BY SRI MARUPAKA NAGESH, MEMBER SECRETARY, TSCOST:

As the Telangana State Council of Science and Technology (TSCOST) embarked on its four-day Training of Trainers (ToT) Program under the DBT - Skill Vigyan Initiative Program, Sri Marupaka Nagesh, Member Secretary, TSCOST, commenced the proceedings with his opening remarks and address. His words set the stage for an enriching and enlightening journey that the participants were about to embark on.

The Member Secretary, TSCOST initiated the program by extending a gracious and warm welcome to all participants, educators, and trainers assembled in the hall. He acknowledged their presence as a testament to their commitment to the fields of biotechnology and intellectual property, underlining the collective responsibility they shared in shaping the future of science and innovation.

In his address, the Member Secretary stressed the paramount significance of biotechnology in the contemporary world. He underscored how these fields are the driving forces behind groundbreaking discoveries, innovations, and the economic and social development of nations. The audience was reminded that biotechnology had become the backbone of several industries, including healthcare, agriculture, and environmental sciences.

The Member Secretary further highlighted the crucial role played by the DBT - Skill Vigyan Initiative Program in fostering innovation and scientific advancement. He commended the program's commitment to imparting practical and theoretical knowledge to educators and trainers, recognizing the need for highly skilled professionals in these domains. The initiative, he explained, aimed not only to educate but to inspire and empower individuals to become agents of change, driving scientific progress and economic growth.

In his address, the Member Secretary encouraged the participants to maximize the opportunities presented by the program. He urged them to embrace the knowledge and skills they would gain in the coming days, emphasizing that these insights were not only for personal growth but, more importantly, for the greater benefit of society. The participants were reminded of their pivotal roles as knowledge disseminators, ensuring that the fruits of their learning reached the broader community.

In conclusion, the Member Secretary's eloquent and motivating words instilled a sense of purpose and enthusiasm among the participants, underlining the transformative potential of knowledge and its application in the fields of biotechnology. His speech set a high standard for the days ahead, inspiring the participants to approach the ToT Program with dedication and a sense of responsibility to society and the future of scientific advancement.

KEYNOTE ADDRESS BY CHIEF GUEST - PROF. VIJJULATHA, VICE CHANCELLOR, TELANGANA MAHILA VISWA VIDYALAYAM, HYDERABAD:

The keynote address and remarks by Prof. Vijjulatha, Vice Chancellor of Telangana Mahila University, Hyderabad, at the Training of Trainers (ToT) Program under the DBT - Skill Vigyan Initiative, were a significant highlight of the event.

In her keynote address, Prof. Vijjulatha began by underscoring the paramount importance of biotechnology in today's ever-evolving world. Drawing from her rich background as an academic leader, she eloquently conveyed how this field had become the lifeblood of innovation, touching upon various aspects of human existence. Her insights resonated profoundly, as she emphasized the pivotal role that educators and trainers played in nurturing the next generation of scientists and innovators who would lead this transformative domain.

Prof. Vijjulatha lauded the DBT - Skill Vigyan Initiative Program for its visionary efforts in promoting scientific knowledge and skills. Her deep-rooted understanding of academia and her role as a university leader added credibility to her praise of the program's comprehensive approach. She acknowledged that the program was not limited to the dissemination of information but aimed to foster critical thinking, problem-solving abilities, and a spirit of innovation among its participants.

Her profession as Vice Chancellor underscored the significance of her endorsement, as it was evident that she recognized the ToT Program's potential to positively impact the academic and research community. Prof. Vijjulatha emphasized the need for universities to collaborate and pool their resources, reiterating her commitment to the program's core objective of equipping educators to be catalysts for scientific progress and innovation.

Prof. Vijjulatha's words left an enduring impression, compelling the participants to view themselves not only as educators but as mentors who could shape the course of scientific advancement and innovation in the years to come.

SYNOPSES OF THE LECTURES:

01. Analytical Techniques and Trends in Pharmaceutical Industry

About the Speaker:

Dr. M V Narendra Kumar Talluri, PhD, MRSC, Director, Knowledge Management & Tech (ARD) - Southeast Asia Daicel Knowledge Centre Shamerpet, Hyderabad. Dr. M.V. Narendra Kumar Talluri is a distinguished professional in the field of pharmaceutical Analysis and research. Dr. Talluri currently serves as the Director and Head of Technical (AR&D) and Knowledge Management at Daicel Chiral Technologies India. He is also the Honorary Secretary for the Royal Society of Chemistry-LSD-India.

With a PhD from CSIR-Indian Institute of Chemical Technology, Hyderabad, Dr. Talluri has an impressive track record of 90 scientific articles to his name. He has successfully guided numerous research scholars and holds various prestigious awards, including the Young Pharmaceutical Analyst Award in 2011 and the Best Research Scientist Award in 2016.

Dr. Talluri's contributions to scientific journals, including recognition by the Journal of Pharmaceutical & Biomedical Analysis, showcase his commitment to advancing the field. He is a respected reviewer for international journals and has an extensive background in examining various national and university-level exams.

In addition to his professional achievements, Dr. Talluri is a trained GLP Inspector, emphasizing his dedication to upholding the highest standards in the industry.

1.1 Introduction

The pharmaceutical industry is committed to the safety and efficacy of drugs, two fundamental aspects of drug therapy. Ensuring the safety of a drug involves an intricate assessment of its pharmacological-toxicological profile and, importantly, the adverse effects that can arise from impurities. Impurities in drugs can introduce unwanted pharmacological-toxicological effects, potentially outweighing the therapeutic benefits. This underscores the necessity for comprehensive characterization of pharmaceutical products to guarantee quality and safety. In the modern pharmaceutical landscape, the analytical activities surrounding impurities in drugs occupy a pivotal role.

1.2 Regulatory Guidelines for Pharmaceutical Analysis

Regulatory authorities have established a framework of guidelines to govern pharmaceutical analysis and quality assurance. These guidelines cover a spectrum of critical aspects, including stability assessment (Q1A-Q1F), analytical method validation (Q2), identification and qualification of impurities (Q3A-Q3D), and compliance with pharmacopoeias (Q4-Q4B). Ensuring the quality and safety of biotechnology products (Q5A-Q5E), setting specifications (Q6A-Q6B), and adhering to Good Manufacturing Practice (Q7) are all integral components of the regulatory landscape. These guidelines not only serve as a compass for regulatory authorities but also provide a critical reference for the pharmaceutical industry in the preparation and validation of test methods.

1.3 Analytical Procedure Development and Validation

The development of analytical procedures within the pharmaceutical industry is a meticulous process. Such procedures must effectively separate impurities from each other and quantify them within dosage forms. To meet this requirement, the method is optimized, taking into account parameters

such as accuracy, precision, specificity, limit of detection, quantitation, linearity, range, and interferences. Validation of analytical methods is not merely a formality; it is an indispensable component of the registration application for a new drug. In the context of chromatographic methods, additional considerations include peak tailing, peak resolution, and analyte recoveries. The International Council for Harmonisation (ICH) guidelines have established a global foundation, emphasizing the necessity of proper validation for both regulatory authorities and the industry.

1.4 Types of Impurities in Pharmaceuticals

Impurities in pharmaceutical products take various forms, each with distinct origins and potential effects. These impurity categories encompass the last intermediates of synthesis, products of incomplete reactions, products of overreactions, impurities originating from starting materials, impurities from solvents, catalyst-derived impurities, products of side reactions, degradation products, enantiomeric impurities, residual solvents, inorganic impurities, and impurities found in excipients.

1.5 Scale of Chromatography in Pharmaceutical Analysis

Chromatography is a cornerstone of pharmaceutical analysis and is applied at various scales to fulfill different objectives. Analytical chromatography is instrumental in compound identification and concentration determination. Semi-preparative chromatography operates at the milligram to gram scale, primarily employed for small-scale compound purification. Preparative chromatography extends its utility to compound purification on a larger scale, from grams to kilograms. At the industrial level, process-scale chromatography is implemented for manufacturing quantities, spanning from kilograms to metric tons.

1.6 Quality by Design (QbD) in Pharmaceutical Development

Quality by Design (QbD) is a systematic approach that has gained prominence in the pharmaceutical industry since its initiation in 2002. This approach has been embraced wholeheartedly, spurred by regulatory initiatives like the U.S. FDA's cGMP for the 21st Century and the introduction of new ICH Q8, Q9, and Q10 regulatory documents. Central to QbD is the incorporation of statistical tools such as Design of Experiment (DoE), multivariate analysis, and Six Sigma methodologies. Design space, a fundamental concept in QbD, empowers pharmaceutical developers with a systematic framework for understanding and controlling the quality of a product. Variables like pH, temperature, and other critical parameters are meticulously monitored and optimized to create a design space. Within this space, it is possible to explore the interaction effects of all variables, which subsequently generates a deeper understanding of the product and the processes involved.

1.7 HPLC Instrumentation and Method Details

In the case study, a Waters Alliance High-Performance Liquid Chromatography (HPLC) instrument is utilized. This instrument, the e2695 model by Waters Corp., is equipped with an integral autosampler and a quaternary gradient pump with an online degasser. To perform the chromatographic analysis, a variety of columns, including Luna C18, Luna C8, Genesis phenyl, and Sun Shell C18, are deployed. Critical parameters such as gradient composition and time, pH, flow rate, and column temperature are precisely controlled during the experiments.

The outcome of the chromatographic experiment is a rich source of data for understanding the interplay of variables and their influence on critical pair resolution. Detailed chromatograms and analysis results unveil the effects of different experimental conditions on the separation of key compounds.

1.8 Method Validation and Validation Parameters

Validation of analytical methods is a critical step in ensuring the reliability and accuracy of pharmaceutical analysis. This involves assessing key validation parameters such as linearity, limit of detection, limit of quantification, accuracy, and precision. The results obtained under different conditions demonstrate the robustness and precision of the analytical method.

1.9 Mass Spectrometry in Pharmaceutical Analysis

Pharmaceutical analysis increasingly incorporates mass spectrometry, a powerful analytical technique. The case study leverages an Agilent 1200 series HPLC instrument coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer. Mass spectrometry data analysis is conducted using Mass Hunter workstation software, offering in-depth insights into the chemical composition of the analytes.

In conclusion, understanding the regulatory guidelines, the importance of analytical method development and validation, and the concept of QbD and design space is crucial for maintaining the quality and safety of pharmaceutical products. The case study illustrates how these concepts are applied in real-world scenarios. As pharmaceutical analysis continues to evolve, future trends may include advanced analytical technologies, enhanced regulatory scrutiny, and a deeper emphasis on data integrity and quality control.

2. Apoptosis Cancer Cell Signalling-Molecular Tools and Methods

About the Speaker:

Dr. Raghu Gogada, Chief Scientific Officer, Scigene Bio Pvt Ltd, M2 - Technology Hub, IDA Uppal, Hyderabad. Dr. Raghu Gogada had pursued his M.Sc. in Biotechnology (Envi.)-2004 with distinction from JNTU-Hyderabad, and completed his dissertation from CCMB, Hyderabad, INDIA. Dr. Gogada received his PGDIPR (2007) from Department of Law and Ph.D. in Biochemistry (May,2005-2010: Membrane Biology) from Department of Biochemistry, Osmania University, Hyderabad, INDIA in collaboration with BARC, Mumbai, INDIA and University of Lyon, FRANCE.

Dr. Gogada worked as a Post-Doc (July 2010 to June 2013), at Roswell Park Cancer Institute (RPCI), Buffalo, NY, USA, where he initiated the study on "Phytochemical role on Apoptosis Cell Signalling and Autophagy on Cancer".

Dr. Gogada started his career as an INSPIRE Faculty-DST (Equivalent to IIT-Assistant Professor) from February 2013 to January 2018, at Department of Biotechnology and Bioinformatics, School of Life Sciences, University of Hyderabad, Hyderabad (T.S), INDIA .As a part of INSPIRE Faculty, he also worked as a Research Visiting Scientist/Fellow (October 2015 to March 2016), at CGC of Max-Planck Institute for Molecular Physiology, Dortmund, GERMANY, and Research Visiting Post-Doc/Fellow with The HJF of Military Medicine (July 2016 to Jul 2017), at Department of Biochemistry, Uniformed Service University of Health Sciences (USUHS), Bethesda, Maryland, USA, respectively.

At CUTM-PKD, Dr. Gogada discharged his duties as an Associate Professor (January, 2018 to October, 2020) and Head of the Department (August, 2018 to December, 2021) and worked as a Professor (November, 2020 to December, 2021) in the Department of Biochemistry and Plant Physiology, MSSSoA, CUTM, Odisha, INDIA

At Popvax Pvt Ltd, AIC-CCMB, Medical Biotechnology Complex, worked as a Principal Scientist (December, 2021 to December, 2022), Presently, he has been working as a Chief Scientific Officer (CSO) at Scigenebio Pvt Ltd, Hyderabad and mainly working on products-based research (i.e., therapeutic peptides synthesis for cancer research, In-vitro Diagnostic kits and Medical Devices coupled with AI-ML data analytics, Anti-hypertensive nutraceuticals, Next Generation Probiotics).

So-far he had published 25 international research articles, 3 research book chapters, and 2 international research books as an editor. At present he is guiding 3-doctoral (Ph.D.) students.

2.1 Introduction

The "Apoptosis Cancer Cell Signalling-Molecular Tools and Methods" offers a comprehensive exploration of apoptosis and its application in cancer research, with a particular focus on Resveratrol.

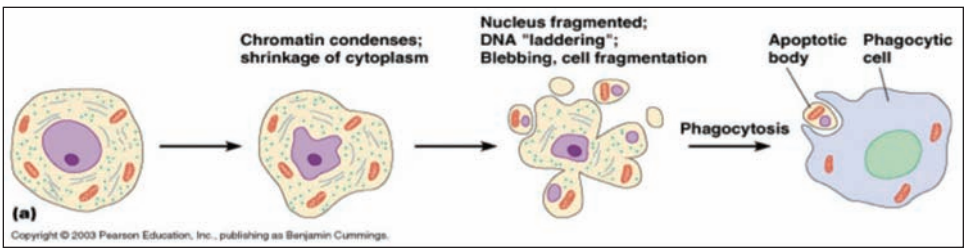
2.2 Types of Cell Death

Cell death can be broadly categorized into two major forms: physiological cell death and programmed cell death. Programmed cell death, with apoptosis being a prime exemplar, is a genetically determined and evolutionarily conserved process. Apoptosis, often referred to as Type I cell death, is orchestrated by a repertoire of intricate molecular machinery.

Apoptosis involves a spectrum of regulatory elements, including caspases, Bcl-2 family proteins, and BH3-only domain proteins. It manifests through two principal pathways, the extrinsic (death receptor-mediated) and intrinsic (mitochondria-mediated) routes. These pathways engage initiator caspases (C-8, 9, and 10) and executioner caspases (C-3, 6, and 7) as crucial players.

2.3 Why Study Apoptosis?

A nuanced comprehension of apoptosis is indispensable owing to its pivotal role in several critical processes. Successful embryonic development, as well as the perpetuation of tissue homeostasis, hinges on the precise orchestration of apoptosis. Deviations from this orchestration can have profound implications for human health, resulting in conditions such as cancer, autoimmune disorders, viral infections, neurodegenerative diseases, immunodeficiency, infertility, and hematologic disorders. Thus, the study of apoptosis assumes paramount importance.



2.4 Methods for Detecting Apoptosis

- Cell Morphology: The discernment of characteristic apoptotic changes through microscopy elucidates phenomena such as cellular shrinkage, nuclear fragmentation, and membrane blebbing.

- Cytochrome c Release: By employing immunolabeling and Western blotting, the release of cytochrome c from mitochondria into the cytosol, a pivotal hallmark of apoptosis, can be determined.
- Immunoprecipitation and Far-Western Blotting: These methods unveil insights into protein-protein interactions, allowing for the identification of critical apoptotic factors.
- Western Blot Analysis: Interrogating caspases and their substrates through Western blotting elucidates their role in the apoptotic cascade.
- DAPI and Annexin-V Staining: Flow cytometry techniques harness DAPI and Annexin-V staining to discern apoptotic cells based on DNA content and phosphatidylserine externalization.
- Detection of Apoptosome: Gel filtration, facilitated by FPLC, unveils the formation of the apoptosome, a multi-protein complex pivotal to caspase activation.

2.5 Resveratrol and Apoptotic Cell Death

Resveratrol, a naturally occurring phytoalexin found in grapes and red wine, emerges as an influential tool for inducing apoptotic cell death in cancer cells. Noteworthy details encompass:

- Resveratrol's multifarious beneficial effects, encompassing anti-aging properties, antioxidant and anti-inflammatory activities, inhibition of platelet aggregation, and suppression of cancer cell proliferation.
- Resveratrol's modus operandi, entailing inhibition of colony formation in cancer cells, induction of caspase-dependent apoptosis, release of cytochrome c from mitochondria into the cytosol, and loss of mitochondrial membrane potential.
- Upregulation of proapoptotic factors, including Bax.
- Induction of autophagy by Resveratrol, with the intriguing observation that inhibiting autophagy accentuates caspase activation.
- Resveratrol's impact on mitochondrial DNA, resulting in its depletion, which, in turn, reinforces its proapoptotic effects.

2.6 Key Takeaways

- Resveratrol is a potent trigger of caspase-dependent apoptosis, a central facet of programmed cell death.
- The release of cytochrome c from mitochondria into the cytosol, coupled with nuclear fragmentation, serves as a defining feature of apoptotic events.
- Mitochondria stand as pivotal organelles in the orchestration of apoptotic signalling.
- Resveratrol stimulates the translocation of Bax to mitochondria and amplifies the presence of proapoptotic factors.
- Autophagy emerges as a cog in the cellular response to Resveratrol, with its inhibition amplifying caspase activation.
- Resveratrol's action extends to mitochondrial DNA, contributing to its apoptotic effects.

3. IP Strategy for Entrepreneurs

About the Speaker:

V. Swapna, Associate Faculty Member and Head of Intellectual property Facilitation Centre at National Institute for MSME (NI-MSME) is an accomplished professional with extensive expertise in intellectual property and various facets of business development. Currently serving as the Associate Faculty Member and Head of the Intellectual Property Facilitation Centre at the National Institute for MSME (NI-MSME), she brings a wealth of knowledge and experience to the field.

Ms. Swapna is a registered Indian Patent Agent and Trademark Agent with the Intellectual Property Office, Government of India, and holds a Master's degree in Textile Technology from the University College of Technology, Osmania University. Additionally, she possesses a Post Graduate Diploma in Patents Law from Nalsar University.

In her role as a Programme Director at NI-MSME, she has successfully executed 15 International Executive Programmes, training approximately 450 international executives from over 50 countries in the areas of Intellectual Property Rights, Quality Management, and Women Empowerment.

As the in-charge of the Intellectual Property Facilitation Centre for MSMEs, Ms. V. Swapna has provided valuable consultancy for patent, trademark, copyright, GI, and design filing and prosecution procedures. Her support has benefited over 250 startups and MSMEs through IP registrations and advisory services.

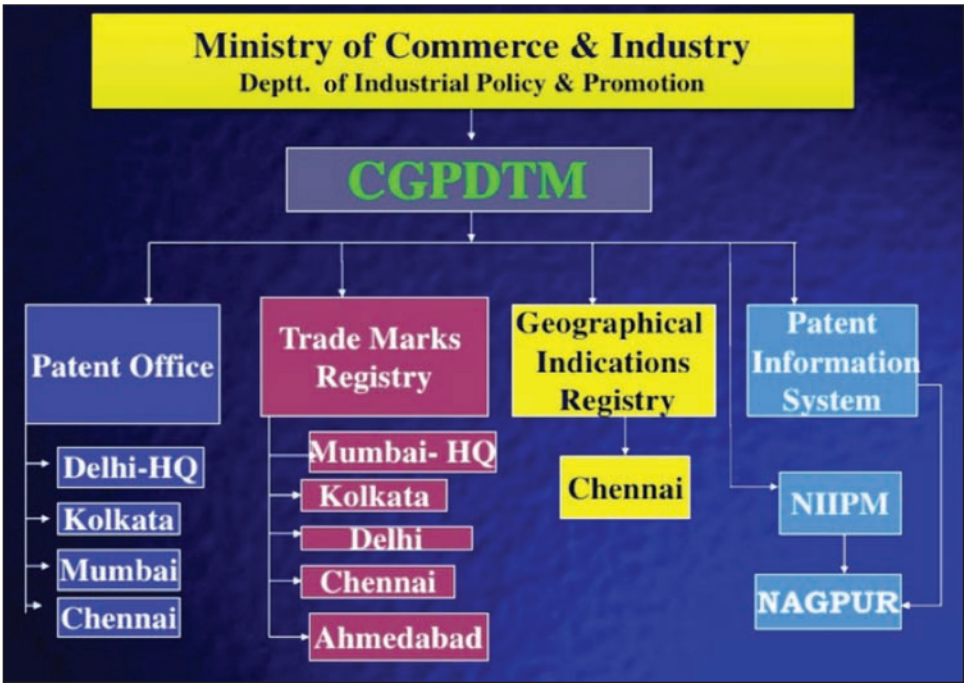
Ms. Swapna is the author of the book "Collection of IP Awardees," which showcases the success stories of entrepreneurs who utilized IPFC services to protect their products and services. She has also published two case studies on IPFC beneficiaries, highlighting their journey from ideation to commercialization.

3.1 Introduction

The core concept of intellectual property (IP) is to grant creators exclusive rights to their creations. These creations encompass a wide range of intellectual work, including inventions, literary and artistic works, and symbols, names, images, and designs used in commerce. IP is essentially about protecting the rights of creators and encouraging innovation by granting them exclusive control over their creations for a specific period.

3.2 IP in India

In India, the Department for Promotion of Industry and Internal Trade, under the Ministry of Commerce & Industry, is responsible for administering intellectual property rights (IPR). This department oversees the management of IP through the Controller General of Patents, Designs, and Trade Marks.



3.3 Categories of Intellectual Property

IP is divided into various categories, each serving a different purpose. These categories include:

- Patents: Patents grant inventors the exclusive right to their inventions, whether they are products or processes, for a specified period (typically 20 years).
- Copyrights: Copyrights protect literary and artistic works, ensuring that creators have control over the use of their creations.
- Trademarks: Trademarks are identifiers of goods or services, such as brand names, logos, or symbols, used to distinguish products or services from those of others.
- Geographical Indications: Geographical indications (GIs) identify the origin of certain products, often associated with specific regions known for their unique characteristics.
- Industrial Designs: Industrial designs encompass the visual elements of an article, such as its shape, pattern, or ornamentation.
- Layout Designs of Integrated Circuits: These protect the layout of electronic integrated circuits, important for electronics and technology industries.
- Trade Secrets: Trade secrets are a category that covers confidential business information, including inventions, formulas, and processes.
- Plant Varieties: This category involves the protection of new plant varieties.

3.4 Patents

A patent is an exclusive right granted to inventors for their inventions, whether they are products or processes. Patents provide inventors with a limited monopoly that typically lasts for 20 years. This right empowers inventors to prevent others from making, using, or selling their patented products or using their patented processes without permission.

What Can Be Patented?

Inventions in all fields of technology, whether products or processes, can be patented if they meet specific criteria. These criteria include novelty (not known to the public prior to the inventor's claim), non-obviousness (inventive step), and industrial application (utility).

Not Patentable Inventions

Some types of inventions are not patentable. This includes frivolous inventions, those against public order or morality, and discoveries of scientific principles. Additionally, the mere discovery of new forms of known substances that do not enhance their known efficacy or the use of known processes, machines, or apparatus (unless they result in new products or employ new reactants) is not patentable.

Patent Filing Procedure in India

Patent rights are obtained by filing a patent application, which can be done physically or through e-filing. The filing process involves submitting a detailed application that describes the invention and its novel features.

3.5 Trademarks

Trademarks are critical for identifying and protecting brands and products. Recognizable examples of trademarks include iconic brands like Coca-Cola, Nike, IBM, and McDonald's, among others. Trademarks are typically registered for a period of 10 years, with the possibility of indefinite renewal.

Trademarks come in various forms, including marks on goods, service marks, certification trademarks, collective marks, well-known marks, and trade names. Each serves a distinct purpose in the market.

Madrid System - International Registration of Trademarks

The Madrid System simplifies the process of trademark registration across multiple countries. Under this system, trademark owners can protect their trademarks in various countries by filing a single application with their national or regional trademark office. Currently, this system includes 96 member countries, making it an effective international solution for trademark protection.

3.6 Industrial Design

Industrial design encompasses the visual appeal of a product. To be registered, designs must meet specific criteria, including being new, not previously disclosed to the public, significantly distinguishable, and not contrary to public order or morality.

3.7 Layout Designs of Integrated Circuits

The exclusive rights granted to the layout designs of integrated circuits include the right of reproduction, importation, sale, and distribution for commercial purposes. To be registrable, a layout design must be original, not commercially exploited in India or convention countries, and significantly distinguishable from other registered layout designs.

3.8 Geographical Indications

Geographical indications (GIs) indicate the source of certain products and are associated with specific regions known for their unique characteristics. Registration of GIs involves detailing distinguishing characteristics and authorized users, including producers and traders.

3.9 Trade Secrets

Trade secrets cover a wide range of confidential business information, including inventions, formulas, and processes. They are preferred when inventions are not patentable, when patent protection is limited, or when reverse engineering is difficult.

3.10 Need of IP Strategy for Entrepreneurs

For entrepreneurs, an IP strategy is invaluable for a multitude of reasons. It enables them to protect their innovations, assign value to their intellectual property assets, position themselves effectively in the market, generate revenue, mitigate risks, gain a competitive edge, plan for the long term, implement defensive measures, and consider global expansion. An IP strategy is a vital tool for entrepreneurs to navigate the complex world of intellectual property rights and maximize their business potential.

4. Expression & Purification of Recombinant Proteins

About the Speaker:

Dr. Anil Pasupulati, Assistant Professor, Dept. Biochemistry, University of Hyderabad. Is an esteemed academic and researcher who has made significant contributions to the field of biochemistry. Dr. Pasupulati is currently an Assistant Professor at the Department of Biochemistry at the University of Hyderabad.

Dr. Anil embarked on his academic journey by earning his Ph.D. from ICMR-NIN, Hyderabad, in 2008. In 2012, Dr. Pasupulati's dedication and research acumen were recognized with the DBT-Welcome Trust Early Career Fellowship and the DST-Inspire Faculty Award. He continued to excel in academia, serving as an Assistant Professor at Osmania University from 2012 to 2014, after which he joined the University of Hyderabad, an Institute of Eminence, in 2014.

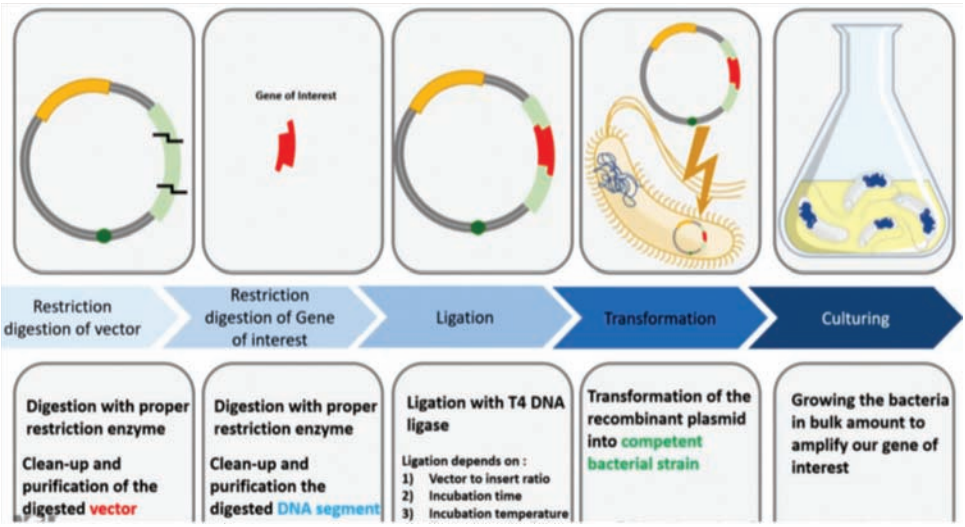
Dr. Anil Pasupulati's research interests are primarily focused on Diabetic nephropathy, chronic kidney disease, and the Bioinformatics of Kidney.

His dedication to excellence has earned him accolades, including the Chancellor's Award from the University of Hyderabad, the Gold Medal from Bionest-UoH, and recognition as a fellow of the Telangana Academy of Sciences.

Dr. Pasupulati's expertise has made him a sought-after speaker, and he has delivered numerous invited talks at various universities in India and abroad, including prestigious institutions such as Harvard, Emory, Pittsburgh, the University of Michigan, JNU, and BHU.

4.1 Introduction

Expression and purification of recombinant proteins are essential processes for various scientific and practical reasons.



These processes are often undertaken for purposes such as structural analysis (utilizing techniques like X-ray crystallography and NMR spectroscopy), understanding enzyme function, exploring interaction partners, conducting biochemical and biophysical studies (e.g., phosphorylation and regulation), functional studies (including cellular localization via confocal microscopy), and potentially for pharmaceutical interventions.

Key considerations when dealing with the expression and purification of recombinant proteins:

4.2 Protein Construct and Expression Host Choice:

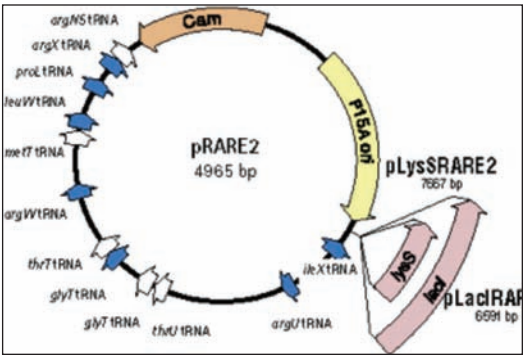
- The choice of protein construct depends on whether you are working with an entire protein or a domain of a larger protein.
- The selection of the expression host depends on the origin of the protein. Bacterial expression is common for proteins of bacterial origin, while non-bacterial proteins might require higher organisms like yeast, insect cells, or mammalian cell lines.

4.3 Expression Host Selection:

- Bacterial expression systems offer advantages such as ease, high expression levels, low protease activity, but might have challenges related to protein solubility and post-translational modifications.
- Eukaryotic expression systems provide advantages like protein solubility and post-translational modifications but are more expensive, yield lower quantities, and can involve protease activity.

4.4 Specialized Bacterial Strains:

- Specialized strains like BL21(DE3) can be used for protein expression, aided by the presence of the lac Repressor and T7 RNAPol.
- Other strains like BL21_pLysS and Rosetta2 can be employed to reduce leaky expression and overcome codon usage deficiency.



4.4 Expression Vector Selection and Fusion Partner:

- The choice of vector and the need for a fusion partner can significantly impact the success of protein expression.
- Fusion partners can improve solubility, but purified proteins may precipitate when cleaved from their fusion partners.

4.5 Ligation-Independent Cloning (LIC):

- LIC is a subcloning method that allows for seamless and efficient gene transfer, reducing the need for traditional ligation and amplifying the chances of success.

4.6 Bacterial Transformation:

- Transformation is the process of introducing plasmid DNA into bacterial cells.
- This can be achieved through a combination of factors, including the use of CaCl2 and heat or electroporation to create pores in the cell wall.

4.7 Small Scale Solubility Experiments:

- Before starting large-scale protein expression, it is crucial to conduct small-scale solubility experiments.
- These experiments involve varying factors such as inducer concentration, expression temperature, and construct variations to ensure successful expression.

4.8 Column Chromatography:

- Purification of recombinant proteins is typically achieved through various chromatography techniques, including size-exclusion chromatography (SEC), ion-exchange chromatography, and nickel-affinity chromatography.

4.9 Protease Cleavage Site and Affinity Tags:

- Protease cleavage sites and affinity tags are important for the purification process.
- Common tags include 6xHis, GST, and MBP.

4.10 Thermal Stability and Buffer Selection:

- The thermal stability of recombinant proteins and the selection of appropriate buffers play a critical role in maintaining protein activity and stability during purification.

The successful expression and purification of recombinant proteins require careful consideration of these factors, depending on the unique characteristics of each protein and the goals of the research or application.

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DAY 02

DATE: 02nd November 2023

VENUE: 06th Floor, Aranya Bhavan, Hyderabad

No. of Participants: 26

Detailed Agenda

Day 2 2nd November, 2023	Particulars
10:30 - 11:00 AM	Inaugural Session - Welcome and Opening Remarks by Dr. Shanti & Dr. Radhika - Open Mic to the Participants
Biology 11:00 - 11:45 AM	Session 1 - Basic Techniques in Genetics and Molecular - Speaker: Prof.Manjula Bhanoori - Affiliation: HOD of Biochemistry OU - Title: CRISPR-Cas9 Genome editing: Genesis and Applications - Presentation and Q&A
11:45 - 12:00 PM	Tea Break
12:00 - 1:00 PM	Session 2 - Workshop on Animal Cell Culture - Speaker: Prof. Sandeepta Burgula - Affiliation: Director CFRD, HOD of Microbiology, OU Hyderabad - Topic: Role of Haptoglobin in Molecular Pathogenesis of SEPSIS - Presentation and Q&A
1:00 - 2:00 PM	Lunch Break
2:00 - 3:00 PM	Session 3 - Awareness on Intellectual property Rights - Speaker: Subhajit Saha - Affiliation: Legal head of resolute 4 IP, Begumpet - Topic: Importance of IPR filing and best practices - Presentation and Q&A
3:00 - 4:00 PM	Session 4 - Workshop on Molecular Docking, Virtual Screening & Computational Biology - Speaker: Dr. Someshwar Sagurthi - Topic: Bioinformatic applications in Advanced research - Affiliation: Assistant Professor, Dept. of Biotechnology, OU - Presentation and Q&A Session
4:00 PM	Day-2 Session Concluding Remarks - Recap of the day's highlights - Announcements for Day 3 - Acknowledgment of Speakers, Sponsors, and Participants - Closing Remarks and Thank You

PARTICIPANTS LIST:

Institution	Sl. No.	Name of the Participants
National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad	01.	Dr. K. Venkat Rao
	02.	Dr. B. Lakshmi
	03.	Ms. Nehal Bhatt
	04.	Ms. Pilli Pushpa
	05.	Mrs. Vijaya Madhyanapu Golla
	06.	Mr. H M Chandra Mouli
	07.	Mr. Arbaz Sujait Shaik
	08.	Ms. Divya
	09.	Ms. Dharipally Harini
Jawaharlal Nehru Technological University (JNTHU), Hyderabad	10.	Dr. A. Uma
	11.	Dr. Ch. Kalyani
	12.	Dr. Venkanna
	13.	Dr. Ranjit
	14.	Dr. Venkateshwara Reddy
Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad	15.	Dr. Anuradha
	16.	Dr. Lakshmi Prasanna
	17.	Dr. SNCVL Pushpavally
Forest College and Research Institute (FCRI), Hyderabad	18.	Prof. M. Mamatha
	19.	Dr. Mohammed Abdul Waseem
	20.	Mr. Bochu Jeevan
	21.	Mr. Pathapelly Rajashekhar
Centre for Cellular & Molecular Biology (CCMB), Hyderabad	22.	Dr. Saloni Kakkar
	23.	Dr. Leela Kumari
Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad	24.	Dr. Rama Krishna
	25.	Dr. Lavyana Tayi
	26.	Ms. Sai Charitha Mullaguri

SYNOPSES OF THE LECTURES:

1. CRISPR-Cas9 Genome editing: Genesis and Applications

About the Speaker:

Prof.Manjula Bhanoori, HOD of Biochemistry, Osmania University is a distinguished academic and researcher with a wealth of experience in the field of biochemistry. Dr. Manjula Bhanoori currently serves as the Professor and Head of the Department of Biochemistry at the University College of Science, Osmania University. Her academic journey includes a tenure as a DST-Woman Scientist at the Centre for Cellular and Molecular Biology, Hyderabad.

Dr. Manjula obtained her doctoral degree from Osmania University in 2000 and subsequently conducted post-doctoral research at the University of Tennessee Health Sciences Centre, Memphis, USA.

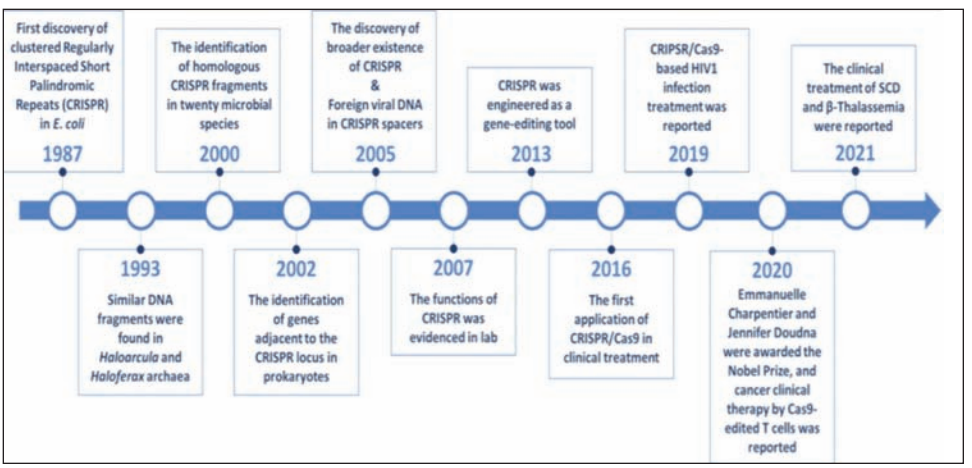
Throughout her career, she has successfully completed several major research projects funded by organizations such as DST and UGC, with an ongoing project supported by ICMR. Dr. Bhanoori has also played an active role in various scientific, academic, and ethical committees and serves as a reviewer for several esteemed international journals. Her expertise and research contributions are notably recognized in the study of gynaecological disorders, particularly endometriosis and PCOS.

In 2020, she was honoured as a Fellow of the Telangana Academy of Sciences (FTAS) and, more recently, received the Best Teacher Award from the Government of Telangana in 2022. Her outstanding work has been further acknowledged with accolades, including the International Research Excellence Award in 2022, the Award for Excellence in Research in the 10th Faculty Branding Awards, the Outstanding Scientist Award in 2022, and the NTR Birth Centenary Award in 2023.

Lecture Synopsis:

1.1 Time Line of CRISPR:

The origins of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology can be traced back to 1987 when Japanese scientist Ishino and his team accidentally discovered repetitive palindromic DNA sequences interspersed with spacers in Escherichia coli. Notably, the 2020 Nobel Prize was awarded to Jennifer Doudna and Emmanuelle Charpentier for their groundbreaking work on CRISPR.



1.2 Nobel Prize in Chemistry 2020

CRISPR operates as a bacterial adaptive immunity system and, astonishingly, as a revolutionary genome editing tool. The transformative year was 2012 when Jennifer Doudna and Emmanuelle Charpentier demonstrated that CRISPR/Cas-9 could edit DNA with precision, speed, efficiency, and cost-effectiveness. The key to this capability is the guide RNA, comprised of CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA), forming a single guide RNA (sgRNA) to target specific genes.

1.3 CRISPR-Cas in Bacteria:

Around 50% of bacteria and 90% of fungi naturally possess CRISPR-Cas systems. The Cas proteins in these systems function as helicases and nucleases. These Cas proteins encompass 93 genes grouped into 35 families based on the sequence similarity of their encoded proteins.

1.4 CRISPR as Bacterial Adaptive Immunity:

CRISPR functions as a bacterial adaptive immunity system, protecting bacteria from viral invasions. It operates through a combination of CRISPR RNA (crRNA) and transactivating CRISPR RNA (tracrRNA). The crRNA, approximately 18-20 base pairs long, specifies the target DNA by binding to it, while tracrRNA serves as a binding scaffold for the Cas-9 nuclease. In genome editing, crRNA and tracrRNA can be synthetically combined to form a single guide RNA (sgRNA) to target specific genes.

1.5 Applications of CRISPR:

CRISPR-Cas9 has become a revolutionary tool for genome editing since its discovery in 2012 by Doudna and Charpentier. The system allows precise, efficient, and accurate editing of genes across various organisms. It has found applications in therapies for diseases such as hemoglobinopathies and has been instrumental in the diagnosis of conditions like COVID-19.

1.6 Success Stories and Controversies:

CRISPR technology has enabled remarkable success stories in treating genetic disorders like sickle cell disease. However, it has also sparked controversies, such as the case of Chinese researcher He Jiankui, who claimed to have created gene-edited twins. The implications and ethical considerations surrounding these developments are subjects of ongoing debate.

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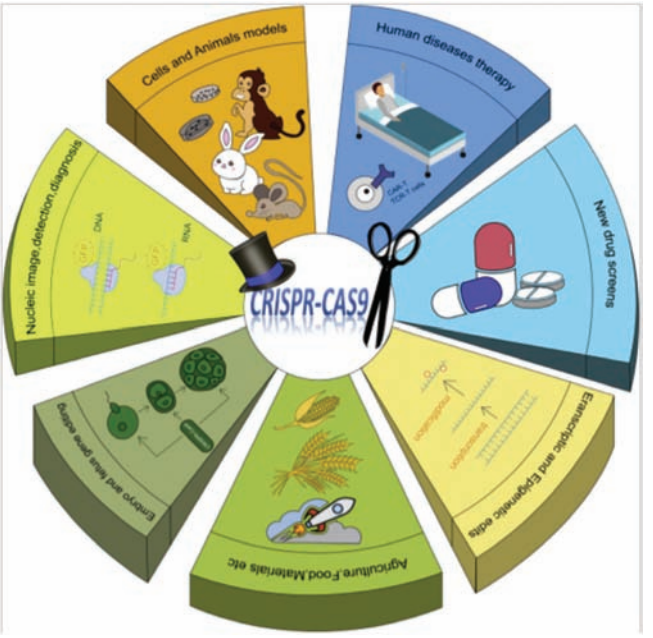


Challenges and Future Directions:

While CRISPR holds enormous promise, challenges remain, including issues related to immunogenicity, delivery, and off-target effects. Future directions involve obtaining regulatory approvals for CRISPR-based therapies, like those for sickle cell disease, and exploring new applications in areas such as Type 1 diabetes treatments.

1.8 Ethical Considerations:

With the continuous expansion of CRISPR applications, ethical considerations are paramount. As we delve deeper into this technology, there is a growing realization that more ethical questions and considerations will emerge, making it essential to stay vigilant in addressing these concerns. This lecture provided a comprehensive overview of the history, functionality, applications, and ethical considerations of CRISPR-Cas9 genome editing, emphasizing its potential to revolutionize science and medicine.

02. Role of Haptoglobin in Molecular Pathogenesis of SEPSIS

About the Speaker:

Prof. Sandeepta Burgula, Director - Central Facilities for Research and Development, HOD of Microbiology, Osmania University, Hyderabad is a distinguished academic and researcher with a strong background in microbiology. Dr. Sandeepta Burgula currently serves as the Head of the Department of Microbiology and the Director of Central Facilities for Research and Development at Osmania University, Hyderabad, India.

Dr. Sandeepta earned her Ph.D. in Biochemistry from Osmania University, and her research endeavours primarily focus on molecular pathogenesis. Her laboratory is dedicated to exploring the role of an acute phase protein, Haptoglobin, in bacterial sepsis. Notably, her team has

developed a cost-effective nanoprobe assay for the early detection of sepsis, a vital advancement in predicting its severity.

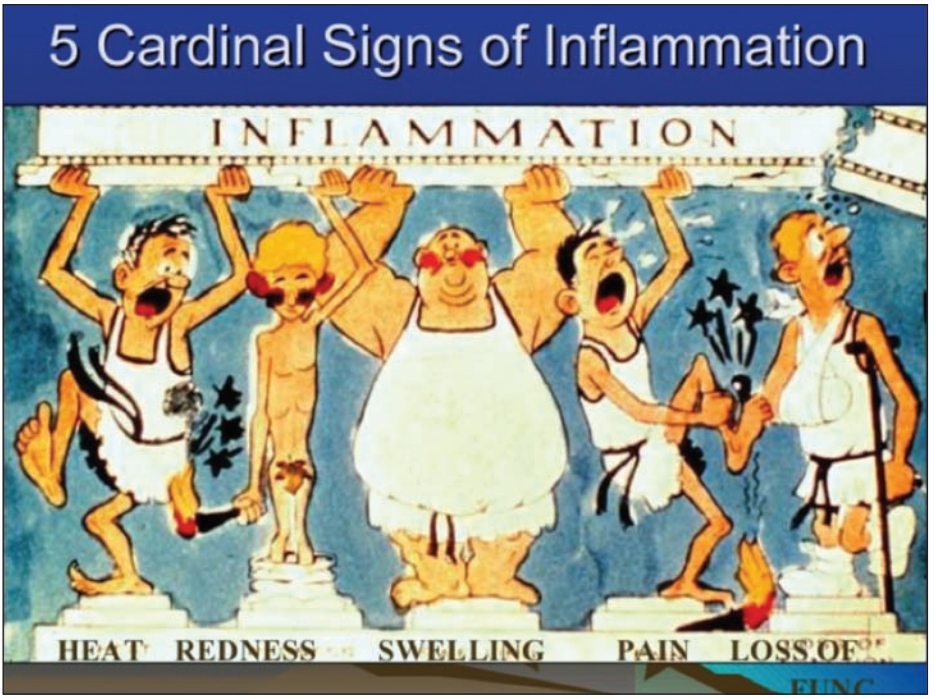
Her contributions to the scientific community have been recognized with several accolades, including her admission as an Associate Fellow of the Telangana Academy of Sciences in 2017, seven awards for paper presentations at national and international conferences, and international travel awards from DBT and ICMR. Dr. Burgula has published 23 research articles and authored three book chapters. She is also a valued reviewer for esteemed international journals, including Toxicology in vitro and the Journal of Ethnopharmacology.

In addition, she holds three Indian patents, one of which has been recently granted, and has guided four Ph.D. students in their research pursuits.

Lecture Synopsis:

2.1 Introduction:

Inflammation is a complex adaptive response to noxious conditions, such as infection and tissue injury, aimed at restoring homeostasis. In response to infection, the body produces various inducers of inflammation, leading to the activation of inflammatory mediators, which include lipids and proteins, particularly cytokines and chemokines.



One of the severe consequences of infection-induced inflammation is sepsis, often driven by a "cytokine storm." Sepsis is characterized by two main facets: the infection itself, which can be treated with antibiotics or antivirals, and the rise in the cytokine storm, which is a major cause of mortality.

2.2 What is Sepsis?

Sepsis, or sepsis syndrome, is a life-threatening medical condition characterized by an overwhelming infection and the body's inflammatory response to that infection. It often manifests as Systemic

Inflammatory Response Syndrome (SIRS) and can be triggered by various sources of infection, including normal flora, Gram-negative bacteria, and more. Sepsis results in organ failure, leading to high mortality, morbidity, and costs.

2.3 Current Diagnosis:

Several diagnostic markers are employed to assess and monitor sepsis. These include:

- WBC Count: Low white blood cell (WBC) count may indicate infection, often used in combination with other markers.
- Serum Lactate: Useful for initial prognosis and monitoring the response to resuscitation, although it doesn't differentiate between survivors and non-survivors.
- C-reactive protein (CRP): A sensitive marker for inflammation and tissue damage but doesn't distinguish between survivors and non-survivors in the early stages.
- Serum procalcitonin (PCT): Correlates with infection but cannot distinguish viral from bacterial infections and doesn't show differences in early-stage survivors and non-survivors.

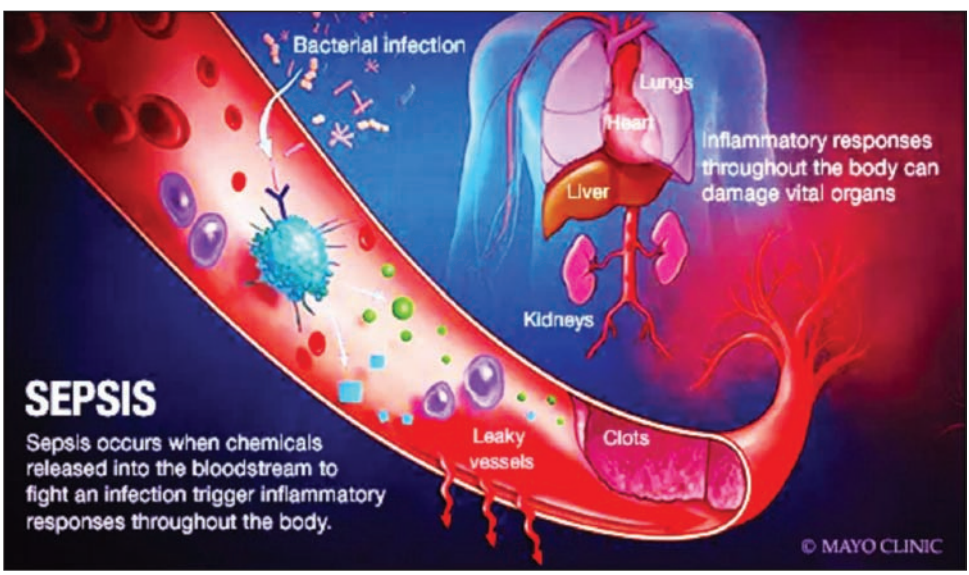
Timely diagnosis of sepsis is crucial, and a combination of PCT, CRP, white blood cell count, and clinical illness scoring is considered an effective diagnostic strategy.

2.4 Sepsis and Haptoglobin:

This section explores the role of various proteins, including haptoglobin (Hp), in sepsis. It identifies specific differentially expressed serum proteins in sepsis survivors and non-survivors from the onset of the disease to recovery or fatality. Notable findings include the delayed expression of certain proteins in non-survivors and the importance of haptoglobin as an anti-microbial peptide secreted in response to LPS stimuli.

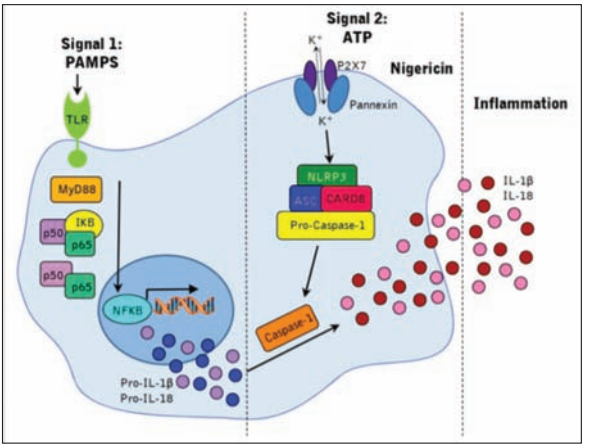
2.5 Inflammasome and Haptoglobin:

This section delves into the role of haptoglobin in inflammasome activation and its impact on caspase-1 and IL-1 cleavage in LPS-primed BMDM (bone marrow-derived macrophages). It reveals how haptoglobin enhances non-canonical inflammasome activation and provides insights into the NLRP3-dependent caspase-1 cleavage.

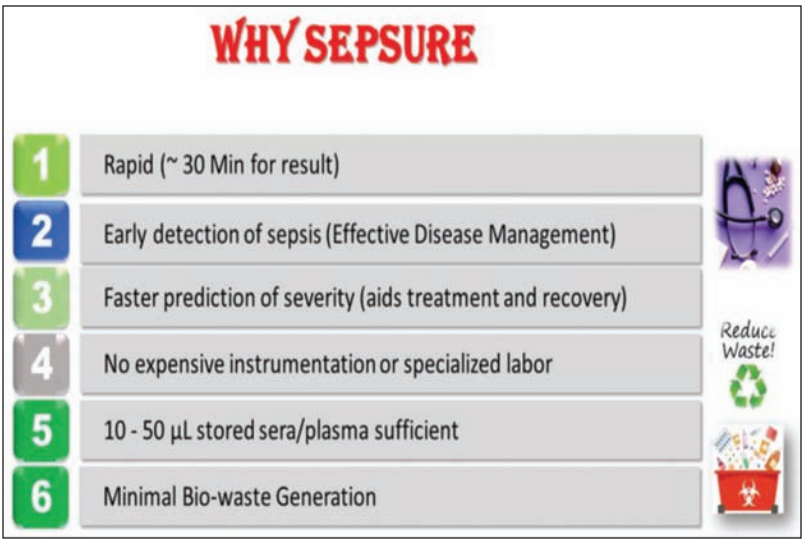


2.6 Haptoglobin in Sepsis Diagnostics:

This section discusses the potential for utilizing haptoglobin as a diagnostic marker for sepsis. It highlights the high incidence of sepsis in India, emphasizing the need for rapid sepsis diagnostics and the advantage of point-of-care tests.



The work mentioned is supported by various publications and grants, including the OU-DST-PURSE grant and the collaboration with Dr. Venkataraman Sritharan from Global Hospitals, Hyderabad.



2.7 Conclusion:

The lecture provides a comprehensive overview of the role of haptoglobin and other proteins in sepsis, their diagnostic potential, and the need for timely and effective diagnostic tools for sepsis, particularly in a high-incidence region like India.

03. Importance of IPR filing and best practices

About the Speaker:

Mr. Subhajit Saha, Legal Head of Resolute4IP, Begumpet, Hyderabad is an accomplished professional with extensive expertise in the legal and intellectual property rights (IPR) domain. Mr. Saha currently serves as the Head of Legal and IPR at the Resolute Group of Companies, where he has been instrumental in their IPR initiatives since December 2021. He is also the Co-founder of Resolute4IP, an IP service arm of Resolute dedicated to delivering affordable IP services to a wide audience.

Mr. Saha holds a postgraduate degree in Microbiology with a PG Diploma in Patent Laws from Nalsar, as well as a PG diploma in Planning and Project Management from the University of Hyderabad. He is a Registered Patent and Trademark Agent with the Indian Patent Office (IPO) and holds a Masters in LLM IPR from Osmania University. His expertise has taken him to 15 countries for lectures and training purposes.

Currently, his focus lies in building IP management portfolios for MSMEs, startups, academia, and grassroots innovators. He is dedicated to assisting them in registering their IP and accessing markets. Additionally, Mr. Saha is actively involved in the GI registration and branding of handicrafts, agriproducts, and textile products from Telangana and other states of India.

Lecture Synopsis:

3.1 Intellectual Property (IP):

Intellectual Property (IP) encompasses various categories: Copyright for expressions of ideas, Patents for inventions, Trademarks for branding, Industrial designs for product appearances, and Trade secrets to protect confidential information. IP matters because it enables creators to earn a living from their work, preventing others from immediately copying their creations. The concept of IP has evolved from mere property to intellectual property rights, covering these categories.

3.2 Patents:

Patents are exclusive rights granted by governments for new, useful, and non-obvious inventions, typically valid for 20 years. They provide the right to exclude others from making, selling, using, or importing the claimed invention. This offers various advantages, including preventing copying, slowing competitors, generating revenue from licensing, and enhancing brand value.

Why Patents?

- Advantages of patents include preventing copying, slowing down competitors, having the freedom to operate, generating revenue from licensing or selling, cross-licensing, raising funds, stimulating joint ventures, and enhancing brand value.

What Can Be Patented?

- Various elements can be patented, such as products, compositions, apparatuses, processes, or improvements on existing patented inventions.

Exclusions:

- Some things are excluded from IP protection, such as abstract ideas, discoveries, mere arrangements or rearrangements, plants and animals, business methods, computer programs, and methods of playing games.

उपलब्धि | किसान ने डीजल जेनरेटर में बदलाव कर कम की लागत, कई बड़ी कंपनियों ने दावे को दी थी चुनौती

गोला के किसान की तकनीक को मिला पेटेंट

■ मनीष मिश्र

गोरखपुर। गोरखपुर के एक किसान ने नामी कंपनियों को पछाड़कर पेटेंट हासिल कर लिया है। किसान ने डीजल जेनरेटर में बदलाव किया। इससे जेनरेटर में डीजल की खपत 20 फीसदी कम हो गई। उसकी आवाज व कंपन कम हो गई। इस तकनीक को ड्राइव अरेंजमेंट्स फॉर फ्यूल इंजेक्शन पंप कहते हैं।

गोला के हटवा दूबे निवासी सत्य प्रकाश दुबे मकैनिकल में डिप्लोमा कर चुके हैं। वह खेती के साथ ही आजीविका के लिए डीजल इंजन की मरम्मत का भी काम करते हैं। उनकी तकनीक से जेनरेटर के निर्माण की लागत भी करीब 10 से 15 फीसदी कम हो गई। उन्होंने इस तकनीक के पेटेंट के लिए वर्ष 2017 में दावा किया था। अब जाकर 5 अगस्त को भारत सरकार से उन्हें पेटेंट प्रमाण पत्र जारी हुआ है। इस पेटेंट को पाने के लिए उन्हें सुजुकी, होंडा व अमेरिका की डेल्फी जैसी नामी कंपनियों की चुनौतियों से भी पार पाना पड़ा। इन कंपनियों ने किसान के दावे को पेटेंट कार्यालय में चुनौती दी थी। किसान द्वारा दिए गए जवाब के बाद कंपनियों ने अपना दावा वापस ले लिया। डीजल इंजन की तकनीक में पेटेंट पाने वाले वह पूर्वी यूपी के पहले किसान हैं।

आवाज हुई कम P04

सत्य प्रकाश दुबे

20 % तक घटी डीजल की खपत जेनरेटर में बदलाव के बाद

20 17 में किया था तकनीक पेटेंट कराने का दावा

सरकार ने जारी किया पेटेंट

सत्य प्रकाश दुबे ने बताया कि जेनरेटर में नई तकनीक का प्रयोग वर्ष 2014 में किया। इसे नवाचार कहते हैं, इसकी जानकारी नहीं थी। वर्ष 2018 में काउंसिल फॉर साइंस एंड टेक्नोलॉजी के कार्यक्रम में वह जेनरेटर को ले गए। वहां एमएमएमयूटी के विशेषज्ञ मिले। उन्होंने नवाचार और पेटेंट की जानकारी दी। जिसके बाद पेटेंट के लिए दावा किया गया था। इसमें एमएमएमयूटी के शिक्षकों ने मदद भी की।

3.3. How Does One Get a Patent?

1. Starting the Process:

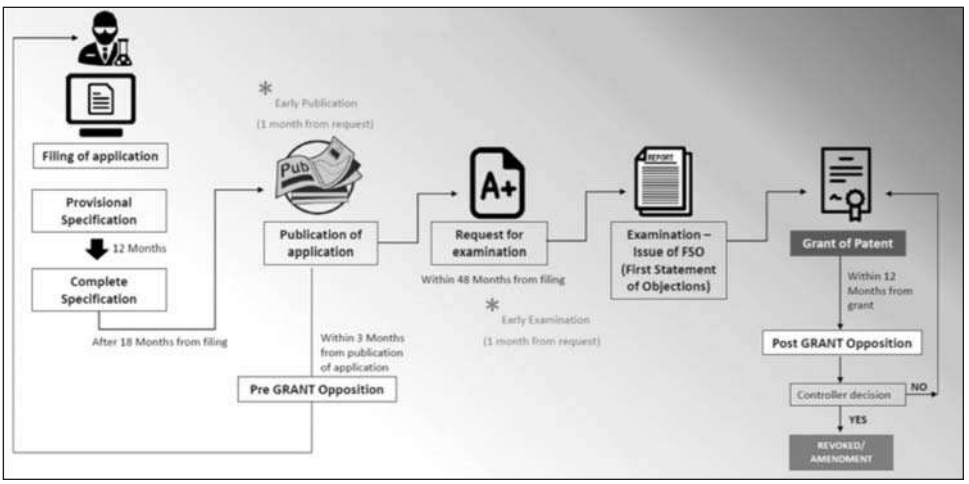
- The patent application process begins with identifying the invention, describing it in the application, and filing the application.

2. Where to File:

- The choice of which patent office to file with depends on the applicant's location, place of business, or service address.

3. Types of Patent Applications in India:

- Provisional Application (which should be converted to a complete specification within 12 months).
- Complete Application.
- Divisional Application.
- Patent of Addition.
- PCT National Phase Application.



3.4 Industrial design

Industrial design protection focuses on the ornamental or aesthetic aspects of useful articles. It offers advantages like preventing copying, slowing competitors, generating revenue through licensing, and enhancing brand value.

3.5 Trademarks

Trademarks serve as brand identifiers, distinguishing one enterprise's goods or services from another. They are registered with national trademark registries and have a 10-year validity, renewable indefinitely.

3.6 Copyright

Copyright protects various original and creative expressions, including literary works, music, films, computer programs, and more. Copyright holders have the right to control various uses of their work, such as reproduction and adaptation.

3.7 Plant Variety Protection

Plant Variety Protection & Farmers' Rights apply a sui generis system to protect new plant varieties in some countries like India, based on criteria like novelty, distinctiveness, uniformity, and stability.

3.8 Trade secrets

Trade secrets protect confidential information with commercial value, providing legal remedies against unauthorized use or disclosure. It's crucial to identify, protect, and enforce intellectual property rights to benefit from innovation and creativity.

04. Bioinformatic Applications in Advanced Research

About the Speaker:

Dr. Someshwar Sagurthi, Assistant Professor, Department of Biotechnology, Osmania University, Hyderabad is a distinguished scientist and researcher with a rich academic background and extensive expertise in biotechnology and crystallography. Dr. Someshwar earned his M.Sc. degree in Biotechnology from Jawaharlal Nehru University, New Delhi, India, in 2001. He continued his academic journey by obtaining a postgraduate diploma in Bioinformatics from the same university in 2002.

His pursuit of knowledge led him to receive a Ph.D. in Crystallography and Biophysics from the prestigious Indian Institute of Science, Bangalore, India, in 2009. Following his doctoral studies, from 2009 to 2013, he served as a Postdoctoral Research Associate at The Scripps Research Institute in San Diego, USA, and at The Samuel Roberts Noble Foundation in Ardmore, USA.

In 2013, Dr. Sagurthi joined the Department of Genetics & Biotechnology at Osmania University, Hyderabad, as an Assistant Professor. Here, he established the Drug Design and Molecular Medicine Laboratory, focusing on protein crystallography, computer-aided drug design, homology modeling, QSAR, bioinformatics, and medicinal chemistry.

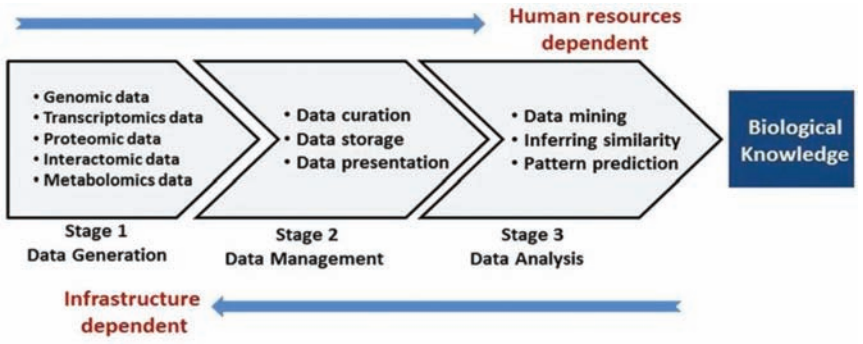
As a researcher and educator, Dr. Someswar embodies a dedication to advancing our understanding of complex biological systems and developing solutions to critical health challenges.

Lecture synopsis:

4.1 Introduction:

The ongoing "Golden Era" of Biology has seen the advent of Genome Projects that have amassed a wealth of biological data, profoundly influencing research and medical practices. This revolution is marked by a paradigm shift, treating biology as an information science rather than merely a field of biochemical technologies. Researchers have transitioned from traditional lab techniques to computational approaches, emphasizing the importance of bioinformatics.

4.2

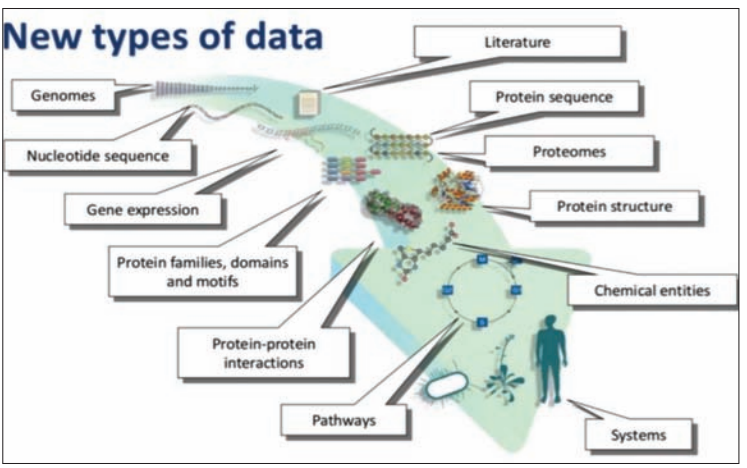


Bioinformatics Tools and Data Acquisition

Bioinformatics tools play a crucial role in data acquisition and treatment. Researchers can obtain sequences of interest from their own experiments or repositories of previously studied materials. These tools standardize data treatment and offer user-defined parameters and conditions for analysis. They also enable the visualization of data through interactive means, including graphs, tables, videos, and 3D visualizations.

Bioinformatics aids in analyzing genomics data by accessing information like accession numbers, protein characteristics, annotations, sub-localization, expression profiles, and 3D folding. These tools are invaluable for discovering conserved regions, identifying similar sequences, and exploring evolutionary relationships among genes.

One critical challenge in bioinformatics is the absence of a single software package capable of performing all functions seamlessly. Researchers often need to utilize a range of tools to address different aspects of genomics analysis effectively.



4.3 COVID-19 and the Role of Bioinformatics

In the context of the global COVID-19 pandemic, bioinformatics has played a pivotal role. Its contribution spans understanding the SARS-CoV-2 genome, analysing massive datasets for predictive modelling, predicting protein structures, and facilitating drug discovery and vaccine development. These applications highlight the significance of bioinformatics in tackling public health crises.

4.4 Protein Structures and Structure Prediction

Understanding protein structures is vital for deciphering their functions. Bioinformatics tools and methods, such as X-ray diffraction, NMR, and cryo-electron microscopy, help determine protein structures, which is particularly challenging when studying complex biomolecules. Protein structures offer valuable insights for rational drug design and enzyme engineering.

4.5 Rational Drug Design and Ligand-Receptor Interactions

Rational drug design, guided by bioinformatics, involves the creation of molecules that selectively target biological entities. Lipinski's Rule of Five, which defines drug-likeness criteria, plays a pivotal role in this process. Additionally, the study of drug-receptor interactions, such as in the context of asthma management, showcases how bioinformatics contributes to personalized medicine.

Bioinformatics empowers researchers to delve into systems biology, providing a holistic view of biological interactions, pathways, and functional annotations. Systems biology offers valuable insights into the genetic basis of disorders, including the study of genetic variations and single nucleotide polymorphisms (SNPs).

4.6 The Evolution of Biological Research

The evolution of biological research reflects the integration of biology, computer science, and information technology into a single discipline. While biologists generate molecular data, bioinformaticians analyse this data to answer crucial biological questions. Computer scientists and mathematicians develop the tools and algorithms that enable the storage, retrieval, and analysis of biological data.

4.7 History and Key Milestones in Bioinformatics

Bioinformatics finds its origins in the 1960s when Margaret Oakley Dayhoff created the first protein database and sequence assembly program. The need for computers and algorithms capable of handling data access, processing, storage, sharing, retrieval, visualization, and annotation underscores the field's growth.

4.8 Advanced Applications of Bioinformatics

Bioinformatics extends its reach into several advanced applications, including next-generation sequencing (NGS) analysis, genome assembly, structural bioinformatics, proteomics, and systems biology. These applications showcase the versatility and significance of bioinformatics in modern biological research.

4.9 Conclusion and Future Prospects

In conclusion, bioinformatics is a transformative force in the realms of biology and medicine. It empowers researchers to unlock the secrets of genomics, protein structures, and disease mechanisms. The future of bioinformatics holds immense promise, with ongoing advancements shaping the landscape of biological research and healthcare, particularly in the context of pandemic preparedness.

DAY 03

DATE: 03rd November 2023

VENUES:

1. SAILife Pharmaceuticals

2. Curia Pharmaceuticals

No. of Participants:

Detailed Agenda

Day 3 3rd November, 2023	Particulars
09:45 AM	Commencement of Industrial Visit
11:00 AM - 01:00 PM	Industrial Visit 01 Name: Sai Life Science Pharmaceuticals, Hyderabad Address: Office # L4-01 & 02, SLN Terminus Survey No. 133, Gachibowli - Miyapur Rd, Gachibowli, Telangana 500032 Nodal Officer: Dr. Ganesh, Associate Vice President, Sai Life Science
01:30 PM - 02:00 PM	Lunch
02:30 PM - 04:30 PM	Industrial Visit 02 Name: Curia Pharma Ltd. Address: Plot #9, MN Park, Turkapally, Shameerpet Genome Valley, RR District, Hyderabad - 500 078, India
06:00 PM	Conclusion of Industrial Visit

REGISTRATION OF PARTICIPANTS:

Institution	Sl. No.	Name of the Participants
National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad	01.	Ms. Nehal Bhatt
	02.	Ms. Pilli Pushpa
	03.	Mrs. Vijaya Madhyanapu Golla
	04.	Mr. H M Chandra Mouli
	05.	Ms. Dharipally Harini
Jawaharlal Nehru Technological University (JNTHU), Hyderabad	06.	Dr. A. Uma
	07.	Dr. Venkanna
	08.	Dr. Ranjit
Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad	09.	Dr. Anuradha
	10.	Dr. Lakshmi Prasanna
	11.	Dr. SNCVL Pushpavally
Forest College and Research Institute (FCRI), Hyderabad	12.	Prof. M. Mamatha
	13.	Dr. Mohammed Abdul Waseem
	14.	Mr. Bochu Jeevan
	15.	Mr. Pathapelly Rajashekhar
Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad	16.	Dr. Rama Krishna
	17.	Dr. Lavyana Tayi
	18.	Ms. Sai Charitha Mullaguri
Telangana State Council of Science & Technology (TSCOST), Hyderabad	19.	Dr. C. V. Ramakrishna
	20.	Dr. L. Shanti Kumari
	21.	Dr. V. Radhika
	22.	Mr. Abhinav B. Kamble
	23.	Mr. K. Krishna
	24.	Mr. I. S. Bhargava
	25.	Mr. K. Shivraju
	26.	Mr. K. Narendra

INDUSTRIAL VISIT 01

SAI LIFE SCIENCE PHARMACEUTICALS, HYDERABAD

Date: 03rd November 2023

Journey Details:

- Starting Point: Aranya Bhavan, Hyderabad

- Departure Time: 09:45 AM

- Arrival Time: 11:00 AM

- Duration of Visit: Approximately 2 hours

1.1 INTRODUCTION

The industrial visit to SAILife Pharmaceuticals Company in Gachibowli, Hyderabad, organized under the DBT - Training of Trainers (ToT) Program, provided a comprehensive look into the operations of this leading pharmaceutical company. The visit involved 26 trainers from various universities, including NIPER, JNTUH, OU - CPMB, PJTSAU, and FCRI. SAILife Pharmaceuticals is a renowned pharmaceutical company that specializes in the research, development, and manufacturing of life-saving drugs. The aim of the visit was to provide an overview and key insights of the SAILife Pharmaceuticals.

1.2 COMPANY OVERVIEW

SAILife Pharmaceuticals is a prominent pharmaceutical company headquartered in Hyderabad, India. It was founded in 1999 and is a leading global pharmaceutical company with a mission to improve the quality of life by developing innovative and affordable healthcare solutions. With a strong focus on research and development, SAILife has emerged as a key player in the pharmaceutical industry, catering to a wide range of therapeutic areas such as oncology, cardiovascular, neurology, and infectious diseases.

SAILife Pharmaceuticals is primarily engaged in service-oriented activities across four main divisions, each of which were explored during the visit.

1.3 WELCOMING OF THE VISITORS

Upon arrival at the IKP Knowledge Park - SAILife Pharmaceuticals, our group was warmly welcomed by Dr. Ganesh, the Associate Vice President of Sai Life Science. We were given a brief introduction to the company's history, vision, and core values. The visit commenced with a briefing on safety protocols by the security head, ensuring that all participants were well-informed and prepared for a secure and educational experience.

2. SAFETY PROTOCOLS

Sai Life Sciences places a strong emphasis on Health, Safety, and Environment (HSE) as a core component of its long-term business strategy to drive sustainable growth. The company's commitment to safety is evident through a comprehensive set of safety protocols and measures that are in place. Few key safety protocols at Sai Life Sciences are mentioned below;

Dos	Don'ts
1. Follow Safety Instructions: Always adhere to safety instructions provided by the facility staff and guides. Pay close attention to safety briefings.	1. Do Not Enter Restricted Areas: Do not enter areas that are marked as restricted, even if you are curious or tempted. These areas are off-limits for safety reasons.
2. Stay in Designated Areas: Remain in authorized and designated visitor areas. Do not enter restricted zones without permission.	2. No Unauthorized Equipment: Do not bring any equipment, tools, or materials into the facility without prior approval.
3. Ask Questions: Feel free to ask questions about safety procedures, emergency protocols, or any other concerns you may have. It's essential to have a clear understanding of the safety measures in place.	3. No Unsanctioned Sampling: Do not sample or handle any substances, powders, or materials without explicit permission and training.
4. Report Hazards: If you notice any hazards or unsafe conditions during your visit, promptly report them to facility staff.	4. Do Not Bypass Safety Measures: Do not bypass or disable safety equipment, alarms, or containment systems under any circumstances.
5. Be Mindful of Containment: Recognize the importance of containment protocols. Avoid touching or tampering with containment equipment, isolators, or other safety devices.	5. Avoid Interfering with Operations: Do not interfere with the ongoing operations of the facility, including machinery or equipment, without authorization.
6. Practice Good Hygiene: Maintain proper hygiene by washing your hands and following any hygiene protocols that are in place.	6. Don't Ignore Emergency Procedures: In case of an emergency or safety incident, do not disregard emergency procedures. Follow evacuation and safety protocols immediately.
7. Stay with Your Guide: Always stay with your designated guide or host. They are there to ensure your safety and provide guidance	7. Don't Bring Contaminants: Avoid bringing contaminants, such as food or drink, into areas where they are prohibited, as these can compromise safety.

After the safety protocol instructions, a guided tour of the manufacturing facilities, research laboratories, and quality control units was initiated by the company's representatives.

3. PROCESS R&D DIVISION

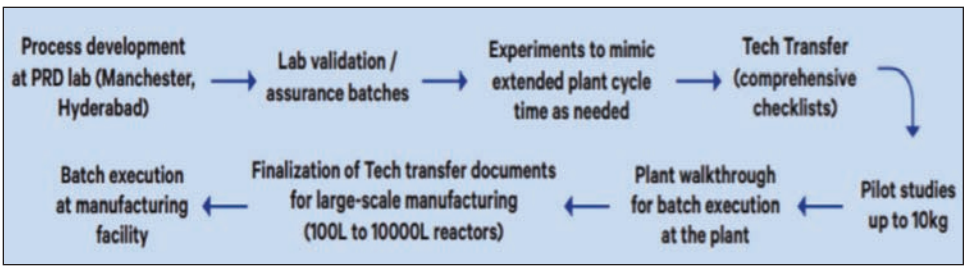
Dr. Prathap, a Chemist, led our exploration of the Process R&D division. This division is integral to Sai Life Science's research and development activities. Dr. Prathap provided a detailed overview of the research processes, equipment, and methodologies used in this division. We gained insights into the company's commitment to innovation and continuous improvement in pharmaceutical development.

Sai Life Sciences boasts a sprawling 12-acre fully integrated R&D campus, a testament to its commitment to cutting-edge research and development. With a scientific team comprising more than

1,200 dedicated members, the campus serves as a hub for innovation and excellence. The facility houses over 100,000 square feet of state-of-the-art laboratories, which are instrumental in driving advancements in pharmaceutical science.

Within these laboratories, various critical functions are meticulously executed. The Discovery division encompasses Biology, Chemistry, DMPK (Drug Metabolism and Pharmacokinetics), and Toxicology, all of which play a pivotal role in the early stages of drug development. This synergy between disciplines is vital for uncovering novel compounds and potential drug candidates.

Sai Life Sciences also boasts world-class Development facilities that encompass Process R&D and Process Safety. These departments ensure that the drug development process is meticulously fine-tuned and that each step adheres to the highest quality and safety standards.



The company's commitment to Process Engineering and Scale-up underscores its dedication to optimizing the production of pharmaceuticals on a larger scale. These capabilities allow for the smooth transition of drug candidates from the laboratory to commercial manufacturing, ensuring that promising therapies reach the market efficiently and safely.

Additionally, Sai Life Sciences excels in Early Phase delivery, where it is equipped to take promising drug candidates through the essential early development phases. With this robust infrastructure, Sai Life Sciences is well-positioned to continue making significant contributions to the pharmaceutical industry and play a pivotal role in the development of new and innovative treatments for various medical conditions.

3.1 Key Activities:

During our visit, we learned that the Process Design team at Sai Life Sciences is engaged in a variety of key activities. These activities encompass basic engineering studies, the evaluation of scale-up parameters using standardized templates, process modelling, simulation, and design. The team excels in determining the best-suited criteria for complex chemistries and unit operations, offering multidisciplinary engineering solutions for complex processes, including catalytic and gas induction reactions.

3.2 Software Enablers:

The use of advanced software tools is integral to Sai Life Sciences' Process Design capabilities. Notably, they rely on Dynochem and Aspen Plus for process simulation, AutoCAD for process instrumentation diagrams, and MixIT for estimating typical mixing scale-up parameters. These software enablers empower their teams to design and refine robust processes effectively.

3.3 Key Deliverables:

The Process Design team at Sai Life Sciences delivers a range of essential outcomes, such as estimating plant-scale reaction kinetics based on lab data, applying simulation tools, conducting rigorous

distillation calculations, and developing mixing profiles for plant-scale batches based on scale-up criteria. The team also conducts heat and mass transfer calculations and assesses the physical and chemical properties of new molecules. They engage in simulation studies, particularly in fractional distillation, heat exchanger rating, and separation efficiency of solvents.

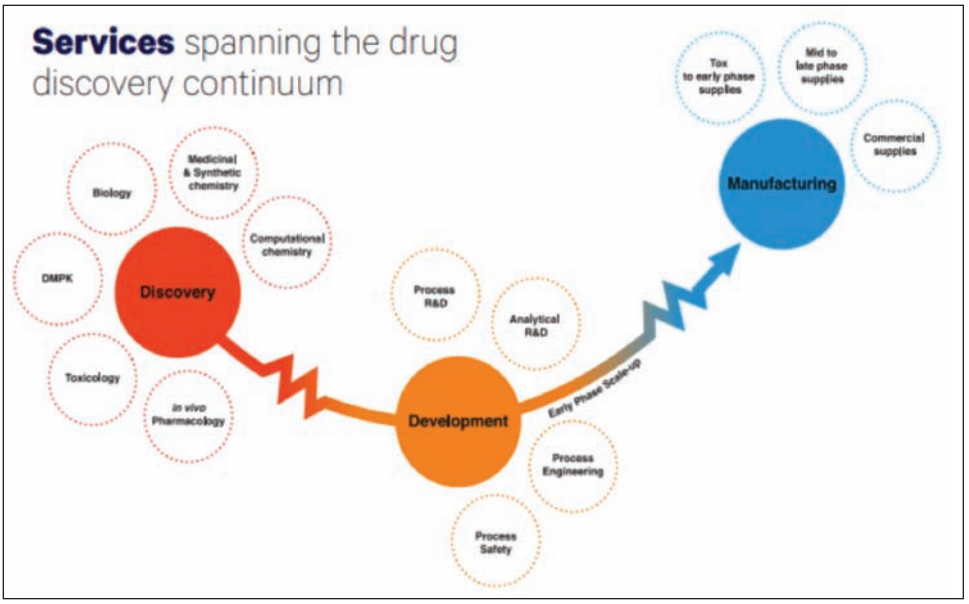
4. NEW TECHNOLOGY LAB

Dr. Devendra, our guide for the New Technology Lab, explained the advanced technologies and equipment utilized for research and development. This lab plays a pivotal role in keeping the company at the forefront of pharmaceutical advancements. Participants were exposed to cutting-edge research methodologies and the significance of staying updated in the field.

Their cutting-edge capabilities in the domains of Discovery, Development and Manufacturing underscore their commitment to delivering high-quality pharmaceutical and biotech products.

4.1 Discovery Lab:

Sai Life Sciences' Discovery phase is a formidable engine of innovation, driving the journey from target identification and validation to the Investigational New Drug (IND) stage. It harnesses the power of Creative Chemistry, High-Quality Biology, Drug Metabolism and Pharmacokinetics (DMPK), and Toxicology. This comprehensive approach ensures that promising drug candidates are thoroughly evaluated and optimized.



The Discovery Phase includes:

a) Integrated Drug Discovery

b) Complex Chemical Synthesis

c) In vitro and in vivo pharmacology

d) Comprehensive ADME (Absorption, Distribution, Metabolism, Excretion) and PK (Pharmacokinetic) profiling

e) Rigorous Toxicology evaluation

4.2 Development Lab:

The Development phase at Sai Life Sciences is marked by the transition from complex chemical synthesis to commercialization. The company boasts advanced laboratories equipped for early, late-phase, and commercial delivery. These facilities incorporate comprehensive analytical capabilities and are complemented by a fully equipped process safety lab, a pilot plant, and an early phase delivery block. Sai Life Sciences also excels in High Potency Active Pharmaceutical Ingredient (HPAPI) capabilities, with containment levels as low as 0.1 _g/m_. They utilize cutting-edge technology platforms, including Bio catalysis, Flow Chemistry, Chemo catalysis, Continuous Extraction, Continuous Distillation, and Lyophilization.

The automation of data capture and robust containment measures further enhance their development capabilities.

4.3 Manufacturing Lab:

Sai Life Sciences' Manufacturing phase operates with a capacity of 450 KL, encompassing reactor sizes ranging from 0.25 to 10 KL in SS, Hastelloy, and Glass-lined materials. The company maintains strict containment levels of 1 _g/m_, with 11 Clean Rooms certified to ISO - 8 standards.

Specialized blocks, including Amidites, HPAPI, Lyophilization at pilot and commercial scales, High-pressure blocks, and chromatography at commercial scale, cater to diverse manufacturing needs. Notably, the company conducts Cryo reactions at 2.5 KL, 4 KL, and 5 KL scales, showcasing their adaptability and versatility in the manufacturing domain.

Our visit to Sai Life Sciences was a revelation of their robust capabilities in Discovery, Development, and Manufacturing. The company's commitment to innovation, quality, and safety is evident throughout these phases, ensuring that pharmaceutical and biotech products meet the highest standards.

5. ANALYTICAL DIVISION

Dr. Shivraj, the head of the Analytical Division, provided a comprehensive tour of this crucial segment of Sai Life Science. The division encompasses various labs, including the WET lab, PANalytical - EMPYREAN, and NMR Testing Lab. Dr. Shivraj elucidated the purpose and functions of each lab, emphasizing the significance of quality control and assurance in the pharmaceutical industry. Additionally, he introduced the software tools like CAD used for analytical purposes. Analytical R&D, in particular, plays a crucial role in validating the integrity and efficacy of pharmaceutical compounds.

5.1 Rotating Bed Reactors: These reactors are crucial for bio-catalysis and are designed to provide an ideal environment for enzyme-driven reactions. They allow for precise control over reaction conditions.

5.2 Horizontal Shaker: The shaker is an essential tool for mixing and agitating cultures, ensuring efficient growth of microbial strains and enzyme production.

5.3 pH-Controlled Peristaltic Pump: Maintaining precise pH levels is critical in enzyme-driven reactions. The peristaltic pump ensures accurate pH control throughout the process.

5.4 Double-Jacketed Reactors: These reactors are versatile and equipped for various types of reactions, allowing for precise temperature control and reaction optimization.

5.5 Column Oven and Vortex Mixer: The column oven and vortex mixer are used for chromatographic separations and mixing, respectively. These instruments play a vital role in purification and analysis.

5.6 Jacketed Reactors: These reactors are essential for process development, enabling precise temperature control and reaction optimization.

5.7 Process Simulation Tools: Sai Life Sciences employs advanced software tools, including Dynochem, Aspen, and MixIT, to facilitate process simulation and optimization, ensuring efficient and economic feasibility.

6. KEY ACHIEVEMENTS

a. They have developed processes using enzymes like Novozyme-435 to achieve the desired chiral purity from small to multi-Kilogram scale.

b. Process development for asymmetric reduction of ketones using keto reductase enzymes has been successfully demonstrated from lab to plant scale, with 150 kilograms as a notable achievement.

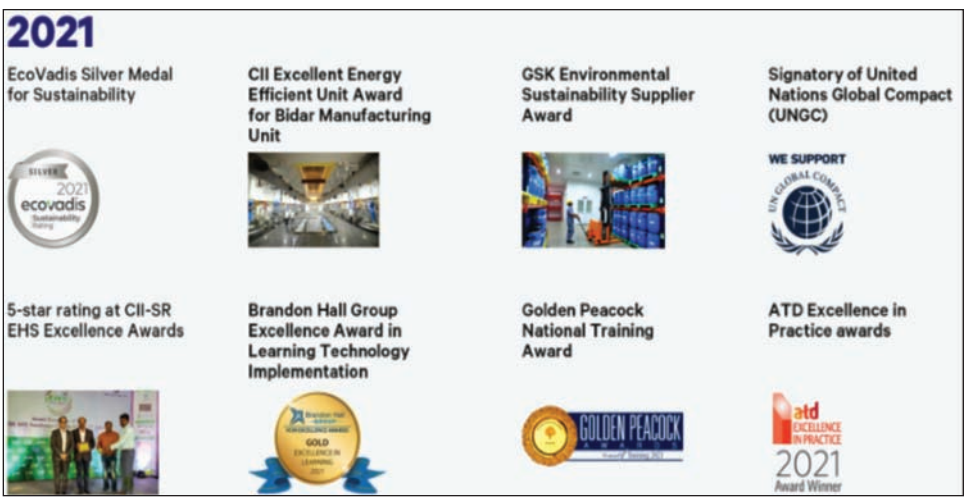
c. Multiple plant-scale campaigns have been executed using transaminase enzymes.

d. The team has excelled in recovery, recycling, and reuse of enzymes such as Lipase-TL.

e. Other achievements include trans-glycosylation on nucleosides and regio-selective acylation of sugar diols, as well as the implementation of flow biocatalysis.

f. Sai Life Sciences excels in High Potency Active Pharmaceutical Ingredient (HPAPI) capabilities, with containment levels as low as 0.1 mg/m_.

g. The ability to conduct Cryo reactions at different scales, including 2.5 KL, 4 KL, and 5 KL, showcases their adaptability in meeting diverse manufacturing requirements.



7. CONCLUSION

After the informative guided tour, Dr. Ganesh, the Associate Vice President of Sai Life Science, addressed the participants and provided concluding remarks. Participants had the opportunity to ask questions and clarify any doubts, ensuring a thorough understanding of the company's operations.

The industrial visit to Sai Life Science Pharma Company proved to be an enriching experience for all the participants. It provided a deep insight into the company's diverse divisions, its commitment to research and development, and its significant role in the pharmaceutical industry.

We left Sai Life Sciences with a deeper appreciation of the complexity and precision involved in the pharmaceutical research and development process. Their state-of-the-art facilities and adherence to strict safety and containment standards serve as a testament to their dedication to delivering high-quality products efficiently and effectively. We are grateful for the opportunity to learn from their expertise and witness their advanced capabilities.

INDUSTRIAL VISIT 02

CURIA PHARMA LTD., HYDERABAD

Date: 03rd November 2023

Journey Details:

- Starting Point: FCRI, Hyderabad

- Departure Time: 02:00 PM

- Arrival Time: 02:30 PM

- Duration of Visit: Approximately 2 hours

1.1 INTRODUCTION

Post lunch at Forest College and Research Institute (FCRI), Hyderabad we started for our Second Industrial visit to the Curia Pharma Company in Hyderabad. This visit was also organized under the DBT - Training of Trainers (ToT) Program, providing an invaluable opportunity to gain insights into the pharmaceutical industry.

1.2 COMPANY OVERVIEW

Curia Pharma Company, also known as Curia Ltd, is a leading Contract Development, Research, and Manufacturing Organization (CDMO) with a remarkable track record of more than 30 years in the pharmaceutical industry. The company has 29 global offices and is dedicated to providing comprehensive drug development services. Curia is a global contract research, development and manufacturing organization (CDMO), offering products and services across the drug development spectrum to help our partners turn their ideas into real-world impact. They partner closely with pharmaceutical and biotechnology companies to boost business performance and improve patients' lives. They also offer state-of-the-art analytical equipment and expertise, making them a vital player in the field.

Curia Ltd. was founded in 1991 as Albany Molecular Research, Inc. (AMRI). Originally known for the discovery and development of the active pharmaceutical ingredient (API) in Allegra, they built a strong foundation over the past three decades. Now known as Curia, they have added a wide array of new

capabilities and developed a comprehensive suite of solutions capable of serving customers across the drug development spectrum.

1.3 WELCOMING OF VISITORS

Upon arrival, the TSCOST team was warmly welcomed by the officials of Curia Ltd. This gracious reception set the tone for an informative and engaging visit. The warm reception was in line with the company's reputation for prioritizing hospitality and professionalism.

2. SAFETY FIRST PROTOCOL AT CURIA LTD.

An essential aspect of Curia Ltd's ethos is the safety of its employees. It was highlighted that the company had successfully completed more than three years in Hyderabad without any accidents. This emphasis on safety underscores the company's commitment to providing a secure and productive working environment.

2.1 Hierarchy of Safety Controls:

Curia Ltd. follows a structured hierarchy of occupational health and safety controls, starting with the elimination of hazards and proceeding through substitution, engineering controls, and administrative controls. This approach ensures a systematic and proactive approach to managing safety.

2.2 Containment Infrastructure:

Curia Ltd. maintains state-of-the-art laboratories, pilot plants, and manufacturing facilities equipped with advanced containment infrastructure. The performance achievement for containment infrastructure is impressive, with facilities designed to achieve 1 $\mu g/m^3$ for hazardous substances. Specialized High Potency Active Pharmaceutical Ingredient (HPAPI) development and manufacturing suites are designed to achieve containment levels as low as 0.1 $\mu g/m^3$. Containment infrastructure includes Isolators, Glove boxes, Drum Containment Systems (DCS), Powder Transfer Systems (PTS), Continuous liner ports, Closed sampling devices, and drum dispensing booths for safe handling of solvents.

2.3 Industrial Hygiene Capabilities:

Curia Ltd. has robust in-house capabilities for the sampling of powders and solvents. An in-house industrial hygiene laboratory is equipped with instruments such as sampling pumps, Heat Stress Meters, Noise Dosimeters, and VOC meters for personal monitoring.

2.4 Validation and Certification:

Standard Operating Procedures (SOP) are in place for the implementation of the industrial hygiene program, control banding, and containment strategy. Guidelines are established for the selection of containment equipment for the safe handling of hazardous agents. Qualitative and quantitative exposure risk assessments are conducted to identify and mitigate potential risks.

These safety protocols and infrastructure at Curia Ltd. reflect the company's dedication to ensuring the well-being of its employees, the environment, and the communities in which it operates while also maintaining the highest standards of quality and performance in the pharmaceutical industry.

3. DISCOVERY WING AT CURIA

3.1 Beacon:

Curia Pharma Ltd. excels in Beacon technologies, a groundbreaking approach to drug discovery. Beacon is a state-of-the-art platform that integrates cutting-edge technologies, data analytics, and scientific expertise to accelerate the identification of novel drug candidates. By leveraging Beacon, Curia enhances its ability to screen and profile compounds efficiently, identifying potential therapeutic leads faster and with greater precision. This technology plays a vital role in the early stages of drug discovery, optimizing the path toward groundbreaking pharmaceutical innovations.

3.2 Molecular Biology:

Molecular Biology is a cornerstone of Curia's Discovery phase. It involves the study of biological processes at the molecular level, encompassing DNA, RNA, and protein analysis. Curia's Molecular Biology capabilities are instrumental in target identification, validation, and understanding the mechanisms underlying diseases. This technology allows the company to manipulate and engineer biological molecules, facilitating the development of innovative therapeutic approaches.

3.3 Viral Vectors & Cell Engineering:

In the realm of drug discovery, Curia Pharma Ltd. harnesses the power of viral vectors and cell engineering to develop novel treatments. Viral vectors are essential tools for delivering genetic material into cells, and Curia's expertise in this field enables precise gene modification and manipulation. This technology is instrumental in gene therapy, regenerative medicine, and the creation of disease models for research.

3.4 Antibody Discovery & Engineering:

Antibodies are crucial components of the human immune system and are increasingly important in the development of therapeutic drugs. Curia's Antibody Discovery and Engineering capabilities allow for the identification and optimization of antibodies for various applications, from cancer treatment to autoimmune diseases. These technologies enhance the precision and efficacy of antibody-based therapies.

3.5 Recombinant Antibody Production:

Recombinant antibody production is a critical aspect of drug discovery. Curia Pharma Ltd. excels in producing therapeutic antibodies using recombinant DNA technology. This process involves genetically engineering cells to produce specific antibodies, ensuring high purity and consistent quality. This expertise positions Curia as a leader in antibody-based therapeutics.

3.6 Recombinant Protein Production:

Recombinant protein production is a fundamental part of the drug discovery process, enabling the synthesis of proteins with diverse functions. Curia's capabilities in this field ensure the production of recombinant proteins for a wide range of applications, from basic research to therapeutic development. These proteins serve as essential tools for studying diseases and potential drug targets.

4. DEVELOPMENT WING AT CURIA

4.1 Stable CHO Cell Line:

The establishment of a Stable CHO (Chinese Hamster Ovary) Cell Line is a pivotal step in biopharmaceutical development. Curia Pharma Ltd. possesses the expertise to create stable cell lines that produce recombinant proteins with consistent quality. These cell lines are essential for large-scale production of biopharmaceuticals and ensure product uniformity.

4.2 Upstream Process Development:

Upstream Process Development is a critical phase in biomanufacturing. Curia specializes in optimizing the processes involved in cell culture, fermentation, and protein expression. This expertise results in increased product yields and efficiency, which are essential for scaling up the production of biopharmaceuticals.

4.3 Downstream Process Development:

Downstream Process Development is focused on purifying and isolating biopharmaceuticals. Curia's capabilities in this area involve refining and streamlining purification processes to ensure product purity and safety. This is essential for meeting regulatory requirements and maintaining product quality.

4.4 Formulation Development:

Formulation development is a crucial step in drug product development. Curia's experts excel in formulating drug candidates into safe and effective dosage forms. They tailor formulations to optimize

drug delivery, stability, and efficacy, ensuring that the final product meets patient needs and regulatory standards.

4.5 Antibody Developability Analysis:

Antibody developability analysis involves evaluating the suitability of antibody candidates for therapeutic development. Curia's experts assess factors such as stability, manufacturability, and immunogenicity to determine the potential of antibody candidates, helping to identify promising drug leads.

5. MANUFACTURING WING AT CURIA

5.1 Mammalian GMP Overview:

Curia Pharma Ltd. adheres to Good Manufacturing Practices (GMP) for mammalian cell culture and biopharmaceutical production. GMP standards ensure the quality, safety, and consistency of pharmaceutical products, and Curia's commitment to GMP underscores its dedication to producing safe and effective therapies.

5.2 Quality Systems:

Quality systems are the foundation of Curia's manufacturing operations. These systems encompass a range of procedures and protocols that ensure the reliability and compliance of manufacturing processes, from raw material procurement to final product release.

5.3 Process Development:

Process development is key to optimizing manufacturing processes. Curia's experts continually refine and improve manufacturing methods, enhancing efficiency and product quality.

5.4 Therapeutic GMP Manufacturing:

Therapeutic GMP manufacturing focuses on producing biopharmaceuticals for therapeutic applications. Curia's manufacturing facilities adhere to strict GMP standards to ensure the safe and consistent production of pharmaceuticals.

5.5 Diagnostic GMP Manufacturing:

In addition to therapeutic manufacturing, Curia specializes in diagnostic GMP manufacturing. This involves producing diagnostic reagents and kits that meet stringent quality and regulatory requirements, providing accurate diagnostic tools for healthcare professionals.

6. ANALYTICAL DIVISION

6.1 WET Lab (Wet Chemistry Laboratory):

The tour commenced with a visit to the WET Lab, one of the analytical divisions of Curia Ltd. Here, we gained insights into the extensive range of wet chemical analyses conducted for quality control and research purposes. The lab's equipment and methodologies were discussed, emphasizing the precision required in pharmaceutical analysis.

A WET lab, short for Wet Chemistry Laboratory, is a specialized facility dedicated to conducting chemical analyses and experiments that involve liquid-phase reactions and manipulations. In contrast to "dry" labs, where work is predominantly theoretical or analytical, wet labs are equipped for hands-on experiments, often involving the use of chemicals, reagents, and various forms of liquid substances.

The primary purpose of a wet lab is to facilitate chemical reactions, including mixing, dilution, precipitation, and other wet chemistry techniques. Wet labs are crucial for sample preparation, where

samples are often processed, dissolved, and extracted for subsequent analysis. They house various analytical instruments and equipment for assessing the chemical composition and properties of substances, such as spectrophotometers, titration equipment, and pH meters. Due to the use of chemicals and potentially hazardous materials, wet labs are equipped with safety features like fume hoods, chemical storage, and personal protective equipment to ensure the safety of researchers.

6.2 PANalytical - EMPYREAN Lab:

Next, we explored the PANalytical - EMPYREAN lab, which featured advanced analytical equipment. The lab's role in characterizing and assessing pharmaceutical substances was highlighted. Participants were introduced to the significance of accurate data for drug development and quality assurance.

PANalytical's EMPYREAN is an advanced X-ray diffractometer that is used in materials science and crystallography to analyse the crystal structure of a wide variety of materials. It is a sophisticated and versatile analytical instrument that provides insights into the atomic and molecular arrangement of crystalline substances.

The EMPYREAN uses X-ray diffraction to investigate the arrangement of atoms in crystalline materials. This enables researchers to determine the crystal structure, phase identification, and crystallographic properties. It is known for its modularity, allowing it to be configured with various accessories and modules for different analytical needs. Also, it can analyse a wide range of materials, including metals, minerals, ceramics, polymers, pharmaceuticals, and more.

The instrument comes with sophisticated data analysis software that enables researchers to interpret diffraction patterns and derive valuable insights about the sample's structure.

6.3 NMR Testing Lab (Nuclear Magnetic Resonance Testing Lab):

The NMR Testing Lab showcased the company's capabilities in nuclear magnetic resonance (NMR) spectroscopy. This technology is pivotal in the structural analysis of pharmaceutical compounds. The visit to this lab provided an understanding of the vital role played by NMR in pharmaceutical research and development.

An NMR Testing Lab, or Nuclear Magnetic Resonance Testing Laboratory, is a facility equipped with NMR spectrometers, which are sophisticated analytical instruments used to study the molecular and structural properties of chemical compounds. NMR spectroscopy relies on the interaction of atomic nuclei with magnetic fields to provide insights into the composition and connectivity of atoms within molecules.

These instruments generate strong magnetic fields and radiofrequency signals to probe the nuclear spin properties of atoms in a sample. NMR labs are used to analyse the chemical structure, composition, and connectivity of organic and inorganic compounds. They can determine factors like molecular weight, configuration, and conformation. NMR is also used for quantitative analysis, such as determining the concentration of specific compounds in a sample.

It plays a crucial role in determining the three-dimensional structure of complex molecules, which is vital for the study of biomolecules, pharmaceuticals, and organic chemistry.

6.4 Other Testing Services:

Curia Pharma Ltd.'s Lab Testing Services encompass a comprehensive array of capabilities designed to support the pharmaceutical and biotech industries. These services are pivotal in the development, analysis, and quality control of pharmaceutical products.

6.4.1 Batch Release & Stability Lot Testing:

Curia Pharma Ltd. conducts batch release and stability lot testing to confirm the consistency and quality of pharmaceutical products. These tests assess various attributes such as potency, purity, and physical properties. Ensuring that each batch meets the predefined standards is vital for regulatory compliance and for delivering products that consistently meet the needs of patients.

6.4.2 Package & Device Testing:

The packaging and devices used in drug delivery are critical aspects of pharmaceutical products. Curia offers testing services to assess the integrity and compatibility of packaging materials and devices with the drug product. Ensuring that packaging does not compromise the stability or safety of the pharmaceuticals is essential for product quality and patient safety.

6.4.3 Preformulation & Material Science:

Preformulation and material science services at Curia are essential in the early stages of drug development. They involve the investigation of drug candidates and their physical properties. Preformulation studies help in selecting the most suitable formulation for a drug, considering factors like solubility, stability, and compatibility with excipients.

6.4.4 Solid State Chemistry:

Solid state chemistry plays a pivotal role in pharmaceuticals, particularly in understanding the crystalline structures and polymorphs of drug compounds. Curia's expertise in this area ensures a thorough comprehension of the solid-state properties of drug candidates, which has a direct impact on formulation, stability, and drug performance.

7. SOFT WARE APPLICATIONS

We were also briefed on the various software applications used at Curia Ltd, including Computer-Aided Design (CAD) and other tools for data analysis and research. The role of these software applications in enhancing efficiency and accuracy was emphasized.

8. CONCLUDING REMARKS

Following the informative and enlightening guided tour of Curia Pharma Ltd., the company's officials delivered valuable concluding remarks that resonated with the essence of the entire visit. Their closing words emphasized a critical aspect that extends beyond the walls of their cutting-edge laboratories and manufacturing facilities. The focus shifted to a broader, industry-wide perspective, addressing the crucial role of education in shaping the future of the pharmaceutical sector.

The officials of Curia Ltd. underscored the ever-evolving nature of the pharmaceutical industry, characterized by continuous advancements in science, technology, and regulatory standards. They stressed that to thrive in this dynamic landscape, it is imperative to have educational curricula that align closely with the emerging needs and demands of companies operating within the pharmaceutical sector.

The importance of educational curricula tailored to meet the industry's evolving requirements cannot be overstated. Such curricula should be designed to equip aspiring professionals with the knowledge, skills, and competencies necessary to tackle the complex challenges and opportunities presented by the pharmaceutical field. This entails not only providing a strong foundation in fundamental pharmaceutical sciences but also staying attuned to the latest developments in areas such as biotechnology, data analytics, regulatory compliance, and quality assurance.

A key takeaway from the visit to Curia Pharma Ltd. was the clear recognition of the pivotal role played by a skilled and knowledgeable workforce in the pharmaceutical sector. The officials emphasized that

the industry's continued growth and its ability to address global health challenges depend on a workforce that can seamlessly adapt to the industry's evolving landscape.

In essence, the visit to Curia Pharma Ltd. served as a reminder of the symbiotic relationship between industry and education.

The pharmaceutical sector thrives on innovation, research, and development, and the creation of innovative drugs and therapies that can transform the healthcare landscape. Educational institutions, therefore, have a significant role to play in nurturing the talent and expertise required to drive this innovation.

The officials of Curia Ltd. left a resounding message: Education is the cornerstone of a vibrant, sustainable, and innovative pharmaceutical industry. By fostering collaboration between industry leaders, educational institutions, and aspiring professionals, it is possible to ensure that the pharmaceutical sector remains at the forefront of scientific discovery and healthcare advancement. This collaborative spirit, as emphasized by Curia Pharma Ltd., will be instrumental in meeting the ever-evolving needs of the pharmaceutical industry and in improving the well-being of individuals around the world.

9. CONCLUSION

The industrial visit to Curia Pharma Company was an educational and eye-opening experience. It provided valuable insights into the pharmaceutical industry, emphasizing safety, advanced analytical techniques, and the importance of aligning educational curricula with industry needs. This visit will undoubtedly benefit the trainers and students from the participating universities as they prepare for careers in the pharmaceutical sector.

DAY 04

DATE: 01st November 2023

VENUE: 06th Floor, Aranya Bhavan, Hyderabad

No. of Participants: 26

Detailed Agenda

Day 4 **4th November, 2023**	**Particulars**
10:30 - 11:00 AM	Inaugural Session - Welcome and Opening Remarks by Dr. Shanti & Dr. Radhika - Open Mic to the Participants
11:00 - 11:45 AM	Session 1 - Quality Assurance in Pharma Industry - Speaker: Mr. K. Raghavendra - Affiliation: Head-QA, Formulations, GVK Biosciences Pvt. Ltd, Hyderabad - Title: Good manufacturing Practices (GMP)
11:45 - 12:00 PM	Tea Break
12:00 - 1:00 PM	Session 2 - Biotechnological tools in crop improvement - Speaker: Prof. B. Rama Devi - Affiliation: HOD Dept. of Botany, Osmania University - Topic: Plant tissue culture and its applications
1:00 - 1:30 PM	ToT Program Concluding Remarks - Recap of the Program's Highlights - Closing Remarks by Member Secretary & Chief Guest - Acknowledgment of Speakers and Participants - Certificates distribution Ceremony - Felicitation of Participants & Guest Speakers - Final Thanks & Program Closing announcement
1:30 - 2:30 PM	Lunch

PARTICIPANTS LIST:

Institution	Sl. No.	Name of the Participants
National Institute of Pharmaceutical Education and Research (NIPER), Hyderabad	01.	Dr. S. Gnanadhamu
	02.	Dr. K. Venkat Rao
	03.	Ms. Nehal Bhatt
	04.	Ms. Pilli Pushpa
	05.	Mrs. Vijaya Madhyanapu Golla
	06.	Mr. H M Chandra Mouli
	07.	Mr. Arbaz Sujait Shaik
	08.	Ms. Divya
	09.	Ms. Dharipally Harini
Jawaharlal Nehru Technological University (JNTHU), Hyderabad	10.	Dr. A. Uma
	11.	Dr. Ch. Kalyani
	12.	Dr. Venkanna
	13.	Dr. Ranjit
	14.	Dr. Venkateshwara Reddy
Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad	15.	Dr. C. V. Sameer Kumar
	16.	Dr. Anuradha
	17.	Dr. Lakshmi Prasanna
	18.	Dr. SNCVL Pushpavally
Forest College and Research Institute (FCRI), Hyderabad	19.	Prof. M. Mamatha
	20.	Dr. Mohammed Abdul Waseem
	21.	Mr. Bochu Jeevan
	22.	Mr. Pathapelly Rajashekhar
Centre for Cellular & Molecular Biology (CCMB), Hyderabad	23.	Dr. Archana B. Siva
Centre for Plant Molecular Biology (CPMB), Osmania University, Hyderabad	24.	Dr. Rama Krishna
	25.	Dr. Lavyana Tayi
	26.	Ms. Sai Charitha Mullaguri

SYNOPSES OF THE LECTURES:

01. Good manufacturing Practices (GMP)

About the Speaker:

Mr. K. Raghavendra, Head-QA, Formulations, GVK Biosciences Pvt. Ltd, Hyderabad is an accomplished professional with a remarkable 23-year career in the pharmaceutical industry, primarily in Quality Assurance. He holds an M. Pharma degree in Pharmaceutical Chemistry from Rajiv Gandhi University of Health Sciences, Bangalore, India.

Mr. Raghavendra has a strong understanding of cGMP guidelines and regulatory requirements, ensuring sustainable compliance with various global regulatory health authorities. He has extensive expertise in developing and managing Quality Management systems, qualification and validation, vendor management, documentation, training, CSV validation, risk management, GxPs, and Quality Remediation for pharmaceutical manufacturing sites. His experience spans across oral solid dosage forms, including tablets and capsules, as well as active pharmaceutical ingredients.

Mr. K. Raghavendra acknowledges the importance of GMP in the pharmaceutical industry. GMP represents a set of guidelines and regulations designed to ensure the quality, safety, and efficacy of pharmaceutical products. Adherence to GMP principles is not only essential but also a legal requirement in many countries.

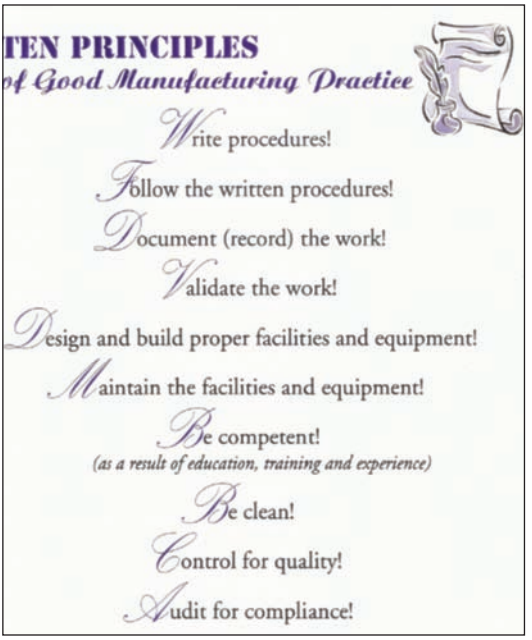
Mr. Raghavendra's extensive experience and expertise in this field make him an ideal speaker to shed light on the significance of GMP in pharmaceutical manufacturing.

1.1 Introduction

In the history and evolution of Good Manufacturing Practices (GMP), we find several pivotal moments that have shaped the pharmaceutical industry. The 1930s witnessed a tragic incident when a US-based pharmaceutical company used Diethylene glycol in an oral elixir of Sulfanilamide, causing the death of 107 children due to the poisonous solvent. As a response, the US government passed the Federal Food, Drug, and Cosmetic Act of 1938 to regulate the industry. In 1941, nearly 300 people lost their lives due to Sulfathiazole tablets contaminated with Phenobarbital. The 1960s brought to light the serious deformities in developing fetuses caused by Thalidomide, resulting in approximately 10,000 cases. Subsequently, in 1963, the first GMPs for finished pharmaceuticals were finalized, and in 1978, 21CFR Part 210 and 211 were established.

The primary objective of GMP is to guarantee the quality of pharmaceutical products, recognizing that it's impossible to test each individual tablet or capsule. While testing the end product is essential, it alone cannot guarantee quality. Quality assurance must be embedded at every stage of the manufacturing process.

GMP also plays a crucial role in ensuring the economy of pharmaceutical production by reducing costs and avoiding product failures. To achieve this, GMP hinges on several core principles, including adherence to pre-determined specifications, consistency in product quality, minimizing contamination, eliminating errors, and ensuring identity, strength, quality, and purity. It also emphasizes the prevention of cross-contamination and product mix-up and, crucially, the strict adherence to written procedures with comprehensive documentation of all activities.



1.2 Critical Elements Influencing GMP

In achieving GMP compliance, four pivotal factors come into play, namely, materials, machines, methods, and personnel. Materials refer to considerations within and between supplies and batches. Machines involve the impact of equipment changes on quality, equipment adjustments, aging, and proper care. Methods are crucial, encompassing the correct and adequate procedures. Lastly, personnel play a fundamental role, where appropriate training and a commitment to quality are paramount.

1.3 cGMP Violations - Consequences

The consequences of violating cGMP standards are severe. They can lead to the shutdown of manufacturing facilities and the recall of products from the market. Such violations can often make headlines, impacting the competitive environment, and forcing organizations to reevaluate their master plans and policies and standard operating procedures (SOPs).

1.4 List of Important Documents in GMP

Within the framework of GMP, various documents play a critical role in ensuring compliance and quality control. These include forms, Master Formula Records (MFR), Batch Manufacturing Records (BMR), Batch Packaging Records (BPR), specifications, and validation protocols and reports. Proper maintenance and utilization of these documents are integral to GMP compliance.

1.5 Principles of GMP

GMP can be understood through several key subparts.

Subpart A lays down the minimum GMP standards for the preparation of human and veterinary drug products.

Subpart B, focusing on organization and personnel, mandates the responsibilities of the Quality Control Unit. This includes their authority to approve or reject all components, containers, closures, labelling, or specifications related to drug products. Personnel qualifications, education, experience,

and training are also crucial in ensuring GMP compliance. Additionally, GMP emphasizes personnel responsibilities such as maintaining clean clothing, adhering to protective apparel, maintaining personnel hygiene, restricting access to manufacturing areas, and reporting illnesses.

Sub Part C - Building and Facilities

GMP compliance extends to building and facilities. This subpart covers component receipt, handling, and storage, in-process storage, and finished product storage. Adequate lighting and ventilation are essential, as are controls on dust, relative humidity, and temperature. Special attention is given to AHU (Air Handling Units) and air filtration systems for production areas, ensuring product quality. The plumbing system is to be free from dead legs to prevent contamination.

Sub Part D - Equipment

In this subpart, the focus shifts to equipment. Equipment must be appropriately designed, adequately sized, and suitably located to facilitate operations. The materials used in equipment construction should be non-reactive to avoid affecting product quality. Importantly, equipment must be designed to prevent any contact between the product and equipment lubricants and coolants. Sections 211.63 and 211.65 outline equipment qualification, while purified water systems, validated with a three-phase process, come into play.

Sub Part E - Control of Components and Drug Product Containers and Closures

Subpart E focuses on the control of components and drug product containers and closures. It underscores the need for written procedures detailing receipt, identification, storage, handling, sampling, testing, and the approval or rejection of components, containers, or closures. It also requires a visual examination upon receipt and before acceptance to check for appropriate labeling, container condition, broken seals, and contamination. Furthermore, it stresses the importance of maintaining control over suppliers and emphasizes testing, retesting, and approval or rejection, with sampling based on statistical criteria.

Subpart F - Production and Process Controls

Subpart F mandates the establishment of written procedures for operations, cleaning, calibration, and maintenance. It calls for the drafting and approval of Standard Operating Procedures (SOPs), along with the creation of Master Batch Records and Master Packing Records. Quality assurance plays a critical role through review and approval by the Quality Assurance (QA) unit. Training, deviation control, and the implementation of change control mechanisms are all part of maintaining effective production and process controls.

Subpart G - Packaging and Labelling Control

In Subpart G, attention turns to packaging and labelling control. It involves materials examination and usage criteria, as well as design and artwork approval. The focus here is on quality at the design and supplier stages, avoiding gang printing, and implementing a robust supplier approval system to ensure consistent product quality. Further, the control of unused labels, electronic scanning, and the physical separation of materials, coupled with lock and key maintenance, contribute to overall quality control.

Subpart H - Holding and Distribution

Subpart H outlines the procedures for warehousing, quarantine, and distribution of drug products. These procedures are essential to ensure that drug products are stored under appropriate conditions of temperature, relative humidity (%RH), and light so that drug product attributes are not affected. Additionally, distribution procedures should be in place to facilitate recall if necessary.

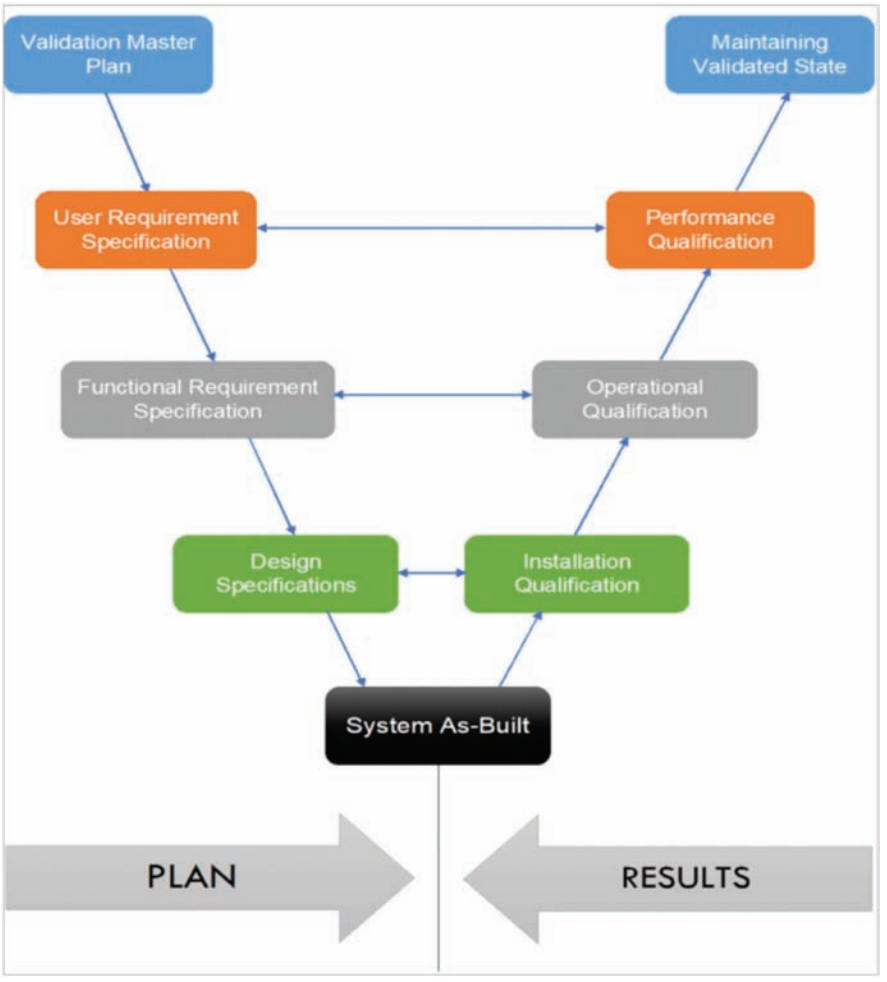
Subpart I - Laboratory Controls

Laboratory controls are addressed in Subpart I. The establishment of specifications, action levels through control charts, instrument calibration, testing and release for distribution, sampling and testing plans, and the necessity for comprehensive method validation are central to ensuring the quality of pharmaceutical products. Subpart I also covers the evaluation of Out of Specification (OOS) results, the analytical failure decision path, stability testing, special testing requirements, and the retention of reserve samples.

Subpart J - Records and Reports

Subpart J outlines the critical requirements for records and reports that must be maintained to ensure GMP compliance. The time periods for retaining records, the manner of storage, their availability for access, and the necessity of reviewing these records are all essential aspects of Subpart J. This section also addresses equipment cleaning and use logs, component, drug product container, closure, and labeling records, production record reviews, laboratory records, and complaint files.

Subpart K - Returned and Salvaged Drug Products



Subpart K is devoted to the inspection and testing of returned and salvaged drug products. It outlines the procedures to be followed when such products are returned and the steps for testing and evaluating the cause of any issues with the returned products. Ensuring the quality of returned drug products is a key aspect of Subpart K.

1.6 Aspects of GMP

In addition to the specific subparts, GMP also encompasses several key aspects that are integral to maintaining product quality. This includes having a robust quality management system in place, conducting product quality reviews, implementing change control mechanisms, investigating deviations, and addressing Out of Specification (OOS) and Out of Trend (OOT) results. Additionally, a well-defined process for handling complaints and recalls and a rigorous self-inspection/internal audit program contribute to overall GMP compliance.

1.7 Good Documentation Practices

The importance of good documentation practices cannot be overstated. Accurate and precise documentation is crucial. Documents should accurately capture the source data, and any corrections or changes must be duly documented. Legibility is equally important; documents should be handwritten clearly and be easily readable. They should not obliterate information when making changes or corrections. Documentation should be complete and contemporaneous, with data recorded at the time the activity occurs. Original data and accurate transcriptions of source data should be maintained. Attributability is another critical element; every document should clearly indicate who recorded the information, ensuring that only designated authorized staff have access to sensitive information. There are various specific practices to follow, such as using legible ink (preferably blue), adopting the correct sequence for date format (DD-MM-YYYY or DD.MM.YYYY), recording time in the 24-hour format (HH:MM), ensuring that the same person does not sign both "Done by/Prepared by" and "Checked by/Reviewed by," and avoiding common issues like overwriting, missing entries, typographical errors, and calculation errors.

02. Plant tissue culture and its applications

About the Speaker:

Prof. B. Rama Devi, HOD - Dept. of Botany, Osmania University, Hyderabad. Professor B. Rama Devi is a distinguished academic and a renowned expert in the field of Botany. Dr. Rama Devi serves as a Professor of Botany at the University College of Science, Osmania University. Her academic journey includes a Ph.D. from Osmania University, Post Graduation in Botany from Kakatiya University, B.Ed. from Kakatiya University, and her undergraduate degree, also from Kakatiya University.

With 21 years of teaching experience and 15 years of research experience, Dr. Rama Devi has made significant contributions to her field. She has published various research papers in national and international journals and has been actively involved in guiding Ph.D. students.

Currently, Dr. Rama Devi is the Head of the Department of Botany at Osmania University, where she continues to inspire and educate the next generation of botanists.

2.1 Introduction:

Plant tissue culture, also known as in vitro culture, is a biotechnology technique that involves the

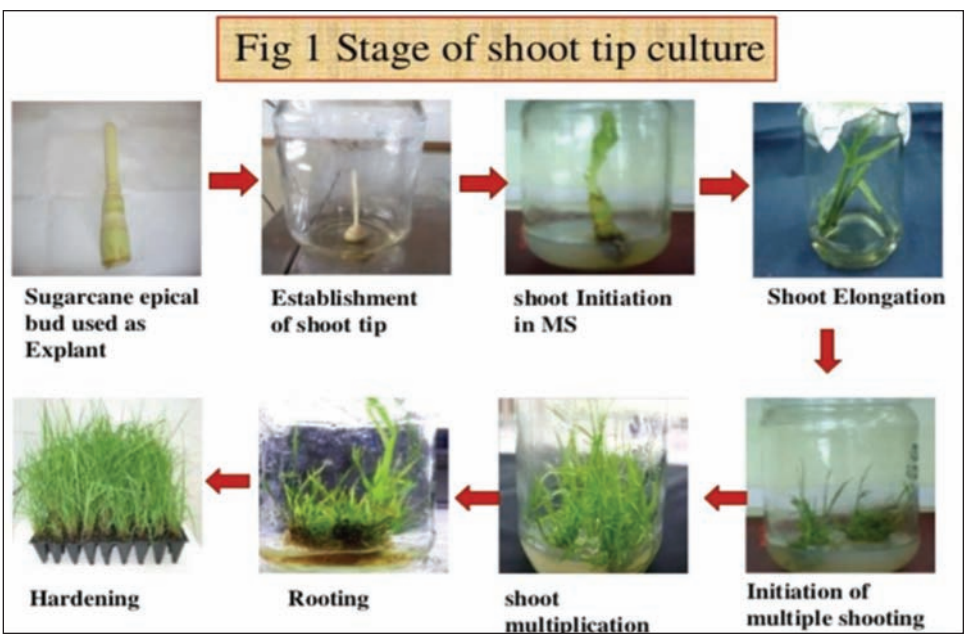
aseptic (sterile) cultivation of plant cells, tissues, or organs in a controlled environment. It allows for the propagation, manipulation, and genetic improvement of plants under laboratory conditions. Plant tissue culture offers several advantages, including the ability to:

1. Clonally Propagate Plants: Tissue culture allows for the production of genetically identical plantlets from a single parent plant, ensuring that the desired traits are maintained in the offspring.
2. Preserve Rare and Endangered Species: It is a valuable tool for conserving and preserving rare, endangered, or difficult-to-grow plant species by storing their genetic material in vitro.
3. Genetic Modification: Plant tissue culture is used to introduce or modify genes in plants, resulting in transgenic plants with improved characteristics, such as resistance to pests or diseases.
4. Secondary Metabolite Production: It is employed for the production of secondary metabolites, like alkaloids or pharmaceutical compounds, in a controlled environment.
5. Somatic Embryogenesis: The technique can produce somatic embryos, which can be encapsulated as synthetic seeds, providing an efficient means for large-scale propagation.
6. Breaking Seed Dormancy: It can overcome seed dormancy and speed up the breeding cycle in some plant species by culturing embryos.

2.2 Hardening of Plantlets

The hardening or acclimatization of plantlets is a crucial step in the plant tissue culture process. It involves transitioning tissue-cultured plants, which have been grown in a controlled environment, to the natural outdoor conditions, making them suitable for eventual transplantation into farmer's fields. This process is typically carried out in several stages.

In the first stage of hardening, tissue-cultured plants are gently removed from the nutrient medium and carefully washed to remove any remaining culture medium residues. These plants are then placed



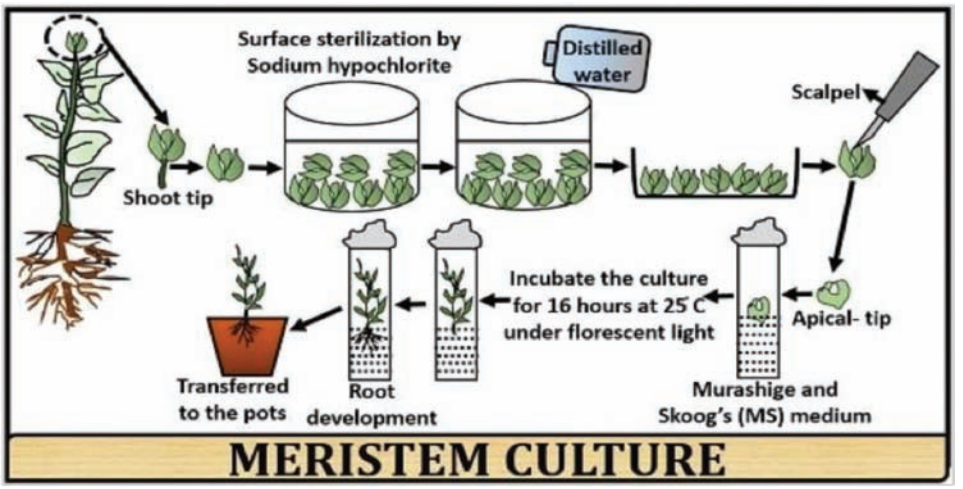
in netted plastic pots containing a liquid nutrient medium. This stage usually takes place in a greenhouse or a shade house. Primary hardening allows the plants to adapt to conditions with higher humidity and controlled lighting, facilitating their transition to the external environment.

After the primary hardening stage, the tissue-cultured plants are further acclimatized in a greenhouse or shaded house during the secondary hardening phase. They are transplanted into polybags filled with a suitable potting mixture. The controlled environment in this stage helps the plants adapt to lower humidity and increased exposure to natural lighting. This step is essential for strengthening the plants and preparing them for the next phase.

In the final phase of hardening, the plants are exposed to even more natural conditions. This stage is often conducted in outdoor settings with reduced shading. The plants become more robust, developing the necessary resistance to environmental stresses such as direct sunlight, temperature fluctuations, wind, and pests. Once the tissue-cultured plants have successfully completed this tertiary hardening stage, they are ready for transplantation into the farmer's fields or their target growth environment.

2.3 Types of Plant Tissue Culture:

1. Meristem Culture: Used for obtaining disease-free plants from apical meristems. More successful in herbaceous plants than woody plants.



2. Callus Culture: Involves the culture of unorganized dedifferentiated cells, which can be used for various purposes.

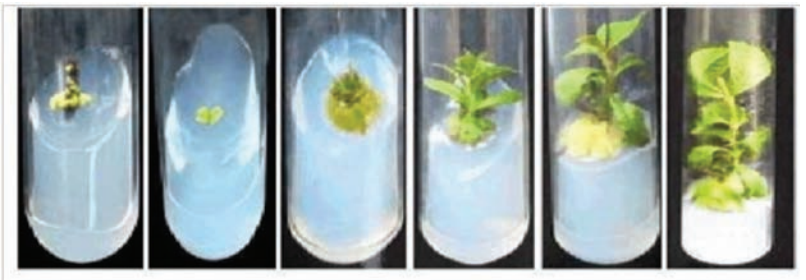
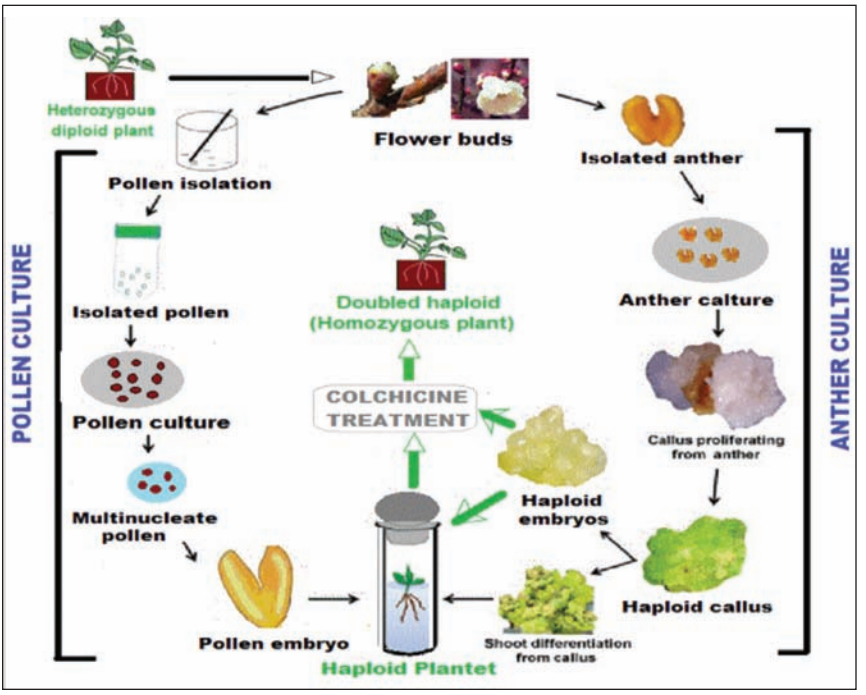


Fig. 1: Picture showing the shoot formation

3. Cell Suspension Culture: Used for producing secondary metabolites like alkaloids, flavonoids, and terpenoids, as well as recombinant proteins.
4. Bud Culture: Utilized for generating new plants from axillary buds or nodal segments.
5. Seed Culture: Used for raising sterile seedlings and obtaining aseptic explants.
6. Anther Culture: Involves the culture of anthers to produce haploid plants.



7. Embryo Culture: Used for obtaining viable plants from immature or mature embryos, overcoming dormancy, and producing interspecific or intergeneric hybrids.
8. Protoplast Culture: Involves culture of plant cells devoid of cell walls and is used for genetic transformation and somatic hybridization.

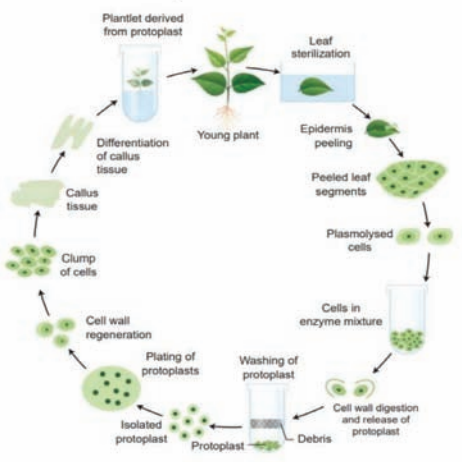


Figure 5.8: Protoplast Culture

10. Applications of Plant Tissue Culture:

- Clonal Propagation and Micropropagation
- Biomass Energy
- Production of Secondary Metabolites
- Genetic Variability (Somaclonal Variation)
- Somatic Embryogenesis and Synthetic Seed
- Breaking Dormancy
- Haploid Plants
- Somatic Hybrids
- Transgenic Plants
- Germplasm Conservation

2.4 Conclusion:

Plant tissue culture has made significant contributions to agriculture, forestry, and biotechnology. It allows for rapid multiplication of plants with desirable traits, the production of secondary metabolites, and genetic improvement of crops. Additionally, it plays a crucial role in conserving plant germplasm, especially for endangered species.

3. RECAP OF THE PROGRAM'S HIGHLIGHTS:

The DBT - Training of Trainers (ToT) Program, organized by the Telangana State Council of Science and Technology (TSCOST) in Hyderabad was concluded and it was essential to provide a comprehensive recap of the highlights and key takeaways from the event, highlighting the value and significance of the knowledge shared during the ToT program.

Throughout the program, esteemed guest speakers covered a wide range of topics, bringing their expertise and insights to the forefront. These topics encompassed various aspects of biotechnology, with a specific focus on the latest developments, research findings, and best practices in their respective fields. The depth of knowledge and experience presented by the guest speakers was exceptional, and participants had the opportunity to engage with them, asking questions and seeking clarification on various aspects of the presented topics.

During the program, a significant emphasis was placed on fostering interaction and knowledge exchange among the participants. This was facilitated through discussions that encouraged participants to share their experiences and insights. The open dialogue led to the exchange of ideas, solutions to common challenges, and the building of a strong network of like-minded professionals and educators.

One noteworthy aspect of the program was the active collection of feedback from the participants. This feedback shall play a crucial role in ensuring the future success of TSCOST's events. It allowed organizers to gauge the effectiveness of the ToT program and identify areas for improvement.

This commitment to continuous improvement is a testament to TSCOST's dedication to providing high-quality educational programs and promoting scientific and technological advancement.

In conclusion, the DBT - Training of Trainers (ToT) Program held by TSCOST in Hyderabad was a resounding success. It provided a platform for the dissemination of cutting-edge knowledge, fostering

collaboration, and creating a vibrant community of educators and professionals. This program not only enriched the participants' knowledge but also paved the way for even more impactful and valuable events in the future.

4. CLOSING REMARKS BY MEMBER SECRETARY:

After the comprehensive recap of the Training of Trainers (ToT) Program, the Member Secretary, TSCOST took the opportunity to highlight the program's significance and express his heartfelt gratitude to all the participants and guest speakers who made the event a resounding success. His concluding remarks reflected on the importance of the program's theme, which revolved around biotechnology. He underlined the critical role of the Department of Biotechnology (DBT), Government of India, in organizing and supporting such vital initiatives.

In his summary, the Member Secretary emphasized that the ToT program was not just an educational endeavour but a catalyst for progress in the fields of biotechnology and intellectual property rights. He recognized that the knowledge shared during the program was essential in fostering innovation, economic growth, and scientific advancement. By equipping trainers and educators with the latest insights, the program played a pivotal role in shaping the future of these fields.

The Member Secretary, TSCOST also emphasized that understanding and effectively managing intellectual property was key to harnessing the potential of biotechnological advancements. In this context, he commended the expert speakers for shedding light on the complex landscape of intellectual property rights and the practical implications for the participants' work and research.

Furthermore, the Member Secretary reiterated the critical support provided by the Department of Biotechnology (DBT), Government of India, in the successful conduction of the ToT Program. He acknowledged the DBT's commitment to promoting scientific and technological education and its dedication to advancing the biotechnology sector in India. The partnership between TSCOST and DBT was pivotal in making this program a reality, and it showcased the collaborative effort towards driving innovation and knowledge dissemination.

As a concluding gesture, the Member Secretary took individual remarks from the participants. This provided a platform for participants to express their views on the program and highlight its relevance to their specific areas of work. The diverse perspectives and experiences shared by the attendees

further underscored the program's effectiveness and its wide-ranging impact on professionals from various backgrounds.

The program not only provided knowledge but also a platform for meaningful discussions and networking, ensuring that its impact would be felt in the participants' work and research long after the event had concluded.

5. CERTIFICATES DISTRIBUTION CEREMONY:

To conclude the Training of Trainers (ToT) Program on a high note and to honour the remarkable achievements of both the dedicated participants and the esteemed guest speakers, a special certificates distribution ceremony was held. This ceremony served as a fitting recognition of the hard work, commitment, and the invaluable contributions made by all those involved in making the program a success.

During this ceremony, certificates were awarded to the participants who had successfully completed the ToT program. These certificates symbolized the participants' dedication to advancing their knowledge and skills in the fields of biotechnology. These certificates were a testament to the participants' journey of continuous learning and development.

In addition to recognizing the participants, the ceremony also served as an opportunity to express gratitude to the guest speakers who had generously shared their knowledge and expertise throughout the program. These experts were presented with certificates of appreciation as a token of gratitude for their significant contributions. The certificates of appreciation acknowledged the invaluable role played by the guest speakers in enhancing the program's quality and educational value. Their expertise and willingness to engage with the participants enriched the learning experience and made the program all the more impactful.

6. Final Thanks & Program Closing Announcement:

The concluding remarks of the Training of Trainers (ToT) program were imbued with sincere gratitude and appreciation for the multitude of participants, organizations, and entities that played pivotal roles in making the program a resounding success. It was a moment of reflection on the collective effort and dedication that had gone into ensuring the program's effectiveness and educational value.

First and foremost, heartfelt thanks were extended to the guest speakers who had generously shared their knowledge and expertise. These experts had selflessly contributed their time and insights, enriching the learning experience for all participants. Their willingness to engage, educate, and inspire was instrumental in the program's success, and their efforts were acknowledged with deep appreciation.

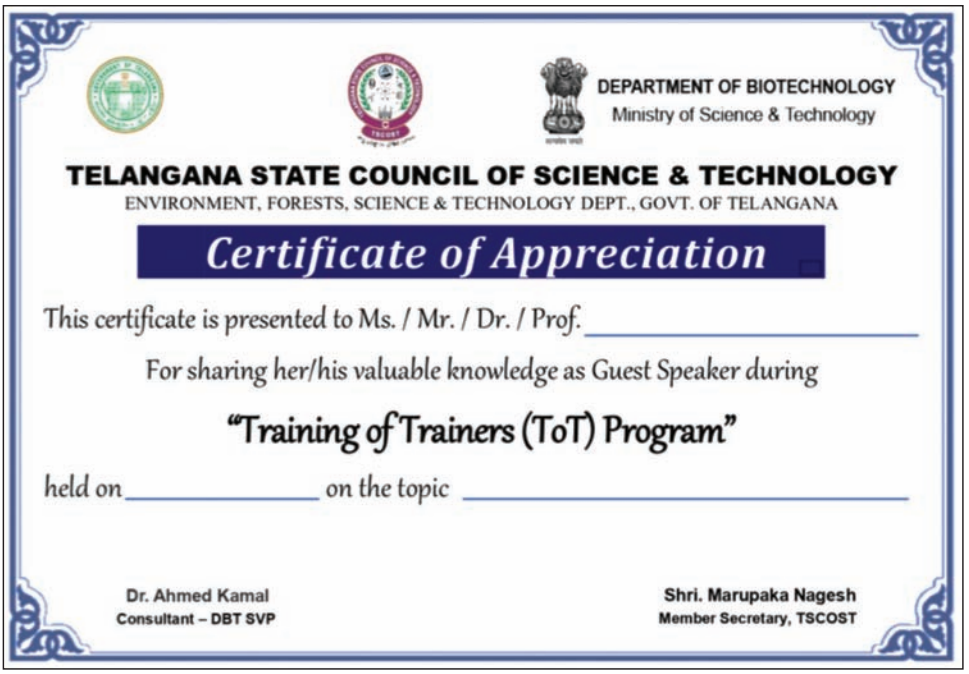
The management of TSCOST, as the driving force behind the program, was also recognized and thanked for their vision and commitment to advancing scientific and technological education. Their unwavering support and dedication to promoting knowledge dissemination and fostering collaboration were integral to the program's success.

Further appreciation was extended to the organizing committee, whose meticulous planning and execution ensured that the program ran seamlessly. The committee's dedication to every detail, from scheduling to logistics, created an environment conducive to learning and collaboration. Their efforts were vital in making the ToT program a well-organized and efficient event.

As the concluding remarks drew to a close, a final announcement marked the official closure of the Training of Trainers program. The program had provided a platform for knowledge exchange, networking, and skill development, and its closure symbolized the participants' readiness to take their enhanced knowledge and expertise back to their respective fields.

In essence, the concluding remarks and final announcement encapsulated the sense of unity and shared purpose that had defined the ToT program. It was a moment of appreciation, reflection, and inspiration, underlining the power of collaboration and the importance of lifelong learning. The event's closure was not an end but a continuation of the journey toward furthering scientific and technological education and innovation.

Certificates issued to the Guest Speakers and the participants



DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology

TELANGANA STATE COUNCIL OF SCIENCE & TECHNOLOGY
ENVIRONMENT, FORESTS, SCIENCE & TECHNOLOGY DEPT., GOVT. OF TELANGANA

Certificate of Appreciation

This certificate is presented to Ms. / Mr. / Dr. / Prof. _____

For sharing her/his valuable knowledge as Guest Speaker during

"Training of Trainers (ToT) Program"

held on _____ on the topic _____

Dr. Ahmed Kamal
Consultant – DBT SVP

Shri. Marupaka Nagesh
Member Secretary, TSCOST

DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology

TELANGANA STATE COUNCIL OF SCIENCE & TECHNOLOGY
ENVIRONMENT, FORESTS, SCIENCE & TECHNOLOGY DEPT., GOVT. OF TELANGANA

Certificate of Participation

Ms. / Mr. / Dr. / Prof. _____

has attended 04-Day "Training of Trainers (ToT) Program"
held from November 01st, 2023 to November 04th, 2023
Organized by Telangana State Council of Science & Technology (TSCOST)
Under the Department of Biotechnology - Skill Vigyan Program.

Dr. Ahmed Kamal
Consultant – DBT SVP

Shri. Marupaka Nagesh
Member Secretary, TSCOST

ANALYTICAL TECHNIQUES AND TRENDS IN PHARMACEUTICAL INDUSTRY

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Quality, safety and efficacy

- The safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy.
- The safety of a drug is determined by its pharmacological-toxicological profile as well as the adverse effects caused by the impurities
- The impurities in drugs often poses unwanted pharmacological-toxicological effects by which any benefit from their administration may be outweighed
- It is quite obvious that the products intended for human consumption must be characterized as completely as possible.
- The quality and safety of a drug is generally assured by monitoring and controlling the impurities effectively.
- Thus, the analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis.

ICH Quality Guidelines

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)

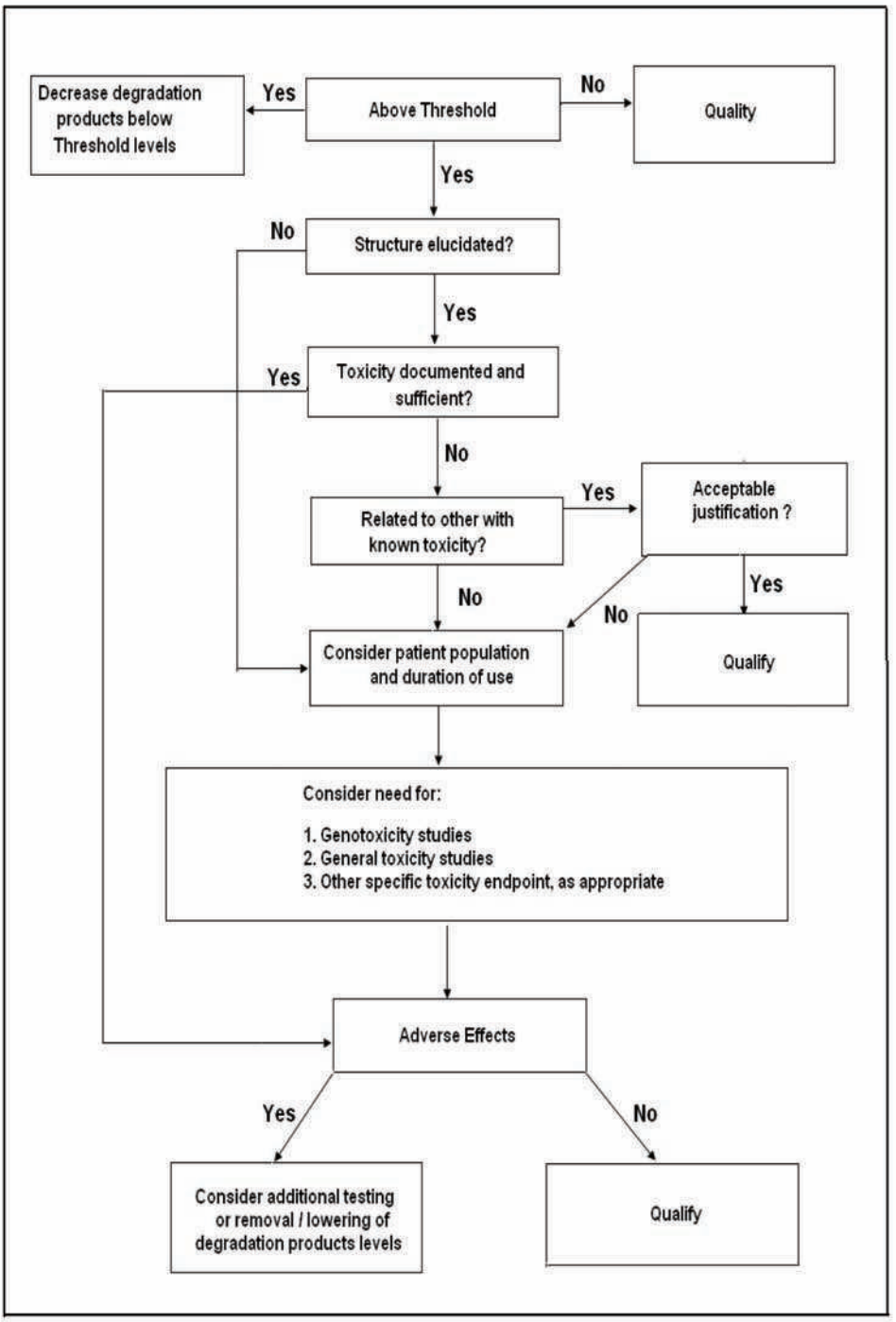
1. Stability Q1A-Q1F
2. Analytical Validation Q2
3. Impurities Q3A-Q3D
4. Pharmacopoeias Q4-Q4B
5. Quality of Biotechnology Products Q5A-Q5E
6. Specifications Q6A- Q6B
7. Good Manufacturing Practice Q7
8. Pharmaceutical Development Q8
9. Quality Risk Management Q9
10. Pharmaceutical Quality System Q10
11. Development and manufacturing of Drug Substances Q11
12. Life Cycle management Q12
13. Continuous manufacturing of drug substances and drug products Q13
14. Analytical Procedure Development Q14

- Analytical procedures should be able to separate all the impurities from each other and the method should be optimized to separate and quantify them in the dosage forms.
- Such methods are to be validated demonstrating the accuracy, precision, specificity, limit of detection, quantitation, linearity, range and interferences
- The validation of analytical procedures i.e., the proof of its suitability for the intended purpose, is an important part of the registration application for a new drug.
- Additional peak tailing, peak resolution and analyte recoveries are important in case of chromatographic methods.
- ICH guidelines serve as a basis worldwide both for regulatory authorities and industry to bring the importance of a proper validation to the attention of all those involved in the process of submission of drug master files (DMF).
- The analytical research and development units in the pharmaceutical industry are responsible for preparation and validation of test methods.

Thresholds

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

Decision tree for qualification of impurities in drugs and pharmaceuticals

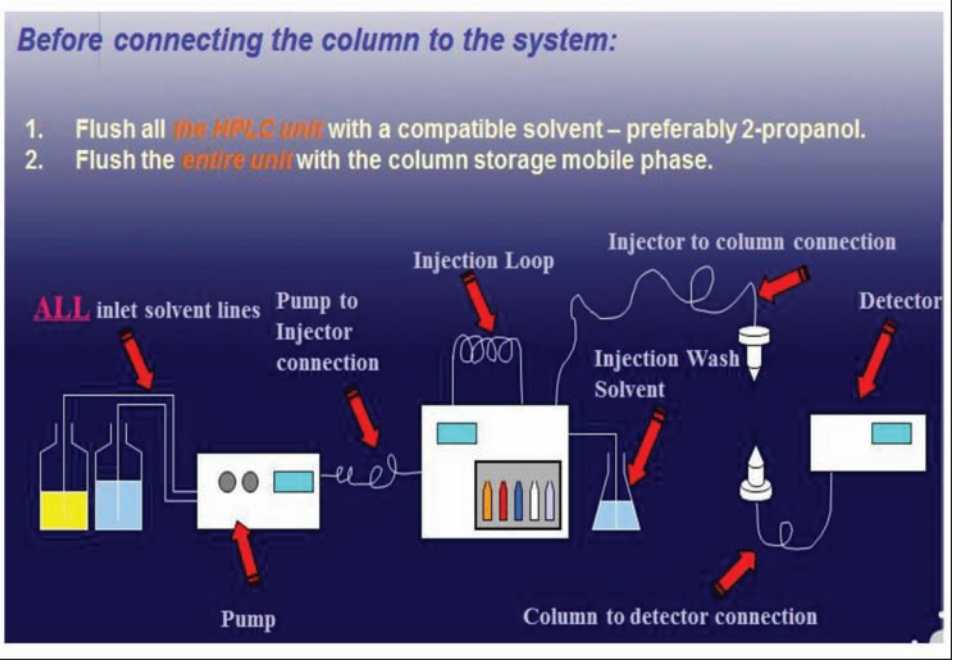


Origin of impurities

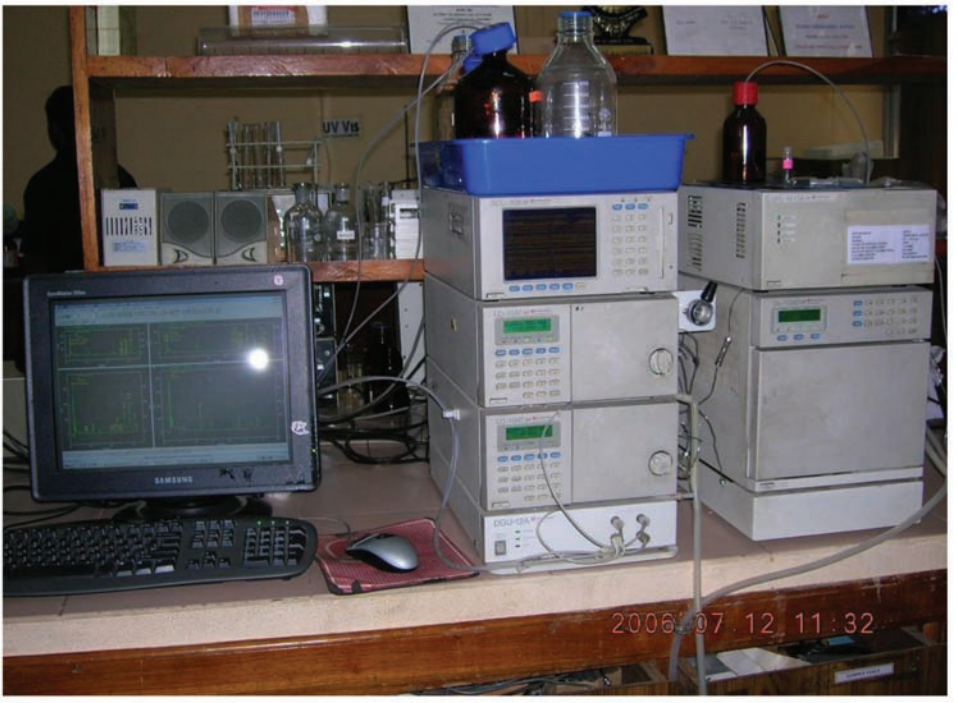
- Last intermediate of synthesis
- Products of incomplete reactions
- Products of overreactions
- Impurities originating from starting materials
- Impurities originating from solvents
- Impurities originating from catalysts
- Products of side reactions
- Degradation products
- Enantiomeric impurities
- Residual solvents
- Inorganic impurities
- Impurities in excipients Principles of Chromatography

- Chromatography is a physical process.
- Chromatography system is composed of three Components :
  - Stationary phase
  - Mobile phase
  - Mixture to be separated
- Chromatography is a dynamic process in which the mobile phase moves in definite direction.
- The stationary phase may be a solid, the mobile phase may be either a gas (GC) or a liquid (HPLC) or Super Critical Fluid (SFC)
- Mobile phase - phase that moves through chromatograph
- Stationary phase - column; phase that is stationary in chromatograph
- Bonded phase - reactive groups imparted to stationary phase in order to achieve selectivity (Chiral Selectors)

Block Diagram of HPLC



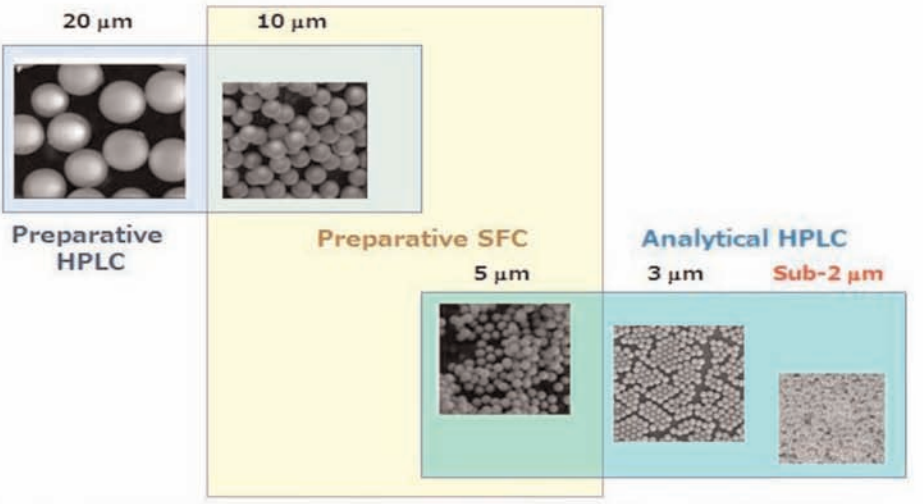
HPLC and Columns



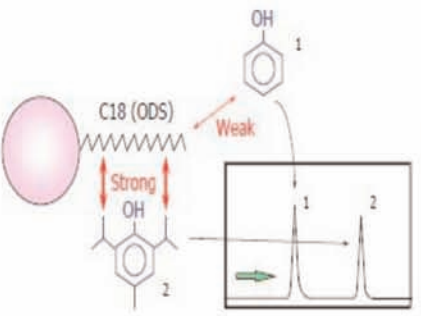
Chromatography categories

Scale	Chromatographic Objective
Analytical	Information: Compound identification and concentration
Semi-Preparative	mg to g: Compound purification (small)
Preparative	g to Kg : Compound purification (large)
Process Scale (Industrial)	Kg to MT: Manufacturing Quantities

Particle size: Analytical Vs Preparative



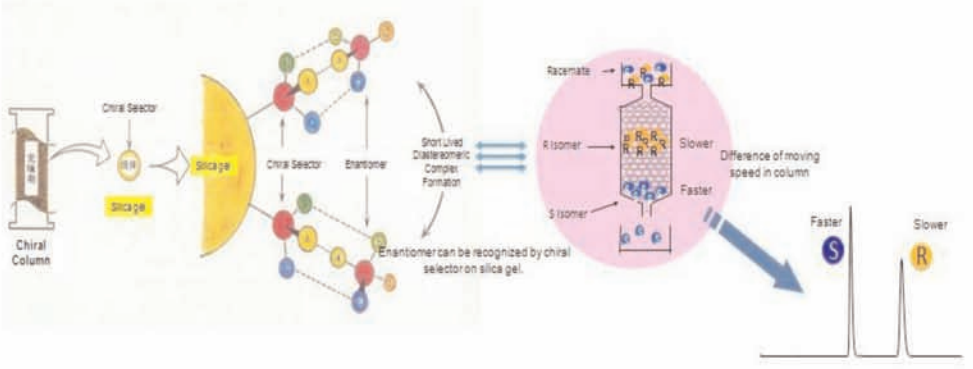
Effect of Stationary Phase



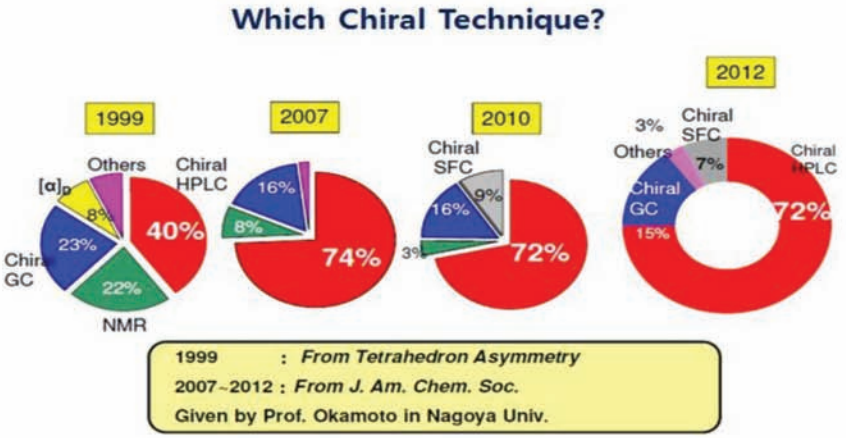
Stationary phase selectivity

S. no.	Stationary phase functionality	Chemical interactivity with solute
1	Amino (tert.)	Basic interactions
2	C18 or C8	Hydrophobic, dispersion forces
3	CN (cyano)	Largely dipolar
4	Phenyl	π–π Interactions, dispersive
5	Amide	Basic and dipolar interactions
6	Ether	Largely basic, some H-bonding interactions
7	Nitro	Strongly dipolar
8	Diol	H-bonding, basic-acidic possibilities
9	Fluoroalcohol	Acidic interactions

Chiral Separation on Chiral (selector) column



Which Chiral Chromatography?



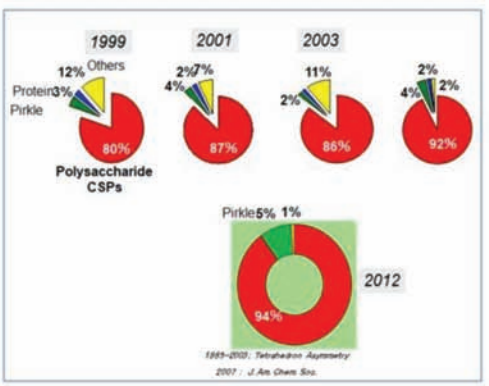
Types of CSPs

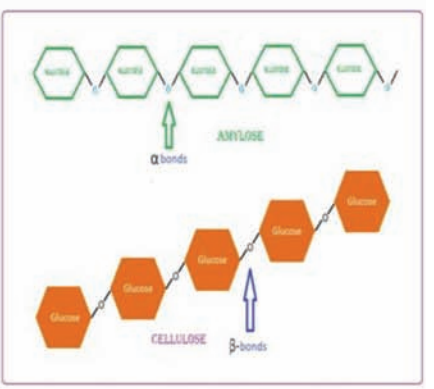
Types of CSPs and their loading capacities

Type	CSPs	Loading capacity (mg solute / g CSP)
I	Pirkle type (Brush type)	1-50
II	Polysaccharide derivatives	**5-150**
III	Macrocyclic type	
	-Cyclodextrins	0.1-5
	-Glycopeptides	0.1-5
	-Chiral Crown ether	0.1-5
IV	Ligand exchange	0.1-1
V	Protein type	0.1-0.2

Drug discovery today, volume 10, Number 8, April 2005

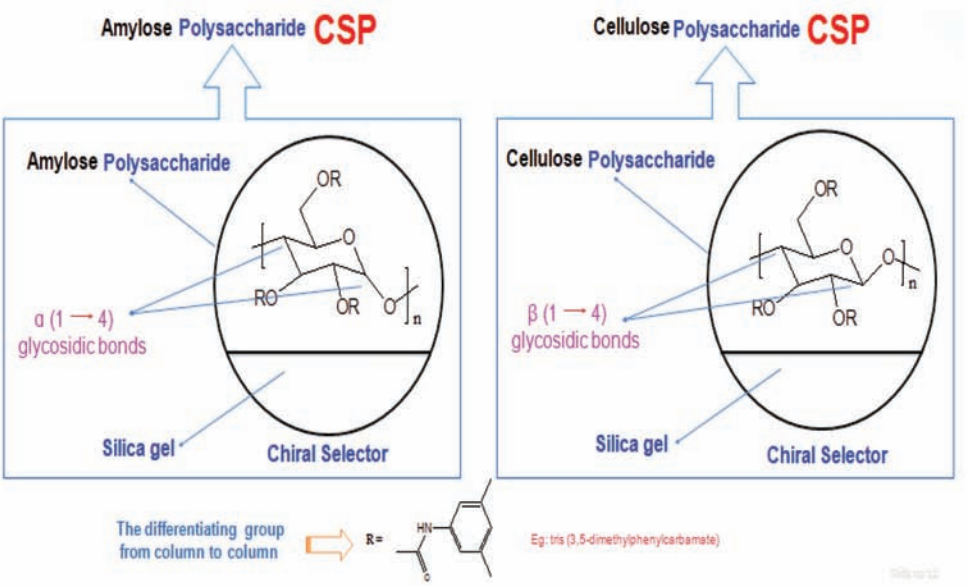
Polysaccharide Derived CSP's



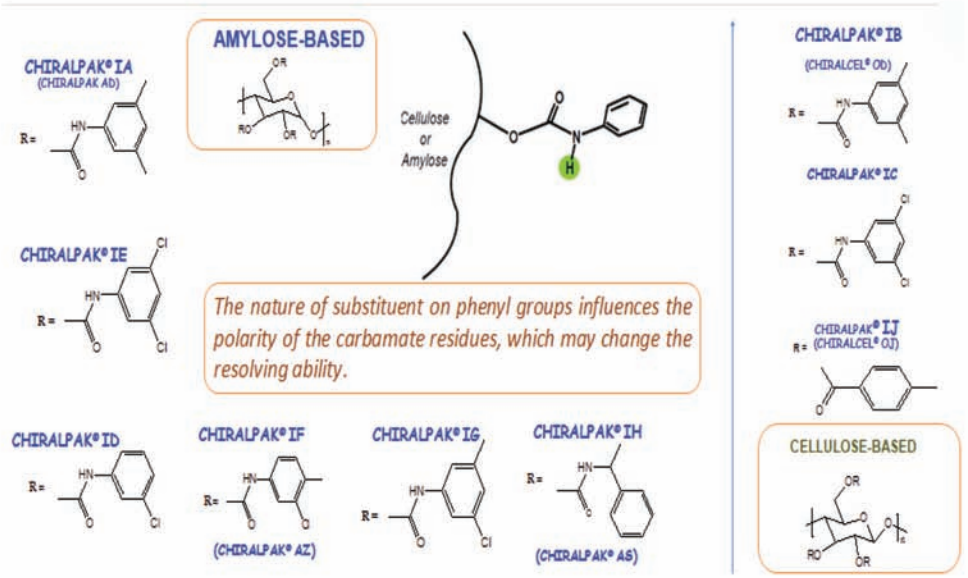


Amylose is better (grooves...helical)> cellulose...

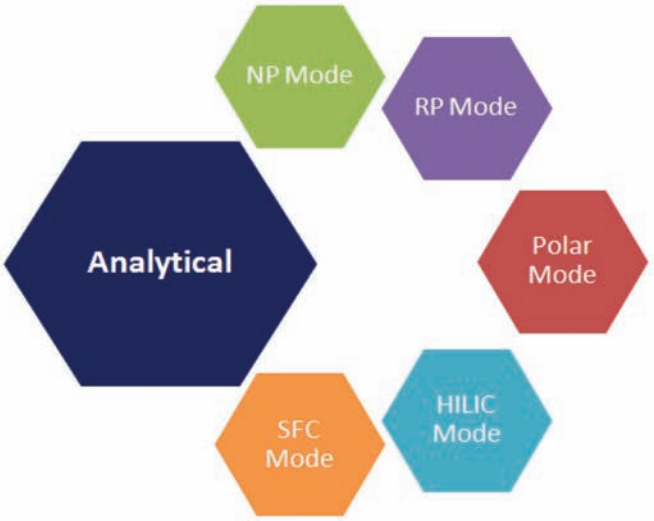
Polysaccharide Derived CSPs



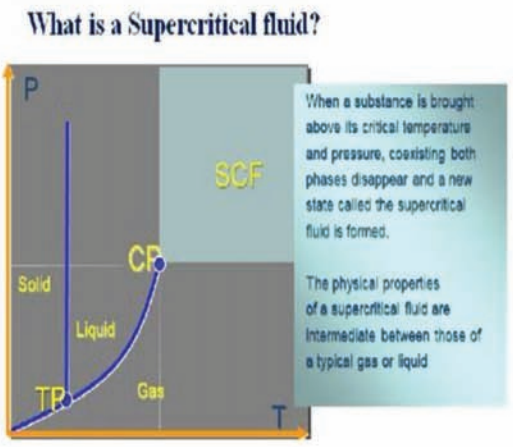
New Generation Immobilized Chiral Selectors

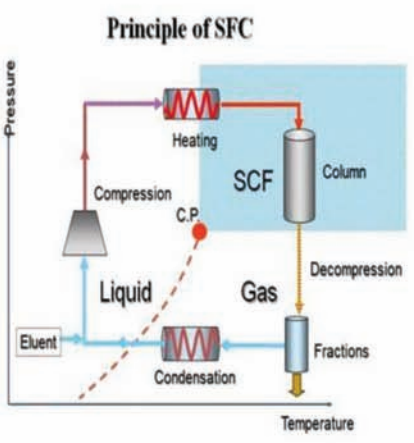


Chiral chromatography Elution Modes

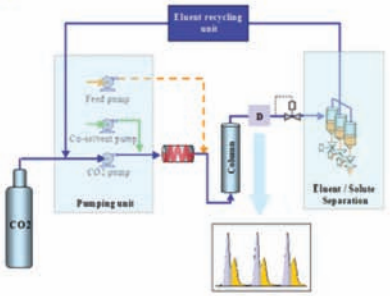


Supercritical Fluid Chromatography





Preparative SFC Flowsheet:



Applicable for Analytical & Preparative separations:

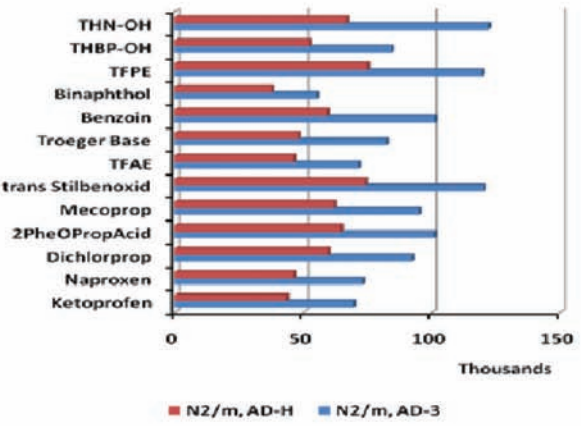
- Complementary selectivity
- Faster separations and high productivity
- Prep separations ranging from g to multi Kg scale
- Green technology
- Easy evaporation of fractions
- Lower operating costs

Eluent system:
CO_2 (60-95%);
Co-solvent: Polar solvents (5-40%)
eg MeOH, EtOH, IPA, ACN

Additives:
Acidic Analyte : No additive
Neutral Analyte : No additive
Basic Analyte (0.1-1%): DEA / EA / IPA / BA

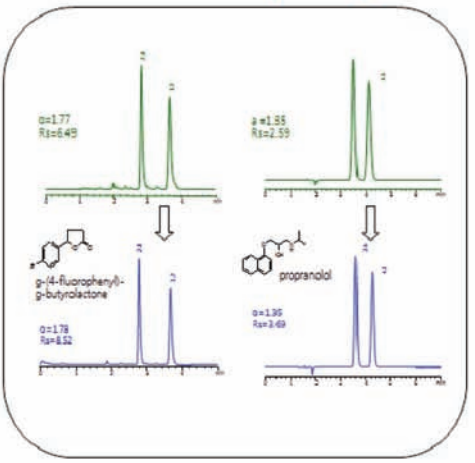
Elution mode: Isocratic / Gradient
Flow Rates : Typically 3-4 times> HPLC flow
Col Temperature: 10-40°C

3µ vs 5µ



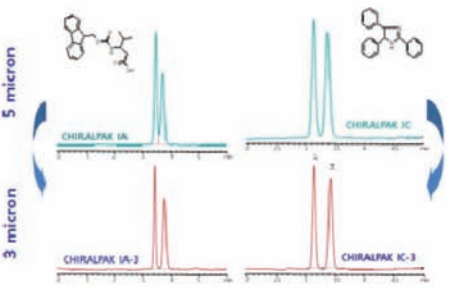
3µ Columns provide Enhanced efficiency (40 - 60%) compared to 5µ Columns

Direct Method Transfer 5 µm to 3 µm



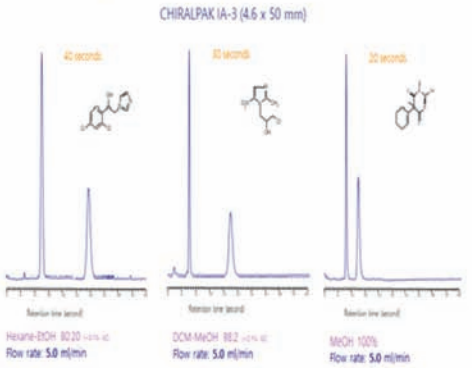
Enhancing resolution for critical separations
Smaller particles

From partial to complete resolution of the enantiomers



Mobile phase: Hexane-DCM 50:50 (+0.1% AE)

Fast Analysis: with sub 2 µm column in <1 min

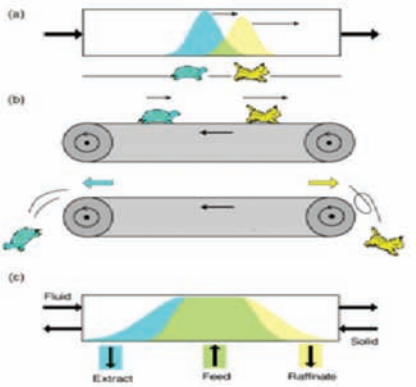


Benefits of 3 & sub 2 µm columns

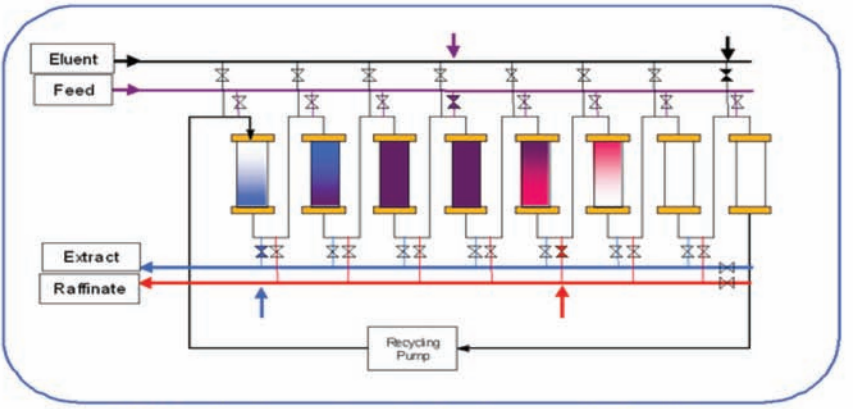
- Reduce analysis time - more no. Of analysis
- Less effort in method development
- Cost reduction
  - Save solvent consumption
  - Save capital investment & operating cost
- Excellent tool for R&D & Drug Discovery CRO

Batch vs Counter current chromatography

A cat and a turtle are placed in the middle of a conveyor belt. Both animals move against the belt in the same direction but at different velocities



Mazzotti. et al., *J. Chromatogr. A* **2009, 1216, 709-738**



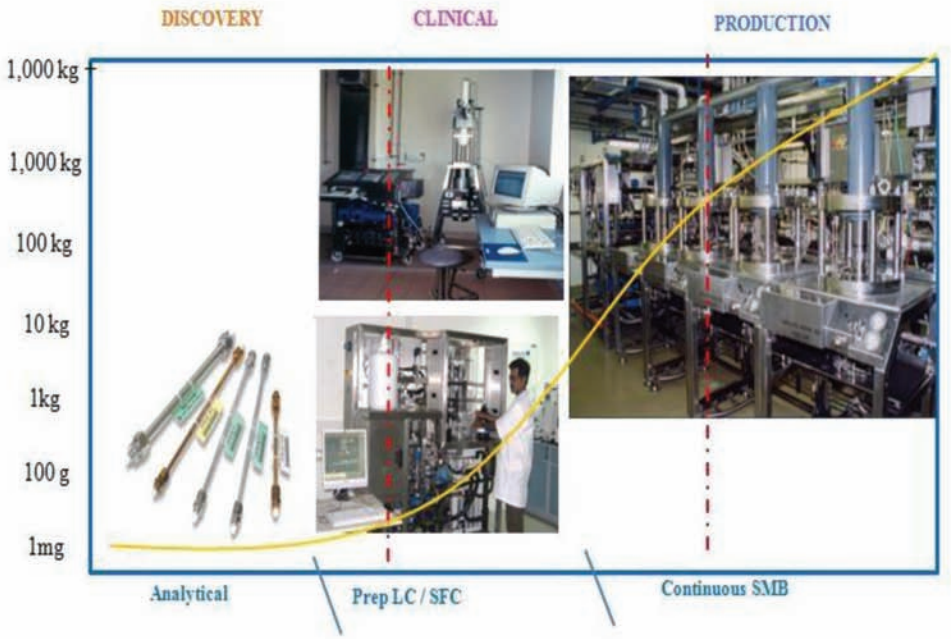
SMB leads to greater yields of separated substances while consuming less eluent and packing material.

Larger scale SMB technologies

30 cm

80 cm

Several 100 cm systems in use.



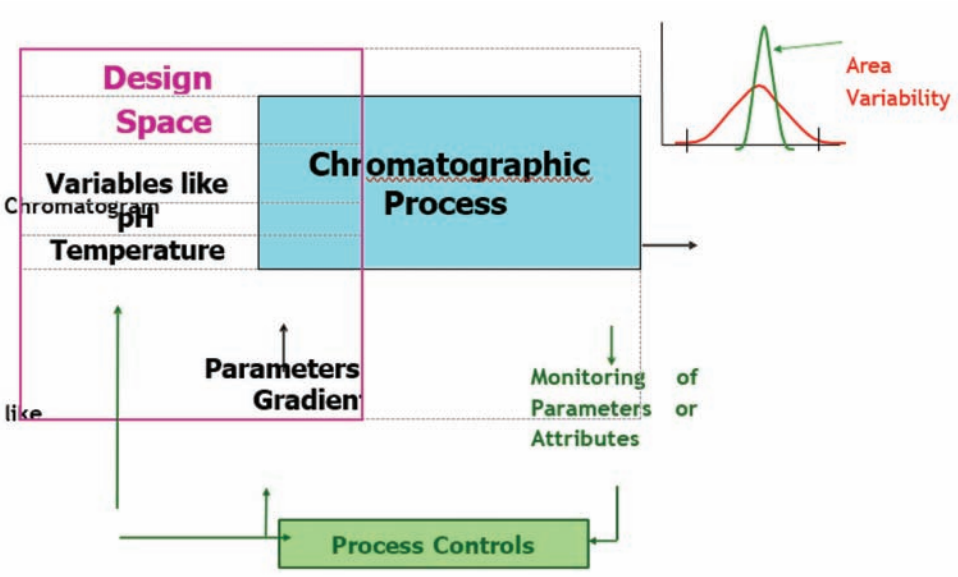
QbD

- QbD has been initiated since 2002 and in Jan-2013 was fully adopted in the pharmaceutical industry
- through several regulatory initiatives such as FDA's cGMP for the 21st Century and New ICH Q8, Q9 and Q10 regulatory documents.
- In QbD approach, many statistical tools are involved like
- Design of Experiment (DoE), multivariate analysis and six sigma methodologies.
- Since last decade the number of publications increased every year based on the experimental design in chromatography.
- QbD is defined in ICH Q8 (R1) guidelines:
- "a systematic approach to pharmaceutical development starting with pre-defined objectives with an emphasis on product and process understanding control"

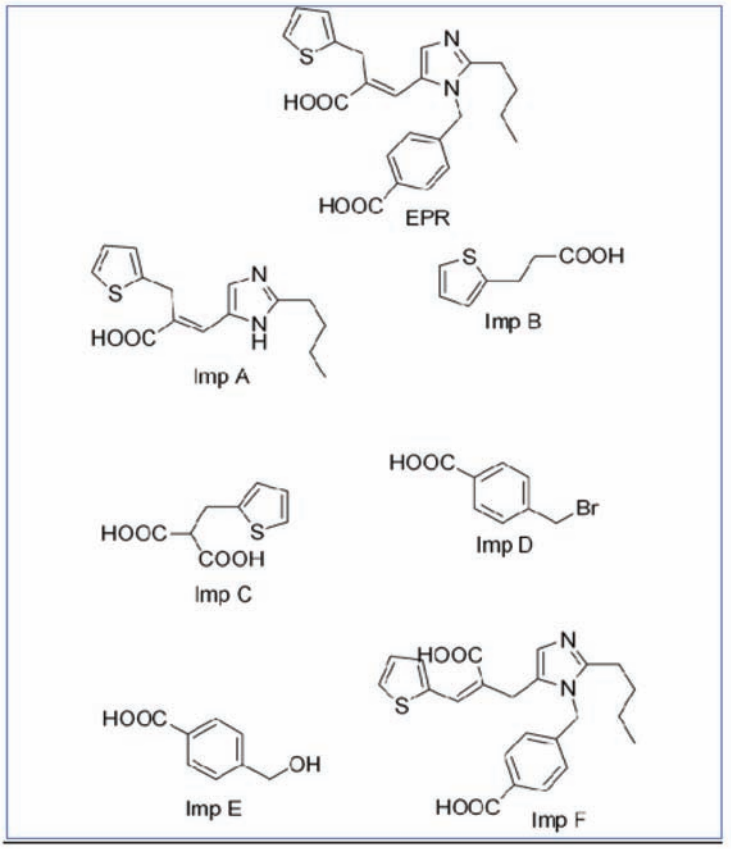
Objectives:

- A rapid, accurate, precise, and robust method
- Interaction effect of all variables
- Generation of Design Space

Reducing Chromatographic Variability



Eprosartan & impurities



A Waters Alliance HPLC instrument- e2695 (Waters Corp., Milford, USA) equipped with integral auto sampler and quaternary gradient pump with an on- line degasser

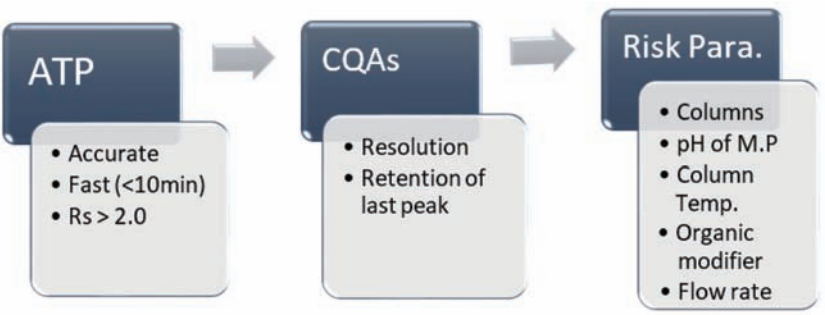
Columns:

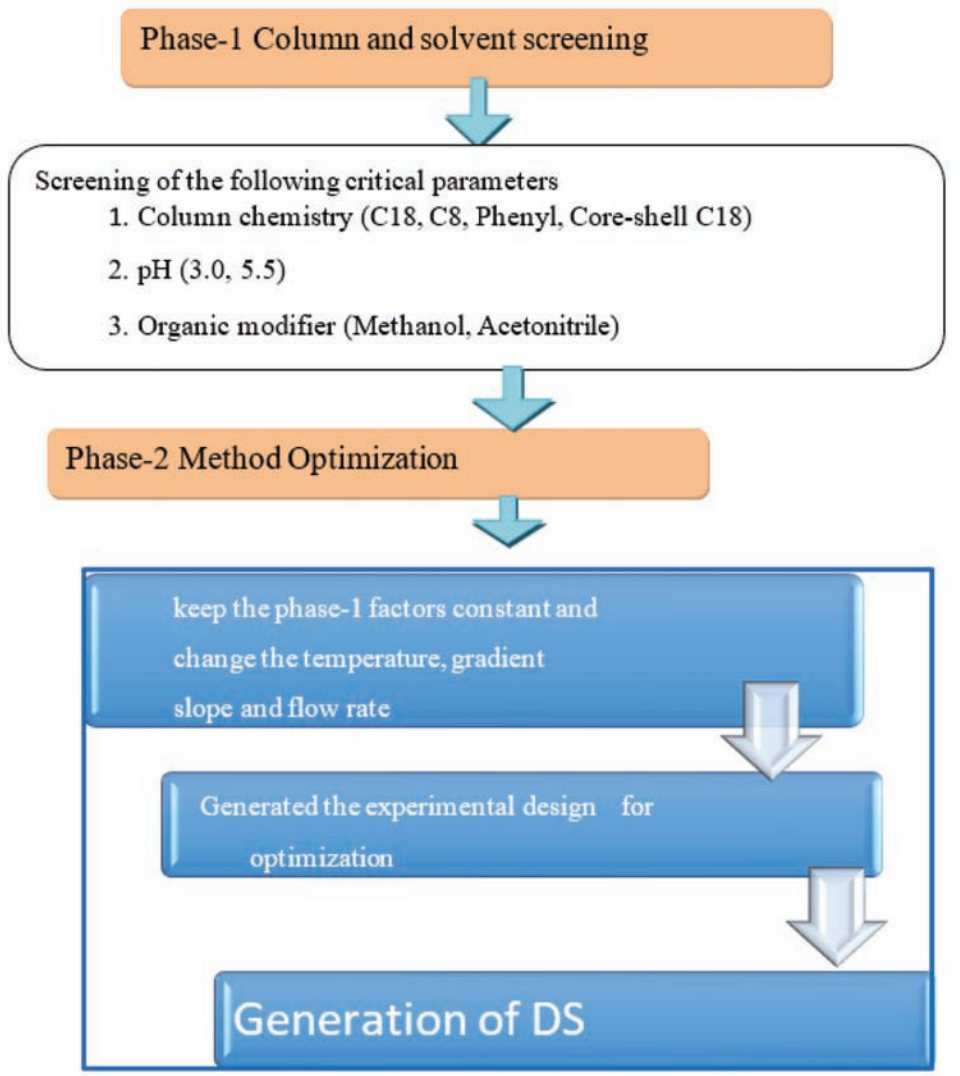
Luna C18 Luna C8 Genesis phenyl

fused core - Sun Shell C18

Software:

Design Expert@ version 8.0.4.1. modeling software (Stat-Ease Inc., Minneapolis, MN)

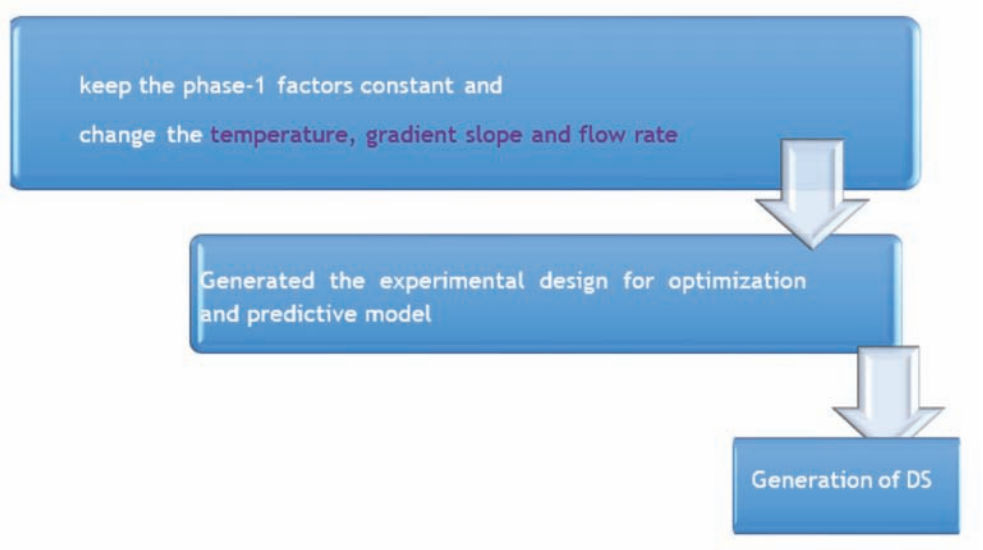




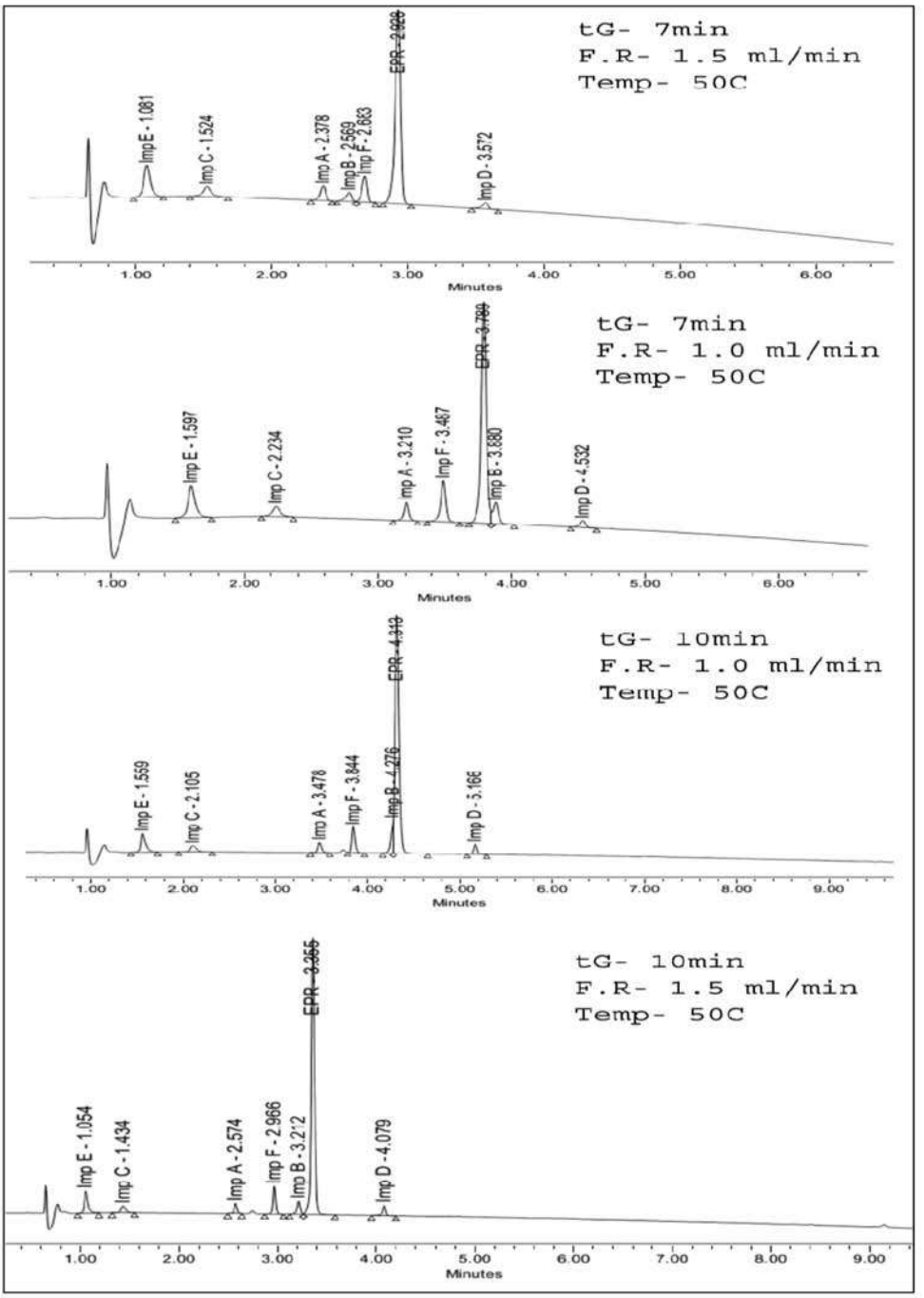
Expt No.	pH of mobile phase	Organic modifier	Columns	Gradient (% of organic phase)	Gradient time (min)
1	5.5	ACN	Grace Phenyl	5-85	20
2	5.5	ACN	Phenomenex C18	5-85	20
3	3	MeOH	Phenomenex C18	10-95	20
4	5.5	MeOH	Grace Phenyl	10-95	20
5	5.5	ACN	Phenomenex C8	5-85	20
6	3	ACN	Grace Phenyl	5-85	20

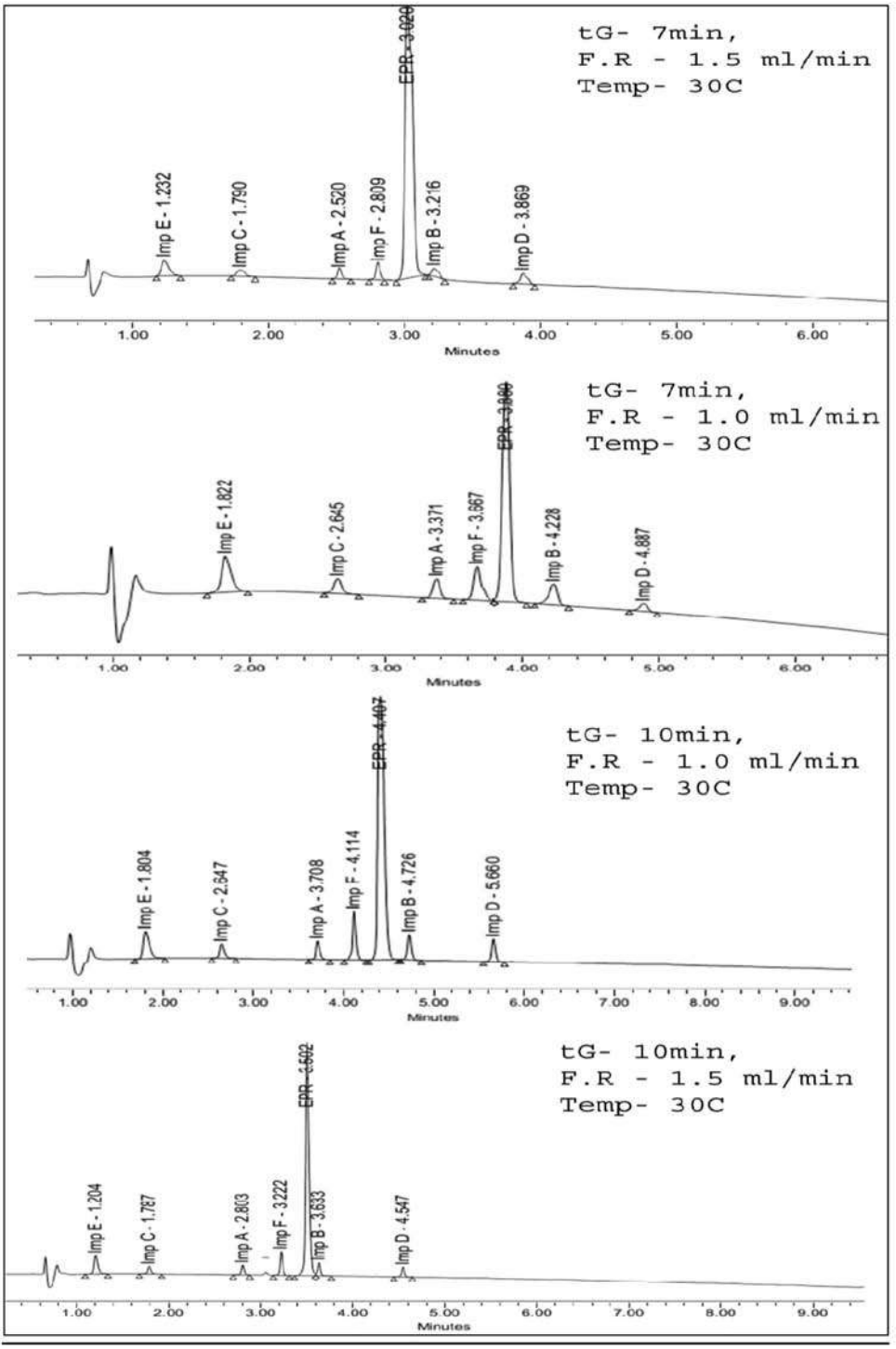
7	3	ACN	Phenomenex C18	5-85	20
8	5.5	MeOH	Phenomenex C18	10-95	20
9	3	MeOH	Grace Phenyl	10-95	20
10	3	ACN	Phenomenex C8	5-85	20
11	3	MeOH	Sun Shell C18	10-95	20
12	3	MeOH	Phenomenex C8	10-95	20
13	3	ACN	Sun Shell C18	5-85	20
14	5.5	ACN	Sun Shell C18	5-85	20
15	5.5	MeOH	Sun Shell C18	10-95	20
16	5.5	MeOH	Phenomenex C8	10-95	20

Expt No.	pH of mobile phase	Organic modifier	Columns	Gradient (% of organic phase)	Gradient time (min)
1	5.5	ACN	Grace Phenyl	5-85	20
2	5.5	ACN	Phenomenex C18	5-85	20
3	3	MeOH	Phenomenex C18	10-95	20
4	5.5	MeOH	Grace Phenyl	10-95	20
5	5.5	ACN	Phenomenex C8	5-85	20
6	3	ACN	Grace Phenyl	5-85	20
7	3	ACN	Phenomenex C18	5-85	20
8	5.5	MeOH	Phenomenex C18	10-95	20
9	3	MeOH	Grace Phenyl	10-95	20
10	3	ACN	Phenomenex C8	5-85	20
11	3	MeOH	Sun Shell C18	10-95	20
12	3	MeOH	Phenomenex C8	10-95	20
13	3	ACN	Sun Shell C18	5-85	20
14	5.5	ACN	Sun Shell C18	5-85	20
15	5.5	MeOH	Sun Shell C18	10-95	20
16	5.5	MeOH	Phenomenex C8	10-95	20



tG (min)	Flow rate (ml/min)	Column Temperature (0C)
7	1.0	30
7	1.5	30
7	1.0	50
7	1.5	50
10	1.0	50
10	1.0	30
10	1.5	30
10	1.5	50


tG- 7min
F.R- 1.5 ml/min
Temp- 50C
Imp E - 1.081
Imp C - 1.524
Imp A - 2.378
Imp B - 2.569
Imp F - 2.663
EPR - 2.929
Imp D - 3.572
Minutes
tG- 7min
F.R- 1.0 ml/min
Temp- 50C
Imp E - 1.597
Imp C - 2.234
Imp A - 3.210
Imp F - 3.467
EPR - 3.788
Imp B - 3.880
Imp D - 4.532
Minutes
tG- 10min
F.R- 1.0 ml/min
Temp- 50C
Imp E - 1.539
Imp C - 2.105
Imp A - 3.478
Imp F - 3.844
Imp B - 4.276
EPR - 4.313
Imp D - 5.166
Minutes
tG- 10min
F.R- 1.5 ml/min
Temp- 50C
Imp E - 1.054
Imp C - 1.434
Imp A - 2.574
Imp F - 2.966
Imp B - 3.212
EPR - 3.355
Imp D - 4.079
Minutes


tG- 7min,
F.R - 1.5 ml/min
Temp- 30C
Imp E - 1.232
Imp C - 1.790
Imp A - 2.520
Imp F - 2.809
EPR - 3.020
Imp B - 3.216
Imp D - 3.869
Minutes
tG- 7min,
F.R - 1.0 ml/min
Temp- 30C
Imp E - 1.822
Imp C - 2.645
Imp A - 3.371
Imp F - 3.567
Imp B - 4.228
Imp D - 4.887
Minutes
tG- 10min,
F.R - 1.0 ml/min
Temp- 30C
Imp E - 1.804
Imp C - 2.647
Imp A - 3.708
Imp F - 4.114
EPR - 4.407
Imp B - 4.726
Imp D - 5.660
Minutes
tG- 10min,
F.R - 1.5 ml/min
Temp- 30C
Imp E - 1.204
Imp C - 1.787
Imp A - 2.803
Imp F - 3.222
EPR - 3.502
Imp B - 3.633
Imp D - 4.547
Minutes

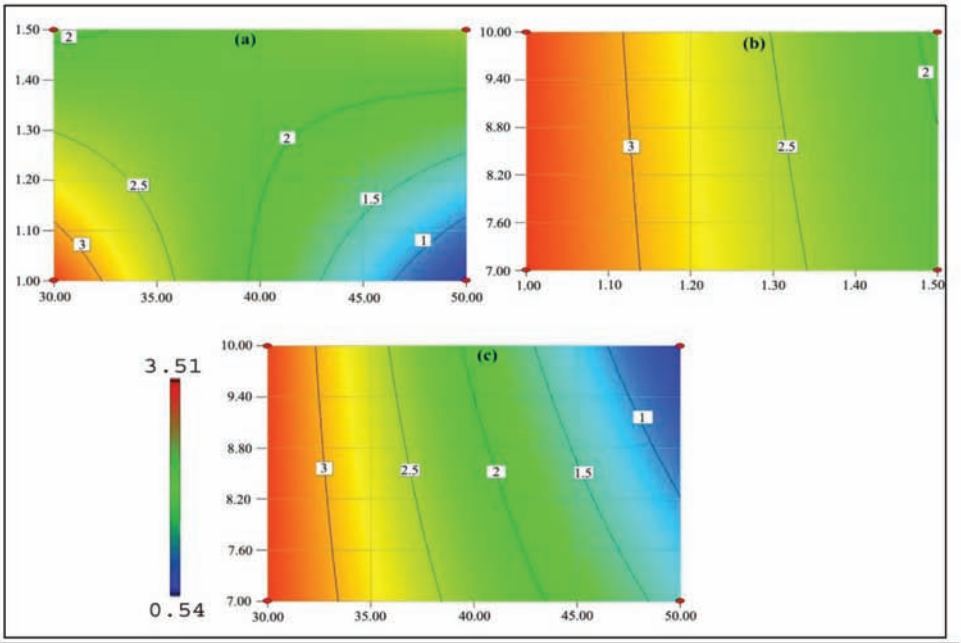


Figure. 2D DS for resolution of critical pair (EPR and impurity B).
(A) The *T (C)—flow rate (mL/min) model for tG 10 min.*
(B) The flow rate (mL/min)—tG (min) model for *T 30C.*
(C) The T (C)–tG (min) DS for flow rate 1.0 mL/min.

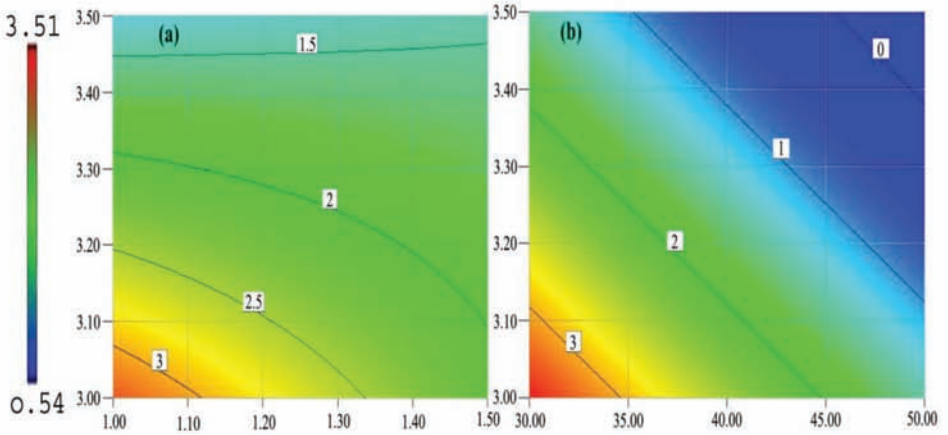
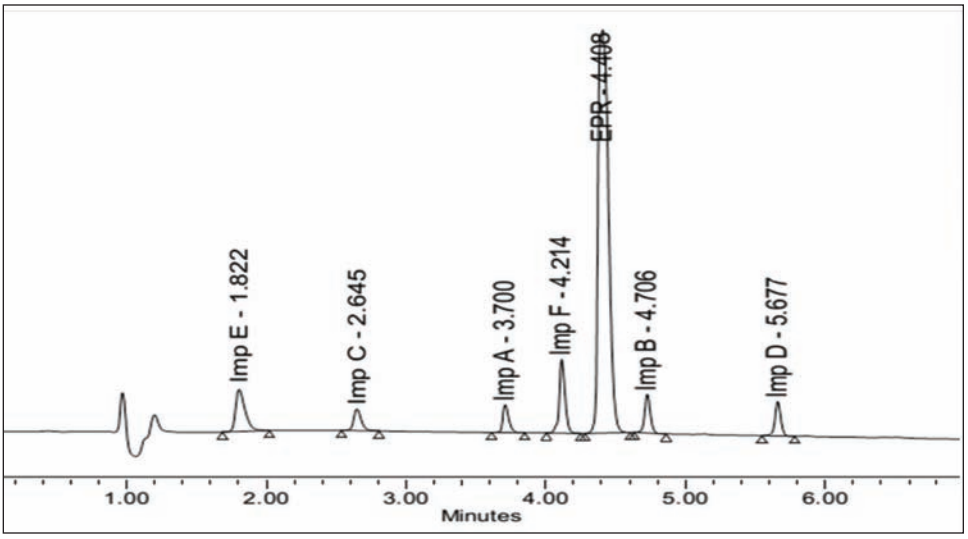


Figure 4. 2D DS for resolution of critical pair (EPR and impurity B).
(A) Flow rate (mL/min)—pH model for tG 10 min and (B) *T (C)—pH* model for tG 10 min.

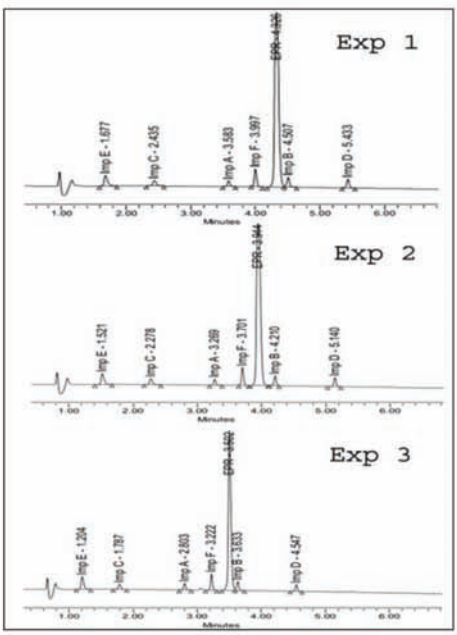
QbD Conditions

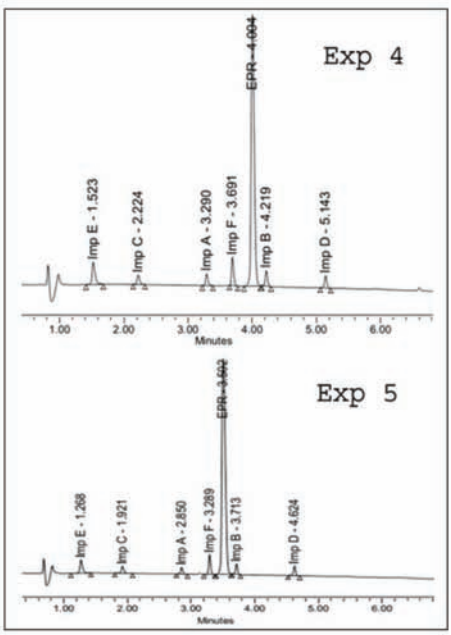
Column	Sun Shell C18; 100mm _ 2.1mm, 2.6μ
Eluent A	10mM ammonium formate pH 3.0 (up to 3.3 pH unit)
Eluent B	100 % Acetonitrile
Gradient	Linear gradient from 15 to 65% for 7 min (up to 10 min), followed by re-equilibration
Flow rate	1.0 ml/min (up to 1.3 ml/min)
Column temperature	300C (up to 350C)
Injection volume	10 μl
Detection wavelength	235 nm



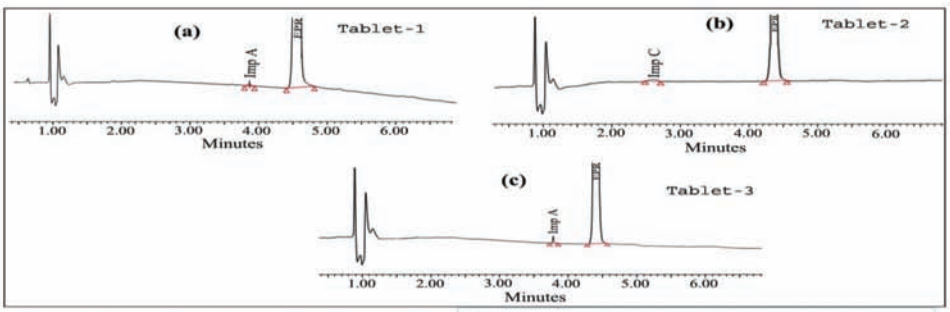
VERIFICATION

	Verification studies														
	Experiment 1 F.R_1.0 ml/min Temp_30⁰C pH_3.2			Experiment 2 F.R_1.2 ml/min Temp_30⁰C pH_3.0			Experiment 3 F.R_1.3 ml/min Temp_35⁰C pH_3.3			Experiment 4 F.R_1.2 ml/min Temp_33⁰C pH_3.0			Experiment 5 F.R_1.3 ml/min Temp_35⁰C pH_3.2		
Peaks	Design Expert [min]	Exp. [min]	% Accuracy	Design Expert [min]	Exp. [min]	% Accuracy	Design Expert [min]	Exp. [min]	% Accuracy	Design Expert [min]	Exp. [min]	% Accuracy	Design Expert [min]	Exp. [min]	% Accuracy
Imp A	3.60	3.58	100.56	3.24	3.27	100.93	2.77	2.80	98.93	3.22	3.29	97.87	2.81	2.85	98.60
Imp B	4.40	4.51	97.56	4.15	4.21	101.45	3.53	3.63	97.25	4.17	4.22	98.82	3.65	3.71	98.38
Imp C	2.48	2.44	101.64	2.21	2.28	103.17	1.8	1.79	100.56	2.27	2.22	102.25	1.89	1.92	98.44
Imp D	5.37	5.43	98.90	5.09	5.14	100.98	4.5	4.55	98.90	5.15	5.14	100.19	4.66	4.62	100.87
Imp E	1.71	1.68	101.79	1.48	1.52	102.70	1.24	1.20	103.33	1.47	1.52	96.71	1.24	1.27	97.64
Imp F	3.89	4.00	97.25	3.68	3.7	100.54	3.26	3.22	101.24	3.66	3.69	99.19	3.37	3.29	102.43
EPR	4.19	4.33	96.77	3.96	3.94	99.49	3.57	3.50	102.00	3.98	4.00	99.50	3.47	3.5	99.14

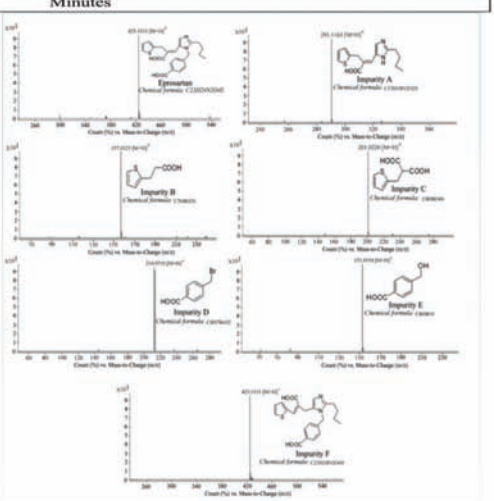




Validation parameters	Test details	Imp A	Imp B	Imp C	Imp D	Imp E	Imp F	EPR
Linearity	6 different concentration was used for linearity	0.3-2 µg/ml	0.3-2 µg/ml	0.3-2 µg/ml	0.3-2 µg/ml	0.3-2 µg/ml	0.3-2 µg/ml	50-500 µg/ml
Slop		26674	20870	14637	21772	45614	35769	22045
Intercept		-696	+439	+111	+142	+734	+1341	+22208
r^2		0.9988	0.9992	0.9984	0.9990	0.9993	0.9990	0.9988
Limit of Detection (µg/ml)	LOD was calculated based on the 3.3* S/N	0.07	0.08	0.09	0.08	0.05	0.06	0.08
Limit of Quantification (µg/ml)	LOQ was calculated based on the 10* S/N	0.22	0.25	0.3	0.25	0.18	0.20	0.25
Accuracy_%RSD	% recovery was calculated (n=3)	99.69_1.13	99.63_1.10	99.37_1.92	99.13_1.11	99.59_0.91	99.55_1.57	100.23_0.36
Intra- day precision (%RSD)	n=3	1.58	1.18	2.04	1.27	1.65	1.49	1.65
Inter-day precision (%RSD)	n=3	1.83	2.14	2.60	1.38	2.71	2.77	2.01
Relative Response Factor (RRF)	RRF= Response of Imp/ Response of drug	0.878	0.832	0.587	0.809	1.660	1.297	1.000



- Agilent 1200 series HPLC instrument (Agilent Technologies, USA) coupled to a quadrupole time-of-flight (Q-TOF) mass spectrometer (Q-TOF LC/MS 6540 series classic G6510A, Agilent Technologies, USA) equipped with an ESI source was used.
- Data analysis was carried out using Mass Hunter workstation software.
- The typical operating source conditions for MS scan of EPR and its impurities in positive ESI mode were optimized as follows:
  - the fragmentor voltage was set at 130 V; the capillary at 3000 V; the skimmer at 60 V; nitrogen was used as the drying (300°C; 9 L/min) and nebulizing (45 psi) gas.



APOPTOSIS CANCER CELL SIGNALLING-MOLECULAR TOOLS AND METHODS: RESVERATROL TARGETS MITOCHONDRIA FOR APOPTOTIC CELL DEATH IN CANCER

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ABSTRACT

Resveratrol, a naturally occurring phytoalexin, is known to induce apoptosis in multiple cancer cell types, but the underly ing molecular mechanisms remain unclear. Here, we show that resveratrol induced p53-independent, X-linked inhibitor of apoptosis protein (XIAP)-mediated translocation of Bax to mitochondria where it underwent oligomerization to initiate apoptosis. Resveratrol treatment promoted interaction between Bax and XIAP in the cytosol and on mitochondria, suggesting that XIAP plays a critical role in the activation and translocation of Bax to mitochondria. This process did not involve p53 but required accumulation of Bim and t-Bid on mitochondria. Bax primarily underwent homo-oligomerization on mitochondria and played a major role in release of cytochrome c to the cytosol. Bak, another key protein that regulates the mitochondrial membrane permeabilization, did not interact with p53 but continued to associate with Bcl-xL. Thus, the proapoptotic function of Bak remained suppressed during resveratrol-induced apoptosis. Caspase-9 silencing inhibited resveratrol-induced caspase activation, whereas caspase-8 knockdown did not affect caspase activity, suggesting that resveratrol induces caspase-9-depen- dent apoptosis. Resveratrol depletes mitochondrial DNA and inhibition of autophagy enhances resveratrol-induced caspase activation. Together, our findings characterize the molecular mechanisms of resveratrol-induced caspase activation and subsequent apoptosis in cancer cells.

INTRODUCTION

Anticancer agents induce cell death in cancer and normal cells via mechanisms including apoptosis and autophagy (1- 4). Therefore, there is a need for alternative anticancer agents that can promote cancer cell death while avoiding killing of normal, non-cancerous cells. Resveratrol (trans-3, 5, 4'-trihydroxystil- bene) is a naturally occurring polyphenolic phytoalexin found at high levels in the skin of grapes and in red wine. It is also present in peanuts and other plant products. Resveratrol has been shown to possess an apoptosis-dependent anticancer activity and minimal toxicity to normal cells (5-11). How resveratrol induces apoptosis or cancer cell death is not clearly known, but available evidence indicates that resveratrol induces p53-dependent signaling, which leads to cell cycle arrest and apoptosis induction (10, 12, 13). Additionally, resveratrol targets mitochondria to induce cytochrome c release and thereby triggers caspase-dependent apoptotic cell death in multiple types of cancer cells (14-18). How resveratrol induces cytochrome c release and caspase activation to execute apoptosis remains unclear. Caspases are activated by proteolytic processing and are broadly divided into initiator caspases (e.g. procaspase-8 and -9) and executioner caspases (such as procaspase-3 and -7) (19- 22). During apoptosis, the released cytochrome c from mitochondria triggers caspase-9 activation, whereas ligation of death receptors on the plasma membrane activates caspase-8. Active caspase-8 generated upon death receptor ligation requires Bid-mediated cytochrome c release to execute apoptotic cell death in epithelial cancer cells (22-25). Proapoptotic BH3-only proteins such as activated Bid/Bim translocate to mitochondria to initiate Bax and/or Bak activation, leading to channel formation on the outer mitochondrial membrane (OMM) and permeabilization of mitochondria (22, 26, 27). Bak localizes on the OMM, whereas Bax mostly exists in the cytosol. Although resveratrol has been shown to modulate the levels of Bax/Bak or other Bcl-2 family proteins (14, 28), it is unclear

whether and how resveratrol activates Bax/Bak to permeabilize the mitochondrial membrane. Here, we demonstrate that resveratrol induced caspase-dependent apoptosis by targeting mitochondria. Our findings indicate that resveratrol may induce Bax oligomerization in the cytosol. Bax activation and its translocation to mitochondria seemed to be regulated by XIAP; however, p53 did not directly participate in the activation of Bax/Bak. Bax recruitment to and its oligomerization on mitochondria were associated with cytochrome c release, caspase activation, and apoptosis. These findings suggest that resveratrol may be a novel inducer of Baxmediated caspase activation and apoptosis in cancer cells, which normally lack p53 activity or harbor mutant p53.

EXPERIMENTAL PROCEDURES

a) **Cells and Reagents:** Colon cancer cells (HCT116, HCT116- Bax-KO, and HCT-p53-KO) were kindly provided by Dr. B. Vogelstein (29, 30) and cultured in McCoy's 5A medium supplemented with 10% FBS. Prostate cancer (PC3 and LNCaP), breast cancer (MDA-MB231, MCF-7, and MDA-MB435), immortalized normal human fibroblast (GM701), Jurkat wild type (WT), Jurkat caspase-8/, mouse embryonic fibroblast WT, and mouse embryonic fibroblast Apaf-1/ cells were obtained from the ATCC or from various investigators and were subcultured as described previously (31-36). The primary antibodies against cytochrome c (monoclonal antibody (mAb)), Apaf-1 and Bax (rabbit polyclonal antibody (Rb pAb)), Bid, caspase-8, XIAP, and Bcl-xL were purchased from BD Pharmingen. Bax N terminus (Rb pAb; Upstate); Bak (Rb pAb; Santa Cruz Biotechnology); p53 (Santa Cruz Biotechnology); cytochrome c oxidase subunit II (Mito Sciences); heat shock protein 60 (Hsp60) (Millipore); Hsp70 (Stressgen); Bak N terminus (Rb pAb; Upstate); VDAC-1 and Bim (Calbiochem); caspase-3 (Rb pAb; Biomol); caspase-9 (Cell Signaling Technology); and poly(ADP-ribose) polymerase, lactate dehydrogenase, and actin (mAb; ICN) were obtained from the indicated suppliers. Secondary antibodies and ECL reagents were acquired from GE Healthcare. Alexa Fluor 594- or 488-conjugated goat anti-mouse or -rabbit IgG (heavy light) and mitochondrial dye (i.e. MitoTracker Orange CMTMRos) were purchased from Molecular Probes (Invitrogen). The fluorogenic caspase substrates DEVD-AFC, LEHD-AFC, general caspase inhibitor Z-VAD-fluoromethyl ketone, and cross-linkers were bought from Enzo Life Sciences. All other chemicals were purchased from Sigma unless specified otherwise.

b) **Subcellular Fractionation and Western Blotting:** The preparation of whole cell lysates and mitochondrial and cytosolic fractions and Western blotting were performed as detailed previously (35).

c) **Quantification of Apoptosis and Caspase Activity Measurement:** Apoptotic cells were counted based on live cell staining with DAPI to label apoptotic nuclei (35). In addition, both live and dead cells were counted using trypan blue dye. DEVDase and LEHDase activities were measured as described previously (35).

d) **Establishment of Cancer Cells Stably Expressing Caspase-9 or Caspase-8 siRNA Using shRNA Lentiviral Vectors:** Green fluorescence protein (GFP)-tagged short hairpin RNAs (shRNAs) specific to caspase-9 and caspase-8 and negative control shRNA were cloned into the pGIPZ (Open Biosystems) lentiviral vector to generate lentiviral particles. The shRNA sequences were as follows: caspase-8, 5'-GACTTCAGCAGAAATCTTT-3'; & caspase-9, 5'-CCAGGCAGCTGATCATAGA-3'. Lentiviral particles specific for caspase-9, caspase-8, and control shRNAs were obtained from the Roswell Park Cancer Institute shRNA core resource and were directly utilized to infect cells at a multiplicity of infection of 3. After 48 h, puromycin (1 mg/ml) was added to the medium to select caspase-8 or caspase-9 knockdown cells (37).

e) **Immunofluorescence:** Cells grown on coverslips were treated with resveratrol and 15 min prior to the end of treatment were incubated live with either DAPI alone (to label nuclei) or MitoTracker Orange (CMTMRos) and DAPI (to label mitochondria and nuclei, respectively). Cells were then fixed, permeabilized, and immunolabeled for cytochrome c (32, 35).

f) **Chemical Cross-linking and Oligomerization Assays:** Freshly harvested cells or freshly purified mitochondria or cytosol (50 mg) was suspended in 45ml of HIM buffer (200 mM mannitol, 70 mM sucrose, 10 mM HEPES-KOH, 1 mM EGTA, pH 7.5) followed by addition of freshly prepared bismaleimidohexane (BMH) or ethylene glycol bis(succinimidylsuccinate) to a final concentration of 2 mM and incubated at room temperature for 30 min. Mitochondria were then mixed with protein sample buffer and subjected to Western blotting (32).

g) **Gel Filtration Analysis:** Various types of cancer cells were treated with resveratrol or vehicle (DMSO) and washed twice in cold phosphate-buffered saline. Cytosolic and mitochondrial fractions were purified as described previously (31, 32, 34) and loaded onto a Superdex 200 HR10/30 column (GE Healthcare). Proteins were eluted at 0.5 ml/min, and fractions (0.5 ml) were collected for Western analyses (38).

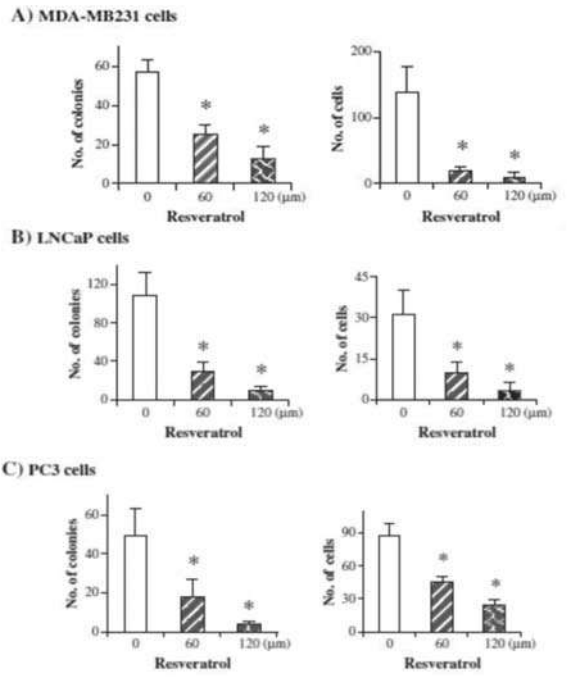
h) **Immunoprecipitation:** Purified mitochondrial lysates or cytosols were precleared with mouse or rabbit (depending on the primary antibodies used) IgG-conjugated agarose beads and incubated with primary antibodies against Bax, Bak, p53, XIAP, or Rb IgG (as control) followed by addition of rabbit or mouse IgG beads. Finally, the beads were washed thoroughly and analyzed by the TrueBlot (eBioscience) Western blotting system (34).

i) **Cell-free Reconstitution Experiments:** Purified mitochondria were incubated with cytosol or homogenizing buffer in a total reaction mixture of 50 ml at 37 °C for 60 min followed by Western blotting to detect XIAP translocation (36).

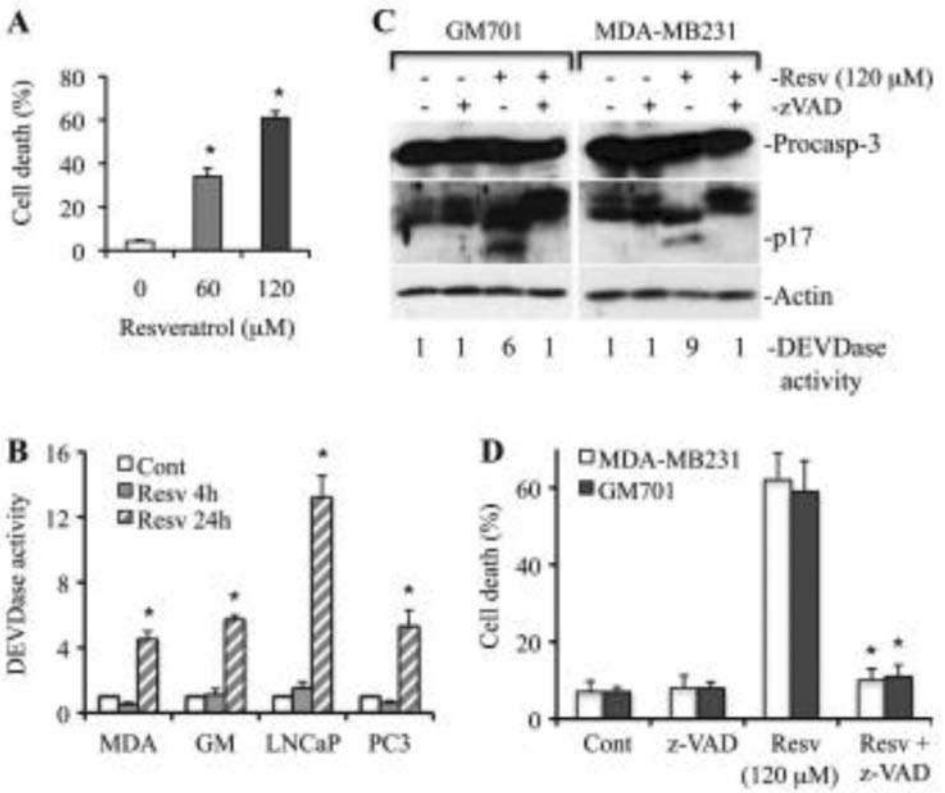
j) **Statistical Analysis:** Results are presented as mean +/-S.D. of data from at least three independent experiments. Statistical analysis was performed by analysis of variance using Sigma Stat. Significant changes ($p < 0.01$) are represented by *.

RESULTS:

a) **Resveratrol-inhibits colony formation in cancer cells:** Prostate (LNCaP and PC3 cells) and breast (MDA-MB231 cells) cancer cells were plated at a density of 500 cells/well in a 6-well plate and treated with 0, 60 and 120 _M of resveratrol for 48 h. After 48 h, the cells were grown in drug-free medium. When colonies became visible (~1-2 weeks), cells were fixed and stained with Giemsa (1:10 in distilled water at room temperature), and counted. Number of cells in individual colony was also counted (right hand side panels). Data are mean±SD, n=3; *Pb0.05.

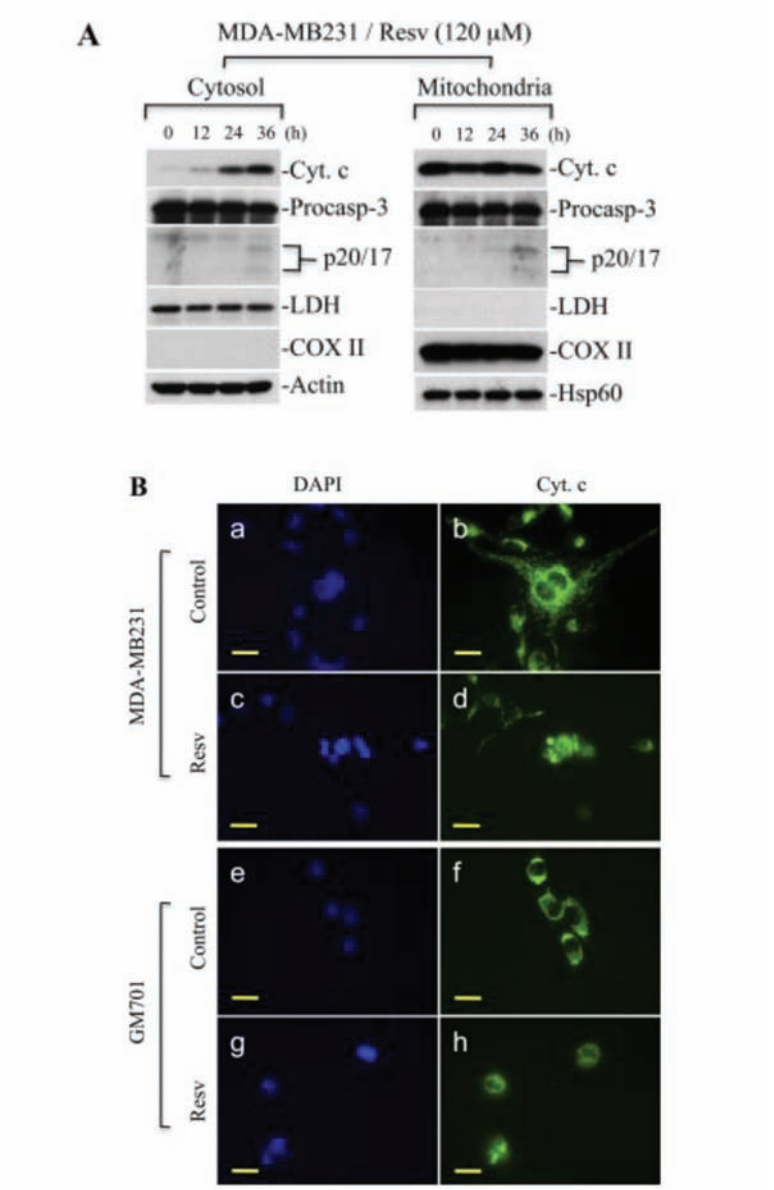


b) Resveratrol induces caspase-dependent apoptosis: A, MDAMB231cells were treated with resveratrol (Resv) for 36 h, and the percentage of cell death (mean S.D. from four independent experiments) was determined using trypan blue dye. *, p0.01. B, MDA-MB231, GM701 (GM), LNCaP, and PC3 cells were treated with resveratrol for the indicated time intervals, and equal amounts of protein (50 ng) were subjected to caspase-3 activity measurements. Caspase activities are presented as values (meanS.D. from at least three independent experiments) relative to those in controls. *, p 0.01. C, GM701 and MDA-MB231 cells were pretreated with the pan-caspase inhibitor Z-VAD (50 M) for 1 h and then treated with resveratrol for 36 h. At the end of treatment, equal amounts of protein were used for Western blotting and DEVDase activity assay. The number in DEVDase activity represents-fold change against untreated control (for example, lane 1 is control for GM701 cells and lane 5 is control for MDA-MB231 cells). Data are the representative of four independent experiments. D, MDA-MB231 and GM701 cells were pretreated with the pan-caspase inhibitor Z-VAD (50 _M) for 1 h and then treated with resveratrol for 36 h. At the end of treatment, the percentage of cell death was determined, and data are represented as meanS.D. from four independent experiments. *, $p<0.01$ as compared with resveratroltreated cells. Cont, control; Resv, resveratrol; MDA, MDA-MB231; Procasp-3, procaspase-3. p17 is a cleaved caspase-3 fragment.

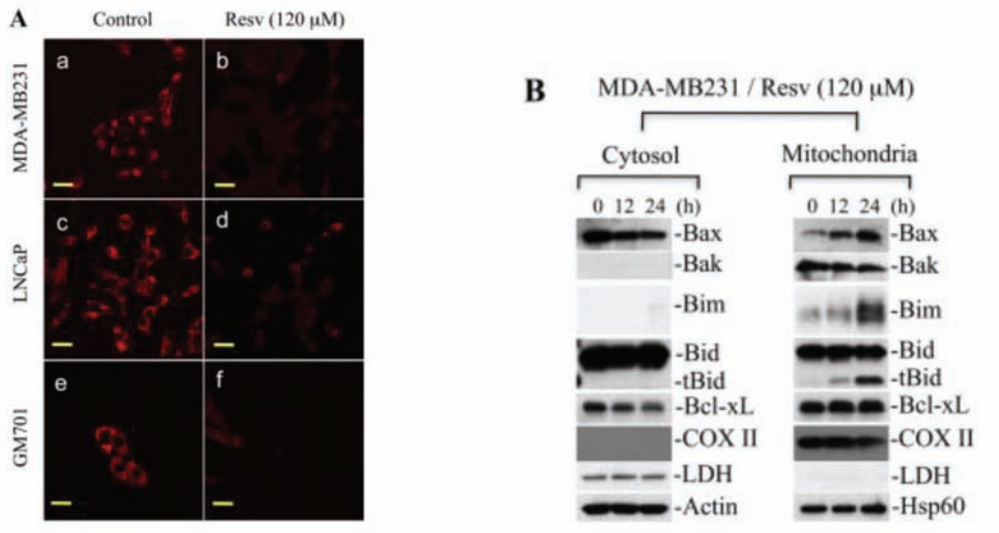


c) Resveratrol induces cytochrome c release from mitochondria into cytosol and is accompanied by fragmentation of nucleus: A, MDAMB231 cells were treated with resveratrol (Resv; 120 M) for the indicated time intervals. At the end of the treatment, cytosolic and mitochondrial fractions were isolated, and equal amounts of protein were subjected to Western blotting for the detection of

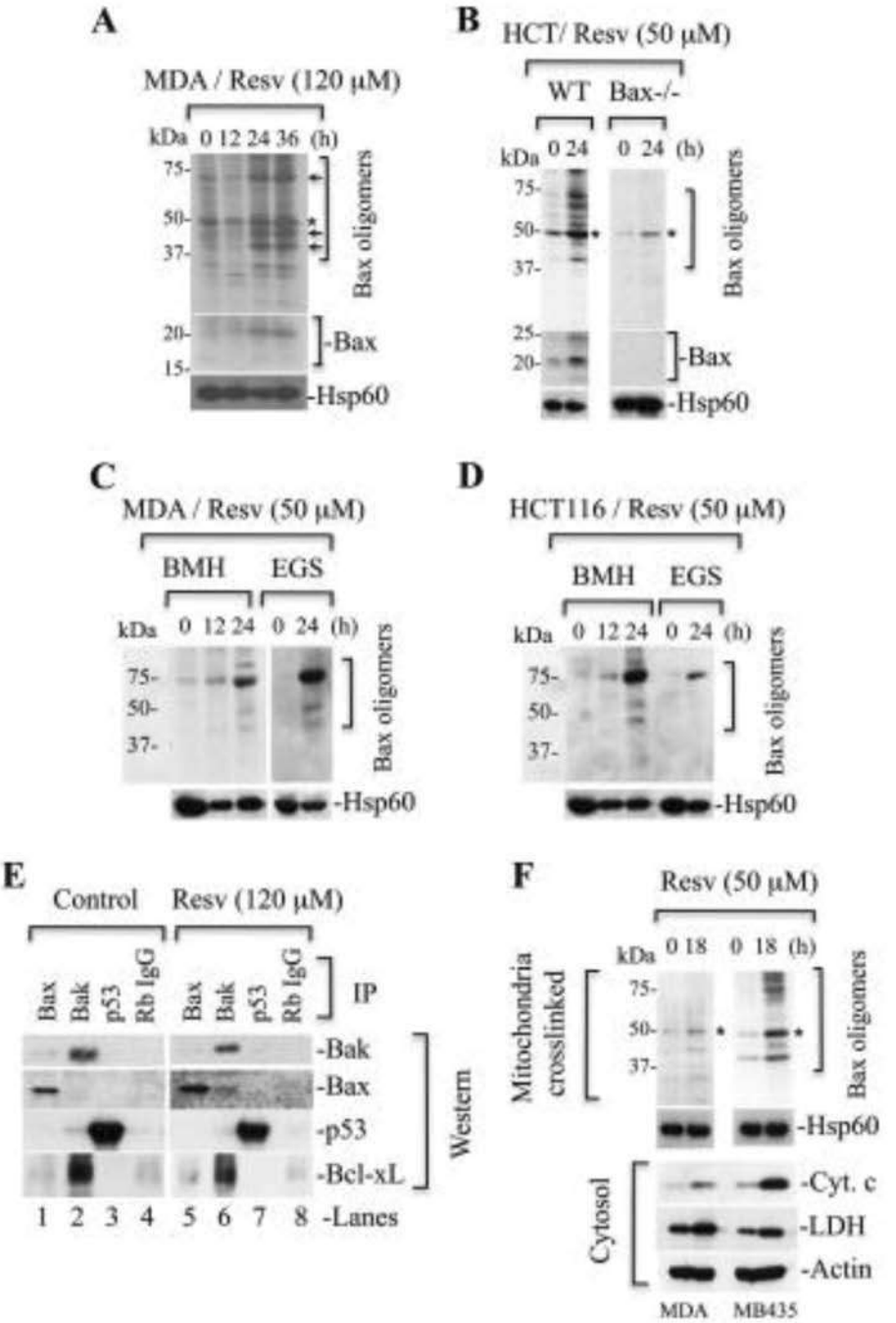
the indicated molecules. Cyt. c, cytochrome c; Procasp-3, procaspase-3; COX II, cytochrome c oxidase subunit II; LDH, lactate dehydrogenase. p20/17 represents cleaved products of caspase-3. Actin and Hsp60 serve as loading controls. B, cells were treated with resveratrol (Resv; 120 _M) for 24 h. At the end of the treatment, live cells were labeled with DAPI (panels a, c, e, and g) and MitoTracker Orange to detect the nucleus and mitochondria (data not shown), respectively. Cells were then immunolabeled for cytochrome c (Cyt. c; panels b, d, f, and h). Representative micrographs are shown, and magnification bars represent 20m. Consistent with the Western blot analysis, the diffuse staining for cytochrome c in individual cells reveals that it was released from mitochondria. Apoptotic cells show fragmented/ shiny nucleus with DAPI staining in the panels c and g. Data are representative of at least three independent experiments.



d) Resveratrol causes loss of mitochondrial membrane potential and induces Bax translocation to mitochondria and up-regulates Bim and t-Bid on mitochondria. A, MDA-MB231, LNCaP, and GM701 cells were treated without (Control; panels a, c, and e) or with resveratrol (Resv; 120 _M for 24 h) (panels b, d, and f) and just prior to fixation were labeled live with Mito Tracker Orange, which accumulates in mitochondria in a membrane potential-dependent manner. The images were captured by microscope, representative micrographs are shown, and magnification bars represent 20m. B, MDA-MB231 cells were treated with resveratrol (Resv; 120 _M) for the indicated time intervals. Cytosolic and mitochondrial fractions were purified followed by Western blotting for the indicated molecules by loading equal amounts of protein. COX II, cytochrome c oxidase subunit II. Actin and Hsp60 serve as loading controls. Data are representative of three independent experiments.

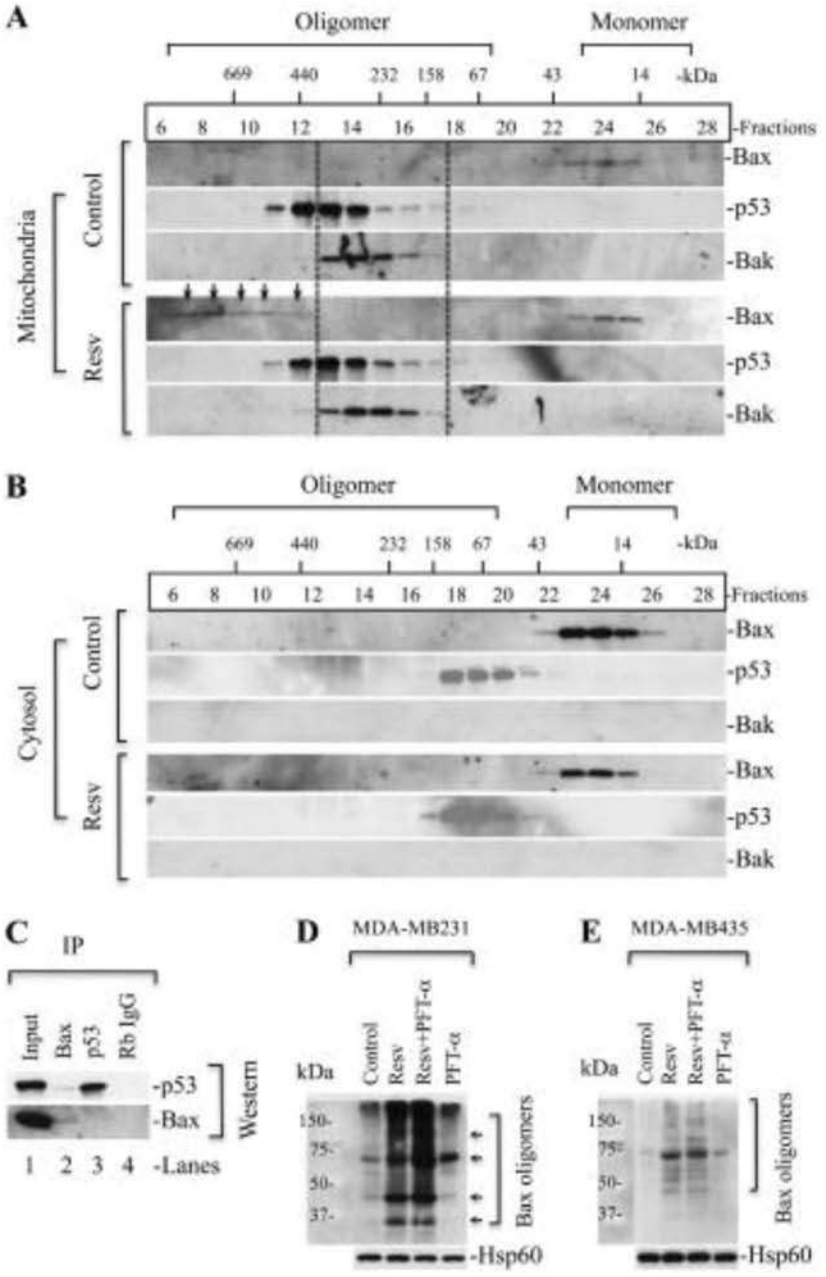


e) Bax undergoes homo-oligomerization on mitochondria, and Bax oligomerization on mitochondria is accompanied by cytochrome c release. A and B, MDA-MB231(A) cells were treated with resveratrol (Resv; 120mM) for the indicated time intervals, and HCT116 WT or HCT116 Bax/ cells (B) were treated with resveratrol (50mM). Purified mitochondria were crosslinked with BMH and subjected to Western blotting for Bax to detect its oligomers. Hsp60 serves as a loading control. C and D, MDA-MB231 and HCT116 cells were treated with resveratrol (50 _M) for the indicated time periods. At the end of treatments, cells were cross-linked with BMH or ethylene glycol bis(succinimidylsuccinate) (EGS) followed by Western blotting for Bax or Hsp60. E, mitochondrial lysates isolated from MDA-MB231 cells treated with resveratrol (120 _M for 36 h) or from unstimulated cells were subjected to immunoprecipitation followed by Western blotting for the indicated molecules. F, MDA-MB231 and MDA-MB435 cells were treated with resveratrol (50 _M) followed by mitochondrial cross-linking and cytochrome c release assayed by Western blotting. MDA, MDA-MB231; MB435, MDA-MB435; Cyt. c, cytochrome c; LDH, lactate dehydrogenase. The asterisks in A, B, and F indicate a nonspecific band. Data are representative of at least three independent experiments.

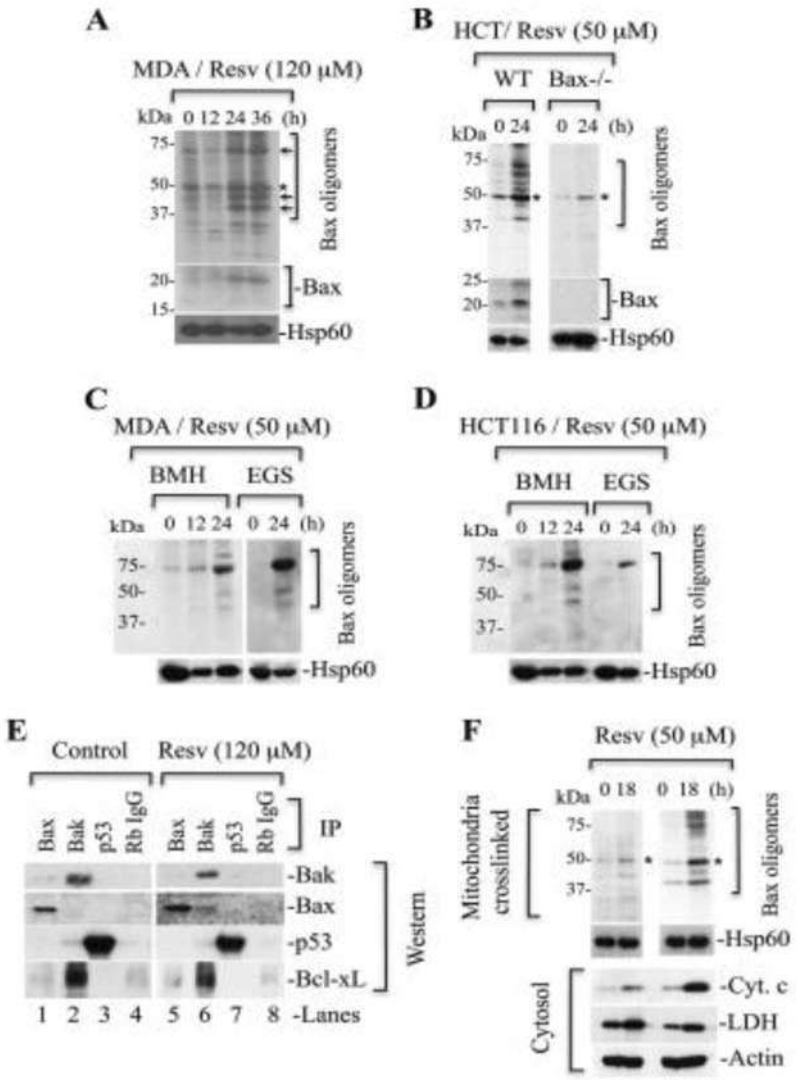


f) Bax undergoes oligomerization on mitochondria; Bax translocation and activation of Bax/Bak do not involve p53. A and B, mitochondrial (A) and cytosolic (B) fractions isolated from MDA-MB231 cells treated with resveratrol (120 _M for 36 h) or unstimulated were fractionated on a Superdex 200 column. Fractions (0.5 ml) were collected, and a portion (20l) of fractions 6-28 was analyzed by Western blotting for Bax, Bak, and p53.Arrows in A indicate Bax oligomers. C, cytosol isolated

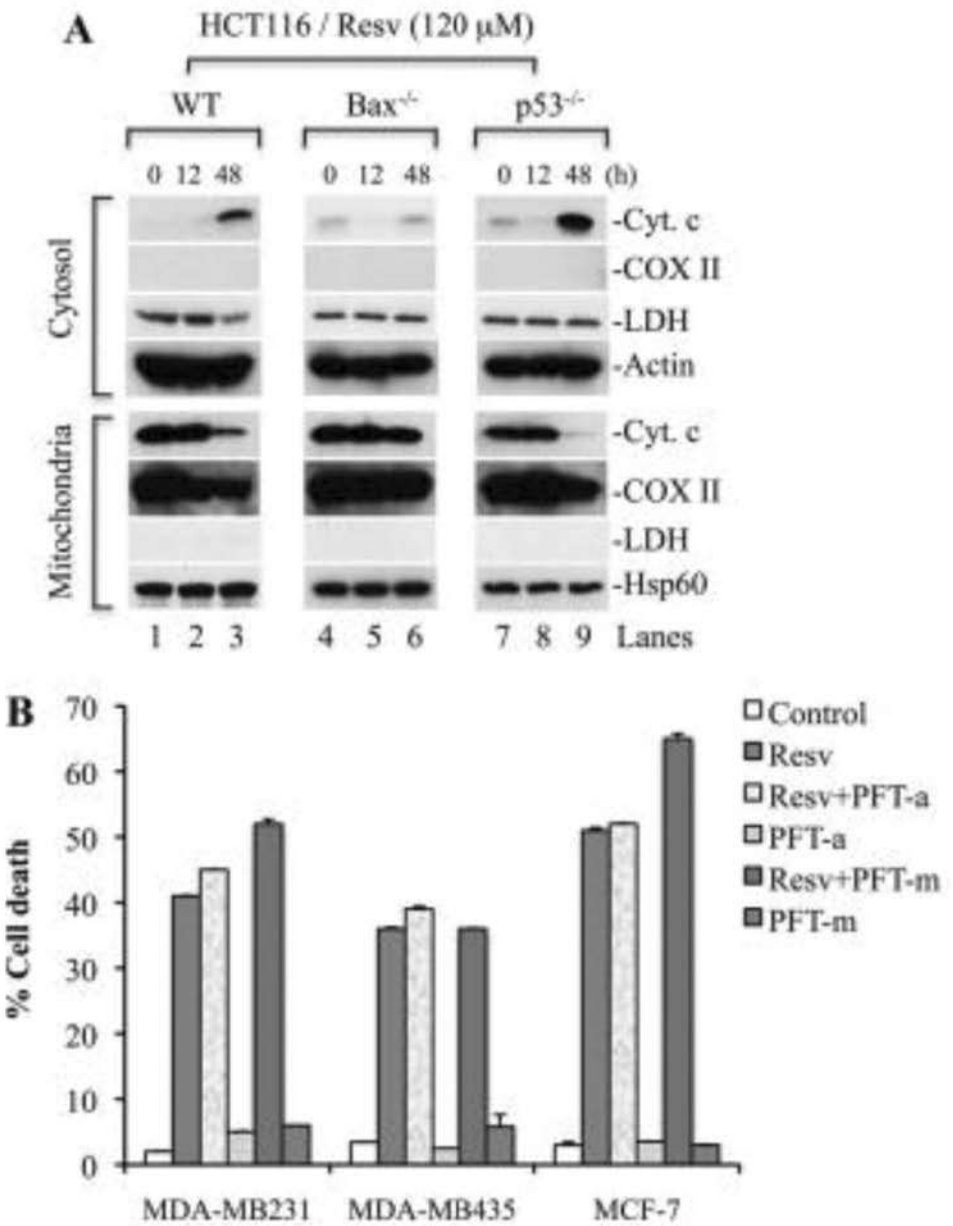
from MDA-MB231 treated with resveratrol (120 M for 36 h) was subjected to immunoprecipitation with p53 or Bax followed by Western blotting for the indicated proteins. D and E, MDA-MB231 (D) and MDA-MB435 (E) cells were pretreated with PFT- (30 _M) followed by resveratrol (50 _M) treatment. Cells were cross-linked and subjected to Western blotting to detect Bax oligomerization. Resv, resveratrol. Data are representative of at least three independent experiments.



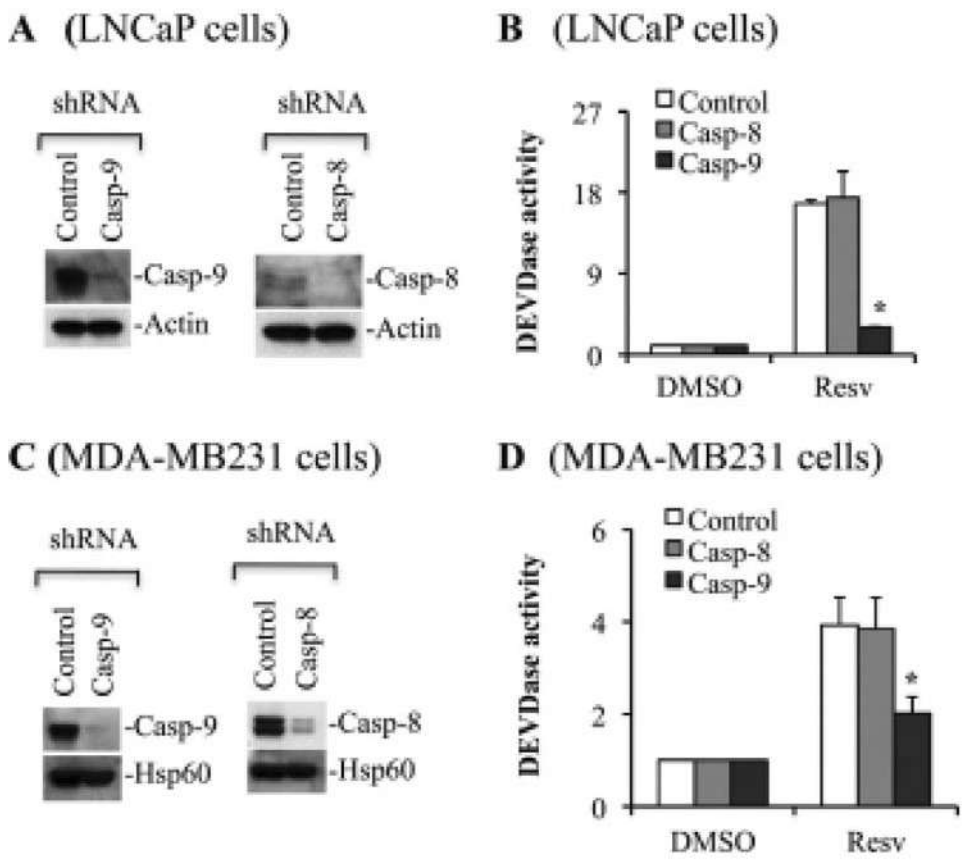
g) Resveratrol induces Bax activation/oligomerization in cytosol, and XIAP interacts with Bax to regulate its activation and translocation to mitochondria. A and B, equal amounts of cytosols obtained from MDAMB231 cells (A) treated with resveratrol (120 _M for 0, 12, 24, and 36 h) or from HCT116 cells (B) treated with resveratrol (50 _M for 0, 12, and 24 h) were cross-linked with BMH followed by Western blotting for Bax. C, purified mitochondria from resveratrol-treated (50 _M for 24 h) or untreated cells were subjected to Western blotting to detect XIAP and Bax translocation to mitochondria. D, purified cytosol and mitochondria obtained from resveratrol treated or untreated MDA-MB231 cells were reconstituted to examine XIAP translocation to mitochondria. E and F, IP was performed using mitochondria (E) or cytosol (F) obtained from MDA-MB231 cells treated with resveratrol (50 _M for 18 h) followed by Western blotting for the indicated proteins. MDA, MDA-MB231; MB435, MDA-MB435; HCT, HCT116 WT; Resv, resveratrol; Cont, control; Cyto, cytosol; Mito, mitochondria. The asterisks in A and B indicate a nonspecific band. Data are representative of at least three independent experiments.



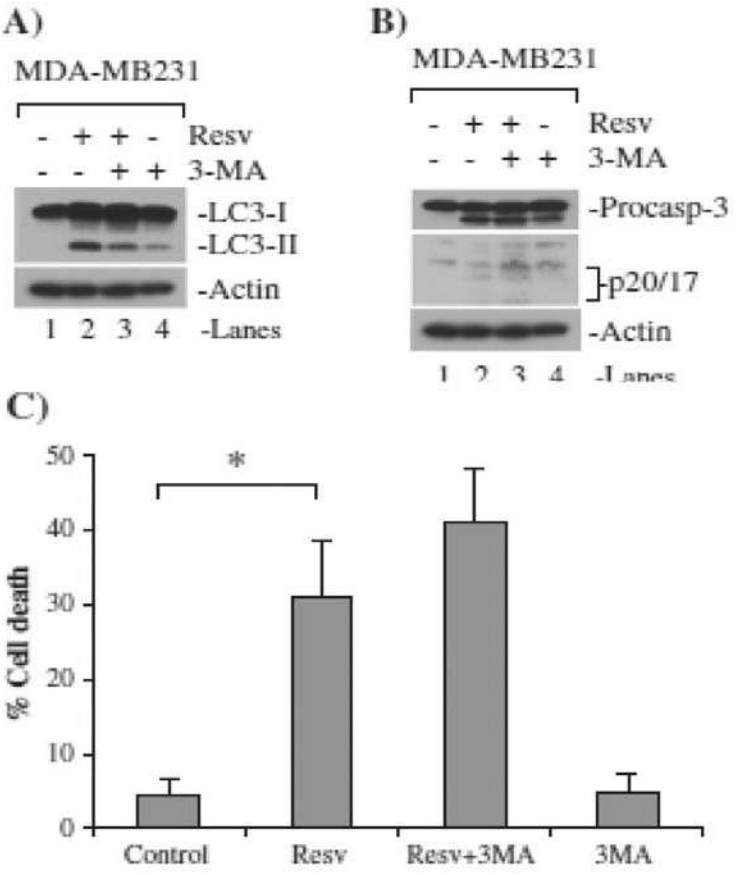
h) Bax plays prominent role to induce cytochrome c release upon resveratrol treatment. A, HCT116 WT, Bax/, and p53/cells were treated with resveratrol (120M for 12 or 48 h). Cytosolic and mitochondrial fractions were subjected to Western blotting to detect cytochrome c release. Resv, resveratrol; Cyt. c, cytochrome c; COX II, cytochrome c oxidase subunit II. Actin and Hsp60 serve as loading controls. Data are representative of three independent experiments. B, MDA-MB231, MDA-MB435, and MCF-7 cells were pretreated for 2 h with PFT- (30 _M) or PFT- (10 _M) followed by resveratrol treatment (50 _M for 24 h). At the end of treatment, the percentage of cell death was quantified. Data are mean S.D. (n 3). PFT-a, PFT-; PFT-m, PFT-.



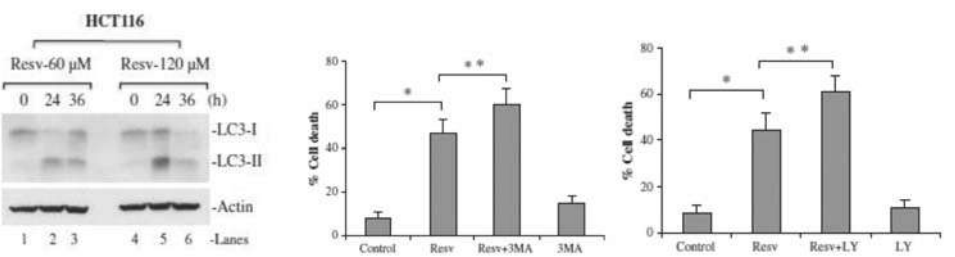
i) Activation of caspase-9 is initiating event in resveratrol-induced apoptosis. A, LNCaP cells were infected with caspase-9, caspase-8, or control shRNA lentiviral particles at a multiplicity of infection of 3. Stable cells were used for Western blotting to detect caspase-9 or caspase-8 silencing. B, caspase-8- and caspase-9-silenced or control (non-targeting) cells were treated with resveratrol (120 _M for 24 h). C, caspase-8 or caspase-9 was silenced in MDA-MB231 cells. D, cells were treated with resveratrol (50 _M for 24 h). At the end of treatment, caspase-3 activity was determined. Resv, resveratrol; Casp-9, caspase-9; Casp-8, caspase-8. Actin and Hsp60 serve as loading controls. Data are mean S.D. (n 3). *, p 0.01 as compared with resveratrol-treated control cells.



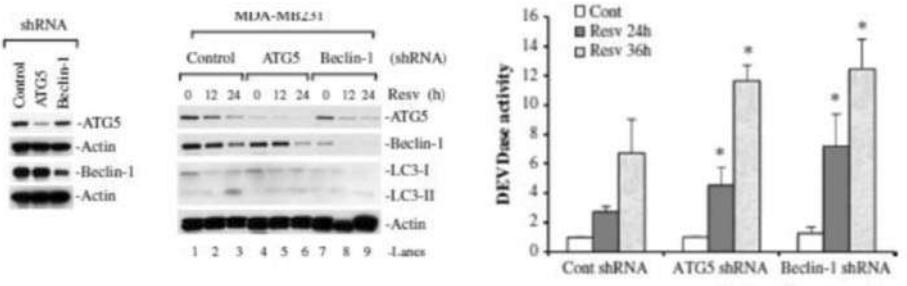
j) Resveratrol induces autophagy in cancer cells: MDA-MB231 cells were pretreated with 3-MA (5 mM) for 1 h followed by treatment with 120 _M resveratrol (Resv) for 24 h or untreated as indicated. (A) Whole cell lysates were subjected to Western blotting for LC3. (B) Whole cell lysates were subjected to Western blotting for determination of caspase 3 processing. Actin serves as a loading control. (C) Percent cell death was quantified using Trypan Blue exclusion assay. Procasp-3, procaspase-3; Resv, resveratrol. Data are mean±SD, n=3; *Pb0.01.



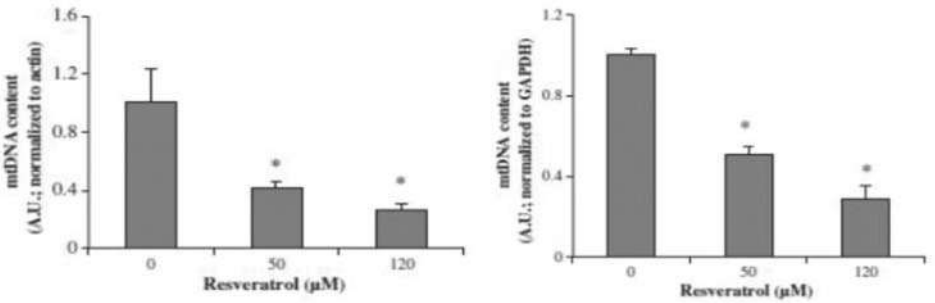
k) Inhibition of autophagy enhances resveratrol-induced cell death. (A) HCT116 cells were treated with resveratrol (60 or 120 _M) for indicated times. At the end of treatment, whole cell lysates were subjected to Western blotting for LC3. Actin serves as loading control. HCT116 WT cells were pretreated with 3-MA (B; 5 _M) or LY294002 (C; 10 _M) for 1 h followed by treatment with resveratrol (120 _M for 24 h) or left untreated. Percentage cell death was quantified using Trypan Blue exclusion assay. Resv, resveratrol. Data are mean±SD, n=3; *Pb0.01; **Pb0.05.



l) Inhibition of autophagy by ATG5 or Beclin-1 silencing enhances caspase activation. MDA-MB231 cells were transduced using control (cont) or ATG5 or Beclin-1 targeted shRNAs in lentiviral vectors. Whole cell lysates were subjected to Western blot analysis for ATG5 and Beclin-1 (A). Stably transduced cells were treated with 120 _M resveratrol for 0, 12, 24, and 36 h. Whole cell lysates were prepared and equal amounts of protein were subjected to Western blotting to detect autophagy related proteins (B), and for caspase-3 activity measurement (C). Cont, control; Resv, resveratrol. Data are mean±SD, n=3; *Pb0.05 as compared to control shRNA cells treated with resveratrol.



m) Resveratrol depletes mitochondrial DNA: ATPase-8 gene encoded by the mitochondrial genome was amplified and quantified by real-time PCR using the SYBR green chemistry on the Applied Biosystems 7300 real-time PCR system. Total DNA was extracted from MDA-MB231 cells treated with DMSO or resveratrol (0, 50 or 120 _M) for 24 h. Mitochondrial ATPase-8 gene specific primers were used to amplify, and values were normalized to actin (A) or GAPDH (B). Data are mean±SD, n=4; *Pb0.01 as compared to DMSO-treated cells.



CONCLUSIONS:

Resveratrol induces caspase-dependent apoptosis. Resveratrol induces cytochrome c release from mitochondria into cytosol and is accompanied by fragmentation of nucleus. Caspases are the main soldiers in the battle field to execute apoptosis. Mitochondrion is the center point for all activities. Resveratrol causes loss of mitochondrial membrane potential and induces Bax translocation to mitochondria and up-regulates Bim and t-Bid on mitochondria. Bax undergoes oligomerization on mitochondria; Bax translocation and activation of Bax/Bak do not involve p53. Resveratrol Induces p53-independent, X-linked Inhibitor of Apoptosis Protein (XIAP)-mediated Bax Protein Oligomerization on Mitochondria to Initiate Cytochrome c Release and Caspase Activation. Resveratrol

depletes mitochondrial DNA and inhibition of autophagy enhances resveratrol-induced caspase activation.

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IP STRATEGY FOR ENTREPRENEURS

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Introduction to IPR

Intellectual Property Rights are statutory rights once granted allows the creator(s) or owner(s) of the intellectual property to exclude others from exploiting the same commercially for a given period of time. It allows the creator(s)/owner(s) to have the benefits from their work when these are exploited commercially. IPR are granted to an inventor or creator, designer in lieu of the discloser of his/her knowledge.

Governing Laws in India for IPR as follows:

1. Patent Act 1970
2. Trade Marks Act (1958 original) 1999
3. The Copyright Act 1957
4. The design Act 2000
5. Geographical Indication of Goods (Registration and Protection) Act 1999
6. Plant Variety and Farmers Right Protection Act 2001

Pressures of globalisation or internationalisation were not intense during 1950s to 1980s, and many countries, including India, were able to manage without practising a strong system of IPR. Globalization driven by chemical, pharmaceutical, electronic, and IT industries has resulted into large investment in R&D. This process is characterized by shortening of product cycle, time and high risk of reverse engineering by competitors. Industries came to realize that trade secrets were not adequate to guard a technology. It was difficult to reap the benefits of innovations unless uniform laws and rules of patents, trademarks, copyright, etc. existed. That is how IPR became an important constituent of the World Trade Organization (WTO).

General Overview on Trade-Related Aspects of Intellectual Property Rights

The WTO Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs Agreement) is the Uruguay Round agreement covering the protection and enforcement of intellectual property rights. Intellectual property rights were a key area of concern for the United States during the Uruguay Round negotiations. From the perspective of the United States, the TRIPs Agreement was a major achievement of the Uruguay Round. The TRIPs Agreement incorporates by reference most of the substantive provisions of two earlier multilateral IPR conventions: the Paris Convention for the Protection of Industrial Property (1967)(covering patents, trademarks, trade names, utility models, industrial designs and unfair competition) and the Berne Convention for the Protection of Literary and Artistic Works (1971) (covering copyrights).

The TRIPs Agreement applies to all WTO Members; it is not a plurilateral agreement (such as the Agreement on Government Procurement).

WTO Dispute Settlement Understanding applies to the TRIPs Agreement (including right of cross-retaliation in products and services).

Dispute Resolution and Parallel Importation ("International Exhaustion of Intellectual Property

Rights"): "For purposes of dispute settlement under this Agreement" (other than regarding national treatment and most favoured nation obligations), "nothing in this Agreement shall be used to address the issue of the exhaustion of intellectual property rights." (Article 6)

The TRIPs Agreement explicitly covers patents, trademarks, copyrights and related rights, geographical indications, lay-out designs (topographies) of integrated circuits (usually called semiconductor mask works in the U.S.), industrial designs and undisclosed information (trade secrets). The WTO Appellate Body has recently held that trade names are also protected by the TRIPs Agreement because of its incorporation by reference of relevant portions of the Paris Convention for the Protection of Industrial Property (1967) (Report of the Appellate Body, United States-Section 211 Omnibus Appropriations Act of 1998, WT/DS176/AB/R 2 January 2002).

Articles 3 and 4 contain national treatment and most favored nation obligations, respectively, concerning the "protection of intellectual property," subject to specified exceptions—particularly those contained in international conventions such as the Berne Convention for the Protection of Literary and Artistic Works (1971), the Paris Convention for the Protection of Industrial Property (1967) and the Rome Convention ("International Convention for the Protection of Performers, Producers of Phonograms and Broadcasting Organizations, adopted at Rome on 26 October 1961.")

Transition Periods:

Developed countries must have fully implemented the TRIPs Agreement by January 1, 1996; Developing countries and countries converting from planned to market economies must have fully implemented by January 1, 2000 (except that developing countries/countries in transition which did not have TRIPs consistent product patent protection (e.g., no product patent protection for pharmaceuticals and agricultural chemicals) have until January 1, 2005 to implement that obligation; those members that availed themselves of the transition period for providing product patent protection for pharmaceuticals and agricultural chemical inventions are obligated to provide a "mail box" system, and exclusive marketing rights for such products as of January 1, 1995.

Least developed countries do not have to fully implement TRIPs until January 1, 2006. The Doha Declaration on the TRIPs Agreement and Public Health further extended the transition period for least developed countries until January 1, 2016 with respect to the TRIPs obligations concerning pharmaceutical product patent protection and the protection of undisclosed pharmaceutical test data against unfair commercial use (Sections 5 and 7 of Part II of the TRIPs Agreement). (WT/MIN (01)/DEC/2 20 November 2001). On June 27, 2002, the WTO's Council for Trade-Related Aspects of Intellectual Property Rights (TRIPs Council) agreed to grant a waiver to least-developing countries concerning the obligation to provide exclusive marketing rights for pharmaceutical products (TRIPs Article 70.9) until January 1, 2016. This waiver is subject to approval by the WTO's General Council. There is no waiver of the obligation to adopt "mail box" provisions (TRIPs Article 70.8) for pharmaceutical product patent applications.

There is no transition period for the national treatment and most favoured nation obligations in Articles 3-5. All WTO Members were required to implement national treatment and most favoured nation obligations by January 1, 1996.

Different Types of Intellectual Property Rights

IP has been generally divided into two main categories

- Industrial Property
- Copyright.

Industrial property consists of rights relating to inventions, trademarks, industrial designs and appellation of origin.

Copyright protects rights related to creation of human mind in the fields of literature, music, art and audio-visual works. The owner of copyright has rights not only in the original work, but also in creative work that is derived from the original work, e.g. its translation or adaptation or the enactment or production of a film based on the original work. Such rights relating to a copyright are called related rights. There are neighbouring rights on copyright, which protect performances of performing artists, phonograms and broadcasts. Related rights and neighbouring rights are terms used interchangeably.

The TRIPS Agreement of the WTO recognises seven types of intellectual property rights (IPRs):

- Copyright and Related Rights;
- Trademarks, Trade names and Service marks;
- Geographical Indications;
- Industrial Designs;
- Patents;
- Layout Designs of Integrated Circuits; and
- Undisclosed Information
- Plant Varieties

Patents

A patent is a form of intellectual property. It consists of a set of exclusive rights granted by a sovereign state to an inventor or their assignee for a limited period of time, in exchange for the public disclosure of the invention. An invention is a solution to a specific technological problem, and may be a product or a process.

Period of Patents - 20 Years

Objective of Patent Law:

The Agreement on Trade Related Aspects of Intellectual Property Rights (TRIPS Agreement) outlines its objectives as follows:

"The protection and enforcement of intellectual property rights should contribute to the promotion of technological innovation and to the transfer and dissemination of technology, to the mutual advantage of producers and users of technological knowledge and in a manner conducive to social and economic welfare, and to a balance of rights and obligations."

Thus, it is clear that the entire IP system, including patent law, is ordered to ensure four broad objectives:

- promotion of technological innovation;
- the transfer and dissemination of technology;
- the advantage of consumers and inventors;
- In a manner conducive to social and economic welfare.

History of Patent Law

1856	ACT VI of 1856 on protection of inventions based on British patent law of
1852.	Certain exclusive rights granted to inventors of new manufactures for a period of 14 years.
1859	The Act modified as ACT XV; Patent Monopolies called exclusive privileges (Making Selling and using inventions in India and authorizing others to do so far for 14 years from the date of filing specification)
1883	Protection of inventions act.
1888	Consolidated as the inventions & design act.
1911	Indian patent & design Act
1972	Patent act (act 39 of 1970) came into force on 20 April 1972
1975	India Joins WIPO
1995	The Indian government became a signatory to TRIPS after it joined the WTO in 1995
1999	on March 26, Patent (Amendment) ACT 2002 came into force
2002	Patent (Amendment) ACT 2002 came into force from 20th May 2003. The Concepts of 20 year patent term, 18 months publication, request for examination, and Inventive step for patent ability were introduced by this amendment
2005	Patent Act 1970 as amended by patent (Amendment) Ordinance 2004 & Patent Rules2003 as amended by the Patent Amendment Rules 2005. Recently Patent Amendment Rules 2016 have been published which included startups & provisions for Startups and the time limits for FER & Refund of Extra Fee paid, etc.

Advantages of patents to inventor:

- Technological inventions provide incentives to monetary reward.
- Exclusive right to use the patent
- Grant licences
- Prevent others from copying the monopoly granted in his favour
- By transferring know how also he can receive consideration

Conditions for patentability:

Inventions in all fields of technology, whether products or processes, if they meet the criteria of,

- Novelty: Invention not known to public prior to claim by inventor
- Inventive Step: Invention would not be obvious to a person with ordinary skill in the art
- Industrial Application: Invention can be made or used in any useful, practical activity as distinct from purely intellectual or aesthetic one.

Non patentable inventions in India:

- An invention, that is frivolous or that claims anything obviously contrary to well established natural laws;

- An invention, the primary or intended use of which would be contrary to law or morality or injurious to public health;
- The mere discovery of a scientific principle or the formulation of an abstract theory;
- The mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant;
- A substance obtained by a mere admixture resulting only in the aggregation of the properties of the components thereof or a process for producing such substance;
- The mere arrangement or rearrangement or duplication of known devices, each functioning independently of one another in a known way;
- A method of agriculture or horticulture;
- Inventions relating to atomic energy.
- Any process for the medicinal, surgical, curative, prophylactic or other treatment of human beings or animals.
- Plants and animals in whole or any part thereof other than microorganisms.
- Mathematical or business method or a computer program per se or algorithms.
- Literary, dramatic, musical or artistic works, cinematographic works, television productions and any other aesthetic creations.
- Mere scheme or rule or method of performing mental act or playing game.
- Presentation of information.
- Topography of integrated circuits.
- An invention which in effect, is traditional knowledge or is based on the properties of traditional knowledge.

Types of Applications filed at the Indian Patent Office

- Ordinary Application - is a first application made for an invention without claiming any priority. This application should be accompanied by a complete specification and claims. (In India, a Provisional Application can be filed too.)
- Convention Application - is an application which claims a priority based on a same or substantially same invention(s) filed in one or more of the convention countries in accordance with Paris convention. The application must be filed within 12 months from the date of first application in the convention country.
- PCT International Application - is an application which is filed in accordance with the Patent Cooperation Treaty (PCT). A PCT Application can be filed within 12 months of the Indian Filing (Priority) Date or directly without filing in India, by filing Foreign Filing License in accordance with the Section 39 of the Indian Patent Act.
- PCT National Phase Application - is an International Application which can enter the Indian National Phase within 31 months from the Priority Date or International Filing Date whichever is earlier.

- Application for patent of Addition - Patent of addition is an application made for a patent in respect of any improvement or modification of an invention described or disclosed in the complete specification already applied for or has a patent.
- In order to be patentable an improvement, should be something more than a mere workshop improvement and must independently satisfy the test of invention. The major benefit is the exemption of renewal fee so long as the main patent is renewed. A patent of addition lapses with the cessation of the main patent.
- Divisional Application - A divisional application is one which has been "divided" from an existing application. The applicant, at any time before the grant of a patent can file a further application, if he so desires or if an objection is raised by the examiner on the ground that the claims disclosed in the complete specification relates to more than one invention. A divisional application can only contain subject matter in the application from which it is divided (its parent), but retains the filing and priority date of that parent. A divisional application is useful if a unity of invention objection is issued, in which case the second invention can be protected as a divisional application.

International Patent Filing - Patent Cooperation Treaty

The patent cooperation treaty (PCT) is a multilateral treaty entered into force in 1978. Through PCT, an inventor of a member country contracting state of PCT can simultaneously obtain priority for his/her invention in all or any of the member countries, without having to file a separate application in the countries of interest, by designating them in the PCT application. All activities related to PCT are coordinated by the world intellectual property organization (WIPO) situated in Geneva

In order to protect invention in other countries, it is required to file an independent patent application in each country of interest; in some cases, within a stipulated time to obtain priority in these countries. This would entail a large investment, within a short time, to meet costs towards filing fees, translation, attorney charges, etc. In addition, it is assumed that due to the short time available for making the decision on whether to file a patent application in a country or not, may not be well founded

Inventors of contracting states of PCT on the other hand can simultaneously obtain priority for their inventions without having to file separate application in the countries of interest; thus, saving the initial investments towards filing fees, translation, etc. In addition, the system provides much longer time for filing patent application in the member countries

The time available under Paris convention for securing priority in other countries is 12 months from the date of initial filing. Under the PCT, the time available could be as much as minimum 20 and maximum 31 months. Further, an inventor is also benefited by the search report prepared under the PCT system to be sure that the claimed invention is novel. The inventor could also opt for preliminary examination before filing in other countries to be doubly sure about the patentability of the invention.

Information for Filing of International Applications for Patent under PCT by Indian Applicants

1	Competent receiving office and Chennai (RO/IN)	1. The Patent Office, at Kolkata, New Delhi, Mumbai, 2. International Bureau (RO/IB), Geneva, Switzerland.
2	Location, Mailing address. Telephone numbers, facsimile ande- mail addresses	1. Kolkata-The Patent Office, Intellectual Property Office Building, CP-2 Sector V, Salt Lake City, Kolkata, India-700091, Phone: 23671945, 1946, 1987, FAX-033-2367-1988, Email: - patentin-pct@nic.in (PCT); kolkata-patent@nic.in Contact: S. KUNDU, Assistant Controller of Patents & Designs 2. New Delhi -The Patent Office, Intellectual Property Office Building, Plot No. 32, Sector 14, Dwarka, New Delhi-110075, Phone: 011-28034304,4305,1053, FAX:011-28034313, Email: delhi-patent@nic.in Contact: Dr. KAVITA TAUNK, Assistant Controller of Patents & Designs 3. Mumbai-Patent Office, Intellectual Property Office Building, S.M.Road, Near Antop Hill Post Office, Antop Hill, Mumbai - 400 037. Phone: 24137701, 24141026, 24150381, 24148165, 24171457 FAX: 24130387 EMAIL: - mumbai-patent@nic.in Contact: SUNITA BETGIRI, Examiner of Patents & Designs 4.Chennai-The Patent Office, Intellectual Property Office Building, G.S.T. Road, Guindy, Chennai- 600032, Phone: 044-22502031- 35, FAX: 04422502066, Email: - chennai-patent@nic.in Contact: J. MEENA, Assistant Controller of Patents & Designs
3	Language of filing	RO/IN: English, Hindi RO/IB: Any language
4	Elements of the International application	i. Request (PCT/RO/101) ii. Description iii. One or more claims iv. Abstract v. Drawings (where applicable) vi Fees vii P.A./G.P.A. (where applicable)
5	No. of copies required	RO/IN: 3 (i to v) to The Patent Office, at Kolkata New Delhi, Mumbai, Chennai and RO/IB : 1

6	Competent International Searching Authorities [ISAs]	Austrian Patent Office (AT) Australian Patent Office (AU) European Patent Office (EP) China Intellectual Property Office (CN) United States Patent & Trademark Office (US) Swedish Patent Office (SE)
7	Competent International Preliminary Examining Authorities [IPEAs]	Austrian Patent Office (AT) Australian Patent Office (AU) European Patent Office (EP) (Only if ISA was AT, EP or SE) China Intellectual Property Office (CN) United States Patent & Trademark Office (US) Swedish Patent Office (SE)

General Precautions for Applicant

The first to file system is employed, in which, among persons having filed the same invention,

First one is granted a patent, therefore, a patent application should be filed promptly after conceiving the invention. It is common experience that through ignorance of patent law, inventors act unknowingly and jeopardize the chance of obtaining patents for their inventions. The most common of these indiscretions is to publish their inventions in newspapers or scientific and technical journals, before applying for patents. Publication of an invention, even by the inventor himself, would (except under certain rare circumstances) constitute a bar for the subsequent patenting of it. Similarly, the use of the invention in Public, or the commercial use of the invention, prior to the date of filing patent application would be a fatal objection to the grant of a patent for such invention, thereafter. There is, however, no objection to the secret working of the invention by way of reasonable trial or experiment, or to the disclosure of the invention to others, confidentially.

Another mistake, which is frequently made by the inventors, is to wait until their inventions are,

Fully developed for commercial working, before applying for patents. It is, therefore, advisable to apply for a patent as soon as the inventor's idea of the nature of the invention has taken a definite Shape. It is permissible to file an application for a patent accompanied by a "Provisional Specification" describing the invention. The application may, therefore, be made even before the full details of working of the invention are developed. The filing of an application for a patent disclosing the invention would secure priority date of the invention, and thereby, enable the inventor to work out the practical details of the invention and to file complete specification within 12 months from the date of filing of provisional specification.

Trademark

Trademark is a name, device, mark or logo which is capable of distinguishing the goods or services of one person from those of others. Trademark may also consist of drawings, symbols, three dimensional signs such as shape and packaging of goods or colors used as distinguishing feature.

What does trademark indicate?

Trade mark is a discrete indicator used by a legal entity such as business house, individual or a firm so that the consumers can easily identify their product or services.

What is the importance of a trademark?

- It identifies the source of the goods/manufacturers.
- It acts as prime instrument in advertising and selling the goods.
- A registered trademark gives the right to exclusive use of the mark.
- A registered trademark serves as a proof of certificate in court proceedings.

What does a trademark guarantee?

- It guarantees consistent quality by showing an organization's commitment to its users and consumers.
- It is a form of communication, a basis for publicity and advertising.

Conditional pre-requisites before a mark are sought to be registered as a trade mark,

- If it is a word mark, or if it is a mark containing at least in part a word, then that word ought to be easy to speak, spell and remember.
- Invented words or coined words that cannot be found in a dictionary as such form the best trademarks of all, since they can seldom be used as a generic word for any purpose.
- One should always be careful to avoid selection of a geographical name precisely for the same reason.
- It is in the best interest of a person not to select as marks descriptive or generic names or names that describes quality of a good such as best, perfect etc., or marks which have become customary in the current language or in the established practices of related trade.
- The mark should not consist exclusively of the shape of goods which results from the nature of the goods themselves, or the shape of goods which necessary to obtain a technical result or the shape which gives substantial value to the goods.
- If the mark is of such a nature as to deceive the public or cause confusion or contains any matter likely to hurt the religious susceptibilities of any class of Indian citizens or any scandalous or obscene matter, it may be refused registration.
- An action that will always satisfy the combined tests of both prudence and sound business sense will be to conduct a thorough market survey and a search at the Trade
- Mark Office before seeking to get a mark registered, so as to know whether similar mark or marks have already been registered or filed for registration.

Marks that can be adopted as trade mark

- Any name (including personal or surname of the applicant or predecessor in business or the signature of the person), which is not unusual for related trade to adopt as a mark.
- An invented word or any arbitrary dictionary word or words, not being directly descriptive of the character or quality of the goods/service.
- Letters or numerals or any combination thereof.
- Devices, including fancy devices or symbols
- Monograms
- Combination of colors or even a single color in combination with a word or device

- Shape of goods or their packaging
- Marks constituting a 3- dimensional sign.
- Sound marks when represented in conventional notation or described in words by being graphically represented.
- All of the aforesaid marks have to be sufficient for the purpose of identification of and distinguishing the goods, in relation to which they are being used, from other goods.

Types of trademarks that can be registered and Classification thereof

- As per the provisions of the Trade Marks Act 1999, following are the types of trademarks that can be registered in India:
- Product trademarks associated with particular good(s).
- Service trademarks associated with a particular kind of service such as insurance, building construction etc

Who can make an application for registration of a trade mark?

- Any person who claims to be the proprietor of a trademark used or proposed to be used by him can apply for the registration of the mark for goods as well services.
- The application for registration has to be made in writing to the Registrar of Trade Marks and it has to be filed in the office of the Trade Mark Registry under whose jurisdiction the principal place of the business of the applicant in India falls. In case the principal place of business is outside India, then the application can be filed in the office of the Trade Mark Registry, under whose jurisdiction the place that has been mentioned in the 'address for service' section of the application (usually office of the lawyer appointed by the person) is located.
- In case of a company about to be formed, anyone may apply for a trademark registration in his name for subsequent assignment of the registration in the company's favor.

Who can use a trade mark?

The right to use a mark can be exercised either by the registered proprietor or a registered user or anybody else who has been duly authorized by the registered proprietor or a registered user.

Legal requirements for registration of trade mark in India

- The selected mark should be capable of being represented graphically (that is in the paper form).
- It should be capable of distinguishing the goods or services of one undertaking in relation to which it is being used from those of others.
- It should be used or proposed to be used in relation to goods or services for the purpose of indicating a connection in the course of trade between the goods or services and some person having the right to use the mark with or without revealing identity of that person.

Duration of trade mark protection available in India

- Term of registration of a trademark is ten years, which may be renewed for a further period of ten years on payment of prescribed renewal fees.
- However, non-usage of a registered trademark for a continuous period of five years is a valid ground for cancellation of registration of such trademark at the behest of any aggrieved party

Copyrights & Related Rights

A copyright can be gained by the author of a literary, artistic and musical works.

- Literary work, for example, includes books, short stories, poems, articles.
- Dramatic work includes plays, screenplays, scripts, narratives, cinema film sequences.
- Musical work includes any musical composition, arrangement or transcription of the work.
- Artistic works include photographs, sculptures, paintings, etc.

Author of a Copyrightable Work

An author in the sense of these works is the playwright, screenplay writer, author of the book/short story/article, music composer, photographer, sculptor, painter etc.

A copyright basically grants protection to an author from having his work:

- reproduced or communicated to the public by way or hearing or
- distributed or benefit any person who is not the author without the author's prior authorization

This protection is also granted to reproduced, rearranged or altered works of music and art, conversion of literary or artistic works into dramatic works and dramatic works into non-dramatic works, known as 'adaptation.

Importance of Registration

A copyright application has to be put into process for protecting your own work or for republishing, reproducing or adapting existing work and seeking protection for the same to avoid any consequential legal battle with the other party/parties as the case may be.

An author has an automatic copyright over his/her work by the very virtue of the work being his creation. However, the law recognizes registered copyrights as sufficient proof when an author is trying to enforce his copyright against the other party.

In case of ownership disputes over adaptations or republications, there must be no objections or legal hassles pertaining to the ownership and usage of the original work. So, if you want to ensure that there is no unjustified benefitting from you work, copyright registration is a must.

When and Who can apply for a Copyright

Before applying for a copyright, the author must ensure that a copyright can subsist in the first place.

A copyright subsists in original literary, dramatic, musical and artistic works and cinematographic films and sound recordings.

No copyright can be granted where the work being copyrighted is fully or partially an infringement of copyright.

In a case concerning two authors, the conditions laid down for the subsistence of a copyright have to be fulfilled by both the authors.

Geographical Indications

Protection of Geographical Indications in India is governed by the legislation namely The Geographical Indications of Goods (Registration & Protection) Act, 1999, which seeks to provide for registration and better protection of geographical indications relating to goods in India. This Act also provides for setting up a Geographical Indications Registry for the purposes of registration of GIs in India.

Applicants

Any association of persons, producers, organization or authority established by or under the law can apply for registration of GIs. The applicant must represent the interest of the producers. Producers are persons dealing with the following three categories of goods.

Producers are persons dealing with the following three categories of goods:

- Agricultural Goods includes the production, processing, trading or dealing;
- Natural Goods includes exploiting, trading or dealing; and
- Handicrafts or Industrial goods include making, manufacturing, trading or dealing

Filing Requirements

An application for registration of a Geographical Indication in India requires the following particulars:

The Details of the Applicant

- Name
- Address
- List of association of persons/producers/organization/authority
- Citizenship
- Legal status and country of incorporation

Specimen of the Geographical Indication

- Six (6) printed specimen of the geographical indication, if the Mark is in Black & White;
- Ten (10) printed specimen of the geographical indication, if the GI is in Color
- Where an application for registration of a geographical indication consists of shape of goods or its packaging, the reproduction furnished shall consist of at least three different views of the geographical indication and a description by word of geographical indication

Industrial Designs

A DESIGN is defined as the features of shape, configuration, pattern, ornament or composition of lines or colours applied to any article by any industrial process or means, whether manual, mechanical or chemical, separate or combined, which in the finished article appeal to and are judged solely by the eye, but does not include any mode or principle of construction or anything which is in substance a mere mechanical device and does not include any trademark or property mark or artistic work.

Design Act protects only designs that are aesthetic in nature. They may be decorative elements added to the article or they may be part of very shape or configuration. Novelty and originality are important criteria in a design for registration. In addition, only those designs that are applied to an article by an industrial process will be protected.

Importance of creating design

It is not a job merely a color or a form of an object. It can be rather defined as the creation of being of a thing and a matter. Through this conduct, design has become an driving force of developing rich societies. Design is capable to create a brief of matters and things. Design makes people's daily lives comfortable and affluent by resolving today's many kinds of problems. A beautiful and easy to use design might innovate our lives, society and economy.

In today's radically digitized social conditions, design's role is more changing. Many countries over the world understand the capability of design, and no small number of nations asset design as a part of nation's primary strategies. There is no doubt that design has played an important and powerful role to promote nation's economy.

Classification

Almost all jurisdictions including India follow Locarno Classification for registration of design comprising 32 classes, numbered 1 to 31 and an additional class 99 to include articles not falling under the aforesaid 31 classes. Most of the classes are further divided into sub classes. Design applications must be filed in a particular class depending upon the predominant material with which the article is made or is capable of being made.

Rights conferred by registration

The registration of a design confers the proprietor copyright in the design for the period of registration. Copyright means the exclusive right to apply the design in respect of the article for which it is registered.

Who can apply for a design?

An inventor or any other person/company assigned by the inventor can apply and obtain the registration for the design.

Industrial Designs Not Registrable before the Indian Patent Office

- Any industrial design being opposing to public moral values.
- Any industrial design describing any process of construction.
- Any industrial design which is not distinguishable from previously registered designs.
- Any industrial design which includes trademark.
- Industrial Designs of following objects - Books, jackets, calendars, certificates, forms and other documents, dressmaking patterns, greeting cards, leaflets, maps and plan cards, postcards, stamps, medals
- Industrial Designs including flags, emblems or signs of any country.
- Industrial Designs of integrated circuits.

Filing and Prosecuting Design Applications

An application for design on Form-1 accompanied by four copies of representation of the design and prescribed fee of Rs. 1000/- is filed at one of the four offices of the Patent Office located at, Kolkata, Mumbai, Delhi and Chennai. The Designs Office initially provides a filing number and filing date and issues a filing receipt, which is sent to the applicant or his attorney. Thereafter the application is formally examined by the Designs Office. Defects will be communicated to the applicant. Once the application is found to be in order it is accepted and the Designs Office issues the registration certificate.

Duration of registration

The term of a design registration is initially for a period of ten years. The renewal is possible for further period of 5 years.

IP strategy for Entrepreneurs

Intellectual property (IP) is a valuable asset for entrepreneurs, as it can help protect their innovations, products, and brand. Here's a simplified IP strategy tailored for entrepreneurs:

Understand Your IP:

Start by understanding what intellectual property is and the different types, such as patents, trademarks, copyrights, and trade secrets. Identify what IP you may already have.

Identify and Prioritize IP Assets:

Determine which aspects of your business are most valuable and innovative. These may include product designs, branding, proprietary software, or unique processes. Prioritize which IP assets are most critical for your business's success.

Search and Research:

Research existing patents, trademarks, or copyrights to ensure your idea are unique. This can help prevent potential legal issues later on. Search for any potential competitors who might have similar IP.

Protect Your IP:

File for IP protection where it makes sense for your business. For instance, if you have a unique invention, consider applying for a patent. If you have a distinctive brand, register a trademark. Work with a qualified IP attorney or consultant to navigate the application process.

Confidentiality and Trade Secrets:

Implement strong confidentiality measures within your organization to protect trade secrets and other sensitive information. Have employees sign non-disclosure agreements (NDAs) when necessary.

Document Everything:

Keep detailed records of the creation and development of your IP. This documentation can be invaluable in proving ownership in case of disputes.

Leverage Your IP:

Consider licensing your IP to other businesses in exchange for royalties. This can generate additional revenue. Use your IP to attract investors, partners, or customers who value your innovation.

Enforce Your IP Rights:

Be prepared to defend your IP if someone infringes upon it. Consult with your IP attorney on the appropriate actions to take. Educate Yourself and Your Team: Ensure that you and your team understand the basics of IP protection and the importance of safeguarding your intellectual property.

Regularly Review and Update:

Periodically review your IP strategy as your business evolves. Consider whether your IP portfolio still aligns with your goals and market conditions.

Budget for IP:

Allocate a portion of your budget for IP protection and maintenance. IP can be a valuable long-term investment.

Legal Support:

Consider working with an IP attorney who can provide guidance and expertise in managing your IP assets and defending your rights.

Remember that as an entrepreneur, your resources might be limited, so it's essential to focus on protecting what matters most and be strategic in your IP efforts. Adapt your IP strategy as your business grows and your needs change.

ADVANCED AREA OF LIFE SCIENCE AND BIOTECHNOLOGY - RECOMBINANT MOLECULAR EXPRESSION TECHNOLOGIES

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Abbreviations:

SD, slit-diaphragm; GFB, Glomerular filtration barrier; CD2AP, CD-2 associated protein; TRPC6, Transient receptor potential cation channel subfamily C member 6; ZO-1, Zonula occludens-1; NEPH, Nephrin-like protein; NS, Nephrotic syndrome; SRNS, steroid-resistant NS; NPHS1 & 2, Nephrotic syndrome-type I and type II; IDRs, intrinsically disordered regions; SEC, Size-exclusion chromatography; MALS, multi-angle light scattering; CD, Circular dichroism.

Abstract

Podocytes are crucial cells of the glomerular filtration unit and playing a vital role at the interface of the blood-urine barrier. Podocyte slit-diaphragm is a modified tight junction that facilitates size and charge-dependent permselectivity. Several proteins including podocin, nephrin, CD2AP, and TRPC6 form a macromolecular assembly and constitute the slit-diaphragm. Podocin is an integral membrane protein attached to the inner membrane of the podocyte via a short transmembrane region (101-125). The cytosolic N- and C-terminus help podocin to attain a hook-like structure. Podocin shares 44% homology with stomatin family proteins and similar to the stomatin proteins, podocin was shown to associate into higher-order oligomers at the site of slit-diaphragm. However, the stoichiometry of the homo-oligomers and how it partakes in the macromolecular assemblies with other slit-diaphragm proteins remains elusive. Here we investi-gated the oligomeric propensity of a truncated podocin construct (residues:126-350). We show that the podocin domain majorly homo-oligomerize into a 16mer. Circular dichroism and fluo-rescence spectroscopy suggest that the 16mer oligomer has considerable secondary structure and moderate tertiary packing.

Keywords: Podocin; Podocyte; Nephrotic syndrome; slit-diaphragm; proteinuria

Introduction:

Vertebrate kidneys regulate electrolyte and water balance to maintain body homeosta-sis. Each kidney is composed of about one million nephrons. The glomerulus and the renal tu-bule are the two major parts of a nephron that work in unison to ensure protein-free ultra-filtrated urine. The glomerular filtration barrier (GFB) offers permselectivity for the filtration of plasma components into the urine. The GFB consists of fenestrated capillary endothelium, glo-merular basement membrane, and podocytes [1]. Podocytes are highly differentiated visceral epithelial cells that encase the glomerular capillaries. A typical podocyte cell consists of a protuberant cell body with primary processes made of actin and microtubules. The primary process further branches into secondary foot processes which interlaces with the neighbouring foot pro-cesses forming a modified tight junction called the slit-diaphragm (SD) [2]. The SD is a negatively charged zipper-like structure bridging the 30-40nm gap between the adjacent foot processes. This structure curbs the passage of albumin and other large molecules from the blood into primary urine thereby tightly regulating the composition of the glomerular filtrate [3, 4]. The intricate structure of the SD is maintained by an array of protein assemblies. Proteins such as podocin, nephrin, CD-2 associated protein (CD2AP), transient receptor potential cation channel subfamily C- member 6 (TRPC6), zonula occludens-1 (ZO-1), and Nephrin-

like proteins 1, 2, and 3 (NEPH) interact to form macromolecular complexes and constitute the structure of SD [5-8].

Mutations in the SD proteins are associated with nephrotic syndrome (NS), which is presented with massive proteinuria, hypoalbuminemia, and edema [9]. Corticoid therapy is the usual recourse to abate NS and based on the patient's response to corticoid therapy NS is divided into steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS). Patients with congenital nephropathies usually belong to the SRNS group since they do not respond to corticoid therapy. Congenital nephropathy typically onsets in infants during 0-3 months of age which eventually progresses to irreversible kidney failure within a decade. Mutations in NPHS1 and NPHS2 that encode for nephrin and podocin respectively result in the majority of congenital SRNS cases [10]. About 18% of the reported SRNS cases are due to mutations in podocin [11, 12]. Mutations in other SD proteins such as TRPC6 and CD2AP have also been observed to cause NS but at a less frequent rate than nephrin and podocin [13-15].

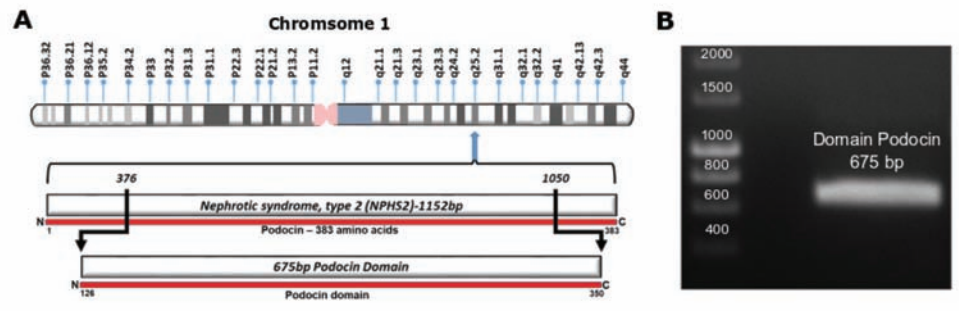
Podocin is a 383 amino acid protein localizing to the lipid rafts along with other SD pro-teins [7, 16, 17]. Podocin shares 44% homology and several structural similarities with stomatin family proteins due to the presence of a highly conserved PHB domain [18]. Indeed, podocin was predicted to share several structural similarities with the stomatin proteins [5, 18]. Podocin adapts a hook-like structure with its cytoplasmic N- and C- terminus as it attaches to the inner side of the plasma membrane via a transmembrane domain at 100-125 residues [5, 12, 19]. Structural characterization of stomatin revealed that different truncations of the protein associ-ated with different oligomeric states and C-terminus of the protein is crucial for homo-oligomerization [20-22]. Studies with truncated C-terminal human podocin revealed that it forms a dimer [23]. Though it was proposed that longer constructs of podocin were capable of associating into higher-order oligomers, it was never demonstrated [23]. Additionally, co-immunoprecipitation studies with nephrin, CD2AP, TRPC6, and NEPH1 indicated that these pro-teins interact majorly with the C-terminus of podocin [6-9, 16, 17, 24]. We reported earlier that these interactions are mediated by the intrinsically disordered regions (IDRs) present in these proteins [18].

Although a large body of evidence suggests a central role for the podocin in the assembly of SD complex, the precise mechanism by which podocin oligomerizes and acts as a scaffolding molecule remains to be elucidated. Importantly, it is not known whether all the other SD pro-teins interact with a single podocin or with homo-oligomers? Therefore, in this study, we at-tempted to understand the oligomeric nature of protein using a truncated construct (residues: 126-350), which encompasses the Prohibitin (PHB) domain, C-terminal oligomerization site, and 4 out of the 6 cysteines present in the native podocin sequence.

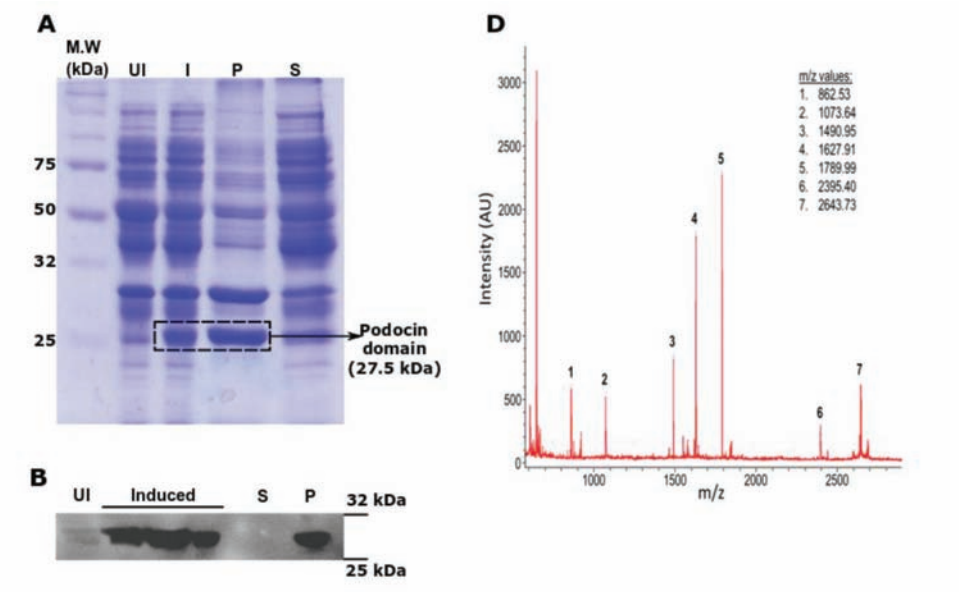
Material and Methods:

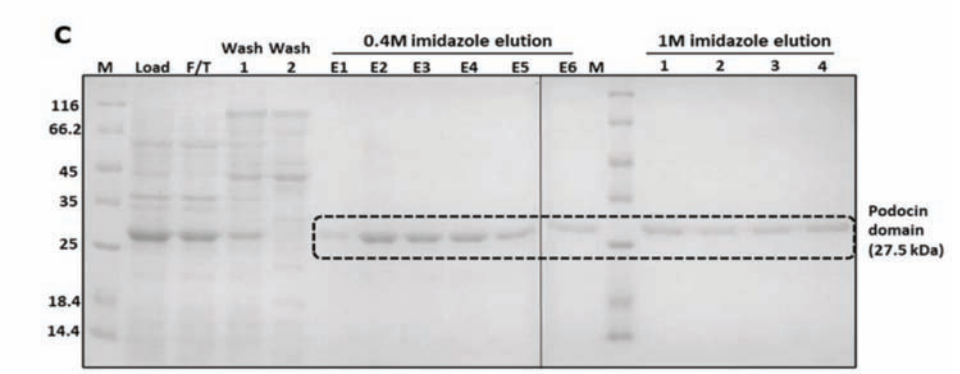
Protein cloning, expression, and purification: The codon-optimized podocin gene (1152bp) was purchased from Gene Art (Life Technologies, USA). The regions encoding the ami-no acids 126-350 (podocin domain) was amplified with the primers 5' CCC GAA TTC G AAA GTG GTG CAA GAA 3' (forward) and 5' GAA CTC GAG CAG ACA ATT CAG CAG ATC 3' (reverse) and cloned into pET22b at EcoRI/XhoI sites. The recombinant construct was transformed into Arctic express (DE3) competent cells (Agilent Technologies, USA). The transformed cells were grown at 37oC in LB media supplemented with 100 μg/ml ampicillin and 10 μg/ml gentamycin. Protein ex-pression was induced with 0.2 mM IPTG and cultured further for 16hrs at 14oC. The cells were harvested by centrifugation (13300 x g, 20 mins, and 4oC) and sonicated in the opening buffer (50 mM potassium phosphate (pH

8.0), 0.3 M NaCl, 5 mM _-mercaptoethanol and 0.1% Triton X-100) followed by clarification by centrifugation at 18000 x g for 45 mins at 4oC. The inclusion bodies were solubilized in 50 mM potassium phosphate (pH 8.0), 0.3 M NaCl, 5 mM _-mercaptoethanol and 8 M Urea followed by clarification at 18000 x g for 1hr at room tempera-ture. The solubilized protein was then purified using Ni-NTA agarose (Qiagen). The purity was confirmed (>98%) on a 12% SDS-PAGE. From the SDS-PAGE gel, the band corresponding to the podocin domain (27 kDa) was excised and subjected to tryptic digestion and analyzed using MALDI-TOF/TOF (Bruker Autoflex III smart beam, Bruker Daltonics, Bremen, Germany) to con-firm the protein sequence.



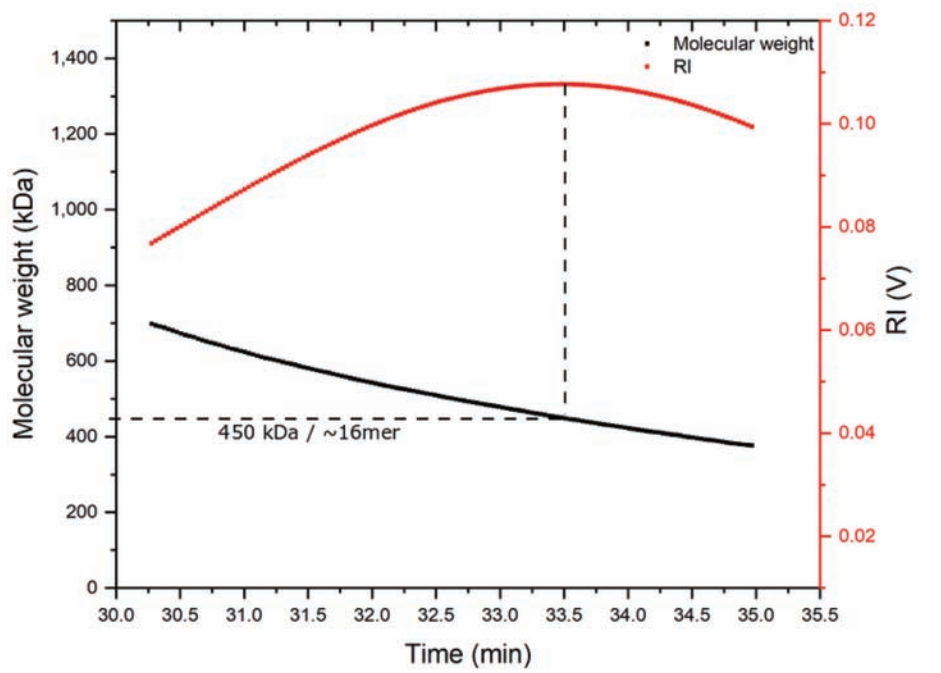
The purified podocin was renatured by rapid dilution at 1:10 into 10mM potassium phos-phate buffer with 150 mM NaCl and 2 mM _-mercaptoethanol (pH 8.0) followed by dialysis in the same buffer to remove traces of urea and imidazole. The same pH and buffer composition are uniformly used in all the subsequent experiments. An excitation extinction coefficient of the 12950 M-1.cm-1 was used for determining the protein concentration on Jasco V-630 UV-Vis spectrophotometer.





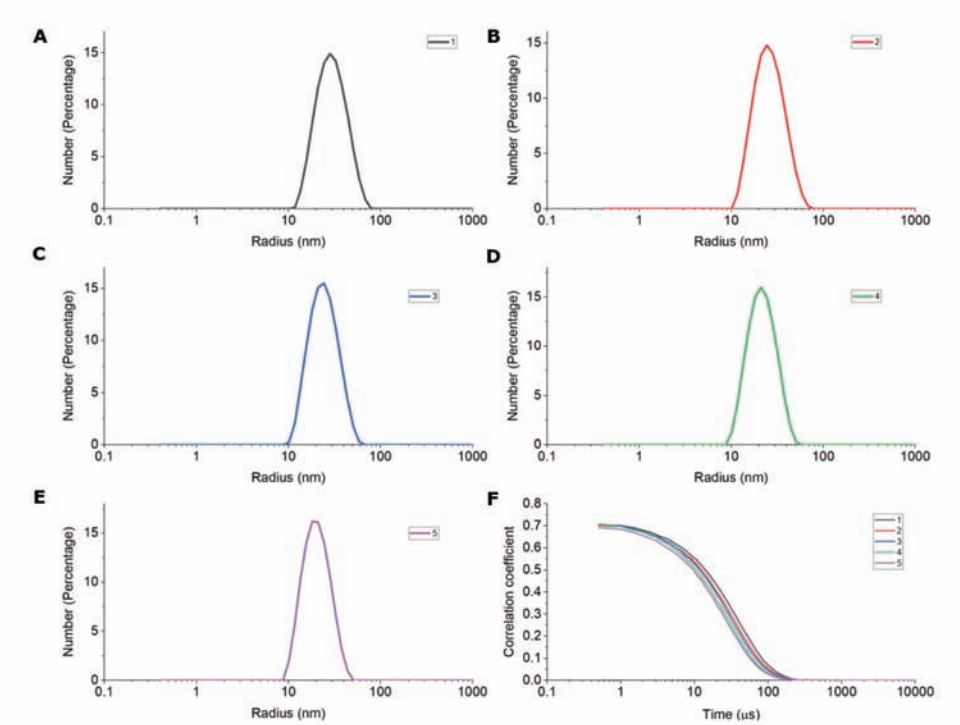
Size exclusion chromatography and multi-angle light scattering analysis (SEC-MALS): MALS in combination with SEC is a sensitive technique to accurately estimate the multiple oligomers within the sample when compared to SEC. We performed SEC-MALS to estimate the oligomeric nature of the podocin domain. SEC-MALS was performed at room temperature by passing 500 μl protein (12μM) at 0.3 ml/min flow rate through a Superdex S200 SEC column (GE Healthcare) pre-equilibrated with 10mM potassium phosphate buffer supplemented with 150mM NaCl and 2mM _-mercaptoethanol (pH 8.0). This column was attached to the MALS system (AF2000- Post-nova) for analyzing the molar mass of the protein. The protein sample from SEC-MALS was next passed through the flow cell equipped Zetasizer Nano ZS90 dynamic light scattering (DLS) device (Malvern Instruments Ltd, UK) equipped with a 4 mW He-Ne laser. The backscattering was meas-ured at 173 nm for analyzing the polydispersity index (PDI) and the hydrodynamic radius of the protein.

Fluorescence spectroscopy: Intrinsic tryptophan fluorescence was measured using Jasco FP-6300 (Japan) equipped with an intense xenon flash lamp as the light source. 287 nm was used as the



excitation wavelength and the 300-450 nm spectral range was used for obtaining the emis-sion spectrum of the sample (12 μM). For accessing the stability of the podocin domain thermal-induced unfolding was performed. Fluorescence emission at 335 nm as a function of increasing temperature was recorded at a bandwidth of 2.5 nm and a scan speed of 200 nm/min with data recorded for every degree rise in temperature in triplicates. All the spectra were buffer cor-rected. The effect of temperature on the protein was analyzed by plotting fluorescence intensi-ty at 335 nm using Origin (pro)-version2020b (Origin Lab Corporation, Northampton, MA).

Circular dichroism (CD) spectroscopy: The CD spectroscopy measurements were recorded on Jasco J_1500 spectropolarimeter (Japan) equipped with a thermoelectric cell holder. The Far-UV (260-195 nm) CD measurements of podocin (12 μM) were recorded using a 0.2 cm path length cell at 2.5 nm bandwidth and a scan speed of 50 nm/min. The Near-UV CD measurements were also recorded for the protein sample at 25 μM concentration using a 0.5cm pathlength cell at the bandwidth of 2.5nm and a scan speed of 100 nm/min. Both for far-UV and near-UV the data was recorded accumulated in triplicates. To assess the effect of temperature and thus the stability of the domain, the sample was subjected to a steady increase in temperature and spectra were recorded at an interval of 5oC over a spectral range of 200 nm-250 nm. The data were plotted using origin lab software after buffer correction.



Calorimetric analysis: Various thermodynamic properties including the melting temperature (Tm) of the podocin domain were obtained from measurements using NANO DSC (TA Instru-ments, USA). Sample containing protein concentration of 12 μM and a volume of 0.650 _L was loaded into the sample capillary and change in heat flow was recorded against reference buffer at a constant pressure of 3 atm, over a temperature range of 293K to 368K, with a scan rate of 1 K/min and a 300-

sec cell equilibration time. Buffer scans were first performed before loading protein for baseline reproducibility. The obtained data was buffer corrected and the analysis data was plotted for peak integration with the peak analyzer option in Origin pro 2020b soft-ware. From the peaks, the transition temperature (T) and the enthalpy of unfolding (_UH) were calculated.

Results:

Purification of Podocin domain: The region from 376bp to 1050bp of human NPHS2 gene encoding 126-350 amino acid residues of podocin was PCR amplified and cloned into the pET22b expression vector (Fig. 1A&B). IPTG induced expression of the construct resulted in the protein to form inclusion bodies which were confirmed by Coomassie blue staining and immunoblotting with anti-His antibody (Fig. 2A&B). The protein was purified from inclusion bodies by Ni-NTA affinity chromatography after solubilization in 8M urea (Fig. 2C). Tryptic digestion of the band corresponding to the podocin domain from the SDS-PAGE gel (Fig. 2C-lane E2) and subsequent analysis of the digested products by MALDI-TOF/TOF revealed five peptide fragments (Fig. 2D and Table. 1). NCBI BLAST search of these peptide sequences against the non-redundant data-base confirmed the purified protein as human podocin covering the region 126-350 amino acids.

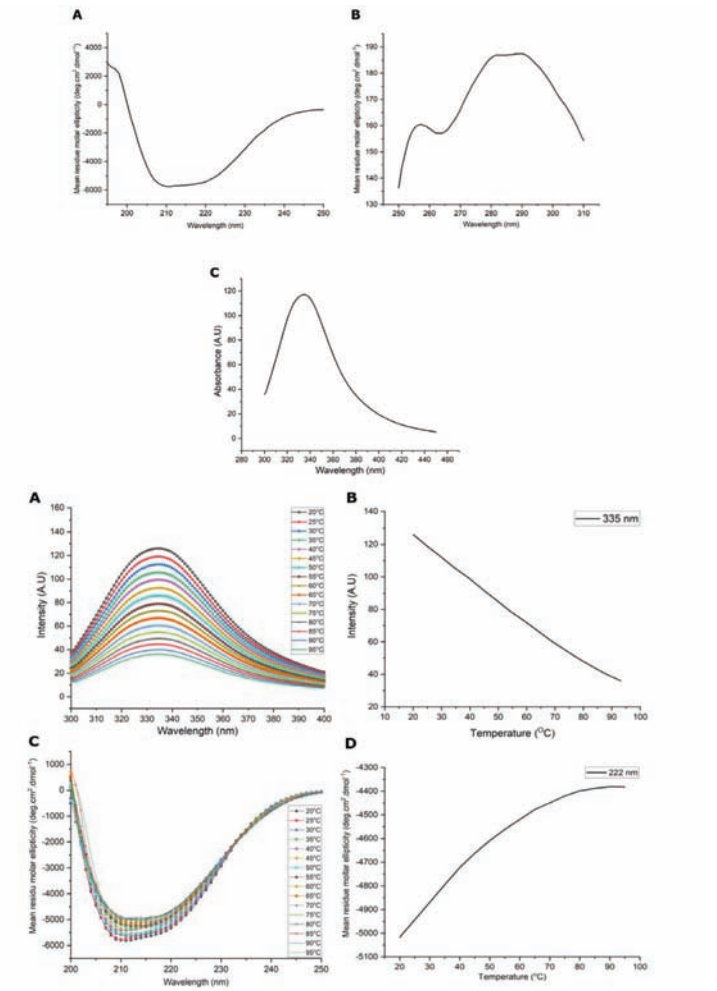


Table 1: MALDI TOF/TOF analysis of the purified protein: Trypsinization of the purified band at 27 kDa and subsequence analysis by MALDI-TOF/TOF showed 5 peptide sequences. BLAST analysis of these sequences against the non-redundant proteins database of NCBI showed 100% similarity with the human podocin sequence. Note: ' ' indicates the site of digestion by trypsin at arginine and lysine residues in the sequence.

Sequence		Observed	Mr	Mr	Expect	Peptide
Start	End		(expt.)	(calc.)		
134	146	1490.95	1489.95	1489.93	2.3e-03	R VIIFRLGHLLPGR A
149	168	2395.40	2394.40	2394.22	9.1e-03	K GPGLFFFLPCLDTYHKVDLR L
263	286	2643.73	2642.72	2642.45	6.1e-04	R IEIKDVRLPAGLQHSLAVEAEAQR Q
270	286	1789.99	1788.99	1788.95	2.4e-06	R LPAGLQHSLAVEAEAQR Q
307	322	1627.91	1626.90	1626.88	3.5e-06	R MAAEILSGTPAAVQLR Y

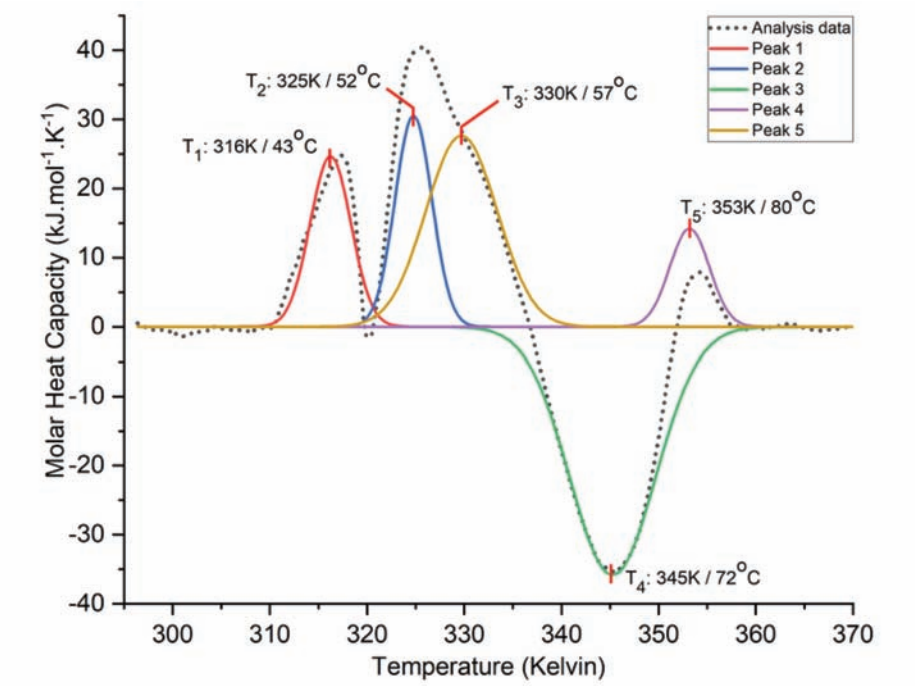
Oligomeric nature of podocin domain:

It was reported that stomatin family members exist as homo-oligomers [20-22]. Since podocin shares significant homology with stomatin, we analysed the oligomeric nature of the podocin domain consisting of the PHB domain. SEC-MALS data is represented as a combinatorial plot of refractive index, and molecular weight versus elution time (Fig. 3). A maximum refractive index value of 0.11 was observed which corresponds to a molecular weight of 450kDa, suggesting that the podocin domain is a 16mer oligomer (monomer = 27.5kDa; therefore; 450 kDa/ 27.5 kDa = ~16mer). In addition to the predominant 16mer species, other oligomeric conformations of the podocin domain ranging from 25mer (refractive index: 0.08, molecular weight: 697kDa) to 13mer (refractive index: 0.10, molecular weight: 376kDa) were also observed, but to a lesser extent. The DLS, which was in tandem with the SEC-MALS analysed the eluted samples for hydrodynamic radius and polydispersity. A hydrodynamic radius range of 13.39 - 9.37 nm corresponding to elusions from 25mer to 13mer was observed respectively (Fig. 4A-E). The average PDI of the sample was found to be 0.14, which suggests that the sample consists of one major population by volume, however, a broad size range within the population implies the presence of different size species. From these results, it is evident that the major species the podocin domains associate into 16mer oligomers while a minor population of other higher-order oligomers also appear to exist in solution.

Structural features of the homo-oligomers of podocin domain: The CD spectra of far UV region (250-195 nm) provides information of the secondary structure while the near UV region (310-250 nm) on the tertiary interactions (Fig. 5A&B). Observation of CD signal in the near UV regions implies the arrangement of aromatic amino acids in a restricted environment(s) and thus implying a folded structure adopted by the polypeptide chain. The far UV CD signal for the truncated podocin domain indicates that the protein adopts a XYZ secondary structure (Fig. 5A). The shape of the spectrum indicates the possibility of the presence of both a helices and b sheet structures. The near UV spectrum of the sample shows a broad peak at 280nm typically contributed from tryptophan residue (W256), and a minor peak from ~250 - 260 nm could be contributed by five-tyrosine and eight-phenylalanine residues. The presence of signals in this region of near UV spectrum implies the tertiary interactions involving these residues.

The intrinsic tryptophan emission spectrum of the podocin domain shows a lmax value at 335 nm (Fig. 5C). The lmax of native proteins is related to the polarity of the environment of the

tryptophan residue and typically can range from 308-350 nm. Unfolded forms with residues in the apolar microenvironment show a blue shift of the lmax [25]. Although, the intrinsic emission may not directly or unequivocally provide structural information, changes in the folded state can be followed by monitoring the changes in the emission intensity which convey the changes in the native state tryptophan environment. The changes in the CD signal and the perturbations of the intensity of the intrinsic fluorescence emission were used to estimate the stability of the folded podocin domain.



Structural stability of the podocin domain: To assess the structural stability, the resistance of the structure to unfold on heating was monitored following changes in the tryptophan fluorescence emission and the far UV CD spectrum. Temperature-induced unfolding was monitored using the fluorescence emission spectra with increasing temperature in the range of 20oC-95oC. The spectra showed a uniform decrease in lmax intensity without either a bathochromic or hypsochromic shift (Fig. 6A). The change in fluorescence intensity as a function of temperature (Fig. 6B) shows a linear transition and does not show a sigmoidal shape typically observed for proteins with a tightly packed tertiary core. The absence of a folded baseline suggests a not so tightly packed tertiary core. Far UV CD spectral changes were also monitored as a function of tempera-ture. The signal intensity showed a gradual loss with increasing temperature (Fig. 6D), and the thermal unfolding monitoring the loss of secondary structure also showed the absence of a native baseline (Fig. 6C) implying that the podocin domain possesses secondary and tertiary inter-actions but lack a tightly packed core.

Calorimetric analysis of podocin domain: Differential scanning calorimetry (DSC) gives the overall enthalpy value (_Hcal) for each structural transition. Therefore, we performed DSC to calculate the thermodynamic parameters such as transition temperature (T) and the enthalpy of transition associated with structural changes of the homo-oligomer. Peak deconvolution of the acquired data revealed 5 transition states, out of which four are endothermic transitions (316 K, 325

K, 330 K, and 353 K) and one is an exothermic transition (345 K) (Fig. 7). The respective val-ues for _H are mentioned in Table 2. The DSC profile suggests that the oligomers in the mixture undergo dissociation via three transition temperatures namely 316 K, 325 K, and 330 K and the presence of exothermic transition at 345 K suggests possible hydrophobic interactions among the constituent oligomers before complete dissociation at 353 K.

Table. 2: Enthalpy values and the transition temperatures as noticed in the dynamic scanning calorimetry.

Temperature range	Transition temperatures		Enthalpy change (DHcal) (kJ/mol)
293K to 368K / (20oC - 95oC)	Endothermic 325 K / 52oC	316 K / 43oC	130
		145	
		330 K / 57oC	73
		353 K / 80oC	254
	Exothermic	345 K / 72 oC	-397

Discussion:

Podocin selectively expresses in the glomerular podocytes and it is instrumental for pre-serving the structural integrity of the SD. Though several mutations in the protein are associated with proteinuria in humans, the structural details of this protein are unclear. Here we report for the first time, the stoichiometry of oligomerization of the truncated human podocin construct. Our investigation indicates that at ambient temperature, and in a reduced environment the podocin domain predominantly adopts a 16mer oligomeric state. However, other oligomeric con-formations ranging from 25mer to 13mer were also observed nevertheless, the population of these states was comparatively less. The polydispersity index we report adds evidence to the presence of multiple oligomeric species. Additionally, through CD and fluorescence spectrums we show that the homo-oligomers have considerable structure and tertiary interactions.

Since stomatin proteins and podocin share significant homology, it expected they may share several structural similarities. Crystallization studies of stomatin protein (Pyrococcus horikoshii; residues: 56-224) revealed that it exists as a trimer and NMR studies of the same pro-tein but with a different truncation size (residues: 66-174) associated into an amalgam of oligo-mers [20]. Earlier reports have suggested that podocin forms homo-oligomers with its C-and N-terminals at the lipid raft micro-domains besides forming a large complex with its neighbouring SD proteins [7, 16, 17, 26]. However, the extent of the oligomer size was not explored. Though our analysis revealed truncated podocin predominantly a 16mer oligomer, we are not sure whether full-length podocin also assemble as 16mer, which is a major limitation of our study. However, in a recent study Straner et.al, reported that podocin C-terminal construct (168-383) can form higher-order oligomers [23]. It was also reported by Straner et. al, that podocin-podocin inter-actions are facilitated via C-terminal helical segments (283-313 and 332-348 residues). Further-more, Huber et. al, reported that podocin (R120X) unable to form podocin dimers suggesting the importance of this region in podocin-podocin interactions (ref). Together these observations suggests that oligomerization of podocin could be effected by mutations these regions that con-tribute interactions. Mutations that cause nephrotic syndrome could possibly distort innate abil-ity of podocin to form oligomers and compromise its ability to act as scafolding molecule. To-gether these molecular deformations at the level of podocin

oligomeric assembly may leads to altered permselectivity of SD and manifest in significant proteinuria. As it was shown that podo-cin interact with several SD proteins, it is less likely all proteins interact with a single podocin molecule. With accumulating evidence that podocin forms homo-oligomers, it could be also be possible that SD proteins interact with a different podocin monomer assembled as homo-oligomers.

We were unsuccessful in expressing and purifying the full-length podocin. This could be due to several reasons including podocin may not be stable outside its native environment, due to the presence of IDRs, or interference of the transmembrane segment with protein expression [5]. We, therefore, cloned and expressed a truncated construct encoding the 126-350 amino ac-ids region of the podocin. The podocin fragment that we consider in this study encompasses the PHB domain and the C-terminal oligomerization site. Though podocin domain forms predomi-nantly a 16mer, SEC-MALS analysis revealed formation of oligomeric states ranging from 25-13mers. These results are further justified by the hydrodynamic radius and the polydispersity index (PDI) measured by the DLS. The reported PDI for podocin domain (0.08 - 0.5) suggests that although it has one major oligomer species by volume, the presence of multiple species could be possible [27]. We were also unable to obtain crystals of the podocin domain for performing X-ray diffraction possibly due to its dynamic oligomeric nature.

Conclusion:

In conclusion, our study, to the best of our knowledge is the first to report the cloning, expression, and purification of the human podocin domain (126-350). We showed that the podocin domain is majorly a 16mer homo-oligomer. However, to a lesser extent, the protein is capable of associating into other oligomeric states. CD and FL analysis of the protein indicated that the podocin domain in isolation attains considerable secondary structure and tertiary packing. However, the significance of the podocin oligomerization in the formation of the large macro-molecular complex and how a mutation in a podocin monomer will affect its oligomerization status need to be addressed. Further structural characterization of podocin and other slit diaphragm proteins is greatly warranted to understand the mechanism of pathogenesis of the nephrotic syndrome.

CRISPR-CAS9 GENOME EDITING: GENESIS AND APPLICATIONS

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Synopsis:

Gene editing is a technology that precisely modifies the genome sequence to induce insertions, deletions, or base substitutions in the genome. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) gene-editing technology is the ideal tool of the future for treating diseases by permanently correcting deleterious base mutations or disrupting disease causing genes with great precision and efficiency. The CRISPR toolbox and its applications have profoundly changed basic and applied biological research. CRlSPR gene editing has long attracted attention in the research community, the media, and the wider public. This peaked in 2020 when the Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry to two leading CRISPR researchers, Jennifer Doudna and Emmanuelle Charpentier. This presentation focuses on: CRISPR-CAS9 time line, Mechanism by which bacterial CRISPR-Cas9 system provide adaptive immunity and resistance to phages (three steps: acquisition, transcription, and interference), designing single guide RNA, success stories and controversies, FDA approved CRISPR therapy and clinical trials, challenges, ethical issues and potential risks associated with CRISPR- CAS9 technology.

Origin: Time line of CRISPR-CAS

Bacterial and archaeal viruses (bacteriophages or phages) display a constant threat to prokaryotic life. In order to withstand phages, prokaryotes have evolved several defence strategies. In the past decade, the prokaryotic immune system CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated) caught increasing attention in the scientific community not only because of its unique adaptive nature, but also because of its therapeutic potential.

Milestones in CRISPR-Cas research:

First described in 1987 as unusual repetitive sequences and initially believed to participate in cellular DNA repair and replicon partitioning processes, first evidence that CRISPR-Cas systems display an adaptive prokaryotic immune system was delivered in 2005. Researchers were surprised as they found that most of the interspersed sequences interspaced between identical repeats derived from extra chromosomal DNA, more specifically from phage genomes and conjugative plasmids.The hypothesis was eventually proven two years later when scientists showed the incorporation of new spacers into a CRISPR-Cas locus of Streptococcus thermophilus after challenging the bacterium with a bacteriophage. The newly acquired spacers always showed perfect complementarity to sequences on the phage genome and conveyed resistance towards that particular phage upon a subsequent infection. Research interest of the CRISPR field soon accelerated, leading to new discoveries that helped to understand the basic mechanisms of the prokaryotic adaptive immune system. In 2008, the processing of the CRISPR transcript into mature crRNAs (CRISPR RNA) that guide the Cascade complex of the E. coli type I-E system was experimentally validated, also giving hints that DNA rather than RNA is targeted. The latter was confirmed in the same year as a study demonstrated that indeed DNA is the targeted molecule. This led scientists to think about the potential role that this prokaryotic immune

system might play as a DNA manipulation tool. Today, CRISPR-Cas9 is a frequently harnessed tool for genome editing purposes and major progress in understanding the underlying biochemical processes in RNA-guided Cas9 was presented in recent years. In 2010, researchers showed that Cas9 creates a single double-stranded break at a precise position on the target DNA. Further insight into the mechanism was delivered 1 year later as the involvement of another small RNA called tracrRNA (Trans-activating CRISPR (tracr) RNA), was shown. The maturation of crRNA requires tracrRNA as well as Cas9 and RNase III. Evidence that the system would function heterologously in other bacteria was demonstrated in 2011, as the S. thermophilus type II CRISPR-Cas system could provide immunity in E. coli. Other research had shown certain elements of the type II system, including the involvement of a PAM (protospacer adjacent motif) sequence in interference but the nature of the cleavage complex remained unknown. In 2012, tracrRNA, which was previously known to be involved in crRNA maturation, was shown to also form an essential part of the DNA cleavage complex, with the dual tracrRNA:crRNA directing Cas9 to introduce doublestrand breaks in the target DNA . Further simplification of the programmed targeting was achieved by creating a single-guide RNA fusion of tracrRNA and crRNA, that guides Cas9 for sequence-specific DNA cleavage. A few months following the description of the CRISPR-Cas9 technology, a number of publications demonstrated its power to edit genomes in eukaryotic cells and organisms, including human and mouse cells [Fig.1].

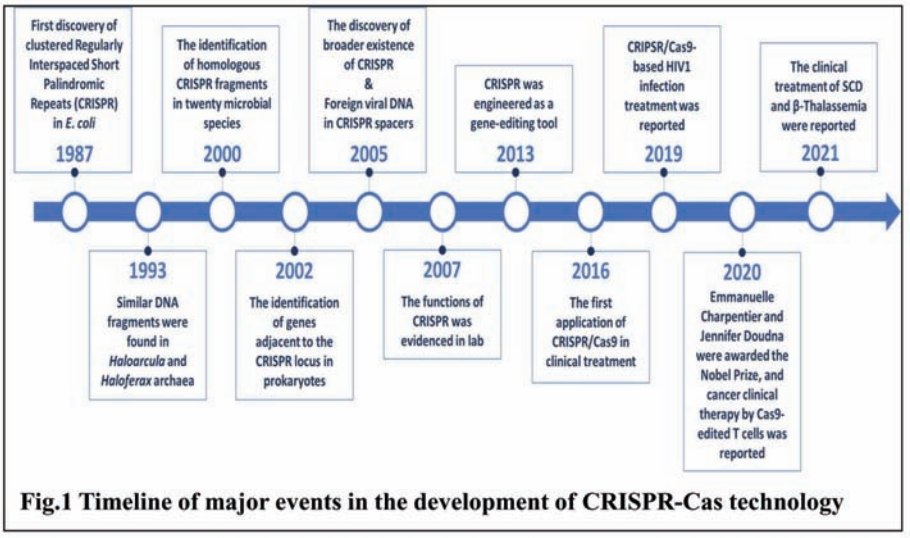

Fig.1 Timeline of major events in the development of CRISPR-Cas technology

CRlSPR gene editing has long attracted attention in the research community, the media, and the wider public which peaked in 2020 when the Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry to two leading CRISPR researchers, Jennifer Doudna and Emmanuelle Charpentier [Fig.2].

Fig.2 Nobel Prize Awarded to Jennifer Doudna And Emmanuelle Charpentier for developing CRISPR-Cas9 technology

CRISPR-Cas is the only adaptive immune system in prokaryotes known so far. In this system, small guide RNAs (crRNAs) are employed for sequence specific interference with invading nucleic acids. CRISPR-Cas comprises a genomic locus called CRISPR that harbours short repetitive elements (repeats) separated by unique sequences (spacers), which can originate from mobile genetic elements (MGEs) such as bacteriophages, transposons or plasmids. The so-called CRISPR array is preceded by an AT-rich leader sequence and is usually flanked by a set of cas genes encoding the Cas proteins [Fig.3].

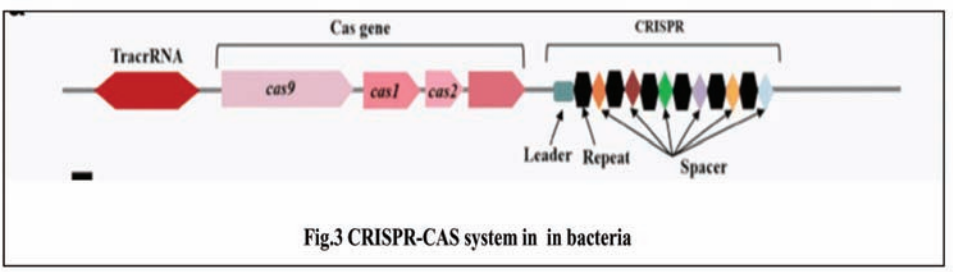

Fig.3 CRISPR-CAS system in in bacteria

To date, CRISPR-Cas systems can be divided into two main classes, which are further classified into six types and several sub-types [Fig. 4]. The classification is based on the occurrence of effector Cas proteins that convey immunity by cleaving foreign nucleic acids. In class 1 CRISPR-Cas systems (types I, III and IV), the effector module consists of a multi-protein complex whereas class 2 systems (types II, V and VI) use only one effector protein.

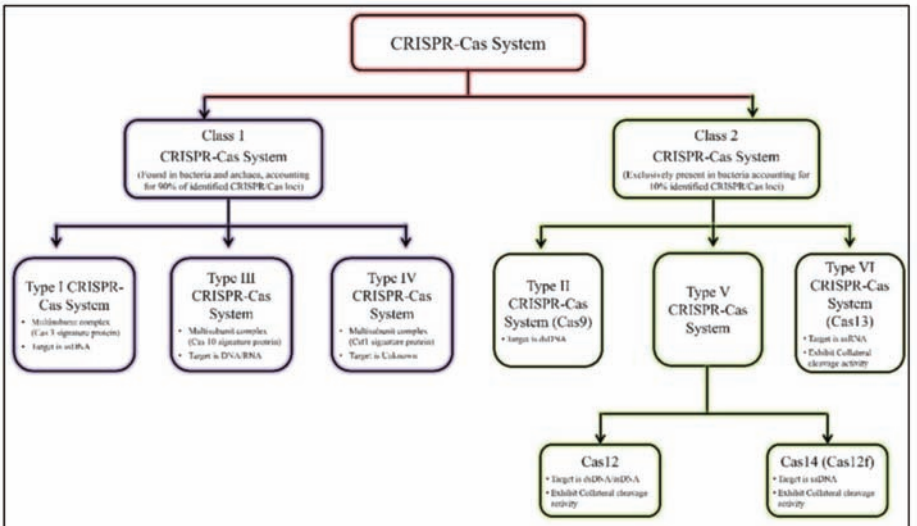

Fig.4 General classification of CRISPR-Cas System

How CRISPR works as bacterial adaptive immune system?

The CRISPR-Cas system acts in a sequence-specific manner by recognizing and cleaving foreign DNA or RNA. The defence mechanism can be divided into three stages: (i) adaptation or spacer acquisition, (ii) crRNA biogenesis, and (iii) target interference [Fig.5]

(i) Adaptation or spacer acquisition

In a first phase, a distinct sequence of the invading bacteriophage called a protospacer is incorporated into the CRISPR array yielding a new spacer. This event enables the host organism to memorize the intruder's genetic material and displays the adaptive nature of this immune system. Two proteins, Cas1 and Cas2, seem to be ubiquitously involved in the spacer acquisition process as they can be found in almost all CRISPR-Cas types. The selection of a target sequence that is integrated into the CRISPR locus is not random. It has been demonstrated that a short sequence, called the protospacer adjacent motif (PAM), is located directly next to the protospacer and is crucial for acquisition and interference. It is believed that after protospacer selection, Cas9 recruits Cas1, Cas2 for integration of the new spacer into the CRISPR array.

(ii) crRNA (CRISPR RNA) biogenesis:

To enable immunity, the CRISPR array is transcribed into a long precursor CRISPR RNA (pre-crRNA) that is further processed into mature guide crRNAs containing the memorized sequences of invaders. In type II systems, tracrRNA (Trans-activating CRISPR (tracr) RNA) is required for the processing of the pre-crRNA. The anti-repeat sequence of this RNA enables the formation of an RNA duplex with each of the repeats of the pre-crRNA, which is stabilized by Cas9. The duplex is then recognized and processed by the host RNase III yielding an intermediate form of crRNA that undergoes further maturation to lead to the mature small guide RNA.

(iii) Target interference

In the last stage of immunity, mature crRNAs are used as guides to specifically interfere with the

invading nucleic acids. To avoid self-targeting, type I, II and V systems specifically recognize the PAM sequence that is located upstream (types I and V) or downstream (type II) of the protospacer. In type II CRISPR-Cas systems, the tracrRNA:crRNA duplex guides the effector protein Cas9 to introduce a double-strand break in the target DNA.

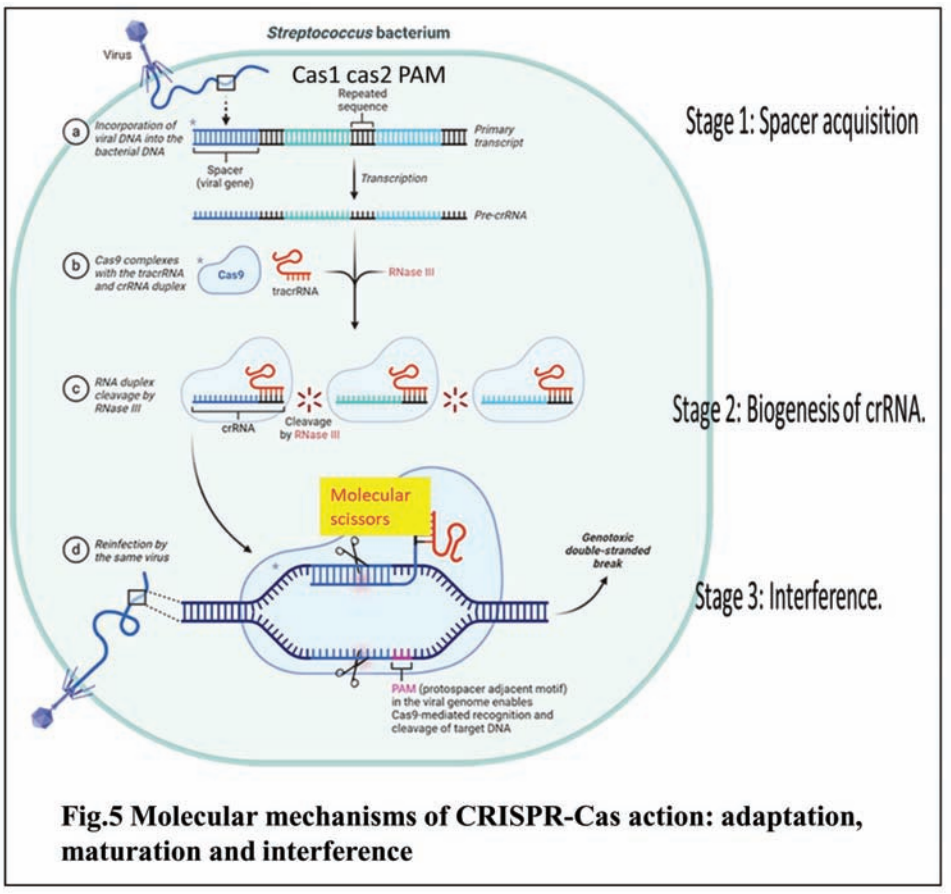

Fig.5 Molecular mechanisms of CRISPR-Cas action: adaptation, maturation and interference

How CRISPR works in genome editing?

Classically, the CRISPR-Cas9 system is utilized by bacteria to destruct foreign DNA that enters the bacteria through horizontal gene transfer. Initially, a unique non-coding RNA trans-activating CRISPR RNA (tracrRNA) (transcribed separately) hybridizes to the precursor CRISPR transcript (pre-crRNA) through repeat sequences for post-transcriptional processing and endonucleolytic cleavage by RNaseIII to generate the CRISPR RNAs (crRNA) and forms a dual RNA hybrid structure. This dual RNA guide directs Cas9 for dsDNA cleavage complementary to the target sequence located adjacent to PAM and cleaves each strand with distinct nucleases (Fig. 5). Engineered sgRNA (single guide RNA) that combines crRNA and tracrRNA can simplify the system. The protospacer adjacent motif (PAM) sequence is NGG for CRISPR/Cas9, which occurs once in every 8 bps or 4 bps of random DNA, enables the design of sgRNA more conveniently (Fig.6). An in vitro double-strand break (DSB) of target DNA caused by the CRISPR-Cas9 foreign DNA destruction programme was a breakthrough and created the basis for its gene-editing toolbox application (Fig.7).

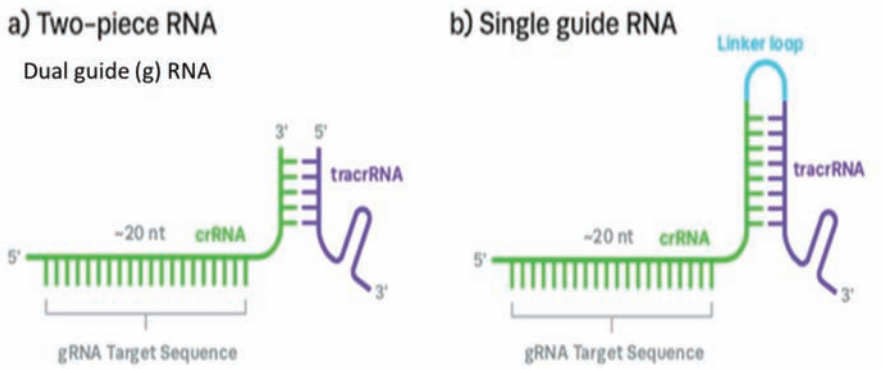

Fig.6 Guide RNA is made up of two parts, CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). The crRNA is an 18–20 base pair in length that specifies the target DNA by pairing with the target sequence, whereas tracrRNA is a long stretch of loops that serve as a binding scaffold for Cas-9 nuclease. In prokaryotes, the guide RNA is used to target viral DNA, but in the gene-editing tool, it can be synthetically designed by combining crRNA and tracrRNA to form a single guide RNA (sgRNA) in order to target almost any gene sequence supposed to be edited. This characteristic of Cas9 activation upon target recognition via gRNA possesses great potential in genotyping and gene detection.

In 2012, George Church, Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang discovered that by designing guide RNA (https://bitesizebio.com/34677/ design-crispr-cas9-experiment-genome-editing/) to target a specific region in the genome, "the CRISPR-Cas9 system can be used as a "cut-and-paste" tool (https://www.sciencemag.org/news/2017/02/how-battle-lines-over-crispr-were-drawn) to modify genomes. As a DNA-editing tool, CRISPR-Cas9 can remove or introduce new genes as well as silence or activate genes. Repurposing the CRISPR-Cas9 system for genome editing exploits the DNA repair mechanisms of eukaryotic cells: after the introduction of a double-strand DNA break, the cell can repair the damage by non-homologous end joining (NHEJ). This process is error-prone and often leads to point mutations, deletions or causes frameshifts that alter the gene product and eventually abolishes its function, which is favoured for genetic knockouts. Precise genome engineering, however, relies on another pathway, termed homology-directed repair (HDR), where a piece of DNA that shows sequence homology to the target site is used to repair the DNA via homologous recombination. This short DNA sequence can harbour any sort of insertion or alteration, allowing the integration of any desirable DNA sequence at the target site (Fig.7).

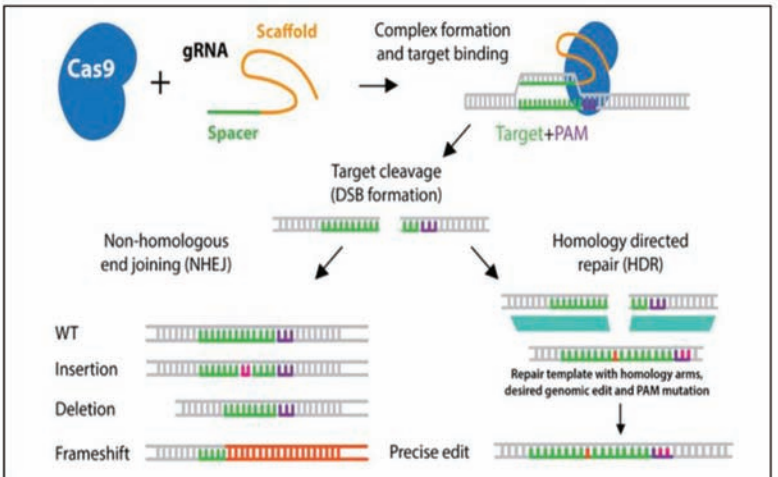

Fig. 7 Mechanism of action of CRISPR-Cas9 techniques: CRIPSPR/Cas9 gene with cleavage activities creating a double-strand break on the target DNA, which are repaired either by non-homologous end joining (NHEJ) or by homology-directed repair (HDR) with the help of Donor DNA provided

Why CRISPR?

Today CRISPR-Cas9 has becomes the most effective, efficient, accurate and frequently harnessed tool for genome editing in all living cells and utilized in many applied disciplines. The CRISPR-mediated genome editing system has become more popular because of its efficiency, precision, simplicity, speed and versatility. In recent years, several engineered versions of CRISPR have been developed mainly due to this technique's programmable nature and minimal requirements. Nowadays, CRISPR based technologies enable efficient targeting and alteration of DNA in living cells from dozens of species (including humans and other eukaryotes) and are widely adopted by the scientific community. Precise editing can be utilized in personalized gene therapy to correct inherited monoclonal diseases or sequence-specific targeting of pathogens to treat infectious diseases, as well as for many other applications (Fig.8).

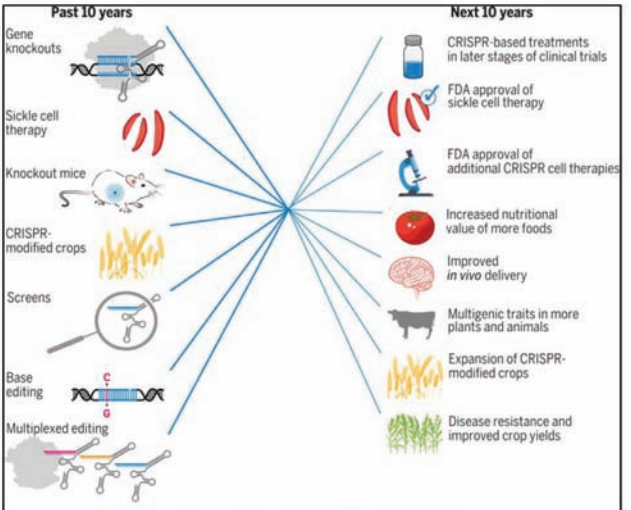

Fig.8 Applications of CRISPR-Cas9 technology

SUCCESS STORIES:

1. Sickle Cell Gene Therapy Using CRISPR

Sickle cell anaemia is a genetic blood disorder that affects haemoglobin, the oxygen-transporting molecule in red blood cells. Sickle cell disease causes the body to produce haemoglobin S, an abnormal form of the molecule that distorts the shape of red blood cells (resembling a sickle), disrupting their function. Individuals affiliated with this disorder produce deoxygenated haemoglobin S molecules, which cause red blood cells to distort and form a rigid, crescent (sickle) shape. The affected cells have a reduced lifespan in the body, resulting in shortages of red blood cells. The rigid, distorted cells obstruct blood vessels and inhibit circulation, causing severe pain and recurrent infections. Obstructions often result in clogs in the spleen, liver, lungs, heart, or eyes. Sickle cell disease causes increasing organ and tissue damage over time and increases the risk of stroke.

Sickle cell anaemia is caused by mutation of a single base in the DNA sequence of the ß-globin gene (HBB). In healthy individuals, position 6 of the resulting amino acid sequence is a glutamic acid (GAG), however, in sickle cell anaemia patients, this is substituted for a valine (GTG). This mutation results in the formation of haemoglobin S, the disease-associated form of the protein

Treatment options for sickle cell anaemia are currently limited. Patients typically require frequent blood transfusions, and medications are typically recommended to relieve the pain attacks or reduce their frequency. The only cure currently available for sickle cell disease is the transplantation of bone marrow from a healthy donor. This approach presents significant challenges, including identifying a suitable donor, immune rejection of the transplant, and graft-versus-host disease (GVHD). It is also typically restricted to children and young people because the associated risks increase with the age of the patient.

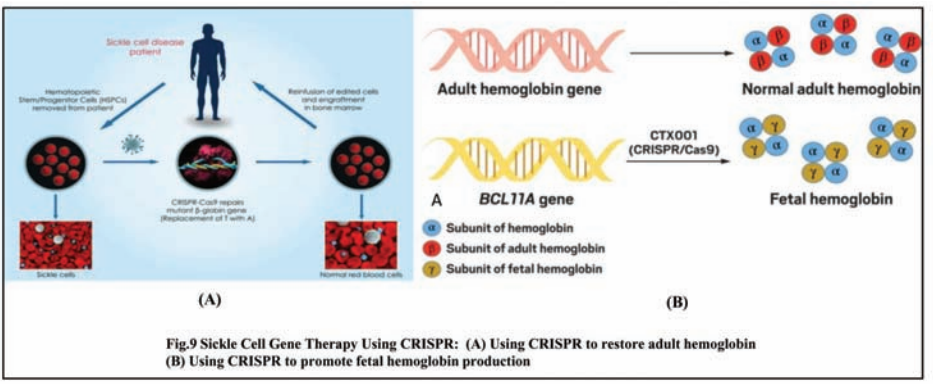

Fig.9 Sickle Cell Gene Therapy Using CRISPR: (A) Using CRISPR to restore adult hemoglobin (B) Using CRISPR to promote fetal hemoglobin production

2. Using CRISPR to restore adult haemoglobin

One of the main approaches to CRISPR sickle cell gene therapy is to repair the mutation in the adult haemoglobin gene responsible for sickle cell disease, causing the healthy, normal form of adult haemoglobin (haemoglobin S) to be produced. Daniel Dever, a research instructor in Matthew Porteus' lab at Stanford University uses this approach in his work involving CRISPR to introduce a DNA break to the ß-globin gene. The site of the break can then be used to introduce a correction to the gene via homology-directed repair (HDR). This is called a gene knock-in; a donor template containing the normal sequence of the gene is introduced so that the mutation is corrected when the cell repairs the DNA break with the template via HDR. The edited cells, now engineered to produce normal

haemoglobin, are re-implanted in the patient's bloodstream. The preclinical development of Dr. Dever's ß-globin gene-editing method is complete, and the method proves to be incredibly efficient and reproducible, allowing for mutation and analysis of precise genomic locations in weeks [Fig. 9A].

3. Using CRISPR to promote foetal haemoglobin production

The second approach to CRISPR sickle cell gene therapy involves a gene knockout, switching off the gene that suppresses foetal haemoglobin. This method causes foetal haemoglobin (haemoglobin F) to be expressed, replacing the mutated adult haemoglobin. This is a highly promising CRISPR-Cas9 sickle cell therapy and one that is closer to a clinical application than ß-globin gene editing. By mutating (knocking out) the BCL11A gene, the expression of haemoglobin F is indirectly promoted. This approach was developed after it was found that sickle cell patients with a natural mutation in their BCL11A gene were resistant to disease symptoms [Fig. 9B].

Victoria Gray was the first CRISPR sickle cell patient, having fought the disease for 34 years. Following the one-time treatment, her blood showed a significant proportion of foetal haemoglobin levels, and she has been able to avoid blood transfusions and pain attacks without any major side effects. Dr. Haydar Frangoul, the medical director of paediatric haematology/oncology at HCA Healthcare's Sarah Cannon Research Institute Centre, treated Ms. Gray [Fig. 10]. A key FDA Advisory Committee met on November 1st,2023 to discuss Vertex Pharmaceuticals' groundbreaking IND application of exa-cel, a CRISPR-based therapy for sickle cell disease (SCD) and FDA Advisory Panel finds Vertex's Off-Target Data Convincing for Approval.

Fig.10 Victoria Gray- First sickle cell patient treated with CRISPR gene-editing Dr. Haydar Frangoul (left) and Victoria Gray (right)

Disadvantages, ethical issues and controversies

However, the application of CRISPR simultaneously brings many practical and technological challenges mainly associated with delivery strategies, the control of repair pathways, off-target and on-target effects, the host immune response toward CRISPR-Cas9 and controversial ethical issues [Fig. 11].

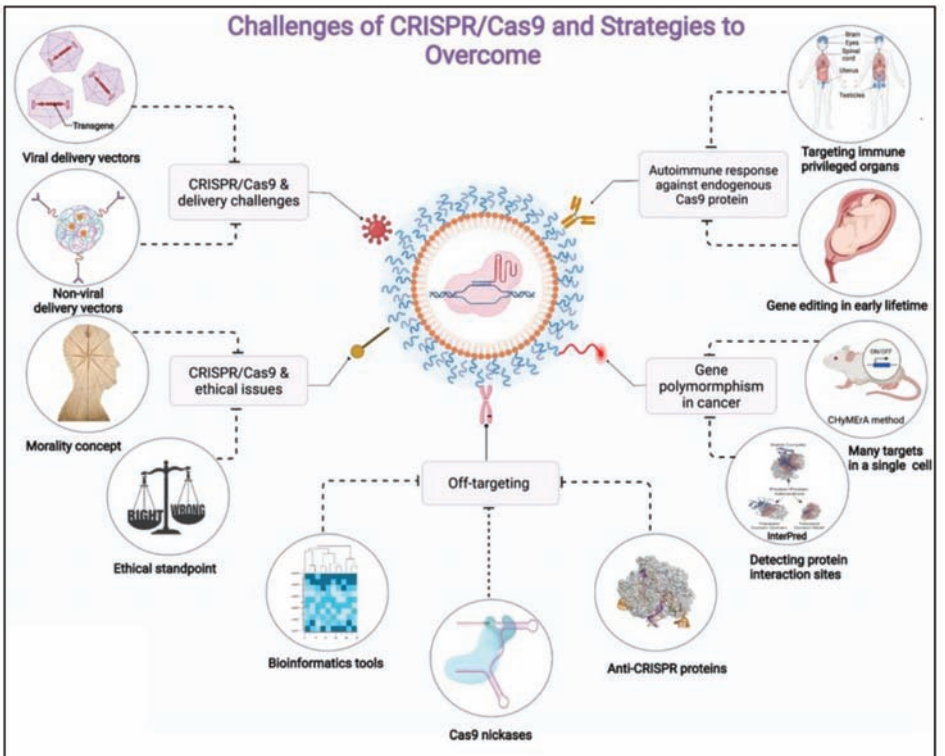

Fig.11 Immunogenicity, off-targeting, polymorphism, delivery technique, and ethical issues are main limitations of CRISPR technology

Practical and technological issues in genome editing

The CRISPR-Cas technology is a simple yet powerful and currently the most reliable tool for editing genomes of various organisms. However, CRISPR is prone to errors (off-target effects) and unintended outcomes (on-target effects); and demands improvements of several aspects, such as the efficiency of HDR and its safety in clinical usage. Until this day, the CRISPR/Cas9 technique is successfully used for the treatment of genetic diseases as muscular dystrophy (Duchenne's syndrome), Cystic fibrosis, Wolfram syndrome, Leber congenital amaurosis, ß-Thalassemia, Sickle-cell disease, Huntington's disease, HIV and others. Finally, yet importantly, this new technology faces ethical severe issues, mainly concerning human genetic engineering (with emphasis on germ line editing).

Off-target and on-target effects

Specificity is essential for all targetable nucleases, mainly when applied in human therapy or food sources. In many instances, the RNA guided nucleases can induce mutations at sites that differ from the intended target region.

CRISPR Technolog y and Patent Ownership dispute

The understandable motive of individual scientists, as well as organizations involved in the study of the CRISPR-Cas9 system, was the possible financial gain potentially obtainable from the use of this promising technology. One of the first patent applications was filed jointly by the University of

California at Berkeley, representing Doudna, the University of Vienna (where one of the two lead authors from the key publication on CRISPR-Cas9 worked, and Charpentier as an individual inventor in accordance with the rules of the University of Umeå (Sweden), where Charpentier worked at the time of publication of the article. This patent application was filed in May 2012, while in December 2012 Zhang and the Broad Institute also submitted a patent application simultaneously with the acceptance of Zhang's paper on human cells' editing for publication in Science [Fig. 12].

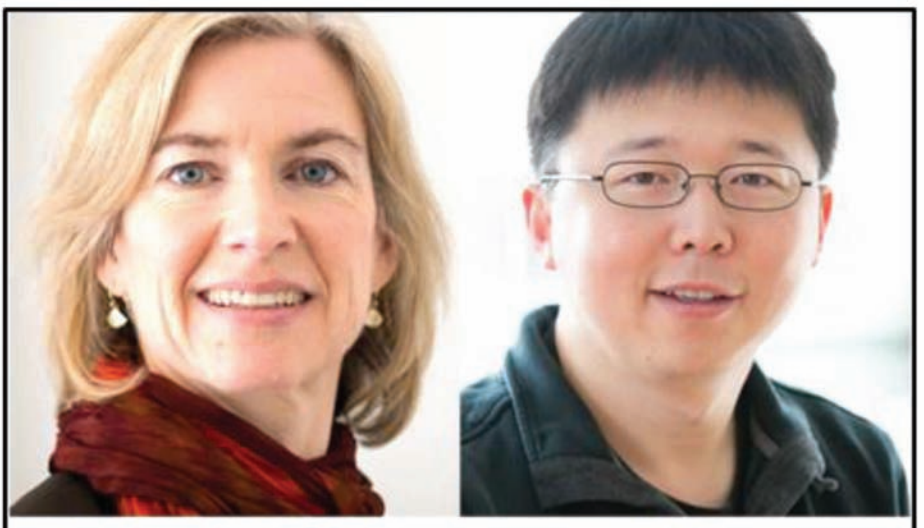

Fig. 12 Jennifer Doudna, left, and Feng Zhang both claim to have made key discoveries of CRISPR gene-editing.

Initially, it was Zhang's application that turned out to be successful and resulted in a patent in April 2014, while Doudna's application was still pending at that time. Doudna's team disagreed with the decision, after which a long dispute between the two parties followed, including appeals and court hearings which ultimately led to an ambiguous situation in CRISPR-Cas9 licensing. Due to the fact that by 2019 both competing parties had patents in this area, some of the biotech companies that used the CRISPR-Cas9 system on human cells received a license from the team of Doudna, while others - from Zhang. However, the U. S. Patent and Trademark Office Appeal Board in February 2022 again confirmed the priority of Zhang and the Broad Institute in the position of the patent holder for the use of CRISPR-Cas9 in human cells, which caused disappointment and frustration from the opposing side, and financial complications for companies licensed by the team of Doudna. Doudna and Charpentier, however, won a similar dispute in Europe, and also hold major patents on the use of technology in the U.K., China, Japan, Australia, New Zealand, and Mexico.

Bioethical controversy

The He Jiankui affair is a scientific and bioethical controversy concerning the use of genome editing following its first use on humans by Chinese scientist He Jiankui, who edited the genomes of human embryos in 2018. He became widely known on 26 November 2018 after he announced that he had created the first human genetically edited babies [Fig 13]. He was listed in the Time's 100 most

influential people of 2019. The affair led to ethical and legal controversies, resulting in the indictment of He and two of his collaborators, Zhang Renli and Qin Jinzhou. He eventually received widespread international condemnation.

He Jiankui, took sperm and eggs from the couples, performed in vitro fertilisation with the eggs and sperm, and then edited the genomes of the embryos using CRISPR-Cas9. The editing targeted a gene, CCR5, that codes for a protein that HIV uses to enter cells. Birth of twins after Genome Editing for HIV Resistance was achieved and the twins, called Lulu and Nana, reportedly had their genes modified before birth using the new editing tool CRISPR. The goal was to make the girls immune to infection by HIV, the virus that causes AIDS.

He Jiankui (right) was found guilty of 'illegal medical practices' and jailed for three years in China

An embryo receives injections at a laboratory in Shenzhen, China

Fig.13 The He Jiankui affair is a scientific and bioethical controversy concerning the use of genome editing of human embryos using CRISPR

He Jiankui sent shock waves across the world of science when he announced in 2018 that he had edited the genes of twin girls, Lulu and Nana, before birth. He was subsequently sacked by his university in Shenzhen, received a three-year prison sentence, and was broadly condemned for having gone ahead with the risky, ethically contentious and medically unjustified procedure with inadequate consent from the families involved.

CRISPR gene therapy and ethical issues associated with it

The haste with which competing laboratories sought to bring their research to the public's attention, as well as the race to patent this technology, were indicators of the significance of this scientific breakthrough. Undoubtedly, one of the main driving forces that motivated many scientists to take part in research using this particular technology was the potential of modifying human cells, both somatic and germ line. However, despite the apparent advantages of the CRISPR-Cas9 system, numerous ethical and technical difficulties stand in the way of researchers who dream of curing life-threatening diseases, especially if the genetic changes resulting from such manipulations can be inherited.

CONCLUSIONS

The discovery of CRISPR-Cas9 as an immune system in prokaryotes at the turn of the 20th-21st centuries a finding at first glance only relevant to microbiology has led to a revolution in the field of genomic manipulations. New opportunities have opened up in multiple areas of biomedicine, such as molecular diagnostics of infectious and non-infectious diseases (e.g., genotyping of bacterial strains, detection of viruses, and identification of genetic mutations in circulating extracellular DNA in

patients with lung cancer, as well as in the development of a potentially new method of immunization, DNA vaccines. One of the more unusual examples of the application of the CRISPR- Cas9 system was the cultivation of brain-like organelles carrying different variants of the important NOVA1 gene characteristic of modern humans, Neanderthals, and Denisovans. The development of CRISPR-Cas9 technology is a good example of how discoveries made in the course of basic research can change entire fields of science and technology, expanding the horizons of the possible. This ground-breaking technique is a worthy continuation of such exciting scientific events as the publication of the double-stranded structure of DNA by Watson and Crick in 1953, the birth of the first child by in vitro fertilization in 1978, and the cloning of Dolly the sheep in 1996. In the coming years the scientific community will watch with interest the development of legislation and ethical principles in the application of the CRISPR-Cas9 system in genome editing, as well as in what other areas of science this promising technology will find its application.

ROLE OF HAPTOGLOBIN IN MOLECULAR PATHOGENESIS OF SEPSIS

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Introduction

The inflammatory response to a variety of systemic infections results in sepsis in susceptible individuals, wherein the mortality rate is an alarming 30 45%. Sepsis constitutes 17% of all admissions to the intensive care unit, of which 45% of admissions end in fatality (1). Severe sepsis takes more lives than cardiovascular disease, breast, colon/rectal, head and neck, throat and prostate cancer combined, and the incidence of sepsis is rising by 1.5 8% annually (2 4), despite advancements in critical care support and equipment. While very little is known about the genetic susceptibility of an individual, the risk of sepsis increases due to various immunosuppressive procedures. Several novel approaches in sepsis prophylaxis and treatment are currently in progress (4). Certain clinical trials have provided contrasting results. For example, when patients with sepsis were treated with two different monoclonal antibodies against endotoxin HA 1A, a human antibody and E5, a murine antibody, no change in mortality was observed in patients with gram negative bacteremia (5). Animal models have also provided conflicting results when compared with human studies (6). Therefore, using an animal sepsis model may not provide conclusive evidence for human application. Immunological response to Gram negative bacterial lipopolysaccharide (LPS) predominantly involves their interaction with Toll like receptors (TLRs) and cluster of differentiation (CD)14 receptors present on monocytes and macrophages, which initiates the production of pro inflammatory mediators, including interleukin (IL) 6, IL 8 and tumor necrosis factor (TNF) _. LPS also induces the production of acute phase response proteins by the liver. Among many known sepsis markers, serum procalcitonin (PCT) is currently the only US Food and Drug Administration approved biomarker for the diagnosis, and as an indicator, of the progression of sepsis, though other acute phase reactants, including C reactive protein (CRP) and serum amyloid A (SAA) protein, are also in use. Several biomarkers are known to be elevated in sepsis (7), although the exact biochemical function and etiology of their overexpression remain unknown. Host immunological response to sepsis is nuanced, and varies in both innate and adaptive responses, which makes diagnosis and therapy a challenge for individuals at risk of mortality. The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non specific nature of the signs and symptoms of sepsis (8). Early diagnosis and prediction of the severity of sepsis is very important, thereby increasing the possibility of starting timely and specific treatment (9,10). Previous studies have shown gender based variation in the pattern of expression of acute phase proteins and sepsis associated mortality (11), wherein mutually opposing observations have been made, which make adjustments with concurrent data while generalizing observations (12). A previous study involving elderly patients revealed that sepsis mortality was independent of gender; however, this was correlated with elevated 17 _ estradiol in both genders, with elevated progesterone in males and elevated testosterone in females (13). An increased risk of acquiring sepsis in surgery patients with higher TNF _ levels due to polymorphism in the N coI region of the TNFB gene has been reported (14,15). However, a generalization in this regard requires study of a larger cohort. Recent advances in understanding sepsis involve various sepsis models to diagnose susceptibility towards sepsis, however, a clear correlation requires a broader and deeper analysis of sepsis response proteins. To address the issue, Kalenka et al (16) analyzed the serum proteome of sepsis patients and successfully identified differences between the proteome of survivors (S) and non survivors (NS) at the end of 28 days from the onset of sepsis(16). Similarly, Su et al (17) studied urinary proteomics of sepsis patients during the 28 days from the onset of sepsis (17). These studies have made significant

contributions to the understanding of serum protein dynamics to assess the differential changes associated with S and NS of sepsis. The present study is a prospective observational longitudinal study, where serum proteome dynamics from early until late stages of sepsis were analyzed in S and NS, from the onset of sepsis as indicated by PCT levels. Since sepsis has a higher incidence in males, and females appear to differ in responses due to hormonal variations, the present study used adult human male samples for homogeneity. The goal of the present study was to target differentially expressed proteins while comparing S and NS, which may be useful, particularly in the early stages to devise strategies to improve chances of patient survival. The present study focused on serum proteome profiles at different phases of sepsis in Indian adult male patients suffering with bacterial sepsis, particularly K. pneumoniae, to eliminate further possibility of heterogeneity in sampling, which may assist with understanding changes in serum acute phase proteins under given conditions, and can be later used to monitor and devise methods of patient specific sepsis management.

Materials and methods

Patients and samples

Blood samples from adult male patients (n=12; S and NS =6 each) diagnosed with sepsis were procured from Global Hospitals (Lakdi ka pul Hyderabad, India; Table I). The patients were carefully monitored up until mortality at day 20 28, and samples were collected daily from the day of clinical diagnosis (onset) until recovery, in the case of S, and 24 h prior to mortality in the case of NS. Criteria for selection of male patients showing signs of severe sepsis or septic shock (endotoxemia) were based on patient serum PCT levels and acute physiology and chronic health evaluation II (APACHE II) scores (Table I). APACHE II is a severity of disease classification system (18), one of several ICU scoring systems. It is applied within 24 h of the admission of a patient to an intensive care unit: An integer score from 0 to 71 is computed based on several measurements; higher scores correspond to more severe disease and a higher risk of death. The APACHE II scoring system has been widely accepted as a measure of illness severity; it has been demonstrated to accurately stratify risk of death in a wide range of disease states, and in different clinical settings (19). Blood samples from healthy males (n=6) were collected with their consent as reference controls. Serum was isolated from blood samples of both patients and healthy controls for further analysis. Whole blood was collected separately from the identical male patients (S=6; NS=6) at early stages (within 24 h of sepsis diagnosis) in EDTA K3 containing tubes for RNA extraction and reverse transcription quantitative polymerase chain reaction (RT qPCR) analysis. Exclusion criteria for sample selection were being 75 years old, patients who were lost at follow up, patients with previous medical history of anti inflammatory drug treatment, chemotherapy and glucocorticoid therapy. Burns patients, subjects with liver diseases, cardiovascular diseases and organ transplant recipients were not enrolled. Samples from each individual patient were collected with the informed consent of the patient or family, and the present study was approved by the institutional ethical committee.

Two-dimensional electrophoresis.

Serum samples for each day from the onset of sepsis until the recovery/death of each patient (n=6) and individual controls (n=6) were subjected to Albumin depletion, according to the manufacturer's protocol (Aurum™ Affi Gel® Blue mini kits and columns; Bio Rad Laboratories, Inc., Hercules, CA, USA). Complete albumin depletion was confirmed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). The albumin depleted elutes were subsequently diluted with ice cold acetone and subjected to acetone precipitation at 15,000 x g at 4°C for 10 min. The pellets obtained were washed with 1 ml acetone and allowed to air dry. The pellets were subsequently resuspended in 75 μl rehydration sample buffer, containing 4 M urea, 2% CHAPS, 1 mM dithioth reitol, 0.2% Biolyte, 3/10 ampholytes (Bio Rad laboratories, Inc.) and traces of Bromophenol blue dye. Once the pellets were completely dissolved in rehydration sample buffer, the total protein content in

resuspended sample was estimated using Bradford protein assay (Sigma Aldrich, St. Louis, MO, USA). A total of 500 μg protein was applied to 11 cm (pH 3 10) immobilized pH gradient (IPG) strips (Bio Rad Laboratories, Inc.) and isoelectric focusing was performed using a Protean IEF unit, according to the manufacturers protocol (Bio Rad Laboratories, Inc.). The IPG strips were subsequently separated by 9 14% gradient SDS PAGE at 16 mA for the stacking gel and 24 mA for the resolving gel. Following electrophoresis, the gels were fixed in a solution of 50% methanol and 10% glacial acetic acid for 1 h and were subsequently stained with colloidal Coomassie blue stain for image analysis.

Image analysis.

The gel images were analysed using Image Master 2D Platinum software (version 7.0; GE Healthcare Bio Sciences, Pittsburgh, PA, USA). Control gels of healthy subjects (n=6) were analysed individually and normalized to be used as the reference gel. The daily sample for S and NS was analysed in duplicate to identify spots with percentage volume variation. Duplicates of each day were combined to give a single representative gel and analysed again to increase the reproducibility and reduce the error rate of analysis. The spots were compared for percentage volume variations.

In gel trypsin digestion. The spots of interest were excised and washed with a 1:1 ratio of 50 mM ammonium bicarbonate and acetonitrile for 15 min. Following two separate washes with ammonium bicarbonate and acetonitrile, a final wash was performed with acetonitrile until the gel pieces were opaque. The acetonitrile was discarded and the gel pieces were vacuum dried. Diluted trypsin was added to the gel pieces and incubated for 1 h at room temperature. The excess trypsin was removed and the pieces were incubated overnight with 25 mM ammonium bicarbonate at 37°C. The digested extract was collected and vacuum centrifuged to concentrate the extract, which was subsequently used for matrix assisted laser desorption ionization time of flight (MALDI TOF; Bruker Daltonics, Leipzig, Germany) analysis.

MALDI TOF analysis. An α-cyano 4 hydroxycinnamic acid (HCCA) matrix (5 mg/ml) was prepared in 70% acetonitrile and 30% 0.1% trifluoroacetic acid. The trypsin digested extract was subsequently mixed with HCCA matrix in a 1:1 ratio and $\sim$2 μl matrix sample mix was spotted onto an anchor chip and ground steel plate (Bruker Daltonics). Once dried, the plates were loaded onto a MALDI TOF mass spectrometer (MS; Bruker Daltonics) at the Central Facilities for Research and Development (Osmania University, Hyderabad, India). Spectra were obtained in the reflectron mode (mass range 500 3,000 Da; 20 keV accelerating voltage; averaging 500 laser shots/spectrum) using a Bruker Autoflex III MALDI TOF/TOF spectrometer (Bruker Daltonics). The spectra were analyzed with Flex Analysis software (version 3.3; Bruker Daltonics) and Biotools software (version 3.2; Bruker Daltonics), with the following parameters: Signal to noise threshold, 6; mass exclu sion tolerance, 0.75m/z; maximal number of peaks, 100; quality factor threshold, 50; monoisotopic peaks (Adduct: H). Matrix and/or auto proteolytic trypsin peaks, or known contaminant ions were excluded. Bioinformatics data mining was performed using the Mascot platform (http://www.matrixscience.com). The resulting peptide mass lists were queried in the Swiss Port 2013_02 database (539,165 sequences; 191,456,931 resi dues). The following criteria were used for search parameters: Taxonomy, Homo sapiens (human); significant protein Molecular Weight Search score at P 500 3,000 Da; 20 keV accelerating voltage; averaging 500 laser shots/spectrum) using a Bruker Autoflex III MALDI TOF/TOF spectrometer (Bruker Daltonics). The spectra were analyzed with Flex Analysis software (version 3.3; Bruker Daltonics) and Biotools software (version 3.2; Bruker Daltonics), with the following parameters: Signal to noise threshold, 6; mass exclu sion tolerance, 0.75m/z; maximal number of peaks, 100; quality factor threshold, 50; monoisotopic peaks (Adduct: H). Matrix and/or autoproteolytic trypsin peaks, or known contaminant ions were excluded. Bioinformatics data mining was performed using the Mascot platform (http://www.matrixscience.com). The resulting peptide mass lists were queried in the Swiss Port

2013_02 database (539,165 sequences; 191,456,931 residues). The following criteria were used for search parameters.

Statistical analysis

Comparisons between groups (S and NS sepsis patients) were performed using either a paired Student's t test and/or a Mann Whitney U test, using GraphPad Prism (version 6.05; GraphPad Software. Inc., San Diego, CA, USA). The expression factors (EFs) are expressed as the mean ± stan dard error of the mean. P≤ 0.05 was considered to indicate a statistically significant difference.

RT qPCR analysis

Blood samples from sepsis patients were obtained and the total RNA was extracted using Total RNA spin columns (Yeastern Biotech, Co., Ltd., Taipei, Taiwan) and treated with RNase free DNase (Macherey Nagel, Inc., Düren, Germany). A total of ~5 μg RNA was reverse transcribed using oligo (dT) primers and reverse transcriptase (Thermo Fisher Scientific, Inc.). qPCR was performed on the cDNA samples.

Results and Discussion

Identification of differentially expressed proteins in S and NS of sepsis. Albumin depleted serum samples from sepsis patients were separated by 2D gel electrophoresis and 2D gel image analysis was performed (Fig. 1). Protein spots representing specific proteins exhibiting an increased or decreased percentage volume and intensity were matched to the corresponding spot in the reference gel (control serum). The proteins exhibiting differential volume percentage with respect to patients with sepsis were then matched between S and NS from the day of onset until recovery/mortality. Approximately 300 spots were analyzed in each gel.

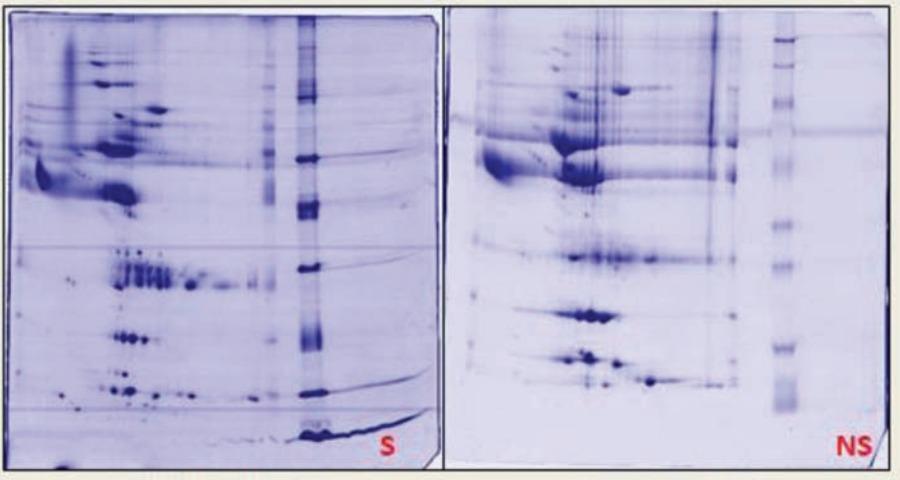


Figure represents (A) serum proteins separated from sepsis survivor recovering from bacterial infection. (B) Serum proteins separated from sepsis non-survivor died of bacterial infection and MOD .

Note : Serum samples from sepsis patients were provided, for standardization of the protocols, by Dr. V. Sritharan lab Global hospital,Hyd

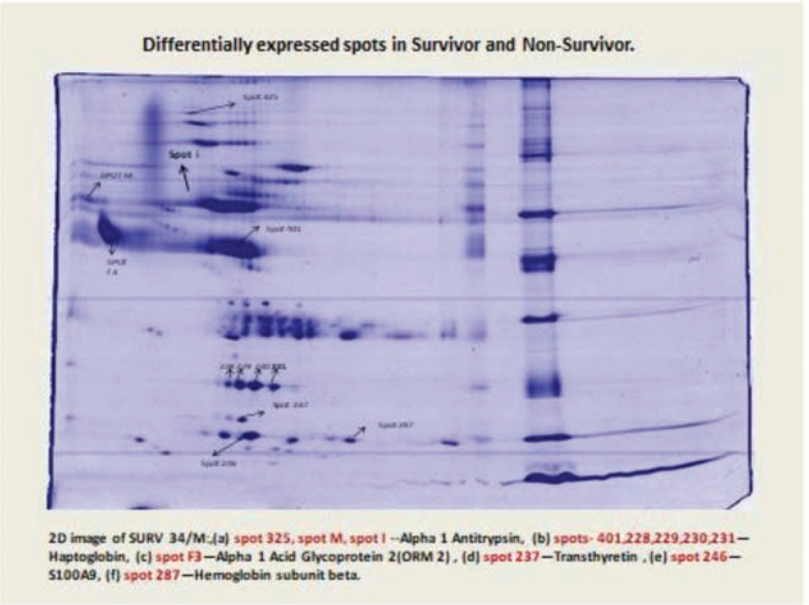


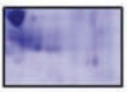
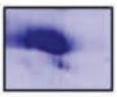
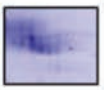
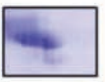
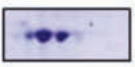
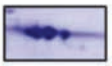
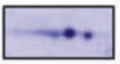
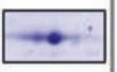
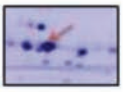
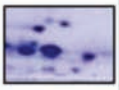
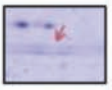
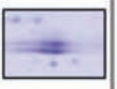
Figure 1: Albumin depleted serum samples of sepsis patients were separated by 2 -Dimensional electrophoresis. Proteins resolved by Isoelectric focusing using 11cm, 3-10 pH range IPG strips were separated by gradient SDS-PAGE (10-14%). The gels were stained with Coomassie Brilliant Blue (G250) and analyzed by Image master 2D Platinum.

The analysis resulted in the identification of 30 differentially expressed spots between S and NS. Identification of the spots by MALDI TOF demonstrated 12 spots with a significant MS/MS score (Table III). Since normalized relative volumes of a spot (%) are independent of variations due to protein loading and staining, the average of the normalized volume percentage of each spot for n=6 patients in each group and n=6 controls were used to calculate the EF of differentially expressed proteins.

MALDI TOF analysis of proteins differentially expressed in sepsis Survivors and Non-Survivors:

Spot #	Protein identified in MALDI-TOF	PI	Mol.Wt (K Da)	Mascot Score	SWISS PORT Accession number and ID	Function/Attributes
F 3	Alpha 1 Acid Glycoprotein	4.9	49	65	P02763 A1AG1_HUMAN,	Acute phase protein, Elevated in inflammation.
237	Transthyretin (Prealbumin)	5.5	15	66	P02766 TTHY_HUMAN,	Negative acute phase Protein
246	S100A9	5.7	13	70	P06702 S10A9_HUMAN	Prominent role in regulation of Inflammatory response
287	Hemoglobin subunit beta	7.6	14	170	HBB_HUMAN	Coagulation and Complement Pathway

Identification of stage specific differentially expressed serum proteins

S.No	Protein ID	control	Surv early stage	Surv late stage	Non-Surv Early stage	Non-Surv Late stage
1.	Hp (45kda) Haptoglobin					
2.	Hp (20kda)					
3.	S100A9					

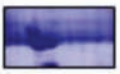
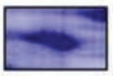
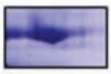
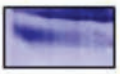
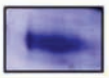
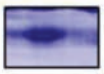
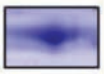
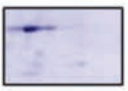
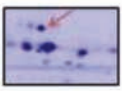
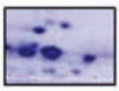
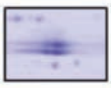
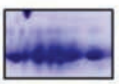
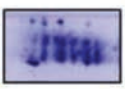
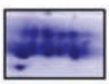
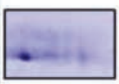
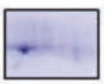
S.no	Protein ID	control	Surv early stage	Surv late stage	Non-Surv Early stage	Non-Surv Late stage
1.	(ORM) Alpha 1 acid glycoprotein					
2.	(A1AT) Alpha 1 anti-trypsin					
3.	(TTY) Transthyreti n					
4.	Immunoglob ulin's					

Table 1: MALDI TOF analysis of Proteins differentially expressed in sepsis Survivors and Non-Survivors

The NS:S ratio was calculated to demonstrate the fold change in protein expression in NS compared with S from onset (day of diagnosis) until recovery/mortality, and early stages of sepsis 24 h day 3 (Table IV). Haptoglobin (Hp; spot 229, P were analyzed, where gene ontology slim analysis

data of differentially expressed proteins in sepsis were obtained using mappings from the gene ontology (GO) consortium website (http://geneontology.org).

These proteins were identified to possess mostly cytoplasmic (23.5%) and extracellular functions (35.2%). Their biological functions predominantly involved the regulation of biological processes (14.6%), response to stimulus (14.6%), defence response (12.1%) and transport (12.1%). The present study sought to identify any possible network interactions between identified proteins using GeneMANIA 3.1.2.6 software (http://www.genemania.org) at the genomic and proteomic level, where GO based weighting was applied to detect maximum connectivity between the input genes, based on their biological process, molecular func tion and cellular component based function. All six proteins, S100A9, SAA, Hp, TTR, SERPINA1, and ORM, were shown to be interacting closely in a protein interaction network, indicating co expression of 91.34% and co localization of 8.66%, where expression was calculated as the Pearson correlation coefficient (Table 1).

Analysis of mRNA expression levels.

RT qPCR analysis of the mRNA isolated from whole blood of six patients revealed a ≥ 2 fold increase in mRNA expression levels (NS:S fold change) of acute phase proteins, S100A9 (2.13), TTR (2.86), SAA (1.84), A1AT (1.4), ORM1 (1.68; P≤ 0.05) and inflammatory markers, interleukin (IL) 6 (2.5), IL 10 (1.70), prepronociceptin (PPN; 1.6; all P≤ 0.0001 during early stages). By contrast, Hp (0.59) and Toll like receptor 4 TLR4 (0.30) exhibited decreased levels during early stages in NS (Fig 1). Hp (protein, 0.59; mRNA, 0.62; P≤ 0.05) and TTR (protein, 3.9; mRNA, 2.86; P≤ 0.05) showed a correlation between protein and mRNA expression levels during the early stages, whereas the other genes exhibited no significant correlation.

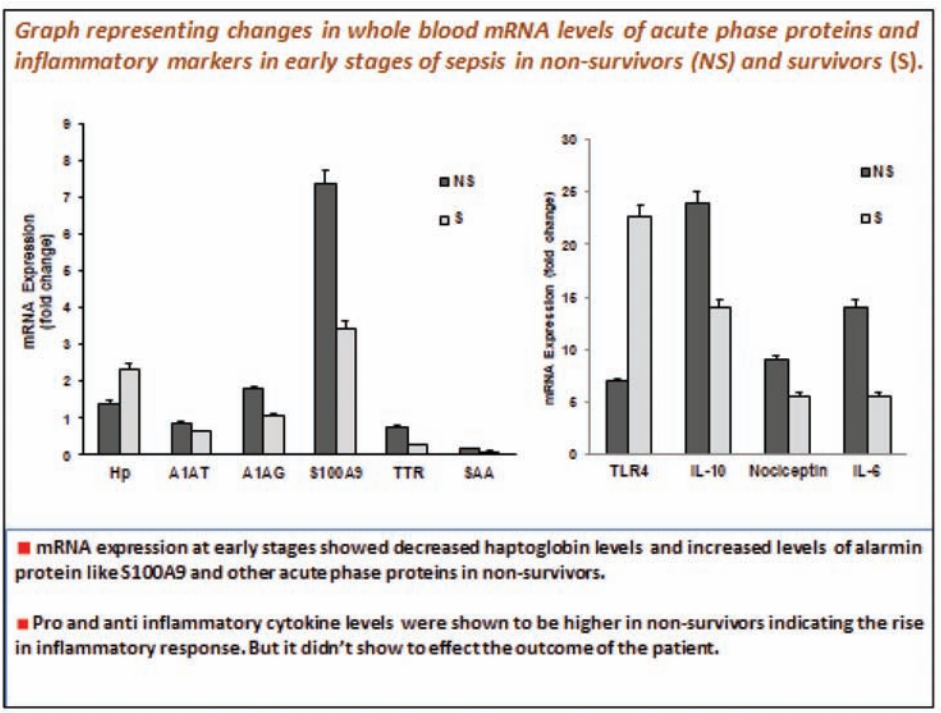


Figure 2

The present study provided a brief insight into possible differential expression and correlation among the proteins involved in sepsis in terms of survivability. It is important to have a laboratory method which is rapid, specific and sensitive enough to predict sepsis mortality at an early stage. A biomarker based algorithm would become predictive if a population specific genetic marker, which predisposes the patients to sepsis, is identified.

Haptoglobin improves acute phase response and endotoxin tolerance in response to bacterial LPS

Sepsis is characterized by delayed acute phase response and lowered immune tolerance in patients. Acute phase serum proteins, like Haptoglobin (Hp), have been associated with increased mortality in bacteria mediated acute lung inflammation and sepsis in neonates. However, it's direct role in modulating the immune response by regulating pro-inflammatory mediators leading to immune tolerant state and if gender affects its expression levels during bacterial infection, especially in blood has not been fully explored. To understand its specific role in endotoxin-mediated immune response, we investigated the correlation between the rise in Hp levels on bacterial infection and its influence on the expression of pro-inflammatory mediators in male and female Whole blood (WHB) and PBMCs. Here, we observed pathogen-specific and gender-specific expression of Hp. Gonadal steroid hormones differentially influenced the Hp expression in LPS-induced WHB, where the addition of Estrogen increased Hp expression, with suppression of TNF_, in both genders. Further on evaluating, the influence of Hp on TNF_ expression in endotoxin tolerance (ET), we show that increased Hp levels directly reduced TNF_ expression in ET models. Interestingly, blockade of secreted Hp significantly reversed the (ET) state, confirmed by a significant rise in TNF_ expression in both ex vivo and in vitro ET models, indicating a possible feedback inhibition by Hp on inflammatory mediators like TNF_.

Findings

Pathogen-specific expression of Hp; The mRNA expression of anti-inflammatory (Hp) and pro-inflammatory mediators (IL-6, S100A9) in whole blood (WHB) upon stimulation with different bacterial LPS were measured to study the possible relationship between disease severity and the cytokine-inducing capacities of these strains. The pattern of Hp expression showed its levels to be elevated in response to K. pneumoniae > E. coli > S. typhi > P. mirabilis - P. aeruginosa, in this order. Hp expression levels were observed to be maintained high up to 12-24 h in response to K. pneumoniae LPS when compared to other strains (Data not shown).

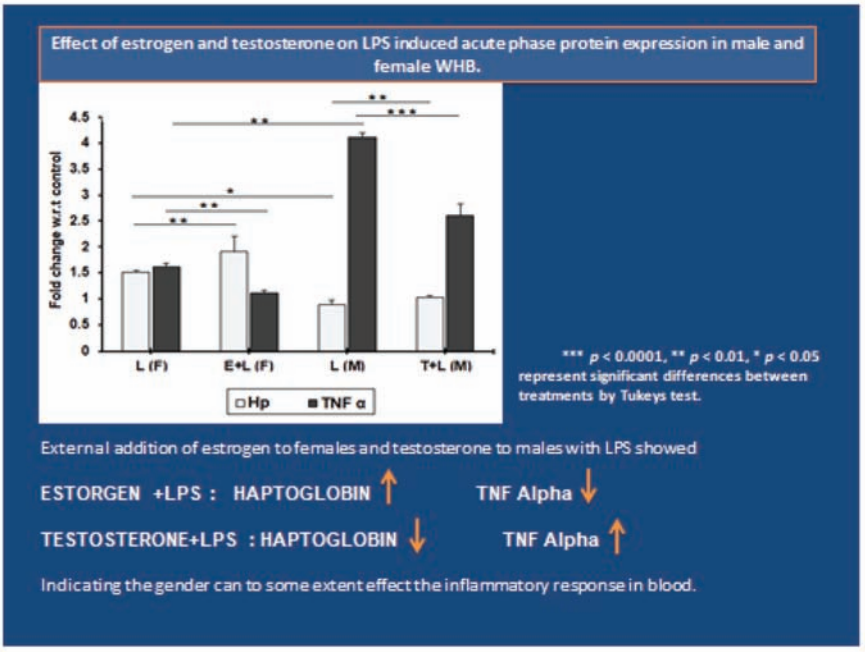


Figure 3

Gonadal steroid hormones tend to regulate the Hp expression in whole blood (WHB).

LPS induced Hp expression on exposure to external estrogen in female WHB and testosterone in male WHB was investigated; mRNA analysis showed that addition of estrogen increased Hp expression (2 fold, $p < 0.01$) in female WHB in presence of LPS. However, TNF_ expression in female WHB in presence of LPS (1.6 fold, $p < 0.01$) was lowered on estrogen exposure (1.1 ± 0.2 fold, $p < 0.01$) (Fig 3). Opposite results were observed for testosterone, where the addition of testosterone to male WHB showed decreased Hp expression (0.9 ± 0.12 fold); with elevated TNF_ expression levels (2 ± 0.60 fold, $p < 0.01$). Immunoblotting performed for Hp and TNF_ protein in serum (Fig. 3) showed, ≥ 2 fold rise of Hp expression in female (175 ± 6.7% of control, $p < 0.001$) than in male (134 ± 5.61% of control, $p < 0.001$) samples on LPS stimuli when compared to control/ unstimulated blood. However, TNF_ expression levels were observed to be (1.5 fold) higher in male samples (Fig. 3). Estrogen pretreatment showed marked rise in Hp expression in females (187 ± 12.3% of control, $p < 0.001$). In males however, Hp levels remained same as in LPS treated samples (113 ± 4.2% of control), whereas TNF_ levels were significantly decreased in presence of estrogen in females (Hp vs. TNF_ - 79 ± 5.2% of control decrease in TNF_ levels) and males (Hp vs. TNF_ -27 ± 3.4% of control decrease). TNF_ was observed to be strongly expressed in testosterone pretreated male samples (136 ± 12% of control; $p < 0.001$), whereas in female samples no change was observed in this condition. However, the addition of LPS in presence of testosterone showed a decrease in Hp level in female (107 ± 9.5%) and male (68 ± 4.8%) WHB when compared to respective controls/ unstimulated blood in both male and female. Estrogen addition was observed to increase Hp levels with a decrease in TNF_ levels, whereas Testosterone addition showed decreased Hp levels with the rise in TNF_ levels in both male and female WHB on LPS stimuli (Fig 4).

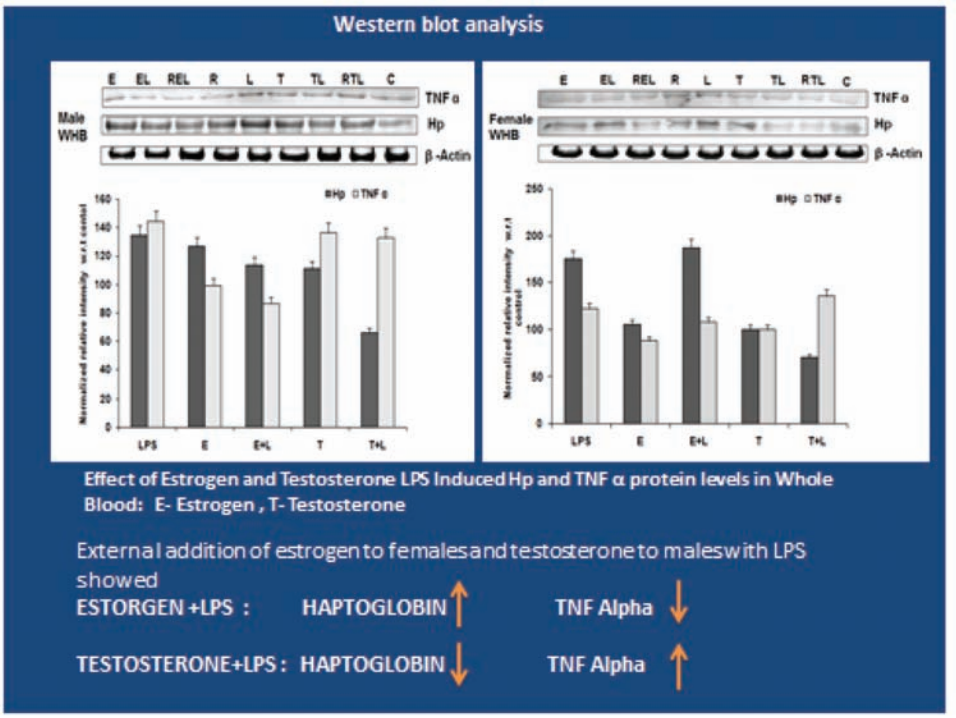


Figure 4 The Effect of Estrogen and Testosterone on LPS Induced Hp and TNF _ protein levels in Whole Blood.

Male and Female whole blood treated with LPS (K.pneumoniae) in presence / absence of 6 h pretreatment with Estrogen /Testosterone followed by LPS stimulation for 12 h were assessed in this experiment. Serum collected at the end of 12H was albumin depleted by modified TCA-Acetone precipitation and 20 μg of protein was subjected to SDS-PAGE. The expression of HP and TNF _ Protein was measured by using western blot analysis. Blot represents (1- Estrogen(E), 2- Estrogen+LPS (E+L), 5- LPS (L), 6- Testosterone (T), 7- Testosterone+LPS(T+L), 9- Control (Con). The graph shows relative HP and TNF _ Protein levels normalized to _ - actin. Results indicate high levels of Hp in females than in males and rise in presence of estrogen on LPS stimulation.

Endotoxin tolerance models show increased Hp expression:

We studied the expression of Hp during endotoxin tolerant state in HepG2 cells and PBMCs. Hp expression was observed to increase in LPS (2 ng + 100 ng) primed cells by 1.5 fold, while TNF_ expression remained unchanged when compared to unstimulated or control cells. Whereas unprimed cells (100 ng LPS) showed ≥ 2.2 fold elevation in the expression of TNF_ with fall in Hp levels by ≤ 0.86 fold when compared to unstimulated or control cells ($p < 0.01$) (Fig. 5). Expression pattern was observed to be similar in PBMC where LPS primed cells (2 ng + 100 ng) showed ≥ 1.7 fold rise in Hp expression associated with fall in TNF_ levels by ≤ 0.85 when compared to control cells ($p < 0.001$) (Fig. 5). Higher Hp expression correlated with low TNF_ expression in LPS primed cells when compared to unprimed cells in both the cell types during endotoxin tolerance.

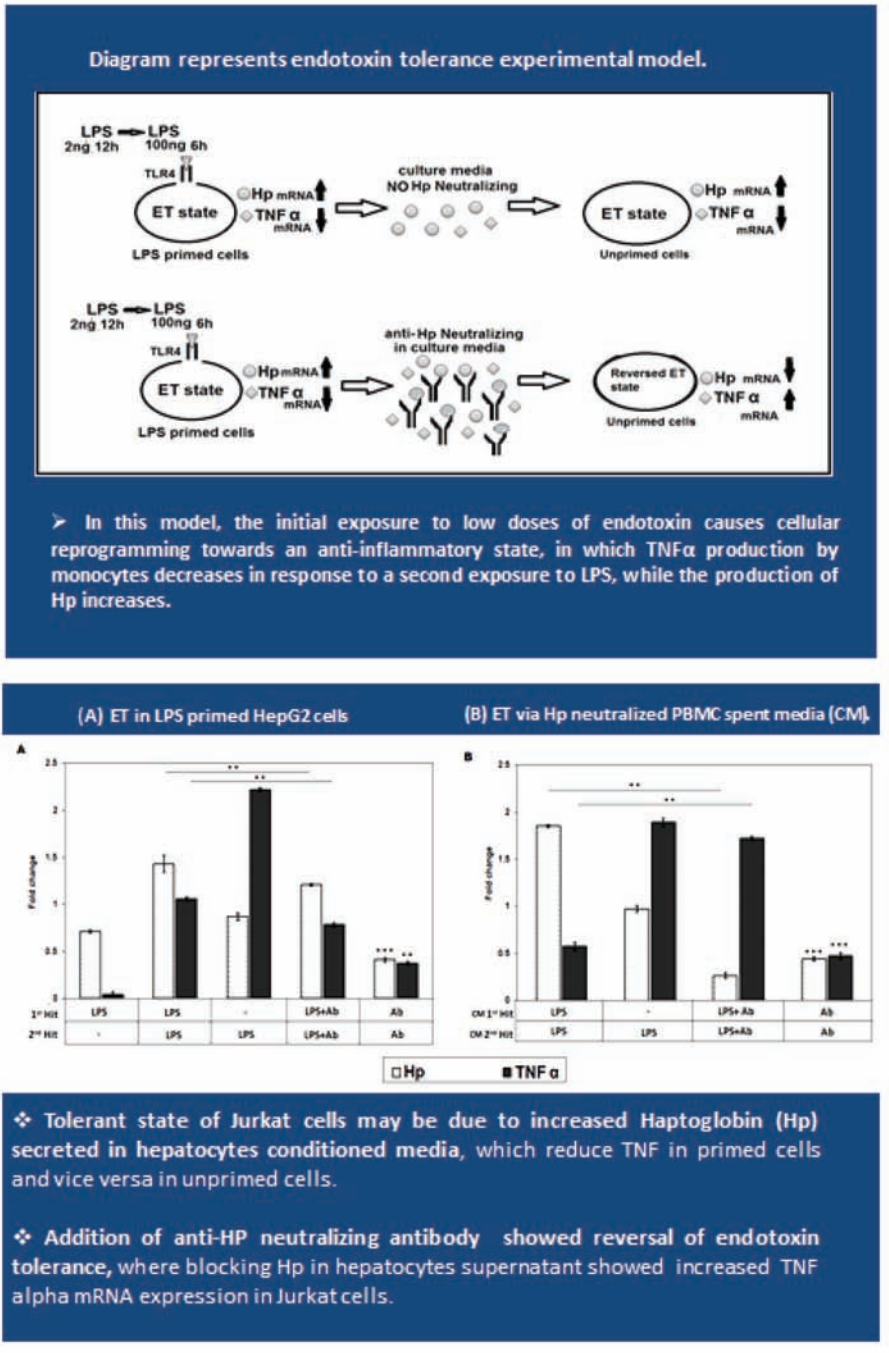


Figure 5

Blocking secreted Hp in LPS treated conditioned media reversed the endotoxin tolerant state

We investigated if the conditioned media from the endotoxin tolerance treated cells were able to produce the ET state in cells without priming them with LPS. When the conditioned media from HepG2 cells was added to Jurkat T cells, we observed that unprimed cells directly entered ET state,

which was shown by ≥ 2 fold rise in Hp levels, with fall in TNF_ levels by ≤ 0.60 in conditioned media from the primed cells (Fig. 3). PBMC too showed a similar pattern where high Hp levels (≥ 2 fold) were associated with low TNF_ (≤ 0.70) in primed cells when compared to the unstimulated or control cells ($p < 0.0001$). Whereas unprimed cells (100 ng) showed ≥ 3 fold rise in TNF_ levels ($p < 0.001$) (Fig. 6). Secreted Hp in the conditioned media was blocked by anti-Hp antibody added during treatment and/ or during incubation. Blocking of Hp did not seem to have much effect on Hp and/ or TNF _ expression when anti-Hp-Antibody was added during treatment to HepG2 and PBMC (first treatment) (Fig 6). However when the Hp neutralized conditioned media from first treatment was added to Jurkat cells and PBMCs (second Treatment), TNF_ expression was observed to rise ≥ 2 fold in Jurkat cells and ≥ 3 fold in PBMC. Neutralization/ blocking of Hp in conditioned media from endotoxin-tolerant cells showed a rise in TNF_ levels indicating the reversal of tolerant state in both Jurkat cells and PBMCs. Hp may thus play a role in maintaining endotoxin tolerant state.

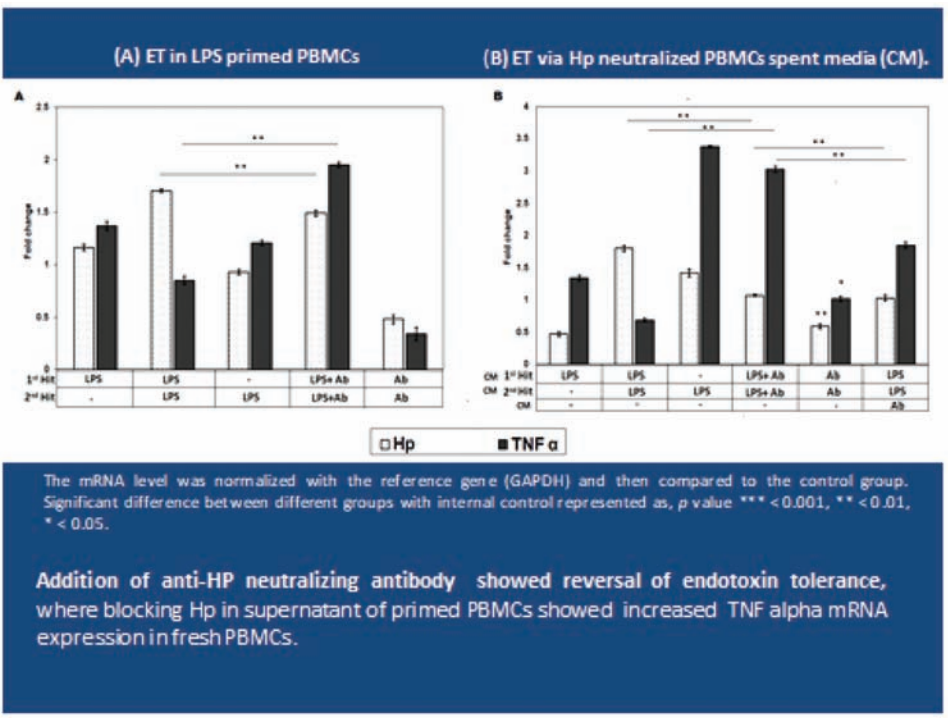


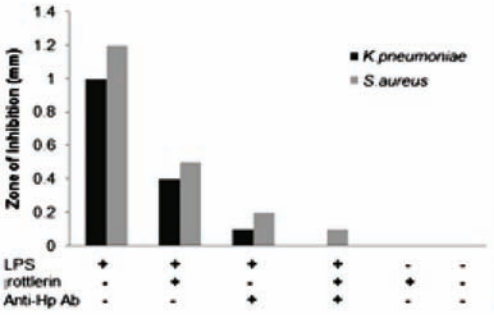
Figure 6

Effect of PKC_ inhibitor rottlerin on LPS stimulated Hp mRNA and protein expression in whole blood

We investigated changes in Hp, IL-6 and PKC_ (PKCD) levels in WHB when treated with rottlerin in presence/ absence of LPS (Fig. 7). We observed that pretreatment of WHB with rottlerin for 1 h followed by LPS (K. pneumoniae and E. coli) showed a decrease in Hp, IL-6 and PKC_ gene expression after 12 h when compared to only LPS treated WHB ($p < 0.005$). Rottlerin decreased the Hp expression in presence of both K. pneumoniae and E. coli LPS in a time-dependent manner. However, as the expression was higher in presence of E. coli LPS, hence the decrease in the expression of all genes in presence of rottlerin was observed more clearly on E. coli LPS treatment when compared to K. pneumoniae LPS. Culture supernatants of PBMC subjected to western blot for Hp showed decreased secretion of Hp on rottlerin pretreatment in presence of LPS in media (Fig. 7B). Western blotting of LPS treated WHB serum showed two fragments of PKC_- 65-72 kDa and 40-45 kDa catalytic

fragment (CF unit) (Fig. 8). The CF unit has been associated to be formed on cleavage of PKC_ by Caspase 3 in response to the apoptotic signal. The CF unit was observed in LPS treated serum, whereas rottlerin treated serum showed the absence of CF unit, indicating that rottlerin inhibits PKC_, by inhibiting the cleavage of CF unit. This may be also related to decreased expression of Hp and pro-inflammatory mediator IL-6 in presence of rottlerin.

Z one of Inhibition (mm) – Treated antisera											
S.No	Pathogen	LPS (100 ng)	LPS + Hp Ab	ROT+ LPS	ROT + LPS + Hp Ab	ROT	Control	LPS (100 ng)	LPS + anti-Hp Ab	LPS + anti-1.2Ngal Ab	LPS + anti-β - actin Ab
1	K.pneumoniae	1	0.1	0.4	No zone	No zone	No zone	1	0.1	NA	NA
2	S.aureus	1.2	0.2	0.5	0.1	No zone	No zone	1.2	0.2	1	1.2



- Results indicate that Hp is one of the major anti-microbial proteins which are secreted in serum on LPS stimulation.
- Hp is partially inhibited by PKCδ inhibitor rottlerin, shown by complete restoration of Gram-negative and partially Gram-positive bacterial growth on blocking Hp.

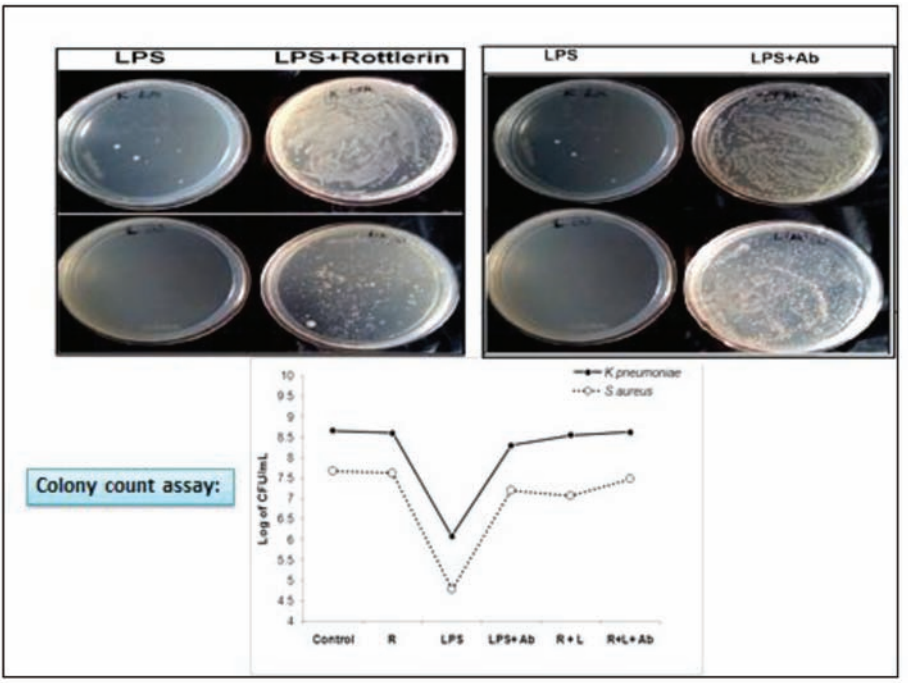


Figure 7

Inhibition of PKC _ and blocking of Hp reduces the anti-microbial activity of serum

LPS treated serum was shown to reduce the bacterial growth, indicated by 1 mm zone of inhibition in K. pneumoniae and 1.2 mm in S. aureus (Fig. 7). However, rottlerin pretreated WHB serum restored bacterial growth shown by reduction in the zone of inhibition from 1 mm (LPS) to 0.4 mm (rottlerin + LPS) for K. pneumoniae and from 1.2 mm (LPS) to 0.5 mm (rottlerin + LPS) for S. aureus. Rottlerin is hence indirectly resulting in the decreased anti-microbial activity of serum, as observed by lower Hp levels. To demonstrate the anti-microbial activity of Hp, secreted Hp was neutralized in the stimulated serum by the anti-Hp antibody, which restored the bacterial growth from 1 mm zone of inhibition (LPS) to 0.1 mm (after neutralizing Hp) and 0.4 mm (rottlerin + LPS) to no zone or complete restoration of K. pneumoniae bacterial growth. Indicating that rottlerin only partially inhibits Hp secretion. Similar results were observed for S. aureus; however, blocking of Hp did not completely restore S. aureus growth. Further, for control experiments incubation of serum with anti- _-Actin antibody and anti- NGAL (Lipocalin) antibody showed that NGAL which is also a known anti-microbial protein was observed to inhibit bacterial growth shown by 1 mm zone of inhibition when compared to _-Actin shown by 1.2 mm zone of inhibition. However, when compared to Hp blocking, both _-Actin (1.2 mm) and NGAL (1 mm), showed minimal / no effect on the antibacterial activity of LPS treated serum (Table 2). These observations were further confirmed by Resazurin dye-based assay (6B) and agar plate colony counting (Fig. 7), where A570/600 values showed lower % reduction of Resazurin (blue) to Resorufin (pink) indicating negative microbial growth or non-viable cells as observed for LPS (15 ± 2.4% reduction) and heat-killed S. aureus (14% reduction) treated sera. This was confirmed by plating the treated cultures, which also showed a decreased number of colonies for K. pneumoniae (Log 6 CFU/ mL) and S. aureus (Log 4.8 CFU/ mL) in presence of LPS treated sera when compared to untreated cultures (Log 7.6-8.7 CFU/mL). Rottlerin pretreated sera indicated increased reduction to Resorufin, for K. pneumoniae (21 ± 3.2%) and S. aureus (63 ± 4.21), showing a rise in the bacterial viability. Increased colony count by Log 6.9 CFU/mL for S. aureus and Log 8.4 CFU/mL for K. pneumoniae was observed on the plates for rottlerin pretreated sera, indicating that rottlerin indeed inhibits secretion of certain anti-bacterial proteins in presence of LPS. Conversely, rottlerin pretreatment did not affect the viability for both K. pneumoniae and S. aureus in presence of serum treated with heat-killed S. aureus indicated by the blue color of Resazurin. Further neutralization of secreted Hp showed restored bacterial growth in both K. pneumoniae (LPS + anti-Hp Ab - 46 ± 1.19%, LPS + rottlerin + anti- Hp Ab - 91 ± 2.87%) and S. aureus (LPS + anti-Hp Ab - 63 ± 2.4%, LPS + rottlerin + anti- Hp Ab - 90 ± 6.9%) indicated by pink color of Resorufin and increased number of colonies on plating by Log 8.2 CFU/mL for K. pneumoniaeand Log 7.1 CFU/mL for S. aureus. Results thus confirm that PKC_ plays a major role in regulating secretion of anti-microbial proteins in serum. Where Hp is one of the major anti-microbial proteins secreted in the serum on LPS stimulation, shown by complete restoration of gram-negative and partially, gram-positive bacterial growth on blocking Hp in sera. This response is conspicuous in case of LPS rather than heat killed S.aureus.

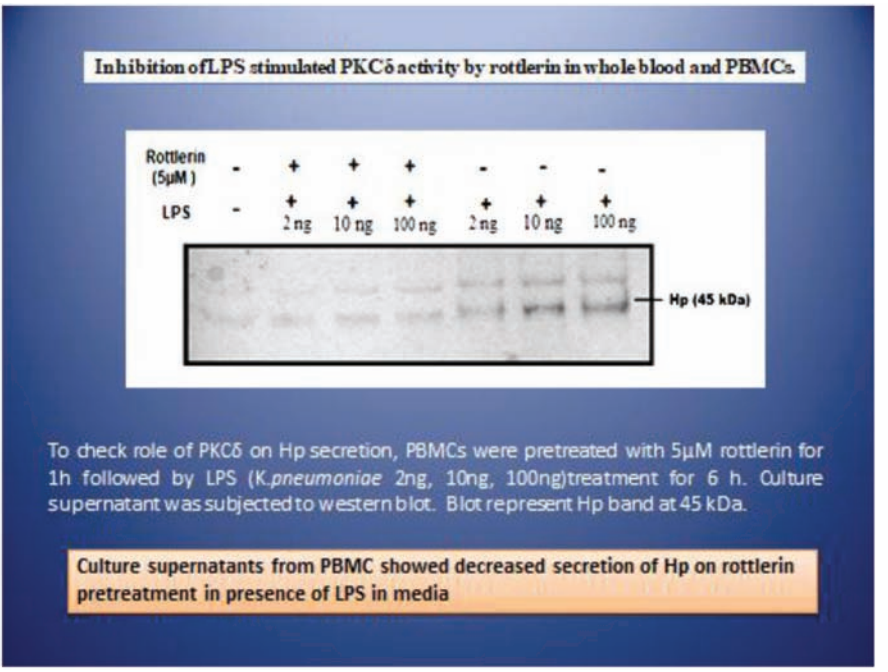


Figure 8

In conclusion, we provide evidence that haptoglobin (Hp) plays a crucial role in balancing the inflammatory response in sepsis by preventing bacterial proliferation and reducing inflammation. We observed higher Hp expression in female blood when compared to male blood on LPS stimulation. This seems to be regulated mainly by estrogen; supporting the gender-specific expression of Hp. PKC_ was shown to regulate the secretion of anti-microbial serum proteins partly including Hp, where its inhibition by rottlerin restored bacterial growth. Also, Hp promotes endotoxin tolerance, as blocking of Hp results in reversal of endotoxin tolerant state both in ex vivo and in vitro conditions, shown by rise in TNF_ levels. These results correlate with our previous patient data, where we observed that non-survivors of sepsis showed lower levels of Hp from onset to death. Overall Hp may prove to be a good prognostic sepsis marker to identify potential nonsurvivors among sepsis patients. This needs to be further validated by individualized trials.

IMPORTANCE OF IPR FILING AND BEST PRACTICES

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Introduction:

Intellectual Property has become a key consideration in day-to-day business decisions. Intellectual Property Rights (IPR) is an essential tool for achieving competitiveness in today's business environment. The term Intellectual Property and IP Management is increasingly in use today but still little understood. The concept of IP management needs to be addressed and demystified on a broader platform among Industry and MSME's in India. Still the mindset in many of the enterprises is that "Intellectual property is for High tech companies and only related to patents". This acts as a huge barrier in the path of development for the Indian MSME's.

Several products and processes developed by Industry and enterprises embody different types of Intellectual Property (Patents, trademarks, designs, copyrights, trade secrets etc.). Companies need to dedicate resources and time for Creation, Protection and Management of Intellectual Property in order to achieve & sustain competitiveness in the marketplace.

IP Management is a very broad aspect which covers all facets like innovation, technology, marketing, finance, corporate governance, industrial economics, and strategy. It's a collective effort to manage IPR of a particular firm or Industry to leverage it for competitiveness. It's a very important portfolio in today's corporate world and has challenges and opportunities like Human Resource Management.

IP management education and research is in a nascent phase in our country. Most business schools and faculties of management science have no IP management education and research programs. Furthermore, there is presently an acute shortage of resources (text books and case studies) and resource persons (lecturers and professors) on this subject. Although the number of research papers presented at various conferences, workshops and meetings have been steadily increasing over the past years but there is still much work to be done to integrate IP as part of mainstream management discipline.

It is very important to align IP with management and make an effort to manage one's IP effectively to get commercial viability. IP management has now emerged as a "Corporate mantra for Success"

Nations have realized the power of IPR and effective IP management can lead to a good economic growth.

Why does Enterprise need IP Management?

A Quote from Josh Billings will be the best to understand why IPR's and IP management is so important.

"Necessity is the mother of invention, but IP right is the father."

This says it all. A firm having inventions, but no IPR and IP management is like a fatherless child struggling for existence.

Imagine a Nation with an effective and latest state of the art -weapon but not able to use it in an effective way for defense is as good as not having one, and moreover the Nation always seems to be unprotected, same is the case of IP management. It is not enough to have IPR but also it is equally

important to learn to use and manage one's IP is such a way that it can give us that cutting edge over others and generate revenue.

It is important that there are proper mechanisms to identify inventions to finally convert them into tangible assets for Enterprises. This mechanism does not come overnight or by heredity to companies. It has to be triggered and is only possible in an environment where an IP culture exists and is slowly built in.

In a country like India where MSME's form the backbone of Indian Industry, the need to manage IPR's effectively is a challenge. MSME's need to respect IPR and manage them effectively to become competitive.

Intellectual Property management means not only protecting a company's Intellectual Property. It involves, in addition to protecting companies' inventions, a company's ability to commercialize its inventions, market its brands, license the know-hows, transfer technologies, conclude JVs and monitor & enforce IPRs. Leading companies and organizations worldwide increasingly consider Intellectual Property management as part of overall business plan and use it effectively in driving the business strategies. The same should be incorporated with time in the Indian Industry and MSME'S to be a Global Leader.

The World Intellectual Property Organization (WIPO) believes that IP is native to all nations and relevant in all cultures, and that it has contributed to the progress of societies.

A case in Example:

IP management helps in establishing an IP culture of identifying and harnessing IP assets and strategically using them, an enterprise can increase its revenue, have an edge over its competitors and position itself well in the market; these are strategies of IP management that may lead to an increased market value of the company.

XYZ, a MSME based out of Hyderabad was in to electronics and instrumentation business for the last 60 years. Over the years they noticed that the market share of their products was decreasing as there were many other players coming into the market. They realized that to stand strong and build goodwill they need to be more innovative and be able to protect their innovations and brands in the market and bring in an **"IP Culture"** in the company. The enterprise then learnt about IPR and IP management. The enterprise chalked out a plan to align IP management into their business. A year after the enterprise had a full-fledged IP management team of its own with a good IP policy in place. IP management helped the company to churn out a lot of products/ process brands and designs that qualified for IPR protection which they were unaware of for a long time. This portfolio of IPR's helped the company to enter into licensing agreement with a US based company and earn a lot of revenue. The enterprise was also able to draw a lot of investors than before. The MSME now claims that IP management has changed the outlook of their enterprise and the way of doing business.

A Road Map for IP Management in Enterprise:

A series of events/happenings lead to the formation of an In-house IP Management Team with an effective IP Management strategy to manage the IPR's of the firm. Different enterprises have different IP management strategy, but the basics remain the same.

The three pillars of IP Management.

IP Promotion, Identification & Protection. These are broadly the three pillars which build a platform of channelising IP into Business which is "IP management".

Let us understand how to carry out an effective IP management:

1. IP Promotion:

It's very important to spread awareness on IPR in the organization in a big way. Everybody in the firm /organization should know about IPR and how it can be useful for the organization. An IP culture needs to be brought in slowly in a phased manner in the organization to take the first step towards IP management.

The firm can hire IP professionals to carry out awareness workshops, training programmes and sensitization programmes for different levels of employees and all the departments.

The important departments which have to be covered are the R&D, Finance and Production of any firm. These three are very much required in forming an in-house IP management Team. It is always preferred that there is representation from these departments in all the IP activities taking place in the organization.

IP promotion in enterprises will bring in IP culture and slowly people in the organization at all levels will be aware of IPR and also understand the need and importance it can be to their existence in the market.

The firm can now select a team of 4-5 employees to form the In-House IP management team. This team will now be trained in all aspects of IPR, with specific modules and intensive focus-based training by IP experts. At the end of this exercise the team is well trained and conversant in IPR and IP issues.

2. Identification:

Every firm /corporate or any MSME will have several products or process, designs, brands which can be considered as an Intellectual asset. These assets need to be first identified as whether they can be brought under the umbrella of IP protection.

To identify one's assets it's very important for the enterprise to carry out an IP Audit. IP audit is very similar to the general audit which a firm carries out every year. IP audit helps the firm to list out all the technologies, brands, and designs which they are in possession and developed over time.

The firm can scan the market and the technologies available and compare them with theirs. This comparison will help the firm to know their stand in the market and thus help to come out with the list of technologies/brands or designs which are new and innovative and can be taken up for IPR protection.

Documentation: Records to be maintained

This is the crux of any IP management. An effective documentation of technologies/processes is a must to bring the ideas/technologies /designs into a tangible form which is needed for IPR protection. It is always advisable that a firm must document all happenings in R&D and production as you never know when it can act as a blessing in disguise to save the firm from litigations to prove itself the rightful owner of the Innovation against the infringers.

IP Protection or IP Valuation: Which needs to be done First?

Many Companies still debate on this as to whether first valuate your IP and then go for protection or to first protect it and then go for valuation to put a tag to it. The answer to this question can be both or we can say strategic. Few Companies look to first do an IP valuation of the brand, technology to analyze what would be the market and then go for protection. If they feel that there will be no market, then why go for protection? But some companies feel the other way round that in this competitive world it's very important to first get yourself protected and then go for the valuation.

3. IP Protection:

All the technologies identified from the audit and documented will be put forward for patent filings by the IP management team. All the brands and promotional material of the firm will qualify for Trademarks and Copyright protection.

The innovative designs of the firm will be protected by filing Industrial designs. Once these are filed the firm can have portfolios in Patents, TM, and Design. This builds the image of the firm and helps itself to be recognized in the market. This will give an edge to the firm to look at technology transfer, licensing, joint ventures and a host of other agreements. All IPR's of the firm must be filed at the Patent, TM and Designs Office. If the firm wishes to go for protection outside one's own country, then it should file the applications separately as per the law of that country. The decision to file depends on the Firm's interest in marketing the products in different countries.

Also, it is very important to note that a single product or service may be protected by various forms of IP rights covering different aspects of that product or service. So, a Firm must consider the best protection package and make sure that all the formal rights are acquired as early as possible but at the same time they should also bear in mind that creating a comprehensive IP portfolio may be a considerable investment. This is particularly the case for patents.

The IP management team of the firm must therefore carefully assess the costs and benefits of patenting on a case-by-case basis and develop a strategy/policy on patent/IPR acquisitions which is appropriate given their budget and market opportunities.

A case in Example:

ABC, a pharma company based out of Hyderabad was doing well in the pharma business and mostly into contract manufacturing and research. The MD of the company once got the opportunity to participate in an IPR workshop and that helped him to understand the need for companies to be innovative and generate their own IPR'S.

The company was keen to explore the new avenue of IPR and opened the doors for IPR learning and awareness. The company now understood the importance of generating self IPR's and the return on investment attached to it.

This triggered the minds of the researchers in the company, they started to develop their own process to synthesize intermediate and Active Pharma Ingredients. The Company had an idea on the processes which presently exist by doing a patent search and how different was their process.

They came out with many new processes based on the analysis and now they were ready to take it up for IP Protection.

The company with the help of IP experts drafted the patent applications and filed it in India. After a while they realized that there is a huge market potential for these processes in the African and American Countries and getting an IP protection in those countries will help them to generate more revenue and, they can look out for joint ventures or any technology transfers.

The ABC Company now selected a few innovations which had a huge market potential in Abroad and decided to file a PCT -International filing of Patent applications under the Patent Cooperation Treaty. The applications were filed for PCT and then within 4-5 months they had got a good search report and written opinion that their invention and process is indeed a novel one and very economical.

Then after 18 months the application went in for international publication and this was the crux as it was like an open advertisement to the world. Based on this Publication the company started getting enquiries by interested parties to take this invention /process and work together to make a good drug,

It was now for the ABC to decide whether to go on their own or have a joint venture. ABC deiced to do a joint venture with a European Pharma company where in the patent rights was assigned to the Foreign Company and the ABC had certain rights in the invention based on the agreement and worked out the royalties and other necessary clauses.

With in a span of 3-4 Years this company which was into contract manufacturing and Research had taken a complete U turn and brought in IP Culture in their business. This IP culture helped the company to boost its revenues and profit margins.

ABC pharma now had a new outlook and this was all possible because of good in time IP management practice and at the end of the day they learnt how to align IP with management and incorporate IPR in to their day-to-day business.

Indian MSME's have huge potential and the need is to learn how to leverage this potential by using IP management as one of the tools to be competitive and come out successful.

IP Valuation:

It's one of the most Herculean tasks for any firm to take up. It is like putting value to your brand or technology or design before launching it into the market. You never know what the best price for your brand /technology will be to launch in the market to be a leader in that product.

It must be a very elaborate and vital exercise. A firm needs to analyze the time, money and effort put in to develop such a product and then put the price on it. Here comes the role of the R&D, Production and Finance department to sit together and give their inputs and help the IP valuation expert to do the valuation.

IP valuation in our country is in a very nascent phase. There is a severe dearth of IP valuation experts who can do a good job of valuating a company's IP. Slowly and steadily IP valuation is picking up momentum in India.IP valuation expertise for an enterprise comes with time and experience.

These conventional methods are followed for IP Valuation:

Cost Based Approach: This takes into consideration the cost incurred for replacing or reproducing the new technology/brand.

Market Based Approach: Comparing the competitive products in the market and then valuating your own brand.

Economic Analysis: This is to see the future profit streams. It takes a lot of factors into consideration from demand, supply, profit, consumers, marketing, manufacturing, sales. This analysis is very cumbersome but a good and reliable one to evaluate one's IP. So once the IP valuation is over.

The enterprise must now look at exploiting its IP assets to generate Return on Investments.

IP Exploitation:

The IP assets may now be exploited in a variety of ways. These may include the commercialization of IP-protected products and services by the firm, entering into licensing or franchising agreements; the sale of IP assets to other firms; the creation of joint ventures; the use of IP to obtain access to other companies' technology through cross-licensing agreements; or the use of IP to obtain business finance. Enterprises should decide in each case how they may best exploit their IP assets both domestically and internationally.

It's very important to exploit one's IP in a proper way and a firm having good IP management strategy will be able to do it.

IP Enforcement:

A firm should keep a constant watch on the market and its competitors to enforce its IPR's in a proper way. The IP management team should try to curb infringement and piracy of its products and initiate legal actions against the other party.

Philips has a very interesting way of enforcing IP. Any employee traveling across the globe on work comes across a duplicate product anywhere must report it immediately to the company with his /her employee code and the product code along with the place where the duplicate product is being sold. In return he/she gets a reward from Philips.

MSME's can also follow such schemes to encourage IP enforcement.

IP Policy:

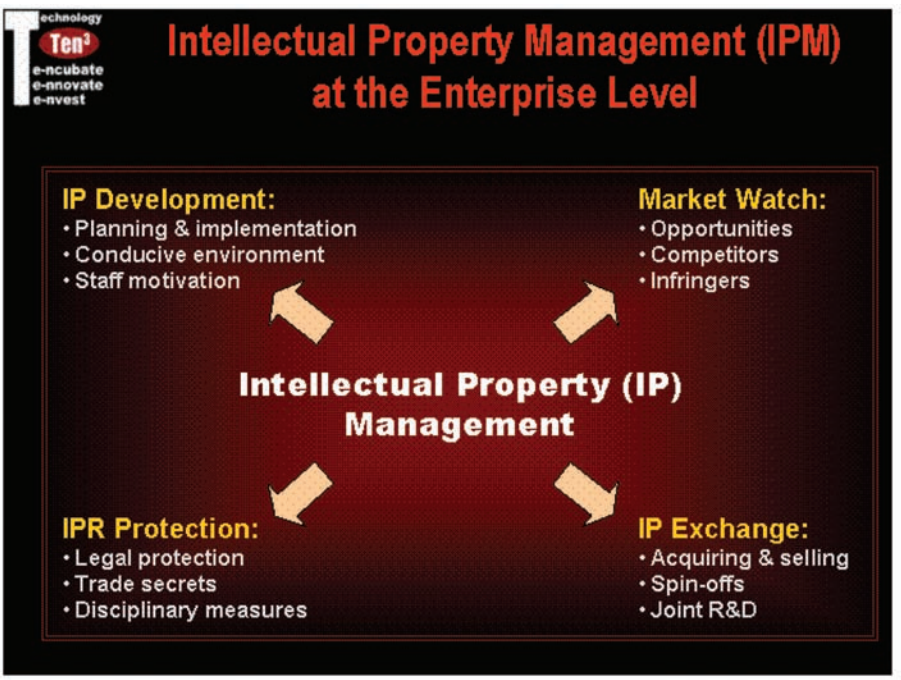
IP policy is a document which has to be drafted by the Firm to safeguard its own interest in the long run. The IP policy will have to take care of everybody's interest. It should be a comprehensive document which helps the employees and customers to understand, follow and respect the implications of IPR. A good IP policy is the base for good IP management. The IP policy makes one and all in the firm understand their role to develop, protect, preserve and exploit the firm's IP.

Few companies in India in their IP policy have a provision to give incentives /rewards to the inventors for fling patents/IPR's. Such culture needs to be imbibed in MSME's to become more innovative.

Once the IP policy is framed all employees from all departments of the organization need to see it, review it and if necessary, make amendments and reach a consensus. Once this is done everybody has to accept the IP policy of Enterprise and ensure to abide by it.

The IP management team implements the IP policy across the Organization. A firm should always be open to new and fresh ideas to be implemented in the IP policy from time to time.

The entire road map or the flow process of IP management is well represented in the below diagram:



Source: 1000 ventures.com

Conclusion:

A good IP management team with an effective IP strategy and good IP policy is the key to success for any enterprise. An enterprise should have a comprehensive IP strategy that flows into almost every aspect of day-to-day business.

The best way to follow IPM (Intellectual Property Management) in a nutshell is IPM itself. I= Identify P=Protect M=Manage.

To sum up let's have a checklist for an effective IP management for enterprise excellence:

Checklist:

- Conducting an IP audit may be a good first step to identify all your company's valuable information and to develop an IP strategy.
- Check IP databases to avoid using an existing IPR's before launching a new product or service or technology/brand in the market.
- Identify your IP's and make sure it is protected early enough to avoid losing the invention to competitors.
- Make sure that IPR's are not shared with others or published before filing an application.
- Make sure that trade secrets are kept within the enterprise and prepare appropriate confidentiality agreements while negotiating and sharing information with business partners to protect trade secrets.
- Use your IP portfolio as an effective tool to convince investors and generate revenue.
- When working on a joint project with other enterprises or research institutes, make sure that there is sufficient clarity on who will own potential IP generated from the research project. This is where the IP policy comes into play.
- Monitor the market and make sure that your IP assets are not being infringed. If violation of your IP rights is detected it may be advisable to seek legal support.

To conclude, Intellectual Property Management is more than just acquiring the formal IP rights through the national IP office. These rights are not worth much unless they are adequately exploited. Enterprises should be proactive and learn how to extract full value from their technology, brands, and designs to take adequate steps to develop an IP strategy.

BIOINFORMATIC APPLICATIONS IN ADVANCED RESEARCH

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INTRODUCTION:

An unprecedented wealth of biological data has been generated by the human genome project and sequencing projects in other organisms. The huge demand for analysis and interpretation of these data is being managed by the evolving science of "bioinformatics". It is the field of science in which biology, computer science and information technology merges to form a single discipline. Storage and analysis are the two important functions. It is the application of tools of computation and analysis to the capture and interpretation of biological data.

HISTORY:

The term "Bioinformatics" was initially coined by Ben Hesper and Paulien Hogewen in 1970 and defined as "the study of informatics processes in biotic systems". This definition placed bioinformatics as a field parallel to biochemistry. Computers became essential in molecular biology when protein sequences became available after Frederick Sanger determined the sequence of insulin in the early 1950s. Comparing multiple sequences manually turned out to be impractical. A pioneer in the field was Margaret Oakley Dayhoff.

The first major bioinformatics project was undertaken by Margaret Day Hoff in 1965, who developed a first protein sequence database called "Atlas of Protein Sequence and Structure".

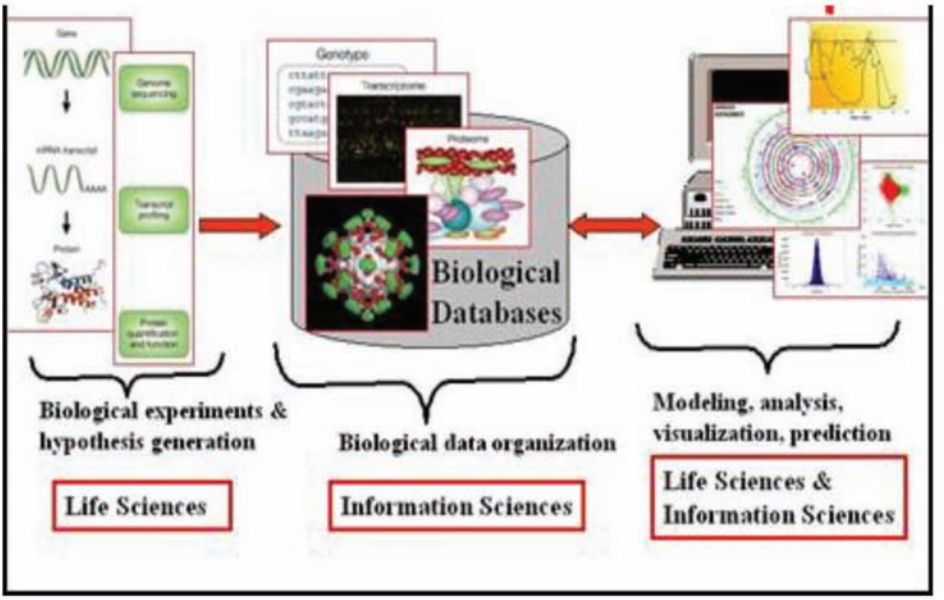
In 1970s the Brookhaven National Laboratory established the "Protein Data Bank" for archiving 3-D protein structures.

In 1980s, the establishment of GenBank and the development of fast database searching algorithms such as FASTA by William Pearson and BLAST by Stephen Altschul and S. Karlin.

OVERVIEW:

Biological data can be described as molecular sequence information and "wet-bench" experimented content of genome and gene product analyses. Being an interdisciplinary branch of the life sciences, bioinformatics targets to develop methodology and analysis tools to explore large volumes of biological data, helping to store, organize, systematize, annotate, visualize, query, mine, understand, and interpret complex data volumes. It harnesses computer science, mathematics, physics, and biology. It is essential for management of data in modern biology and medicine.

The ultimate goal of bioinformatics is to better understand a living cell and how it functions at molecular level.



BIOLOGICAL DATABASES:

A database is a computerized archive used to store and organize data in such a way that information can be retrieved easily via a variety of search criteria.

Based on their contents, biological databases can be roughly divided into three categories: "primary databases, secondary databases, and specialized databases".

- Primary databases contain original biological data that archive the raw sequence and structural data. for example, GenBank, European Molecular Biology Laboratory (EMBL), DNA Data bank of Japan (DDBJ), Protein Data Bank (PDB).
- Secondary databases contain computationally processed or manually curated information. For example, SWISS- Prot and Protein Information Resource (PIR).
- Specialized datagbase normally serve a specific research community nor focus on a particular organism. For example Flybase, WormBase, AceDB, TAIR and Microarray Gene Expression Database at European Bioinformatics Institue (EBI).

Prospects in this field include:

- To uncover the wealth of biological information hidden in the mass of sequence, structure, literature and biological data.
- It is a logical and technical means by which we can not only solve the biological problems but also can predict the new aspects.
- Its future contribution to functional understanding of the human genome, leading to enhanced discovery of drug targets and individualized therapy.
- Gain a better understanding of gene analysis, taxomony and evolution.
- In agriculture to produce high yield, low maintenance crops.
- Benefitting environment by identifying waste and clean up bacteria

APPLICATIONS

a. Structural Bioinformatics - Prediction of structure from sequence (Secondary structure)

b. Homology modeling- two or more sequences have common ancestor. Also knows as comparative modeling of protein, refers to constructing a model of target protein from its amino acids sequence and an experimental 3Dstructure of a related homologous protein

c. Analysis of 3D structure

d. Sequence Alignment (Comparative genomics) - Sequence comparison lies at the heart of bioinformatics analysis; it is an important first step towards structural and functional analysis of newly determined sequences. The fundamental process in this type of comparison is sequence alignment. This is the process by which sequences are compared by searching in common character patterns and establishing correspondence among related sequences. It can be pair wise sequence alignment (aligning 2 sequences) and multiple sequence alignment.

e. Functional bioinformatics- Prediction of function from structure

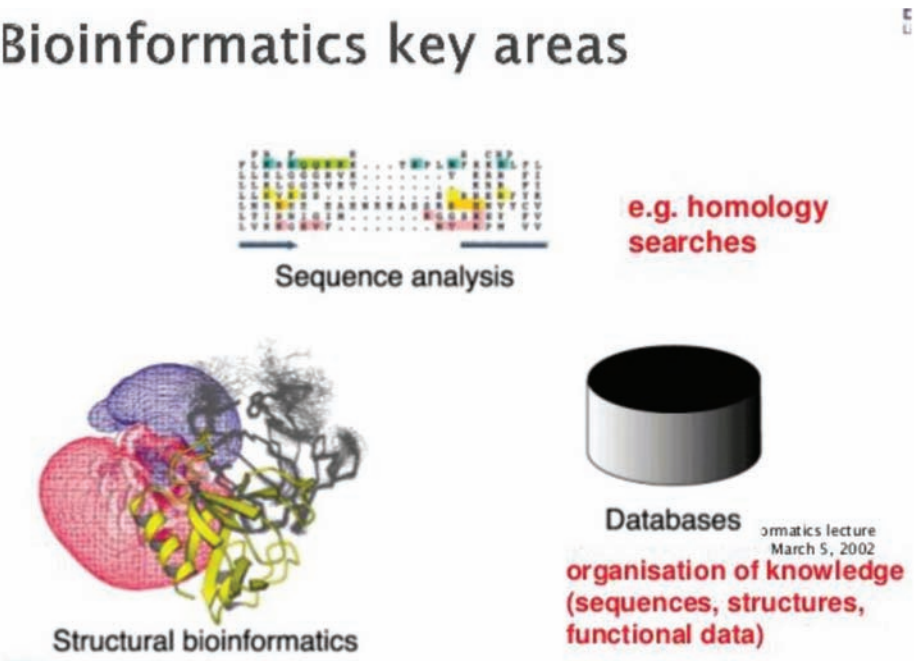
f. Molecular phylogenetics- is a fundamental aspect of bioinformatics. Similarities and divergence among related biological sequences revealed by sequence alignment are visualized in the context of phylogenetic trees.

g. Molecular mechanics/ molecular dynamics

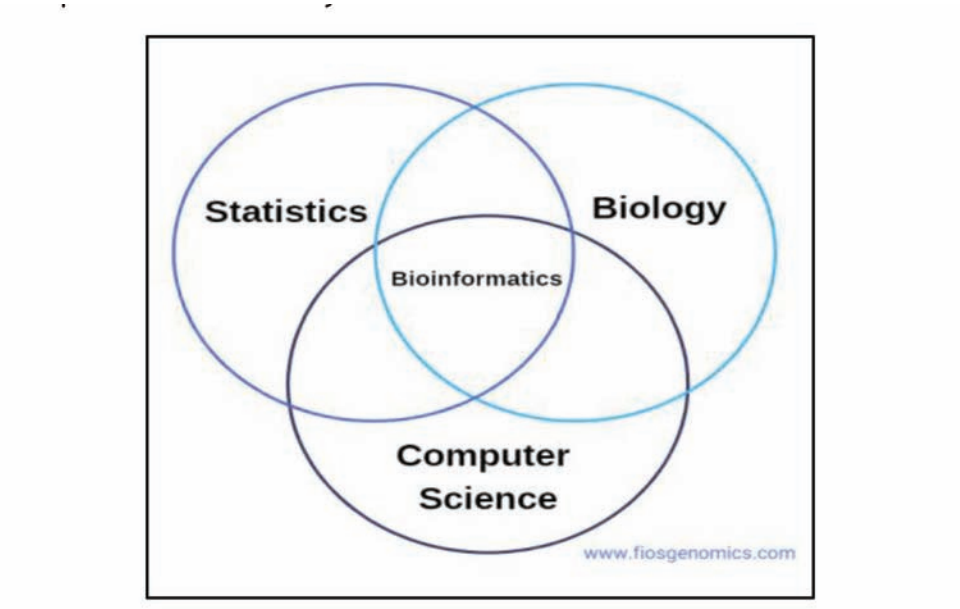
h. Prediction of molecular interactions

i. Genome annotation- the genomes are marked to know the regulatory and protein coding. It is a very important part of human genome project as it determines the regulatory sequences.

j. Health and Drug Discovery- the tools of bioinformatics are helpful in drug discovery, diagnosis and disease management.



- Bioinformatics is an interdisciplinary field mainly involving molecular biology and genetics, computer science, mathematics, and statistics.
- In the beginning of the 1970s, Ben Hesper and I started to use the term "bioinformatics" for the research we wanted to do, defining it as "the study of informatic processes in biotic systems".

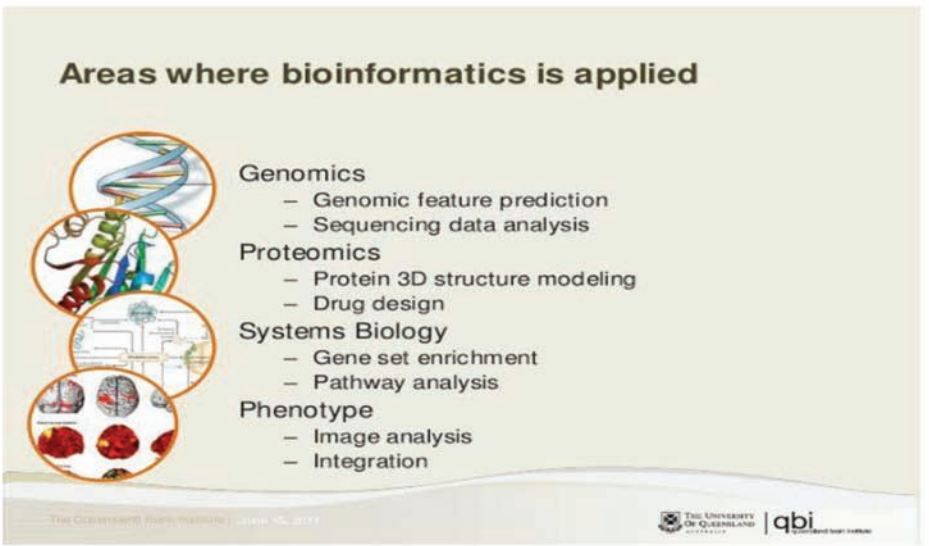


- Biological networks such as gene regulatory networks, metabolic pathways, and protein-protein interaction networks are usually modeled as graphs and graph theoretic approaches are used to solve associated problems such as construction and analysis of large-scale networks.

- Bioinformatics employs computers and information technology to large molecular biology data sets.
- Bioinformatics is seen as a cutting-edge branch of the biotechnology sector, used for novel drug discovery and personalized medical treatments.

- For example, in medicine, bioinformatics can be used to identify links between specific diseases and the gene sequences that cause them.
- The field of pharmacogenomics uses bioinformatics data to tailor medical treatments to the patients who take them, based on their DNA.

APPLICATIONS OF BIOINFORMATICS



The main uses of bioinformatics include:

- Bioinformatics plays a vital role in the areas of structural genomics, functional genomics, and nutritional genomics.
- It covers emerging scientific research and the exploration of proteomes from the overall level of intracellular protein composition (protein profiles), protein structure, protein-protein interaction, and unique activity patterns (e.g. post-translational modifications).
- Bioinformatics is used for transcriptome analysis where mRNA expression levels can be determined.
- Bioinformatics is used to identify and structurally modify a natural product, to design a compound with the desired properties and to assess its therapeutic effects, theoretically.
- Cheminformatics analysis includes analyses such as similarity searching, clustering, QSAR modeling, virtual screening, etc.
- Bioinformatics is playing an increasingly important role in almost all aspects of drug discovery and drug development.
- Bioinformatics tools are very effective in prediction, analysis and interpretation of clinical and preclinical findings.
- Its major applications include in the following fields:

1. Molecular medicine

   The human genome will have profound effects on the fields of biomedical research and clinical medicine.

2. Personalised medicine

   Clinical medicine will become more personalised with the development of the field of pharmacogenomics.

   This is the study of how an individual's genetic inheritence affects the body's response to drugs.

3. Preventative medicine

   With the specific details of the genetic mechanisms of diseases being unravelled, the development of diagnostic tests to measure a persons susceptibility to different diseases may become a distinct reality.

4. Gene therapy

   Gene therapy is the approach used to treat, cure or even prevent disease by changing the expression of a person's genes.

5. Waste cleanup

   Deinococcus radiodurans is known as the world's toughest bacteria and it is the most radiation resistant organism known.

   Scientists are interested in this organism because of its potential usefulness in cleaning up waste sites that contain radiation and toxic chemicals.

6. Forensic analysis of microbes

   Scientists used their genomic tools to help distinguish between the strain of Bacillus anthracis that was used in the summer of 2001 terrorist attack in Florida with that of closely related anthrax strains

7. Comparative Studies

   Analysing and comparing the genetic material of different species is an important method for studying the functions of genes, the mechanisms of inherited diseases and species evolution.

   Bioinformatics tools can be used to make comparisons between the numbers, locations and biochemical functions of genes in different organisms.

8. Veterinary Science

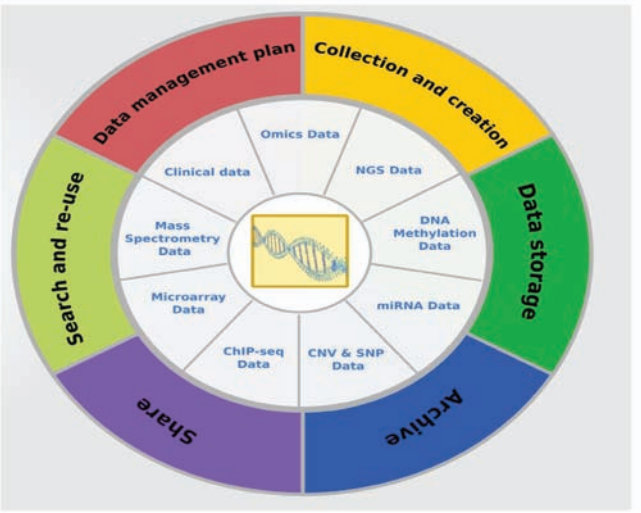
   Sequencing projects of many farm animals including cows, pigs and sheep are now well under way in the hope that a better understanding of the biology of these organisms will have huge impacts for improving the production and health of livestock and ultimately have benefits for human nutrition.

9. Crop improvement

   Comparative genetics of the plant genomes has shown that the organisation of their genes has remained more conserved over evolutionary time than was previously believed.

   These findings suggest that information obtained from the model crop systems can be used to suggest improvements to other food crops.

   At present the complete genomes of Arabidopsis thaliana (water cress) and Oryza sativa (rice) are available., etc.



The main components of bioinformatics are

- The development of software tools and algorithms and
- The analysis and interpretation of biological data by using a variety of software tools and particular algorithms.

STORAGE AND RETRIEVAL OF DATA

In bioinformatics, data banks are used to store and organize data. Many of these entities collect DNA and RNA sequences from scientific papers and genome projects. Many databases are in the hands of international consortia. For example, an advisory committee made up of members of the European Molecular Biology Laboratory Nucleotide Sequence Database (EMBL-EBI Bank) in the United Kingdom, the DNA Data Bank of Japan (DDBJ), and GenBank of the National Center for Biotechnology Information (NCBI) in the United States oversees the International Nucleotide Sequence Database Collaboration (INSDC).

APPLICATIONS OF BIOINFORMATICS

The primary goal of bioinformatics is to increase the understanding of biological processes. What sets it apart from other approaches, however, is its focus on developing and applying computationally intensive techniques to achieve this goal. Examples include: pattern recognition, data mining, machine learning algorithms, and visualization. Major research efforts in the field include sequence alignment, gene finding, genome assembly, drug design, drug discovery, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, genome-wide association studies, the modeling of evolution and cell division/mitosis.

a. In the field of medicine in particular, a number of important applications for bioinformatics have been discovered. For example, it is used to identify correlations between gene sequences and diseases, to predict protein structures from amino acid sequences, to aid in the design of novel drugs, and to tailor treatments to individual patients based on their DNA sequences (pharmacogenomics).

b. Bioinformatics plays a vital role in the areas of structural genomics, functional genomics and nutritional genomics.

c. It covers emerging scientific research and the exploration of proteomes from the overall level of intracellular protein composition (protein profiles), protein structure, protein-protein interaction, and unique activity patterns (e.g. post-translational modifications).

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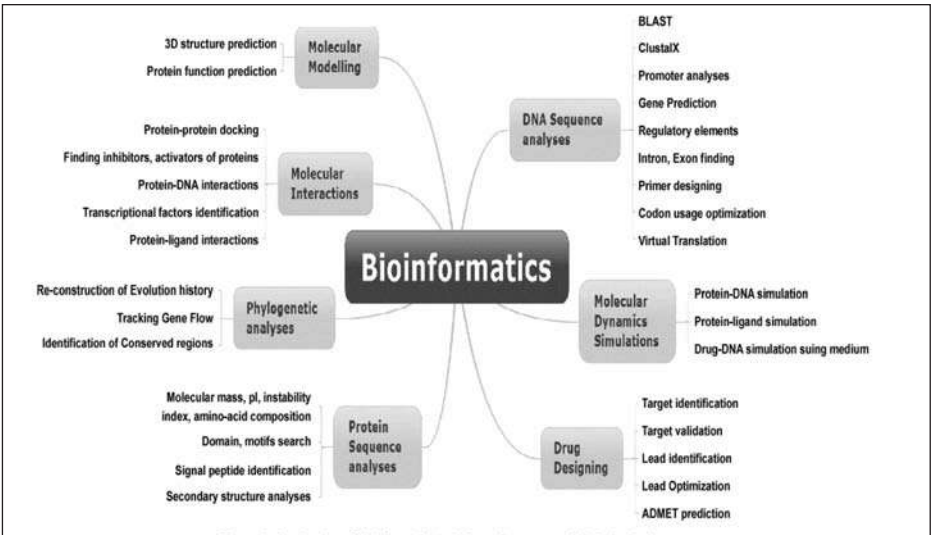

Figure 1: Application of bioinformatics tools in various areas of biological sciences.

GOOD MANUFACTURING PRACTICES (GMP)

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What is GMP?

Good Manufacturing Practices or GMP is a system that consists of processes, procedures and documentation that ensures manufacturing products, such as food, cosmetics, and pharmaceutical goods, are consistently produced and controlled according to set quality standards. Implementing GMP can help cut down on losses and waste, avoid recall. Overall, it protects both company and consumer from negative food safety events.

GMPs examine and cover every aspect of the manufacturing process to guard against any risks that can be catastrophic for products, such as cross-contamination, adulteration, and mislabeling. Some areas that can influence the safety and quality of products that GMP guideline and regulation address are the following:

- Quality management
- Sanitation and hygiene
- Building and facilities
- Equipment
- Raw materials
- Personnel
- Validation and qualification
- Complaints
- Documentation and recordkeeping
- Inspections & quality audits

What is the difference between GMP and cGMP

Good Manufacturing Practices (GMP) and current Good Manufacturing Practices (cGMP) are, in most cases, interchangeable. GMP meaning the basic regulation promulgated by the US Food and Drug Administration (FDA) under the authority of the Federal Food, Drug, and Cosmetic Act to ensure that manufacturers are taking proactive steps to guarantee their products are safe and effective. cGMP, on the other hand, was implemented by the FDA to ensure continuous improvement in the approach of manufacturers to product quality. It implies a constant commitment to the highest available quality standards through the use of up-to-date systems and technologies.

What are the 5 Main Components of Good Manufacturing Practice?

It is paramount to the manufacturing industry to regulate GMP in the workplace to ensure consistent quality and safety of products. Focusing on the following 5 P's of GMP helps comply with strict standards throughout the entire production process.

1. People

   All employees are expected to strictly adhere to manufacturing processes and regulations. A current GMP training must be undertaken by all employees to fully understand their roles and responsibilities. Assessing their performance helps boost their productivity, efficiency, and competency.

2. Products

   All products must undergo constant testing, comparison, and quality assurance before distributing to consumers. Manufacturers should ensure that primary materials including raw products and other components have clear specifications at every phase of production. The standard method must be observed for packing, testing, and allocating sample products.

3. Processes

   Processes should be properly documented, clear, consistent, and distributed to all employees. Regular evaluation should be conducted to ensure all employees are complying with the current processes and are meeting the required standards of the organization.

4. Procedures

   A procedure is a set of guidelines for undertaking a critical process or part of a process to achieve a consistent result. It must be laid out to all employees and followed consistently. Any deviation from the standard procedure should be reported immediately and investigated.

5. Premises

   Premises should promote cleanliness at all times to avoid cross-contamination, accidents, or even fatalities. All equipment should be placed or stored properly and calibrated regularly to ensure they are fit for the purpose of producing consistent results to prevent the risk of equipment failure.

What are the 10 Principles of GMP?

1. Create Standard Operating Procedures (SOPs)
2. Enforce / Implement SOPs and work instructions
3. Document procedures and processes

4. Validate the effectiveness of SOPs
5. Design and use working systems
6. Maintain systems, facilities, and equipment
7. Develop job competence of workers
8. Prevent contamination through cleanliness
9. Prioritize quality and integrate into workflow
10. Conduct GMP audits regularly

Regulations

GMP regulations are mandated by manufacturers' respective national governments to regulate the production, verification, and validation of manufactured products and ensure that they are effective and safe for market distribution.

For example, in the United States, GMP is enforced by the US FDA through Current Good Manufacturing Practices (CGMP) which cover a broader range of industries such as cosmetics, food, medical devices, and prescription drugs. The FDA conducts facility inspections to assess if a manufacturing company complies with CGMP regulations. If any serious violations are found during the inspection, FDA recalls all products, which is problematic for manufacturers in terms of both profit and business operations.

The quality of manufactured products is highly regulated as it can pose negative health risks to consumers and even the environment. Poor hygiene, temperature-control, cross-contamination, and adulteration in any step of the manufacturing process are some examples of how a manufactured product that doesn't follow GMP regulations can bring fatal consequences to consumer.

Standards

GMP standards are developed to enhance the safety of manufactured products, especially pharmaceutical goods, and to ensure consumers get the highest quality possible. Adherence to GMP standards not only positively impacts the reputation of manufacturing companies but also reduces batch recalls and negative reports from consumers. Below are 4 measures you can follow to uphold GMP standards:

1. Quality team

   Have a team of skilled workers that will focus on improving current manufacturing procedures and complying with GMP. Members will perform quality assessments on operations to identify problems and develop appropriate corrective measures. Part of the team's responsibility will also be performing scheduled monitoring of instruments, equipment, processes, and staff skills.

2. Validation

   Validation is the documented act of demonstrating instruments, processes, and activities that are regularly used or done. This is done to check if they function according to expectations. GMP can involve a number of things to be validated, but it's good to focus on the following processes:

- Process validation
- Cleaning and sanitation validation
- Computer system validation
- Analytical method validation

3. Surprise Audits

   A surprise audit every now and then can help gain a more accurate insight into what goes on in the facility. Identify real root causes of non-compliance and take action before it progresses into a larger issue.

4. Compliance Training

   Providing compliance training to staff is the best way to ensure compliance with GMP standards. Help staff gain a better understanding of GMP and continually improve operations or systems in place to ensure standards are GMP-compliant. All employees should receive training on recordkeeping, sanitation, proper equipment handling, and labelling, and SOPs to minimize errors and maintain compliance.

Guidelines and Basic Concepts

GMP guidelines are a set of principles that help manufacturers implement an effective manufacturing process and ensure that quality is built into the organization and the processes involved. GMP guidelines are customarily flexible, with countries having their own legislation to comply with local GMP guidelines and principles. But almost all regulations are derived from the basic concept and guidelines which are:

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Sanitation and hygiene

Sanitation and hygiene are vital in every aspect of the manufacturing process. It covers anything that can cause contamination such as personnel, the premises, equipment, containers, and production materials. All potential sources of contamination should be identified and eliminated with a comprehensive sanitation and hygiene program.

Building and facilities/premises

As a principle, the premises should be situated in an environment that is suitable for its operations and one that is free from risks of contamination of materials and products. The premises should also be designed to minimize errors in operations and should be easy to clean and maintain.

Equipment

Same with the premises, equipment should be designed, located, and maintained to function according to its intended use. Additionally, it should be cleaned and stored according to procedures. In the event of a defect or malfunction, it should be removed or labelled as defective.

Raw materials

All materials used for production should be stored properly according to the appropriate conditions which are set by the manufacturers. There should be a proper stock management system implemented to ensure that all incoming materials are correct and of high quality.

Personnel

The success of GMP compliance heavily relies on the people implementing it. For this reason, it is vital that all personnel are qualified and trained to do the job. They should be aware of the principles of GMP and receive continued training, hygiene instructions, and other tools relevant to their needs. Respective managers should be clear on job descriptions for each worker to avoid misunderstandings and reduce the risk of issues like overlapping responsibilities.

Validation and qualification

Qualify systems, premises, and equipment if they are fit/ready for their intended use and validate if processes and procedures can repeatedly produce high-quality products. Critical steps in the manufacturing process should be verified to ensure that product quality is consistent and maintained at a high level. According to the WHO (World Health Organization), qualification and validation should establish and provide documentation stating that:

- the premises, supporting utilities, equipment, and processes have been designed in accordance with the requirements for GMP (design qualification or DQ)
- the premises, supporting utilities, and equipment have been built and installed in compliance with their design specifications (installation qualification or IQ);
- the premises, supporting utilities, and equipment operate in accordance with their design specifications (operational qualification or OQ); and a specific process will consistently produce a product meeting its predetermined specifications and quality attributes (process validation or PV, also called performance qualification or PQ)

Complaints

Handling complaints is also part of GMP, therefore all manufacturing companies should have a well-designed GMP complaint system. Ideal complaint handling should have a ready solution to provide for all contingencies.

Documentation and recordkeeping

Good documentation and record keeping are an essential part of the quality assurance system and are required in compliance with GMP requirements. Accurate recordkeeping can help managers and supervisors keep track of the historical record of manufacturing procedures and corrective measures implemented. Below are general requirements for documentation:

- Documents must be designed, prepared, reviewed, and distributed with care.
- Documents should be clear and legible.
- Documents must be approved, signed, and dated by appropriate and authorized personnel.
- Documents must have unambiguous contents such as title, nature, and purpose.
- Documents must be regularly reviewed and updated.
- Documents must not be handwritten.
- Any corrections made to a document or record must be signed or initialed and dated. The reason for the correction should also be recorded (where appropriate).
- Record each action taken for traceable activities such as manufacturing and control of products.

Inspections & quality audits

Inspections should be regularly performed to monitor if GMP is implemented and complied with. Document what areas need more work and provide corrective measures for continuous improvement. Quality audits are done to assess the quality systems implemented by the manufacturing company. GMP audit checklists can help companies comply with GMP guidelines set by regulatory authorities.

How to Comply with Guidelines

GMP guidelines and regulations address different issues that can influence the safety and quality of a product. Meeting GMP or cGMP standards helps the organization comply with legislative orders, increase the quality of their products, improve customer satisfaction, increase sales, and earn a profitable return of investment.

Conducting GMP audits play a big part in assessing the compliance of the organization to manufacturing protocols and guidelines. Performing regular checks can minimize the risk of adulteration and misbrand. A GMP audit helps improve the overall performance of different systems including the following:

- Building and facilities
- Materials management
- Quality control systems
- Manufacturing
- Packaging and identification labeling
- Quality management systems
- Personnel and GMP training
- Purchasing
- Customer service
- Ensure consistency in quality and safety with GMP training

Good Manufacturing Processes, or GMP, make sure that products are controlled in quantity and produced in such a way that meets quality standards. GMP involves many factors such as hygiene, environmental conditions, and more. Through this process, any flaws or anomalies can already be identified earlier on which wouldn't be detected when the final product is tested or already out in the market. By making sure that your products go through GMP, you'll have high-quality end products that are safe for use or consumption which, in turn, will give better customer satisfaction and yield higher revenue.

Compliance and Your Organization

GMP compliance is crucial in the manufacturing industry. Providing quality-assured products can secure the health and safety of the public. Robust processes and easy-to-use systems that enforce and monitor standards can strengthen GMP compliance in your organization. With technological advances in the industry, food manufacturers have more opportunities to transform reactive company culture, into a proactive, predictive workforce equipped for continuous improvement.

PLANT TISSUE CULTURE AND ITS APPLICATIONS

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Plant tissue culture:

Plant tissue culture was a new addition to plant breeding strategies that emerged around the 1950s. Because traditional breeding procedures were unable to meet crop demand, tissue culture emerged as a significant advancement in breeding processes. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites.

Small pieces of tissue (explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled. Growing the plant cells, tissues and organs on a artificial, synthetic medium under controlled conditions is called plant tissue culture. Plant tissue culture has become a major thrust area in plant biotechnology.

Concept of Plant tissue culture:

The basic concept of plant tissue culture is totipotency, differentiation, dedifferentiation and re differentiation.

1. Totipotency: The inherent potential of any living plant cell to develop into entire organism is called totipotency. This is unique to plant cells.
2. Differentiation: The meristematic tissue is differentiated into simple or complex tissues.
3. Dedifferentiation: Reversion of mature tissue into meristematic state leading to the formation of callus is called dedifferentiation.
4. Re differentiation: The ability of the callus to develop into shoot or root or embryoid.

The origin and development of plant tissue culture:

The beginning of plant tissue culture was made as early as 1898, when a German Botanist G. Haberlandt successfully cultured individual plant cells, isolated from different tissues. But only during 1934 to 1939, a foundation of plant tissue culture was laid down by three scientists (Gauthret, White and Nobecourt) due to discovery of plant growth regulators such as auxins and vitamins. During next twenty years (1940 to 1960) a variety of growth regulator such as cytokinins were identified for their effect on cell division, growth and differentiation.

After 1960, in vitro culture of plant cells, tissues and organs was reasonably well developed. Research in this area was initiated in early 1960s by Prof. P. Maheshwari and Prof. S. Narayanaswamy at the Department of Botany, University of Delhi in India. Consequently, media and culture techniques for a variety of plant materials became known, which are now extensively utilized in all areas of plant improvement programmes.

History of Plant Tissue Culture:

Let's have a look at the successive timeline for the development of the tissue culture technique:

1902: Gottlieb Haberlandt proposed the theoretical basis of plant tissue culture. He is known as the father of plant tissue culture. He experimented with isolated photosynthetic leaf cells but was unsuccessful in inducing any growth. However, he predicted that one can obtain artificial embryos from vegetative cells using this culturing technique and established the concept of totipotency.

1904: Henning isolated embryos of some crucifers and successfully grew on mineral salts and sugar solutions.

1922: WJ Robbins and W. Kotte independently cultured small root tips of peas and maize. This led to the development of the concept of Organ culture.

1934: Gautheret cultured cambium cells of Salix caprea, and Populus nigra on Knop's solution containing glucose and cysteine hydrochloride. The cultures survived a few months.

1939: Gautheret obtained the first established continuously growing tissue cultures from carrot root cambium. At this same point, P. R. White Demonstrated indefinite culture of tomato roots on subculturing in a liquid medium.

1941: J Van Overbeek demonstrated that coconut milk is essential for the growth and development of very young Datura embryos.

1942: P.R. White and A.C Braun began studying crown galls and tumor formation in plants.

1952: G. Morel and Martin C demonstrated that virus-free plants can be recovered from infected plants using shoot meristem culture.

1957: F. Skoog, and C.O. Miller proposed that a particular auxin-cytokinin ratio can regulate shoot and root initiation in cultured callus.

1959: G. Melchers and Bergmann L cultivated haploid tissues other than pollen for the first time.

1960: E.C Cocking isolated and cultured protoplasts after digesting the cell walls enzymatically and showed new cell wall regeneration on tomato fruit locule protoplasts. This same year L. Bergmann first obtained callus by transferring cells from suspension cultures on to solid medium and G. Morel developed a method of producing virus-free Cymbidium progenies through meristem culture.

1966: S G Guha and S C Maheshwari cultured anthers and pollen and produce haploid embryos.

1973: I. Potrykus attempted the first chloroplast and nucleus transfer from Petunia hybrida into albino protoplasts of the same species.

1974: J P Nitsch cultured microspores of Datura and Nicotina, doubling their chromosome number and harvesting seed from homozygous diploid plants within five months. At the same time, Mursahige developed the concept of developmental stages in cultures in vitro: Stage I: Establishment; Stage II: Multiplication; and Stage III: Rooting and hardening.

1975: G. Morel established cold storage of regenerated plants for a year.

1978: A. Zelcer, D. Aviv, and E. Galun devised a method for transferring organelles from one plant to another called Donor - Recipient protoplast fusion.

1981: P. J. Larkin and W. R. Scowcraft developed the concept of somaclonal variation. At the same time D. Wilson, G. Patnaik, G., and E. C. Cocking regenerated a whole plant from a single free cultured tobacco protoplast.

1986: J. D. Hamill, A. J. Parr, R. J. Robins, and M. J. C. Rhodes established hairy root cultures of Beta vulgaris and Nicotiana rustica following infection with Agrobacterium rhizogenes. Compared to in

vitro roots of the same variety, the transformed cultures synthesized their characteristic secondary products.

1991: C. Sautter, H. Waldner, and G. Neuhaus developed a novel method for the acceleration of microprojectiles, called micro-targeting.

Basic requirements of tissue culture laboratory:

1. Equipment and apparatus
2. Washing and storage facilities
3. Media preparation room
4. Sterilization room Aseptic chamber for culture
5. Culture rooms or incubators fully equipped with temperature, light and humidity control devices.
6. Observation or recording area well equipped with computer for data processing.

Equipment and Glassware:

a. Incubating chamber or laminar airflow cabinet with UV light fitting for aseptic transfer.
b. Incubator with temperature control ± 0.5°C generally temperature recommended for most tissue culture studies is 36°C.
c. Autoclave-for sterilization of glassware, media etc.
d. Refrigerators and freezers-For storage of reagents, tissue culture stock solutions, chemicals etc.
e. Hot air oven-for dry sterilization of glassware, media etc.
f. Microscope-Simple and special microscope with a provision to take camera are required. The stage of this microscope should be large enough to accommodate large roller bottles in specific cases.
g. pH meter- for adjusting the pH of the medium A spirit burner or gas micro burner for flame sterilization of instrument

Steps in Plant Tissue culture technique:

1. Selection and Sterilization of Ex plant
2. Preparation and Sterilization of Culture Medium
3. Inoculation (Transfer of ex-plants into culture vessels)
4. Incubation of cultures
5. Sub culturing
6. Transfer of Plant let

SELECTION AND STERILIZATION OF EX PLANT:

Selection of ex-plant plant:

Selection of mother plant with all desirable characters like high yield, good quality and disease resistance plays crucial role in tissue culture. A cut portion of plant organ or tissue used for initiation of an in vitro culture is called an ex-plants. These can be shooting tips, root tips, leaves, tuber etc. Shoot tips are generally preferred.

Sterilization of ex-plant:

Plant material which is to be cultured, should be surface sterilized to remove the surface borne microorganisms. This procedure is done in front of a laminar air flow or inside the inoculation chamber before the plant material is inoculated onto the culture medium. Thoroughly washed plant material or ex- plant in tap water is immersed in 5% v/v solution of liquid detergent such as 'Teepol' for 10-15 minutes. Then wash the material thoroughly in tap water and finally in distilled water. This step can be done in the general laboratory. Subsequent steps are done in front of a laminar air flow or the pre-sterilized inoculation chamber. Dip the ex-plants in 70% ethyl alcohol for 60 seconds. Immediately transfer the material into an autoclaved jaw bottle and pour 0.1% mercuric chloride (HgCl2), 5-10% Sodium hypochlorite (v/v) solution. Keep them for 10- 15 minutes. During that period, the bottle is frequently swirled for shaking so that all surfaces of plant material come equally in contact with sterilant. After 10-15 minutes, decant the sterilant and wash the ex-plants thoroughly with several changes of autoclaved distilled water to remove all traces of sterilant. Then the ex-plants are ready for culture.

Preparation and Sterilization of Culture Medium:

Several media formulations are commonly used for the majority of all cell and tissue culture work includes those described by White, Murashige and Skoog, Gamborg et. al., Schenk and Hilderbrandt, Nitsch and Nitsch, and Lloyd and McCown.

Preparation of Culture Medium:

In vivo plant cells, tissues and organs get their appropriate nutrient and growth requirements from the intact plant body for their organized growth and development. Isolated cell, tissues and organs also need nutrients for their in vitro growth and development. So, nutrients are supplied artificially according to the medium formulated by several workers. The main objective of medium preparation is to culture the cell, tissue and organ in vitro.

Dissolve 30gms Sucrose in 200 ml DDH2O. Take DDH2O in another flask and add stocks of majors, minors, KI, Fe-EDTA, vitamins, glycine and meso-inositol in the appropriate amounts. Pour filtered sucrose solution and majors, minors, vitamins, amino acid, hormone solution mixture into a one litre measuring cylinder. Make the final volume to one litre with DDH2O. Shake well to mix up uniformly.

Desired concentrations of auxin and/or cytokinin are added from stock solution. Adjust the pH of the liquid medium 5.6-5.8 with the aid of 0.1(N) HCl or 0.1(N) NaOH. This operation is done using the pH metre. Add 5% to 8% agar to the liquid medium to make solid medium. Heat to 60°C to dissolve the agar completely. Otherwise, with_out adding agar, liquid medium can be used for culture. Dispense the culture medium into culture tube (20 ml/tube) or wide mouth conical flask (25-40 ml/flask). Insert non-absorbent cotton plug wrapped with gauge cloth. Cover the plug with the help of brown pa_per and rubber band. Medium is finally sterilized by autoclaving.

Sterilization of Media and other non-living Articles:

The culture medium, especially when it contains sugar, will also support the growth of micro-organisms like bacteria, fungi etc. So if they come in contact with medium either in cellular form or in spore form, the micro-organisms grow faster than the higher plant tissue due to their brief life cycle and will kill the tissue. The micro-organisms may come from glass vials, instruments, nutrient medium used for culture and even from plant material itself. Therefore, the surface of plant tissue and all non-living articles including nutrient medium should be sterilized.

The routine sterilization procedure of non-living articles such as nutrient medium, glass goods, distilled water, instruments (wrapped with brown paper) is by autoclaving under steam at a pressure of 15 lb/in2 and a temperature of 120°C for 15 minutes. Thermolabile compounds are often added in the medium and such medium is sterilized either at room temperature or in cold by passing through bacterial filter. An alternative method of sterilizing glass goods and instruments is by heating in an oven at 150°C for 3-4 hrs.

Inoculation (Transfer of ex-plants into culture vessels containing nutrient media):

This is done by experienced person in inoculation room under sterilized conditions. The sterilized ex-plants are transferred to culture vessels containing culture media with the help of fore ceps. Such culture vessels are kept in growth room where there will be controlled air, light, humidity and temperature. When the tissues are placed on the culture medium within a few days a mass of cells called callus will develop due to active division of the tissue cells and within 3-6-week multiple shoots in bunches will appear from the callus.

Incubation of Cultures:

High temperatures are likely to lead to dissociation of the culture medium and tissue damage while at very low temperatures tissue growth is slow. Again some tissues grow well in dark while others need both light and dark conditions. Low humidity causes the quick desiccation of culture medium and high humidity is favourable for the contamination of culture medium. There_fore, cultures are incubated in a culture room where light, temperature and humidity are con_trolled. After inoculating the tissue onto the culture medium, cultures are incubated on culture rack at 25-28°C constant temperature. Culture tubes are placed at 30-45° inclined position. Illumination is provided by cool-white fluorescent light placed about 18 inches above the culture to give a light intensity of 4 - 10 x 103 lux for 16 hours. If light is not necessary, then put off the light and cover the whole rack with a black cloth.

Method of sub-culture and production of plants:

Multiple shoots are separated and transferred to the flask containing media in laminar airflow chamber. The medium is modified in such a way that roots and vegetative parts develop quickly. In one to three weeks young plants may develop from the cultured tissue.

Transfer of plant lets (Method of hardening):

This is a method in which the tissue culture plants developed in artificial media are habituated to grow in natural environment. Firstly, these plants are taken out from nutrient media and washed thoroughly with water. Then these plants are grown in netted plastic pots filled with liquid nutrient medium and kept in green house for 6 - 8 weeks. This is called Primary hardening. Afterwards the plants are transferred to poly bags filled with potting mixture and grown under shaded house for 6 - 8 weeks. This is called Secondary hardening. After secondary hardening the plants are suitable for growing in farmer's fields.

Types of Plant Tissue Culture:

1. Meristem culture
2. Callus culture
3. Cell suspension culture
4. Bud culture

5. Seed culture
6. Anther Culture
7. Embryo culture
8. Protoplast culture

Meristem Culture:

The apical meri stem of shoots of angiosperms and gymnosperms can be cultured to get the disease-free plants. Temerity tips, between 0.2-0.5 mm, most frequently produce virus-free plants and this method is referred to as meri stem-tip culture. This method is more successful in case of herbaceous plants than woody plants. In case of woody plants, the success is obtained when the ex-plant is taken after the dormancy period is over. After the shoot tip proliferation, the rooting is done and then the rooted plant let is potted.

Callus Culture:

Callus is basically more or less nu-organized dedifferentiated mass of cells arising from any kind of ex plant under in vitro cultural conditions. The cells in callus are parenchymatous in nature, but may or may not be homogenous mass of cells. They are meristematic tissue, under special circumstances they may be again organized into shoot primordia or may develop into somatic embryos.The callus tissue from different plant species may be different in structure and growth habit. The callus growth is also dependent on factors like the type of ex plant and the growth conditions. After callus induction it can be sub-cultured regularly with appropriate new medium for growth and maintenance.

Cell Suspension Culture:

The growing of cells including the culture of single cells or small aggregates of cells in vitro in liquid medium is known as cell suspension culture. The cell suspension is prepared by transferring a portion of callus to the liquid medium and agitated using rotary shaker instrument. The cells are separated from the callus tissue and used for cell suspension culture.

Production of Secondary Metabolites: Cell suspension culture can be useful for the production of secondary metabolites like alkaloids, flavonoids, terpenoids, phenolic compounds and recombinant proteins. Secondary metabolites are chemical compounds that are not required by the plant for normal growth and development but are produced in the plant as 'byproducts' of cell metabolism. For Example: Biosynthesis and isolation of indole alkaloids from Catharanthus roseus plant cell culture. The process of production of secondary metabolites can be scaled up and automated using bio-reactors for commercial production. Many strategies such as biotransformation, elicitation and immobilization have been used to make cell suspension cultures more efficient in the production of secondary metabolites.

Bud Culture:

Buds contain quiescent or active meristems in the leaf axils, which are capable of growing into a shoot. Single node culture, where each node of the stem is cut and allowed to grow on a nutrient media to develop the shoot tip from the axil which ultimately develops into new plantlet. In axillary bud method, where the axillary buds are isolated from the leaf axils and develop into shoot tip under little high cytokinin concentration.

Seed Culture:

Seeds may be cultured in vitro to generate seedlings or plants. It is the best method for raising the sterile seedling. The seed culture is done to get the different kinds of explants from aseptically grown plants which help in better maintenance of aseptic tissue.

Anther Culture:

An important aspect of plant tissue culture is the haploid production by another culture or pollen culture which was first established by Guha and Maheswari (1964, 1966) in Datura. During the last few decades, much progress has been made in different crops like rice, wheat, maize, mustard, pepper and others. The anthers bearing the uni-nucleate microspores are selected and allowed to grow in medium to produce callus from the pollen mass. Then the triggering of these androgenic calli is directed to produce the embryos and haploid plants are developed from these androgenic embryos. The anther culture can be done with the isolated anthers on solid medium where anther wall will break open and the androgenic calli will be formed from the pollen. In pollen culture, microspores of uni_-nucleate stage are collected in liquid media and can be grown in suspension culture. In suspension, the uni-nucleate pollens may give rise to calli mass or the globular mass from which the plants can be raised either through embryogenic or organogenic path_way.

Embryo Culture: Embryo culture is the sterile isolation and growth of an immature or mature embryo in vitro with the goal of obtaining a viable plant. In some plants seed dormancy may be due to chemical inhibitors or mechanical resistance, structures covering the embryo. Excision of embryos and culturing them in nutrient media help in developing viable seedlings. Embryo developed from wide hybridization between two different species may not mature fully due to embryo-endosperm incompatibility. So, the isolation and culture of hybrid embryos prior to abortion help in overcoming the post-zygotic barrier and pro_duction of interspecific or inter-generic hybrids.

Protoplast Culture:

It is the culture of plant protoplasts i.e., culture of cells devoid of cell wall. Isolated protoplasts are usually cultured in either liquid or semisolid agar media plates. Protoplasts are isolated from soft parenchymatous tissue by enzymatic method and then viable proto_plasts are purified and cultured. The protoplast culture is aimed mainly to develop gene_tically transformed plant where the transgenic is put successfully within the plant protoplast and the transgenic plant is regenerated from that transformed protoplast. Another aspect of protoplast culture is somatic hybridization of two plant species through proto_plast fusion. Protoplasts are cells without a cell wall, but bounded by a cell membrane or plasma membrane. Using protoplasts, it is possible to regenerate whole plants from single cells and also develop somatic hybrids. The steps involved in protoplast culture.

i. Isolation of protoplast:

   Small bits of plant tissue like leaf tissue are used for isolation of protoplast. The leaf tissue is immersed in 0.5% Macrozyme and 2% Onozuka cellulase enzymes dissolved in 13% sorbitol or mannitol at pH 5.4. It is then incubated over-night at 25°C. After a gentle teasing of cells, protoplasts are obtained, and these are then transferred to 20% sucrose solution to retain their viability. They are then centrifuged to get pure protoplasts as different from debris of cell walls.

ii. Fusion of protoplast:

   It is done through the use of a suitable fusogen. This is normally PEG (Polyethylene Glycol). The isolated protoplast is incubated in 25 to 30% concentration of PEG with Ca++ ions and the protoplast shows agglutination (the formation of clumps of cells) and fusion.

iii. Culture of protoplast:

MS liquid medium is used with some modification in droplet, plating or micro-drop array techniques. Protoplast viability is tested with fluorescein diacetate before the culture. The cultures are incubated in continuous light 1000-2000 lux at 25°C. The cell wall formation occurs within 24-48 hours and the first division of new cells occurs between 2-7 days of culture.

iv. Selection of somatic hybrid cells:

The fusion product of protoplasts without nucleus of different cells is called a cybrid. Following this nuclear fusion happen. This process is called somatic hybridization.

Applications of Plant tissue culture:

Clonal Propagation and Micro-Propagation:

Plant population derived from a single donor plant is called a clone and the multiplication of genetically identical copies of that cultivar is called clonal propagation which may be a useful tool to get a large population of plant species having desirable traits. Micro-propagation is achieved through multiplication of shoot tips or axillary buds cultured in vitro. This technique is very much used in horticulture and silviculture in the plants which have long seed dormancy, tree species, orchids and many fruit plants. This micro-propagation technique is also helpful for supplying the plant material throughout the year involving large scale multiplication i.e., grower and breeder gets a large number plant stocks irrespective of seasonal variation.

In tissue culture from a callus mass large numbers of shoot meristems can be regenerated within a very short time and space. As a result, a large number of plantlets can be produced from such callus tissue. The most obvious advantage of this technique is the large-scale production of plants of same gene_tic stock.

Biomass Energy:

In recent years, the interest has aroused in commercializing the in vitro propagation of forest trees.Micro-propagation has been successfully done in many trees of economic importance like Acacia nilotica, Albizia lebbeck, Azadirachta indica, Butea monospermous, Dendrocalamus strictus, Shorea robusta, Tectona grandis and Cedrus deodara, Cryptomeria japonia, Picea smithiana, Pinus sylvestris. All these plant species are useful in forestry for biomass energy production. Development of automated procedure, plant delivery systems using somatic embryos and artificial seeds are also in progress.

Secondary Metabolite production:

Production of many useful compounds like alkaloids (Codeine, Vincristine, Quinine, etc.), Steroids (Diosgenin), Glycosidic compounds (Digoxin) and many other essential oils (Jasmine), flavouring and colouring agents (saffron) can be done by plant cell culture. This aim can be achieved by selection of specific cells producing high amount of desired compounds and development of a suitable medium. In general, secondary metabolites produced by plant cell cultures are rather small in amount but by clonal selection the particular high yielding clone of cells can be isolated. Sometimes the plant cell culture may provide the helpful way for more production of secondary metabolite by feeding the culture with inexpensive product precursors (biotransformation) or by manipulating their biosynthetic control mechanisms.

Specialized Strategies for the Production of Secondary Metabolites:

Supply of Precursors: Although, plant cells are totipotent in carrying out secondary metabolic pathways, plant cells in culture generally shows low-level production of secondary metabolites when compared to the natural plants. Only scanty information is available on the exact factors which control metabolite production.

One of the in vitro constraints is that secondary metabolite pathways are blocked by one or more deficient intermediates. To overcome the lacuna, intermediates are supplied in culture media to set right the pathways. Timing of precursor supply is very important because precursor when fed initially may inhibit both cell growth and metabolite production. In callus culture of Capsicum Capsaicin production is enhanced by supplying immediate precursors like vanillylamine and isocarpic acid.Production of diosgenin is accomplished by feeding cholesterol (100 mg/L) to Dioscorea culture. Similarly, feeding of phenylalanine to the culture of Coleus blumei enhanced the production of rosemarinic acid.

Supply of Elicitors:

Elicitors are the triggering factors which can elicite the production of secondary metabolites. These are the substances originally derived from microorganisms and actively participate in secondary metabolism.

Elicitors are of two types - abiotic and biotic elicitors;

Abiotic elicitors: These are light and metallic co-factors and others. Certain particular wavelengths of the light (Ultraviolet (UV) has a significant influence in the induction of secondary metabolites, for example, flavon glucoside synthesis is most sensitive to UV light at wavelength below 300 nm. Similarly, induction of anthocyanine takes place at the peak of 312 nm and 438 nm. Certain metallic co-factors such as gold, copper and silver are added into the medium which can induce secondary metabolite production by acting as abiotic elicitors. These metabolic co-factors can be a part of the enzymatic activity involving secondary metabolic pathways.

Biotic elicitors: These are simply the extracts of fungi and fungal cell wall material, which are generally included in the medium. Soyabean cell culture when inoculated with the strain of Pseudomonas syringa, triggered glyeollin (phytoalexin) production. Addition of culture filtrate of micromucor to the cell culture of Catharanthus roseus enhances tryptomine biosynthesis. Elicitors are also probably involved in controlling gene expression. As a result, increased level of enzyme production can stimulate the synthesis of compounds which are new to the cells.

Bio-transformations:

A bioconversion can be defined as the conversion of one chemical into another, i.e., of a precursor (or substrate) into a final product, using a cell suspension acting as biocatalyst. The biocatalyst can be microorganisms, plant or animal cells, either growing or in a quiescent state, or an extract from such cells or a purified enzyme.The biocatalyst may be free, in solution, immobilized or on solid support or entrapped in a matrix. In bioconversion by whole cells or extract a single enzyme or several enzymes may be involved. By means of recombinant DNA technology genes encoding relevant enzymes can introduced in the host cells.

Hairy Root Culture:

The potentials of hairy root cultures from plant transformation by Agrobaderium rhizogens for the biosynthesis of secondary metabolites have been well documented. Hairy roots can be produced

by incubating a piece of plant tissue in Agrobacterium rhizogens solution. Transfor_mation takes place due to the transfer of T-DNA from bacteria into the plant cells. The Ri plasmid of A. rhizogens contains auxin-related genes in T-DNA. The successful integration of T-DNA inside plant DNA resulted in the expression of auxin-related genes and consequently, over production of IAA. As a result, numerous hairy roots are induced from the explant. Several examples on the secondary metabolite production by hairy root culture have been reported.

Production, accumulation and release of nicotine and nicotine related alkaloids are facilitated by hairy root culture of Nicotiana rustica and the accumulation of betacyanine and betaxanthin in Beta vulgaris. There have been reports on the production and secretion of novel therapeutic protein using hairy root culture. Hairy root cultures are novel possible potential sides for the synthesis and easy secretion of recombinant proteins. Thus, avoiding expensive downstream processing.

Genetic Variability:

The variability generated by the use of a tissue culture cycle has been termed as somaclonal variation by Larkin and Scowcroft. This genetic variability is due to cells of various ploidy levels and genetic constitution of the initial explant or also may be developed due to different cultural conditions. The chromosomal instability in the cultured cells play an important role in polyploidization of cells and genetically variable plants can be raised. Such kind of variations may show some useful characters such as resistance to a particular disease, herbicide resistance, stress tolerance, etc. and also some agronomi_cal traits like tiller number, panicle size, flowering time, plant height, lodging resistance, yield, nutrient content and different kinds of morphological variations in leaf.

Somatic Embryogenesis and Synthetic Seed:

Direct or indirect somatic embryogenesis may be achieved from pro-embryonic cell of the direct explant or the embryoids developed within the callus tissue from induced embryogenic cells. The potential application of this technique is the mass production of adventitious embryos which ultimately develop into complete plantlet in maturing media. These somatic embryos can be encapsulated with suitable nutrient containing alginate medium which are called artificial seeds or synthetic seeds. As the somatic embryos are derived from a single cell, this method is very much useful for production of disease free propagule. This artificial seed production is also desirable in case of asexually propa_gating plants.

Breaking Dormancy:

Using embryo (zygotic) culture technique the seed dormancy period can be reduced or eliminated and the breeding cycle can be shortened in many of the plants like Malus sp, Ilex sp. and Telia americana etc. The life cycle of Iris was reduced from 2-3 years to less than one year. It was possible to obtain two generations of flowering in Rosa sp. Embryo abortion in unsuccessful crosses may be recovered by culture of immature embryo of different hybrids.

Haploid Plants:

Haploid plants can be obtained through anther or pollen culture (androgenesis) or through ovaries or ovule culture (gynogenesis). The anther culture and haploid plant production has been attempted in many of the crop plants, where these haploids are of immense importance for production of homozygous diploid or polyploid lines by colchicine treatment within a very short period specially in case of fruit trees.These androgenic haploids can also be used for production of

different kinds of aneuploids like monosomic, nullisomic, trisomic, etc. and also for the induction of mutagenesis and doubling of those mutated lines. Many of the recessive traits can be made expressed in double haploids such as low glucosinolate content in Brassica, salt tolerance and disease resistance in rice, etc.Generation of exclusively Y chromosome containing plant is possible also through haploid production as in case of Asparagus. The triploid or poly_ploid can also be produced by using protoplast fusion technique of this kind of androgenic haploids which may be used for different breeding programmes.

Somatic Hybrids:

Isolation and regeneration of plant from the protoplasts in vitro has opened up a new avenue in various fields of plant breeding and in plant biotechnology. Somatic hybri_disation, i.e., the asexual hybridisation using isolated somatic protoplasts is a new tool to make the wide hybridisation successful. Products of fusion between two protoplasts (heterokaryon) could be cultured to regenerate a new somatic hybrid plant of desired genotype. This technique has been mainly used for introgressing many useful criteria from the wild genotype to cultivated crop variety. Success has been achieved obtaining somatic hybrid plants between sexually compatible and incompatible plants.

Production of cybrid, i.e., the fusion between two protoplasts-one partner with nucleus and another partner with cytoplasm, is also of immense importance in the plant breeding programme, mainly for production of male sterile line with the help of extra-nuclear genome.

Transgenic Plants:

The genetically modified (GM) plants, in which a functional foreign gene has been incorporated by biotechnological method, are called transgenic plants. A number of transgenic plants have been produced carrying genes for different traits like insect resistance, herbicide tolerance, delayed ripening, increased amino acid and vitamin content, improved oil quality, etc. The different methods of introduction of foreign genes, direct (electroporation, microinjection or particle bombardment) or indirect (Agrobocterium mediated), have been applied either in plant tissue culture method such as embryogenic or organogenic plant development from different plant parts or in protoplast culture system.

The direct DNA uptake through protoplast is the most ideal method for production of transgenic plants. Any gene of interest that may be of eukaryotic or prokaryotic origin can be used for this purpose but should be expressed.

Germplasm Conservation:

Many of the important crop species produce recalcitrant seeds with early embryo degeneration. Also many of the plants are vulnerable to insects, pathogens and various climatic hazards. Maintenance of these plants are very difficult. Mainly the plant species which are endangered, rare and threatened with extinction are needed to be conserved by ex-situ method of germplasm conservation. Plant tissue culture may be applied for this purpose. In vitro germplasm storage collection provides a cost-effective alternative to growing plants under field conditions, nurseries or greenhouses. Furthermore, the cryopreservation of cells and tissue, revival of these tissue and regeneration of plants from tissue through tissue culture technique really effective in conservation biotechnology. Cryopreservation involves storage of cells, tissues, etc. at a very low temperature using liquid nitrogen.