



**Compendium**



**34<sup>th</sup> IAUA Convention of Vice-Chancellors**

*and*

**National Symposium**

*on*

**Application of Bio-Nanotechnology in  
Agricultural and Animal Sciences for Food Security**

**7-8 December, 2009**

*Sponsored by*

**Indian Agricultural Universities Association, New Delhi**

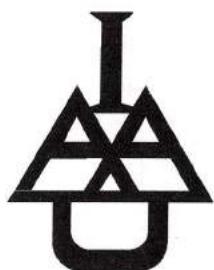
*Organised by*

**National Dairy Research Institute**

(Indian Council of Agricultural Research)

(Deemed University)

Karnal-132 001 (Haryana), India



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*Published by:*

**Dr. A.K. Srivastava**, Director & Vice Chancellor  
National Dairy Research Institute  
Karnal - 132 001

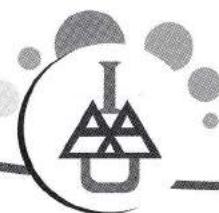
*Compiled & Edited by:*

**Dr. (Mrs.) Meena Malik**  
**Dr. Rajeev Kapila**  
**Dr. Rameshwar Singh**

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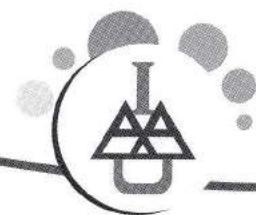


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## Preface

It is indeed a matter of great honour for us to organize the 34<sup>th</sup> Vice Chancellor's Convention under the aegis of Indian Agricultural Universities Association alongwith the National Symposium on "**Application of Bio-Nanotechnology in Agricultural & Animal Sciences for Food Security**" from 7<sup>th</sup> -8<sup>th</sup> December 2009. This is the first time that National Dairy Research Institute Karnal is organizing the Convention of Vice-chancellors of State Agricultural Universities and Veterinary Universities to deliberate on this new and emerging area of science and technology.



The role of the National Dairy Research Institute (NDRI) as the apex R&D Institution for spearheading the growth of dairying is very significant and substantial. As the country's premier Dairy Research Institution, NDRI has developed considerable expertise over the last eight decades in different areas of Dairy Production, Processing, Management and Human Resource Development. Information generated and services offered by the Institute have contributed enormously to the growth of Dairy Industry and well being of millions of milk producers. Realizing the challenging need of globalized Dairy Trade, the Institute is continuously working to develop its R&D and HRD programmes to serve the nation in terms of food security, employment generation, and poverty alleviation.

Today, the application of Bio-Nanotechnology is emerging as an important tool to boost the productivity in agriculture and allied sectors. Though the application of Bio-Nanotechnology in food and agriculture is in its very nascent stage, yet it is visualized to be a rapidly evolving field. The application of nanotechnology has the potential to revolutionize food processing, food packaging and food safety practices. It is believed that nanotechnology may prove to be a boon to change the entire agriculture sector and food industry chain from production, preservation, processing, packaging, transportation to waste management. Bio-Nanotechnology is sure to be a part of the future veterinary medicine and animal health care, and security of animal food products. The Bio-Nanotechnology has also various applications in monitoring the quality of agricultural products. Nano based biosensors could be used for disease diagnosis and be integrated into packaging materials to monitor the freshness of the food. Spoiling of the food could be indicated by the change in colour of the sensor and even traces of residual chemicals and/or even presence of few bacteria and viruses could be detected with ease on bio-selective surfaces.

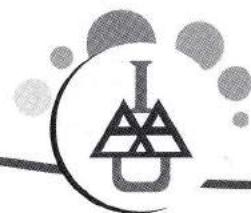


Keeping in view the scope and applications of Bio-Nanotechnology in various fields, Govt. of India has also launched the Nano Technology Mission at the National level. However, there are more gaps in application of Bio-Nanotechnology in the food and agriculture sector in the country. Therefore, IAUA has rightly chosen to deliberate on this issue of national importance to come out with a road map for its effective utilization and implementation in meeting new challenges in agriculture. Further, this Annual Convention also brings an excellent opportunity for sharing of the knowledge pool and practical experience of the galaxy of Vice-Chancellors of State Agricultural and Veterinary Universities, Directors and Deans of SAUs, and Directors of ICAR Institutes for taking up this programme at the national level.

Once again, I express my sincere thanks to the President, Executive Secretary and the Executive Committee of IAUA for reposing their trust in us and providing us the opportunity to hold this 34<sup>th</sup> Vice Chancellor's Convention and the National Symposium on Bio-Nanotechnology. I sincerely hope that this convention will serve as an apex academic platform to promote research and teaching in this emerging hi-tech area of science and facilitate fruitful interaction of scientists and academicians.

  
(A. K. Srivastava)





**34<sup>th</sup> IAUA Convention of Vice-Chancellors**

*and*

**National Symposium**

*on*

**Application of Bio-Nanotechnology in Agricultural  
and Animal Sciences for Food Security**

**DECEMBER 7-8, 2009**

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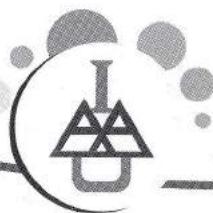
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**8<sup>th</sup> December, 2009**

2.45 PM – 3.30 PM

**Plenary Session**

**Session Coordinator:** Dr. Rajan Sharma, NDRI

**Chief Guest:** Prof. Ved Parkash, Vice-Chairman, UGC, New Delhi

**Guest of Honour:** Dr. M. M. Pandey, National Director & DDG (Engg), ICAR

**Chairman:** Dr. Anwar Alam, President IAUA & VC, SKUAST-K, Srinagar

**Executive Secretary, IAUA, New Delhi:** Dr. R. P. Singh

**Programme:**

Welcome Address by Dr. R. P. Singh, Executive Secretary, IAUA

Presentation of Reports of Different Technical Sessions

Remarks by Co-Chairman and Chairman

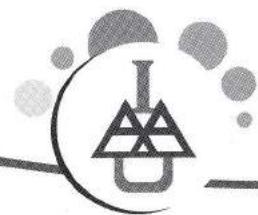
Address by Guest of Honour

Address by Chief Guest

Wrap-up and Vote of Thanks by Dr. A. K. Srivastava, Director & VC, NDRI

Tea





**8<sup>th</sup> December, 2009**

12.30 PM – 2.00 PM

**Technical Session - IV**

**Nanotechnology in Food Processing, Packaging & Value Addition**

**Session Coordinator:** Dr. Suman Kapila, NDRI

**Chairman:** Dr. Tej Pratap, VC, CSKHPKV, Palampur

**Co-Chairman:** Dr. H. C. Pathak, VC, NAU, Navsari

**Co-Chairman:** Dr. Basant Ram, VC, NDUAT, Faizabad

**Rapporteur:** Dr. G. R. Patil, JD(A), NDRI, Karnal

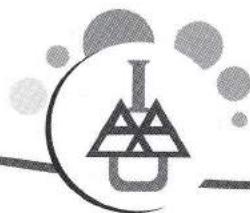
**Speakers:**

1. Dr. C. S. Pundir, Professor, M D University, Rohtak  
*"Nano-Biosensors based on Multiwalled Carbon Nanotubes for Determination of Ascorbate and Oxalic Acid in Food Stuffs"*
2. Dr. A. K. Das, VC, UBKV, Cooch Behar, West Bengal  
*"Nanotechnology and its Application in Agriculture and Food Industry- An Overview"*
3. Dr. Y. S. Rajput, PS, ABC, NDRI, Karnal  
*"Nanomaterials and their Potential Applications in Food Processing"*

**Panelists:**

1. Dr. S. S. Kadam, VC, MAU, Parbhani
2. Dr. V. M. Mayande, VC, DPDKV, Akola
3. Sh. K. R. Viswambharan, IAS, VC, KAU, Thrissur
4. Dr. M. L. Chaudhary, VC, RAU, Pusa
5. Dr. K. K. Baruah, Director, NRC on YAK, Dirang
6. Dr. Alok Jha, Prof. Food Science & Technology, BHU, Varanasi
7. Dr. Er. Ram Kishore, Registrar, NDUAT, Faizabad
8. Dr. Bakshi Ram, Head, Sugarcane Breeding Institute (Regional Station), Karnal
9. Dr. S. N. Sinha, Head, IARI, Regional Station, Karnal
10. Dr. (Mrs.) Rita Singh Raghuvanshi, Dean Home Science, GBPUAT, Pantnagar

**2.00 PM – 2.45 PM      Lunch**



## Nanotechnology and Prospects of its Application in Agriculture and Allied Fields

Prof. Anwar Alam\*

Sher-E-Kashmir University of Agricultural Science & Technology of Kashmir, Srinagar  
President, Indian Agricultural Universities Association

His Excellency the Governor of Jammu & Kashmir, Shri N.N. Vohra; Dr. A.K. Srivastava, Director and Vice-Chancellor of National Dairy Research Institute & Chairman, Organizing Committee; Dr. C.D. Mayee, Chairman ASRB; Dr. R.P. Singh, Executive Secretary IAUA; Vice-Chancellors from State Agricultural Universities and State Veterinary Universities; Joint Directors, NDRI and Dr. Rameshwar Singh, Organizing Secretary of Conference on "Application of Bio-Nano Technology in Agricultural and Animal Sciences for Food Security". Let me first express my gratitude to HE Shri N.N. Vohra Ji for agreeing to be the Chief Guest at the Inaugural Session. I am thankful to Dr. Srivastava for agreeing to hold 34<sup>th</sup> Annual Convention of Vice-Chancellors of State Agricultural and Veterinary Universities at NDRI - Karnal. NDRI received Deemed University status two decades back and is hosting now Annual Convention of Vice-Chancellors. Perhaps, it is first such occasion where Annual Convention of Vice-chancellors of State Agricultural and Veterinary Universities is being held in ICAR Institute. This Annual Convention brings Vice-Chancellors of State Agricultural and Veterinary Universities & ICAR Institute closer to each other and allow sharing of knowledge in agricultural, animal and dairy research towards improving productivity of crops and animals.

The 20<sup>th</sup> century is called the century of physics, electronics and communication; the 21<sup>st</sup> century is being visualized as century of mainly dominated by biological revolutions including nanotechnology. Nanotechnology is an exciting and rapidly growing technology allowing us to work, manipulate and create tools, materials and structures at the molecular level. Nature has been performing nanotechnological feats for millions of years. We are just beginning to understand nanoscale methods used in nature to create self-replicating, self-monitoring, self-controlling and self-repairing tools, materials and structures.

In spite of all scientific and technological developments, we have not been able to resolve if we and world around us was created or it evolved through some evolutionary processes over a period of time. I have a hunch life on planet earth has evolved over millions of years starting at nanoscale. Many Anthropologists hold view that it all began from a organism like cyanobacteria which was self-replicating that grew on muddy wet surface and the mother nature provided it energy through sun and thunderbolts.

Nanotechnology is the design, characterization, production and application of structures, devices and systems by controlling shape and size at nanoscale. Eight to 10 atoms span is one nanometer (nm). Human hair is about 70,000 - 80,000 nm thick. Nano science is the world of atoms, molecules, macromolecules, quantum dots and macromolecular assemblies. Through application of nanotechnology a wide range of materials with distinct properties - optical, electrical, magnetic can be fabricated. Nano particles take advantage of their dramatically increased surface area to volume ratio.

\*Vice-Chancellor



The roots of nanotechnology go back to the 1959 talk presented by Nobel Prize winning physicist, Richard Feynman (1961) at a meeting of the American Physical Society that led to the discovery of tube-like structures of carbon atoms which is basically a rolled up sheet of carbon that has outstanding properties.

Bio-nanotechnology is a new and exciting field of research in which recent advances in nanotechnology is integrated into the realm of biology, in particular into molecular biology and cell biology. Bionanotechnology requires interdisciplinary collaboration, especially between the life, physical and chemical sciences with input and nanotools from the engineering sciences.

Biological and medical research scientists have already exploited unique properties of nanomaterials for various applications such as marker assisted biological tests, labeling of specific molecules, structures and microorganisms. In agriculture marker assisted selection (MAS) by plant breeders has become quite common. The overall drug consumption and side effects can be significantly lowered by drug delivery in morbid region only and just right dose, and treatment of cancer. Nano technology can help to reproduce or repair damaged tissue – “tissue engineering”. It can substitute organ transplant requirements. Bio-nanotechnology will leave no field untouched by its ground breaking scientific innovations. The agricultural industry is no exception. It can augment agricultural production and boost food processing industry through application of its unique properties. Advances are being made in reproductive science and technology, energy conversion of agricultural waste, enzymatic processing, nano membrane separation etc.

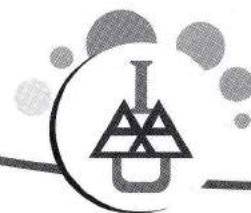
Bio-nanotechnology is sure to be a part of the future veterinary medicine and animal health care, molecular and cellular breeding, animal history and security of animal food products. Nanocomposites can play very important role in animal food product packaging, it can increase or decrease gas permeability of different fillers.

Chemical catalysis and filtration technology are two prominent applications where nanotechnology plays important role. Nanoporous membranes are suitable for a mechanical filtration through small pores smaller than 10 nm. It is used for removal of certain ions or separation of two different fluids.

Advances in application of bio-nanotechnology in agriculture and animal sciences are many,

- Nano encapsulation of vitamins, antioxidants, antimicrobials, flavorings and preservatives.
- Nanolamination particles from carbohydrates, proteins and lipids for protection against moisture, gases, lipids that also serve as carrier of colours, flavors, antioxidants, super foods etc.
- Crop improvement by modifying genetic constituents, induced mutations
- Plant disease diagnostics using rapid testing technologies and biosensors
- Nucleic Acid Bioengineering – Dr. Hargovind Khurana received noble prize for synthesizing monostructured nucleotides stringing them in to DNA.
- Smart treatment delivery systems





- Nanobioprocessing of new biochemicals and pharmaceutical products.
- Bioanalytical nanosensors capable of detecting biotic and abiotic allergens and toxic pollutants at very minute levels
- Nanomaterials – man-made or those that exist in nature, smart fabrics, study of soil as nanocomposites.
- Bioselective surfaces on which most chemicals and biological interactions occur.

Before, I conclude, let me say that we are at extremely exciting time in the evolution of science and engineering where convergence of major technologies – nanotechnology, biotechnology, information technology and cognitive science is poised to a new agriculture and veterinary science. It is this convergence that will truly be key to solving our future requirement of food and nutrition and infrastructure. Applications of nanotechnology have the potential to change the entire agriculture sector and food industry chain from production, conservation, processing, packaging, transportation and waste management. Speakers are now going to cover whole range of gamut of bio-nanotechnology in different technical sessions and come out with recommendations that are going to impart new dimensions to Indian agriculture and rural economy.

As I conclude, I would like to thank HE the Governor of J&K and Chief Guest of this function to spare his valuable time, Director, NDRI for hosting 34<sup>th</sup> Annual Convention of IAUA and all the Vice-Chancellors and their representatives who could make to the Convention to participate and enrich its deliberations.

## Nanotechnology and Agriculture

Lalit M Bharadwaj\*

Biomolecular Electronics & Nanotechnology, Central scientific Instruments Organization  
(Council of Scientific & Industrial Research), Sector 30-C, Chandigarh-160030.(India)  
*lalitmbharadwaj@hotmail.com, www.csio.res.in*

### Introduction

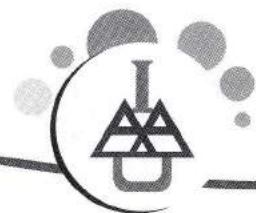
Nanotechnology is a field which deals with materials and systems having at least one dimension in the range of 1-100 nanometer (1 nanometer is  $10^{-9}$  meter and diameter of human hair is about 70,000 nanometer) While MicroElectroMechanical Systems (MEMS) includes all those devices, which have dimensions in microns and has both electronic and mechanical components put together. Nanosystems are fabricated using the processes which have fundamental control over the physical and chemical attributes of atoms or molecules mainly using self assembly processes while MEMS are fabricated in the same way as semiconductor devices using lithography techniques.

Nanotechnology & MEMS being new enabling technologies for development of devices, system, components and materials for every sector of the industry and it is predicted that this century will be dominated by industrial revolution through hybrid devices based on MEMS and Nanotechnology. BioMEMS and BioNanotechnology take advantage of innumerable molecular and nano devices working in all living systems and have been perfected over billions of years under harsh conditions. These molecules can be exploited for interfacing them with micro and nano-structures to design and develop commercially viable products with higher performance and selectivity. The development of specific devices, however need core competence in the domain of interfacing functional and intelligent biomolecules with active surfaces including characterization for their wide ranging applications in healthcare, agriculture, defense, material science, etc.

The market for these technologies is growing very fast with development of new materials and devices. Nanotechnology market has already crossed US \$100 billion mark and for MEMS this figure is at US \$12 billion. According to James Murday & Mike Roro' National Nanotech Initiative' the Nanotechnology market is expected to touch US \$1 trillion by 2015 out of which materials, electronics and pharmaceuticals will account for 70% of total market. Some of the areas of active research are novel foods, medical devices, chemical coatings, personal health testing kits, sensors for security systems, water purification units, manned space craft, displays for hand-held computer games, and high-resolution screens. In US there are more than 400 companies in business in these areas and majority of these are startup companies, Europe and Japan are lagging behind but putting all efforts to increase their share in the world market. Thus MEMS and Nanotechnology have opened unlimited opportunities for fundamental & applied research and innovative high performance miniaturized product development for all sectors of economy. The Ministry of Information Technology, Govt. Of India initiated research in this area in the country and the work started at most of the leading academic and R & D institutions. CSIO also initiated the work on Biomolecular Electronics and Nanotechnology with particular focus on sensor development. State of art infra structure has been developed and several programs related

\* Add. Director (Scientist G) & Head





to broad topics such as DNA as sensing device, Bio-MEMS, Carbon Nanotubes, Bio-molecular motors etc. The talk opens with broad coverage on international scenario of R & D carried out and reported by various researchers on nanomaterials, Bio-nanotechnology, Nanoelectronics and Bio-MEMS. The author further describes nanotechnology work being carried out at CSIO and a brief touch on future trends in this field. This is expanding fast and is continuously drawing the attention of researchers all over the world.

### **Nanotechnology Paradigm**

Nanotechnology being highly interdisciplinary in terms of science and technology, calls for convergence of various areas of expertise both in technology and application. Nanotechnology can be broadly categorised into following:

- Nanoelectronics
- Nanomaterials
- Bionanotechnology

Nanoelectronics deal with miniaturization of present submicron semiconductor technology with feature size below 100 nm. Nanomaterials take advantage of entirely different chemical, physical, optical and electronic properties of nano-particles than bulk material. Nano-Biotechnology deals with interfacing functional biomolecules with available devices for development of devices with higher performance in terms of selectivity, sensitivity and economics.

The emerging fields of nanoscale science, engineering, and technology - the ability to work at the molecular level, atom by atom, to create large structures with fundamentally new properties and functions - are leading to unprecedented understanding and control over the basic building blocks of natural and man-made materials. The convergence of nanotechnology with information technology, modern biology and social sciences will reinvigorate discoveries and innovation in almost all areas of applications. The long-term research on the manipulation of matter at the atomic and molecular levels will give an unprecedented ability to create building blocks for advanced products such as new classes of devices as small as molecules and machines as small as human cells.

### **Nanomaterials**

Nanomaterials (nanocrystalline materials) are materials possessing grain sizes of the order of a billionth of a meter. They manifest extremely fascinating and useful properties, which can be exploited for a variety of structural and non-structural application. The unique properties of nanomaterials and structures on the nanometer scale have sparked the attention of materials developers. Nanomaterials are already in the market in some specific applications for product performance improvement, for example, as fillers in plastics, as coatings on surfaces, and as UV-protectants in cosmetics. The technology holds more promise for the future and is expected to bring more disruptive changes to both products and markets in the area of medicine, plastics, energy, electronics, space, etc. Nanomaterials are synthesis by sol-gel, inert gas condensation, mechanical alloying or high-energy ball milling, plasma, epitaxial growth, laser ablation, electro-deposition, etc. Driven by what is already high demand for nanotechnology research and development, it is expected to see a trend toward increased industry acceptance and large-scale availability of raw





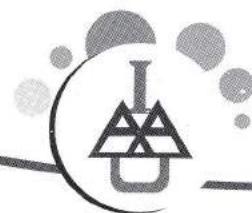
nanomaterials. Today a large portion of the work being done in nanomaterials is yielding fascinating results that just don't yet meet the rigors of large-scale commercialization; invention of a great product is only the first hurdle: advancements in scalability, reliability and affordability are necessary.

Nanoparticles are also produced as agricultural by-products: airborne dust and aqueous runoff that cause air and water pollution. Controlling these nanoparticles is in the best interest of efficient, cost-effective and environmentally responsible agriculture. Soils are aggregates of nanoparticles, layered particles, organisms and water. The environmental impact (biodegradability) of agricultural by-products in soils needs further research. Viewing soil as a nanocomposite, and applying the paradigms and technologies of nanoscale science to it, can lead to more efficient and environmentally friendly agriculture.

### **Bio-nanotechnology**

The nature has created large variety of molecular devices which are working in living system ranging from micro-organism to human beings. Nature creates these devices by self-assembly process and does not requires billions of dollar lithography based fabrication facilities used in VLSI devices. The life processes and materials have been perfected by nature in millions of years. These devices will find applications in electronics, life sciences, medicine, space, defense, bio-warfare, security, etc. Thus, the best way is to directly utilize large number of molecular devices working in the living systems for engineering applications by interfacing with non-biological materials and devices. These hybrid devices will have higher performance and perfection as molecular devices, such as, DNA, biomolecular motors, proteins, enzymes have well established dedicated functions to perform under given set of environmental conditions. At the same time, these molecular devices can be isolated from the living system or synthesized in simple molecular biology laboratory without huge investments as required in semiconductor industry. The system that works inside our bodies, in our cells, and in the nature that surrounds us is full of examples of nanoscale systems. Whether it's our genetic makeup that works for storing and transferring of information about our looks, personality or susceptibility to diseases or increased understanding of the calcium carbonate nanostructures used in seashell construction. Nanotechnology thus holds massive potential for the health care and life sciences market. Nanodevices enable drug delivery mechanisms that can send biologically active materials directly to the location where they are beneficial and reduce side effects to make pharmaceuticals more effective. Some important healthcare areas are self-assembly, biointerface, implants, nano-robots, tissue engineering. Nucleic Acid Diagnostics/Molecular Diagnostic Assays, Point-of-care testing, Genomics, Proteomics, DNA biochips, and microarrays, regenerative medicine, the healthcare environment, drug discovery tools, liquid handling, separation and filtration, nanopore sequencing and separations etc.. Bionanotechnology will bring new kind of sensors for applications in biology and engineering based on nanoparticle tagging, nanotube and nanowire, quantum dots, piezoelectric acoustic assays/rupture event scanning, enzymatic electrochemical detection, single-molecule sequencing and analysis, Lab-on-a-tape, nanoarrays, dip pen nanolithography, nano mass spectrometry, Nanogrid etc. these devices will play important role in early warning and treating life-threatening conditions, including cancer and heart disease in the form of body implants to deliver smart drugs or carry new





cells to repair damaged tissue. Bionanotechnology also provides new tools/sensors for agriculture for improving the efficiency of crop production, food processing, food safety and environmental consequences, storage and distribution, remote control etc.

### **Nanoelectronics**

The electronics and semiconductor industries have been one of the biggest components of the global high-tech economy and have continuously blazed the path with breakthrough innovations. Nanotechnology, with new materials and processes, has the potential to fundamentally alter these industries by enabling new devices, architectures and applications. There has been a lot of speculation and discussion on the role nanotechnology that will play in the future development of electronics.

The demands for more sophisticated electronics and the production of electrical components smaller than the limits presently offered by traditional lithographic techniques have led to rapid growth in the development of new processing techniques at the nanoscale. It is vital that properties measured on such small scales are accurately measured and traceable to accepted macroscopic standards. In addition in nanostructures the electrical properties can be markedly different from their macroscopic equivalents thereby revealing many novel effects. Emerging techniques for the fabrication and study of structures on the nanoscale are being employed to construct devices and explore their potential as modern standards.

Electrical nanostructures have many applications as metrological quantum standards or as tools for nanoscale metrology. Well-established examples are the quantum Hall effect for resistance and the Josephson Effect for voltage. Nanostructures work includes the fabrication of atomic wires, the study of spin-polarised electronics and magnetic nanostructures. Future applications could range from quantum computing and so-called "safe" quantum communication, to devices for single-particle sensor technologies, nanoscale frequency standards and the study of atom-surface interactions.

The quantum effects on which most of these devices are based are very weak and the measurement technology is of paramount importance. Areas being studied include the fabrication of atomic wires; single electron tunneling (SET) devices and atto-farad structures; and the study of spin-polarised electronics and magnetic nanostructures - all of which are likely to play important part in future electronic devices. A study of the thermal motion of an isolated surface-trapped atom is also being studied towards its potential as a nanoscale noise thermometer.

Work in the area of quantum-based devices for nanoscale metrology is also in line towards fabrication of ultra-small SQUID for applications in single-particle detection. The fabrication of such a device will be of significant achievement, and should prove important in areas such as future nanoscale frequency standards, emerging quantum computer and single-particle sensor technologies and in the study of atom-surface interactions.

### **Micro Electro Mechanical Systems (MEMS)**

As the name suggests Micro Electro Mechanical Systems (MEMS) includes all those devices, which have dimensions in microns and has both electronic and mechanical components put together. It is an enabling technology that combines the processing capability of electronics with the work capacity of mechanics to achieve functions which



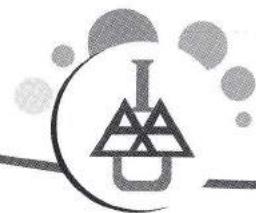
have special relevance. MEMS offer special features such as low power consumption, feasible signal measurement, electronics put along with the sensor providing high S/N ratio, array structure for integrated measurements, etc. Various MEMS based sensors are already reported by researchers and vendors which includes Pressure, temperature, chemical, and magnetic parameters, accelerometers, rate gyros, fluidic sensors, actuators, micro valves, thermal switches, variable emissivity surfaces, microthrusters, synthetic jet actuators, micropower generators, microrelays switches, micro RF switches, variable capacitors, inductors, micro-opto-electro-mechanical devices, integrated devices, springs, bearings, gears, connectors. Such Sensors find abundant applications in almost all fields of science & technology.

BioMEMS, a further extension of MEMS combine the power of MEMS technology with the bio-molecular interactions of anti-body and anti-gens for the specific measurements in health, food and environmental fields.

### **Agriculture and Food System**

Nanotechnology, has the potential to revolutionize agriculture and food systems. Including security, early disease diagnosis, treatment delivery & eradication, environmental protection .Agriculture has long dealt with improving the efficiency of crop production, food processing, food safety and environmental consequences of food production, storage and distribution. Nanotechnology provides a new tool to pursue these historically relevant goals. Today in agriculture if a plant or animal becomes infected with disease, it can be days, weeks, or months before disease presence is detected by whole-organism symptoms. By that time infection may be widespread and entire herds/fields might need to be destroyed. Nanotechnology operates at the same scale as a virus or disease-infecting particle, and thus holds the potential for very early detection and Nanotechnology holds out the possibility that "Smart" treatment delivery systems could be activated long before macro symptoms appear. For example, a smart treatment delivery system could be a miniature device implanted in an animal that samples saliva on a regular basis. Long before a fever develops, the integrated sensing, monitoring and controlling system could detect the presence of disease and notify the farmer and activate a targeted treatment delivery system. Smart treatment delivery systems are envisioned for biology and bioactive systems such as drugs, pesticides, nutrients, probiotics, nutraceuticals and implantable cell bioreactors. In agriculture, the fundamental life processes are explored through research in molecular and cellular biology. New tools for molecular and cellular biology are needed that are specifically designed for separation, identification and quantification of individual molecules. This is possible with nanotechnology and could permit broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful by-products through enzymatic nanobioprocessing, disease prevention and treatment in plants and animals. New materials that have special characteristics at the nanoscale could offer a tremendous breakthrough for pathogen and contaminant detection. Materials that have self-assembly and self-healing properties can find a multitude of applications in agriculture. Packaging of food in self-healing containers could prevent food microbial contamination and facilitate food preservation, storage and distribution. Protection of the environment through the reduction and conversion of agricultural materials into valuable products can be made easier by nanotechnology. New nanocatalysts to convert vegetable oils into biobased fuels





and biodegradable industrial solvents. Keeping this in view the Institute will take R&D to address following immediate problems:

- Early pathogen detection nano-devices for applications in agricultural security and food safety.
- Retrieval nanosystems for sampling specific components (from air, plant and animal organisms, water, soil)
- Non-invasive plant health-monitoring device for “early stress detection” in hydroponic plant growth systems (less complex than soil-based systems) based on detection of changes in plant metabolism, respiration, root-zone excretions and root zone microbial ecology.
- Delivery systems for biological and bioactive systems (drugs, pesticides, nutrients, probiotics, nutraceuticals and implantable cell bioreactors).
- Design food nanostructure, oral delivery matrices, particulates, emulsions and nanodevices for enhanced food flavor and digestibility.
- Develop a health monitoring device for large animals utilizing saliva as a non-invasive indicator.
- Efficient water, fertilizer and pesticide use, decreased pollution and greater economy in destroying only the diseased part of a crop at an early stage of infestation.
- Nano-materials development from various agriculture products and waste.
- Nano-catalyst for conversing agriculture products and waste into fuel and other useful solvents and chemicals.

### **Bio-nanotechnology & BioMEMS research at CSIO**

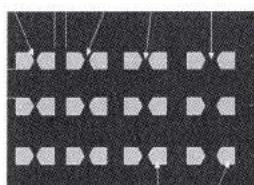
CSIO is actively involved in development of biomolecular and nanodevices for engineering and medical applications. CSIO, over the last five years, has generated state-of-art expertise and facilities in focused research for development of specific devices for biomolecular electronics, disease diagnosis and targeted drug delivery based on molecular devices working in living systems. A cross multi-disciplinary team comprising scientists and Ph.D. Students from physics, chemistry, molecular biology, biochemistry, and information technology have been knit together for understanding and to do application oriented research in this area.

The group is taking multi-disciplinary integrated approach to various R & D activities related to broad areas such as nanowire, Bio-MEMS for disease diagnosis, biomolecular motors for nano electronic switching etc. Studies towards Carbonnanotubes and DNA are combined towards new sensor development.

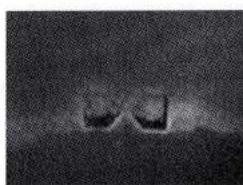
**DNA Nanowire based Devices:** DNA is the best molecular electronic device produced by nature which can store, process and provide information for growth and maintenance of any living system. Single cell production carries with it all requisite information and processing capability to produce living species by taking materials from environment. DNA can be synthesized in any desired sequence of four bases A, T, G, C to act as information storage. Encryption and decryption techniques can be applied which qualify this to be secure device. With large possibilities available with four bases, hence large data storage can be possible.



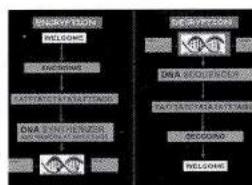
As a typical illustration, CSIO developed DNA based encryption where all the 256 ASCII characters are defined in terms of four bases (A,T,G,C) and using a dedicated software developed for this. Basic number systems with arithmetical operations in terms of DNA sequences were also attempted which can handle up to rational numbers. Work on complex number is in progress. This will lead towards molecular ultra-high density memory devices. For example, National Flag can be coded in terms of a DNA sequence of 7924 bases i.e. in our single genome (containing 6.4 billion bases) tricolor can be copied almost a million times. Space occupied by a genome is only few  $\text{nm}^3$ . Work is on towards studying electrical behavior of various segment of  $\lambda$ -DNA to understand their information processing capability using Femto range ( $10^{-15}$ ) IV-CV measurements. This will help in sensor development for very low concentrations. DNA sequences can be bound on micro/nano electrodes to study such characteristics.



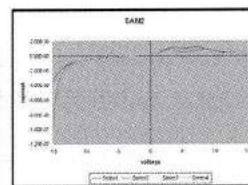
Microelectrodes with varying spacing from 5-25 micron



Binding of DNA in between the electrodes



DNA encryption

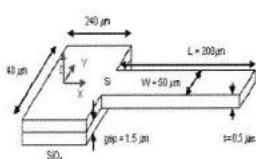


Current-voltage plot of DNA between the microelectrodes

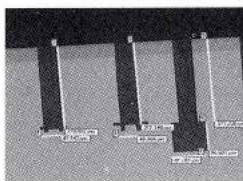
**Bio-MEMS based Microdiagnostic Kits :** The Bio-MEMS based sensors are the most recent development that provide specific molecular direction. The high specificity of the sensors is provided by the biomolecular recognition characteristics of antigen-antibody, probe DNA-target DNA, protein-receptor and enzyme-substrate. The change in mass on the microcantilever surface due to the binding of the analyte molecules is directly proportional to the deflection of the microcantilever.

CSIO carried out significant work towards development of disposable microdiagnostic kits for diseases by developing innovative technique for binding antibodies on cantilever with surface coverage greater than 65%. With this achievement it will be possible to have sensitive device based on 0.5 micron thick cantilever design. This concept has been validated by theoretical simulation.

**Nanotubes based Sensors and Targeted Drug Delivery:** Carbon Nanotubes (CNT) are tiny molecular tubes made up of Hexagonal carbon rings joined together. Dimensions of these tubes range from 1 nm to 10s of nm depending upon their structure i.e. single wall or multi-wall. These tubes have wonderful electrical behavior which can be tailored by changing orientation or angle of bending. Carbon nanotubes are one of the most dominant materials for fabrication of molecular electronic devices with ultra high packing density and performance. CNTs filled with medicines are well suited for targeted drug delivery.



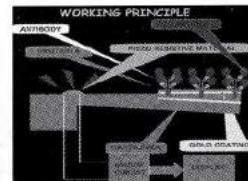
Schematic of cantilever



Cantilever fabricated at SCL

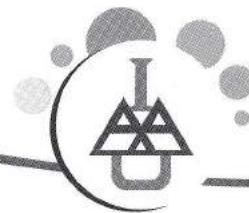


Antibody coated cantilever



Working principle of cantilever

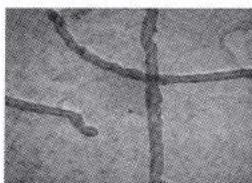




Nanotubes have been synthesized from large number of materials such as vanadium oxide, boron nitride,  $B_xC_yN_x$ ,  $WS_2$ , Rossette,  $NiCl_2$ ,  $NbS_2$ ,  $Ti_2O$ ,  $MoS_2$  etc. Organic, inorganic and bionanotubes will be studied for their applications in engineering and medical applications.

CSIO has functionalized carbon nanotube with functional groups such as  $COOH$ ,  $NH_2$  and  $OH$  for binding biomolecules such as DNA, antibody-antigen, bioreceptors and proteins for developing nanoimplantable sensors and actuators. On this basis we are attempting towards a targeted drug delivery system. The schematic diagram describes carbon nanotubes which can be filled with drug and encapsulated using disease receptors based biomembranes.

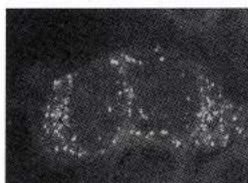
Interfacing biomolecules with carbon nanotube will be of immense importance in development of highly sensitive and selective miniaturized biocompatible sensors and actuators with dimensions in micro and nano range. These will revolutionize in vivo early diagnosis and targeted drug delivery.



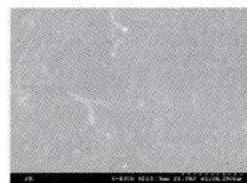
*Dispersed fCNTs*



*DNA-CNT binding  
(fluorescent image)*



*Quantum Dots - CNT  
uptake in Cell*



*Quantum Dots CNTs  
(TEM)*

**BioMolecular Motors:** In living system various kinds of movements of vital organs and muscles take place through different systems of molecular motors, such as, myosin-actin, microtubules-kinesin, microtubules-dynein, microtubules-bacterial flagella motor etc. It is possible to extract motors from their native environment, prepare assemblies of them on a plane surface. For these motors to be useful in nanofabrication, it is essential that external control can be applied to the following properties: (a) translocation speed; (b) translocation direction; (c) activation and arrest. These characteristics can be controlled by altering the chemical and/or the physical environment of the motors.

At CSIO research is on towards in-vitro studies on myosin actin systems which are responsible for our muscular movements to control the movement of actin against myosin in predictable and reproducible manner using myosin coated channels on silicon wafer. Study of this system under various electrode potentials, buffer, electrolyte, pH, etc. will help in following:

- Targeted drug delivery
- Designing molecular switches with ultra-high density, using low cost fabrication technique of molecular biology
- Understanding muscular movement and their diagnosis and cure

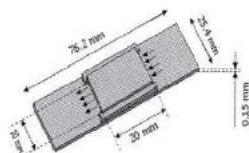
The development of commercially viable innovative bio-nano devices for high density memory, nano electronic devices, micro disposable & implantable diagnostic devices, targeted drug delivery etc. will revolutionize information processing, communication and health care.

Myosin was immobilized on different solid substrates like gold-coated glass slide, mica, silicon which involve both covalent binding using cross linkers like EDC, APTES,

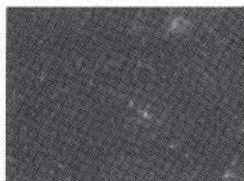




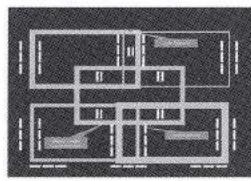
silanes and non-covalent binding employing Protein A, surface adsorption. Atomic force microscope was used to investigate topological characteristics of different surfaces.



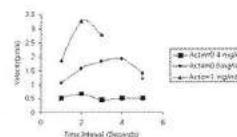
Schematic of flow cell for molecular motors



Motility of actin filament



In-vitro control of molecular motors



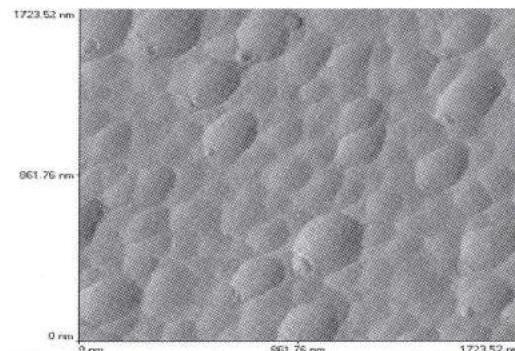
Motility of actin filament

The velocity of actin filaments sliding over myosin heads is calculated using tracking program developed in MATLAB. Velocity of actin filament on myosin tracks immobilized on gold coated glass surface is found to be  $1.81\mu\text{m}/\text{sec}$  on mica surface is  $2.99\mu\text{m}/\text{sec}$  and on glass slide cleaned with 0.1M KOH in ethanol is  $3.65\mu\text{m}/\text{sec}$ . In-vitro studies show that average velocity of the actin molecules was ranging from 1.0-1.5  $\mu\text{m}/\text{sec}$  at pH 8.0. Microchannels are fabricated on gold coated glass surface by ablating the surface using laser micro dissection system to control the position of the actin filaments.

Nanorobotic application of the system has been demonstrated by transportation of carboxylated polystyrene microbeads attached to actin filament. Experiments are conducted to control directionality as well as the movement of actin filaments, by varying environmental conditions in terms of electrical and magnetic field, pH, and temperature for transport of nanoparticles, drugs, quantum dots, bioconjugates etc.



Movement of actin filament in Microchannel

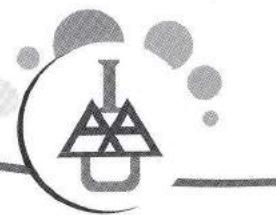


AFM image of myosin on Glass Surface

### Future Program at CSIO

During last few years CSIO has been able to generate unique expertise for undertaking product oriented research in the domain of Bionanotechnology. Presently CSIO is conducting further focused research for the development of bionanosensors for health care and agriculture applications. This includes development of early diagnostic techniques, targeted drug delivery, bionanosensors for pathogens etc. These devices will find applications besides healthcare and agriculture, in defense, space, environment etc. also. To initiate a major CSIO Golden Jubilee project in the area of BioNano Photonics with judicious combination of expertise available at CSIO in the area of Biomolecular Electronics & Nanotechnology, Photonics, Holography. Image Processing, Thin Film





,materials science and out sourcing of microfluidics . Efforts will be made for in-vitro and in-vivo techniques with emphasis on non invasive methods. Initial goal will be for disease diagnosis and clinical analysis in health care and agriculture but these can be extended to other applications.

### Acknowledgements

The talk has high lightened the work carried out at the Biomolecular Electronics and Nanotechnology Division at CSIO by multidisciplinary team consist of students, scientist with basic degree in physical , biological and engineering sciences. Thanks are due to all government agencies such as DRDO, DST, DBT, DIT for their financial support.

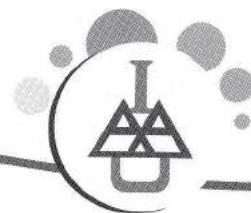
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## Application of Nanotechnology in Agriculture

P. Murugesha Boopathy\*

Tamil Nadu Agricultural University, Coimbatore – 641 003

Nanotechnology is an emerging field with applications spanning all areas of science and technology. It has the potential to revolutionize the scientific world by allowing scientists to manipulate matter at the atomic or molecular level. Among the many advancements in science, nanotechnology is visualized as a rapidly evolving field that has the potential to revolutionize agriculture and food systems (Roco, 2003; Kuzma and Verhage, 2006).

As Nanotechnology is seen as the new industrial revolution, developed and developing countries are investing enthusiastically to secure a market share. United States has invested 3.7 billion US dollars through its National Nanotechnology Initiative (NNI). The US is followed by Japan (750 million USD) and the European Union (1.2 billion USD) which have both committed substantial funds (EU Commission, 2005). China's share of academic publications on a nanoscale Science and Engineering, rose from 7.5 % in 1995 to 18.3 % in 2004. In India, the importance of research and development in nanotechnology has received paramount importance. The Department of Science and Technology (DST), Government of India, launched a Nanomaterials Science and Technology Initiative (NSTI) in 2001 under the leadership of Prof C.N.R Rao with investments of over Rs 1000 crores for the period between 2005 -10. Under this initiative, over 100 research projects on the synthesis and assembly of ceramic nanoparticles, nanotubes, nanowires, nanoporous solids, nanostructured alloys and DNA chips have been supported along with establishment of a number of shared facilities and infrastructure.

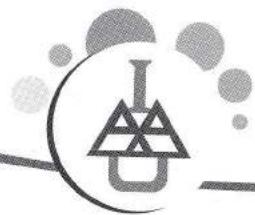
The application of nanotechnology in the agricultural and food industry is still in its infancy, but is predicted to transform the entire industry in the coming years. Therefore, agriculture should take advantage of the powerful tools of nanotechnology, in order to assure food security. In this paper we shall visit the research achievements of scientists in the field of nanotechnology and also analyse the areas of agriculture that can benefit through nanotechnological interventions.

### Review of Developments in Nano Science

- Feynman (1961) an eminent physicist was the first man to dream of Nanoscience. He mentioned the distinguished concepts of Nanotechnology in the book "There's Plenty of Room at the Bottom". In this book he described the process of manipulating individual atoms and molecules using one set of precise tools to build and operate another proportionally smaller set, and so on down to the  $n$ th scale. In the course of this, he noted scaling issues would arise from the changing magnitude of various physical phenomena: gravity becomes less important, whereas surface tension and Van der Waals attraction would become more important. To put it in his own words "..... A biological system can be exceedingly small. Many of the cells are very tiny, but they are very active, they manufacture various substances, they walk around, they wiggle, and they do all kinds of marvelous things--- all on a very small scale. Also they store information. Consider the possibility that we too can make a thing very small which does what we want --- that we can manufacture an object that maneuvers at that level".

\*Vice Chancellor





- In 1980's, the basic idea of this definition was explored by Drexler (1986, 1999).
- In 1981, Scanning Tunneling Microscope (STM) was invented. The first instrument that is able to "see" atoms.
- In 1982, Scanning Probe Microscope (SPN) was invented to "see" different properties at the nanometer scale.

### **Application of Nanotechnology in Agriculture**

Today's research is tomorrow's technologies. Scientific knowledge must be fully exploited to increase national productivity and bring prosperity. Nanotechnology application in the agricultural and food industry was first addressed by the US Department of Agriculture (USDA, 2003). It has been identified that the nanotechnology applications in the following areas of agriculture will play a significant role in improving the agricultural productivity.

#### **1. Nano-biotechnology**

The credit for the term "nanobiotechnology" goes to Lynn W Jelinski, a biophysicist at Cornell University, USA. Cell machinery and biological molecules are the nanostructures and nanomachines designed by nature.

##### **a. Next Generation computers**

DNA has been used not only to build nanostructures but also as an essential component of nanomachines. Most appropriately, DNA, the information storage molecule, may serve as the basis of the next generation of computers. As microprocessors and microcircuits shrink to nanoprocessors and nanocircuits.

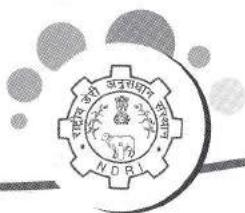
DNA molecules mounted onto silicon chips may replace microchips with electron flow-channels etched in silicon. (Prasanna, 2007).

Biochips are DNA-based processors that use DNA's extraordinary information storage capacity. Biochips exploit the properties of DNA to solve computational problems, in essence, they use DNA to do mathematics. Scientists have shown that 1,000 DNA molecules can solve in four months computational problems that require a century for a computer to solve.

Other biological molecules are assisting in our continual quest to store and transmit more information in smaller places. For example, some researchers are using light-absorbing molecules, such as those found in our retinas, to increase the storage capacity of CDs a thousand-fold.

##### **b. Nanofabricated Gel-free Systems and High Throughput DNA Sequencing**

As a central process, DNA sequencing needs to be improved in terms of its throughput and accuracy. Nanofabrication technology will be critical toward this goal both in terms of improving existing methods as well as delivering novel approaches for sequence detection. The scaling down in size of the current sequencing technology allows the process to be more parallel and multiplex. Research in nanobiotechnology is advancing towards the ability to sequence DNA in nanofabricated gel-free systems, which would allow for significantly more rapid DNA sequencing. (Prasanna, 2007).



## 2. Crop Variety Development

### a. Mutation breeding

Nanotechnology has given a new dimension to mutation research. Nanotechnology can modify the genetic constitution of the crop which is until now done through chemical and physical mutagen. In Thailand, Chiang Mai University's Nuclear Physics Laboratory, using nanotechnology, the scientists changed the colour of the leaves and stem of Khao Kam from purple to green and the grain becomes whitish. The research involves drilling a nano-sized hole through the wall and membrane of a rice cell in order to insert a nitrogen atom. The hole is drilled using a particle beam, (a stream of fast-moving particles, not unlike a lightning bolt) and the nitrogen atom is shot through the hole to stimulate rearrangement of the rice's DNA. This newly derived organism through the change at the atomic level is designated as Atomically Modified Organisms (AMOs) (Warad and Dutta, 1995.).

### b. Nanoparticle mediated gene delivery

Nanoparticle is a potential non-viral vector which has the ability to incorporate genetic materials such as plasmid DNA, RNA, and siRNA into functionalized nanoparticles. Nanoparticles with little toxicity demonstrates a new era in pharmacotherapy for delivering genes selectively to tissues and cells (Sha Jin et al., 2009). Non viral vectors are relatively easy to prepare, less immunogenic and oncogenic; besides, there is no potential for virus recombination and no limitation on the size of a transferred gene.

## 3. Seed Management

### a. Assessment of seed viability

Quantum dots specific to aliveness of embryonic cells can help to identify live and dead seed, similar to the results obtained through destructive methods of Tetrazolium test or Quick Viability test. Quantum Dots are nano-sized semi-conductors made of cluster of electrons which can be fixed on the seeds. When a laser beam passes on the QDs, the electrons get excited and loses energy and exhibit spectral variations. This will indicate the internal biochemical changes in the seed (eg dehydrogenase enzymes, pH, hydrogen peroxide, free fatty acids etc.,) which can be used as a signal to precisely screen the viable seeds.

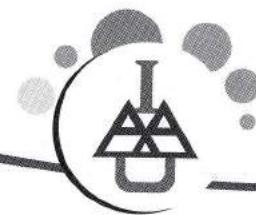
### b. Assessment of quality of packed seeds.

Electronic nose (E-nose) which can sense the 'aldehyde' levels produced inside sealed seed pouches may serve as indicators of potential deterioration level of the seed lot.

## 4. Soil Health Maintenance

Soil health maintenance is a key issue in sustaining crop productivity. In fact major portion of nutrient ions gets fixed in the broken edges of the clay particles, thereby reducing the nutrient availability. Nanoparticles can be spread over the field so that it can adsorb on to the clay lattice and prevent nutrient fixation. This will ensure that nutrients are readily released into the solution for better utilization by the plants.





### **5. Slow Release Fertilizers**

Nutrients released at a slower rate throughout the crop growth enable maximum fertilizer use efficiency. Slow release of nutrients in the environment could be achieved by using zeolites that are a group of naturally occurring minerals having a honeycomb-like layered crystal structure. Its network of interconnected tunnels and cages can be loaded with nitrogen and potassium, combined with other slowly dissolving ingredients containing phosphorous, calcium and a complete suite of minor and trace nutrients. Zeolite acts as a reservoir for nutrients that are slowly released "on demand."

Fertilizer particles can also be coated with nanomembranes that facilitate slow and steady release of nutrients. Coating and cementing of nano and subnano-composites are capable of regulating the release of nutrients from the fertilizer capsule (Liu et al., 2006). A patented nano-composite consists of N, P, K, micronutrients, mannose and amino acids that increases the uptake and utilization of nutrients by grain crops (Jinghua, 2004).

### **6. Plant Disease Diagnostics**

Today in agriculture by the time a disease infection is detected, the infection may be widespread and entire herds/fields might need to be destroyed. Nanotechnology holds the potential for very early detection and eradication. Implanted nanosensors could determine the presence of plant viruses in plants and help us to undertake suitable remedial measures well before the malady causes yield reduction in crops.

Nanocides are nanoscale pesticides which are encapsulated for controlled release of active ingredient. Nanocides are already in the market. BASF of Germany, has developed pesticide formulation namely 'Nanoparticles Comprising a Crop Protection Agent' with an active ingredient of 10 and 150 nm size. The advantage is that the pesticide dissolves more easily in water; it is more stable with higher killing capacity. Bayer Crop Science of Germany has developed an emulsion with nanoscale drop droplets in the range of 10-400 nm termed 'micro emulsion concentrate'. Advantage claimed is reduced application rate, a more rapid and reliable activity and extended long-term activity.

The "Smart treatment delivery system" can be a nanoscale device that would have the capability to detect and treat an infection, nutrient deficiency, or other health problems long before symptoms are evident. "Smart delivery systems" can possess any combination of the following characteristics: time-controlled, spatially targeted, self-regulated, remotely regulated, preprogrammed, or multifunctional characteristics to avoid biological barriers to successful targeting. (USDA, 2003).

### **7. Nanoherbicides for Effective Weed Control**

Nano-encapsulated herbicide molecules can destroy weeds even when they are buried in soil. The polymer coating of herbicides split open only in the presence of moisture in the soil and release the chemicals which circumvent weed seeds from germinating.

### **8. Precision Farming**

Precision farming makes use of computers, global satellite positioning systems, and remote sensing devices to measure highly localised environmental conditions thus determining





whether crops are growing at maximum efficiency or precisely identifying the nature and location of problems. One of the major roles for nanotechnology-enabled devices will be the increased use of autonomous sensors linked into a GPS system for real-time monitoring. Nanosensors can act as mini laboratories to precisely detect the availability of nutrient and water. It can monitor temporal and seasonal changes in the soil-plant system. These nanosensors could be distributed throughout the field to monitor soil conditions and crop growth (Tiju Joseph and Mark Morrison, 2006)

## 9. Post Harvest Technology

### a. Food Preservation

The nanotechnology intervention is used in the food processing, from nutrition delivery to intelligent packaging. Food can be microencapsulated to preserve its flavour and nutritional qualities like vitamin and volatiles. Researchers at Leeds University have developed nanoparticles with antimicrobial properties for safer food packaging. Nanoparticles of titanium oxide, zinc oxide and magnesium oxide as well as a combination of them, once functionalized can efficiently kill microorganisms. They are cheaper and safer than metal based nanoparticles.

Nanosensors can be placed in food packaging or the food itself to detect all kinds of food pathogens like E.coli, Campylobacter and Salmonella by attaching them selves to the pathogens. A single nanosensor can have thousands of nanoparticles that can detect the presence of any kind of pathogen, accurately and rapidly. Nano-sensors can be tailor made to fluoresce into different colours.

In food and beverage industry, added micronutrients, antioxidants, substances like polyphenols and resveratrol present in most foods are degraded or oxidized when exposed to air. Nanocochleates solve early oxidation by individually capturing and wrapping them in a phospholipids wrap and maintain the internal nutrients secure from water and oxygen. BioDeliry Sciences International have developed nanocochleates, which are 50 nm coiled nanoparticles used to deliver vitamins, lycopene and omega 3 fatty acids to cells without affecting taste or odour.

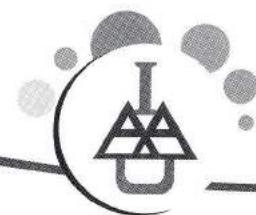
### b. Food Packaging

Nanofilm technology will help in efficient packaging to extend the shelf life of packed food. In 2005, Bayer Ltd., introduced a new packing material made of plastic material 'Durethan', containing nano particles. The embedded particles have a maze like arrangement in the plastic, acting like barriers, which makes it difficult for gases, like oxygen, to pass through the packaging. They actually increase the distance the gas molecules have to travel by causing those molecules to zig zag around the silicate plates thereby increasing the amount of time taken for the molecules to completely penetrate. (<http://www.research.bayer.com/median/pages/2999/polyamides.pdf>).

### c. Nano Bar Codes and Identity Preservation

The bar code is an electronic data depicting several parameters such as date of production, place of packaging, prices etc. and reading this code requires an electronic data reader. With the advent of nano technology, nano based bar codes are also available which can do the same function.





Each day a huge amount of shipments of livestock and other agricultural products are moved all over the world and it is becoming increasingly difficult to keep track on critical control points of the production, shipment and storage processes. Nano-based Identity Preservation (IP) has the potential to revolutionize the entire agri-based industry as it can continuously track and record the history of a particular agricultural product. The nanoscale monitors linked to recording and tracking devices can improve the IP of food and agricultural products. The keys are biodegradable sensors for temperature and other stored data containing the history of stored food for both physical and biological parameters. (Prasanna, 2007).

### **10. Environmental Safety**

Nanoscale particles represent a new generation of environmental remediation technologies. Nanoscale iron particles with large surface areas and high surface reactivity can effectively detoxify common contaminants like chlorinated organic solvents, organochlorine pesticides and PCB. Rapid and complete dechlorination can be done with nanoparticles. For example Pd/Fe nanoscale particle dose at 6.25 gL<sup>-1</sup>, could reduce chlorine levels to below detectable limits. Ethane could also be removed with nanoparticles with in 24 h. Zero valent Iron (ZVI) is used as chemical reductant to treat chlorinated and nitroaromatic compounds. Excessive use of herbicides causes damage to the succeeding crops. Application of silver modified with nanoparticles of magnetite stabilized with CMC nanoparticles recorded 88% degradation of herbicide atrazine residue under controlled environment (Susha et al. 2009).

### **11. Nanotechnology Solutions to Climate Change**

Most of the warming that has occurred over the last 50 years is very likely to have been caused by human activities. Nanotechnology can intervene in possible areas to reduce the harmful greenhouse gas emissions : a) the development of hydrogen powered vehicles b) enhanced and cheaper photovoltaics or solar power technology c) new generation of batteries and super capacitors d) improved insulation of buildings and e) fuel additives to enhance the energy efficiency of motor vehicles. These technologies are being developed elsewhere in the world contemporarily to reduce the dependence on fossil fuels and consequently begin the process of decoupling carbon dioxide emissions from energy.

### **Nanotechnology Research in Tamil Nadu Agricultural University**

Tamil Nadu Agricultural University, Coimbatore is taking a pioneering effort to use nanotechnology to improve the soil health, crop productivity, food preservation besides environmental safety. A course on Nanotechnology has been included in the Undergraduate Curriculum. The University is in the process of constructing state-of-the art facility encompassing sophisticated equipments such as Scanning Electron Microscope, Transmission Electron Microscope, Atomic Force Microscope and Scanning Tunneling Microscope worth over Rs.10 crores. Twelve faculty members have been trained at Cornell University, United States. Further, Tamil Nadu Agricultural University has invested Rs 207 lakh on eight projects. The projects under progress are :

1. Controlled release of nanoherbicides for weed management in rainfed agriculture





2. Nano-fertilizer formulations for promoting balanced crop nutrition and sustainable soil productivity
3. Design and fabrication of nanobiosensor for detecting pathogens in banana
4. Development and evaluation of high barrier nano-composite films for food packaging and technology development for nanoencapsulation and control delivery mechanism for functional food ingredients
5. Application of nanotechnology to enhance the bioavailability of phytochemicals in health foods
6. Customizing nano particles and quantum dots for monitoring live and dead seeds
7. Biosynthesis of nanoscale zero valent iron (Fe<sup>0</sup>) for the remediation of polluted soil and ground water habitat
8. Neem based Nano Pesticide for the management insect pest

Nanotechnology is primarily a multiplier for other technologies, proving enhanced performance and reliability. The technological convergence across the fields of physics, engineering, chemistry, biology, agriculture and food sciences is the essential core of development of nanotechnology. More than individual attempts, the consortium of faculty is important to achieve anything substantial. Therefore, efforts are needed to enhance collaboration between scientists of basic science like physics, chemistry and engineering with scientists of agriculture to realize viable interventions in the field of agriculture .

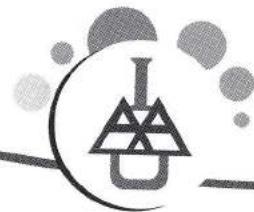
### **Risks and Ethical Considerations**

Technological advances have always been two – edged sword. All materials and products eventually come to the end of their useful life. This means that engineered nano-materials will ultimately enter the waste stream and find their way into land fills and incinerators and eventually into air, soil and water. As a result , it is important , in the development of nanotechnology, to consider how various forms of nano-materials will be disposed off. “If technology has applications, it has limitations too. Right from the beginning it is advisable to have a national regulatory commission on nanotechnology so that people don’t get into litigation later,” cautions the renowned scientist M. S. Swaminathan,

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## Application of Bio-Nanotechnology in Agricultural and Animal Sciences for Food Security

N. C. Patel\*

Junagadh Agricultural University, Junagadh-362001

### Introduction

Food security refers to the availability of food and one's access to it. It is a condition in which all community residents obtain a safe, culturally acceptable, nutritionally adequate diet through a sustainable food system that maximizes community self-reliance and social justice. Food insecurity exists when people do not have physical or economic access to food as defined above.

The food section has four dimensions viz. Availability (production, imports, stocks, food aid), Access (both physical and economic – levels of poverty, purchasing power, marketing and transport infrastructure, food distribution systems), Stability (supplies and access – weather, price fluctuations, natural and human induced disasters), Safe and healthy food utilization (care and feeding, food safety and quality, access to clean water, health and sanitation).

### Factors Affecting Food Security

Factors governing access to food are listed below :

- Declining crop / animal productivity
- Low income from traditional crops
- High dependence on imported food
- Growing incidence of food related diseases
- Increasing incidence of extent of poverty

Existing research has clearly demonstrated the feasibility of introducing nano-shells and nano-tubes into animal systems to seek out and destroy targeted cells. Nano-particles smaller than one micron have been used to deliver drugs and genes into cells. Thus, some building blocks do exist in isolation and are expected to be Paper submitted for IAUA 34<sup>th</sup> Convention being held at NDRI, Karnal during Dec. 7-8, 2009.

integrated into systems over the next 10 to 15 years. It is reasonable to presume over the next couple of years that nano-biotechnology industries will revolutionize animal health and medicine.

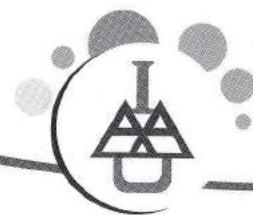
There are only twelve crops which have major share in world food basket. The yield of these crops attained plateau. The stagnation of yield is perhaps due to limitations of classical crops and animal breeding. Further, fillip in the production has to come through biotechnology and nanotechnology or bio-nanotechnology.

### Biotechnology

Biotechnology is an interdisciplinary science that blends various subjects—Biology, Mathematics, Physics, Chemistry and Engineering and applies them together to living cells

\*Vice-Chancellor





to produce a certain product or to improve upon it. Biotechnology is a precision science that enables us to find the most beneficial traits, in terms of added nutrition, increased flavour, or greater ability to fight pests or diseases, and incorporate them into various organisms.

### **Bio-Nanotechnology**

The credit for term "Bio-Nanotechnology" goes to Lyne W. Jelinski, a biophysicist at Cornell University, U.S.A. Bionanotechnology is the intersection of biology and nanotechnology. Molecular biologist helps nanotechnologists understand and access the nanostructures and nano-machines designed by 4 billion years of natural engineering and evolution- cell machinery and biological molecules. It is the intersection of biology and nanotechnology.

### **Applications of Bio-nanotechnology in Agriculture**

The bio-nanotechnology has various applications in Agricultural and Animal sciences like crop improvement, animal health, precision farming, fisheries, disease detection, post harvest technology, green houses, solar energy utilization, water purification, nano-biosensors based disease detection, *etc.*

The bio-nanotechnology has also various application in monitoring quality of agricultural products like increasing solubility in water, sensors could be integrated into packaging materials to monitor the freshness of the food, spoiling of the food could be indicated by a colour change of the sensor and minute amount of chemicals and even presence of bacteria and viruses can be detected with ease on bio-selective surfaces, *etc.*

The bio-nanotechnology has various application in crop improvement like to modify the genetic constitution, mutations –both natural and induced, new white-grained rice variety, changed the colour of the leaves and stems, to insert a nitrogen atom and rearrangement of the rice's DNA, *etc.*

The bio-nanotechnology has various applications in plant disease diagnostics like the detecting of the exact stage of virus, application of some therapeutic to stop the disease, increase the speed of detection and increase the power of the detection, *etc.*

The bio-nanotechnology has various applications in protein micro-arrays which includes discover protein biomarkers that indicate disease stages, assess potential efficacy and toxicity of pesticides (natural and synthetics) and measure differential protein production across cell types and developmental stages, in both healthy and diseased stages, study the relationship between protein structure and function and evaluate binding interactions between proteins and other molecules, *etc.*

The bio-nanotechnology has various applications in animal health like *nano vaccines* for prevention of disease in advance by developing antibody against the particular pathogen, for genetic manipulation, and *nano-apoptosis* which kills the cancer cells and tumor and measurement of changes in the level of estradiol in the blood, *etc.*

The bio-nanotechnology can also be used for the production of plant made vaccines or edible vaccine like for biopharmaceuticals. It is called the next generation of vaccines. This edible vaccine is sub- unit vaccines and mucosal targeted. The advantages of plant made edible vaccines are low cost, needle free shot, easily scale up, no refrigeration





requirements, no risk of provoking infection, elicit mucosal as well as systemic immunity, effective distribution in developing countries, easy consumption by children and no purification required.

Besides Agriculture, the bio-nanotechnology has applications in medical science field.. It includes pharmaceuticals, diagnostic devices and sensors, food preservation and packaging, burn and wound therapy and in stress analysis.

Lastly in the field of energy and environment, the bio-nanotechnology can also be used for water purification, brown field remediation, catalysts, filters, solar cells and fuel cells, *etc.*

### **Ethical and Environmental Consideration**

Gravity and size barriers are negligible at nano-scale. Their thermal motion is significant and water environment is excellent for bio-nano particles. Thus bio-nano particles can expand, travel and penetrate extensively up to red blood cell of human and most of the cell of living organisms. Thus, nano-bio molecules are potential danger to all the facts of environment provided we work wisely and respect the life on this planet.

### **Conclusions**

Precision farming, with the help of bio-nanotechnology, will allow enhanced productivity in agriculture by providing accurate information, thus helping farmers to make better decisions. Finally, it may be possible one day to manufacture food from component atoms and molecules, so-called "Molecular Food Manufacturing". Already some research groups are exploring this, but still from a top-down approach, using cells rather than molecules.

Although the practical application of such technology is far into the future, it is expected that this could allow a more efficient and sustainable food production process to be developed where less raw materials are consumed and food of a higher nutritional quality is obtained. Agriculture, according to the bio nano-vision, needs to be more uniform, further automated, industrialized and reduced to simple functions. In our molecular future, the farm will be a wide area bio-factory that can be monitored and managed from a laptop and food will be crafted from designer substances delivering nutrients efficiency to the body.

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## Nanotechnology and its Application in Agriculture and Food Industry- An Overview

Prof. A. K. Das\*

Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal.

### Abstract

*Nanotechnology applications in food and agriculture is in its nascent stage. This may be used as tools and techniques to detect contamination in food and agricultural products. Currently a lot of work is being carried on nano-sensors targeting improved pathogen detection in food system. These sensors can detect very low levels of molecular signals of spoilage and foodborne pathogens within minute of exposure. It can also be used to improve packaging materials.*

*This technology can also be used in the agricultural insect pest management without leaving any residual toxicity. Water purification is another field of application of nanotechnology. Some of these important aspects are discussed here.*

### Introduction

Nanotechnology is a creation and exploitation of materials with structural features in between those of atoms and bulk materials. At the nanoscale, fundamental properties change. For example, a nanoscale wire or circuit component does not necessarily obey Ohm's law. When we reach nanoscale, everything will change including the gold's colour, melting point and chemical properties. The reason for this change has to do with the nature of the interaction among the atoms that make up the gold. Interactions that are averaged out of existence in the bulk material. Nanogold does not act like bulk gold.

Any material containing particles with size ranging from 1 to 100 nm is called nanomaterial and in this particle size range, those material show peculiar properties, which can not be adequately explained with our present day knowledge.

### Application of Nanotechnology

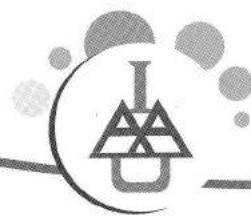
#### (i) In Food Industry

Nanotechnology has been a domain of Physics, Chemistry, electrical engineering and material science. Macroscopic World properties do not vary considerably from the microscopic World, but at the nanometer scale ( 100nm) materials can altogether behave differently. Many biological materials are nanoparticles such as protein is a nanoparticle consisting of a compacted polypeptide nanowires. DNA also has the structure of a compacted nanowire.

Nanotechnology has the potential to improve food quality and safety significantly. Currently a lot of work is being carried out on nanosensors targeting improved pathogen detection in food systems. Many electronic companies have been investigating electrically conducting polymers. These same materials can also be used to manufacture sensors that can detect very low levels of molecular signals of spoilage and foodborne pathogens within

\*Vice-Chancellor





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9. Harald F. Krug. (2005) Nanotechnology: Environmental Aspects. Willy-VCH Verlag GmbH & Co, New York.

**10. Web Resources**

- [www.jnanobiotechnology.com](http://www.jnanobiotechnology.com)
- [www.nanotechproject.org](http://www.nanotechproject.org)
- [www.nanoforum.org](http://www.nanoforum.org)
- [www.bio-scope.com](http://www.bio-scope.com)
- [www.lifescience.org.in](http://www.lifescience.org.in)
- [www.infibeam.com](http://www.infibeam.com)
- [www.springerlink.com](http://www.springerlink.com)
- [www.thehindu.com](http://www.thehindu.com)
- [www.doaj.org](http://www.doaj.org)
- [www.waset.org](http://www.waset.org)
- [www.science.gov](http://www.science.gov)
- [www.safenano.org](http://www.safenano.org)



minutes of exposure. Scientists at the Kopelman Laboratory at the University of Michigan are working on non-invasive bioanalytical nanosensors that could perhaps be placed in an animal's (i.e. cow's) saliva gland to detect a single virus before it has had a chance to multiply and develop disease symptoms. Researchers at the University of Connecticut are working on an "electronic tongue" that detects minute amounts of a huge range of chemicals. This sensor uses tiny electrodes coated with a conductive polymer. According to the researchers involved in the project, this device can detect parts per trillion and costs about 50 cents to produce. It is also expected that the tongue technology could potentially be incorporated into food packages, such as meat starting to spoil. Scientists at the University of Bonn in Germany are working on nanoscale level dirt-repellent coatings. This concept could have important applications at food production sites, in particular abattoirs and meat processing plants. Nanoscale monitors could be linked to recording and tracking devices to monitor temperature changes and detect pesticides and genetically modified crops within the food system. It is expected that such machines/sensors will appear on production lines within a few years.

A recent study from Helmut Kaiser Consultancy, which looked into nanotechnology in the food industry, estimates that nanofood market will expand from \$2.6 billion to \$20.4 billion by 2010. According to the same source, worldwide sales of nanotechnology products to the food and beverage packaging sector jumped to \$860 million in 2004 from \$150 million in 2002. It is believed that about 200 companies around the world are active in research and development in nanotechnology. It is expected that nanotechnology is going to change the whole packaging industry. Nanotechnology enables designers to alter the structure of packaging materials at the molecular level. For example, plastics can be manufactured with different nanostructures to gain various gas and moisture permeabilities to fit the requirements of specific products such as fruits, vegetables, beverage and wine. As a result, shelf-life and flavor and color preservation of the products can be improved. Nanostructured films and packaging materials can prevent the invasion of pathogens and other microorganisms and ensure food safety. Nanosensors embedded in food packages will allow the determination of whether food has gone bad or show its nutrient content. By adding certain nanoparticles into packaging material and bottles, food packages can be made more light- and fire-resistant, with stronger mechanical and thermal performance and controlled gas absorption.

### **(ii) In Agriculture**

Nanotechnology can improve our understanding of the biology of different crops and thus potentially enhance yields or nutritional values. In addition, it can offer routes to added value crops or environmental remediation.

Particle farming is one such example, which yields nanoparticles for industrial use by growing plants in defined soil. For example, research has shown that alfalfa plants grown in gold rich soil, absorb gold nanoparticles through their roots and accumulate these in their tissues. The gold nanoparticles can be mechanically separated from the plant tissue following harvest.

The major problem in the agricultural insect pest management today is the development of suitable agents which can kill insects physically. This has become immensely important



considering the fact that, in EU countries, all kinds of chemicals and biologically active pesticides have been banned in the urban agricultural and horticultural settings. So, there is an urgent need for discovering purely physically active bio-pesticides. One of the plausible ways of killing insects is to puncture insect cuticle and allow the Fick's law of diffusion to operate so that insect loses water from its body and finally die via desiccation. Insect cuticle has a thick layer of lipid, which if could be punctured simply by physiosorption of these particles and then it would act as effective biopesticide. Nanosilica particles have been found to be very effective for this purpose. A range of insect pests, both in tropical as well as temperate climates, has been tested by nanosilica particles. For example, coconut mite is causing havoc devastation. Some of nanosilica have shown excellent efficacy against mite and it is expected that these will be effective against coconut mite as well.

A large number of pure plant molecules (e.g. lectins, flavonoids, terpenoids, phyto-cholesterols, phyto-esters, phyto-aldehydes etc.) have been generated. These plant molecules, when attached to nanosilica particles, will facilitate tissue specific delivery of the compound. These works gained immense importance on the following points :

- (i) to generate ecofriendly pest control strategies against mite and other insect pests to be incorporated in the existing IPM.
- (ii) as natural nanosilica kills insects by physiosorption and therefore it acts by physical means.

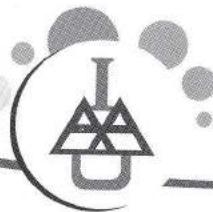
Nanotechnology can also be used to clean ground water. The US company Agronite is using 2mm diameter aluminium oxide nanofibres (nanoCerum) as a water purifier. Filters made from these fibres can remove viruses, bacteria and protozoan cysts from water. While some companies are working on water filtration, others are following a purification approach. Nanocheck, prepared by Altairnano, contains lanthanum nanoparticles that absorb phosphates from aquatic environments. Applying these in ponds and swimming pools effectively removes available phosphates and as a result prevents the growth of algae. The Company expects this product to benefit commercial fish ponds which spend huge amount of money to remove algae.

Research at Lehigh University in the US shows that an ultrafine, nanoscale powder made from iron can be used as an effective tool for cleaning up contaminated soil and ground water – a trillion dollar problem that encompasses more than 1000 still untreated Superfund sites (uncontrolled or abandoned places where hazardous waste is located in the U.S). The iron nanoparticles catalyse the oxidation and breakdown of organic contaminants such as trichloroethane, carbon tetrachloride, dioxins and PCBs.

### Acceptance in the Society

Nanotechnology is still an emerging technology. Experiences from the genetically modified organism debate clearly indicate that public support and consumer acceptance of this technology will depend on the behavior of institutions responsible for development and regulation of technological innovations and risk assessment. One way to secure public support for this technology is to dedicate resources to further research on widely shared goals, such as clean and renewable energy and public health intervention to ensure development of healthy and nutritious foods and crops. Distribution of expertise





and benefits and availability of choices worldwide would help public acceptance of the technical innovations derived from nanotechnology and nanoscience. Currently, there are a number of on-going studies that attempt to detail, analyze and assess the potential problems and benefits, pitfalls and known and unknown issues related to developments in nanoscience and technology. However, health, environmental and workers health risk analyses, and a regulatory framework for nanotechnology and nanoscience are some of the issues that have to be addressed as the technology matures.



## Role of Gene Revolution in Rice and Wheat Research for Food Security

B. Mishra\*

Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (J&K)

The current global population of 6.4 billions is expected to reach 7.5 billions by 2020 and 9.0 billions by 2050 AD. Most of this population increase will occur in developing countries of Asia and Africa. *Rice and wheat are the most important crops in India and both occupying second rank in production in the world.* Globally rice is cultivated now on 155.8 million hectares with annual production of around 659 million tons and average productivity of 3.6 tons/ha. More than 90% of the rice is produced and consumed in Asian countries. The other continents in which rice is grown are Africa (7.78% of the global area), South America (6.4%) and North America (1.4%). In India, rice was cultivated in an area of 44.6 million hectare with a production of 134 million tons of paddy, average productivity being 3.01 t/ha. Globally, wheat is grown in 214 million hectares with production of around 606 million tones and productivity of 2.8 tons/ha. The wheat producing major countries are China, India, USA, the European Union, Soviet Union, Canada, Australia, Turkey and Argentina. India produces 80.6 million tones of wheat from 27.8 million hectares of area. Large scale food shortages were experienced in India and in several neighbouring countries in Asia during late 50s and early 60s. Frequently there were dire warnings of impending widespread famines. During this grim scenario, the semi-dwarf, fertilizer responsive, high yielding genotypes of rice and wheat were introduced, which led to spurt in their production and productivity. The food situation in many Asian countries progressively became better. This phenomenal turn around on food front from scarcity to self sufficiency and in few cases even to exportable surplus is referred to as "Green Revolution".

During the last five decades the rice and wheat production trend has kept in pace with population growth trend. Rice exports from India have steadily grown from 1.8 million tonnes during 2001 to 5.0 million tons during 2007-0. India now occupies second position in rice export, next only to Thailand, among the rice trading countries of the World. This has been mainly possible due to the contributions made by the green revolution technologies. However, the surplus production scenario has no room for complacency. Keeping in view the average annual population growth rate of 1.5% and per capita consumption estimate demand for rice and wheat is expected to be 140 M tons and 10gmt by 2025 to maintain the level of self-sufficiency. Is there a need for a paradigm shift in rice and wheat research to meet the challenges of the future decades for ensuring food security? Do we need to adopt the gene revolution technologies? After a brief review of rice and wheat research in India and considering the gains obtained through green revolution technologies and hybrid rice technology, the possibilities and prospects of utilizing the gene revolution technologies (i.e. biotechnology) are considered for further enhancing the production and productivity of rice and wheat for not only ensuring food security but also nutritional security.

\*Vice-Chancellor



### **Initial Phases of Rice Improvement in India and Ushering of the 'Green Revolution'**

Systematic rice research in India was initiated in 1911 in the then provinces of Bengal and Madras. Initial research work involved collection of land races and their systematic evaluation. Pure line selection was practiced with these land races and more than 300 pure line varieties were developed in various parts of the country during the first 40 years. Recombination breeding was initiated subsequently. Prior to sixties, most of the rice varieties cultivated were tall and leafy with a grain biomass ratio of 3:7 (harvest index - the ratio of grain to the total biological yield of the crop - of 0.3) and were susceptible to lodging with a productivity of less than 1.0 tonne/ha. Breeders soon realized that to increase the yield potential it was necessary to promote nitrogen responsiveness of the rice plant by developing lodging resistance. This was accomplished by reducing the plant height through incorporation of a dwarfing gene *sd-1* conferring short stature, from a Chinese variety, *Dee-geo-woo-gen*. The presence of this single gene ensured that the varieties were semi-dwarf, had good response to the applied fertilizers, non-lodging and better in yield, thus raising the productivity to more than 4-5 tonnes/ha compared to the traditional Indian rice cultivars. The change in plant architecture brought out by dwarfing gene *sd-1* has now been elucidated to be a single gene mutation which makes the plant non-responsive to endogenous Gibberellin. Scientists at the International Rice Research Institute (IRRI) developed IR8 in 1966, a semi-dwarf variety with sturdy stems that became quickly popular with farmers and ushered the green revolution in rice in Asia. It has a harvest index of 0.5, and with appropriate crop management doubled the yield potential of varieties. Being photo-insensitive could be planted at any time of the year and hence the rice crop could fit in well with any cropping system that farmers would find profitable. This land-saving technological innovation enabled farmers to increase food production without recourse to extending cultivation to marginal land. Adoption of semi-dwarf high yielding rice varieties on a large scale, however, increased farmers' dependence on chemical fertilizers. It also required control of water regimes on rice farms and therefore supplementary investment was needed to develop irrigation facilities. In the early years of introduction of High Yielding Varieties (HYV), before resistance to pests was developed, there was a need to rely on insecticides for pest management. The evolution and spread of semi-dwarf rice varieties, nonetheless, is an excellent example of concerted efforts of breeders that has ensured country's food security. The ever-increasing population coupled with depleting soil and water resources and transformation in dietary habits necessitates a change in our focus on rice improvement and deployment of altogether new strategies to accomplish. The semi-dwarfing gene has been recently cloned in rice and identified to be a mutation in gibberellin response pathway in the plant. Efforts are on by rice scientists to identify novel mutants for gibberellin biosynthetic and response pathway in order to better semi-dwarf rice lines.

### **Breeding Strategies in Rice for Post Green Revolution Era**

Most traditional varieties in tropical and subtropical Asia grown during 1960s matured in 160-170 days and many were photoperiod sensitive. These were suitable for growing one crop of rice a year during the rainy season. Considering the pressure exerted by the demand for food for the population, plant breeders subsequently developed varieties that matured early in 100 to 110 days without sacrificing the yield. The key to the success was



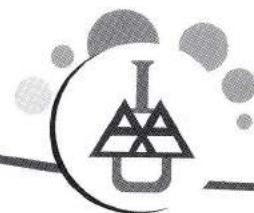
the selection of the genotypes with rapid vegetative vigor at the earlier growth stages. This helped farmers to grow two rice crops during the year in areas where there are good irrigation facilities, or to introduce a non-rice crop in the rice-based system under rainfed condition. Most widely grown early duration rabi rice varieties like Krishnahamsa, IR 64 are examples. As the profitability in rice farming has increased with new varieties, a relatively small number of improved varieties have replaced thousands of traditional ones, thereby reducing the genetic variability of the rice crop. The reduction in biodiversity, coupled with vegetative growth and continuous cropping, increased the vulnerability of the rice crops to insects and diseases. Scientists addressed this problem by incorporating resistance to major insects and diseases in newly released modern varieties. Large germplasm collections were screened and donors for resistance identified. Utilizing these donors, improved varieties with resistance to four major diseases (blast, bacterial blight, sheath blight and tungro) and four insects (brown planthopper, green leafhopper, gall midge and stem borers) have been developed. Large-scale adoption of varieties with a broader genetic-base has helped stabilize rice yield and reduce the use of pesticides, which are known to have serious adverse effects on human health and on water quality.

### **Hybrid Rice Technology and its Applications**

Despite the tremendous progress witnessed in terms of increase in the rice production and productivity over the past few decades, which was possible mainly due to exploitation of plant-type based improvement strategies and reduction of pest disease incidences, the plateauing of yield levels is a matter of concern for rice breeders and economists. Unless radical changes are ushered in rice improvement programmes, there may not be sufficient rice to feed the burgeoning population by 2035 A.D. One strategy, which has the potential to boost rice production, is the development and release of rice hybrids.

Rice being a self-pollinated crop, it was thought earlier that heterosis levels might be too low for commercial production of hybrids. The developments in Hybrid rice in China predominantly due to the efforts of a single individual – Prof. L.P. Yuan (who is regarded as Father of hybrid rice technology) during the 1970s have significantly changed the course of rice production. Through dogged determination, the Chinese have demonstrated that Hybrid rice technology could be commercially viable and a means to address the concerns related to rice yield stability and plateauing. The development and utilization of cytoplasmic male sterile (CMS) lines from the WA cytoplasm background have resulted the CMS lines currently used all over the world like IR58025A, IR62829A, ZhenshanA etc in the popular three-line hybrid rice technology. Heterosis to the tune of 15-20% has been successfully demonstrated on a commercial scale in self-pollinated crop like rice through the three-line hybrid system in India as well. Many elite hybrids like Sahyadri, KRH2, DRRH1 and 2, CoRH2, PusaRH10 etc. have been released by public sector and these hybrids are being cultivated in many locations across the country. The interest and zeal of public section in developing hybrids has been matched by private sector and many private bred hybrids have been developed and released in the country. As on date more than 20 different companies have invested in hybrid rice, which are being grown widely in states like Chattisgarh, Jharkhand, Eastern Uttar Pradesh, Bihar etc. Significantly bulk of the areas where hybrids are being cultivated in these states are prone to abiotic stresses and hybrids have been documented to perform exceedingly well as compared to inbred varieties in such adverse ecosystems. Due to these concerted efforts by





both public and private sectors, the area under hybrid rice has increased from a meager 0.4 Mha in 2002 to around 1.5 M ha in 2008.

### **Initial Phases of Wheat Improvement in India and Advent of “Green Revolution”**

The three species of wheat namely, *Triticum aestivum* (bread wheat), *Triticum durum* (macaroni wheat) and *Triticum dicoccum* (Emmer or Khapli) grown on commercial basis in the Indian subcontinent from pre-historic times are of spring type. Systematic wheat improvement in India started during 1905 and since then has undergone many developmental changes after pioneering works of imperial botanists Howard and Howard at Pusa (Bihar) in the beginning of twentieth century. The contributions made by the unknown and unsung Indian farmers in preserving enormous variability in form of land races of wheat through knowingly/unknowingly selection need special mention. The pure line selection practiced by earlier wheat workers in local land races which happened to be mixtures resulted in the development of several quality wheat varieties including NP 4, NP 6, NP 12, Pb8, Pb 8A, Pb 9D, Pb 11, K13, K46, AO13, AO 85, AO 90, Bansi, Motia, Gulab etc amongst which NP 4 won international award for its grain quality and also became popular in countries like Australia, South Africa and Hungary. Thereafter, recombination breeding between pure lines led to the development of varieties like Pb C 518, Pb C 591, NP 52, NP 80-5, NP 120, NP 125, NP 165, Niphad 4, AO 68, AO 113 and AO 115 however, with little emphasis on disease resistance. Following this Prof. K.C. Mehta and Dr. B.P. Pal initiated pathological research in wheat leading to the development of disease resistant NP 700 and NP 800 series. Notably NP 809 being the first Indian wheat variety resistant to all three rusts was a classical achievement. Some other popular old varieties were Pb C 228, C 273, C 281, C 519, Hyb 11, Hyb 23, Hyb 38, RS 31-1, Kenphad 28 etc. During 1947-48, total wheat production was just 5.6 million tons with an average yield of 0.8 tons per hectare as most of the Indian wheat varieties were tall in stature and prone to lodging, susceptible to diseases and less responsive to inputs. Despite of all possible efforts, India could not harvest beyond 12.3 million tons of wheat till 1964-65 crop season.

The beginning of growing dwarf wheat cultivars was made by introducing the seeds of four varieties, Sonora 63, Sonora 64, Mayo 64 and Lerma Rojo 64 along with 613 segregating lines from CIMMYT, Mexico. This provided the base material for development and commercial release of another five important varieties namely PV 18, Kalyan Sona, Sonalika, Chhoti Lerma and Safed Lerma thereby ushering in the Green Revolution in India. These dwarf wheats were highly responsive to inputs, were non-lodging types and possessed desired level of disease resistance. Due to these qualities, the dwarf wheat varieties could yield exceptionally high under best management production conditions especially in Indo-Gangetic Plains (IGP). Later in 1970-75, it was realized that semi-dwarf, high yielding and input responsive lines should be crossed with local wheats to combine attributes from both the groups.

It was sheer coincidence that the advent of dwarf wheat and establishment of the All India Coordinated Wheat Improvement Project (AICWIP) happened concurrently in the year of 1965. This was an important milestone that brought about systematic developments in wheat research and had resulted in the real breakthrough in wheat productivity. Since then the wheat



research had made spectacular advances in yield potential, productivity and sustainability of wheat system. Since the initiation of the Green Revolution in the mid sixties, India achieved remarkable increase in production and productivity of wheat. This is indicative from the fact that the area, productivity and production of wheat to the tune of 123%, 226% and 630% respectively by the year 2006-07 as compared to 1965-66. This achievement in India's wheat production has been perhaps the most important and unparalleled in the history of developing world as stated by the Nobel Laureate Dr. N.E. Borlaug. The launch of the Green Revolution and rise in production coincided with productivity growth in the magnitude of 2-3% at national level saving crores of rupees to Indian treasury for importing wheat from other countries. Few land mark wheat varieties like Sonalika, Kalyan Sona, HD 2009, WL 711, WH 147, UP 262, Lok 1, HUW 234, HD 2285, HD 2329 and PBW 343 dominated wheat areas for reasons of wider adaptability, high yield potential, disease resistance, grain quality, maturity duration, plant height and other desirable agronomic traits.

India became second largest wheat producing country during 1997-98, and achieved a record production of 76.4 million tons during 1999-2000. The total production increased to the magnitude of more than six folds from roughly 12.3 million metric tons in 1964-65 to an estimated 76.8 million metric tons in 2007-08.

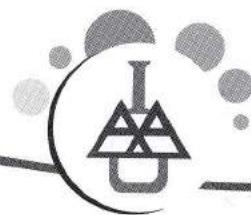
Some of the major landmarks in wheat improvement at global level were the introduction of new dwarf plant type utilizing Norin 10 genes and spring x winter wheat hybridization culminating into recombination breeding leading to the development of 'Veery' germplasm (1B/1R), development and use of long spike bultre gene pool for improving spike length and grain number and finally the development and use of synthetic wheats for improving a number of traits like resistance to biotic stresses, tolerance to abiotic stresses along with adaptability to varying environments. The wheat programme since its inception (1965) has released 344 wheat varieties (291 bread, 46 durum, 4 *dicoccum* & 3 triticale) for cultivation under different production conditions in all the six wheat growing zones.

### **Achievements Made in Wheat Varietal Improvement**

The success of these varieties can be gauged from the fact that the area, productivity and production of wheat have gone up by 123%, 226% and 630% respectively during last 42 years. During last decade India is occupying the second position in the list of wheat producing countries in world, next to only China. India in particular has not faced any rust epidemic since last three and half decades because of proper deployment of rust resistance genes in wheat breeding programmes. Taking clue from recently conducted yield tests in the All India Coordinated Trials in North Western Plains Zone (NWPZ) which is presently covered by the highest yielding variety PBW 343, a yield gain of 11.93% has been seen in case of DBW 17, an improved variety released in 2006. DBW 17 has not only given higher yield over PBW 343 but also possessed inbuilt resistance against rust diseases including the important stripe rust virulences like 78S84 and 46S119. Similarly, the new wheat variety GW 366 gave a yield advantage of 5.9% over long-term check variety GW 322 in Central Zone. In addition, 110 wheat genetic stocks carrying novel genes/traits have been developed and registered. These stocks are being used in the hybridization programme.

However, it is a matter of great concern that for the last several years plateau in wheat productivity is being observed. There is a very little scope for increasing the area under wheat





cultivation, so the major challenge is to break the yield barriers. Since a yield jump as observed in 60s does not appear to be possible now, there is need to follow an approach for gradual gain in yield in the breeding populations in order to break the yield plateau. For the purpose, already known high yielding diverse lines are being used as base material for incorporating some useful traits from other unadapted cultivars or lines. In this direction, the winter x spring wheat hybridization, interspecific/wide hybridization for introgression of useful genes from related wheat species is playing an effective role. In addition, other unexploited germplasms such as *buitre* germplasm for grain number and spike size, synthetic hexaploids for thousand kernel weight, resistance to heat stress, Karnal bunt, rusts and waterlogging and Chinese sub compactoid germplasm for lodging resistance, noodle quality and high yield potential are being utilized, as these unexploited materials have shown promise in Indian wheat improvement programme. The development of hybrids in wheat is another promising approach to break the yield barriers and to get the quantum jump. A basic research to re-address hybrid wheat development through CMS and CHA approach was initiated in 1995 in a network mode. Though initially hybrids were developed using the CHA approach, these could not be commercially exploited due to non-sterile late tillers, residual effect of the chemicals as well as unstable and low heterotic levels over locations. Hence the emphasis has now been shifted to development of hybrids through CMS based genetic system.

Increase in wheat production in the country can be either through area expansion or increase in productivity. There is hardly any scope for area expansion in the country, such expansion in some place would be offset by reduction in other places. However there is considerable scope for raising production through increase in productivity per unit of land. Accordingly, the strategy is to sustain production in NWPZ, raise productivity substantially in NEPZ and promote production of quality wheat in Central and Peninsular zone. The NEPZ and CZ regions, which have low level of wheat yield and hold high potential for growth, have comparatively weak R&D and infrastructural facilities. Thus steps should be taken to develop proper strategy for increasing the output in these regions.

In view of the changing environmental conditions, emerging new RCTs, need for diversifying cropping systems, availability of major and minor nutrients, scarcity of water for irrigation, threat of new virulence of diseases and pests and quality needs of wheat based industries, it is imperative to re-orient and infuse new innovations in varietal improvement programme. To achieve 109 million ton target by 2025, we need to concentrate and work on long term and short-term strategies. Among these the short-term strategies give more emphasis to the effective transfer of available technology to the farmers. While, the long term strategies are to address the anticipated threats like climate change, water scarcity, need for tillage specific varieties, improving yield potential of future genotypes and so on.

### **Wheat Quality - An Area to Develop Product Specific Genotypes**

Developing product specific varieties, increasing protein content, sedimentation value and grain hardness to match international standards, improving beta-carotene and protein content and semolina recovery in durumms and biofortification are some of the issues that relates to wheat quality.

Annually wheat grain samples are collected and analyzed to identify product specific varieties/areas and classify/grade the Indian wheat varieties based on various parameters



(physico-chemical, electrophoretic, rheological, HMWGS and baking evaluation). Thus, varieties C 306, PBW 175, K 9107, NW 1014, Sujata, Lok 1, HW 2004 etc. (good for chapatti); HD 2285, HI 977, HD 2189, DWR 162 etc. (good for bread); Sonalika, UP 2425 (good for biscuit) and PDW 233, WH 896, HI 8498 etc. (good for pasta products) have been identified.

### Promising genotypes for specific end product quality

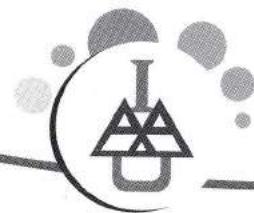
Quality product	Promising genotypes
Chapatti (Score >8.0/10)	C 306, LOK 1, SUJATA, RAJ 3765, HD 2285, PBW 373, PBW 533, HUW 234, K 9107, MACS 6145, MACS 6164, NW 1014, HI 1500, UP 262, HW 2004, DL 788-2, GW 273, GW 322, HD 2833, GW 322 and GW 173
Bread (>575 ml bread loaf volume)	HI 977, HS 240, VL 738, HD 2285, HD 2733, LOK 1, GW 120, GW 173, GW 190, HD 2189, MACS 2496, NI 5439, K 9107, HD 2733 and NIAW 917
Biscuit (>7.5 biscuit spread factor)	Sonalika, UP 2425, WH 542, HD 2687, Raj 3765, PBW 373, and DBW 16
Pasta	PDW 233, WH 896, HI 8498, HD 4672, RAJ 1555, A-9-30-1, MACS 2846, DDK 1009 and NP 200

The promising varieties have also been identified for individual quality parameters like protein content, sedimentation value, extraction rate, wet/dry gluten, gluten index, beta-carotene, iron, zinc, copper and manganese content, which can be used in the breeding programme for the improvement of wheat quality.

### Promising genotypes for protein, $\beta$ -carotene and micro-nutrients in bread an durum wheat

Parameters	Promising genotypes	
	<i>T. aestivum</i>	<i>T. durum</i>
Protein	Sonalika, K 9107, GW 173, DL 788-2, Lok 1, MP 1010 and NIAW 34	HI 8664, RKD 130, HI 8498, CDW 04 and HI 8663
$\beta$ -carotene	UP 2645, HS 240, VL 804, HS 420, DBW 17, HD 2687, PBW 343, PBW 502, PBW 373, 306, HD 2824, HI 1531 and HD 2891	PDW 300, PDW 233, WH 896, HI 8663, HI 8664, MACS 3572 and AKDW 4155
Iron	NW 1014, HUW 234, NIAW 34 and HW 5001	NIDW 295, MACS 3444, MACS 1967 and HD 4672
Zinc	K 8027 and GW 322	MACS 3444, MACS 1967 and HD 4672
Copper	NW 1014, HUW 234 and GW 173	PDW 300, PDW 291, HI 8498, HD 4672, NIDW 295 and AKDW 2997-16
Manganese	VL 829, Sonalika, NW 2036 and HUW 234	PDW 291, MACS 3444 and MACS 1967





For improving the wheat quality for chapatti, bread and biscuit, experimentation is on and sufficient material is available exhibiting chapatti quality, bread loaf volume and other products. The combination of both soft grain characteristics and weak gluten is useful in developing varieties suitable for biscuit making. Nap Hal, an Indian land race of wheat possessing double null trait has been found as potential donor for improving the biscuit making quality to a great extent.

The Indian wheat programme has a strong multi-disciplinary team across the country and it can be concluded that the synergy between research, developmental and extension workers, can lead India to archive the projected demand of wheat in time to come.

### **Rice and Wheat Improvement – Challenges of the Future**

The efforts of traditional, hybrid rice *and wheat* breeders have no doubt brought the rice and wheat yield to such a stage where at least for the present; food production rates will outrace population growth. But we should not be complacent as the vagaries of monsoon disturbing trend with respect to soil health *and predicted adverse effects of global warming* are bound to destabilize rice production and we must therefore be ready to face the challenges of the future, which are as follows:

- Bridging the yield gap
- Rapidly changing climate
- Deteriorating soil and water quality and availability
- Fast changing pest and disease scenario
- Improving nutritional quality of rice and wheat to combat malnutrition

### **'Gene Revolution' to Meet Future Rice and Wheat Production challenges**

For meeting the future demands and challenges, one of the feasible option is judicious and pragmatic application of biotechnological tools, which have ushered in a 'gene revolution' in rice *and good progress in wheat*. A concerted effort for development and application of biotechnological tools in rice *and wheat* improvement is the need of the hour. The complete sequencing of rice genome and availability of the sequence in public domain in 2005 has opened new vistas. Like other countries, India has begun its focus on rice *and wheat* biotechnology in the right earnest.

From a breeder's perspective, biotechnology helps to add precision in the breeding process to become more target oriented and purposeful compared to traditional breeding. Biotechnology can help in improving rice *and wheat* breeding through:

1. Transfer of economically important traits across genus/species barrier into the rice *and wheat* gene pool (i.e. Broadening the genetic base)
2. Manipulation of target trait without disruption to the non target regions of the genome (i.e. Increasing efficiency in selection)
3. Shortening the breeding cycle

The three broad applications of rice and wheat biotechnology that are expected to contribute both directly and indirectly towards improvement efforts in India are:



- DNA marker technology
- Genetic engineering
- Application of genomic tools

### 1. DNA Marker Technology

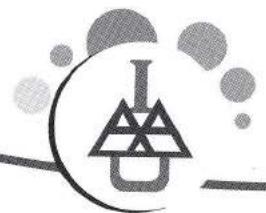
This refers to the application of DNA based markers in breeding programmes to improve the selection efficiency. Selection for segregants carrying desired traits has always been the hallmark of plant breeding activities since the beginning of crop improvement. Plant breeders generally use phenotype as the basis along with morphological markers and statistical methods to select superior segregants. But selection based on morphological markers has many limitations like influence of the environment on the expression of the trait phenotype, less abundance of morphological markers and stage specific expression of traits. As compared to morphological markers, analysis of polymorphism at DNA level can lead to better inferences. Various techniques are applied to detect polymorphism at DNA level and these polymorphic regions in the DNA are referred to as molecular markers or DNA markers. Like any other genetic markers, DNA markers which are located near a gene controlling a trait co-segregate with the trait phenotype across generations and because of this property DNA markers are highly useful. Breeders can use these markers to complement classical breeding techniques and can select segregating plants based on the DNA marker genotype rather than waiting to observe the phenotype. Many molecular genetic linkage maps have been constructed and a multitude of DNA markers have been identified to be linked to traits of agronomic importance. Among the different types of DNA markers used in rice and wheat genetics and breeding, PCR based markers, which utilize the technique of polymerase chain reaction (PCR) are the most useful due to their simplicity, robustness and speed of assay. Many PCR based markers like randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter-simple sequence repeats (ISSRs) and simple sequence repeats (SSRs) are being used. Of these, SSRs are the most popular among breeders due to their cost effectiveness, repeatability, robustness, simple handling and most importantly co-dominant nature of their amplification pattern which helps to discriminate homozygous individuals from heterozygous ones.

Three important application of DNA markers have been visualized in rice and wheat genetics and breeding. They are:

- to determine the allelic status of genes [i.e., determining whether homozygous or heterozygous] conferring identical phenotypes,
- use in marker assisted selection and
- map based cloning of genes.

Molecular markers have been used for tagging and mapping of many agronomically important genes. These include some major genes in rice conferring resistance to bacterial blight, blast, gall midge, brown plant hopper and rice tungro virus. Using molecular markers, breeders at IRRI, Philippines had earlier developed near isogenic lines (NILs) of bacterial blight resistance genes in the genetic background of IR24 and blast resistance genes in the genetic background of Co39. Using the PCR based molecular markers linked to three major bacterial blight resistance genes, molecular breeders at IRRI, PAU-





Ludhiana, IARI-New Delhi and DRR-Hyderabad have introgressed these genes into the genetic background of elite rice varieties IR24, PR106, Pusa Basmati-1 and Samba Mahsuri respectively. The introgressions into Pusa Basmati-1 and Samba Mahsuri represent the first successful products coming out of rice biotechnology programme in India.

Molecular markers are not only useful in marker assisted breeding but also for assessing genetic purity of seeds of rice hybrids, their parental lines and elite varieties. DRR in collaboration with CCMB has developed a molecular marker based strategy for purity assessment of seeds of rice hybrids and parental lines. Recently, at DRR, a mitochondrial SSR marker which can distinguish WA-CMS lines of rice from their maintainers has been developed and this marker will be highly useful for assessing purity of CMS lines and is expected to have a lot of commercial value. Another potential application of molecular markers in hybrid rice technology is for indirect selection of fertility restorer lines. Many PCR based markers linked to fertility restorer genes have been identified in rice which will be useful in quick identification of restorers in germplasm lines and segregating material, thus avoiding costly and time consuming process of making test crosses and their evaluation which takes almost a year. The marker technology requires just one week time.

Molecular markers which are very closely linked ( $< 0.5$  cM) to agronomically important genes are useful for map based cloning of these genes. A typical example is the major bacterial blight resistance gene *Xa21*. Using molecular markers closely linked to *Xa21*, the research group at University of California, Davis analyzed genomic libraries and identified the clones possessing *Xa21*. Many other genes have been cloned with the help of closely linked molecular markers. These include the major recessive bacterial blight resistance genes *xa13* and *xa5*, dominant bacterial blight resistance genes *Xa1*, *Xa26* and *Xa27* and blast resistance genes *Pi-ta* and *Pi-kh*.

Hence the emphasis has now been shifted to development of hybrids through CMS based genetic system. Biotechnological interventions such as marker assisted selection are now being used in the gene pyramiding programme for developing wheat varieties resistant to rusts as well as for superior quality and nutritional parameters. A research programme in the form of a "Network project on gene pyramiding for resistant to multiple biotic stresses in crops" has been initiated with the objective of improving disease resistance in existing cultivars. PCR based markers linked to disease resistance genes are being used for marker assisted pyramiding for improving the most popular varieties such as LOK 1, HUW 234, PBW 343, HD 2687, HD 2733 and WH 147. Future research at DWR and other centres of excellence are given priority to explore potential of functional genomics to elucidate genes and their functions associated with resistance to biotic stresses, tolerance to abiotic stresses and nutritional quality of wheat.

The most serious constraints to wheat production are a host of biotic stresses. Among the major biotic stresses, control of rusts and foliar blight are more critical for achieving higher yields. However, the threat of a new black rust race (Ug99) looms large as most of the wheat lines available in India carrying the *Sr31* gene are susceptible to this race. Fortunately, timely steps have been initiated by the Council and also through the launching of 'Global Rust Initiative' (GRI) to combat this impending problem. The identified genetic stocks have then been used in our crossing block at DWR, Karnal. All these stocks carrying *Sr24* and *Sr25* were





also sent to wheat breeders of the country for their inclusion in crossing block and now form an integral part of NGSN, where it has been reported to be most utilized. Subsequently, in 2006 a set of 102 lines consisting of elite advance lines along with released varieties were sent to Kenya for screening against Ug99 and a number of wheat varieties viz., GW 273, GW 322, HI 1500, HD 2781 and MP 4010 expressed resistance to Ug99. Interestingly, these resistant wheat varieties are recommended for cultivation in the central and peninsular regions of the country where the likelihood of epiphytotic of Ug99 is more. The Sr-genes like *Sr25*, *26*, *27*, *32*, *33*, *35*, *40*, *43*, *Sr39/Lr35* have been found effective against this new virulence. During the current summer 318 wheat lines consisting of notified varieties, genetic stocks and elite materials have been screened against Ug99 at Kenya.

Besides this, a new race of yellow rust, 78S84, has been observed on the most widely grown variety PBW 343, hence an urgent need for an alternative is imperative. A new wheat variety DBW 17 which has been developed and released recently, possesses adult plant resistance to this new race with superiority in yield also. The Directorate and its cooperating centres have initiated breeding programmes for using new and more effective rust resistance genes like *Yr5*, *Yr10*, *Yr15* for yellow rust, *Lr24*, *Lr28*, *Lr35*, *Lr37* for leaf rust and *Sr24*, *Sr25*, *Sr26* for stem rust. The problem of leaf blight is more prominent in the north eastern region and is being addressed through our linkages with CIMMYT-South Asia and cooperators located in the region. Other problems like Karnal bunt, powdery mildew, head scab and pests (aphids, termites, nematodes etc.) are also receiving due attention in the wheat improvement programme. The entire plant protection strategy is moving towards the integrated pest management mode focussing on surveillance, biological control and other eco-friendly methods.

## 2. Genetic Engineering

Genetic engineering refers to the process of mobilization of useful genes from one organism to another. The most important advantage of genetic engineering is capacity to mobilize many genes into the primary gene pool of rice and wheat without much disruption to their genome. Genes of other organisms, i.e., transgenes can be introduced into the rice and wheat genome in two different ways:

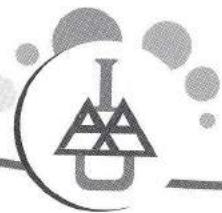
1. through biolistic transformation
2. through Agrobacterium mediated transformation.

Among these, the methodology of Agrobacterium mediated transformation is preferred in rice due to the limited copy number of transgene integration, well characterized integration pattern and minimum or no disruption to the transgene construct.

Many transgenes of agronomic importance have been introduced in rice. These can be classified based on their trait as follows:

1. biotic stress resistance genes [e.g., *Cry1Ac*, *Cry1Ab*, *Cry1Aa*, *Chitinase*, *Xa21*, *Gna lectin*, *Thaumatin like protein* etc],
2. abiotic stress tolerance genes [e.g., *DREB*, *Gly1*, *Gly2*, *TPSP fusion gene*, *Glycine betaine synthase* etc] and
3. Nutritional enhancement [e.g., *Phytoene synthase*, *Phytoene desaturase*, *Ferritin*, *lactoferrin* etc].





### Similarly many examples exist in wheat too

The technology of genetic engineering can enhance the capacity of breeder's manifold and manipulation of trait phenotypes like stem borer resistance and sheath blight tolerance which have not been so far possible due to limitations of traditional breeding. It is now possible with the help of transgene technology. Transgenic rice varieties with different versions of *Bt* genes like *Cry1Ab*, *Cry1Ac* and *Cry1Aa* have shown excellent field resistance to stem borer in many National and International trials carried out under biosafety conditions. Genetic engineering work for introducing alien genes for resistance to stem borer, BPH and abiotic stress such as tolerance to salinity and drought in rice and wheat is under progress at various research laboratories across the country.

Transgenic technology is also being employed to attempt to convert rice from C3 to C4 plant. It is hoped that through this the photosynthetic efficiency and consequently, the yield can be increased tremendously. The researchers at the Washington State University have made efforts in engineering C4 photosynthesis pathway, using an *Agrobacterium*-mediated transformation system. They have independently introduced into rice three maize genes encoding the C4 photosynthetic pathway enzymes: phosphoenolpyruvate carboxylase (PEPC); pyruvate orthophosphate dikinase (PPDK); and NADP-malic enzyme (ME). The transgenic rice plants expressed high levels of these genes and the maize enzymes remained active in rice plants. Most importantly, PEPC and PPDK transgenic rice plants exhibit higher photosynthetic capacity than untransformed plants, mainly due to an increased stomatal conductance (i.e., more atmospheric CO<sub>2</sub> becomes available for fixation). Preliminary field trials conducted in China and Korea also show 10-30% and 30-35% increases in grain yield for PEPC and PPDK transgenic rice plants, respectively. A further enhancement of the photosynthetic capacity of rice will require engineering a limited C4 pathway of photosynthesis by simultaneously expressing the three previously mentioned key enzymes in proper cellular compartments. Ultimately, for the most efficient operation of the pathway to concentrate CO<sub>2</sub> around Rubisco in the leaf, the concomitant installation of Kranz leaf anatomy will be essential. Various transgene deployment have been reported in rice and wheat for various traits.

### 3. Genomics

Similar to DNA marker technology and transgenics, genomics is another area full of prospects. The developments in the last five years have been explosive and we now have a complete sequence of the rice genome in the year 2005. As the rice genome is being completely sequenced, biotechnologists have started a systematic assessment of the phenotypes resulting from the disruption of putative gene sequences with genetic resources such as mutants, near isogenic lines, permanent mapping populations, and elite and conserved germplasm. Functional genomics, to a large extent, is analogous to the extensive germplasm screening that has allowed the extraction of useful traits in conventional breeding programs, yet with DNA sequence level precision on a global genome scale. The judicious utilization of the sequence information through functional genomic analyzes will certainly offer solutions to many a breeding problems through means hitherto not thought of. The availability of rice genome information is the foundation for the identification of orthologous genes in cereals and also facilitates the sequencing of other cereal genomes.



An international collaboration was established for completion of rice genome sequencing and to coordinate the concerted utilization of sequence information for the benefit of humankind. This initiative called the International Rice Genome sequencing Project (IRGSP) publicly funded and has 8 countries as its members. IRGSP has recently released completion of rice genome sequencing to ten-fold redundancy.

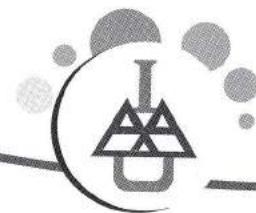
The science of genomics has two components: (i) Structural genomics and (ii) Functional genomics. Structural genomics refers to a systematic reading of all the sequences of the rice genome. Structural genomic efforts by the International rice genome sequencing project has been completed recently and the complete rice genome sequence of a Japonica rice variety called Nipponbare has been shared among rice researchers free of cost. Structural genomics, by itself is not an end. Through structural genomics, a set of ~30,000 genes have been identified in rice. The task now for rice molecular biologists is to decipher the function of each of these genes. The process of understanding the function of each of the sequenced genes is called as Functional genomics. India has contributed to both structural and functional genomic efforts and has successfully sequenced a portion of chromosome 11 of rice. Similar research initiatives are under progress with respect to functional genomics of rice in India. DRR has a sponsored collaborative network of projects on rice functional genomics by DBT project aimed towards understanding the functionality of genes associated with biotic stress resistance and grain yield. Ingenious utilization of information derived from functional genomic efforts can offer durable solutions to many breeding problems hitherto not available. The fields where genomic tools are expected to have significant impact include yield improvement, conversion of rice from a  $C_3$  to  $C_4$  plant and abiotic stress tolerance.

### Conclusions

The trinity of DNA marker technology, genetic engineering and genomics will certainly accelerate rice and wheat improvement programmes across the world including India. Through a judicious application of all these three technologies, development of a designer rice and wheat plant which is high yielding, using lesser nutrients from soil, with tolerance to biotic and abiotic stress and with enhanced nutritional quality may be possible in the near future. There is an urgent need to integrate the biotechnological work with mainstream plant breeding to derive maximum benefits from the wonderful science of biotechnology.

For India this requires development of necessary human resources, infrastructural facilities and interdisciplinary collaboration among plant breeders, molecular biologists, plant protection scientists, agronomists, physiologists, soil scientists and others. Thus to keep winning the war on food and nutrition front, the green revolution technologies need to be supplemented and complemented by the nascent gene revolution technologies. We need to develop and effectively utilize gene revolution technologies for ushering in an evergreen revolution.





## Applications and Implications of Nanobiotechnology: Issues and Concerns

C Devakumar\*

Indian Council of Agricultural Research, Krishi Anusandhan Bhawan-II  
New Delhi-110012, cdevakumar@gmail.com

### Abstract

*Nanobiotechnology is the convergence of engineering and molecular biology leading to a new class of multifunctional devices and systems for biological and chemical analysis with better sensitivity and specificity and a higher rate of recognition. It is being predicted that the value of nano-products will total \$2.6 trillion in 2014. Applications of nanoporous materials include photonic crystals, tissue engineering, bio-implants and sensors. Genomics, choice of suitable therapies/doses, food production /food safety, theranostics and implantable biosensors are some of the fields in which nanotechnologically improved biochips can play an important role within the next decade. One can envision a "toolbox" of capabilities, which can be assembled to create probes tailored to explore specific biological questions at near-molecular scale. NAIP has at least three projects on nanomaterials. There are certain priority issues such as basic research on structure-property-processing relationship at the molecular level, computer modelling and simulation at the nanoscale, metrology, developing a standard regulatory framework and common approval procedures, improving collaboration between academia and industry and technology transfer, providing education and skills both for young researchers and co-workers, addressing health, safety and environmental issues and dialogue among stakeholders about benefit and risks. We can mirror the Nano Mission of Government of India launched in May 2007 in the field of agricultural research and education for early success and impact. The education of this technology will see convergence in a larger number of areas cutting across disciplines. Given the range of products and applications described here, future applications of nanotechnology will be many and will excite the scientist and consumer alike.*

Nanotechnology is the design, synthesis, manipulation and application of functional materials, devices, and systems through control of matter at the atomic and molecular levels corresponding to the nanometer scale (1–100 nanometers), and the exploitation of novel phenomena and properties of matter at that scale. It is being predicted that the value of products incorporating nanotechnology will total \$2.6 trillion in 2014 and the value of basic nanomaterials in the order of \$13 billion. Healthcare and life sciences applications will be the driver of this significant movement during 2010-14.

The key characteristics of nanomaterials include the following:

- High surface area
- High activity
- Catalytic surface
- Adsorbent
- Prone to agglomeration
- Range of chemistries

\*Assistant Director General  
(Education, Planning & Development)



- Natural and synthetic
- Wide range of applications

The Nanoroadmap Project (<http://www.nanoroadmap.it/>), co-funded by the European Commission as a part of their Framework initiative has produced a document in 2005 entitled *Roadmaps at 2015 on Nanotechnology Application in the Sectors of Materials, Health & Medical Systems, Energy: Synthesis report*. A two cycle Delphi-like approach was used for the roadmap exercise. The result has been condensed in 12 roadmaps (4 for each sector) and the three sectors are: materials, health and medical systems and energy.

The following are the common 12 product types:

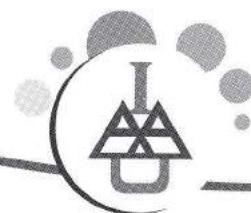
1. Nanoporous materials
2. Dendrimers
3. Nanostructured materials
4. Nanoparticles / nanocomposites
5. Nanocapsules
6. Nanofibres
7. Fullerenes
8. Nanowires
9. Carbon Nanotubes
10. Molecular Electrics
11. Quantum Dots
12. Thin Films

**Nanoporous materials** are natural or synthetic, organic or inorganic, hybrid materials, with holes less than 100 nm in diameter (those with a diameter of 2 to 50 nm are called mesopores and macropores those with a 50 to 100 nm diameter). Nanoporous materials can have open (interconnected) or closed pores and amorphous, semi-crystalline or crystalline frameworks. Examples of applications include photonic crystals, electrodes, tissue engineering, bio-implants and sensors. Nanoporous materials are usually divided into bulk nanoporous materials and membranes. Examples of bulk materials are carbon, silicon, silicates, polymers, metal oxides, organometalics, organo-silicon, while specific for membranes are zeolites followed by ceramics (silica, alumina, zirconia) and polymers. They combine the advantages of the porous structure with the physicochemical-biological functionality which can be enhanced or inhibited by the nano scale dimension. Increased specific surface area, improved sieving (including selectivity), reduced weight, thermal insulation and photonic properties are their characteristics.

### Researchable Issues

- Basic research for a better understanding on how materials work at a molecular or atomic scale is necessary to improve the ability.
- Studies on structure-properties relationships and design of modelling/simulation and software tools.





- Exploration of suitable raw materials (namely templates) both in quality and price.
- Design and development of equipments for robust and quick characterisation at nanoscale.
- Development of environment-friendly materials and green processes.
- Control over pores' characteristics and pores' functionalisation as well as thermostability over time.

### **Dendrimer**

It is a macromolecule with highly branched 3D structure built around a central multi-functional core molecule, with branches and end-groups that provides a high degree of surface functionality and versatility. Constituent and basic elements of a dendrimer can be anything that can branch (metal atoms, organometallic groups, or purely organic materials). The principal difference between a dendrimer and other hyper-branched polymers is that each of the monomer units in the dendrimer has at least one functional unit that allows further branching. Their main characteristics are;

- polyvalence,
- defined architecture, size and shape control,
- monodispersity (consistency of shape and form between molecules),
- loading capacity,
- biocompatibility,
- transfection properties (transporting genetic material into cell interiors health care/ medical (for ex. diagnostics or targeted drug delivery),
- consumer/industrial goods (for ex. inkjet inks-printing toners or dyes and paints),
- environment (for ex. decontamination agents),

### **Biochips**

These consist of the miniaturization of a variety of biological substrates and their deposition onto computer chip-like substrates for automated, high throughput analysis. Their preparation relies both on traditional techniques of microlithography and new microarraying (spotting and *in situ* synthesis) technologies. Up to now, studies using microarrays have served to advance the understanding of biological processes and to accelerate knowledge gains about fundamental biochemical processes. With the evolving of the knowledge, these technologies will become an essential tool for clinical medicine for diagnostics of a large spectrum of diseases, offering reproducibility, low cost and speed. Nanotechnology will lead to improved biochip devices, such as lab-on-a-chip devices with built-in nano-optical, mechanical and electronic intelligence, capable of performing reactions, separation and detection on a single platform.

Some of the fields in which nanotechnologically improved biochips can play an important role within the next 10 years are:

- Gene identification, gene-sequencing
- Basic research



- Choice of suitable therapies/doses
- Food production /food safety
- Theranostics Sophisticated portable lab-on-a-chip devices
- Implantable biosensors and
- Inexpensive whole genome arrays

According to the experts' estimations, there are three mainstream applications which should emerge in the next ten years: sophisticated portable lab-on-a-chip devices by 2010 and implantable biosensors and whole genome arrays by 2015.

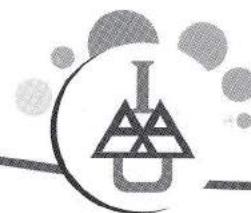
Nanotechnology related to biochips is still at its early stage and there are numerous problems and challenges still to overcome.

1. Cost: The medical practice demands for cheap products with increased throughput to reach the commercial standards as well as chips with broader applications.
2. The challenge of establishing robust, reliable and precise methods and instrumentations for standardisation of the assays is formidable. This is particularly important when genetic diagnostic applications are at stake and important clinical decisions are to be based on the interpretation of gene chip readouts.
3. In implantable biochips, biocompatibility, the stability of biomolecules which are linked to the sensors and thus the lifetime of such an implant, are critical.
4. With regard to cell-based biochips, cell compatible surfaces and of tools for cell manipulation and characterization are the challenges.

### **Biosensors**

The combination of nanotechnology, biology, microtechnology and advanced materials will offer new devices which will be able to detect and manipulate atoms and molecules. The small size of these sensors will lead to reduced weight, low power requirements, greater sensitivity and thus, to a totally new medical diagnosis at the cellular/ molecular level. Biosensors are highly integrated analytical devices, incorporating a biological or biomimetic sensing element (receptor or recognition system), a signal converter or transducer and an amplifier. In a way they act like noses by specifically detecting certain molecules with recognition units that are based on biological components. The integration of a biochemical recognition element and a transducer is the main aspect which distinguishes biosensors from other bioanalytical configurations. Two basic sensor types can be distinguished, termed after the respective recognition reaction: affinity sensors and catalytic sensors. Affinity sensors are based on the specific bonding capacity of biological molecules and subsequent modifications of electron densities, light absorption, layer thickness, surface stress or refraction index can be detected by the appropriate methodology. Catalytic biosensors, on the other hand, are based on the molecular recognition of substrates by biocatalysts and their subsequent conversion into products which are detected via an enzyme electrode. For in vitro diagnostics the impact of biomolecular sensors is expected to derive from miniaturization and new, highly sensitive and selective biosensor arrays, which could be integrated as multiple components in single, manageable devices to be used in clinical laboratories and home self-diagnostics (point of care diagnostics). Nanobased





biosensors are expected to find their way also in food production to improve quality control in production processes and hence food chain management as well as consumer protection, providing confidence in the products quality.

### **Fluorescent Proteins**

Increasing in size from single dye molecules, green fluorescent proteins (GFP), such as those originally isolated from *Aequorea* jellyfish are of great interest to biologists for their ability to indicate genetic expression levels in living systems by the addition of the GFP sequence genes to the host's genome. A variety of related fluorescent proteins have been generated, covering much of the visible spectrum, permitting simultaneous labeling of multiple genetic activities within the same system. Although their fluorescence emission is similar to organic dyes, fluorescent proteins are fundamentally different from other labels, as their synthesis and localization are dependent primarily upon the gene transcription activity of the host cell or organism, rather than any external targeting. Similar to dyes, the fluorescent proteins tend to exhibit excited state interactions which can a variety of functionalities have been introduced that demonstrate the versatility of silica as a host material for fluorescent dyes for many applications, specifically in the fields of nanobiotechnology and the life sciences. The power of silica as a host material is twofold. First, encapsulation of organic dyes in a silica matrix can enhance their stability and performance (e.g. brightness). Second, the ability to synthesize particles with core-shell architectures allows multiple functions to be brought together in a single vehicle, separated in different shells, for example, to minimize interference towards creating highly functional particles. The fluorescent core-shell silica nanoparticle design concepts exemplified by the sensors hint at the possibilities available to researchers in the future by combining such functionalities as antibody-mediated targeting, mesoporosity and chemical sensing already demonstrated individually, a "single-particle laboratory" or "lab on a particle" can be envisioned that would, for example, (a) seek out a specific cellular location, (b) deliver a payload of therapeutics and (c) subsequently monitor the cell's response. These particles would provide high-content feedback on the activity of the therapeutic agent, rather than simply monitoring whether or not the cells survived treatment, as is commonly the case today.

With the development of these and other functionalities, one can envision a "toolbox" of capabilities, which can be assembled in different shells to create probes tailored to explore specific biological questions at near-molecular scale. The versatility of silica as a host material and the modularity of the core-shell architecture thus provide a wide and open space for innovative research towards creating highly-integrated and highly-functional nanomaterials for nanobiotechnology and beyond.

### **NAIP initiatives**

NAIP has at least three projects on nanomaterials:

1. Designing, and studying mode of action and biosafety of nanopesticides with the following objectives:
  - Preparation and characterization and study of release characteristics of fungicides (Elemental sulphur and hexaconazole), insecticides (acephate and fly ash) and fumigants in nano and nano-encapsulated forms following standard operating procedure (SOP)



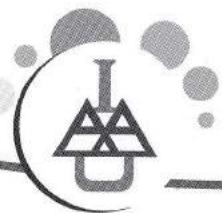


- Studies on bioefficacy and mode of action of the nanocides against the insects; Cotton bollworm (*Helicoverpa armigera*), Red flour beetle (*Tribolium castaneum*), Pulse beetle (*Callosobruchus maculatus*), Red spider mite (*Tetranychus urticae*), and the fungi, *Erysiphe necator* causing powdery mildew in grapes and *Rhizoctonia solani* causing sheath blight in rice
  - Biosafety studies of effective nanocides on rats, honeybee and *Trichoderma viride* as models
  - Residue dynamics of effective nanocides in soil and effect on soil microflora
2. Nano-technology for Enhanced Utilization of Native - Phosphorus by Plants and Higher Moisture Retention in Arid Soils with the following objectives
- Enhancing the utilization of native phosphorus by plants using nano-particles of Mg, Zn and Fe.
  - Enhancement of gum production for soil binding and moisture retention by microbes through nano-particle (Mg, Zn, Fe, P) stimulation.
  - Synthesis and application of nano-granules of phosphorus from rock phosphate for enhancing its utilization.
3. The NAIP project "Detection and mitigation of dairy pathogens and detection of adulterants using chemical biology" envisages addressing the following objectives:
- To develop high affinity nucleic acid aptamers for the detection of food spoilage and dairy pathogens as per legal document, viz., *Escherichia coli* including pathogenic strain 0157, *Listeria monocytogenes*, *Salmonella typhi*, *Bacillus cereus*, *Clostridium perfringens* and *Enterobacter sakazaki*.
  - To develop high affinity nucleic acid aptamers and chemical based detection methods for common adulterants of milk viz., urea, detergents, and whey.
  - To screen drug like molecules for mitigation of gram-negative bacteria and separation of the dead/inactivated bacteria and associated drug molecules from milk during processing.

At IARI, New Delhi, we have prepared zinc oxide coated urea which has shown enhanced efficiency of zinc utilization by rice.

The use of nanotechnology will enable totally new production processes with integrated bottom-up assembly of structures at a molecular level to realize highly miniaturized diagnostic systems. The advantages offered by nanotechnology when compared to existing or alternative technologies will be lower costs, improved device performance and reliability, extreme miniaturization. Other advantages include the ability to enhance sensitivity, improve equipment compatibility, reduce energy consumption and allow reactions, detections and identifications not otherwise available. The vast knowledge about the complex cellular processes could imply genetic testing of individuals to forecast a genetic susceptibility to future diseases. Nanotechnology is expected to offer the solution to these problems and the tremendous variety of biochips, which derives also from the wide range of possible detection methods such as, for example, field effect transistors, microelectromechanical systems (MEMS), optical or thermal devices/methods, radio labelling or mass spectrometry, will expand the range of applications.





Nanobiotechnology is the convergence of engineering and molecular biology leading to a new class of multifunctional devices and systems for biological and chemical analysis with better sensitivity and specificity and a higher rate of recognition. Moreover, nanotechnology will be used as a tool for genetic information and research, facilitating genome sequencing and nuclear transfer with "smart" nano-devices that have some independence and learning capabilities. Both technological movements involve tiny science and large imagination. Both fields involve smaller and smaller scales and manipulation of nano-sized materials.

**Implications and concerns:** Anything affecting society, certainly affects the law and legal frameworks. We need to examine social, ethical and legal implications. Inherent in the promise of nanotechnology is the creation of superior products and services at a much-reduced cost. The effect of such creation, by itself, will perhaps take decades to manifest in society, spinning off environmental, social, economic and educational implications. Within these spheres, we will find "a fundamental tension of civilization – the tension between humanity's quest for more control over nature and the future, and our equally strong desire for stability and predictability in the present."

Many legal implications of nanotechnology will become clear as the technology develops, but some predictions have emerged already. For instance, legal concepts of property, intellectual property and privacy will probably change as nanotechnology integrates into society. From a national security standpoint, limitation and control in cases where resources get consumed too quickly, random viruses are produced, machines no longer respond predictably to humans, or inexpensive products are being used for mass indoctrination or mass destruction weaponry. A conversation between the public and the nanotechnologists as a kind of accountability and transparency mechanism, and also as a way to bridge the information barrier between those two groups is essential. The trail of regulation also runs persistently through the proposed Foresight Institute Guidelines on Molecular Nanotechnology. While the Guidelines speak of the opportunities in nanotechnology, they recognize that "Along with these new capabilities come new risks, and new responsibilities. The acceptance of these responsibilities is not optional." The strongest suggestion comes in the area of self-regulation, including "professional guidelines that are grounded in reliable technology, and knowledge of the environmental, security, ethical and economic issues." Safety measure suggestions also appear, so that nanotechnologies will include such mechanisms as built-in termination dates, artificial fuel sources (rather than those found in nature), and human-dependent subassemblies. While the Foresight Guidelines are currently far from binding, the existence of such a structure indicates how, even in the early stages of this technology, awareness about regulation and ethical, social and legal issues may compatibly accompany the development of nanotechnology.

*A good place to start addressing the ethical implications of nanotechnology is to examine the experience we have gleaned from biotechnology and information technology because these fields, are likely to share some ethical issues with nanotechnology.* Many of nanotechnology's issues mirror those of genetics. Our society's experience with the genetics revolution can be a valuable model for nanotechnology development, in terms of assessing potential benefits and problems. The National Institutes of Health in the United States established ELSI (Ethical Legal and Social Implications of Human Genetics Research) to accompany the genetics revolution. Canada had numerous similar projects, including Genome Canada's current GELS (Genetics, Ethics, Law and Society) initiative.



## Exposure Metrics

Two areas require the urgent attention of exposure scientists. The first is metrology and developing tools to characterize and measure relevant attributes of nanomaterials, including particle size, number, and surface area. The second is lifecycle analysis of nanomaterials in consumer goods and their transformation and degradation in products throughout the lifecycle of materials.

There is a lack of generally accepted, acknowledged, and/or validated measurement methods, reference data, and standards for the determination of the nature and properties of engineered nanomaterials. Standard reference materials would be necessary for measuring, testing, and characterizing engineered nanomaterials in the various human exposure media.

US Environmental Protection Agency (EPA) cites challenges of monitoring nanoscale materials including the small size of the analyte and the need for monitoring equipment to be sensitive and specific as well as durable, affordable, and able to operate in a range of environments. The equipment should be able to monitor continuously and analyze in real-time, measure in a variety of media, and measure several analyte properties in parallel.

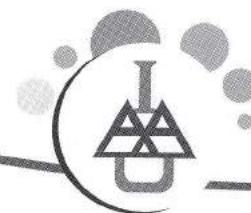
With regard to occupational exposure, there are currently no convenient methods for monitoring the surface area of particles in the workplace or in a worker's breathing zone, where instruments would have to be small enough to attach to a worker. NIOSH has also recognized the need for the development of new direct-reading instruments to measure size distribution, surface area, and mass concentration of nanoaerosols in the workplace.

Methods are also not available for the characterization of nanomaterials in biological media or their ionic states within tissue. Development of reliable tools to measure characteristics of nanomaterials within cells and tissues is necessary for successful bio-effects research. These methods will enable study of the relationships between exposure, absorbed dose, and effective dose. Other complications for measurement of engineered nanomaterials involve the potential for intra- and inter-batch variation in physicochemical properties.

Currently, there is no established nomenclature to differentiate a compound at various sizes and agglomeration states. Researchers will need to identify nanomaterial properties and determine the potential impact of these factors on fate and transport through the environment, human exposure and dose, disposition in the body, and ultimate health effect.

Exposure measurements for nanoscale particles, more appropriate exposure metrics can include particle number, particle size, surface area, shape, crystal structure, surface charge, porosity, or other surface physical and chemical properties. When the dose of TiO<sub>2</sub> was expressed as particle surface area, the inflammatory response for both size particles fit the same dose-response curve. These findings suggested that particle surface area for different sized particles of the same chemical composition is a better dose metric than particle mass or particle number. However, more recent studies suggest that this dose-response may not follow for very small TiO<sub>2</sub> nanoparticles below 10 nm because of size-dependent physicochemical changes in properties seen for these smaller nanoparticles. Surface chemistry of nanomaterials can affect surface reactivity which can in turn affect toxicity. Modification of the surface of nanomaterials with surfactants or polymers has been shown





to reduce toxicity *in vitro* and alter half-life and tissue deposition *in vivo*. Researchers have also discussed the importance of particle number concentration as a dose metric in toxicity of nanomaterials.

In a study comparing the effects of carbon nanotubes and carbon black in rodent lungs, the investigators found that four nanotube products were able to produce granulomas in mice or rats, while granulomas were not observed in rodents exposed to carbon black. The study results point to the fundamental difference between the unique physicochemical properties of nanotubes and those of carbon black. In addition to their unique surface chemistry, the fibrous structure of carbon nanotube products must be considered.

Recent studies have also found that the aspect ratio, the ratio of a compound's length to diameter, and agglomeration state may play an important role in the potential health effects of carbon nanotubes.

### Priority Issues

1. Basic research for understanding structure-property-processing relationship at the molecular level;
2. Computer modelling and simulation at the nanoscale;
3. Metrology;
4. Developing a standard regulatory framework and common approval procedures;
5. Improving collaboration between academia and industry and technology transfer;
6. Providing education and skills both for young researchers and co-workers;
7. Addressing Health, Safety and Environmental (HSE) issues;
8. Dialogue among stakeholders about benefit and risks.

**Mission on Nano Science and Technology (Nano Mission)** was launched in May 2007 with an allocation of Rs. 1000 crore for 5 years by the Department of Science and Technology. The following components are envisaged:

- Basic Research Promotion
- Infrastructure Development for Nano Science & Technology Research
- Nano Applications and Technology Development Programmes
- Human Resource Development
- International Collaborations

It is steered by a Nano Mission Council (NMC). The technical programmes of the Nano Mission are also being guided by two advisory groups, viz. the Nano Science Advisory Group (NSAG) and the Nano Applications and Technology Advisory Group (NATAG).

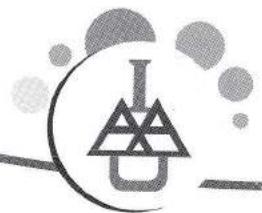
Nanobiotechnology will certainly provide opportunities for developing new materials and methods that will enhance our ability to develop faster, more reliable and more sensitive analytical systems. A gradual, rather than explosive incorporation of these new discoveries into molecular recognition is predicted. Being interdisciplinary it brings together life scientists and engineers. This, in turn, fuels further growth of ideas, which would not occur without these interdisciplinary interactions. As this technology is a unique combination



of engineering, physics, chemistry, biology, computer and materials sciences, the field will require a force of multidisciplinary experts leading to more collaboration and more interdisciplinary flexibility. The education of this technology will see convergence in a larger number of areas. At the same time, the technology is expected to evolve so rapidly that the students will be valued for their ability to think and learn quickly, rather than for their technical expertise alone.

Nanobiotechnology is still at its early stages of development; however, the development is multi-directional and fast-paced. Universities are forming nanotechnology centres and the number of papers and patent applications in the area is rising quickly. The nanotechnology 'tool-box' is quickly being filled with nanotools. Given the range of products and applications described here, future applications of nanotechnology will be many and will excite the scientist and consumer alike.





## Application of Nanotechnology for Diagnosis of Infectious and Non-infectious Diseases

Amit K. Dinda\*

Department of Pathology

All India Institute of Medical Sciences, New Delhi - 110029

akdinda@hotmail.com

Nanotechnology may be defined as, "the technology of nanoscale materials or devices". At nanodimension every material acquires unique properties which is different from its mass physical and chemical properties. This unique phenomenon in the recent scientific development has revolutionized the advancement of medicine and galvanized almost every specialty of science engineering and business community. Nanotechnology also has a tremendous potential of application in diagnostic pathology. This technology has empowered us to detect even a single bacteria or virus in a sample which is beyond the scope of existing detection systems<sup>1</sup>.

The specialty of nanotechnology is relatively young in comparison to other scientific fields. The idea of nanotechnology was born on December 29, 1959 in the annual meeting of American Physical Society, at the California Institute of Technology (Caltech), when Richard P. Feynman delivered an invited talk entitled, "*There is plenty of room at the bottom*" (<http://www.zyvex.com/nanotech/feynman.html>). Till early 90's there was not much work or interest in this field. However in last 10 years it has become one of the most sought after specialties for research and innovation across the world<sup>2</sup>.

The unique properties of nanosized material or devices are being utilized to develop complex intelligent and interactive systems which can be used at molecular level detection in diagnostic pathology as well as research in pathobiology<sup>3</sup>.

The potential applications of nanotechnology in diagnostics can be enumerated as follows:

- Nanotechnology for designing various biochips
- Nanoparticle technology
- Nanopore technology
- Cantilever arrays
- DNA nanomachines for molecular diagnostics
- Nanoparticle-based immunoassays
- Resonance light scattering technology
- Nanosensors

With in last few years, the biological and biomedical applications of micro- and nanotechnology (Biomedical or Biological Micro-Electro-Mechanical Systems [BioMEMS]) have become increasingly popular and have a potential for widespread use in a wide variety of diagnostics applications. Biochips, constructed with microelectromechanical systems on a micron scale, use micromanipulation, whereas nanotechnology-based chips

\*Additional Professor & Sub Dean (Examination)



are on a nanoscale acts on nanomanipulation. The size of this integrated “lab-on-a-chip” will be small enough to carry like a pen or watch and enable the patients to carry out the tests at any place and time of their choice. The day will come when there will be no need of samples to be sent to laboratory ushering the era of personalized medicine or medical care. The concept of “lab-on-a-chip” has become a reality today<sup>4</sup>.

Development of Cadmium sulphide nanoparticles, “Quantum Dots” (QD), with unique stable luminescent capability has helped to develop labeling and advanced imaging techniques for detection of very small quantity of marker molecules and even few microorganisms which can not be detected by conventional methods is available at present. Qdots are fluorescent semiconductor nanocrystals, a few nanometers in diameter whose size and shape are precisely controlled by the duration, temperature, and ligand molecules used during synthesis. Compared with conventional fluorophores, the nanocrystals have a narrow, tunable, symmetric emission spectrum and are photochemically stable. QD bioconjugates raise new possibilities for studying genes, proteins and drug targets in single cells, tissue specimens and even in living animals and enable visualization of cancer cells in living animals<sup>5</sup>.

Nanobarcodes are submicrometer metallic barcodes with striping patterns prepared by sequential electrochemical deposition of metal ions. The differential reflectivity of adjacent stripes enables identification of the striping patterns by conventional light microscopy. This readout mechanism does not interfere with the use of fluorescence for detection of analytes bound to particles by affinity capture, as demonstrated by DNA and protein bioassays<sup>6</sup>.

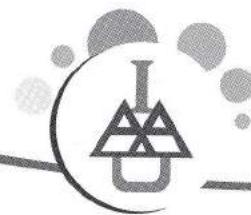
SEnsing of Phage-Triggered Ion Cascade (SEPTIC), uses a nanowell device with two antenna-like electrodes to detect the electric-field fluctuations that result when a bacteriophage infects a specific bacterium and then identifies the bacterium. This method had a 100% success rate in detecting and identifying strains of E coli quickly and accurately<sup>7</sup>.

Advances in nanotechnology are providing nanofabricated devices that are small, sensitive and inexpensive enough to facilitate direct observation, manipulation and analysis of single biological molecule from single cell or infective organism. This opens new opportunities and provides powerful tools in the fields such as genomics, proteomics, molecular diagnostics and high throughput screening<sup>8-10</sup>.

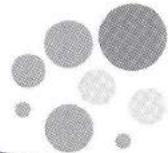
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## Nanobiotechnology and its Applications in Agriculture

B.M. Prasanna\*

Division of Genetics, Indian Agricultural Research Institute (IARI)  
New Delhi - 110012; E-mail: bmprasanna@gmail.com

### Introduction

Nanotechnology is the creation and utilization of materials, devices and systems through the control of the properties and structure of matter at the nanometric scale. Nanobiotechnology is an exciting branch of nanotechnology, in which recent advances in nanotechnology are integrated into the biology realm, in particular into molecular biology and cell biology.

All organisms, from microbes to humans, are powered by highly evolved molecular and cellular machines that operate at the nano level. Nature has been performing 'nanotechnological feats' for millions of years. Nanobiotechnology is a highly interdisciplinary field of research and is based on the cooperative work of chemists, physicists, biologists, medical doctors and engineers. We are just beginning to understand the nanoscale methods used in nature to create self-replicating, self-monitoring, self-controlling and self-repairing tools, materials and structures.

### Nanobiotechnology: Molecular Biology Complementing Nanotechnology

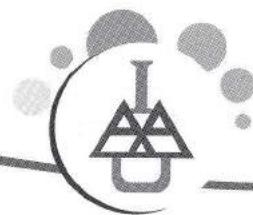
The credit for the term "nanobiotechnology" goes to Lynn W. Jelinski, a biophysicist at Cornell University, USA. Nanobiotechnology joins the breakthroughs in nanotechnology to those in molecular biology. Molecular biologists help nanotechnologists understand and access the nanostructures and nanomachines designed by 4 billion years of natural engineering and evolution – cell machinery and biological molecules.

Exploiting the extraordinary properties of biological molecules and cell processes, nanotechnologists can accomplish many goals that are difficult or impossible to achieve by other means. For example, rather than build silicon scaffolding for nanostructures, DNA's ladder structure provides nanotechnologists with a natural framework for assembling nanostructures; and its highly specific bonding properties bring atoms together in a predictable pattern to create a nanostructure. Nanotechnologists also rely on the self-assembling properties of biological molecules to create nanostructures, such as lipids that spontaneously form liquid crystals.

DNA has been used not only to build nanostructures but also as an essential component of nanomachines. Most appropriately, DNA may also serve as the basis of the next generation of computers. As microprocessors and microcircuits shrink to nanoprocessors and nanocircuits, DNA molecules mounted onto silicon chips may replace microchips with electron flow-channels etched in silicon. Such biochips are DNA-based processors that use DNA's extraordinary information storage capacity. Conceptually, they are very different from the DNA chips discussed below. Biochips

\*ICAR National Fellow & Principal Scientist





exploit the properties of DNA to solve computational problems; in essence, they use DNA to do math. Scientists have shown that 1,000 DNA molecules can solve in four months computational problems that require a century for a computer to solve. Other biological molecules are assisting in our continual quest to store and transmit more information in smaller places. For example, some researchers are using light-absorbing molecules, such as those found in our retinas, to increase the storage capacity of CDs a thousand-fold.

Nanobiotechnology, thus, is an emerging area of opportunity that seeks to fuse nano/microfabrication and biosystems to the benefit of both. It relates to all applications of genomics including mammalian, plant and microbial. It provides the basic tools and subsequently the technology for gathering sequence information and designing innovative devices to probe questions related to the biological importance of the genomic information and the application of this knowledge in diverse fields, particularly medicine and agriculture. The impact of nanobiotechnology may be immediately felt in the following areas:

### **Nanofabricated Gel-free Systems and High Throughput DNA Sequencing**

As a central process, DNA sequencing needs to be improved in terms of its throughput and accuracy. Nanofabrication technology will be critical toward this goal both in terms of improving existing methods as well as delivering novel approaches for sequence detection. The scaling down in size of the current sequencing technology allows the process to be more parallel and multiplex. Research in nanobiotechnology is advancing toward the ability to sequence DNA in nanofabricated gel-free systems, which would allow for significantly more rapid DNA sequencing.

Currently, there are three widely deployed deep sequencing platforms in hundreds of research laboratories and in some core facilities worldwide, the Genome Sequencer FLX from 454 Life Sciences/Roche, Illumina Genome Analyzer, and Applied Biosystems SOLiD. Each instrument essentially massively parallelizes individual reactions, sequencing hundreds of thousands to hundreds of millions of distinct, relatively short (50–400 bases) DNA sequences in a single run (Lister et al., 2009). Thanks to such instrumentation, the past two decades have witnessed spectacular advances in genomics, leading to genome sequencing of a wide array of organisms

Coupled with powerful approaches such as association genetic analysis, DNA sequencing data of the crop germplasm, including the cultivated crop gene pool and the wild relatives can potentially provide highly useful information about molecular markers associated with agronomically and economically important traits. Thus, nanobiotechnology can enhance the pace of progress in molecular marker-assisted breeding for crop improvement.

### **Expression Profiling**

One of the first nanobiotechnologies in the market is micro-chips for DNA or protein sequencing (bio-chips). This technology is a good example of how a technology issued from traditional microelectronic industry is combined to a recently developed biotechnology. Another technology under development concerns microfluidic bio-chips, also known as



lab-on-a-chip devices. They are all based on manipulation of minute bio-objects immersed in fluids, allowing on-chip biochemical processing (sampling, mixing, amplification, separation, detection and analysis). The application area of biochips and microfluidic chips is very broad, ranging from high throughput screening, cell analysis, drug discovery to portable devices for minimal-invasive therapy, precision surgery as well as drug delivery.

Microarray-based hybridization methods allow to simultaneously measure the expression level for thousands of genes; this is referred to as 'expression profiling'. Such measurements contain information about many different aspects of gene regulation and function, and indeed this type of experiments has become a central tool in biological research. The development of novel formats for sequence determination and patterns of genomic expression which can have significantly higher throughput than current technologies is vital. Thousands of DNA or protein molecules are arrayed on glass slides to create DNA chips and protein chips, respectively. Recent developments in microarray technology use customized beads in place of glass slides.

The fundamental principle underlying the microarray technology has inspired researchers to create many types of microarrays, including DNA microarrays and protein microarrays, to answer scientific questions and discover new products. Our lab at IARI is intensively engaged in using such arrays for analysis of gene expression in maize under important biotic and abiotic stresses, such as downy mildews and drought.

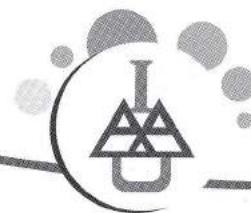
Overall, nanofabrication techniques can be used, for example, to pattern surface chemistry for a variety of biosensor and biomedical applications, for (i) determination of new genomic sequences; (ii) scanning of genes for polymorphisms that might have an impact on phenotype; and (iii) comprehensive survey of the pattern of gene(s) expression in organisms when exposed to biotic or abiotic stress.

### **Disease Diagnostics**

Diseases are one of the major factors limiting crop productivity. The problem with the disease management lies with the detection of the exact stage of prevention. Most of the times pesticides are applied as a precautionary manner leading to the residual toxicity and environmental hazards and on the other hand application of pesticides after the appearance of disease leads to some amount of crop losses. Among the different diseases, the viral diseases are the most difficult to control, as one has to stop the spread of the disease by the vectors. But, once it starts showing its symptoms, pesticide application would not be of much use. Therefore, detection of exact stage such as stage of viral DNA replication or the production of initial viral protein is the key to the success of control of diseases particularly viral diseases. Nano-based viral diagnostics, including multiplexed diagnostic kit development, have taken momentum in order to detect the exact strain of virus and stage of application of some therapeutic to stop the disease.

Detection and utilization of biomarkers that accurately indicate disease stages is an important area of research. Measuring differential protein production in both healthy and diseased states leads to the identification of the development of several proteins during the infection cycle. The nano-based diagnostic kits not only increase the speed of detection but also increase the power of the detection.





## Post-Harvest Management and Food Biotechnology

**Nano Bar Codes and Identity Preservation:** Usage of bar code is the essential characteristics for selling almost all commodities both in the international market as well as in the national market. This bar code is essentially a sticker having a number of black and white bars with certain digits written at the bottom. The bar code is nothing but an electronic data depicting several parameters such as date of production, place of packaging, prices etc. and reading this code requires an electronic data reader. With the advent of nano technology, nano based bar codes are also available which can do the same function as that of conventional bar codes, thereby helping in tracking and controlling the quality of food product and give all relevant details in minute.

Each day a huge amount of shipments of livestock and other agricultural products are moved all over the world and it is becoming increasingly difficult to keep a track on critical control points of the production, shipment and storage processes. Lack of finances also limits the number of inspectors that can be employed at these critical control points. An identity preservation (IP) system can be installed that creates increased value by providing consumers with information about the practices and activities used to produce an agricultural product and it is possible to provide stakeholders and consumers with access to information, records and supplier protocols regarding the farm of origin, environmental practices used in production, food safety and security, and information regarding animal welfare issues.

Nano-based identity preservation has the potential to revolutionize the entire agri-based industry as it can continuously track and record the history of a particular agricultural product. The nanoscale monitors linked to recording and tracking devices can improve the IP of food and agricultural products. The keys are biodegradable sensors for temperature and other stored data containing the history of stored food for both physical and biological parameters. The future of the meat industry may well depend on an ability to track all stages in the life of the product, including the birth of the animal, its medical history, and its movements between the ranch, the slaughterhouse and the meat-packing plant, right through to the consumer's table.

**Monitoring Quality of Agricultural Products:** Nanotechnology also has applications in the agri-food sector. Many vitamins and their precursors, such as carotinoids, are insoluble in water. However, when formulated as nanoparticles, these substances can easily be mixed with cold water, and their bioavailability in the human body also increases.

Many lemonades and fruit juices contain these specially formulated additives, which often also provide an attractive colour. The world market potential of such micronized compounds is estimated at \$1 billion. In the future bio and gas sensors could gain importance. These sensors could be integrated into packaging materials to monitor the freshness of the food. Spoiling of the food could be indicated by a colour change of the sensor. Several concepts have already been developed for such applications based e.g. on silicon or polymer thin film sensors.

Bioselective surfaces are the new innovation of nano science technology with a principle that surfaces are the environment and location on which most chemical and biological interactions occur. A bioselective surface has either an enhanced or reduced ability to bind



or hold specific organisms or molecules. With this bioselective surfaces minute amount of chemicals and even presence of bacteria and viruses can be detected with ease. These surfaces are important to the development of biosensors, detectors, catalysts and the ability to separate or purify mixtures of biomolecules.

### **Biosafety and Ethical Issues**

Although nanobiotechnology has high potential to benefit agriculture and food systems, there is an ethical responsibility to apply it wisely, and to recognize that there are potential unforeseen risks that may come with the tremendous positive potential. For instance, nanocapsules and microcapsules are the ideal nano technology based tools which are very much stable even within the plant system. These vehicles have the capability to deliver toxic chemicals in a very minute amount and remain be inactive until any trigger is given. In addition, because of their increased bioavailability only a small quantity of the chemical is needed. Once the trigger in the form of infrared rays or magnetic field is applied the toxic chemicals get released in to the system, leading to the suppression of growth and even death of the plant system. Therefore, this nanocapsulated toxic chemical has the potential to kill weeds and pests with ease. However, the same system may be exploited as a bioweaponry.

Secondly, there are some concerns expressed about the negative impact of nanoparticles to human health. The US Department of Agriculture is presently assessing the impact of application of nanotechnology to the food chain, its implications for food security and farmers' rights.

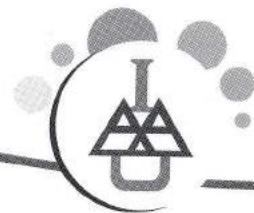
Thirdly, there are apprehensions regarding monopolistic tendencies of the institutions that may be engaged in nanobiotechnology R & D, as these highly powerful technologies are also highly cost-intensive. These might, therefore, encourage broad-spectrum intellectual property claims that could have a major dampening effect on the pace and type of innovations being developed by the scientific community. Therefore, of major importance to the development of nanotechnologies will be a thoughtful, thorough and balanced assessment of the benefits and risks. There must be a gradual and intensive drive for generating better public awareness about various applications of nanotechnologies, and their potential benefits and risks.

### **The Way Ahead**

India, undoubtedly, has the required talent in the individual fields of computational science, biology, physics and engineering. However, there is a highly limited number of institutions, mostly working in isolation, which have a research programmes linking these vital components. To effectively utilize the power of nanotechnology for addressing some major problems in agriculture and also for undertaking basic and strategic research on prioritized areas, it would be to launch multi-institutional research and education programmes on nanotechnology. I would suggest three specific action points in this regard:

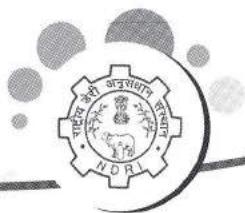
1. A National Nanotechnology Initiative in Agriculture may be launched by ICAR/ DARE, in partnership with national/international institutions, for conducting basic and strategic research on nanobiotechnology in relation to agriculture and for





developing technologies such as (i) nanosensors for monitoring quality of agricultural produce; (ii) nanofertilizers; (iv) nanopesticides; and (v) disease diagnostics. Nanoscale technologies should also be utilized to reveal the dynamics of biologic processes, such as insect movement, pheromone dynamics, and soil-atmosphere gas fluxes.

2. Research on biosafety of nanoparticles, especially in food, must be undertaken by relevant institutions in a mission mode, in collaboration with the Department of Biotechnology (DBT), Govt. of India.
3. Capacity building and human resource development would assume great significance to tap the potential of nanotechnology. Necessary institutional adjustments may also have to be made with reference to faculty hiring and reorientation of our education programmes to generate adequate and well-skilled human resources in this cutting-edge technology.



## **Synthesis and Application of Biocompatible and Environment Friendly Controlled Release Nanomaterials, in Medicine and Agriculture**

**Susmita Mitra\***

Amity Institute of Nanotechnology, Amity University

In the context of technological advancements, medicine and agriculture are two specific areas that have a direct bearing on human life. From the beginning of human civilization, the focus on developing new technology has been for improving crop yield, safeguarding the agricultural produce, and enhancing the quality and life span of plants and animals. With the advent of nanotechnology, the immense possibilities of utilizing the nanomaterials in both these areas has led to the emergence of novel research fields such as nanomedicine and nanoagriculture. Nanotechnology being an enabling technology has the potential to revolutionize medicine, agriculture and food systems. Along with nanomedicine the research areas in the field of agriculture include: pathogen and contaminant detection through the use of bioanalytical nanosensors, smart treatment delivery systems for plant and animal disease conditions, smart systems integration for agriculture and food processing, nanodevices for molecular and cellular biology, environmental and agricultural waste disposal through the design of nanocatalysts for waste bioprocessing etc., to name a few of the vast areas of application possibilities. Research in the area of controlled delivery nanomaterials has made inroads in medical science in the past two decades. Futuristic applications in agriculture could have an impact on improved nutrient and pesticide delivery, based on these regulated delivery systems. In this respect, the use of environment friendly materials as nanocarriers has to be considered, keeping in mind the toxic effects on plant and animal life that could result from the carriers themselves.

### **Nanomedicine: Controlled Delivery and Interventions by "Smart" Nano-objects**

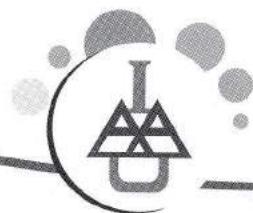
Various materials are being developed for controlled drug delivery, and there has been increasing interest to employ nanostructured materials for this purpose. Nanoparticles of polymers, micelles and inorganic materials have been tailored to achieve sustained and targeted delivery of drugs, genes, and proteins. Nanoparticles are attractive since they are small enough to escape macrophage detection, and they provide for enhanced absorption, retention and bioavailability. Besides, these materials are further engineered with stimuli responsiveness, so that administration of the therapeutics can be controlled by changes in temperature and/or pH, and by magnetic forces. These provide for the possibility of targeting the drugs to specific organs and tissues. Future challenges in this area include the tunable delivery of multiple biologics, such as growth factors, for tissue engineering, with distinct kinetics to better mimic the natural tissue regeneration process over extended periods.

### **Polymeric Nanocarriers**

For controlled delivery, a polymer whether natural or synthetic, has to be judiciously combined with a drug or other active agent in such a way that the active agent is released

\*Professor





from the material in a predesigned manner. The release of the active agent may be constant over a long period, it may be cyclic over a long period, or it may be triggered by the environment or other external events. The purpose behind controlling drug delivery is to achieve more effective therapies while eliminating the potential for both under- and overdosing. Other advantages of using controlled-delivery systems can include the maintenance of drug levels within a desired range, the need for fewer administrations, optimal use of the drug in question, and increased patient compliance. Natural and synthetic hydrogel polymers that are being used for controlled delivery of drug include chitosan, alginate, dextran, gelatin, albumin, poly(N-vinyl pyrrolidone), poly(acrylic acid), poly(vinyl alcohol), polyacrylamide, poly(N-isopropylacrylamide).<sup>1</sup> Nanocarriers can be formulated from these materials utilizing water-in-oil emulsions and microemulsions, coacervation, phase separation, etc. Some of the other synthetic polymeric materials include poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(ethylene-co-vinyl acetate), poly(ethylene glycol), poly(methacrylic acid), polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides, polyorthoesters. PLA and PLGA polymers are biodegradable and biocompatible polymers, and can be formulated to encapsulate various types of therapeutic agents including low molecular weight drugs. The polymeric matrix prevents the degradation of the drug, is able to sustain therapeutic drug levels for prolonged periods of time and also allows precise control over the release kinetics of the drug from nanoparticles. Moreover, the duration and levels of drug released from the nanoparticles can be easily modulated by altering formulation parameters such as drug: polymer ratio, or polymer molecular weight and composition. PLGA nanoparticles are generally formulated using 'water-in-oil-in-water' double emulsion solvent evaporation techniques, using poly vinyl alcohol (PVA) as emulsifier <sup>2</sup>.

### **Inorganic Nanomaterials for Controlled Release**

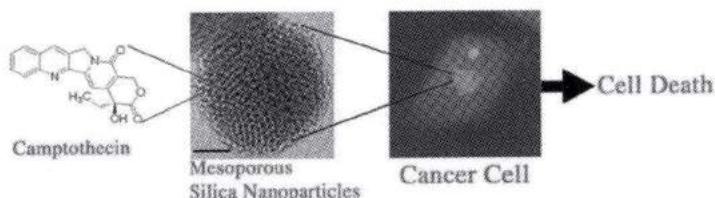
Inorganic nanomaterials entrapping biomolecules have demonstrated diverse applications in material science. Their excellent storage stability in comparison to organic materials and the added advantage of protection from microbial attack has contributed to their importance in various applications <sup>3</sup>. Inorganic nanoporous materials have demonstrated promising therapeutic utility as controlled delivery materials, specifically in implants such as stents, scaffolds for tissue growth, as well as nanoparticles for tissue specific drug delivery. Nanoporous silicon and alumina have emerged as important materials in this respect. Porous ceramics such as calcium phosphate-based materials are used for filling bone defects. The ability to control porosity and solubility of some ceramic materials offers the possibility of use as drug delivery systems.

#### **Porous Silicon**

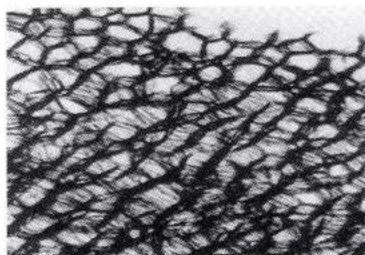
A form of the chemical element silicon, has nanopores in its microstructure, rendering a large surface to volume ratio in the order of  $500\text{m}^2/\text{cm}^3$ . The primary application of porous silicon has been in controlled and localised delivery of drugs and other therapeutic agents. The high porosity of the nano-structured silicon confers a high capacity for loading with a therapeutic agent (up to 95%). It can be very conveniently produced as micro-particles, which are loaded with the therapeutic agent, and can be injected using a fine needle. In this way treatment is administered via a minimally invasive route, directly to the site of interest for treatment.



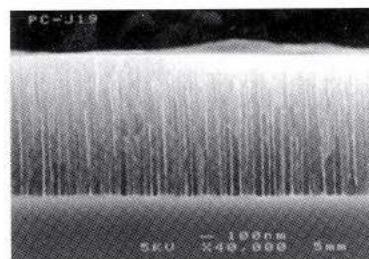
On entering the site of treatment, the porous silicon slowly dissolves, releasing the therapeutic agent at a controlled rate. The resulting treatment thus becomes very efficient in both use of potentially expensive therapies and addressing the exact location of the disease. It is possible to introduce drugs with poor aqueous solubility into the porous structure, thus increasing the dissolution/ solubility of the drug in the aqueous, physiological environment up to approximately 10-fold.



This new level of reactivity, resulting from the nanostructure, has been developed as BioSilicon by a nanotechnology company, pSivida 4. The company is exploring ophthalmic implants from both a drug delivery perspective and a tissue engineering aspect. Initial constructs involve biodegradable implants for drug delivery from the tissue surrounding the eye. The rate of dissolution can be tuned so that delivery can be achieved over days or months. The eye is a particularly favored target due to the safety of BioSilicon. Unlike the degradation products from polymers like lactides and glycolides, silicic acid, the product from BioSilicon, is a very mild acid, expected to cause less irritation.



TEM image showing the highly porous



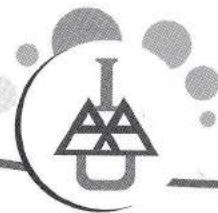
BioSilicon™ membrane, showing porous structure of BioSilicon™ surface layer on the underlying silicon wafer

Medically biodegradable (resorbable) forms of BioSilicon™ dissolve in bodyfluids into orthosilicic acid, the natural form of silicon that we take up from our diet. This diffuses freely through the circulatory system and body tissues and is excreted in urine via the kidneys. In the environment, BioSilicon™ would degrade through hydrolysis to the same corrosion product, which would thus return the material to the natural state of the element, present in the sea, soil and rivers. BioSilicon™ is being developed for several applications, including time-released delivery of nutrients and actives in nutritional products, high-performance functional packaging, and personal care, including oral care.

### Porous Alumina

Nanoporous anodic alumina, which has been widely used as anticorrosion coating to improve the mechanical properties of aluminum, is now attracting renewed attention as an indispensable part of the nanoscience and nanotechnology. The porous anodic alumina films possess uniform cylindrical pore sizes and high pore density, which are known to be important membrane attributes. These nanosized pores can serve as a perfect controlled





release platform for different drugs / chemicals, depending on the surface modification that is carried out. The porous anodic alumina, can be fabricated through the relatively simple and low-cost anodization process. When a sheet of aluminum is electrochemically anodized in a certain acid electrolyte under specific conditions, an almost perfectly regular ordered porous alumina film with controlled pore size and thickness forms on a thin barrier layer alumina covered aluminum substrate<sup>5</sup>. Using alumina templates, both nanotubes and nanotestubes open on only one end, have been synthesized from many different materials. These have great potential as drug delivery vehicles for biomedical applications. The tunable alumina template allows one to dictate both pore diameter and length. The nanotubes can be differentially functionalized on their inner and outer surfaces. Corked nanotesttubes can also be designed by covalent capping to prevent premature payload leakage. Porous alumina is useful as an alternative for the formation of drug releasing stents, as they are more wear resistant and highly biocompatible. Drug-eluting stents are used as support for blood vessels during angioplasty, and coating the stents with polymers impregnated with clot-busting drugs has helped reduce complications and new blockages. But these polymeric materials are not durable and tend to crack easily, making way for newer materials such as porous alumina.

### **NanoAgriculture: Nanotechnology for Controlled Environment Smart Delivery Systems**

In the future, nanoscale devices with novel properties could be used to make agricultural systems "smart". For example, devices could be used to identify plant health issues before these become visible to the farmer. Such devices may be capable of responding to different situations by taking appropriate remedial action. If not, they will alert the farmer to the problem. In this way, smart devices will act as both a preventive and an early warning system. Such devices could be used to deliver chemicals in a controlled and targeted manner in the same way as nanomedicine has implications for drug delivery in humans.

Technologies such as encapsulation and controlled release methods have revolutionized the use of pesticides and herbicides. Many companies make formulations which contain nanoparticles within the 100-250 nm size range that are able to dissolve these chemicals in water more effectively than existing ones (thus increasing their activity). Other companies employ suspensions of nanoscale particles (nanoemulsions), which can be either water or oil-based and contain uniform suspensions of pesticidal or herbicidal nanoparticles in the range of 200-400 nm. These can be easily incorporated in various media such as gels, creams, liquids etc, and have multiple applications for preventative measures, treatment or preservation of the harvested product. One of the world's largest agrochemical corporations, Syngenta, is using nanoemulsions in its pesticide products<sup>6</sup>. Another encapsulated product from Syngenta delivers a broad control spectrum on primary and secondary insect pests of cotton, rice, peanuts and soybeans. Marketed under the name Karate® ZEON this is a quick release microencapsulated product containing the active compound lambda-cyhalothrin (a synthetic insecticide based on the structure of natural pyrethrins) which breaks open on contact with leaves. In contrast, the encapsulated product "gutbuster" only breaks open to release its contents when it comes into contact with alkaline environments, such as the stomach of certain insects. In other areas, scientists are working on various technologies to make fertiliser and pesticide delivery systems which can respond to environmental



changes. The ultimate aim is to tailor these products in such a way that they will release their cargo in a controlled manner (slowly or quickly) in response to different signals e.g. magnetic fields, heat, ultrasound, moisture, etc., such that there's less fluctuation in the quantity of fertiliser present in the soil, less waste, and less residue. New research also aims to make plants use water, pesticides and fertilizers more efficiently, to reduce pollution and to make agriculture more environmentally friendly.

### **Nano-emulsion for Controlled Delivery**

Nano-emulsions are highly stable systems that show little coalescence of particles, nor sedimentation or creaming. They can consist of lipid or polymeric vesicles or particles, in the size range of 20-200 nm. The organic phase is non-toxic and can be made from food grade components. Through encapsulation, the active ingredient is afforded some protection against atmospheric and environmental conditions, such as oxidation, and is released slowly. Nano-emulsions can be produced by both high energy (mechanical process using rotators, ultrasound, or pressure homogenisers) and low energy (either spontaneous emulsification, due to solvent diffusion as a result of mixing or dilution; or by phase inversion temperature, a process which is controlled by specific surfactants, such as polyethoxylated surfactants, in response to temperature change) means. In each process the active elements can be added during the synthesis stage (e.g. agrochemicals) so that they are encapsulated by the nanoparticle or vesicle. High energy systems offer more control of size distribution and composition of the resultant nanoscale vesicles or particles.

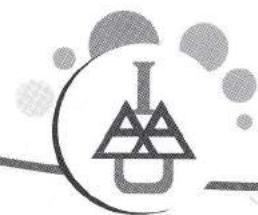
In terms of agricultural applications, nano-emulsions can be used for hydrophilic and hydrophobic pesticides, but are largely being developed for those that are poorly water soluble. For example, pyrethroids such as  $\gamma$ -cyhalothrin and  $\beta$ -cypermethrin have been successfully formulated as lipid nano-emulsion, obviating the need for the organic solvents such as benzene and toluene normally required for its formulation, as have more ecologically friendly pesticides such as *Artemisia arborescens* L essential oil. In addition to exhibiting greater stability, nano-emulsions demonstrate increased coating of leaves and uptake through plant cell walls, as a result of low surface tension.

The main advantages to using nano-emulsions are therefore- solubilisation of hydrophobic pesticides (hence no need for toxic organic solvents), no precipitation or creaming (therefore no need for constant mixing), increased stability (protect against oxidation), improved uptake<sup>7</sup>.

### **Nanoparticulate Carriers**

The fungicides tebuconazole and chlorothalonil have been successfully incorporated into polymeric nanoparticles (100-250 nm), using polyvinylpyridine and polyvinylpyridine-co-styrene employed as the polymer matrix. By introducing the biocides into solid wood by incorporating them within polymeric nanoparticles, they have been successfully used to treat the wood decay fungus *Gloeophyllum trabeum* in southern pine sapwood samples. Once in the wood, the polymer matrix served as a reservoir for the biocide and controlled its release rate into the wood<sup>8</sup>. Porous hollow silica nanoparticles (PHSNs) have been studied in relation to pesticide release. PHSNs of diameter 100 nm with various shell thicknesses in the range of 5-45 nm and a pore diameter of about 4-5 nm, were synthesized via a sol-gel route using inorganic calcium carbonate nanoparticles as templates, and were subsequently employed as pesticide carriers to study the controlled release behaviour





of avermectin. A multi-stage sustained-release pattern from the Av-PHSN samples was noted. Increasing pH or temperature intensified the avermectin release, and the shell thickness also had a significant impact. Increasing the shell thickness in the range of 5–45 nm led to a more sustained release by decreasing the release rate of the pesticide from the nanocarriers<sup>9, 10</sup>.

### Future Outlook

The diverse nanomaterials available for controlled release have opened the possibilities of various applications entrapping chemical entities such as drugs, pesticides, fertilizers, to name a few. Already research has made headway, taking nanomaterials to the market for application in medicine as well as agriculture. With the development of methodologies that would be highly cost-effective and conducive to large-scale manufacture of environment friendly nanomaterials, their future wide-spread use seem a distinct possibility, specially in the arena of agriculture.

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## Nano-Biosensors based on Multiwalled Carbon Nanotubes for Determination of Ascorbate and Oxalic Acid in Food Stuffs

C S Pundir\*

Deptt. of Biochemistry, MD University, Rohtak-124001, Haryana, India

E-mail: pundircs@rediffmail.com, pundircs@gmail.com

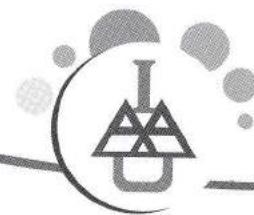
Nanobiotechnology, a branch of nanotechnology with biological and biochemical applications is immersing as a frontier area for future research. The world market in 2010 for nanomaterials, nanotubes, nanodevices and nanobiotechnology is expected to be over \$200 billion. Carbon nanotubes (CNT), discovered in 1991 by S. Iijima are long, thin cylinders of carbon large macromolecules, which are unique for their size, shape and broad range of electronic, thermal & structural properties that change depending on wall, single cylindrical wall (SWCNTs) and multiple walls (MWCNTs)-cylinder inside the other cylinder. Biosensors are fast developing tools for simple, rapid, selective & highly sensitive determination of compounds. The sensitivity of these biosensors has been improved recently with the use of nanomaterials. We have constructed nano-biosensors by electrodepositing carboxylated multiwalled carbon nanotubes (c-MWCNT) & conducting polymer, polyamine (PANI) onto Au/Pt electrode and then immobilizing covalently bottle guard fruit ascorbate oxidase/sorghum leaf oxalate oxidase onto this hybrid electrode. This working electrode along with calomel standard electrode & Pt wire as auxiliary electrode were connected through potentiostat to construct nano-biosensor. When three electrode system was immersed into buffered ascorbic acid/oxalic acid solution & polarized at high voltage, it generated current, which was directly proportional to ascorbic acid/oxalic acid measure. Linear range, response time, and detection limits of these biosensors were studied. The sensors showed optimum response in acidic pH range at room temperature within 10-20 second. The sensors were employed for the determination of ascorbic acid and oxalic acid level in various food stuffs. There was good correlation between the level of ascorbic acid and oxalic acid obtained by standard methods and present biosensors. The electrodes were used 100 times a period of two months, without any significant loss of activity, when stored at 4°C.

Nanotechnology is the study, manipulation, creation and use of a wide variety of nanoparticles with dimensions smaller than 100 nm (normally in the range of 1-100nm). Due to their small size, nanoparticles exhibit unique chemical, physical and electronic properties that are different from those of bulk materials and can be used to construct novel and improved sensing devices, in particular electrochemical sensor & biosensors. A variety of nanoparticles of different sizes and compositions are now available which facilitate their use in electroanalysis. The important functions provided by nanoparticles include the (1) immobilization of biomolecules (2) the catalysis of electrochemical reactions (3) the enhancement of electron transfer between electrode surfaces and proteins, (4) labeling of biomolecules and (5) even acting as reactant.

Various nanostructures have been studied to determine their properties and possible applications in biosensors. These structures include nanotubes, nanofibers, nanorods,

\*Professor





nanoparticles and thin films. The tubular nanostructures have been used in biosensors to increase the quality and activity of immobilized biomolecules. In view of their unique properties, the tubular nanostructures provide opportunities for development of novel designs of biosensors.

### **Carbon Nanotubes**

Since carbon nanotubes (CNT) were discovered by S. Iijima (1991), these nanostructures have attracted great attentions as nanoscale building blocks for microdevices. CNTs are long, thin cylinders of carbon large macromolecules. The nanodimensions, graphite surface chemistry and broad range of electronic, thermal properties of CNT make them an ideal material for use in chemical & biochemical sensors. Depending on their structure i.e. walls, single cylindrical wall nanotubes (SWCNT) and multiple walls carbon nanotube (MWCNT)- containing cylinder inside the other cylinder, have been used in biosensors.

### **Synthesis of Carbon Nanotubes**

Carbon nanotubes are synthesized by (1) laser evaporation, (2) carbon arc method and chemical vapour deposition. In laser evaporation, a quartz tube containing argon gas and graphite are heated at 12000C. A water cooled copper collector is contained in the tube outside the furnace while graphite target contains a small amount of cobalt and nickel, which act as a catalytic nucleation sites for formation of CNT. An intense pulsed laser is incident on target evaporating carbon the graphite. The argon sweeps the carbon atoms from high temperature zone to colder copper collection on which they condense into nanotubes. This method is suitable of SWCNTs having a diameter of 10-20 nm & length of 100µm. In Carbon Arc method, carbon atoms are evaporated by energetic plasma of the gas, ignited by passing high centers through carbon electrodes. The operating connections include use of carbon rod electrodes of 5-20mm diameter separated by ~1mm with a voltage of 20-25V across the electrodes and continuous DC electric current of 50-120A flowing between electrodes. The arc is operated at 500 Torr He with a flow rate of 5-15ml/sec for cooling. For synthesis of MWCNTs, no catalyst is used, while for production of SWCNTs, catalysts such as transition metal (Co, Ni, Fe) and rare earth (Y, Gd) are used. The method can produce SWCNT of diameter 1-5nm with 1µm length. In chemical vapour deposition method, a catalyst material is heated upto high temperature in a furnace with a flow of hydrocarbon gas through tube reactor. The general nanotube growth mechanism involves the dissociation of hydrocarbon molecules catalysed by transition metal and dissolution and subsequent of carbon atoms in metal nanoparticle. In case MWCNT, ethylene or acetylene is used as carbon feedstock at 550-750oC along with Fe, Ni or Co as catalyst, while for SWCNT, methane is used as carbon feed stock at 850-1000oC along with suitable catalyst materials (A. George, 2006).

### **Carboxylation & Dispersion of CNT**

The carboxylation of CNT is done by heating it into mixture of conc. H<sub>2</sub>SO<sub>4</sub> and HNO<sub>3</sub> in 1:3 ratio, followed by ultrasonication in a water bath at 35oC for 48h. The resulting suspension is diluted in distilled water in 1:1 ratio and again ultrasonicated for 24 hr. The dispersed CNT solution is again dissolved in the same H<sub>2</sub>SO<sub>4</sub> - HNO<sub>3</sub> mixture in 1:10 ratio to obtain a uniform carboxylated MWCNTs (c-MWCNT) solution.



### **Deposition of c-MWCNT & Conduction Polymer on Electrode**

Dispersed c-MWCNT and conducting polymer (e.g. Aniline) in 1N HCl (in 1:200 ratio) are electrodeposited onto metal/nonmetal electrode (e.g. Pt/Au/ITO coated glass plate) using potentiostat-galvanostat to construct a working electrode. An electrochemical cell containing of calomel as reference and Pt wire as counter electrode are also used. The electropolymerization is achieved by chronoamperometry by applying 8 polymerization cycle at 0.6-0.8V with a constant current (150 $\mu$ A) for 960s with intermittent shaking after every 120s (Arora et al 2007). The resulting electrode is washed thoroughly with distilled water to remove unbound matter.

### **Construction & Testing of Working Electrode (Enz/MWCNT/PANI/Metal)**

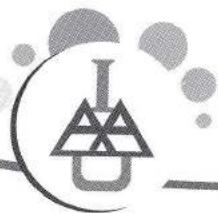
The c-MWCNT/PANI/Metal electrode is activated by spreading 0.5% glutaraldehyde on activated surface for 4 hr. The excess of glutaraldehyde is removed from electrode by washing it many times with distilled water. The enzyme solution is placed onto surface of glutaraldehyde activated c-MWCNT/PANI/Metal electrode and kept overnight for incubation. After immobilization of enzyme, the electrode is washed with reaction buffer to remove unbound enzyme from the surface of electrode and protein concentration is measured in washing. The resulting enzyme /c-MWCNT/PANI/Metal is used as working electrode. The three electrode system (Working electrode; Ag/AgCl reference and Pt wire as counter electrode) is immersed into buffered substrate and electrode system is polarized at high volts and the current generated is measured in mA. Optimal current is achieved at a particular voltage for a particular enzyme sensor. Further experiments are carried to study whether current measured is directly proportional to the substrate concentration. Once it is confirmed, the other kinetic properties of electrode are studied viz optimum pH, temp, response time etc.

### **Use of CNT in Biosensors**

In one glucose biosensor, glucose oxidase (GOx) was immobilized by coating onto the surface of SWCNT, without any loss of activity, which resulted into enhanced performance of the sensor, mainly due to high enzyme loading and partly because of better electrical communication ability of the CNT (Azamian et al 2002). Use of SWCNT has made possible a direct electron transfer with the redox active centers of absorbed oxidoreductase enzymes (Guisseppi-Elie et al 2002). Both FAD and GOx were found to spontaneously adsorb to unannealed CNT that had been cast onto GC electrodes and to display quasi-reversible one electron transfer. Similarly horsereddish peroxidase (HOPD) adsorbed on a CNT microelectrode exhibited direct transfer of electrons to the electrode and retain its catalytic activity towards H<sub>2</sub>O<sub>2</sub> (Zhao et al 2002).

Use of CNT in electrochemiluminescence (ECL) biosensors for assay of  $\alpha$ -fetoprotein has been reported (Wohlstadter et al 2003). In such biosensor, they are not only conducting, can act as electrodes and can generate ECL signal but also functionalized for the immobilization of biomolecules.





### Use of MWCNT in Biosensors

Aligned MWCNTs grown on Pt substrate have been employed for construction of amperometric biosensor (Sotiropoulou et al 2003). In these CNT array based biosensors, two array systems were either acid treated or air treated. This chemical etching was more effective in opening the CNT and allowing the enzyme to enter the inner channel. The oxidation of the array introduced -COOH groups at the opening ends to provide a stabilizing hydrophilic environment that allowed for adsorption and insertion of the enzyme into cavity of the nanotubes. The immobilization of enzyme within nanotubes may permit a mediated direct electron transfer to Pt electrode (Jianrong et al 2004). A disposable biosensors based on acetyl choline esterase (AChE)-functionalized acid purified multiwall carbon nanotubes modified thick film strip electrode for organophosphorous (OP) insecticides was developed. The degree of inhibition of AChE by OP compounds was determined by measuring the electrooxidation current of thiocholine generated by AChE catalysed hydrolysis of acetyl thiocholine (ATCh) (Joshi et al 2005). A composite of multiwalled carbon nano Aubes-Chitoson (MWCNT-CHIT) was used as a matrix for entrapment of LDH onto a GC electrode to fabricate lactate biosensors (Tsi et al 2007). Recently a highly sensitive amperometric glucose biosensor was developed using glucose oxidase (GOx) co-immobilized/absorbed onto MWCNT film on Au disc at low potential glucose sensing properties were studied using CV and chronoamperometric technique (Rahman et al 2009).

The use of nanobiocomposite of c-MWCNT-PANI in improving the performance of the enzyme sensors for determination of ascorbic acid & oxalic acid in food stuffs will be presented.

### Conclusion

Nanotechnology has recently revolutionized the construction of biosensors. The unique chemical and physical properties of nanomaterials make them extremely suitable for designing new and improved biosensors. The advantage of use of nanomaterials & nano-structure in sensing devices include immobilization of biomolecules, catalyse electrochemical reaction enhancement of electron transfer between electrode surface and proteins labeling of biomolecules and even acting as reactants. New many more nanomaterials & nanostructures requires to be explored for improve performances of biosensors.

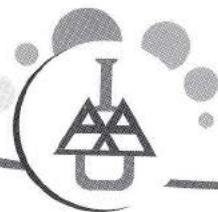
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## Nanocides and Nanodrugs in the Realm of Nanobiology: Applications in Agricultural and Veterinary Sciences

Arunava Goswami\*

Agricultural and Ecological Research Unit, Biological Sciences Division,  
Indian Statistical Institute, (Institute of National Importance), Kolkata, India

### Abstract

*Minimization of loss of produced crops and mortality caused by insect pests and pathogens to veterinary animals using nanotechnology will be important for maintaining higher food and animal production rate in this century. Biologically active nanoparticles deal with particles in the domain of 1-100 nm. Poly-disperse naked nanoparticles produced by top down (ball mill) are highly active in nature but they tend to aggregate in the natural conditions with time. Capped nanoparticles produced bottom up (sol gel) are mono-disperse in nature and depending on the chemical nature of the capping layer, aggregation of nanoparticles could be reduced to minimum. Capping layer provides an intermediate platform through which surface free energy of the nanoparticles is transmitted to the environment. Thus nanoparticle surface provides activation site for supra-molecular forces to perform a variety of novel functions. We have used a large number of nanoparticles which could be of interest to Agriculture and Veterinary Sciences. In this paper, we provide a brief summary of our work so far.*

### Origin

Naturally occurring diatomaceous earth (DE) used for centuries contains nano-porous micron size hydrophilic natural silica particles. These particles are effective in killing insects in the dry climatic conditions. As nanopores are filled with water in humid conditions of the tropics, their efficacies as pesticides are greatly reduced. We used surface functionalization for making hydrophobic and lipophilic DE and this improved the efficacy of the DE significantly. Our previous work on these mesoporous silica materials led us to study nanoscience to a greater depth later (A. Goswami, I. Mewis and C. Ulrichs).

### BmNPV: Combating with hydrophobic alumino-silicate nanoparticles

Commercial silkworms, reared by small silk farmers in India are often 100% decimated by the lethal *Bombyx mori* nuclear polyhedrosis virus (BmNPV). The disease, called 'grasserie' is highly virulent in nature and within 24-30 hours of infection all the organs are destroyed as the virus can replicate in all kinds of larval tissues. Ten types of alumino-silicate nanoparticles (~4-6 nm in size; AL series) were tested for their efficacy as drugs. Cocktail of AL60102 and 100% heat inactivated virus could save 35-40% of the affected larvae. Further biochemical and biophysical examination showed that nanoparticles and inactivated virus act via multiple mechanisms. First, nanoparticles attach to the viral assembly and inactivate the virus by making hierarchy of lesser energy structures compared to its original form. Second, nanoparticles absorb lipoprotein-lutein complex in hemolymph essential for growth of the virus. Third, inactivated viruses present in hemolymph induce naturally occurring innate immunity in silkworm larvae and aid nanoparticles in providing

\*Associate Professor



better protection. Use of special kinds of needle shaped nano-TiO<sub>2</sub> along with AL60102 and inactivated virus is in progress to further augment the efficacy of the nano-drug (A. Goswami and A. Rahman).

### **Nanosilica: hydrophilic, hydrophobic and lipophilic: Crop protection**

Naked hydrophilic nanosilica prepared through wet route was found to be efficacious on insects but for a brief period of time (~ 7-10 days) as aggregation limits its activity. This result is in agreement with our previous results where naked hydrophilic silica nanoparticles were produced by using high energy ball mill. In a series of studies, hydrophilic, hydrophobic and lipophilic nanosilica were prepared using different capping agents. Hydrophilic nanosilica now absorbs less water than its natural counterparts due to the special capping agent used and exhibit insecticidal property for a longer period of time. Hydrophobic and lipophilic nanosilica exhibited inherent property of absorbing lipids from the outermost cuticular layer of insects. These nanoparticles worked as efficient 'physically active pesticides and break 'water barrier' of insects. This opened a new vista in the realm of eco-friendly pesticide generation principle. We have produced large quantities of these nanopesticides now and have found that they are extremely efficient as pesticide against a large number of stored grain pests and mustard aphid. Different kinds of formulations have been prepared and patent applications are in process with DBT, GOI (A. Goswami and N. Debnath).

### **Nano-ZnO: Lipophilic formulations: Tea pests**

ZnO is used in cosmetic industry for centuries. Hydrophobic, hydrophilic and lipophilic nano-ZnO nanoparticles were made and tested on several tea pests. Due to heavy rainfall in tea growing belts of Assam, novel formulation strategies were made to stabilize nano-ZnO. Lipophilic ZnO was found to be highly effective against looper caterpillar [*Biston suppressaria* or *Buzura suppressaria*] and other tea pests. Mechanisms of action of these nanoparticles are currently being studied in the laboratory (A. Goswami).

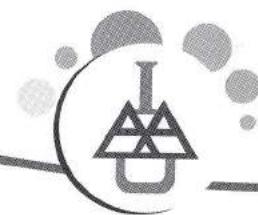
### **Dsethvasan: Novel micro-nano drug for Malaria and Trypanosomes**

Sub-micron size silica nanoparticles (FS) and pure nano-AL (~4-6 nm) series when injected in *Trypanosoma evansi* infected mice alone; no significant increase in life span of host was observed. However, when particular combinations of sub-micron and nano-size nanoparticles were tested for their efficacy as drug on *T. evansi* infected mice and *Plasmodium gallinaceum* infected Vencobb chicken, results were encouraging. In both the cases, micro-nano drug (Dsethvasan) increased the life span of the host significantly and reduced the parasite burden. Mechanisms of action of these particles against malaria parasite and trypanosomes have been studied in detail and have been published recently (A. Goswami and D. Seth).

### **Nano-gold: Quantum nanoparticles for modulation of photosynthesis**

A series of different sizes of capped gold nanoparticles have been prepared. 2 nm size particles (among 25 nm, 15 nm, 10 nm, 5 nm, 4 nm and 2nm sizes prepared) behaved as





classical quantum nanoparticles and are expected to help us in modulation of photosynthesis of crop plants in very low dose (in ppb level) (A. Goswami, S. Das and A. Datta).

### **BSA Capped Nano-silver: Antibacterial against drug resistant microbes**

Nano-silver was prepared using a large number of protocols. BSA capped nano-silver was found to be more stable than PVP-capped nano-silver as antibacterial at different pH and temperature. BSA capped nano-silver showed excellent anti-bacterial activity on a number of drug resistant bacteria. Mechanism of action studies showed that novel pathways are used by the nanoparticles to kill the bacteria (A. Goswami and D. Seth).

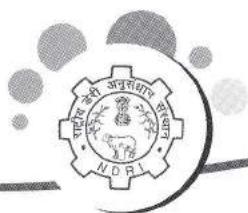
### **Emerging Nanocides: Nano-sulfur, Nano-fly ash, Nano-acephate, Nano-hexaconazole**

Elemental sulphur was introduced to Indian agro-chemical market long ago. The product could not be commercialized due to high price and high volume of use. Nano-sulfur was prepared with via four protocols. Two types of nano-sulfur have shown excellent fungicidal activity against food pathogen, *Aspergillus niger* at 1 ppm. Furthermore, kinetics of spore formation is greatly reduced in presence of nano-sulfur while not in presence of elemental sulphur (A. Goswami and S. Roy Choudhury). Nanoacephate and nanohexaconazole produced by encapsulation of the acephate and hexaconazole molecule in biocompatible capping agents produced nanocides which were found to effective against a number of pests and fungi of commercial importance. Further studies on efficacy and mechanisms of action are currently in progress in the laboratory scale (A. Goswami, S. Pradhan and S. Roy Choudhury). Fly ash is considered an eco-toxic by product of thermal power industry. Large quantities of fly ash from Kolaghat thermal power station were taken to nano-size domain (20-80 nm) by a series of intelligent ball milling in presence of eco-friendly solvents. Resultant nano-fly ash were mixed with commercial enamel (600 nm and 1200 nm size) and deposited on solid support. Further studies on the production of solid supports in combination of 42 different kinds of mesoporous nano-carriers are in progress. These solid supports will be used for slow release of volatiles such as fumigants, anti-microbials and anti-fungal agents at a commercial scale. Use of such tiles in the Agricultural and Veterinary industry are estimated to usher a new research area countrywide (A. Goswami and P. Patra).

### **Nanobiology: Organic and inorganic world**

Nanosize particles present in different biological systems with a view to evolutionary developments have been studied in detail. Serum or biological fluids in different organisms have reasonably higher concentration of lower size particles (~10-50% in the domain of 4-20 nm). Characterization and utilization of these particles for Agriculture and Veterinary sciences is active area of research in the laboratory (A. Goswami and R. L. Brahmachary; unpublished data)

Two riverine systems in India have been mapped for nanoparticles of geological origin. Naked nanoparticle and nanocomposites are currently being studied in the laboratory with the view to use in ion transport and plant nutrition (A. Goswami and R. Sen; unpublished data)



## Summary

A large number of nanoparticles have been developed using novel top down and bottom up approaches. In most of the cases, modulation of transfer of large surface free energy of nanoparticles to surroundings were done using a variety of top down and bottom up approaches. Efforts towards understanding mechanisms of action of the nanoparticles produced so far using biophysical and DNA microarray has yielded novel pathways of action of these nanoparticles. Nanoparticles produced so far in my laboratory have yielded efficacious nanocides against agriculturally important insect pests, fungi and microbes. Some of these particles have shown excellent activity as nano drug against pathogens of Veterinary importance. Challenges before my laboratory is now to design nanoparticles where supramolecular forces present on the nanoparticle surface could be more efficiently transferred to cells and organs for better productivity in Indian Agriculture and Veterinary industry. In a recent set of experiments we have utilized nano-silica as high surface area platform for delivering N-P-K to the crop plants. Preliminary results show that at least 20% increase in vegetative growth could be achieved in crop plants when ~ 0.2 ppm of such nanoparticles is mixed with commercial fertilizers. Biosafety issues of engineered nanoparticles are important. So far, we have deliberately used very low concentration of nanoparticles so that collateral damage to environment could be minimized. Also, a large number of experiments using DNA microarray have been done in my laboratory with the nanoparticles described earlier in the text. So far, no significant differences in level of gene expression profiles between control and nanoparticle treated insects have not been observed. Organ and tissues toxicity studies on beneficial insects and mammals are currently in progress.

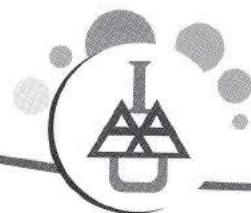
## Acknowledgements

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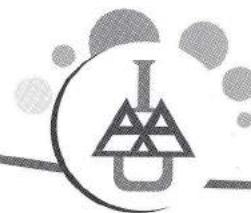


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## Applications of Nanotechnology in Fisheries & Aquaculture Development

S.D. Singh\*

Central Institute of Fisheries Education, Mumbai 400061

### Nanotechnology

Nanotechnology is the ability to work at the atomic, molecular and supramolecular levels (on a scale of 1- 100 nm) in order to understand, create and use material structures, devices and systems with fundamentally new properties and functions resulting from their small structure. The term "Nanotechnology" was coined by Japanese scientist Professor Norio Taniguchi in 1974 (Tokyo Science University). Nanotechnology involves the production and application of substances and structures at the nanoscale: within this size range substances can have very different properties when compared to their larger counterparts, reflecting surface properties and quantum effects that become important at the nanoscale. There are different terms being used nowadays very commonly like Nanoscience, Nanoengineering and nanotechnology. These terms deal with things smaller than 100 nanometers (especially with the manipulation of individual molecules). Nanotechnology is defined by its scale - the nanometer (nm) or one billionth ( $10^{-9}$ ) of a meter. Conceptually, nanotechnology refers to the ability to control the composition of molecules and atoms, within the range of 100nm down to 1 nm, potentially enabling scientists to create specific molecular structures and devices.

Rearranging matter at the nanoscale using 'weak' molecular interactions, such as van der Waal forces, hydrogen bonds, electrostatic dipoles, fluidics and various surface forces requires low-energy consumption and allows for reversible or other subsequent changes. Such changes of usually 'soft' nanostructures in a limited temperature range are essential for bioprocesses to take place.

Smalley- a famous researcher from USA in Nanotechnology, classified nanotechnology into two categories: 'wet' nanotechnology or Nanobiotechnology (including living biosystems) and 'dry' nanotechnology. Research on dry nanostructures is now seeking systematic approaches to engineer man-made objects at the nanoscale and to integrate nanoscale structures into large-scale structures, as nature does.

### Nanomaterials

Nanomaterials are those materials which have structured components with at least one dimension less than 100nm. Materials that have one dimension in the nanoscale (and are extended in the other two dimensions) are layers, such as a thin films or surface coatings. Some of the features on computer chips come in this category. Materials that are nanoscale in two dimensions (and extended in one dimension) include nanowires and nanotubes. Materials that are nanoscale in three dimensions are particles, for example precipitates, colloids and quantum dots (tiny particles of semiconductor materials). Nanocrystalline materials, made up of nanometre-sized grains, also fall into this category. Some of these materials have been available for some time; others are genuinely new.

\*Principal Scientist



An attempt has been made to introduce the various aspects of both basics and applied aspects of Nanotechnology and the significant foreseeable applications of some key nanomaterials in several fields of human concern including in fisheries and aquaculture.

Two principal factors cause the properties of nanomaterials to differ significantly from other materials: increased relative surface area, and quantum effects. These factors can change or enhance properties such as reactivity, strength and electrical characteristics. As a particle decreases in size, a greater proportion of atoms are found at the surface compared to those inside. For example, a particle of size 30 nm has 5% of its atoms on its surface, at 10 nm 20% of its atoms, and at 3 nm 50% of its atoms. Thus nanoparticles have a much greater surface area per unit mass compared with larger particles. As growth and catalytic chemical reactions occur at surfaces, this means that a given mass of material in nanoparticulate form will be much more reactive than the same mass of material made up of larger particles.

Intentionally produced (i.e. manufactured or engineered) nanoparticles are an important sector of nanotechnologies, representing a diverse range of substances currently produced, i.e. from metal and metal oxide-based nanoparticles to carbon-based nanotubes and fullerenes. In fact, nanoparticles are far from new and occur widely, from volcanic dust to atmospheric pollution. What is new is the scale of manufacture of novel nanoparticles and other nanomaterials that exploit the enhanced properties of materials when produced at this very small size range. Such enhanced properties of nanomaterials can offer huge potential socio-economic, health and environmental benefits, which has in turn fuelled a rapid increase in current nanomaterials manufacture. Potential benefits to the environment include the promotion of sustainable production and consumption (e.g. lightweight but strong materials, fuel cells and fuel additives) and remediation of contaminated groundwater and land.

In addition, Nanotechnology – a form of molecular engineering – promises significant social benefits, including enhancements in medical diagnosis and health treatments, more efficient energy sources, and lighter, faster and cheaper materials and electronic products. While intentionally produced nanoparticles offer significant potential benefits, there are also considerable uncertainties with regards to potential risks to the environment and human health.

Thus, worldwide emergence of nanoscale science and engineering was marked by the announcement of the National Nanotechnology Initiative (NNI) in USA in January 2000. Recent research on biosystems at the nanoscale has created one of the most dynamic science and technology domains at the confluence of physical sciences, molecular engineering, biology, biotechnology and medicine. This domain includes better understanding of living and thinking systems, revolutionary biotechnology processes, the synthesis of new drugs and their targeted delivery, regenerative medicine, neuromorphic engineering and developing a sustainable environment. Nanobiosystems research is a priority in many countries as it very clear from rising trend in their R &D expenditure on Nanotechnology shown below in tables & graph and its relevance within nanotechnology is expected to increase in the future.



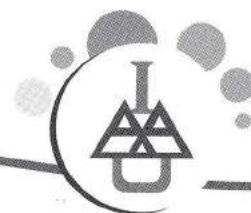
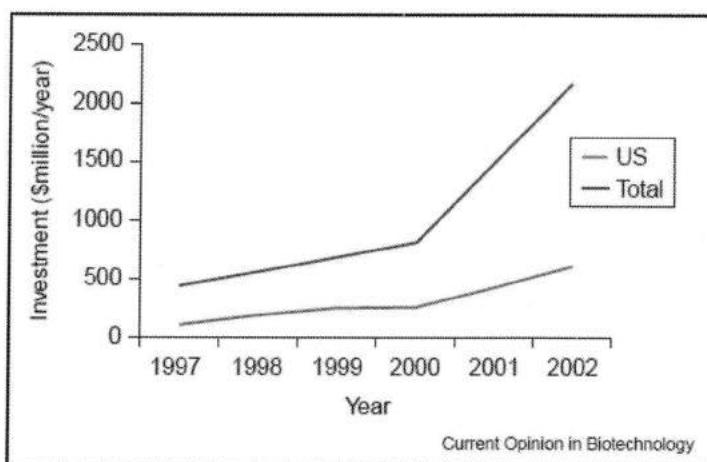


Table 1

Estimated government nanotechnology R&D expenditure (in \$ millions/year).

Area	1997	1998	1999	2000	2001	2002	2003	2004
Western Europe	126	151	179	200	~225 (270) <sup>1</sup>	~400	~600	
Japan	120	135	157	245	~465	~700	~810	
USA*	116	190	255	270	422 (465) <sup>1</sup>	600 (653) <sup>1</sup>	774	849
Others	70	83	96	110	~380	~550	~750	
Total	432	559	687	825	1580	2303	2934	
% of 1997	100%	129%	159%	191%	365%	533%	679%	

\*'Western Europe' includes countries in the EU and Switzerland. The rate of exchange \$1 = 1.1 Euro in 2002 and \$1 = 0.95 Euro in 2003; Japanese rate of exchange \$1 = 120 Yen in 2002. 'Others' includes Australia, Canada, China, Eastern Europe, FSU, Israel, Korea, Singapore, Taiwan and other countries with nanotechnology R&D. \*A financial year begins in the USA on October 1 of the previous calendar year and in most other countries six months before. <sup>1</sup>Denotes the actual budget recorded at the end of the respective FY. Estimations use the nanotechnology definition as defined in NNI (see [1\*\*]), and include publicly reported government spending.



Government investment in nanotechnology between 1997 and 2002 (total worldwide investment including US, blue; US investment, red).

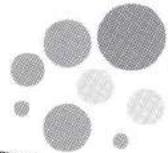
Table 2

Examples of NNI centers of excellence with biosystems research and education.

Center name	Funding agency	Institution
Nanoscience in Biological and Environmental Engineering (Nanoscale Science and Engineering Center - NSEC)	NSF	Rice University
Integrated Nanopatterning and Detection (NSEC)	NSF	Northwestern University
Directed Assembly of Nanostructures (NSEC)	NSF	Rensselaer Polytechnic Institute
Nanobiotechnology, Science and Technology Center	NSF	Cornell University
Institute for Cell Mimetic Space Exploration	NASA	UCLA
Institute for Intelligent Bio-nanomaterials and Structures for Aerospace Vehicles	NASA	Texas A&M
Bio-inspection, Design and Processing of Multifunctional Nanocomposites	NASA	Princeton

(Source: - Nanotechnology: convergence with modern biology and medicine Roco, M.C. www.current-opinion.com Current Opinion in Biotechnology 2003, 14:337-346)





## Nanoparticles from Noble Metals

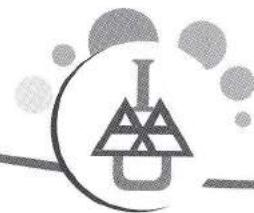
Noble metal nanoparticles (Ag, Au and their alloys) have unique optical properties, such as surface plasmon resonance (SPR), showing the dependence of optical properties on their size, shape, surrounding environment, and dielectric constant of the embedding medium. Electroless plating permits deposition of metals from solutions onto surfaces without the need to apply an external electrical potential. The method is based on chemical reduction of metal ions to deposit metals onto surfaces and is therefore easy to perform in common laboratories. This method has been successfully used for the deposition of metals, such as copper, silver, gold, nickel, rhodium and cobalt, on solid surfaces to produce fine metal patterns or micro- and nano-metallic structures. The studies on the electroless deposition of gold by a completely solution-based method have been reported. Conductive gold films were prepared on glass via NH<sub>2</sub>OH-mediated growth of gold colloidal monolayers. The electrochemical properties of the resultant gold films were investigated and the result showed that these films yielded excellent voltammetry. The use of photopolymerizable self-assembled monolayers (SAMs) has been reported on nanoparticles for electroless plating of gold films. More recently, the fabrication of gold films suitable for SPR measurements has also emerged. Compared with gold films prepared by conventional film fabrication technologies such as vacuum deposition and sputtering method, the electroless-plated gold films as reported herewith exhibit the following distinguishing characteristics. (1) The method is simple, cost-effective. (2) The route offers good control over gold film thickness and morphology. (3) The whole fabrication process is operated entirely in solution without any use of expensive instruments, so batches of identical films can be prepared simultaneously. Among the past researches, the electroless-plated gold films have been widely studied and used as the substrates for electrochemical detection and formation of photopolymerizable self-assembled monolayer. These gold plated nano particles have numerous applications in diagnosis and detection of pathogenic organisms as given by Yun Lei et al (1) in following discussions.

## Tools of Nanotechnology

Measurements of the intermolecular mechanics of a single protein molecule, polymer molecule or 'soft' nanoparticle have been performed with atomic force microscopy (AFM). Spatial and temporal interaction among cells, including intracellular forces has also been measured. AFM has been used to obtain the intermolecular binding strength between a pair of molecules in physiological solutions, providing the quantitative evidence of their cohesive function. Flow and forces around cells have been quantitatively determined and the mechanics of biomolecules are now better understood. It is accepted that cell architecture and macro behavior is determined by small-scale intercellular interactions. Fluorescent semiconductor nanoparticles, or quantum dots, have been developed for use in imaging and have been employed as markers for biological processes. These nanoparticles offer several advantages over fluorescent dye molecules, as they photobleach much more slowly and their emission wavelength can be finely tuned.

Nanoscience investigative tools have helped us to understand self-organization, supramolecular chemistry and assembly dynamics, and have furthered our knowledge of the self-assembly of nanoscopic, mesoscopic and even macroscopic components in living systems.





Investigative methods of nanotechnology have made inroads into uncovering fundamental biological processes, including self-assembly, cellular processes, and systems biology (such as neural systems). It has also been possible to measure the chemical composition within a single cell in vivo. Nanoscale instrumentation has also allowed measurements of small RNAs (also called 'nanoRNAs' or short stretches of RNA ranging in length between 21 and 28 nucleotides) and their significant effect on gene expression.

Dendrimers used in drug delivery and visualization were prepared by the divergent method, in which growth starts at the core and proceeds radially, and the convergent method where growth starts at what will become the periphery. The resulting shapes and conformations of dendrimers

The commercial production of nano-scale applications has already begun, and hundreds of products incorporating nanotechnology are commercially available. Products currently incorporating nanotechnology include simple passive nano-scale particles, compounds and composites for use in foods, pesticides, sunscreens, cosmetics, stain resistant clothing, automotive paints and coatings, sporting goods and digital cameras.

## **Nanotechnology Solutions for Biotechnology**

### **Confluence of Biology and Nanotechnology**

Nanobiotechnology is defined as a field that applies the nanoscale principles and techniques to understand and transform biosystems (living or non-living) and which uses biological principles and materials to create new devices and systems integrated from the nanoscale. The integration of nanotechnology with biotechnology, as well as with infotechnology and cognitive science, is expected to accelerate in the next decade.

Nanotechnology provides the tools and technology platforms for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Thus, Nanotechnology provides the tools to measure and understand biosystems.

### **Nanotechnology Solutions for Biomedicine and Agriculture**

Nanotechnology offers new solutions for the transformation of biosystems and provides a broad technological platform for applications in several areas: bioprocessing in industry; molecular medicine (e.g. for the detection and treatment of illnesses, body part replacement and regenerative medicine, nanoscale surgery, synthesis and targeted delivery of drugs); investigating the health effect of nanostructures in the environment (e.g. pollution by nanoparticles and eco-toxicology improving food and agricultural systems (e.g. enhancing agricultural output, new food products, food conservation); and improving human performance (e.g. enhancing sensorial capacity, connecting brain and mind, integrating neural systems with nanoelectronics and nanostructured materials).

Nanotechnology has also enabled the development of biochips and has a role in green manufacturing (e.g. biocompatibility and biocomplexity aspects). Other applications have included the design of sensors for astronauts and soldiers, biofluidics (for handling DNA and other molecules), in vitro fertilization of live stock, nanofiltration, bioprocessing 'by design' and traceability of genetically modified food. It might also find application in





designing pharmaceuticals as a function of patient genotypes and in applying chemicals to stimulate production as a function of plant genotypes. The synthesis of more effective and biodegradable chemicals for agriculture and the production of implantable detectors could be aided by nanotechnology. Employing this technology it should also be possible to develop methods that use saliva instead of blood for the detection of illnesses or that can perform complete blood testing within one hour. Broader issues include economic molecular medicine, sustainable agriculture, conservation of biocomplexity, and enabling emerging technologies. Biosystems offer models of inspiration for nanotechnology.

### **Linking Nanosensors to Drug Delivery Systems for Animals and Humans**

The notion of linking in-built sensors to in-built smart delivery systems has been called "the fuel injection principle" since it mimics the way modern cars use sensors to time fuel-delivery to the engine. The closest applications to market are implantable insulin-delivery devices or "drug chips" that will be linked with glucose sensors for (human) diabetics to automatically regulate blood sugar levels. Over time, this could become the model for all drug delivery, in both humans and animals.

### **Nanotechnology for Fisheries and Aquaculture Development**

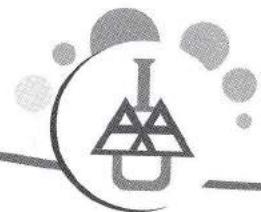
Aquaculture is the cultivation of fish shell fish, crustaceans or aquatic plants in natural or controlled marine or freshwater environments. Nanotechnology has multiple applications in fisheries and aquaculture development viz.

1. Production of more effective fish feed
2. Improving of the physical, chemical and nutritional quality of feed and their respective ingredients by application of Nanotechnology in the different steps of their manufacturing.
3. New materials in the different aspects of fisheries and aquaculture. These include: antifouling in fishing and aquaculture nets,
4. Antibacterial substances for aquaculture tanks and new packaging materials
5. New devices for Nanofood Market detection of shelf life of sea products, etc.

### **Use of Nanoparticle in Detection of Pathogens in Fish /Fishery Environment**

White spot syndrome virus (WSSV) is a shrimp viral pathogen that has severely impacted the worldwide shrimp culture industry (Chang et al., (2). WSSV is an enveloped DNA virus that causes 100% mortality in cultured shrimp within 3–4 days and the economic loss has been estimated at nearly one billion dollars per year since 1994. In recent years, the pathological effects of and preventive measures for this virus have been extensively studied in an attempt to control possible massive disease outbreaks. Many diagnostic tools, such as gene probes, polymerase chain reaction (PCR), flow cytometry, and immunological methods have been developed to control the spread of WSSV from parent shrimps and imported shrimps. However, these analyses are usually expensive, lengthy and labor-consuming. Furthermore, the immunoreactions cannot be monitored in real time. Hence, there is a need for the development of rapid, sensitive and practical techniques for identification and/or quantification of WSSV.





Infact, very recently, Yun Lei et al (1) have reported a very simple, quick and sensitive method for detection of WSSV virus in as low as 2.5 ng/mL in 2% shrimp hemolymph, based on nanotechnology principles of Electroless-plated gold films and surface plasmon Resonance. The plating condition for obtaining films suitable for SPR measurements was optimized. Gold nanoparticles adsorbed on glass slides were characterized by transmission electron microscopy (TEM). Detection of the WSSV was performed through the binding between WSSV in solution and the anti-WSSV single chain variable fragment (scFv antibody) preimmobilized onto the sensor surface. Specifically, an anti-WSSV single chain variable fragment (scFv) from an antibody phage display library was covalently attached to carboxyl-modified gold films and subsequently used for the SPR detection of WSSV. Both the scFv surface coverage and the WSSV sample composition were finetuned so that WSSV at trace levels can be analyzed without extensive sample pretreatments and modifications.

### **Chitosan Based Nanoparticles for Oral Delivery of DNA Vaccine in Fishes**

In recent years, attention has been focused on the possibility of utilizing DNA vaccines in fish aquaculture. A successful regime for intramuscular injection of naked DNA into fish has been developed and novel methods to deliver this DNA to fish are under investigation. The potential of chitosan as a polycationic gene carrier for oral administration has been explored since 1990s. In the present work, Kumar et al (3) have examined the potential efficacy of DNA vaccine against *Vibrio anguillarum* through oral route using chitosan nanoparticles encapsulation. The porin gene of *V. anguillarum* was used to construct DNA vaccine using pcDNA 3.1, a eukaryotic expression vector and the construct was named as pVAOMP38. The chitosan nanoparticles were used to deliver the constructed plasmid. In vitro and in vivo expression of porin gene was observed in sea bass kidney cell line (SISK) and in fish, respectively by fluorescent microscopy. The cytotoxicity of chitosan encapsulated DNA vaccine construct was analyzed by MTT assay and it was found that the cytotoxicity of pVAOMP38/chitosan was quite low. Distribution of gene in different tissues was studied in fish fed with the DNA (pVAOMP38) encapsulated in chitosan by using immunohistochemistry. The results indicate that DNA vaccine can be easily delivered into fish by feeding with chitosan nanoparticles. After oral vaccination Asian sea bass were challenged with *Vibrio anguillarum* by intramuscular injection. A relative percent survival (RPS) rate of 46% was recorded. The results indicate that Sea bass (*Lates calcarifer*) orally vaccinated with chitosan-DNA (pVAOMP38) complex showed moderate protection against experimental *V. anguillarum* infection. Therefore, chitosan may represent a potentially safe and efficient oral vaccine carrier against bacterial and viral pathogens of fish. Based on the data presented here, this study recommends the use of chitosan as a plasmid DNA carrier since it is more economical and practical for mass immunization in commercial fish farming.

### **Effects of Nanoparticle based Fish Feeds on Growth Performance of Food Fishes**

Selenium (Se) is an essential trace element for human and animal health. It was found to be an integral part of the glutathione peroxidase enzyme, Rotruck et al (4). Glutathione peroxide takes part in the cellular defence against oxidative damage of cytoplasmic structures by





catalyzing the reduction of hydrogen peroxide and lipid peroxides, Watanabe et al., (5). Selenium has been studied in rainbow trout ([Hilton et al., (6), [Hilton et al., (7), [Bell et al., (8) and [Vidal et al., (9)], catfish (Gatlin and Wilson, (10), Atlantic salmon (Lorentzen et al., (11) and juvenile grouper (Lin and Shiau, (12). Selenomethionine is a predominant chemical form of organic selenium in feedstuffs due to their excellent bioavailability and has been reported to have higher bioavailability than inorganic Se (sodium selenite) for Atlantic salmon ([Bell and Cowey, (13) and [Lorentzen et al., (11) and channel catfish (Wang and Lovell, (14). Recently Zhou et al (15) have designed a fish feed to evaluate the application of different Se sources, including Nano-Se and selenomethionine, as feed additives in diets for crucian carp- a valuable freshwater fish species of China.

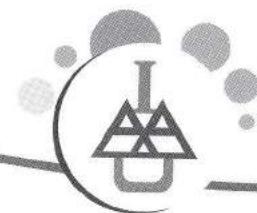
Scientists from the Russian Academy of Sciences have reported that young carp and sturgeon exhibited a faster rate of growth (30% and 24% respectively) when they were fed nanoparticles of iron. (Source: 'Down on the Farm: the Impact of Nano-Scale Technologies on Food and Agriculture', ETC Group Report, November 2004).

#### **Use of Zebrafish as Model to See the Toxic Effects of Nanoparticles on Environment**

Zebrafish (*Danio rerio*) have unique advantageous features over other vertebrate model systems (e.g., mouse, rat, human) (16-20). For example, its early embryonic development is completed rapidly within 120 h with well-characterized developmental stages. The embryos are transparent and develop outside of their mothers, permitting direct visual detection of pathological embryonic death, mal-development phenotypes, and study of real-time transport and effects of nanoparticles *in vivo*. Therefore, zebrafish embryos offer a unique opportunity to investigate the effects of nanoparticles upon intact cellular systems that communicate with each other to orchestrate the events of early embryonic development. Genetic screens of zebrafish phenotypes indicate similarities to human diseases, and protein sequences of drug-binding sites in zebrafish and human show a high degree of identities. (16-20). Thus, zebrafish have served as a vital model system for screening drug targets for curing human diseases. Large numbers of embryos can be generated rapidly at low cost, which can serve as an ideal *in vivo* assay for screening biocompatibility, pharmacological efficacy, and toxicity of nanoparticle probes. Fish are renowned for their ability to bioconcentrate trace contaminants in the environment. Human consumption of fish suggests a direct impact to human health by potential releases of nanomaterials into the environment.

Nanomaterials possess unique physical and surface properties, which have inspired plans for a wide spectrum of applications, such as target-specific vehicles for *in vivo* sensing, diagnosis, and therapy (e.g., nanomedicine, drug delivery) (21-25). These unique properties may also incite toxicity, damaging *in vivo* systems of interest and posing risks to human health and the environment (26). Currently, fluorescent probes, such as fluorescent dyes and proteins, are commonly used probes for *in vivo* imaging. Unfortunately, fluorescence probes suffer photodecomposition, offering limited lifetime for probing dynamic events of interest. Recently, nanoparticle probes such as semiconductor quantum dots (QDs) and noble metal nanoparticles are becoming popular and powerful probes for living cellular and *in vivo* imaging. (27-32). Therefore, Lee et al (33) have selected an effective *in vivo*





model system (zebrafish embryos) and one type of nanomaterials (Ag nanoparticles) and focused on probing the transport mechanism and dose-dependent biocompatibility of the nanomaterials in vivo, targeting the initial entry step of nanomaterials into embryos, and aiming to demonstrate its potential applications and address its potential adverse effects. They have further observed individual Ag nanoparticles inside embryos at each developmental stage, and in normally developed, deformed, and dead zebrafish, showing that biocompatibility and toxicity of Ag nanoparticles and types of abnormalities of zebrafish highly depend on the dose of Ag nanoparticles with a critical concentration of 0.19 nM.

### **Potential Risks to Microbial Diversity by Engineered Nanoparticles**

In addition to adverse effect of nanoparticles to animal and plant kingdom, they may also influence the microbial species as posed by First UK Govt report on this topic

(source:-[www.defra.gov.uk/environment/nanotech/nrcg/reports/](http://www.defra.gov.uk/environment/nanotech/nrcg/reports/)).

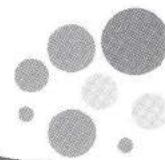
Examples of fundamental questions that might be addressed include

1. Interaction: do nanoparticles influence the fate, behaviour or ecotoxicology of other naturally occurring and anthropogenic substances present in the environment via microbial interactions? Does this interaction impact the microbial communities themselves (e.g. at the rhizosphere, in groundwaters and surface waters, in soils or sediments)?
2. Are microbial effect dose response relationships influenced by particle size, number, surface area or shape?
3. How do pH, ionic strength, the presence of organic matter and other factors influence the interaction of nanoparticles with microbial communities in water bodies?
4. Are substances in their nanoform within environmental matrices more persistent, bioaccumulative or toxic to microbial communities when compared to the substance in bulk or dissolved form?
5. How do nanoparticles interact with microbial biofilms and what are factors ?
6. Are interactions and impacts of nanoparticles to microbes governed by specific properties exhibited at the nanoscale (e.g. specific surface properties), properties that are both measurable and generalisable to certain or all classes of particles?
7. Can we model the interaction of nanoparticles with microbes?

### **New Emerging Nanotechnology Applications in Aquaculture Sector**

#### **Cleaning Fish Ponds with Nanotechnology Devices**

USA Nevada-based Altair Nanotechnologies makes a water cleaning product for swimming pools and fishponds called 'NanoCheck.' It uses 40 nm particles of a lanthanum-based compound which absorbs phosphates from the water and prevents algae growth. NanoCheck is currently undergoing large-scale testing in swimming pools and Altair is expected to launch a swimming pool cleaner in early 2005. Altair has its eye on a potentially large demand for NanoCheck for use in thousands of commercial fish farms worldwide



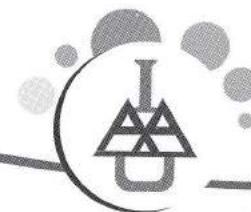
where algae removal and prevention is costly at present. According to Altair, the company plans to expand its tests to confirm that its nanoparticles will not harm fish, but no mention is made of the tests that will be undertaken to examine the impacts of nanoparticle-laden run-off on human health or on the environment.

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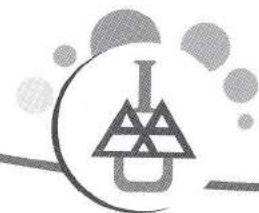


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## Application of Bio-Nanotechnology

Debu Chaudhary\*

Central Avian Research Institute, Izzatnagar, UP-243 122, India

People throughout the world say "Think big and dream big." Today I have come to prove Think small, think as small as you can. This small will become the biggest thing in no time. This small will make us meet the biggest challenges of science. Nano Technology which is called as 'The Alchemist of 21st century' has come to our living. Nano Technology is the latest technology that makes new materials by controlling atoms and molecules of Nano meter size i.e.  $1 \times 10^{-9}$ . It is the technology to make new materials, components and machineries based on the theory of that any atoms and molecules become to have different physical and electrical characters once its size becomes smaller to Nano sizes.

The United States is not the only country to recognize the tremendous economic potential of nanotechnology. While it is difficult to measure accurately, but estimates from 2005 showed the European Union (EU) and Japan invested approximately \$1.05 billion and \$950 million, respectively in nanotechnology. Behind them were Korea, China, and Taiwan with \$300 million, \$250 million, and \$110 million, respectively while India initiated with approx. \$35 million in nanotechnology research and development. The United States of America 2009 budget request provides \$1.5 billion for the National Nanotechnology Initiative, reflecting steady growth in the investment.

India revised the nanoscience and nanotechnology allocation to \$254 million in 2007. Dr A.P.J Abdul Kalam, Ex President of India" has rightly imagined India's vision (Next ten years will see nano technology playing a dominant global business role with technology going beyond the estimates and cross the figure of one trillion USD). Though India may have missed many a 'technology bus' over the decade we cannot afford to miss the 'Nano bus' as it is the future of the World- rightly indicated by C.N.R Rao, Chairman of the scientific advisory committee to prime minister

### **Nano materials Act as Natural Antibiotics and Preservative Against Infections**

They act as catalyst and disable the enzyme (Disulfide bonds(-S-S-) play an important protective role for bacteria as a reversible switch, that turns a protein on or off when bacterial cells are exposed to oxidation reaction) that one celled bacteria, viruses and fungi needed for their oxygen metabolism. They suffocate organism without causing harm to host enzyme or body chemistry. Nano materials can kill 99.9 % of MRSA bacteria within few seconds

### **Nano Materials as Disinfectants**

10 PPB of nano materials in drinking water system and within 24 hrs the colliform count in drinking water was brought to zero

### **Nano Materials as Immune Enhancer**

Nano materials assist in developing T- cells (T helper cells), Th1 participate in cell-mediated immunity, essential for controlling intracellular pathogens

\*Principal Scientist

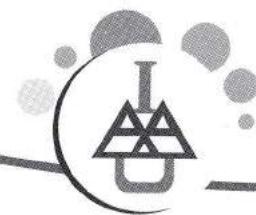




**Nano Materials is the Answer to Variety of Ailments. These are a list of just a few:-**

- Alleviating Angina.
- Improving the health of arteries.
- Helping lowering blood pressure in people afflicted with hypertension.
- Helping prevent Raynaud's Disease.
- Reducing the incidence of Gangrene in persons with Raynaud's.
- Blocking the replication of the HIV virus by stimulating the body's production of Interferon, increasing the body's production of Macrophages and NK-Lymphocytes.
- Helping suppress some forms of Cancer.
- Helping prolong the survival time of persons afflicted with Colon Cancer.
- Helping prolong the lifespan of persons afflicted with Liver Cancer.
- Significantly inhibiting the development of Lung Cancer.
- Inhibiting the growth of some forms of Detrimental Fungi:
- Inhibiting the growth of Candida albicans.
- Activating resting Macrophages and converting them to cytotoxic (killer) Macrophages
- Stimulating the production of Suppressor T-Cells.
- Lowering total serum Cholesterol levels.
- Enhancing the body's utilization and facilitation of Oxygen.
- Lowering the requirement for Oxygen consumption by Organs.
- Protecting against Carbon Monoxide asphyxiation.
- Alleviating various Eye Ailments:
- Retarding the progression of Cataracts (by preventing the cross-linking of the lens).
- Treating Detached Retinas.
- Alleviating Glaucoma.
- Rejuvenating the Blood Vessels that supply the Retina of the Eyes.
- Preventing decreased Bone strength, and increasing lowered bone density caused by Osteoporosis.
- Temporarily alleviating Epilepsy.
- Very effectively alleviating Pain.
- Successfully helping fight Diabetes Mellitus.
- Successfully helping fight Amyloidosis.
- Acting as sexual enhancer.
- Improving male libido movement.
- Regulating the functioning of thyroid gland.





### **Nanobiotics:**

Several Nanomaterials available with Amazon Bio Nanolabs have been identified to possess antibacterial properties and will be used in the form of Nanobiotics to eliminate several Bacteria, Viruses and Fungi. Small doses, greater results are the mantra behind Nanotechnology. These Nanomaterials have unique biological properties. The material that possesses toxicity at ionic level is absolutely non-toxic at Nano level. The risk of developing resistance and the transmission of resistant micro-organisms to humans through food products will be greatly reduced.

Future lies in the hands of Small. Future lies in hands of Nanotechnology research. There will be no single human or no single area that will remain untouched from this phenomenon. Nanotechnology is here to stay. The benefits of nanotechnology are being recognised worldwide and while there may be some public reservations over the technology. The best has come to serve our future generations. The science has become Nano and Nano has been synchronised to make us super humans.

### **Work on Nano-biotics in India**

Effect of oral administration of different levels of nano-materials (germanium and selenium) on growth and production performance of Japanese quails and production of premium nano-riched quail eggs has been achieved.

Sterilization of ICU and Operation Theater with less than 50 ppm concentration within 2 hours.

Use nanomaterial for anticestodal activities in relation to neurogastrointestinal disease specially in hydrated organisms.

A Nano particle on PPR virus replication has been studied.

### **Future Work Plan:**

Role of Nano silver in prevention and cure of diseases in chickens.

Nano selenium as immunomodulator in chickens

### **Research on Nano Stem Cells**

- a. Neural stem cell to combat epilepsy and neural disease
- b. On muscle cells to enhance growth pattern so broiler meat can be obtained at lower age (at 4 wks)
- c. On bone cells to make strong legs to bear the body weight at marketable age



## Nanomaterials and their Potential Applications in Food Processing

Y.S. Rajput\* and Rajan Sharma\*\*

National Dairy Research Institute, Karnal

### Introduction

The word 'nano' is used to indicate one billionth of meter or  $10^{-9}$  meter. Therefore nanoscience encompasses science of small particle in the size range of 1 to 100 nanometers. The applications of nanoparticles existed before scientists could realize that nanoparticles exhibit different physical, chemical and optical properties. These particles are now on focus of study because of potential applications in widely different field. Still considerable attention is being paid to develop protocols for synthesis and functionalization of nanoparticles. Nanoscale devices are often manufactured to imitate natural nanosize molecules, structures found in nature such as proteins, DNA, membrane. Nanotechnology in food is new compared to biomedical, information technology and manufacturing industries where nanotechnology is in use for fabrication of nanomaterials. In a survey performed by Israel and USA in year 2006 it was observed that over 200 manufacturers were involved in business of nanoproducts. Only 9% of nanoproducts were for 'food and beverages' (Sozer & Kokini, 2009). As of now, most of research on nanotechnology is focused on electronics, medicine, and automation sector. The knowledge gained from these sectors can be applied in food and agriculture products especially in food safety, water purification and delivery of nutrients.

### Nanomaterial Preparation

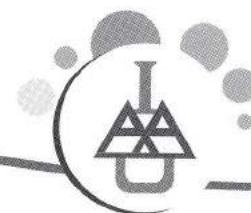
Nanomaterials can be prepared by either 'top-down' or bottom-up' approach. 'Top-down' approach involves physically breaking materials to nanometer range employing processes such as grinding, milling. Commercial scale production of nanoparticles largely involves 'top-down' approach. As the science of nanoparticles is developing, there is possibility of more use of 'bottom-up' approach. Size determines functionality of food materials. 'Top-down' involves reduction of size by application of force. A smaller size means bigger surface area and is good for water absorption, flavour release, better bioavailability and improved rate of catalysis. The particles of narrow size distribution are desirable for control of functionality and product quality. Different forces such as compression, impact and shear are used for nanomaterials in 'top-down' approach.

Ball milling and jet milling are commonly used for reduction of size in food (Sanguansri & Augustin, 2006). A ball mill is a cylindrical device used in grinding materials like ores, chemicals. Ball mills rotate around a horizontal axis, partially filled with the materials to be grounded plus grinding medium such as stainless steel balls. An internal cascading effect reduces the materials to a fine power. Industrial ball mill can operate continuously meaning that materials can be fed at one end and discharged at other end. High quality ball mills can grind to produce particles as small as 5 nm. The grinding works on principal of critical speed. Critical speed is that speed after which steel balls start rotating along the direction of the cylindrical device and thus causing no further grinding. Planetary

\*Principal Scientist

\*\*Senior Scientist





ball mills are smaller than common ball mill and mainly used in laboratories for grinding samples to very small sizes. A planetary ball mill consists of at least one grinding jar which is placed eccentrically on a sun wheel. The direction of sun wheel is kept opposite to that of grinding jar. The grinding balls in grinding jars are subjected to superimposed rotational movements. The difference in speeds between the balls and grinding jars produces an interaction between frictional and impact forces and resulting energy is used for size reduction.

Jet milling is fluid energy impact-milling technique used for producing materials between 1 to 10 microns. These are available in vertical as well as horizontal designs. A jet mill typically requires (i) source for fluid energy such as compressor, compressed gas generating system eg. evaporation of liquid nitrogen (ii) a means to meter the material to the pulverizer at a constant rate of feed and (iii) a means to separate the ground product from spent air eg. simple free hanging filter bag. This technique has been tested to produce high intensity sweetener sucralose with 5-10 micron particle size (Jackson et al., 1988). Production of low protein-starch has also been reported (Letang et al., 2002).

For the liquid product, high pressure homogenization and microfluidization are used and in these processes the product is subjected to very high shear stress causing the formation of very fine emulsion droplets (Sanguansri and Augustin, 2006). The shear is caused by sudden restriction of flow under high pressure through restrictive valve. Microfluidization process overcomes limitation of conventional processing technologies by utilizing high pressure streams that collide at high velocities in precisely defined microchannels. A combined effect of shear and impact act upon product to create finer, more uniform dispersions. Microfluidization produces a tighter particle size distribution than that of valve homogenization. It enhances mouth-feel and product texture (Sanguansri & Augustin, 2006). Its use in salad dressing, syrups, chocolate and malted drinks, flavour oils emulsions, cream and yoghurts has been demonstrated (Swientek, 1990).

Ultrasound emulsification occurs when two immiscible liquids are introduced into ultrasonic energy field in presence of surfactant. Ultrasonic is frequency beyond hearing range (>18 kHz). Bubbles formed in ultrasonic field cause intense waves in the surrounding liquid and formation of liquid jet at high velocity creates emulsion droplets. The droplet size is dependent on oil volume fraction, emulsification time, surfactant concentration and ultrasonic power (Sanguansri & Augustin, 2006). Emulsion in the range of 300 to 1000 nm can be prepared. The presence of stabilizer reduces the particle size of emulsion. Operating cost of ultrasound is lower in comparison to that of high pressure homogenization.

Supercritical fluids such as carbon dioxide can be used for production of 10 to 200 nm particle size. Rapid expansion of supercritical solutions (RESS) involves solubilization of product in supercritical fluid, and then depressurizing the solution through nozzle. This results in nucleation of the product. The technique is useful for production of microcapsules with active ingredient inside a carrier. (Sanguansri & Augustin, 2006).

The bottom-up approach relies on self-assembling properties of molecules under thermodynamic control to build supramolecular structures and microstructures to produce functional materials. Self assembly is dependent on attractive and repulsive forces between two molecules as building blocks. The interaction between building blocks can be influenced by temperature, concentration, pH, ionic strength etc. The example of self-



assembled nanostructures in food is casein micelles. When two or more immiscible liquids are stabilized by an adsorbed film at liquid-liquid interface forming an oil-in-water or water-in-oil emulsion. Nanoemulsions are between 50 to 100 nm in diameter and can have lipophilic cores separated from the aqueous phase by monomolecular layer of surfactant materials making it suitable for encapsulation of oil based bioactive compound.

### **Application of Nanoparticles in Food Packaging**

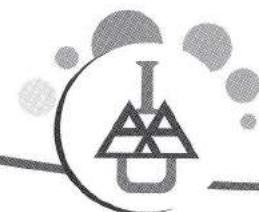
The use of protective coatings and suitable packaging by the food industry has become a topic of great interest because of their potentiality for increasing the shelf life of many food products. By means of the correct selection of materials and packaging technologies, it is possible to keep the product quality and freshness during the time required for its commercialization and consumption. Nowadays, most materials used for food packaging are practically undegradable, representing a serious global environmental problem. New bio-based materials have been exploited to develop edible and biodegradable films as a big effort to extend shelf life and improve quality of food while reducing packaging waste. However, so far the use of biodegradable films for food packaging has been strongly limited because of the poor barrier properties and weak mechanical properties shown by natural polymers. Nanotechnology has potential to produce packages with stronger mechanical and thermal performance with better barrier for water and oxygen and at the same time are biocompatible. Further, nano-sensors may be embedded in the packaging to alert consumers if a food product is no longer safe to eat.

### **Nano-reinforcements**

The use of nanoscale fillers in the polymer composites (mixtures of polymers with inorganic or organic additives) is leading to the development of polymer nanocomposites and represents a radical alternative to these conventional polymers composite. A uniform dispersion of nanoparticles in the packaging material leads to a very large matrix/filler interfacial area, which changes the molecular mobility, the relaxation behavior and the consequent thermal and mechanical properties of the material and thus called 'nano-reinforcements'. Fillers with a high ratio of the largest to the smallest dimension (i.e., aspect ratio) are particularly interesting because of their high specific surface area, providing better reinforcing effects. Although several nanoparticles have been recognized as possible additives to enhance polymer performance, the packaging industry has focused its attention mainly on layered inorganic solids like clays and silicates, due to their availability, low cost, significant enhancements and relative simple processability. The concept of polymer-clay nanocomposites (PCN) was developed in the late 1980s, and firstly commercialized by Toyota (Sorrentino et al., 2007), but only since the late 1990s researches have been published on development of PCN for food packaging (Ray et al., 2006). The layered silicates commonly used in nanocomposites consist of two-dimensional layers, which are 1 nm thick and several microns long depending on the particular silicate. Its presence in polymer formulations increases the tortuosity of the diffusive path for a penetrant molecule, providing excellent barrier properties (Adame & Beall, 2009).

In contrast with the tactoid structure predominating in conventional composites (microcomposites), in which the polymer and the clay tactoids (stacks of clay) remain





immiscible, resulting in agglomeration of the clay in the matrix and poor macroscopic properties of the material, the interaction between layered silicates and polymer chains may produce two types of ideal nanoscale composites. The intercalated nanocomposites result from the penetration of polymers chains into the interlayer region of the clay, resulting in an ordered multilayer structure with alternating polymer/inorganic layers at a repeated distance of a few nanometers. The exfoliated nanocomposites involve extensive polymer penetration, with the clay layers delaminated and randomly dispersed in the polymer matrix. Exfoliated nanocomposites have been reported to exhibit the best properties due to the optimal interaction between clay and polymer. The most widely studied type of clay fillers is montmorillonite (MMT), a hydrated alumina-silicate layered clay consisting of an edge-shared octahedral sheet of aluminum hydroxide between two silica tetrahedral layers. MMT is an effective reinforcement filler, due to its high surface area and large aspect ratio (50–1000).

Since clay layers constitute a barrier to gases and water, forcing them to follow a tortuous path, the introduction of nanoclays into polymer biostructures has been shown to greatly improve barrier properties (Adame & Beall, 2009), minimizing one of the main limitations of biopolymer films. The most widely known theories to explain the improved barrier properties of polymer–clay nanocomposites are based on a theory developed by Nielsen (1967), which focuses on a tortuous path around the clay plates, forcing the gas permeate to travel a longer path to diffuse through the film. The increase in path length is a function of the high aspect ratio of the clay filler and the volume% of the filler in the composite.

Nanocomposite material is generally prepared by one of the following two approaches (de Azeredo, 2009): In the first approach, also identified as “in situ polymerization”, the nano-filler is swollen within the liquid monomer so as the polymer formation can occur between the intercalated sheets. Polymerization can be initiated by heat or radiation, by the diffusion of a suitable initiator, or by an organic initiator or catalyst fixed through cationic exchange inside the interlayer before the swelling step by the monomer. In the second approach, the layered inorganic is mixed with the polymer matrix in either the molten state or a solvent in which the polymer is soluble. Under these conditions, and if the layer surfaces are sufficiently compatible with the chosen polymer, the polymer can crawl into the interlayer space and form either an intercalated or an exfoliated nanocomposite.

Recently, several research groups started the preparation and characterization of various other kinds of biodegradable polymer nanocomposites showing properties suitable for a wide range of applications. So far, the most studied biodegradable nanocomposites suitable for packaging applications are starch and derivatives, polylactic acid (PLA), poly (butylene succinate) (PBS), polyhydroxybutyrate (PHB), and aliphatic polyester as polycaprolactone (PCL).

### **Antimicrobial Nanocomposites**

The incorporation of antimicrobial compounds into food packaging materials has received considerable attention. Packaging material with antimicrobial activity could help control the growth of pathogenic and spoilage microorganisms. An antimicrobial nanocomposites film is particularly desirable due to its acceptable structural integrity and barrier properties imparted by the nanocomposite matrix, and the antimicrobial properties contributed by





the natural antimicrobial agents impregnated within. Materials in the nanoscale range have a higher surface-to-volume ratio when compared with their microscale counterparts. This allows nanomaterials to be able to attach more copies of biological molecules, which confers greater efficiency. Nanoscale materials have been investigated for antimicrobial activity so that they can be used as growth inhibitors, killing agents or antibiotic carriers.

The most common nanocomposites used as antimicrobial films for food packaging are based on silver, which is well known for its strong toxicity to a wide range of microorganisms, with high temperature stability and low volatility. The antimicrobial property of silver nanoparticles are due to its adhesion to the cell surface, interaction of Ag<sup>+</sup> with cytoplasmic components and nucleic acids, and to interfere with the membrane permeability. Silver ions are believed to interact with the ribosomes, inhibiting the expression of enzymes related to ATP production (Fernandez et al., 2009).

Chemical reduction (e.g. using sodium borohydride) is the most common method for preparation of silver nanoparticles (Ag-NPs) as stable, colloidal dispersions. The reduction of Ag<sup>+</sup> in aqueous solution produces colloidal silver with particle diameters of several nanometers (Wiley et al., 2005). Initially, the reduction leads to the formation of silver atoms (Ag<sup>0</sup>) and their subsequent aggregation into oligomeric clusters, which leads to the formation of Ag particles. The synthesis is often performed in the presence of stabilizers in order to avoid undesirable agglomeration of colloids. A correlation has been found between the aggregation stability and antibacterial activity. Ag-NPs have been successfully tested as an antimicrobial material. Smaller Ag-NPs, having larger surface area available for interaction with microbial cells, result in better bactericidal effect than larger Ag particles. In one of the recent study (Fernandez et al., 2009), Ag-NPs have been incorporated into absorbent pads which could then be used for the preservation and for maintaining the aseptic conditions during the storage of food products such as meat, poultry and fish.

### **Oxygen Scavenging Packaging Material**

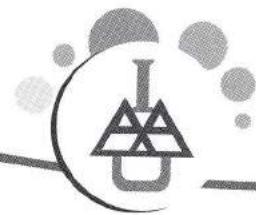
Oxygen (O<sub>2</sub>) is responsible for the deterioration of many foods either directly or indirectly. Direct oxidation reactions result in browning of fruits and rancidity of vegetable oils, to name a few examples. Food deterioration by indirect action of O<sub>2</sub> includes food spoilage by aerobic microorganisms. The incorporation of O<sub>2</sub> scavengers into food package can maintain very low O<sub>2</sub> levels, which is useful for several applications. Attention has particularly focused on the photocatalytic activity of nanocrystalline titania (TiO<sub>2</sub>) under ultraviolet radiation.

Oxygen scavenger films were successfully developed by Xiao-e et al. (2004), by adding titania nanoparticles to different polymers. The authors suggested their use for packaging a wide variety of oxygen-sensitive products. As TiO<sub>2</sub> act by a photocatalytic mechanism, its major drawback is the requirement of UV light.

### **Nano-based Sensors**

Nanoparticles can be applied as reactive particles in packaging materials. The so-called nanosensors are able to respond to environmental changes (e.g., temperature or humidity in storage rooms, levels of oxygen exposure), degradation products or microbial contamination. The food expiration date is estimated by industries by considering distribution and storage





conditions (especially temperature) to which the food product is predicted to be exposed. However, it is not known that such conditions are always the real ones, and foods are frequently exposed to temperature abuse; this is especially worrying for products which require a cold chain. Moreover, micropores or sealing defects in packaging systems can lead food products to an unexpected high exposure to oxygen, which can result in undesirable changes. When integrated into food packaging, nanosensors can detect certain chemical compounds, pathogens, and toxins in food, being then useful to eliminate the need for inaccurate expiration dates, providing real-time status of food freshness.

**Gas Sensors:** Based on applied studies of the surface properties of materials, several types of gas sensors have been developed, which translates chemical interactions between particles on the surfaces into a response signal. Metal oxide gas sensors are one of the most popular types of sensors because of their high sensitivity and stability. Food spoilage is caused by microorganisms, whose metabolism produces gases which can be detected by conducting polymer nanocomposites (CPC) or metal oxides, which can be used for quantification and/or identification of microorganisms based on their gas emissions. Sensors based on CPC consist on conducting particles embedded into an insulating polymer matrix. The resistance changes of the sensors produce a pattern that corresponds to the gas under investigation (Arshak et al., 2007). The authors have demonstrated that three bacteria (*Bacillus cereus*, *Vibrio parahemolyticus* and *Salmonella* spp.) could be identified from the response pattern produced by the sensors.

Another class of material used for detection of gases is conducting polymers (CP) or electro active conjugated polymers, which can be synthesized either by chemical or electrochemical oxidation. Such materials electrical, electronic, magnetic and optical properties, which are related to their conjugated  $\pi$  electron backbones. Polyene and polyaromatic CPs such as polyaniline (PANI), polyacetylene, polypyrrole (PPy) have been widely studied (Ahuja et al., 2007). Electrochemically polymerized CPs have a remarkable ability to switch between conducting oxidized (doped) and insulating reduced (undoped) state, which is the basis of many applications.

**Oxygen sensors:** Oxygen allows aerobic microorganism to grow during food storage. There has been an increasing interest to develop non-toxic and irreversible oxygen sensors to assure oxygen absence in oxygen-free food packaging systems, such as packaging under vacuum or nitrogen. An UV-activated colorimetric oxygen indicator, which uses nanoparticles of titania ( $\text{TiO}_2$ ) to photosensitize the reduction of methylene blue (MB) by triethanolamine in a polymer encapsulation medium has been developed (Lee et al., 2005). Upon UV irradiation, the sensor bleaches and remains colorless, until it is exposed by oxygen, when its original blue color is restored. The rate of color recovery is proportional to the level of oxygen exposure. In another technique, Gutierrez-Tauste et al (2007) deposited MB/ $\text{TiO}_2$  nanocomposite thin films on glass by liquid phase deposition (LPD), a soft chemical technique which has been applied for deposition of oxides to several substrates. This technique could be used to develop oxygen indicator packaging systems for a variety of oxygen-sensitive foods. Mills & Hazafy (2009), have used nanocrystalline  $\text{SnO}_2$  as a photosensitizer in a colorimetric  $\text{O}_2$  indicator comprising a sacrificial electron donor (glycerol), a redox dye (methylene blue - MB), and an encapsulating polymer (hydroxyethyl cellulose). Exposure to UV light led to activation (photobleaching) of the



indicator and photoreduction of MB by the SnO<sub>2</sub> nanoparticles. The color of the films varied according to O<sub>2</sub> exposure – bleached when not exposed, and blue upon exposed.

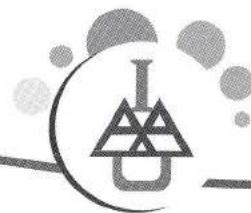
### Concluding Remarks

Applications of nanotechnology in food science are still in infancy. The technology is being used to develop biodegradable packaging material with high mechanical strength, better thermal properties, diminished permeability and antimicrobial property. The development of nano-based sensor as indicator of pathogen, pesticide & other contaminants and adulterants offers good promise for assessing quality of food in short duration. Considerable efforts have not so far placed in evaluating possible toxic effects of nanoparticles and therefore people at large are reluctant to consume nanoparticles based food products.

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## Role of NDRI as the Apex R&D Institution for Dairy Development

Meena Malik\*, Rameshwar Singh\*\* and A. K. Srivastava\*\*\*

National Dairy Research Institute, (Deemed University), Karnal-132001

### Abstract

*National Dairy Research Institute as the premier Dairy Research Institution is marching ahead with its research, teaching and extension activities with the sole aim of augmenting dairy development in the country. Being the National Institute under the aegis of Indian Council of Agricultural Research, it continues to conduct basic and applied research with the objective to enhance animal productivity and also to develop cost effective technologies for the benefit of the consumers, the industry and the farmers. The Institute, having the status of Deemed University, is contributing significantly to the Human Resource Development through its Undergraduate and Post-graduate teaching /training programmes. Looking at the present monumental set-up of the National Dairy Research Institute, the Premier Institution of Dairying in Asia, one just cannot dispense with the historical way of looking at its pristine grandeur. Like any other great Institution, NDRI has a great history. The flowering glory of this Institution germinating from the dreams of the great visionaries requires proper nurturing and care in equal measures by the present generation of its custodians. As the country's premier Dairy Research Institution, NDRI has developed considerable expertise over last eight decades in different areas of Dairy Production, Processing, Management and Human Resource Development. Information generated at the Institute and services offered have contributed to the growth of Dairy Industry as a whole and well being of millions of milk producers and consumers of milk and milk products. Realizing the challenging need of globalized Dairy Trade, the Institute is continuously working to develop its R&D and HRD programmes to better serve the nation in terms of food security, employment generation, and poverty alleviation.*

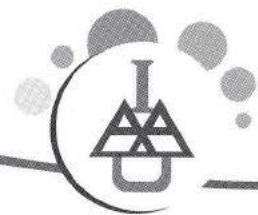
Dairying in India has witnessed tremendous growth during the last few decades and has come to be recognized as a potent instrument to bring about socio-economic changes in the urban and rural sectors alike. With an annual increase of 4.7%, milk production has increased from 17 million tonnes in 1971 to approximately 104.9 million tones in 2008-2009. Livestock sector contributes 5.59% to National GDP and 36.6% to Agricultural GDP. Dairying contributes a major share, which is more than paddy and wheat, in the total GDP of the agriculture sector. This remarkable success of the Indian Dairy Sector can be attributed to the constant efforts of the scientists, planners and millions of farming families across the country. The strength of Indian Dairy sector lies in the fact that despite limited investments, it has shown consistent and sustainable growth. Planning Commission estimate suggests that by the end of XI plan, milk production will touch 120 million tonnes, growing at 4.8%, which is nearly 4 times the global rate. Currently, consumption of fresh liquid milk accounts for about 60 -70% of the total milk production and the rest is processed, of which 16-17 % is processed by the organized sector.

The role of The National Dairy Research Institute (NDRI) as the apex R&D Institution for spearheading the growth of dairying on the sound scientific lines is quite significant and substantial. The foundation stone of the edifice of National Dairy Research Institute was laid with the establishment of Imperial Institute for Animal Husbandry & Dairying

\*Assistant Professor (English)

\*\* Registrar

\*\*\* Director and Vice Chancellor



at Bangalore on July 1, 1923. This was the first concrete step undertaken towards the organised development of cattle and dairy industry in the country. In fact the idea of having such an Institute was conceived in 1916 itself, when Government of India (GOI), implementing the recommendations of the Board of Agriculture appointed William Smith, Assistant Director of Military Farms as Imperial Dairy Expert. The assigned mandate given to Mr. William Smith was to help establish dairy industry on commercial lines, to study and improve the existing methods of dairying, to start dairy education programmes and to develop good dairy breeds of cattle and buffaloes. Mr. William Smith travelled extensively throughout the country and identified major problems to be undertaken on high priority. Two major problems identified by him were the lack of technical personnel trained in scientific method of dairy production and processing, and acute shortage of stock of good germplasm of cows and buffaloes for establishing the foundation herds at dairy farms.

Mr. William managed the transfer of Military Farms of Bangalore, Karnal and Wellington (Nilgiris) to the Imperial Department of Agriculture in 1923. He selected Bangalore as its headquarters and named Karnal and Wellington farms as its substations. The Central Institute was, thus, located on a farm of about 100 acres at Bangalore. A herd of crossbred cattle, which had been taken over along with farm, was retained. To this, a herd of pure Red Sindhi cattle was added. Meanwhile, facilities for teaching were developed and also dairy machinery and appliances were procured. The staff of the already existing Animal Nutrition Section at Bangalore offered courses to train dairy managers and employees of Military Dairy



*Imperial Institute of Animal Husbandry & Dairying established at Bangalore in 1923*

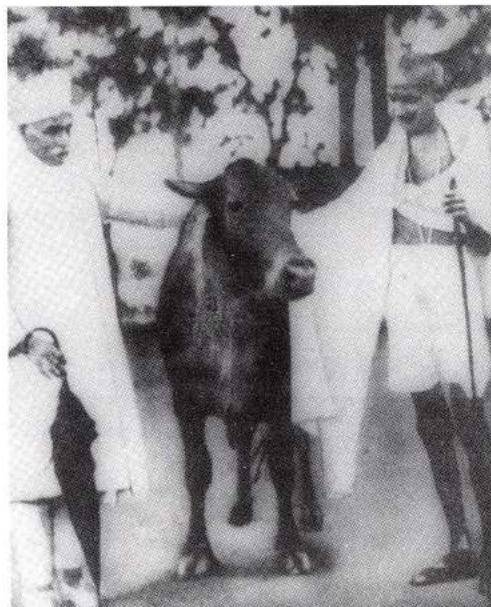
Farms. The Physiological Chemist's Section located at Pusa, Bihar was also transferred to Bangalore Institute. All these facilities, thus pooled at Bangalore, constituted the core infrastructure and were utilized as the main Educational and Experimental Centre. Hence, the Imperial Institute for Animal Husbandry & Dairying came into existence in 1923.

Since its inception at Bangalore, Imperial Institute for Animal Husbandry & Dairying started a two-year training course for the award of Indian Diploma in Dairying (IDD) on the lines of National Diploma in Dairying given by the Royal Agricultural Society of England. Later in 1924, two other courses, viz., (i) a 15 month Post-graduate course (subsequently designated as Associateship course) for imparting higher knowledge in dairying and research techniques and (ii) a 3 month short course on practical training in dairy farming for the benefit of the personnel in the Industry, were initiated. Subsequently, special training courses for imparting instruction to Cooperative Inspectors and Vocational Training Course for soldiers and ex-service personnel were also started. A galaxy of pioneers,



who were passed out from this Institute, steered several projects in dairy development in different states of the country.

During the early stage of its progress from 1923 to 1932, the activities of the Institute were mainly confined to the training of students apart from providing technical advice and guidance to the industry and the State Governments. Dr. Z.R. Kothawalla was recruited as Assistant Imperial Dairy Expert in 1925. Subsequently in 1931 when Mr. William Smith retired, he was elevated to the post of Imperial Dairy Expert. The most important event in the history of the Imperial Institute for Animal Husbandry & Dairying was the training imparted to Mahatma Gandhi and Pandit Madan Mohan Malviya in 1927. They came to the Institute for study training and to get acquainted with modern methods of cattle management. Both of them spent two weeks discussing and learning the technicalities and complexities of problems pertaining to cows and buffaloes in India. Mahatma Gandhi evinced great interest in the work of the Institute and wrote several articles in 'Young India' and 'Harijan' on the importance of dairying and scientific cattle management.



*Mahatma Gandhi and Pt. Madan Mohan Malviya at Imperial Dairy Research Institute, Bangalore*

In 1936, the Karnal Military Animal farm was handed over to the Imperial Institute of Agricultural Research, New Delhi. At the same time, the Physiological Chemist's Section was transferred to Izatnagar under the Imperial Veterinary Research Institute. The Imperial Dairy Experts' Section along with the Bangalore Dairy Institute was separated from the administrative control of the Imperial Agricultural Research Institute, New Delhi, and placed directly under the erstwhile Department of Education, Health and Lands (presently the Ministry of Agriculture). This change helped the Institute in securing greater attention from the Government in its development. The name of the Institute was changed to "**Imperial Dairy Institute**".



*Mr. William Smith  
1920-31*



*Dr. Z.R. Kothavalla  
1931-39 & 41-44*



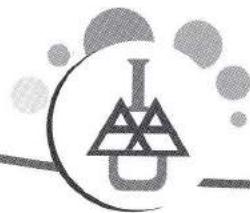
*Dr. W.L. Davies  
1939-41*



*Dr. K.C. Sen  
1944-57*

*Directors of Imperial Institute of Animal Husbandry & Dairying*





In 1939, Dr W. L. Davies, a distinguished Dairy Chemist (the author of book Chemistry of Milk) was appointed as the Director of Dairy Research Institute, which government of India had established on the recommendations of Dr. N. C. Wright, Director of Hannah Dairy Research Institute, Ayer, Scotland. The government had appointed him to examine and make recommendations for putting Indian dairy industry on sound lines. Dr Davies established the Divisions of Dairy Chemistry and Dairy Bacteriology and located them temporarily in IARI premises. He also surveyed available sites for the location of new Dairy Research Institute and was about to decide in favour of a site at Ferozshakotla grounds, but unfortunately, he fell ill and died in 1941. Dr. Z. R. Kothawalla was appointed to succeed him.

In 1941 itself, Dr. Z. R. Kothawalla shifted the headquarters of the Director of Dairy Research Institute to Bangalore and brought back the Divisions of Dairy Chemistry and Dairy Bacteriology from IARI to Bangalore. In August 1944, Dr. Kothawalla was transferred to New Delhi as Dairy Development Advisor, a post sanctioned under the "Grow More Food Drive".

In September 1944, Dr K. C. Sen Officer-in-charge of Animal Nutrition Section at IVRI, Izatnagar was appointed as Director of the Institute. Dr. Sen strengthened Animal Nutrition studies at Bangalore Institute where Prof. Worth, Physiological Chemist had already started work on Nutrition & Physiology of Dairy animals since 1936 itself. Later Dr. Sen was deputed on study tour to visit Dairy Research Laboratories in UK, Europe and USA. After his return to India in 1946, a new section of Dairy Technology was opened in the Institute. Then the research and training activities of the Institute were intensified and managed under four research sections i.e. Dairy Bacteriology, Dairy Chemistry, Dairy Husbandry and Dairy Technology. Later, in 1947, the fifteen months' Associateship course was discontinued and in its place the Institute admitted a limited number of Honorary Research Workers for taking up post-graduate studies leading to M. Sc. and Ph.D. Degrees from different Universities.

In the mean time, the proposal for reorganization of the Institute was again taken up in 1946, when Prof. H. D. Kay, Director of the National Institute for Research in Dairying, Reading (England) was invited to review the existing set up and make recommendations. Prof. Kay recommended the early establishment of a National Dairy Research Institute at or near Delhi along with a Dairy Science College for starting degree and post-graduate courses in dairying and to continue the existing Institute at Bangalore as Regional Station to cater to the requirements of the Southern States of India. In 1954, the Government of India decided to establish the National Dairy Research Institute together with Dairy Science College at Karnal in the premises of the Cattle-cum-Dairy farm (old Karnal farm), which had been functioning under the direct control of the Union Ministry of Food and Agriculture since 1951.

The headquarters of the Director of Dairy Research Institute was shifted from Bangalore to Karnal in June 1955. To assist in the establishment and organization of the new Institute, a number of senior research and technical staff was also transferred to Karnal. Sri Ajit Prasad Jain, the then Union Minister for Food and Agriculture, laid the foundation stone for the National Dairy Research Institute on 7th August 1955. Dr. K.C. Sen, who had held the post of Director of Dairy Research Institute since 1946, retired in January 1957. He was



succeeded by Dr. K.K. Iya, who was working as Dairy Bacteriologist at Bangalore.

After the establishment of NDRI, the main task was to start the Dairy Science College along with other many Divisions such as Animal Husbandry, Dairy Technology, Dairy Chemistry, Dairy Bacteriology and Dairy Engineering besides Service Sections like Economics, Statistics and Library. This proposal was included in 2nd Five Year Plan



*National Dairy Research Institute (NDRI) at Karnal*

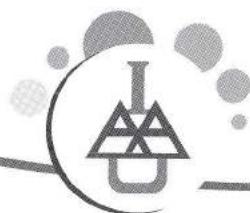
of the Ministry of Agriculture, GOI and Rs. 2 crores were allotted for it. Then the work started for providing basic infrastructure of water, electricity, roads, sewage disposal plant, fodder farm, cattle yard etc. Subsequently, the construction work of different divisions, administrative block, library, students' hostels, guest house and residential quarters was initiated. And this was done with the cooperation of all central government departments & ministries/ agencies of Panjab Government and local officials.

The Dairy Science College was then affiliated to the Punjab University. The first B. Sc (Dairying) course was started in July 1957 with only 10 candidates selected from all over India. The degree programme offered broad training in the field of Dairy Science covering both dairy production and processing. Dr. Noshir N. Dastur, Head of the Dairy Chemistry Division, was appointed as the first Principal of the College. In 1961, the composite B.Sc. (Dairying) course was bifurcated into B.Sc. (Dairy Technology) and B.Sc. (Dairy Husbandry) courses. The M. Sc. course in different branches of dairy science was started in 1961 and facilities were also provided to research scholars for conducting research work for the Ph.D. degree from different Universities. A Dairy Engineering Diploma course was also started in 1961. Later, the IDD Course was also bifurcated into IDD (Dairy Technology) and IDD (Dairy Husbandry).

The Southern Regional Station at Bangalore was strengthened with the setting up of a new dairy building and reorganization of its programmes. The Western Regional Station at Bombay and the Eastern Regional station at Haringhatta (Kalyani) near Calcutta were established in 1962 and 1964, respectively. However in 1984, the Western Regional Station (WRS) at Bombay was closed down. In 1966, the Institute's management was weaned away from the Ministry of Agriculture and brought under the umbrella of Indian Council of Agricultural Research (ICAR). This was



*Visit of Smt. Indira Gandhi, Prime Minister of India to NDRI on 21st Nov., 1970*



mainly done to provide greater operational autonomy in research management functions. NDRI flourished enormously during the era of Dr D. Sunderasan who steered the Institute for more than a decade. It was during his tenure that NDRI made its visibility in farming communities in the surrounding villages through the Operational Research Project (ORP). Under this project, in 1975 a cluster of villages was adopted for translating the research conducted at NDRI into reality. A landmark of this era was the development of two strains of cattle, namely Karan Swiss and Karan Fries by crossbreeding, followed by selection. For further augmenting the Dairy Education programmes of the Institute, in 1976, the Department of Human Nutrition and Dietetics was also established. In 1979, M. Sc. and Ph. D. Programmes in Dairy Engineering were introduced.

Dr R. Nagarcenkar, who was appointed as Director of the Institute in June 1984 gave further impetus to the Transfer of Technology programmes through setting up of the "Farm Advisory Bureau" and "Industrial Consultancy Cell" in 1985. The research in the area of Biotechnology got boost and the "Embryo Biotechnology Centre" was established in the Livestock Farm premises in 1987. The Institute also got recognition as Centre of Excellence in Biotechnology. The B. Sc Dairying (Dairy Technology) was renamed as B.Tech (Dairy Technology). A few postgraduate courses viz., M.Sc. (Human Nutrition), M.Sc. (Forage Production) and M.Sc. (Quality control) added earlier were, however, discontinued. Diploma course in Dairy Engineering was also discontinued in the year 1984. Another short course on Dairy Management, started at SRS Bangalore, was dropped after a few sessions only. IDD, which had been closed at Bangalore in the year 1986, was, however restarted in 1996 as National Dairy Diploma (NDD). An important milestone in the history of NDRI was added in 1989 when the Institute was conferred the status of Deemed University for further strengthening the academic programmes for human resource development. In 1994, The Institute got recognition as a Centre of Advanced Studies in Dairy Technology and Dairy Cattle Breeding disciplines to further strengthen the research and training components.

Research efforts at NDRI resulted in a number of scientific breakthroughs. The production of ten calves from a single cow through ETT in one calendar year and first in vitro fertilized buffalo calf "Pratham" in 1991 were the few significant achievements that the Institute has made. Through scientific breeding and progeny testing programme, Murrah Buffalo bulls with superior milk producing ability were produced.

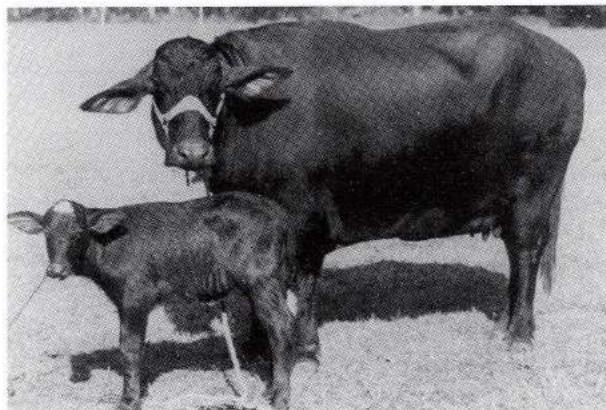
Detailed research on endocrine profiling of animals, metabolism in buffalo spermatozoa during storage and cryopreservation was carried out. The research efforts in ruminant nutrition resulted in enrichment of nutritive value of poor quality roughages, balanced feed and feed block formulations, and bypass protein technology. These findings have



*The Institute granted Deemed University status by the then Union Minister for Agriculture, Sh. Bhajan Lal*



proved very useful in raising the animal productivity further. During this period the Institute received sufficient grants through World Bank funded National Agricultural Research Project (NARP). The decade of 1990s also registered many scientific and technological achievements in the field of Dairy Processing. Many new technologies, such as kulfi mix powder, accelerated ripening of cheese, whey powder & WPC using membrane technology, long life paneer curry using hurdle technology & retort processing, cake mixes using milk byproducts, long-life kheer, rasogolla mix powder, lassi powder, cheddar cheese flavour base, whey based fruit juices in dry & concentrated form, coffee & tea complete powder, instant rice-kheer mix, shelf-life modeling of condensed milk & soy beverage were developed. Dairy Engineering Division of NDRI designed the prototype of a machine that could prepare khoa on continuous basis. A milk adulteration kit was also developed. In the year 1999, the Institute got funding to the tune of 266.25 lakhs through 9 NATP Projects.

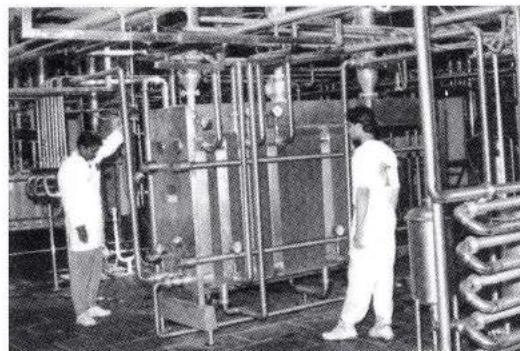


*First Buffalo Test Tube Calf Pratham (1991)*

This decade of nineties will be remembered for creation of many new facilities at NDRI such as 20 bedded Hospital Complex in 1991, a state-of-the-art Auditorium having seating capacity of 950 and 2 conference rooms and 2 meeting rooms in 1997, and the construction of a modern Cafeteria with a seating capacity of 150 in front of the boys' hostels in 1998. Another landmark added to the infrastructure of the Institute was setting up of a commercial Model Dairy Plant, in collaboration with NDDB, with a capacity to process 60,000 lit. of milk per day. The mandate of this state-of-the-art milk plant is to impart practical training to the students of NDRI University and also to serve as an interface between Institute and Industry.

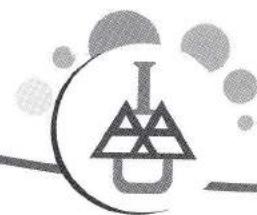


*Model Dairy Plant at NDRI*



*Students during their In-plant Training at Model Dairy Plant*

The first decade of new millennium of 2000 has also been quite productive for the Institute. Progeny testing of crossbred cattle, Sahiwal cattle and Murrah buffaloes continued



through associated herd and All India Coordinated Project. Six sets of Karan Fries bulls, four sets of Sahiwal bulls and three sets of Murrah buffalo bulls have been evaluated till date. Protocols for transferring IVF goat embryos to synchronized recipient goats using laparoscopy were standardized resulting in the birth of first in vitro fertilized goat kid in the country at NDRI. Further, the Institute continues to provide high quality manpower to help meet the human resource requirements for the overall dairy development in the country. The Institute has also introduced Ph. D. Programme in Animal Biotechnology since 2002-03. The participation of the Southern Regional Station (SRS) at Bangalore in academic programmes of the Institute has been further strengthened by offering M.Sc. and Ph.D. Programmes in a few disciplines of Dairying since 2002-03. In the recent past, Academic Programmes of NDRI Deemed University have been duly accredited by the Accreditation Board of ICAR for a period of 5 years. The Institute is also carrying out extension programmes for transferring the know-how from the laboratory to the farmers' fields.

Some new infrastructures have been added to meet the challenges in dairy science. A new state of the art imposing building of Animal Biotechnology Centre having 60,000 square feet laboratory space came up and is serving as a nucleus for biotechnological research. Animal Genomics Laboratory has been established under NICHE Project on Buffalo Production and Reproduction Genomics (funded by Education Division of ICAR). Construction of International



*Research Scholars working in the lab*

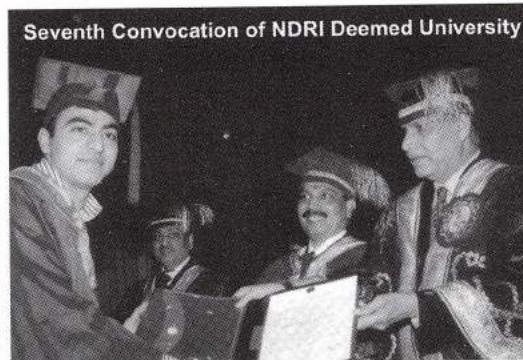
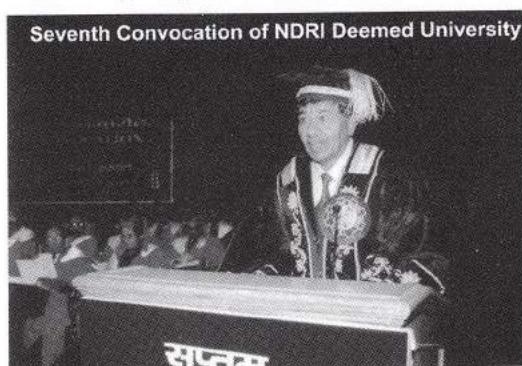
Students' Hostel equipped with modern facilities and amenities is another feather in the cap of the Institute. Mini-Auditorium equipped with audio visual aids now meets the requirement for organization of workshops. A video conferencing facility has been created to make use of IT for better communication. A fan- and mist- cooling system has been installed in recently created shelter system for housing 120 elite cows and buffaloes at the Livestock Farm. Establishment of Technology Business Incubator (TBI) Facility to promote entrepreneurship has been another step ahead. To give thrust to research & development efforts, research laboratories have been upgraded and equipped with latest precision analytical instruments for carrying out research in the most advanced areas of Dairying. Computerized herd management and data management systems have been successfully installed at the Livestock Farm of the Institute. Feed Quality Control Laboratory has also been established. The facilities in certain specialized laboratories i.e. Animal Genomics, protein engineering, Nutraceuticals, Downstream Processing and Real Time PCR and Reproduction Physiology have also been upgraded. Further, keeping in view the significance of ICT vis-à-vis TOT in current scenario, Agriculture Technology Information Center (ATIC) has been created. This centre is serving as 'a single window' for all ICAR Institutes located at Karnal. It helps farmers, entrepreneurs, extension workers,



development agencies, non-government organizations (NGOs) and private sector organizations in providing solutions to their problems in Agriculture and Dairying.

In the year 2009, World Bank through NAIP has funded nineteen research projects (with an outlay of Rs. 32.53 crores) in Consortium Mode wherein NDRI is either a Lead Institute or a Consortium Partner. Research grants received from NAIP will boost the research in the areas of buffalo reproduction, proteomics, stem cell, embryo cloning, bio-sensors, nano particles, semen cryopreservation, functional foods, nutraceuticals, agroweb and e-learning. Also, a Network Project with an outlay of rupees 9 crores has been initiated at NDRI to address the burning issue of impact adaption and vulnerability of Indian Agriculture to climate change. Signifying the importance of indigenous dairy products, a Network Project initiated in 1997 has resulted in development of number of traditional-products technologies. In the recent past, NDRI has been successful in getting external funding from almost all leading national funding agencies i.e. Dept. of Biotechnology (DBT), Department of Science and Technology (DST), National Dairy Development Board (NDDB), National Bureau of Agriculturally Important Microorganisms (NBAIM), National Communication to United Nations Framework Convention on Climate Change (NATCOM-UNFCCC), Ministry of Food Processing Industries, National Fund for Basic and Strategic Research on Agriculture (NFBSRA), and Haryana State Council of Science and Technology (HSCST).

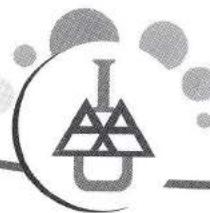
The Institute continues to attract international students from Nepal, Bangladesh, Afghanistan, Iran, Iraq, Myanmar, Mauritius, Sri Lanka, Vietnam, Ethiopia, Rawanda, Holland, Egypt and many other countries for training and education in Dairying. Advanced training imparted to the faculty members under various collaborative programmes has immensely helped in improving the quality of research, teaching and consultancy.



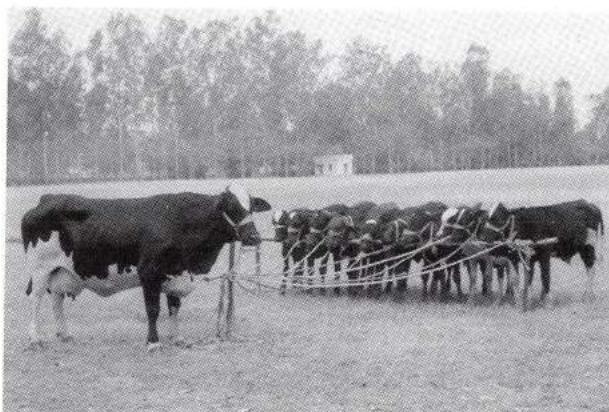
The R&D activities of the Institute mainly focus on three fundamental facets of dairying i.e. how to produce animals with better productivity, to innovate suitable milk processing technologies and equipments and to provide the dairy farmers and entrepreneurs information about existing market demands and practical management inputs for making dairying a self sustaining profitable business. Research, both in basic and applied aspects in various disciplines constitute the core activity of the Institute.

Continued research efforts on genetic improvement of dairy animals has resulted into development of superior strains of dairy cattle which are suitable for Indian agro-climatic conditions both at organized farm as well as field level. These synthetic strains have been





evolved by crossing the Sahiwal and Tharparkar as indigenous breeds with Holstein-Friesian and Brown Swiss, using scientific breeding methodologies. The young bulls of Sahiwal and Karan-Fries cattle and Murrah buffaloes produced through nominated matings are progeny tested to select genetically superior bulls with higher breeding values. As a part of the germplasm dissemination programme, frozen semen from high pedigreed bulls is distributed to different state govt.



*Ten Calves Produced in a Year through Embryo Transfer Technology at NDRI*

establishments, NGOs and other developmental agencies. Advanced genome techniques are used to identify animals with better production and reproduction attributes. Molecular interventions for augmenting reproduction, clean milk production and post-milking improvement of milk quality, development of suitable animal shelter management systems and environmental aspects of dairy development have helped to work out well defined package of practices for different production categories of animals. Faster multiplication of superior animals using Embryo Transfer Technology and other Embryo Biotechniques constitute the core research efforts in the area of Animal Biotechnology at the Institute. By refinement of this technology, it was made possible to produce multiple numbers of calves from an elite female in one calendar year. NDRI has the distinction of producing first test tube buffalo calf of the world using a highly sophisticated technique of in vitro fertilization and Embryo Transfer. The technology has now been extended to develop goat as a model animal for advanced research in transgenic animal production. The country's first in vitro goat kid was born at NDRI.

The very recent breakthrough in the history of NDRI is the production of the world's first cloned buffalo calf through development of the landmark technique called 'Hand-guided cloning' technique. This technique is modification of the 'conventional cloning technique' used for the production of the world's first cloned sheep 'Dolly'.

Research on animal physiology has helped in detailed endocrine profiling of animals to ameliorate heat stress, better reproduction management and inducing lactation which essentially augment the production level of animals. Using chemical and physical methods, significant improvement has been



*The Cloned Buffalo Calf "Garima" produced at NDRI by New "Hand Guided Cloning Technique" Transfer Technology at NDRI*



achieved in the nutritive value of poor quality roughages and agro industrial by-products as animal feed. Improved varieties of fodder crops and their cultivation methods have been developed at the Institute. Research on balanced feed formulation has resulted into development of complete and enriched feed/ mineral blocks for sustaining production level in high producing animals. Research is being carried out for manipulation of rumen fermentation and increasing the efficiency of nutrient utilization by animals. Bypass protein technology has been developed for meeting the nutrient needs of high yielding animals.

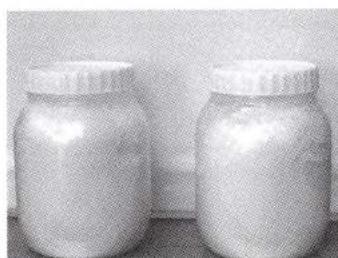
Towards Dairy Processing related research, the Institute has developed technologies for the manufacture of a variety of indigenous dairy products like khoa and khoa based sweets, chhana and chhana based sweets, srikhand, rabri, paneer etc. Several innovative ready-to-reconstitute formulations for the manufacture of khoa, gulabjamun, rasogolla, kulfi, rasmalai, basundi, kheer, dalia and paneer curry are available for adoption at industrial scale. The formulated foods evolved at this Institute include whey based lassi and flavoured drink, weaning foods based on whey/skim milk, soy-butter milk, malted milk food, whey based soups and low fat spreads. New functional dairy products such as probiotic cheese, dahi, sports drinks, low cholesterol ghee, herbal ghee, ice-cream and burfi for diabetics have also been developed with the potential to improve human health. A food-grade bacteriocin based bio-preservative formulation has also been developed for enhancing the shelf life of paneer and khoa. A Kit developed for the detection of various adulterants in milk is in high demand among dairymen across the country. The Institute maintains a National Collection of Dairy Cultures (NCDC) that supplies starter cultures to the industry and institutions across the country. Equipments have been designed for both small scale dairy operations and mechanized production. Some of the equipments suitable for adoption by the Indian dairy industry are continuous paneer, ghee, khoa and rasogolla making machines.



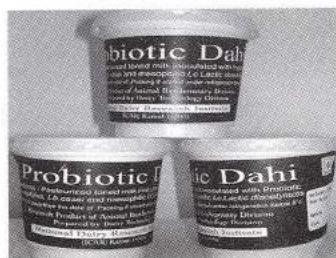
*Long-life Milkcake*



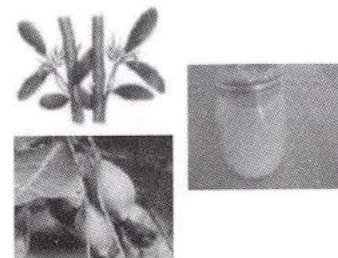
*Instant Rasmalai Mix*



*Low Cholesterol Ghee*

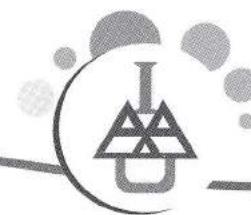


*Probiotic Dahi*



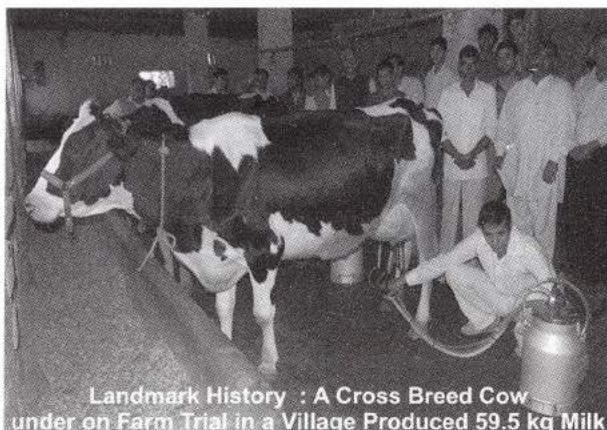
*Arjuna Herbal Ghee*





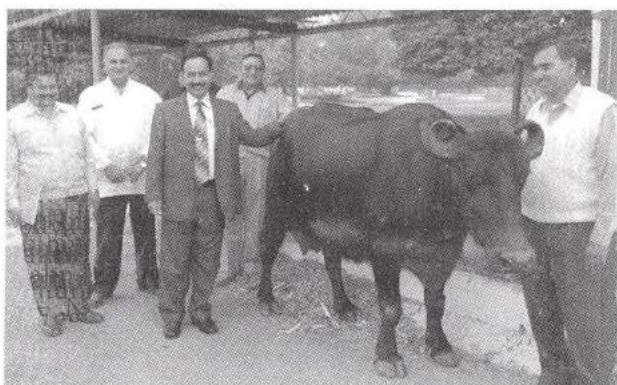
Recent developments in Biotechnology have opened up new and exciting possibilities both in Dairy Production and Processing. Some of the magic tools of Biotechnology such as Genetic Engineering, Somatic Cell Cloning, Establishment of Stem Cells, Hybridoma Technology, Transgenics and Bioprocess Engineering are currently being explored to develop commercial products and processes that use living systems to provide value added, clean, wholesome and nutritious high quality products which are within the reach of entire population. The success of these programmes would prepare India's Dairy Industry to face the newer challenges of global competition. It would permit greater value addition to processed products by improving the nutritional and therapeutic attributes and ensure higher returns to the farmers.

Dairy Farmers who have played crucial role in ushering white revolution in the country remain central to all the R&D activities of the Institute. The farming system research approach used by NDRI in adopted villages has made significant contributions to the economic prosperity of the farming communities. New scientific know-how on animal husbandry, milk and crop production have been transferred by means of Grameen Dairy Melas, Calf



Landmark History : A Cross Breed Cow under on-Farm Trial in a Village Produced 59.5 kg Milk

Rallies, Veterinary Camps, Women Agriculture Days, Field Days and various on farm demonstrations. A major Pilot Project on Technology Assessment and Refinement through Institution - Village Linkage Programme (IVLP) has also been completed to identify available resources, production systems, problems in agriculture and animal husbandry and gender related issues affecting rural development. Krishi Vigyan Kendra (KVK)



Murrah buffalo which yielded 23.3 kg milk in a day at NDRI Livestock Farm

and Dairy Training Centre (DTC) at NDRI conduct regular training programmes for farmers and rural women to acquaint them with modern dairy farming practices. Since its inception in 1976, more than 3000 training programmes for various categories of end users have benefitted more than 57,000 rural youths, school drop-outs, farm women, farmers and ex-servicemen.

The Institute continues to share its innovative dairy processing technologies with the Indian Dairy



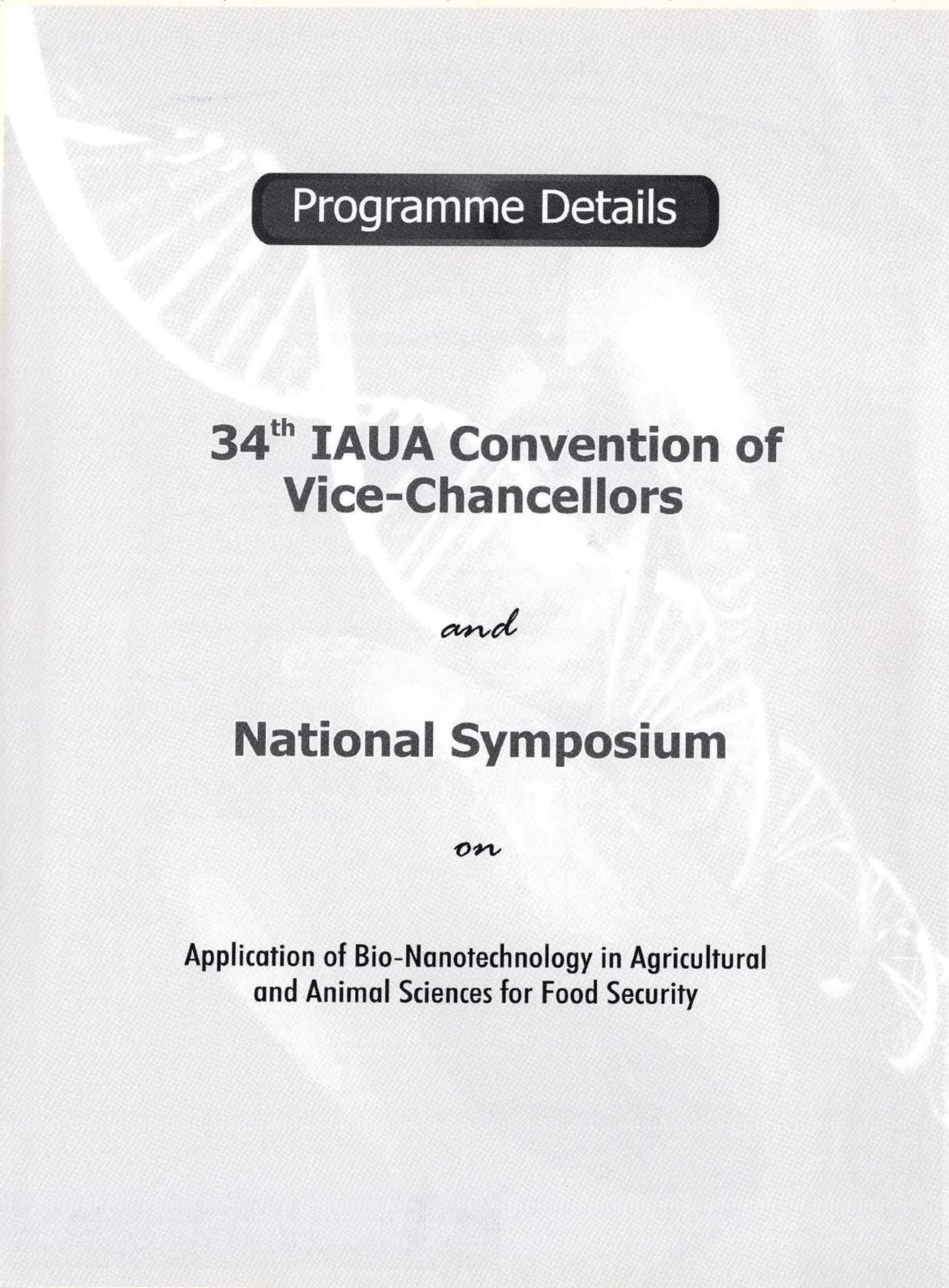
Industry through the consultancy cell. Some of the technologies that have been recently transferred are know-how for the manufacture of long- life paneer, whey- jaljeera drink, instant banana milk mix, payasam mix, gulabjamun mix, calcium enriched milk, emulsifier mix for frozen desserts, herbal ghee etc.

At last the old saying that 'Rome was not built in a day' holds true for NDRI also. The golden history of NDRI, showing each and every milestone of its growth does not stop here. Notwithstanding its glorious past, the canvas of the present times is soon going to be painted as history... continuously motivating each one of us to contribute one's mite in its own individualistic way, thus, adding yet another shade of hue and drop of fragrance to already well-bloomed flower of its existence till date.

### **Acknowledgements**

The article is based on information gathered from various sources of information such as letters written by Dr. K. K. Iya, Former Director NDRI; NDRI Annual Reports; Souvenirs; Brochures; Golden Jubilee Commemorative Volumes and personal interactions. The contributions made by one and all are gratefully acknowledged. The Part of the article has been reproduced from the feature entitled, "The Saga of National Dairy Research Institute" already published by Meena Malik and A. K. Srivastava in July 2009 issue of Indian Dairyman.





**Programme Details**

**34<sup>th</sup> IAUA Convention of  
Vice-Chancellors**

*and*

**National Symposium**

*on*

**Application of Bio-Nanotechnology in Agricultural  
and Animal Sciences for Food Security**

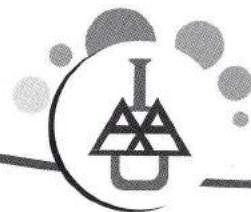


## Summary of Programme

Day		Event
7 Dec	Morning	Inaugural Session*
	Afternoon	Technical Session – I (Concept and applications of Bio-Nanotechnology)**
		Technical Session – II (Nanotechnology in Agriculture)** General Body Meeting of IAUA**
Evening	Cultural Programme*	
8 Dec	Morning	Technical Session – III (Nanotechnology in Animal, Veterinary and Fisheries Sciences)**
		Technical Session – IV (Nanotechnology in Food Processing, Packaging & Value Addition)**
	Afternoon	Plenary & Concluding Session**

**Venue: NDRI Auditorium\***  
**Auditorium (Admn. Block)\*\***





**7<sup>th</sup> December, 2009**

10 AM – 12.00 Noon

**Inaugural Session**

**Session Coordinator:** Dr. Ritu Chakravarty, NDRI

**Chief Guest:** His Excellency the Governor of J&K Hon'ble Sh. N. N. Vohra

**Guest of Honour:** Dr. C. D. Mayee, Chairman, ASRB, New Delhi

**Chairman:** Dr. Anwar Alam, President, IAUA & VC, SKUAST-K, Srinagar

**Chairman Organizing Committee:** Dr. A. K. Srivastava, Director & VC, NDRI

**Executive Secretary, IAUA, New Delhi:** Dr. R. P. Singh

**Secretary, Organizing Committee:** Dr. Rameshwar Singh, Registrar, NDRI

**Programme:**

- 10.00 AM Inauguration of Exhibition on Agricultural and Dairy Development by His Excellency the Governor of J&K Hon'ble Shri N. N. Vohra
- 10.25 AM Arrival of Chief Guest His Excellency the Governor of J&K Hon'ble Shri N. N. Vohra at NDRI Auditorium
- National Anthem, Floral Welcome & Lighting of Lamp
- 10.30 AM Welcome Address by Dr. A. K. Srivastava, Director & Vice-Chancellor, NDRI Deemed University and Chairman, Organizing Committee
- 10.45 AM Brief Report about IAUA - Dr. R. P. Singh, Executive Secretary, IAUA
- 10.55 AM Address by Dr. Anwar Alam, President, Indian Agricultural Universities Association on "Theme of the Conference"
- 11.10 AM Address by Dr. C. D. Mayee, Chairman, ASRB, New Delhi
- 11.20 AM Inaugural Address by His Excellency the Governor of J&K Hon'ble Shri N. N. Vohra
- 11.50 AM Book Release and Presentation of Mementoes
- 12.00 Noon Vote of Thanks by Dr. Rameshwar Singh, Organizing Secretary
- 12.05 PM National Anthem
- Tea



7<sup>th</sup> December, 2009

12.30 PM – 2.00 PM

**Technical Session - I**

**Concept and Applications of Bio-Nanotechnology**

**Session Coordinator:** Dr. Smita Sirohi, NDRI

**Chairman:** Dr. M. C. Varshaneya, VC, AAU, Anand

**Co-Chairman:** Dr. R. B. Deshmukh, VC, M P K V, Rahuri

**Co-Chairman:** Dr. B. Mishra, VC, SKUAST, Jammu

**Rapporteur:** Dr. R. P. Narwal, Director Research, CCSHAU, Hisar

**Speakers:**

1. Dr. Lalit Bhardwaj, Additional Director, CSIO, Chandigarh  
*"Nanotechnology and Agriculture"*
2. Dr. Devakumar, ADG (EPD), Education Divn., ICAR  
*"Applications and Implications of Nanobiotechnology: Issues and Concerns"*
3. Dr. N. C. Patel, VC, JAU, Junagadh  
*"Application of Bio-Nano Technology in Agricultural and Animal Sciences for Food Security"*

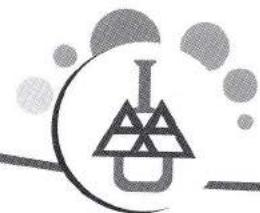
**Panelists:**

1. Dr. P. G. Chengappa, VC, UAS, Bangalore
2. Dr. R. K. Samanta, VC, BCKVV, Mohanpur
3. Dr. K. R. Dhiman, Dr. YSPUH&F, Nauni, Solan
4. Dr. A. K. Das, VC, UBKV, Coochbehar
5. Dr. Suresh Honnappagol, VC, KVAFSU, Bidar
6. Dr. V. S. Tomar, VC, RVSKV, Gwalior
7. Dr. C. S. Prasad, ADG, ICAR, New Delhi
8. Dr. S. R. Singh, Director School of Agril., BHU, Varanasi
9. Dr. A. K. Mishra, Project Director, PDC Meerut
10. Dr. B. P. Singh, Director, CARI, Izatnagar

2.00 PM

Lunch





7<sup>th</sup> December, 2009

03.00 PM – 5.00 PM

### Technical Session - II

#### Nanotechnology in Agriculture

**Session Coordinator:** Dr. Latha Sabikhi, NDRI

**Chairman:** Dr. N. N. Singh, VC, BAU, Ranchi

**Co-Chairman:** Dr. C. S. Chakrabarti, VC, WBUFAS, Kolkata

**Co-Chairman:** Dr. R. C. Maheshwari, VC, SDAU, Dantiwada

**Rapporteur:** Dr. R. S. Dalal, Registrar, CCSHAU, Hisar

#### Speakers:

1. Dr. Arunava Goswami, Assoc. Professor, ISI, Kolkata  
*"Nanocides and Nanodrugs in the Realm of Nanobiology: Applications in Agricultural and Veterinary Sciences"*
2. Dr. Sushmita Mitra, Professor, Amity University, Noida  
*"Synthesis and Application of Biocompatible and Environment Friendly Controlled Release Nanomaterials, in Medicine and Agriculture"*
3. Dr. B. Mishra, VC, SKUAST-J, Jammu  
*"Role of Gene Revolution in Rice and Wheat Research for Food Security"*
4. Dr. B. M. Prasanna, National Fellow, IARI, New Delhi  
*"Nanobiotechnology and its Applications in Agriculture"*
5. Dr. P. Murugesu Boopathi, VC, TNAU, Coimbatore  
*"Application of Nanotechnology in Agriculture"*

#### Panelists:

1. Dr. M. S. Kang, VC, PAU, Ludhiana
2. Dr. M. P. Pandey, VC, IGKV, Raipur
3. Dr. P. M. Boopathi, VC, TNAU, Coimbatore
4. Dr. B. V. Patil, VC, UAS, Raichur
5. Dr. K. S. Khokhar, VC, CCSHAU, Hisar
6. Dr. N. C. Patel, VC, JAU, Junagarh
7. Dr. B. C. Bhowmik, Director, Extn. Edu., AAU, Jorhat
8. Dr. Gurbachan Singh, Director, CSSRI, Karnal
9. Dr. S. S. Singh, Project Director, DWR, Karnal
10. Dr. R. L. Savaliya, Director, Extn. Edu. Junagarh

5.00 PM - 6.30 PM      **General Body Meeting of IAUA**

6.30 PM - 8.00 PM      **Cultural Programme**

8.00 PM - 10.00 PM      **Dinner**





8<sup>th</sup> December, 2009

9.30 AM – 12.30 PM

**Visit of NDRI, Karnal**

**Technical Session - III (11.30 AM - 12.30 PM)**

**Nanotechnology in Animal, Veterinary and Fisheries Sciences**

**Session Coordinator:** Dr. Meena Malik, NDRI

**Chairman:** Dr. R. B. Lal, VC, AAIDU, Allahabad

**Co-Chairman:** Dr. Dilip Kumar, Director & VC, CIFE, Mumbai

**Co-Chairman:** Sh. K. R. Viswambharan, IAS, VC, KAU, Thrissur

**Rapporteur:** Dr. J. P. Mishra, Dean, NDUAT, Faizabad

**Speakers:**

1. Dr. Amit K. Dinda, Professor, AIIMS, New Delhi  
*"Application of Nanotechnology for Diagnosis of Infectious and Non-infectious Diseases"*
2. Dr. S. D. Singh, PS, CIFE, Mumbai  
*"Applications of Nanotechnology in Fisheries & Aquaculture Development"*
3. Dr. Debu Chaudhary, PS, CARI, Izatnagar  
*"Application of Bio-Nanotechnology"*

**Panelists:**

1. Dr. V. K. Taneja, VC, GADVASU, Ludhiana
2. Dr. H. S. Gupta, Director & VC, IARI, New Delhi
3. Dr. M. C. Sharma, Director & VC, IVRI, Izatnagar
4. Dr. K. M. L. Pathak, Director NRC on Camel, Bikaner
5. Dr. B. K. Joshi, Director, NBAGR, Karnal
6. Dr. R. K. Sethi, Director, CIRB, Hisar
7. Dr. R. K. Singh, Director, NRC on Equines, Hisar
8. Dr. Anubrata Das, Project Director, NRC on Pigs, Guwahati
9. Dr. K. P. R. Vittal, Director NIAM, Baramati
10. Dr. S. L. Goswami, Joint Director (Res.), NDRI, Karnal

Tea





## **National Dairy Research Institute**

(Indian Council of Agricultural Research)

(Deemed University)

Karnal-132 001 (Haryana), India

Website : [www.ndri.res.in](http://www.ndri.res.in)

E-mail : [dir@ndri.res.in](mailto:dir@ndri.res.in)