

# Applications of Nanobiotechnology - II

**R.R.Rao**

Professor & Head, Department of Microbiology, Kamineni Academy of Medical Sciences & Research Center, L.B.Nagar, Hyderabad-500068, Telangana State, India

Nanomaterials are the leading edge of the rapidly developing field of Nanobiotechnology. The technology deals with nano-materialized objects. Their unique size dependent properties make these materials superior or indispensable to many areas of human activity. Nanobiotechnology is developing at several levels such as materials and systems. Currently, the level of nanomaterials is the most advanced both in scientific knowledge and its commercial application.

Living organisms are built of cells that typically measure about 10  $\mu\text{m}$  across. However, the cell parts and the organelles are much smaller and they fall in the sub-micron domain.

The proteins are even smaller and measure just 5 nm in size which is comparable with the dimensions of smallest man-made nanoparticles. Because of their small size they can be used as very small probes that would allow us to study at the level of cellular machines without much interference.

Nanotechnology is the understanding of biological processes on the nanoscale level. Hybrid bionanomaterials can also be applied to build novel electronic, optoelectronic and memory devices. There are a number of potential applications of nanomaterials to biology and Medicine in the form of a) fluorescent labeling, b) drug and gene delivery, c) biodetection of pathogen, c) detection of proteins, d) probing of DNA structure, e) tissue engineering, f) tumour destruction through hyperthermia, g)

separation and purification of biological molecules and cells, h) MRI contrast enhancement and i) phagokinetic studies and many more.

The fact that the size of nanoparticles is same as that of proteins makes the nanomaterials suitable for bio-labeling. Apart from the size of the nanoparticles, in order to interact with the biological target, a biological or molecule coating or layer that acts as bio-inorganic interface should be attached to the nanoparticle in the form of biological coating with antibodies, biopolymers like collagen or monolayers of small molecules that make nanoparticles biocompatible. In addition, widely available optical detection techniques can be used to make the nanoparticles to fluoresce or change their optical properties.

Nanoparticle is the core of nanobiomaterial. It can be used as a convenient surface for molecular assembly and may be composed of inorganic or polymeric materials. It can also be in the form of nano-vesicle surrounded by a membrane or layer. The shape is more often spherical or cylindrical but plate-like or other shapes are also possible to make. The size and size-distribution is important in some cases, if penetration through a pore structure of cellular membrane is required. The size and size-distribution becomes extremely critical when quantum-sized effects are used to control material properties. A tight control of the average particle size and narrow distribution of sizes allow creating very efficient fluorescent probes that emit light at narrow wave length in a wide range of wave lengths. This helps in producing biomarkers with many and distinguished colors. The core itself must have several layers and be multi-functional like combining magnetic or luminescent layers so that one can detect both and manipulate the

**\*Corresponding author :**

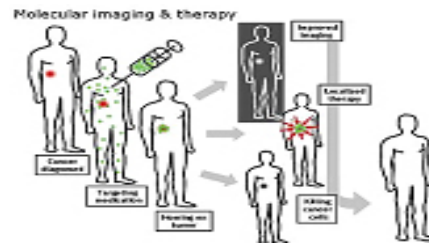
Dr. R.R.Rao, MD, M.A.M.S., FICpath  
Professor & Head, Department of Microbiology , Kamineni  
Academy of medical Sciences & Research Center,  
L.B.Nagar, Hyderabad-500068  
drrrrao@yahoo.com

particles. The core particle is protected by several monolayers of inert material like silica. Organic particles those are adsorbed or chemisorbed on the surface of the particle are also used for this purpose. The same layer might also act as a biocompatible material. More often addition or deletion of layers of linker molecules is required to proceed with further functionalization. These linear linker molecules required for functionalization possess reactive groups at both the ends. One group attaches the linker to the nanoparticle and the other group can be used to bind various moieties like biocompatible antibodies, fluorescent dyes depending upon the function required with the application.

Tissue engineering is an upcoming field where nanobiotechnology has significant role to play. The natural bone surface quite often contains features that are about 10 nm across. If the surface of an artificial bone implant were left smooth the body would reject it because the smooth surface of the implant is likely to cause production of fibrous tissue covering the surface of the implant. This reduces the bone-implant contact which may result in loosening of the implant and further inflammation. If nano-sized features are created on the surface of the hip or knee implant, the chances of rejection can be minimized and also the production of osteoblasts can be stimulated. The effect can be demonstrated with polymeric, ceramic or even with metal materials. More than 90% of the human bone cells from suspension are adhered to metal surface and only 50% in the control samples. These findings are in favour of designing more durable and longer lasting hip or knee replacements and reduce the chances of implant getting loosened and thereby reduce the degree of inflammation.

In dentistry an artificial hybrid material prepared from 15-18 nm ceramic nanoparticles and poly co-polymer (methyl methacrylate) using tribiology approach, vesico-elastic behaviour of human tooth was demonstrated. An investigated hybrid material was deposited as a coating on the tooth surface, improved scratch resistance as well as possessed

healing behaviour similar to that of natural tooth.<sup>1</sup>

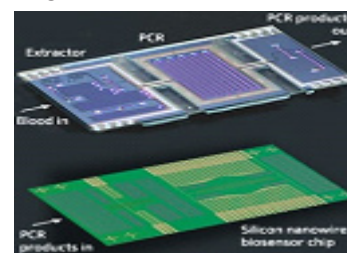


**Fig. 1: A schematic representation as how nanoparticles and cancer drugs might be used in treating the cancer**

Laser generated atomic oxygen is cytotoxic and is capable of destroying cancer cells and this technique is known as photodynamic cancer therapy (Fig. 1).<sup>2</sup>

Nanobiotechnology can be applied for manipulation of cells and biomolecules. Functional magnetic nanoparticles are being used in cell separation and probing. Most of the magnetic particles studied are spherical which somewhat limits the possibilities to make these nanoparticles multifunctional. Alternatively cylinder shaped nanoparticles can be created by employing electro-deposition into nanoporous alumina template in the range of 5-500 nm radius while their length can be as big as 60  $\mu\text{m}$ . By sequentially depositing various thicknesses of different metals the structure and the magnetic properties of individual cylinders can be tuned widely.

Nanobiotechnology is being explored to detect proteins which are important part of cell's language, machinery and structure. Understanding of the functionalities of proteins is extremely important for further progress in human well being.



**Fig. 2: Nanotechnology in the diagnosis of viral infections like Dengue**

Another area where Nanobiotechnology application has significant development is the availability of sensors for the detection of pathogenic microorganisms in particular the point of care viral diagnostics (Fig:2). Direct and label-less detection of viruses is possible with interferometric biosensor immunoassay. The technology comprises of monochromatic light from a laser source coupled to a channel wave guide and is guided into four parallel channels. One of them acts as a reference channel and the rest three channels can be used to detect three different viruses simultaneously as the individual channels are coated with specific antibodies. On exciting through the channels, the probe light is interfered, generating a phase change on a monitor screen. The interference pattern provides the information on the concentration of virus in the reaction channel. The detection of avian influenza virus through whole-virus capture on a planar optical wave guide has been described. The response is based on the index of refraction changes that occur after binding of virus particles to haemagglutinin-specific antibody on the wave guide surface.<sup>3</sup>

Nanobiotechnologies are clinically applicable and are potentially useful for laboratory diagnosis infections in general and viral infections in particular. Functionalized nanoparticles covalently linked to antibodies, peptides, proteins and nucleic acids have been developed as nanoprobe for the detection of the pathogen at molecular level. These functionalized nanoparticles can directly detect viruses rapidly with high sensitivity.

Additional emerging technologies include the triangulation identification for genetic evaluation of risk (TIGER), that uses high performance electro-spray mass spectrometry to identify the base composition of Polymerase Chain Reaction (PCR) products and the matrix assisted laser desorption-ionization-time of flight-mass spectrometry (MALDI-TOF) which generates information on PCR product size and composition based on mass charge ratios. Rapid RT-PCR followed by electro-spray ionization mass spectrometry (ESI-MS) analysis

has been used for identification of all species of influenza viruses with clade-level resolution, identify mixed viral populations and monitor global spread and emergence of novel genotypes. A new nanoparticle based amplification (BCA) has been developed for early detection of HIV-1 capsid P24 antigen. The anti-p24 antibody coated micro plates capture the viral antigen (p24) and are linked to a detection monoclonal antibody with avidin label as detection probe. This immune complex is detected by streptavidin-coated nanoparticle based barcode DNAs for signal amplification. The signal is detected by a chip-based scanometric method. The sensitivity of this system is as low as 0.1 pg/ml and it is 150-fold more sensitive than conventional Enzyme Linked Immuno Sorbent Assay (ELISA). The technique may serve as an alternative to HIV-RNA detection.

Viral diarrheas are major public concern and newer agents with potential for large food-borne out-breaks have been identified in recent times. Noroviruses are the leading cause of gastroenteritis in many parts of the world. A matrix assisted laser desorption ionization and nanospray mass spectrometry was developed and evaluated for the detection of Noroviruses. Peptide sequencing using nanospray tandem mass spectrometry was found to be the best method for identification of 250 fM or more of the capsid protein in stool extracts.

In summary the use of nanoparticles as tags or labels allows for the detection of infectious agents in small sample volumes directly in very sensitive, specific and rapid format at an affordable cost in near future compared to existing technology.

Health and Environmental Concerns:

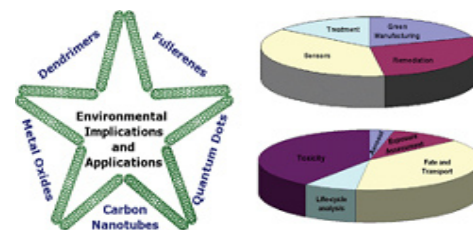


Fig. 3: Environmental implications of Nanotechnology

It has been observed that when rats breathed in nanoparticles, the particles settled in brain and in the lungs which in turn showed significant increases in biomarkers for inflammation and stress responses. In hair less mice nanoparticles induced skin aging through oxidative stress. A two year study on experimental mice at UCLA School of Public health, the mice consuming nano-titanium dioxide exhibited DNA and chromosomal damage to a degree linked to all big killers of man such as cancer, heart diseases, neurological diseases and aging. A published study in Nature Nanotechnology suggests that some forms of carbon nanotubes could be as harmful as asbestos if inhaled in sufficient quantities. Some other forms have the potential to cause mesothelioma (Fig.3). Silver nanoparticles found in biological waste might be harmful to bacteria that are critical components of natural ecosystems, farms and waste treatment processes. Currently researchers are more positive for nanotechnologies for energy applications than for health applications.<sup>4</sup>

### The Future

Applications of Bionanotechnology seem to be extremely wide spread and it holds promises to create biological pathways and mechanisms in a form that are useful in other ways. Nanobiotechnology is helping modern medicine progressing from treating symptoms to generating cures and regenerating the biological tissues (Fig.4). American patients received cultured bladder constructed through Nanobiotechnology.

In experimental animals the uterus has been grown outside the body which was then re-implanted back to produce litters. There is also funding for research into allowing the people to have new limbs in place of prosthesis. With the advances in Nanobiotechnology artificial proteins might also be available to manufacture without the need of harsh chemicals and expensive machines. In near future computers may be made out of biochemical and organic salts<sup>5</sup>.

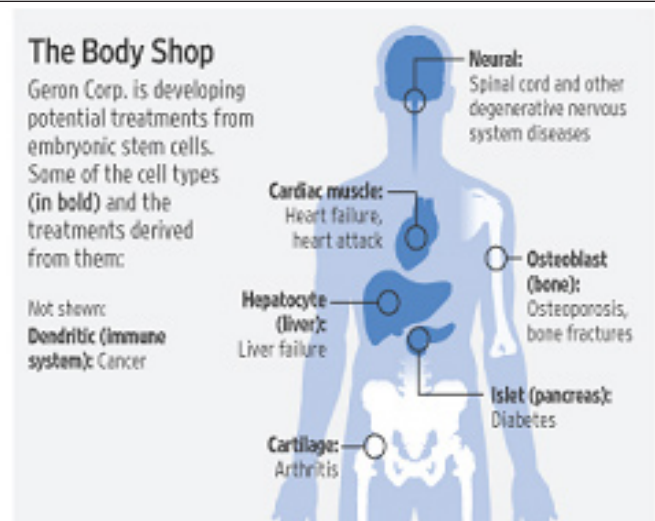


Fig. 4: The future trends in nanotechnology

Experiments are in progress to design polymers where fluorescence is quenched as they encounter specific molecules. Different polymers would detect different metabolites. The polymer coated spheres could become a part of many biological assays. It is possible to introduce these nanobiparticles into the body and track down metabolites associated with cancer and other health problems. Nanobiotechnology although is still at its infancy there are a lot of promising methods that are going to rely on this technology in near future. Natural evolution has optimized the natural form of nanobiology over millions of years. The Nanobiotechnology is best described as 'organic merging with synthetic'. Self assembly nanoparticles coupled with rhodopsin would facilitate optical computing process and help with the storage of biological materials. DNA, the software for all living things can be used as structural proteomic system- a logical component for molecular computing. DNA nanotechnology is one of the best examples of Bionanotechnology. The utilization of the inherent properties of nucleic acids like DNA to create useful materials is a promising area of modern research. The study of membrane properties enable in future to generate synthetic membranes. Research in understanding of protein folding is of high importance and could prove fruitful for Bionanotechnology in future.

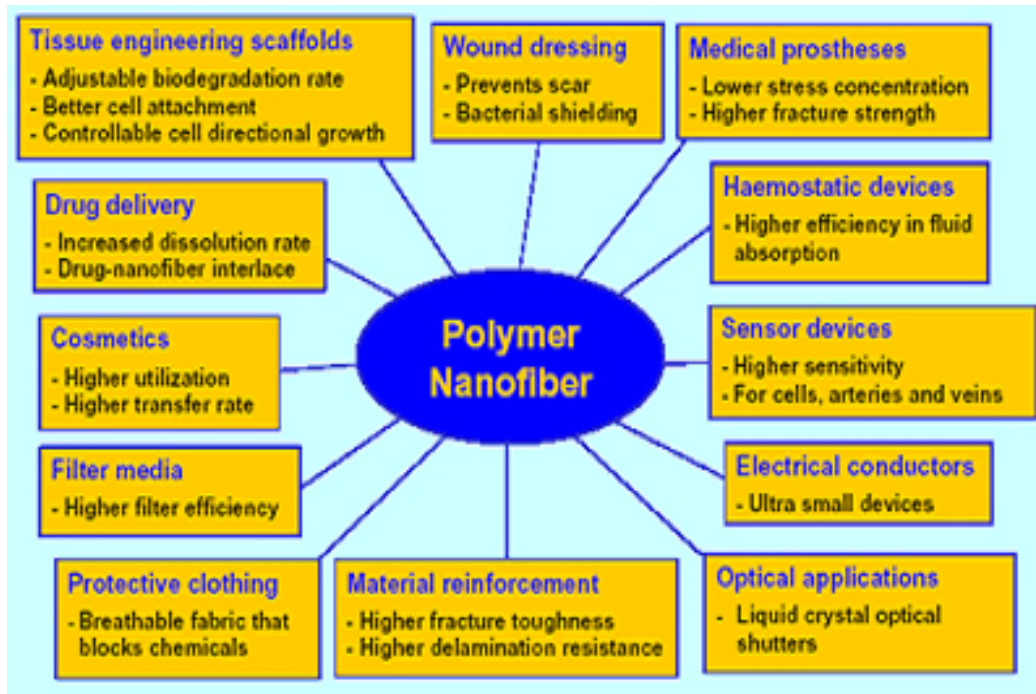


Fig. 5: Applications of nanotechnology

Approaches those seek to arrange smaller components into more complex structures include a) DNA nanotechnology that utilizes the specificity of base-pairing to construct well defined structures out of DNA and other nucleic acids, b) classical chemical synthesis aims at designing molecules of well-defined shape (e.g bis-peptides), c) molecular self-assembly seeks the use of concepts of supramolecular chemistry and molecular recognition in particular to cause single molecule components to automatically array themselves into some useful conformation, d) Atomic force microscopy can be used as a nanoscale 'write heads' to deposit a chemical upon a surface in a desired pattern through a process called dip pen nanolithography which is a developing laser sub field of nanolithography, e) nanomaterials like titanium dioxide is used in summer screens, cosmetics, surface coatings and in some food products and carbon allotropes are used to produce gecko tape, f) silver is used in food packaging and bandages are being infused with silver nanoparticles to help healing of cuts and wounds faster (fig. 5).

## References

1. Freitas RA., Jr Nanodentistry. J Am Dent Assoc.2000;131:1559-66.
2. Labhasetwar,V.Nanotechnology for drug and gene therapy. The importance of understanding the molecular mechanisms. Curr opin Biotechnol 200516:674-80
3. Cui,Y.,Wei,Q.Park,H.Leiber,CM. Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species.Science 2001:293:1289-93
4. Nel A, Xia T.Madler L,Li N.Toxic potential of materials at the nano level. Science 2006:311:622-7
5. Schulze E,Ferrucci JTjr, Poss K, Lapointe L,Bagdanova A,Weisseleder R,Cellular uptake and trafficking of a prototypical magnetic iron oxide label in vitro. Investi Radiol 1996:30:604-10