

Emerging trends of Nanotechnology and Nanoscience in food safety

N. VIJAYA SREE, P. UDAYASRI, V.V. ASWANI KUMAR Y and N. RAMA RAO

Department of Biotechnology, Acharya Nagarjuna University, Guntur - 522 510 (India).

(Received: January 19, 2010; Accepted: February 18, 2010)

ABSTRACT

Nanotechnology and nanoscience refers to the technology development and basic research at the atomic or molecular scale, leading to the controlled manipulation and study of structures, objects and devices with length scales in the 1- to 100-nanometers range. These studies of the nanoworld have been possible due to the simultaneous discovery of new instrumentation methods. Although the impact of nanotechnology has already been felt in engineering and medical fields, its presence in developing nanofoods and in food safety are on the steady rise. Nanocapsules controlled release and adsorption of nutrients as desired by the customer has facilitated the growth of nano foods with immediate impact. In the converging fields of microbiology, biotechnology and nanotechnology emphasis has been given on nanoprotocols for pathogen detection and identification coupled with the use of instrumentation. New tools for early intervention against pathogens are now possible. The uses of nanoparticles as reporters with reference to quantum dots and gold nanoparticles have increased our understanding of the nanoworld. S-layers from various *Bacillaceae* have been used for isoporous ultrafiltration membranes as well as immobilization matrices for binding of monolayer of functional molecules which would be used in development of bioanalytical sensors and immunoassays. The number of reports and publications in this area is on the annual increase. Here we bring to the attention of the readers some recent developments in the area of nanofoods and pathogen identification incorporating nanoscience and nanotechnology which would have a positive impact on the society and economy.

Key words: Nanoscience, Nanoparticles, Pathogen identification.

INTRODUCTION

The research disciplines in academia often focus on questions appreciated by peer groups, leading to greater depth within the disciplines. However, depth within a given discipline does not represent the only path to discovery of major importance. In addition, with the evolving strength of individual disciplines, the fertile frontier of discovery across disciplines has been somewhat neglected. Nanotechnology is one subject area which tries to find solutions across the knowledge boundaries, including, studying individual nanoparticles and the unique ways in which they interact.¹

The word nano means 'a dwarf' in Latin and physically it has dimensions in the order of 10^{-9} nanometres. The concept of nanoparticle got a fillip during Dr Feynman's lecture series in Southern California in 1959. The term nanotechnology was not used until 1974, when Norio Taniguchi, a researcher at the University of Tokyo, used it to refer to the ability to engineer materials precisely at the nanometer level. A quick search of the internet with Google has yielded 10, 100, 1000 hits for nanotechnology and only 1,400,000 hits for nanoscience, indicating the growing popularity of the two fields.² Nanotechnology involves experimenting and manipulating with particles, called nanoparticles that have dimensions in the

scale of a nanometer, and create structures using the mentioned materials. The properties of these products depend on how those atoms are arranged and interact. The different particles which exist in the nano scale include atoms, viruses, antibodies, water molecules etc. The physical and biological structures so created/formed have properties unique to them and are not found in the original matter or material from which the nanoparticle has been formed.³

The phenomenal growth in instrumentation and the ability to make nanodevices and structures fuelled the growth of the nanotechnology industry during the last few decades. The phenomenal success at the nanolevel is unparalleled in the recent times with the growing number of papers, patents and the steady rise in the nano business contributing to the economic growth. Over a period of 21 years from 1980 and 2000; 80 incumbent pharmaceutical firms generated 15,607 biotechnology patents, and 249 firms across a diverse set of industries were granted a total of 3236 nanotechnology patents.³ An example is the Israel nanotech industry which has grown more than 150% over a period of 3 years. A business worth 250 million \$ was generated and the number of companies in the market grew from 45 to 75.⁴ This review attempts to compile and place the developments of nanotechnology and nanoscience with relevance to food, water and pathogen detection. Research undertaken in food and water systems within the domain of nanotechnology are in food security and safety, molecular cell biology, new materials for pathogen and contaminant detection.

Nanofoods

Functional materials in nanofoods

Nano-emulsions

The use of high-pressure valve homogenizers or microfluidizers often causes emulsions with droplet diameters of less than 100 to 500 nm. In modern literature such emulsions are often referred to as "nano-emulsions." The use of multiple emulsions can create novel delivery system with novel properties. The most common examples of this are oil-in-water-in-oil (O/W/O) and water-in-oil in- water (W/O/W) emulsions.⁵ Functional food components could be encapsulated within the inner water phase, the oil phase, or the outer water phase,

thereby making it possible to develop a single delivery system that contains multiple functional components. Encapsulating functional components within the droplets often enables a slowdown of chemical degradation processes.⁶

Nano structured multilayer emulsions

Recent studies have shown that the use of multilayer emulsions can create novel delivery systems. These systems typically consist of oil droplets (the core) surrounded by nanometer thick layers (the shell) comprised of different polyelectrolytes. These layers are formed by a layer-by-layer deposition method that involves sequential adsorption of polyelectrolytes onto the surfaces of oppositely charge colloidal particles.⁷ The thickness and porosity of shells can change with exposure to pH and ionic strength. Functional ingredients in the nanoemulsions formed are released in response to specific environmental triggers by altering the solution conditions to induce complete particle dissolution or changes in particle porosity.

Nanolaminates

Nanotechnology provides food scientists with a number of ways to create novel laminate films suitable for use in the food industry. Edible coatings and films are currently used on a wide variety of foods, including fruits, vegetables, meats, chocolate, candies, bakery products, and French fries. A nanolaminate consists of two or more layers of material with nanometer dimensions that are physically or chemically bonded to each other. One of the successful methods is based on the layer-by-layer deposit in which the charged surfaces are coated with interfacial films consisting of multiple nanolayers of different materials.⁸ The nanolaminated coatings could also be created entirely from food-grade ingredients (proteins, polysaccharides, lipids) by using simple processing operations such as dipping and washing. These coatings or films could serve as moisture, lipid, and gas barriers. Alternatively, they could improve the textural properties of foods or serve as carriers of functional agents such as colors, flavors, antioxidants, nutrients, and antimicrobials. Realistically, the currently available edible films (protein, lipids, and carbohydrates) do not satisfy all the properties for both moisture retention as well as gas exchange for which, additives such as

polyols, fibres etc. are added to increase their beneficial properties.⁹ The choice of the type of adsorbing substances and the preparation conditions for each layer, number and sequence of different layers will determine their overall mechanical, physical barrier, environmental sensitivity. A report from the Helmut Kaiser consultancy predicts that the nanofood market will surge from 2.6 billion USD to 20.4 billion USD by 2010. The report suggests that with more than 50% of the world population in Asia, the largest market for nanofood in 2010 would be Asia lead by China¹⁰.

The definition of nanofood is that nanotechnology techniques or tools are used during cultivation, production, processing, or packaging of the food. Nestle, Kraft, Heinz, and Unilever support specific research groups are also working to develop new "on demand" foods, which will remain dormant in the body and deliver nutrients to cells when needed. A key element in this sector is the development of nanocapsules that can be incorporated into food to deliver nutrients. The concept is that thousands of nanocapsules containing flavor or color enhancers, or added nutritional elements (such as vitamins), would remain dormant in the food and only be released when triggered by the consumer.¹¹ One of the leading bakeries in Western Australia has been successful in incorporating nanocapsules containing tuna fish oil (a source of omega 3 fatty acids) in their top selling product "Tip-Top" Up bread. The microcapsules are designed to break open only when they have reached the stomach, thus avoiding the unpleasant taste of the fish oil. Omega-3 fatty acids are one of the success stories in health industry. However their use is limited in foods by their low solubility in water and their sensitivity to spoilage by oxygen. Nano-encapsulation was one method for protecting the oils. Researchers at the Israel Institute of Technology in Haifa have investigated the potential of beta-lactoglobulin a whey protein to simultaneously bind to omega-3 fatty acid docosahexaenoic acid (DHA) and act as a carrier for the fatty acid. The new complex along with pectin produced a transparent dispersion with extended shelf life for the product¹².

The Israeli company Nutralease utilizes nanosized self-assembled liquid structures (NSSL)

technology to deliver nutrients in nanosized particles to cells. The particles are expanded micelles (hollow spheres made from fats, with an aqueous interior) with a diameter of approximately 30 nm. The nutrients or "nutraceuticals" are contained within the aqueous interior. Nutraceuticals that have been incorporated in the carriers include lycopene, beta-carotene, lutein, phytosterols, CoQ10 and DHA/EPA. The Nutralease particles allow these compounds to enter the bloodstream from the gut more easily, thus increasing their bioavailability.¹³ The technology has already been adopted and marketed by Shemen Industries to deliver Canola Activa oil, which it claims reduces cholesterol intake into the body by 14%, by competing for bile solubilisation.

A number of chemical companies are researching additives which are easily absorbed by the body and can increase product shelf life. Biodelivery Sciences International a New Jersey based firm have developed nanococheates, which are 50 nm coiled nanoparticles and can be used to deliver nutrients such as vitamins, lycopene, and omega fatty acids more efficiently to cells, without affecting the colour or taste of food.¹⁴

Royal Bodycare, a Texas based firm utilizes nanotechnology in nutritional sciences. It has marketed a new product called NanoCeuticals which is a colloid (or emulsion) of particles of less than 5 nm in diameter. The company claims the product will scavenge free radicals increase hydration and balance the body's pH. The company has also developed NanoClustersTM, a nanosize powder combined with nutritional supplements to enhance the absorption of nutrients.

The US based Oil fresh Corporation has marketed a new nanoceramic product which reduces oil use in restaurants and fast food shops by half. As a result of its large surface area, the product prevents the oxidation and agglomeration of fats in deep fat fryers, thus extending the useful life span of the oil.¹ An additional benefit is that oil heats up more quickly, reducing the energy required to cook. The German company Aquanova has developed a new technology which combines two active substances for fat reduction and satiety into a single nano-carrier (micelles of average 30 nm

diameter), an innovation said to be a new approach to intelligent weight management called NovaSOL. To sustain, it uses CoQ10 to address fat reduction and alpha-lipoic acid for safety.

Water

Water purification systems, equipped with nanomaterial in different kinds of membrane technologies, nanoclays, nanoscale metals, nanofibres could provide safe drinking water, water desalination, waste water treatment. Nanoscale metals can be used to render the compounds which could have toxic effect ineffective. These include mercury, arsenic, lead and perchlorate. Nanotechnology can also be used to clean ground water. The US Company Argonide uses 2nm diameter aluminium oxide nanofibres (NanoCeram) as a water purifier. Filters made from these fibres can remove viruses, bacteria and protozoan cysts from water. Similar projects are taking place elsewhere, particularly in developing countries such as India and South Africa¹⁵. The German chemical group BASF's future business fund has devoted a significant proportion of its 105 million USD nanotechnology research fund to water purification techniques.

Centre for Biological and Environmental Nanotechnology (CBEN) based at Rice University, Texas has shown that nanoscale iron oxide particles called 'nanorust' are extremely effective at binding and removing arsenic from groundwater something which affects the water supply of millions of people in the developing world. Nanorust is used in sand filters to treat groundwater from wells. This also has a huge potential in places where people develop toxicity due to the high persistence of arsenic in the drinking water supplies and in open water sources for which there is no existing solution. The collateral benefit of the nanorust filters is that they may also help to remove water borne viruses causing gastrointestinal diseases.¹⁵

Nanomethods for pathogen detection

S-layer protein and pathogen detection

Crystalline bacterial cell surface layer (S-layer) proteins represent the outermost envelope component of many bacteria and archaea. S-layers are composed of a single protein or glycoprotein species, with molecular weights ranging from 40 to

200 kDa. Isolated S-layer proteins frequently show the ability to recrystallize into monomolecular protein lattices on solid supports, langmuir lipid films, or liposomes. The crystalline arrays exhibit oblique, square or hexagonal lattice symmetry. One morphological unit, also referred to as the unit cell, may consist of one, two, three, four or six identical protein subunits. The unit cell dimensions are in the range 3–30 nm, with a mean thickness of 5–10 nm. Pores in the crystalline protein meshwork are of well-defined size and morphology, with a mean diameter of 2–6 nm.¹⁶

S-layers from various *Bacillaceae* were shown to be suitable for the production of isoporous ultrafiltration membranes with well-defined molecular weight cutoffs. S-layers proteins are being used as immobilization matrices for binding of monolayers of functional molecules (e.g., enzymes, antibodies, and immunogens) in a geometrically well-defined way. Their application potential has been exploited for the production of bioanalytical sensors, immunoassays for diagnostics, affinity microparticles, and affinity membranes¹⁷.

Another line of development is directed towards the use of S-layer self-assembly products in suspension as a combined carrier-adjuvant system against infection with pathogenic bacteria and in antiallergic immunotherapy. S-layers of pathogenic organisms were identified to be essential for virulence. Whole-cell preparations or partially purified cell products are currently used as attenuated vaccines against fish pathogens. S-layer-stabilized lipid membranes mimic the supramolecular structure of those archaeal envelopes which possess S-layers as exclusive wall components. They can be used in diagnostics, as vaccines, for drug targeting or deliveries, and for gene therapy.¹⁷

Many S-layer proteins specifically recognize a distinct type of secondary cell wall polymer (SCWP) as the proper anchoring structure to the rigid cell wall layer. These heteropolysaccharides present on the cell wall will bind to the S-layer region of the fusion protein which would facilitate the foreign sequence to be stabilized on the outer most surface. They are used for recrystallization to generate functional

monomolecular protein lattices. They are currently being exploited as sensing layers for label free detection systems, as affinity matrices for binding immunoglobulins, or in the case of liposomes, as novel targeting and delivery systems¹⁸.

Identification of pathogens using nanoparticles and quantum dots

Nanoparticles have been used as quantitation tags in biological assays. The driving force behind the use of nanoparticles as tags in biological assays is to eliminate the need for either organic fluorophores or radioactive labeling, both of which have shortcomings. The majority of the work has been focused in two areas, quantum dots and metallic nanoparticles. An active area of research is the development of surface coatings that can be derivatized with biomolecules without changing the optical properties of the quantum dots (QD)¹⁹.

Nanotechnology-based chips have been designed at a nanoscale level and are related to nanomanipulation. Nanotechnology on a chip is one more dimension of microfluidic/lab-on-a-chip technology. Biological tests measuring the presence or activity of selected substances become quicker, more sensitive, and more flexible when certain nanoscale particles are put to work as tags or labels. Magnetic nanoparticles, bound to a suitable antibody, are used to label specific molecules, structures, or microorganisms. Magnetic immunoassay techniques have been developed in which the magnetic field generated by the magnetically labeled targets is detected directly with a sensitive magnetometer²⁰. Gold nanoparticles tagged with short segments of DNA can be used for detection of genetic sequence in a sample. Multicolor optical coding for biological assays has been achieved by embedding different-sized QDs (nanocrystals of cadmium selenide) into polymeric microbeads.

A potentially revolutionary tag has been successfully synthesized using water-soluble 'nanodots' of gold and silver. A poly (amidoamine) dendrimer cage stabilizes and solubilizes single nanodots ranging in size from two to eight silver atoms. These are the smallest tags yet developed at 0.8 nm. They have large quantum yields, are more

robust than organic dyes, are water soluble, and therefore are expected to be important in single-molecule detection applications¹⁹.

Nanoparticles that act as signal transducers show the most promise in diagnostic assays, due to the elimination of the need to tag a biological sample. The sample preparation steps are reduced or eliminated. The diagnostic test will become more robust and less expensive. We define signal transduction as a system in which a change occurs either to the location of nanoparticles relative to one another, or as a perturbation to the biological component of the assay—both of which in turn lead to a change in a measurable signal. Researchers have developed an assay in which antibody concentration was determined as a function of aggregation of antigen-coated gold particles.¹⁹

The rapid and sensitive determination of pathogenic bacteria is extremely important in food safety, early detection of diseases and infectious agents. Researchers at the University of Florida have reported a bioconjugated nanoparticle-based bioassay for *in situ* pathogen quantification down to single bacterium within 20 min. The bioconjugated nanoparticle provides an extremely high fluorescent signal for bioanalysis and can be easily incorporated with biorecognition molecules, such as antibody. The antibody-conjugated nanoparticles can readily and specifically identify a variety of bacterium, such as *Escherichia coli* O157:H7, through antibody-antigen interaction and recognition. The single-bacterium-detection capability within 20 min has been confirmed by the plate-counting method and realized by using two independent optical techniques²¹.

Currently QDs are popular as probes because their emission spectra are narrow, symmetric and tunable according to the particle sizes and material composition of the QD and they show excellent photostability. Earlier some workers demonstrated a rapid method for the detection of *E. coli* O157:H7 using QDs as a fluorescence marker coupled with immunomagnetic separation. They used magnetic beads coated with anti-*E. coli* O157 antibodies to selectively attach target bacteria, and biotin-conjugated anti-*E. coli* antibodies to form sandwich immuno complexes after magnetic

separation. The immuno complexes were labeled with QDs via biotin-streptavidin conjugation²².

Agromicon based at Wanchai, Hong Kong have developed the nanobioluminescence detection spray which contains a luminescent protein that has been engineered to bind to the surface of microbes such as *Salmonella* and *E. coli*. When bound, it emits a visible glow, thus allowing easy detection of contaminated food or beverages. The company aims to market the product under the name Biomark and making products for bioterrorism. Also, Toxin Alert (Ontario, Calif., U.S.A) was developing a diagnostic system called toxin guard that incorporates antibodies into plastic packaging films to detect pathogens²³.

Another interesting application worth mentioning is that of superparamagnetic crystalline $\text{Fe}_2\text{O}_3/\text{Fe}_3\text{O}_4$, caged in epichlorohydrin cross linked dextran, and has been used as nanosensors in DNA hybridization experiments. The presence of a particular DNA sequence brings the particles coated with the complementary probes in close proximity, leading to a significantly enhanced spin-spin relaxation of the resulting clusters. As a result, networks of these particles were formed and were stabilized long enough to report the presence of specific DNA targets.

This approach has potential in pathogen detection¹⁹.

Many magnetic bacteria produce nano-sized magnetite with species- or strain-specific morphologies. Magnetic bacteria are gram negative, secrete magnetosome also called BMP (bacterial magnetic particle) favoring their usage in pathogen diagnostics. The lipid bilayer membranes covering the BMPs confer easy dispersion of the magnetic particles in aqueous solutions; therefore they can be easily separated from a heterogeneous mixture by simply applying a magnetic field. The use of BMPs in immunoassays enables the separation of bound and free analytes in a magnetic field.²⁴

Nanoparticles can serve as substrates for multiplexed biological assays in solution. Compared with microarray-based methods, these 'suspension arrays' offer, in principle, greater flexibility, more rapid assay times, greater reproducibility, and can

potentially use less sample and reagent. Mixtures of quantum dots have been embedded in micron-sized polymer particles as a multiplexing method in a solution-based array format. Typically, analyte quantitation involves the use of a quantitation tag (typically an organic fluorophore). The proof-of-principle was demonstrated for a DNA hybridization experiment using a suspension of polymeric colloids preencapsulated with three kinds of quantum dots mixed at various ratios for probe identification. Subsequently, the surfaces of these particles were conjugated to specific oligonucleotide probes for molecular recognition, and an organic dye molecule was used to directly label the target DNA molecules for analyte quantitation.¹⁹

Individual microorganisms, even those in "clonal" populations, may differ widely from each other in terms of their genetic composition, physiology, biochemistry, or behavior. Methods for identification, characterization, and/or physical separation of individual microorganisms are needed for the detection of pathogens and for the identification and selection of strains. Single-cell techniques have been key in probing microbial viability phenomena that are beyond the resolution of culture-based approaches with special reference to viable but nonculturable bacteria which may be alive but showing a low metabolic activity. It is also useful in elucidating the mechanisms of pathogenesis & the invasive forces of individual cells.²⁵

Pathogenic bacteria have the ability to harvest iron from human transferrin. The presence of receptors for human transferrin is highly correlated with virulence. The transferrin proteins labeled with fluorescent labels (QD) conjugates provides a method of identifying pathogenic bacteria and elucidating the biochemical processes involved in bacterial iron acquisition. Transferrin labeling is contingent upon the bacteria being alive and metabolically active. *S. aureus* was studied from iron-deprived conditions indicating that the transferrin function was not compromised by its attachment to the nanocrystal. Firstly, the ability to utilize human transferrin indicates that a strain is pathogenic, and this may be used as a rapid test for invasive *Staphylococci*. Secondly, other iron-binding proteins and siderophores may be

conjugated with QDs, allowing study of uptake and trafficking of these molecules²⁶.

Nanoprotocols coupled with instrumentation for pathogen identification

Major breakthroughs in the instrumentation field like the Scanning Tunnel Microscope, Atomic Force Microscope, Raman Spectroscopy has resulted in the study of single molecule single organism which has been used in early pathogen detection and prevention of diseases. As nanobiotechnology progresses, sensors to detect pathogens or their constituents become smaller and more sensitive. Owing to the nature of these nanoscale sensors, the sample size from which the detection is being made is typically a microliter or smaller. Therefore, the challenge for scientists developing detection methods for pathogens in foods is in the sample preparation. Although the sample preparation requirements will vary from one food product to another, research into this step is required to bridge the emerging field of nanosensors with the food industry. The sample preparation will not only depend on the food matrix, but on the pathogen as well. Pathogenic viruses, bacteria and parasites might all exist within the same food production²⁷.

RAMAN spectroscopy methods

In the development of metallic nanoparticles as quantitation tags, there are many varieties of detection approaches being developed, including electrochemical, optical, microscopic and mass spectrometric. Nanoparticles functionalized with different Raman dyes have been used to detect DNA sequences from various infectious diseases and bioterrorism agents with a femtomolar detection limit. The technology developed at Northwestern University has a number of advantages over current molecular biology techniques for DNA detection and has been licensed to Nanosphere, Inc. for commercialization. Gold (Au) nanoparticles, 13 nm in diameter, functionalized with different Raman dyes (eg. Cyanin 5 dye) and short DNA molecules, are designed to bind to specific target DNA on the surface. After a silver (Ag) enhancement step, surface-enhanced Raman scattering (SERS) spectroscopy can detect signals from the dye molecules bound to the target DNA. The different Raman labels act as 'spectroscopic fingerprints' to

distinguish multiple DNA sequences in a given sample simultaneously. Multiplexing can be reached in solution as well as in microarray on a chip. The high sensitivity, speed, and selectivity of this approach are much better than comparable biological techniques using fluorescent probes. The number of Raman dyes available is also greater than the number of discernable fluorophores. The group was able to detect many specific DNA sequences within mixtures of DNA targets from anthrax, HIV, hepatitis A and B, Ebola, and smallpox viruses²⁸.

Label-less detection: nanocapacitors, nanopores, nanochannels and nanomechanics

Nanogap capacitors (50 nm electrode spacing) were developed and fabricated using silicon nanolithography. The ssDNA probe is immobilized on the electrode surface. The dielectric properties of the ssDNA probe and dsDNA formed on hybridization with the target are different and can be measured through capacitance measurements in these nanogap capacitors. An immediate prospect is large two-dimensional arrays of capacitors that could be used to provide capacitive, label-free simultaneous measurement of nucleic acid targets in a sample. These techniques avoid the tagging methods on the analyte²⁹.

Nanomechanical deflections on micromachined silicon cantilevers have also been used to recognize the occurrence of molecular events such as DNA hybridization and protein binding. In the case of DNA hybridization, ssDNA is immobilized on the surface of a cantilever and subsequently target ssDNA is introduced. The cantilever acts as a miniature 'balance' and deflects proportionally to the amount of hybridized target. The deflection is measured accurately using optical detection method. Extension of this method to multiplex recognition and prevention of non-specific binding is still a challenge – however, the method is a clever demonstration of making use of the power of sophisticated microfabrication and assay methods²⁹.

Nanochannels that would stretch out the DNA molecule and simplify the sequencing

are also in the investigational stage. Estimates for the sequencing rate that could be achieved using nanopores range from a conservative 1000 bases per second to an optimistic 10 000 bases per second, which greatly exceeds the current rate of 30,000 bases per day using conventional sequencers²⁹.

Nanofluidic systems are so small that these channels approach molecular dimensions and the well-characterized rules of microfluidics may no longer apply. Nanofluidics has been applied to DNA sizing and has been demonstrated for the direct reading of the nucleotide sequence of a single-stranded DNA (ssDNA). In this method, the ssDNA is pulled through a nanopore slightly larger than the diameter of the ssDNA molecule. Changes in the electrical current that flows through the pore are correlated with the movement of the different bases (A, G, C, or T) of the DNA molecule as they pass through the opening. Agilent Technologies (Palo Alto, CA) and Harvard University's Daniel Branton are codeveloping a nanopore sequencer on the basis of this idea that DNA can be shuttled through a 10^{-9} m hole at a rate of a million bases per second. With this method, it would take less than 2 hours to sequence an entire genome³⁰.

Biological Micro-Electro-Mechanical Systems (BioMEMS)

BioMEMS are systems construction & analysis of biological and chemical entities at micro/nanoscale. The devices and integrated systems using BioMEMS are also known as lab-on-a-chip or micro-total analysis systems (micro-TAS). BioMEMS for diagnostic applications are also sometimes referred to as 'BioChips'. Conductance techniques are attractive due to their simplicity and ease of use since measurement of impedance (or admittance) was used to measure the metabolic activity of microorganisms within micro-fluidic biochips. As bacterial cells are grown within micro-fluidic channels and wells, the impedance changes in the medium can be detected using electrodes placed appropriately within the channels. These devices are used to detect cells, microorganisms,

viruses, proteins, DNA and related nucleic acids, and small molecules of biochemical importance³¹.

Recent advances in fluorescence detection technology have enabled single molecule detection. Fluorescence-based detection in BioMEMS has been applied to detection of cells within micro-chips, using antibody-based (ELISA type) assays. Majority of the detection schemes in microarray and numerous lab-on-a-chip devices and applications utilize optical detection schemes. Disposable plastic fluidic biochips have been developed with on chip air pressure sources for fluidic movement and electrochemical detection of metabolic parameters for point of care health monitoring applications and using magnetic-bead based biodetection of DNA and proteins³¹.

Striped metal nanoparticles (Nanobarcodes particles)

Striped metal nanoparticles have been described as substrates for multiplexed assays. In contrast to the quantum dots described above, for which fluorescence measurements are needed both to identify the particles and to quantitate an analyte, these particles are intrinsically encoded by virtue of the difference in reflectivity of adjacent metal stripes. The large number of striping patterns available, and the development of automated software for particle identification should facilitate development of very highly multiplexed assays (i.e. hundreds to thousands), although optimization of assay performance is in the early stages¹⁹.

Dielectrophoresis

Dielectrophoresis provide another means of using electric fields for manipulating particles such as DNA, viruses, proteins, and cells. Electrodes are used to generate a nonuniform alternating current (AC) in a channel with particles and fluid. The nonuniform field induces an electrical polarization on uncharged particles to produce a dielectrophoretic force that can be used to collect them. Dielectrophoresis has been demonstrated in a microfluidic device as an effective method of discriminating between infected and uninfected blood cells at concentrations of 1 infected cell in more than 10^5 normal cells³⁰.

The ability of AFM to reveal native surface

structures of pathogens at nanometer resolution and under physiological conditions offers exciting prospects in basic and applied research. *Saccharomyces cerevisiae*, *Phanerochaete chrysosporium*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Pinnularia viridis*, Lactic acid bacteria, *Bacillus* spores, *Salmonella* species, *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium bovis*, Vaccinia virus and human rhinovirus are just a few examples of microbes that have been imaged in physiological conditions³².

Microorganisms produce a range of characteristic volatile compounds that may be useful as well as harmful to human beings. Bacteria are the most common cause of food rotting; the presence of foul odor is an indication of food rotting. Electronic Nose (E-Nose) is a device mimicking the operation of the human nose, which uses a pattern of response across an array of gas sensors to identify different types of odors. The main purpose of the E-nose is to identify the odorant, estimate the concentration of the odorant and to find characteristic properties of the odor as might be perceived by the human nose. The main component of the electronic nose is its gas sensors that identify odors. These gas sensors are composed of nanoparticles (e.g. Zinc oxide nanowires) whose resistance changes when a certain gas is made to pass over it. This change in resistance generates a change in electrical signal that forms the fingerprint for gas detection. The fingerprint pattern derived from the sensor is used to determine the type, quality and quantity of the odor being detected. The advantage of using nanoparticles is that they have improved surface area for better gas adsorption.³³

Nanotechnology- Concerns and Economics

The lifecycle of products containing nanoparticles is difficult to establish, since the degradation process of nanomaterials and components is only estimated. The identified hazards with regard to health are chiefly related to the absorption of nanoparticles by the human body and their distribution as well as the risk of accumulation in organs. Carbon nanoparticles, for instance, have been shown to induce lipid peroxidation in the brain cells of fish and pulmonary inflammation in rats.³⁴ The antimicrobial properties

of nanoparticles have led to concerns that they may shift into microbial populations and disrupt signaling between nitrogen-fixing bacteria and their plant hosts. Any significant disruption of nitrogen fixing could have serious negative impacts for the functioning of entire ecosystems. High levels of exposure to engineered nanoparticles of aluminium (currently used in face powders and sunscreen) have been found to stunt root growth in five plant species. Airborne nanoscale material was seen as a potential inhalation threat. Prolonged exposure to airborne carbon nanotubes led to lesions and inflammation of the lung tissue.

It is further unknown, how the human (and animal) metabolism will react to the intake of nanoengineered food and nanoparticles, since once dispersed in the ecosystem, these will enter the food chain. However, recent results obtained suggest that the benefits far outweigh the risks (e.g. applied nanotechnology techniques for water purification systems). Techniques related to nanobiotechnology do not require large investments and infrastructure and can therefore be developed on the site of application itself in a cost effective manner. US National Science Foundation (NSF) estimates the total global market for nanotechnology related products and services to reach US\$ 1 trillion by 2015. In India, the Department of Science and Technology (DST) has launched the "Nanomission" in the Nanoscience and Technology Initiative with a financial outlay of Rs. 1000 crores from May 2007 for a period of 5 years.

CONCLUSION

Nanoscience and nanotechnology as a scientific and technological thrust encompasses the best of many opportunities afforded to the scientific, engineering, and industrial communities. Some of the nano technologies that show good promise in food and food safety in the near future are a nanopore size sequencer for whole genome sequencing in less than 2 hours; detection of different nanoparticles labeled with Raman dyes by spectroscopy with an aim to obtain a specific Raman spectroscopic fingerprint; self assembled molecules of the S-layer proteins which are being used as sensing layers for label free detection systems and as affinity matrices for binding immunoglobulins.

However, there are still fundamental questions about these materials that must be answered if nanotechnology has to be translated from the lab to the field on a larger scale. There is a need for better characterization of nanotechnology constructs, production and evaluation of uniform "reagent-grade" nonmaterial. Standardized assays to facilitate rigorous evaluation of nanomaterials in terms of their toxicity and efficacy in animal experiments are of immediate priority. Whether actual or perceived, the potential health risks

associated with the manufacture, distribution, and use of nanoparticles must be balanced by the overall benefit that nanotechnology had to offer. Biologists are using these innovative technologies to overcome barriers which would have a common applicability in food, agriculture, environment, cell biology, clinical medicine, nanomaterial development, production, bioinformatics, and modeling and simulation tools. As new frontiers are being explored, new paths would be laid out and new horizons reached.

REFERENCES

1. Sun, Y.D., Chen, Z.M., Wei, H., Liu, C.X., Nanotechnology challenge: safety of nanomaterials and nanomedicines. *Asian Journal of Pharmacodynamics and Pharmacokinetics*, **7**: 17-31 (2007).
2. Norman, R.S., Hongda, C., A National Planning Workshop: Nanoscale Science and Engineering for Agriculture and Food System, November 18-19, Washington, USDA (2002).
3. Rothaermel, F.T., Thursby, M., The nanotech versus the biotech revolution: Sources of productivity in incumbent firm research. *Research Policy*, **36**: 832-849 (2007).
4. Tolles, W.M., Rath, B.B., Nanotechnology, a stimulus for innovation. *Current Science*, **85**: 1746-1759 (2003).
5. Ritzoulis, C., Scoutaris, N., Papademetriou, K., Stavroulias, S., Panayiotou, C., Milk protein-based emulsion gels for bone tissue engineering. *Food Hydrocolloids*, **19**: 575-581 (2005).
6. McClements, D.J., Decker, E.A., Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science*, **65**: 1270-1282 (2000).
7. McClements, D.J., Weiss, J., Lipid emulsions. In "Bailey's Industrial Oil and Fat Products," Wiley-Interscience, New York, 457-502 (2005).
8. McClements, D.J., Decker, E.A., Weiss, J., *Novel procedure for creating nanolaminated edible films and coatings*, University of Massachusetts, U.S. patent application: UMA 05-27 (2005).
9. Cagri, A., Ustunol, Z., Ryser, E.T., Antimicrobial edible films and coatings. *Journal of Food Protection*, **67**: 833-848 (2004).
10. Helmut Kaiser Consultancy, Study: Nanotechnology in Food and Food Processing Industry Worldwide 2003-2006-2010-2015. Helmut Kaiser Consultancy. www.hkc22.com/nanofood.html (2004).
11. Taylor, T.M., Davidson, P.M., Bruce, B.D., Weiss, J., Liposomal nanocapsules in food science and agriculture. *Crit. Rev. Food Sci. Nutr.*, **45**: 1-19 (2005).
12. Patricia, Z., Yoav, D.L., Beta-lactoglobulin and its nanocomplexes with pectin as vehicles for ω -3 polyunsaturated fatty acids. *Food Hydrocolloids*, **23**: 1120-1126 (2008).
13. Chen, H., Weiss, J., Shahidi, F., Nanotechnology in nutraceuticals and functional foods. *Food Technol.*, **60**: 30-36 (2006).
14. Sanguansri, P., Augustin, M.A., Nanoscale materials development - A food industry perspective. *Trends Food Sci. Technol.*, **17**: 547-556 (2006).
15. Si, D.Y., Liang, W., Sun, Y.D., Cheng, T.F., Liu, C.X., Biomedical evaluation of nanomedicines. *Asian Journal of Pharmacodynamics and Pharmacokinetics*, **7**: 83-96 (2007).
16. Dietmar, P., Sleytr, U.B., The application of bacterial S-layers in molecular nanotechnology. *Nanotechnology*, **17**: 1-12 (1999).

17. Sara, M., Sleytr, U.B., S-Layer Proteins. *Journal of Bacteriology*, **182**: 859-868 (2000).
18. Huber, C., Egelseer, E.M., Ilk, N., Sleytr, U.B. Sara, M., S-layer-streptavidin fusion proteins and S-layer-specific heteropolysaccharides as part of a biomolecular construction kit for application in nanobiotechnology. *Microelectronic Engineering*, **83**: 1589-1593 (2006).
19. Penn, S.G., He, L., Natan, M.J., Nanoparticles for bioanalysis. *Current Opinion in Chemical Biology*, **7**: 609-615 (2003).
20. Baeumner, A., Nanosensors identify pathogens in food. *Food Technol.* **58**: 51-55 (2004).
21. Zhao, X., Hilliard, L., Mechery, S., Wang, Y., Bagwe, R., Jin, S., Tan, W., A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles. *Proceedings of National Academy of Sciences*, **101**: 15027-15032 (2004).
22. Su, X.L., Li, Y., Quantum dot biolabeling coupled with immunomagnetic separation for detection of *Escherichia coli* O157:H7. *Anal Chem*, **76**: 4806-10 (2004).
23. Chatterjee, A., Kanawjia, S.K., Radio frequency identification: The technological aspects and applications in food industry. *Indian Food Industry*, **27**: 48-55 (2008).
24. Matsunaga, T., Okamura, Y., Genes and proteins involved in bacterial magnetic particle formation. *Trends in Microbiology*, **11**: 536-541 (2003).
25. Byron, F.B.S., Eric, A.J., Single-cell microbiology: Tools, technologies and applications. *Microbiology and Molecular Biology Reviews*, **68**: 538-559 (2004).
26. Kloefer, J.A., Mielke, R.E., Wong, M.S., Nealon, K.H., Stucky, G., Nadeau, J.L., Quantum dots as strain- and metabolism-specific microbiological labels. *Applied and Environmental Microbiology*, **69**: 4205-4213 (2003).
27. Nugen, S.R., Baeumner, A.J., Trends and opportunities in food pathogen detection. *Anal Bioanal Chem.*, **391**: 451-454 (2008).
28. Cao, Y.C., Jin, R., Mirkin, C.A., Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection. *Science*, **297**: 1536-1540 (2002).
29. Fortina, P., Kricka, L.J., Surrey, S., Grodzinski, P., Nanobiotechnology: the promise and reality of new approaches to molecular recognition. *Trends in Biotechnology*, **23**: 168-173 (2005).
30. Deirdre, R.M., Mark, R.H., Microscale bioanalytical systems. *Science*, **297**: 1197-1198 (2002).
31. Bashir, R., BioMEMS: state-of-the-art in detection, opportunities and prospects. *Advanced Drug Delivery Reviews*, **56**: 1565-1586 (2004).
32. David, A., Etienne, D., Claire, V., Guillaume, A., Vincent, D., Yves, F.D., Nanoscale imaging of microbial pathogens using atomic force microscopy. *Nanomedicine and Nanobiotechnology*, **1**: 168-180 (2009).
33. Hossain, M.K., Ghosh, S.C., Boontongkong, Y., Thanachayanont, C., Dutta, J., Growth of zinc oxide nanowires and nanobelts for gas sensing applications. *Journal of Metastable and Nanocrystalline Materials*, **23**: 27-30 (2005).
34. Scott, E.M., Nanotechnology for the biologist. *Journal of Leukocyte Biology*, **78**: 585-594 (2005).