CHAPTER

Introduction into nanotechnology and microbiology

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1 The world of 'microbiology'

The discipline of 'Microbiology' deals with the microscopic analysis of living and non-living entities such as bacteria, viruses, yeast, fungi and protozoans, which are organisms that are invisible to naked human eyes. Bacteria, viruses and yeast represent three types of microorganisms, which form a significant component of biota, exhibiting unique functions due to their peculiar cell structure. For example, yeast are eukaryotic, while bacteria belong to the prokaryotic group. However, viruses are obligate intracellular parasites which are considered non-living organisms. Due to the genetic diversity of microorganisms, it is believed that microbes were the first life recorded on Earth, long before the existence of any plant or animal. Some microorganisms have been studied for their unique ability to survive at extreme environmental conditions. For example, some species of microbes can survive well in extreme cold regions of Antarctica, while others survive in hot water springs at 90 °C and above, in places with highly alkaline soils, high concentrations of heavy metals and Sulphur and in locations where no other forms of life can survive. Millions of microbial species exist in the natural environment but only 5% of these (totalling 160,000 known species) have been identified so far (Minocheherhomji, 2016).

The field of microbiology began with the advent of the first microscope in the year 1676 when Antony van Leeuwenhoek visualized bacteria for the first time. The visual microbiological world was further extended in the 1880s by the combined work of Ernst Abbe and Carl Zeiss further advancing the field of light microscopy. Ernst Ruska made it possible to study the structure of viruses with the advent of the electron microscope in 1931. Subsequently, crystallization of tobacco mosaic virus (TMV) was carried out by Wendall Stanely in 1935. More recently, the atomic force microscope (AFM) has opened a new path for the investigation and manipulation

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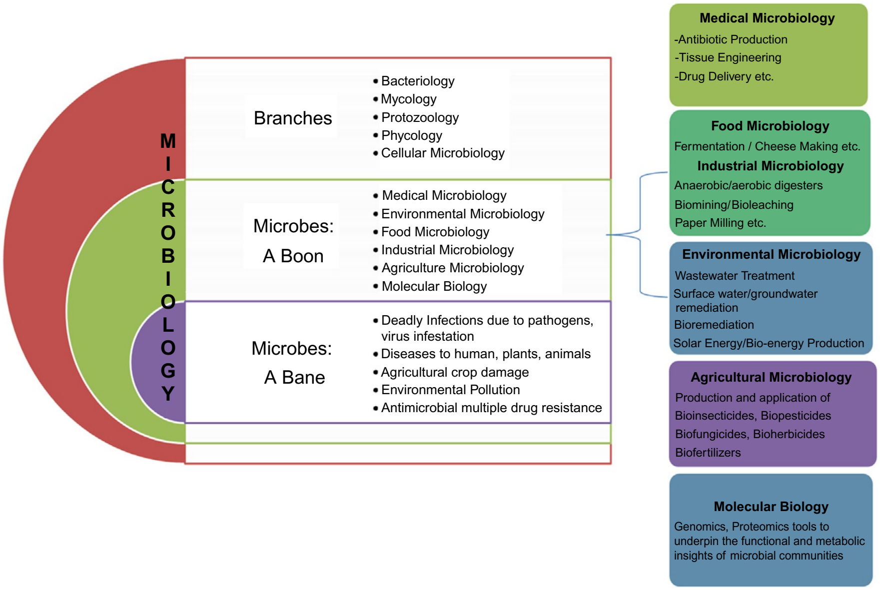

FIG. 1

An overview of the interdisciplinary scope of microbiology.

of structures on a very small scale in the study of biological structures by providing high resolution images under physiological conditions, and to investigate the binding forces between microorganisms and target surfaces. Microbiology mainly deals with visual characterization of various microbial groups (Fig. 1).

2 The boom of nanotechnology

The era of Nanotechnology, based on the precise development, manipulation and application of materials at a nanoscale (10^{-9}m) is evolving at a very rapid pace. The U.S. National Nanotechnology Initiative (NNI) defines nanotechnology as the research and development efforts at the atomic or molecular level to create structures and systems applicable in diverse aspects (Balzani, 2005; Drexler & Peterson, 1989). The history of nanotechnology lies in the 4th and 5th centuries BC, when traditional medicinal practitioners in India and China succeeded in preparing gold colloids (known as 'Suwarna Bhasma' in ancient ayurveda in India) for therapeutic purposes (Paul & Chugh, 2011). During the middle ages in Europe, Paracelsus applied colloidal gold in treating mental disorders and syphilis (Dykman & Khlebtsov, 2012). With the periodical advancement in 1618, a book on the preparation and therapeutic applications of colloidal gold was published by the Philosopher and Doctor, Francisco Antonii. The first scientific publication of colloidal gold was published by Michael Faraday in 1857. However, real interest in the field of nanomaterial science was inspired by Richard Feyman during his famous talk 'There's **Plenty** of Room at the Bottom' in 1959 at Caltech, USA (Feynman, 1960). This concept of nanomaterial science was further popularized by Eric Drexler through his book *Engines of Creation: The Coming Era of Nanotechnology* in the 1980s. Currently, the field of Nanotechnology has spread its wings in medical, pharmaceutical, industrial, food and agriculture, and environmental sectors with a wide range of applications (Fig. 2). It is now possible to build materials atom by atom and impose desired characteristics for numerous applications in almost every area, such as composite materials development, electronics, nano-electro-mechanical systems (NEMS), biomedical technologies, renewable energy solutions and environmental remediation (Navya & Daima, 2016). According to the Business Communication Company (BCC), the global market of nanomaterials used in the biomedical, pharmaceutical and cosmetic industries has increased from $170.17 million in 2006 to $684.4 million in 2012 with an expected compound annual growth rate of 27.3% by 2020 (https://www.bccresearch.com/market-research/nanotechnology). The invention of characterization and manipulation tools such as scanning tunnelling microscope (STM) from 1981 to recent advances with transmission electron microscope (TEM), X-ray diffraction (XRD), Fourier transmission infrared (FTIR) has caught the interest of many researchers from various disciplines in developing nanomaterials and systems for widespread use.

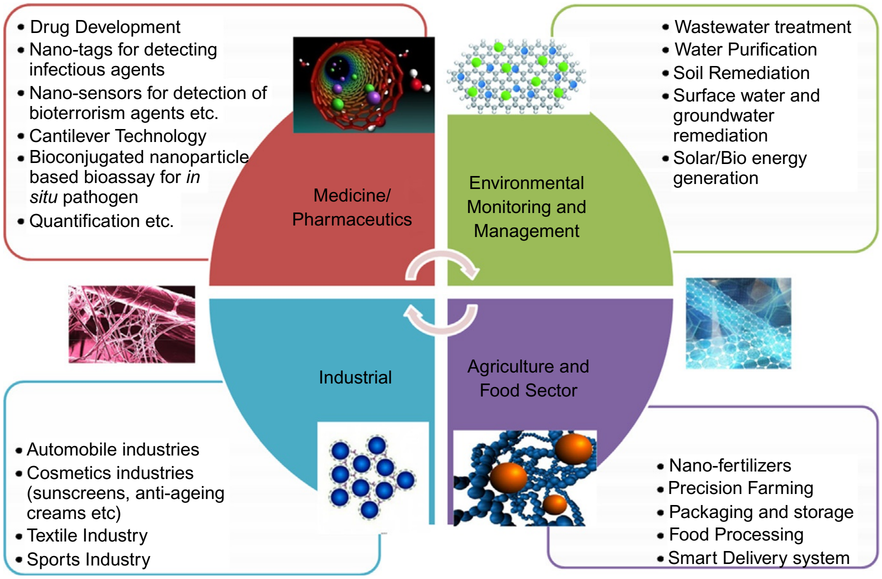

FIG. 2

Applications of nanotechnology in various sectors.

Table 1 Major groups of nanomaterials in application.

Class	Category	Examples
i.	Metal based nanomaterials	Gold, silver, iron oxide, gadolinium.
ii.	Ceramic nanomaterials	Alumina, zirconia, hydroxyapatite tricalcium phosphate, silicon nitride
iii.	Semiconductor based nanomaterials (quantum dots)	Cadmium sulphide, cadmium telluride, cadmium selenide
iv.	Carbon based/organic nanomaterials	Carbon nanotubes (multi walled; single walled), carbon quantum dots, graphene, fullerenes.
v.	Organic-inorganic hybrid nanomaterials	SiO_2, Ag_2S, CuS/PVA, gold/polyethylene hybrid, phytase-SiO_2, phytase-gold
vi.	Silica based nanomaterials	Monoliths, rod-like particles, fibres, hollow or solid nanospheres, silica nanotubes, mesoporous silica nanoparticles
vii.	Polymeric nanomaterials and nanoconjugates	Poly (lactic acid), poly (glycolic acid), poly (hydroxyl butyrate), proteins (collagen, silk), polysaccharides (starch, chitin/chitosan, alginate).
vii.	Biological nanomaterials	Lipoprotein or peptide-based nanomaterials, nano-cellulose
ix.	Self-assembled/biologically directed materials	Virus derived nanomaterials (cowpea mosaic virus or CPMV)

Modified from Sitharaman, B. (2011). Nanobiomaterials handbook*. Boca Raton, FL: CRC Press.*

Nanomaterials are classified into nine major groups based on their shape, size, composition, surface charge, aggregation and chemical nature. Table 1 gives an overview of these nine nanomaterial classes.

3 The coming together of science and technology: Microbiology and Nanotechnology

The disciplines of Microbiology and Nanotechnology individually have strengthened the field of science and technology by delivering novel solutions for human well-being and maintaining environmental and ecological balance. However, due to instances where the frequent use of drugs has led to antibiotic/multidrug resistance in microorganisms, and the delivery of metal nanoparticles is impacting the food chain, it is now necessary to develop interdisciplinary approaches combining Microbiology and Nanotechnology to combat secondary human health, and environmental and ecological damage. The amalgamation of these fields offers innovative and sustainable solutions in a rational manner. In the present chapter, we highlight the relationship between these disciplines identifying the great potential resulting from interdisciplinary research.

Nanoscience impacts several areas of Microbiology. It allows for the study and visualization at the molecular-assembly levels of a process. It facilitates identification of molecular recognition and self-assembly motifs as well as the assessment of these processes. Specifically, there are three areas where microbiologists use nanotechnological techniques:

- Imaging single molecules
- Manipulating nanoscale objects (laser traps and optical tweezer) and;
- Determining spatial organization in living microbes (AFM, near/far field microscopy)

Below we present a number of key examples illustrating the application of nanotechnology to microbiology.

3.1 Applications of nanotechnology in water microbiology

As stated by the World Health Organization (WHO, 2016), around 1.40 billion people in the world do not have access to safe drinking water, leading to 9300 deaths per day due to waterborne diseases. It has taken $23 billion to bring safe drinking water and sanitation to everyone over 8–10 years, especially in African and Asian countries. An important challenge is therefore the rapid, specific and sensitive detection of waterborne pathogens in water sources. Presently, microbial detection is based essentially on time-consuming culture methods like most probable numbers, defined substrate method, antibody-based detection and others. However, newer enzymatic, immunological and genetic methods are being developed to replace and/or support classical approaches to microbial detection. Moreover, innovations in nanotechnology and nanosciences are having a significant impact in wastewater or sewage wastewater decontamination.

For example, a nanomembrane is a porous thin-layered material that allows water molecules to pass through while restricting the passage of bacteria, viruses, salts, and metals. Electrospun nanofibres and nanobiocides have shown potential in the improvement of water filtration membranes. Biofouling of membranes caused by the bacterial load in water reduces the quality of drinking water and has become a major problem. Several studies showed inhibition of these bacteria following exposure to nanofibres with functionalized surfaces. Nanobiocides such as metal nanoparticles and engineered nanomaterials have been successfully incorporated into nanofibres showing high antimicrobial activity and stability in water (Botes & Cloete, 2010).

Carbon based surfaces (CNTs) as well as metal based (FeO, TiO_2, Al) nanoadsorbents with a highly specific surface and excellent adsorption capacity have been shown to remove organic and inorganic contaminants and bacteria during industrial or sewage wastewater treatment (Kunduru et al., 2017). For example, monodispersed nanosilver bioconjugate particles (15 nm), synthesized using fungal mycelia (*Rhizopus oryzae*) have been shown to be effective adsorbents and antimicrobial agents, significantly reducing (85–99%) the concentration of pesticides (parathion and chlorpyrifos) in wastewater. In addition, the amount of *Escherichia coli* (initial concentration—60 cells/mL) was negligible after treatment.

Similarly, Nano-Ag and CNTs prevent membrane biofouling due to their antimicrobial properties. Inhibition of bacterial attachment or biofilm formation was observed with doping or surface grafting of nano-Ag on polymeric membranes (Mauter et al., 2011). In addition, photocatalysis is an advanced oxidation process employed in the treatment of water and wastewater which is based on the oxidative elimination of micropollutants and microbial pathogens using titanium oxide nanoparticles (Gehrke, Geiser, & Somborn-Schulz, 2015).

Current disinfection methods applied to the treatment of drinking water can effectively control microbial pathogens. The commonly used chemical disinfectants in the water industry are chlorine, chloramines, and ozone. However, they can react with other constituents in the water and generate harmful disinfection byproducts (DBPs). Most are carcinogenic (Hossain, Perales-Perez, Hwang, & Roman, 2014); there have been more than 600 DBPs reported, such as halogenated DBPs, carcinogenic nitrosamines and bromate (Krasner et al., 2006). UV-disinfection represents an alternative for oxidative disinfection, since they generate fewer DBPs, but a high dosage is required for certain viruses, such as adenoviruses. All these limitations suggest the need for the development of alternative methods that can enhance the robustness of the disinfection process, while avoiding DBP formation (Li et al., 2008). Materials such as nano-Ag, nano-ZnO, nano-TiO_2, CNTs and fullerenes exhibit antimicrobial properties without strong oxidation because they have a lower tendency to form DBPs (Hossain et al., 2014; Li et al., 2008). The antimicrobial properties of such nanomaterials has already proved to be effective for surface, drinking and waste-water treatment (Table 2).

Although nanotechnology is proving beneficial for sewage or industrial wastewater treatment, there are human and environmental challenges, risk and safety issues that need to be addressed. Many applications of nanomaterials have been developed and demonstrated in the laboratory; however, scaling up for commercialization remains a significant hurdle. This is due to a lack of clear understanding of the fate of nanoparticles (NPs) when entering the natural environment with the notable exceptions of TiO_2, FeO and nanofibres. Long term risk assessment of engineered nanoparticles (ENPs) is crucial when applying nanotechnology to freshwater, groundwater or wastewater treatment. In addition, the costs associated with the large-scale production of NPs hinders the widespread application of NPs. Cost efficiency could be achieved by reusing or recycling nanomaterials. Overall future research must be aimed towards safety evaluation, large scale production facilities, safe disposal practices, cost and energy efficiency for NPs production and disposal to address the key issues related to scaling up of nanomaterials for environmental benefit.

Table 2 Antimicrobial nanomaterials used in wastewater disinfection and microbial control.

Antimicrobial action of nanoparticles	Properties	Reference
TiO_2	Generated ROS destroys the cell membrane, damaging DNA and protein, releasing hazardous ions leading to cell malfunction, disrupting electron transfer, and hampering the respiration process.	Hossain et al. (2014)
Ag	Nanosilver releases silver ions in water binding to −SH groups in vital enzymes and damaging them; it interferes with DNA replication and induces structural changes in the cell envelope.	Qu, Alvarez, and Li (2013); Xiu, Ma, and Alvarez (2011); Xiu, Zhang, Puppala, Colvin, and Alvarez (2012)
ZnO	Photocatalytic generation of H_2 and O_2 are responsible for the antimicrobial action of ZnO; in view of its easy dissolving nature, ZnO applications in drinking water purification are limited.	Franklin, Rogers, Apte, Batley, and Casey (2007)
Carbon nanotubes (CNTs)	CNTs effectively remove bacteria by size exclusion, and viruses by depth filtration; MWNTs directly oxidize adhering bacteria and viruses; decontaminate water within seconds by using small intermittent voltage.	Rahaman, Vecitis, and Elimelech (2012)
Nano polymers	Polymeric nanoparticles kill microorganisms either by releasing antibiotics, antimicrobial peptides, and antimicrobial agents or by contact-killing cationic surfaces, such as quaternary ammonium compounds, alkyl pyridiniums, or quaternary phosphonium.	Beyth, Houri-Haddad, Domb, Khan, and Hazan (2015); Jain et al. (2014)
Nanochitosan (chitosan-based nanoparticles)	Broader spectrum of activity against bacteria, viruses, and fungi, and less toxicity towards animals and humans; positively charged chitosan particles that interact with negatively charged cell membranes, causing an increase in membrane permeability, and eventual rupture and leakage of intracellular components	Li et al. (2008)

3.2 Applications of nanotechnology in food microbiology

3.2.1 Food spoilage is a global problem affecting our economy, society and environment

One-third of the food produced in the world for human consumption (approx. 1.3 billion tonnes p.a.) gets wasted through spoilage and poor handling. In Australia alone the cost of food waste to the economy is more than $8 billion each year.

As a result of intensification of food production over the past 50 years there are now four major food safety challenges including: (i) emergence of new food pathogens, (ii) adulteration of food materials, (iii) unknown effects from long term consumption of genetically modified food and (iv) the presence of significant amounts of chemical contaminants/pollutants in our food (Yuan et al., 2015). Numerous factors are contributing towards an increase in different food safety issues. These include the industrialization and mass production of agricultural products, the growing number of imported food products and variations in food consumption patterns due to changes in consumer lifestyle (Motarjemi, Stadler, Studer, & Damiano, 2008). Moreover, consumers are leaning towards ready-to-eat meals and fast foods to save cost and time. As a result many new food borne pathogens have emerged or existing pathogens have re-emerged because of these new types (e.g. raw and fast food) of transmission vehicles. As a result, many foodborne outbreaks have occurred in the last 20 years by bacteria, viruses and protozoa, and many more pathogens are being introduced via food contamination every year (Marušić, 2011). The annual cost of medical treatment, lost productivity, and illness-related mortality is estimated at $55.5 billion in the United States alone (Kowitt Fortune magazine, May 2016).

Nanotechnology is becoming increasingly important for the agri-food sector to (i) improve shelf life, food quality and safety, control delivery, storage and packaging and (ii) in the development of nanosensors for contaminated food detection (Ranjan et al., 2014). As conventional food technologies are proving inefficient with growing consumer demand, the nanofood sector is proving an ideal option for fulfilling ever-increasing consumer demands.

For example, essential oils are bioactive compounds and proven antimicrobial agents which have been shown to increase the shelf life of food. However, the same bioactive compounds in nanoemulsion form exhibit enhanced antimicrobial activities compared to their conventional forms due to their minute droplet size (Ranjan, Dasgupta, Ramalingam, & Kumar, 2016). Nanoemulsions of various essential oils such as carvacrol, cinnamaldehyde and limonene have shown significant antimicrobial activity against three microorganisms *E. coli*, *Saccharomyces cerevisiae* and *Lactobacillus delbrueckii* (Ranjan et al., 2016).

In addition, several nanoparticles, such as silver, gold, zinc, copper, and cerium have been applied based on their antimicrobial activity (Ismail, Pinchuk, Pinchuk, & Pinchuk, 2008). Among all, silver acts differently from other antimicrobials and showed broad-spectrum toxicity to numerous strains of bacteria, fungi and algae and possibly to some extent for some viruses (Rai, Yadav, & Gade, 2009). Nanosilver causes significant structural changes in bacterial cell wall and cell membrane due to its binding to key proteins causing cell disruption and eventually death. The binding of nanosilver to DNA and RNA can also cause death by inhibiting bacterial replication. Hence, silver nanoparticles are widely applied as a sterilizer of freeze-dried foods and an antiripening agent by food companies. Two other antimicrobial mechanisms exhibited by nanoparticles were proposed by Rabea, Badawy, Steven, Smagghe, and Steurbaut (2003), based on the chelation of trace metals by chitosan, inhibiting microbial enzyme activities, and (in fungal cells) penetration through the cell wall and membranes to bind DNA and inhibit RNA synthesis. Carbon nanotubes (CNTs) have also been reported to have antibacterial properties as direct contact with aggregates of carbon nanotubes can kill *E. coli*, possibly due to the puncturing of microbial cells by the long, thin nanotubes, causing irreversible damage and leakage of intracellular material.

Oxygen (O_2) is responsible in many ways for food deterioration, such as oxidative reactions causing browning reactions and rancid flavours, etc. Food deterioration by the indirect action of O_2 includes food spoilage by aerobic microorganisms. In such circumstances, O_2 scavengers could inhibit the spoilage process by the incorporation of O_2 scavengers into food packaging systems to maintain very low O_2 levels. Such O_2 scavenging films were developed by Xioa-e, Greens, Haque, Mills, and Durrant (2004) by adding TiO_2 NPs to different polymers. Nanocomposite materials can also be used as packaging film for a variety of oxygen sensitive food products. The role of nanosensors incorporated in food packaging in the detection of pathogens and toxins in food products represents a key emerging field of application (Bhattacharya, Jang, Yang, Akin, & Bashir, 2007). The application of fluorescent nanoparticles for the detection of pathogens and toxins in food is of particular interest (Burris & Stewart, 2012); foodborne pathogenic bacterial species such as *Salmonella typhimurium*, *Shigella flexneri* and *E. coli* can be detected by quantum dots coupled with immunomagnetic (due to Fe_2O_3 nanoparticles) separation in milk and apple juice (Zhao et al., 2008). Light scattering is another way of detecting the presence of *E. coli* in food products. The technique works on the principle that a protein of a known and characterized bacterium set on a silicon chip can bind with any *E. coli* present in the food sample resulting in nanosized light scattering, detected by analysing the digital image (Horner, Mace, Rothberg, & Miller, 2006). A similar technique was reported by Fu, Huang, and Feng (2008) for the detection of *Salmonella* in food products. Silicon/gold nanorods were incorporated with anti-*Salmonella* antibodies and fluorescent dye, which became visible when attached to bacteria.

Despite the advantages of nanotechnology in the agri-food sector, food nanotechnology represents a relatively new concept and issues relating to product labelling, potential health risks and lack of unifying regulations currently hinder the development of the sector (Handford et al., 2014). Consequently, more research on the application of nanomaterials to the food sector is required. Specifically:

- A clear, strict definition of nanoparticles is needed.
- Validated methods for in situ detection and characterization of nanomaterials in complex food matrices should be developed.

- Precise toxicology studies should be conducted.
- The long-term health consequences of ingesting nanoparticles via food have to be investigated.
- Finally, adequate regulations on nanotechnology applications for food and related products should be developed by food regulatory authorities.

3.3 Applications of nanotechnology in medical biology and immunology

Recent advances in the nanotechnological world have drawn considerable attention on the new horizons of the application of nanoscience to the fields of medical biology, pharmaceutics and immunology. Miscellaneous nanoscopic vectors including polymeric nanoparticles, liposomes, micelles, dendrimers, nano-emulsions, polymeric conjugates, carbon/metal and bio-based nanoparticles have been engineered to deliver promising diagnostic effects (Fig. 3). Numerous therapeutic substances such as drugs, genes, proteins, siRNA, peptides are being delivered in a smarter way for precise targeting to diseased tissue. Nanomedicine is paving the way in early detection, cure and diagnosis of cancer, Parkinson's disease, Alzheimer's disease, tuberculosis and ophthalmology.

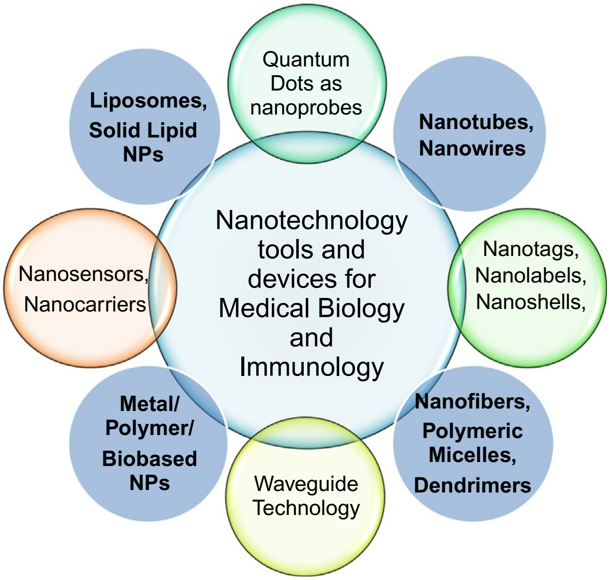

FIG. 3

Schematic diagram of various bio-nanotechnological tools and devices in medical biology and immunology.

Today diarrhoea-causing viruses represent a major public health concern. Norovirus is a leading cause of gastroenteritis in many parts of the world. Peptide sequencing using nanospray tandem MS has been reported to be the best method for the identification of capsid protein in stool extracts at a concentration as low as 250 fM (Colquhoun, Schwab, Cole, & Halden, 2006). The biosensing method most explored in virus detection is surface-enhanced Raman spectroscopy (SERS). It has shown great promise for the detection of even a single virus particle. Recently it has been shown that a silver nanorod array, fabricated using oblique-angle deposition (OAD) acts as an extremely sensitive SERS substrate.

Nanotechnology is functional in the design of biochips as they enable the diagnosis at the molecule and single cell level and hence serve as a great advance in molecular diagnostics. Recently, functionalized NPs, covalently linked to biological molecules such as antibodies, peptides, proteins, and nucleic acids have been developed as nanoprobes for molecular detection. These functionalized NPs can provide a direct, rapid, highly sensitive virus detection method (Tang et al., 2007; Tripp et al., 2007).

Waveguide technology is another nanomedicinal tool for rapid pathogen detection. The application of the technology was demonstrated for the detection of herpes simplex virus type 1 (HSV-1) by coating one of the waveguide channels with the appropriate herpes antibodies. The technique detected the virus over a range of concentrations ranging from as low as 10^3/mL to 10^7/mL. The technology is claimed to have wide application, as any antibody can be used to coat a channel for detection (Jain, 2005). This sensor can be extended to any virus including human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS) corona virus, HBV, HCV, or the avian influenza virus (H5N1), among others (Ymeti et al., 2005). The HBV, HCV, and HBV/HCV gene chips together with the gold/silver NP staining amplification method were shown to be useful in detecting these viruses in patient samples. A method for the respiratory syncytial virus (RSV) consisting of functionalized NPs conjugated to monoclonal antibodies was shown to be used to rapidly and specifically detect RSV in clinical samples with a great degree of sensitivity (Tripp et al., 2007).

Another area of development is nanobiosensors, in which antibody-based piezoelectric nanobiosensors are applied for point-of-care viral diagnostics (Jain, 2005) based on interferometric biosensor immunoassay for the direct and label-less detection of viruses. Monochromatic light from a laser source is coupled to a channel waveguide and is guided into four parallel channels (one reference channel and three measuring channels). This facilitates simultaneous detection of three different viruses as the individual channels were coated with specific antibodies (Ymeti et al., 2007, 2005). The detection of avian influenza virus through whole-virus capture on a planar optical waveguide has already been described (Xu, Suarez, & Gottfried, 2007). The assay response is based on the index of refraction changes that occur upon binding of virus particles to unique antigen-specific (haemagglutinin) antibodies on the waveguide surface.

Quantum dots (QD) is another aspect of nanobiotechnology, composed of fluorescent semiconducting nanocrystals which have broad excitation spectra and narrow emission spectra with good stability on exposure to light (Chan et al., 2007). These are effective nanolabels and have been successfully used to study RSV pathogenesis using antibody or oligoRNA probes. Research is now ongoing to develop QD mixes to simultaneously detect multiple respiratory viruses (Demidov, 2004; Jain, 2005). A new nanoparticle-based biobarcode amplification (BCA) assay has been developed for early and sensitive detection of HIV-1 capsid (p24) antigen.

Cantilever technology, the recently emerging tool in nanomedicine uses microscale and nanoscale cantilever beams as highly sensitive mass detectors with antibodies efficiently bound on cantilevers. This technology has been successfully demonstrated for the detection of a single vaccinia virus particle (average mass of 9.5 fg). These devices can be very useful as components of biosensors for the detection of air-borne virus particles (Abraham, Kannangai, & Sridharan, 2008).

Likewise, nanomaterials have high surface area and nanoscale effects, and are therefore being employed as a promising tool for the advancement of drug and gene delivery, biomedical imaging and diagnostic biosensors. In future, it will enable researchers to customize immune responses in new and unexpected ways. Through the absorption of NPs that have unique combinations of antigens and cytokines, rapid activation of innate and adaptive immune responses is allowed within hours for improved protection against the outbreak of endemic viruses (Smith, Simon, & Baker, 2013).

There is a very bright future for nanotechnology, by its merging with other technologies and the subsequent emergence of complex and innovative hybrid technologies for early detection, control and diagnosis of human and animal diseases (Nikalje, 2015). Biology-based technologies intertwined with nanotechnology are already used to manipulate genetic material. Furthermore, nanotechnology-based screening strategies using silicon nanowires in combination with siRNA and transcriptional profiling over time has shown promise for the selective perturbation of the immune response. Medicine, regenerative medicine, stem cell research and nutraceuticals are among the leading sectors that will be modified by nanotechnology innovations.

3.4 Biosynthesis of nanomaterials using microorganisms

There has been tremendous development in the field of nanotechnology in terms of nanoparticle synthesis pathways, their mode of action, characterization techniques and applications. Physical and chemical methods of nanoparticle synthesis have proven to be efficient, less time consuming and effective. However, an increasing production of metal nanoparticles and oxides by chemical and physical methods are showing ecotoxicological effects when released into the environment. Hence, ‘Green synthesis’ or ‘Biosynthesis’ of nanoparticles using microorganisms (bacteria, fungi, viruses), yeasts, algae is gaining considerable attention. Different microorganisms have different mechanisms of forming nanoparticles (Pantidos & Horsfall, 2014). However, nanoparticles are usually formed via:

(i) metal ions are first trapped on the surface or inside microbial cells;
(ii) the trapped metal ions are then reduced to nanoparticles in the presence of enzymes.

Nowadays, a variety of inorganic nanoparticles with well-defined chemical composition, size, and morphology have been synthesized using different microorganisms, and their applications in many cutting-edge technological areas have been explored including targeted drug delivery, cancer treatment, gene therapy, DNA analysis, antibacterial agents, biosensors, enhancing reaction rates, separation science and magnetic resonance imaging (MRI) (Hulkoti & Taranath, 2014). Recently, scientists have become more and more interested in the interaction between inorganic molecules and biological species. Studies have found that many microorganisms can produce inorganic nanoparticles through either intracellular or extracellular routes (Luo, Shanmugam, & Yeh, 2015).

Semi-conductive QDs are the most promising potential materials used in biomedical diagnosis. Xiong et al. (2014) designed targetable cellular beacons for diagnosis of pathogens using the biosynthesis strategy coupled with the antibody-combination ability of *Staphylococcus aureus*. Taherkhani, Mohammadi, Daoud, Martel, and Tabrizian (2014) presented another facile strategy in an application for cancer therapy using the magnetosomes derived from the bacteria *Magnetospirillum marinus* MC-1. Ghosh et al. (2012) used engineered M13 virus combined with phage display technique and magnetic nanoparticles for targeting diagnosis of prostate cancer. Furthermore, quasi-biosynthesized Ag_2Se QDs were good candidates for use as low-toxicity fluorescent tags for in vivo imaging diagnosis (Gu, Cui, Zhang, Xie, & Pang, 2012). Overall, these cases provide selected examples of the biomedical application of biosynthesized nanomaterials. However, more comprehensive studies using biosynthesized nanoparticles as nanomedicine have yet to be explored.

With the prevalence and increase of microbial resistance to multiple antibiotics, the application of silver-based antiseptics have been examined in recent years. Silver nanoparticles have been biosynthesized using the fungus *Trichoderma viride* (Fayaz et al., 2010). Durán, Marcato, De Souza, Alves, and Esposito (2007) showed that extracellularly produced silver nanoparticles using *Fusarium oxysporum* can be incorporated into textile fabrics to prevent or minimize infection with pathogenic bacteria such as *S. aureus* (Durán et al., 2007). Spherical selenium nanoparticles formed by *Bacillus subtilis* with diameters ranging from 50 to 400 nm have been reported (Wang, Yang, Zhang, & Liu, 2010) with a high surface-to-volume ratio, good adhesive ability and biocompatibility that were employed as enhancing and settling materials for use in a HRP (horseradish peroxidase) biosensor. Furthermore, magnetic particles conjugated with biological molecules, which represent attractive materials for building assay systems, have been proposed for use as a biological label. Competitive chemiluminescence enzyme immunoassays using antibodies immobilized onto BacMPs were developed for the rapid and sensitive detection of small molecules, such as environmental pollutants, hormone, and toxic detergents (Matsunaga, Ueki, Obata, et al., 2003; Tanaka, Takeda, Ueki, et al., 2004). Nanoparticle biosynthesis is gradually attracting considerable interest because of its simplicity, cost effectiveness and eco-friendliness in various sectors. However, cellular, molecular and biochemical mechanisms that mediate the synthesis of biological nanoparticles should be studied in detail to increase their application rates in various sectors.

4 Conclusion

The wealth of information gained from the science of microbiology and nanotechnology is enormous and despite the fact that it is a recent interdisciplinary science, benefits have already appeared to human, environmental and ecological sciences. Nanotechnology has been effectively combined with microbiology in areas such as medicine, pharmaceutical, industrial, agricultural and environmental applications to overcome existing microbiological limitations. Further innovations are inevitable as this interdisciplinary science develops. However, though the application of nanotechnology and microbiology represents an exciting and formidable combination, the toxicity of the engineered nanomaterials/nanoparticles (ENPs) to the environment and human health could raise serious concerns. Therefore, it is important to ensure that potential ecotoxicity, health and environmental impacts of ENPs in terms of the fate of ENPs when released into the environment through various pathways is fully considered. No matter the numerous avenues of nanoscience applied in medical, immunology, agriculture and the food sector, the positive and negative roles of NMs needs to be investigated prior to in vivo studies. There is an urgent need to understand their mechanism of action, life cycle, targeted and non-targeted delivery system and fate in the food chain of living organisms. Adequate regulations on nanotechnology applications in food and related agri-food products, surface, fresh or waste-water, drug development and delivery should be developed and monitored stringently. Once these limitations have been solved, approved nanotechniques can be used safely and effectively in various arenas making the most of their excellent properties for human well-being, environmental sustainability and for the maintenance of ecological balance.

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