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IMPORTANCE & APPLICATIONS OF NANOTECHNOLOGY

Microbial Nanotechnology

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Abstract

The era of nanotechnology, which is based on the precise design, production, development and application of materials at a nanoscale (10^{-9m}), is evolving at an unprecedented rapid pace. The areas of nanotechnology and microbiology separately have delivered novel solutions for human well-being and keeping the right ecological and environmental balance. Yet, owing to instances where the frequent/inappropriate use of drugs has led to emergence of multidrug resistance in microorganisms, and the nanoparticle delivery is affecting the food cycle, it is urgently required to develop interdisciplinary research practices combining nanotechnology and microbiology to provide innovative solutions for the human health, and environmental and ecological damage.

In the present chapter, we underline the relationship between these disciplines highlighting the promising potential resulting from interdisciplinary research. Figure 1 showed the main fields correlated to microbial nanotechnology that will be covered in this chapter.

Microbial nanotechnology in industrial applications

In general, nanomaterials describe materials with structural components sized between 1 and 100 nm. Nanomaterials are developed to exhibit novel physical, chemical, and biological characters that make them suitable for use in various applications, including degradation of pollutant and wastewater treatment [1].

Recently, there is a rising demand for using green approaches in industrial applications. Microbial nanotechnology can be used in manufacturing of nanomaterials, which could be applied in degradation of pollutants from industrial wastewater [2,3]. There is a current research potential toward the eco-friendly remediation of pollutants thanks to the production of green nanomaterials from microorganisms and extracts of other organisms. Pollutant sensing is another important application of microbial nanotechnology in nanocatalysis. The traditional methods of sensing of pollutants suffer from time consuming,

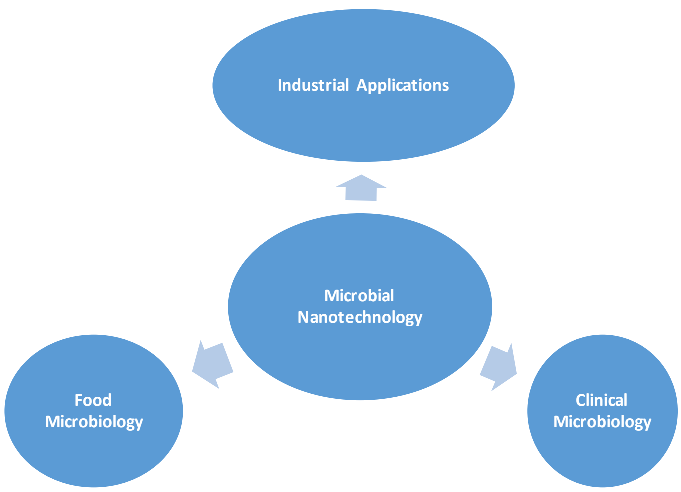

Figure 1: Schematic illustration showing the main fields correlated to microbial nanotechnology that will be covered in this chapter.

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high cost, requirement of well-trained personnel and tedious laborious procedures [4,5]. It is also challenging to determine the precise composition and nature of the pollutant under field circumstances with the traditional sensors. In biological samples such as soil, air, and water, conventional sensors can detect the chemical components of pollutants at low levels up to ppm and ppb. But, even at very low concentrations, nanoparticle sensors could detect heavy metals, microbial pathogens, and organic compounds thanks to advancement of nanotechnology [6].

Carbon nanotubes, carbon nanocomposites, metals and their oxides-based nanomaterials have been applied for effluent removal from wastewater [1]. The chemically synthesized nanoparticles may suffer from some issues in relation to the use of chemicals and self-agglomeration in aqueous solution. Fabrication of nanomaterials using microbes makes nanotechnology more sustainable and eco-friendly. Therefore, the green synthesis of nanomaterials from plans, fungi, and bacterial enzymes could be a novel and promising solution, acting as reductive factors for the metal complex salt and generating metallic nanoparticles. These nanoparticles have high solidity in an aqueous media due to co-precipitation or putting proteinaceous and bioactive materials onto the outer nanoparticle face [3]. In a recent study, removal of Pb (II), Cu (II), Ni (II), and Zn (II) from wastewater was successfully performed using bio fabricated ferromagnetic, spherical, monodispersed, and crystalline iron oxide nanoparticles using manglicolous fungi *Aspergillus tubingensis* (STSP 25) isolated from rhizospheric sediment samples of *Avicennia officinalis* in Sundarbans, India [7]. The metals were chemically adsorbed on the nanoparticle surface in endothermic processes. The researchers found that the synthesized nanoparticles remove more than 90% of heavy metals [Pb (II), Ni (II), Cu (II), and Zn (II)] from aqueous matrix with a regeneration capability till five adsorption/desorption cycle.

In another recent study, exopolysaccharides derived from *Chlorella vulgaris* were immobilized in iron-magnetic nanoparticles, and used as non-toxic nano approach for wastewater treatment [8]. They reported that, under optimum conditions (3.5 g/L of Fe3O4@EPS, pH 7.0 and 13 h of incubation), the nanocomposite has removed 91% of PO_4^{3-} and 85% of NH_4^{+}. The biogenic nanoparticles could acts as photocatalysts for azo dye degradation and treatment of textile effluents. There is a huge amount of azo dyes and heavy metals in textile wastewater, which could deteriorate the agricultural field through negatively impacting its phyisco-chemical/biological and nutritional characters if directly went to agricultural lands without previous treatment. Owing to the unique physico-chemical and biological properties, low cost and environmental sustainability of biogenic copper nanoparticles, there is a considerable attention to use these biogenic copper nanoparticles for photocatalytic degradation of wastewater pollutants. In a recent study, biogenic copper nanoparticles synthesized from a native copper-resistant bacterial strain *Escherichia* sp. SINT7 were successfully used as photocatalysts for azo dye degradation and treatment of textile effluents [9]. At 25 mg L^{-1} dye concentration and after 5 h of sunlight exposure, the reduction of reactive black-5, direct blue-1, malachite green, and congo red was 83.61, 88.42, 90.55%, and 97.07 respectively, while this was reduced at 100 mg L^{-1} dye concentration to 76.84, 62.32, and 31.08%, and 83.90 respectively. Thus, these biogenic nanoparticles are expected to lead to the development of cost-effective and eco-friendly process for large-scale wastewater treatment. Biogenic iron-sulfur nanoparticles synthesized from *Pseudoalteromonas* sp. CF10-13 (without extra sulfur) showed promising results in decolorization and degradation of metal complex Naphthol Green B at wide ranges of dye concentration, salinity, and alkalinity under anaerobic conditions [10].

Application of nanotechnology in food microbiology

Nanotechnology improves the food bioavailability, texture, taste, and consistency, achieved via modification of particle size, possible cluster formation, and surface charge of food nanomaterials [11]. Nanotechnology not only helps in increasing the shelf life of various types of food materials, but also in reducing the level of food wastage because of microbial spoilage [12].

Food additives are currently carried out on nanocarriers without modifying the basic morphology of food products. Ideal delivery system should be able to precisely deliver the active compound to the target site, guarantee the availability at an exact time and specific rate, and efficiently keep active constituents at appropriate levels for long time periods as in the case of food storage process. Since nanotechnology are being used in the production of emulsions, encapsulation, biopolymer matrices, simple solutions, and association colloids, it can provide efficient delivery systems with all the above-mentioned characters [13]. Use of nanotechnology in delivery systems facilitates the deep penetration into different tissues because of the small size of nanoparticles, allowing excellent delivery of active components to their target sites in the body [14].

Nanoencapsulations have excellent characters and high release efficiency compared with conventional encapsulation methods. For example, nanocapsules can mask tastes or odors, manage interactions of active components with the food matrix, control the release of the active chemicals, guarantee the availability at an exact time and specific rate, and protect them from heat, moisture, biological or chemical decomposition during processing, storage, and utilization, and also ensure compatibility with other components in the food matrix [15,16].

Anthocyanins are highly reactive and unstable plant pigments with various biological activities. As a way to overcome their highly reactive characters and increase their photostability, a group of scientists encapsulated cyanidin-3-O-glucoside molecules within the inner cavity of Apo recombinant soybean seed H-2 subunit ferritin (rH-2) [17]. They found that encapsulation not only improved the thermal stability and photostability, but also increased transport efficiency of cyanidin-3-O-glucoside molecules suggesting that a nanoplatform may play a pivotal role in the nutrition field.

Owing to the subcellular size of nanoparticles, they can improve bioavailability of nutraceutical compounds, increasing drug bioavailability. Although rutin is a common dietary flavonoid with excellent pharmacological characters, it has limited application in the food industry due to its poor water-solubility. Recent study reported that the nano-scale ferritin cage not only enhanced its water-solubility, but also improved its thermal and UV radiation stability [18]. A group of scientists fabricated vitamin E fortified nanoemulsions using natural biopolymers such as protein isolate and gum arabic [19]. They found that, at low concentrations, smaller droplets are produced from whey protein than gum arabic. Nanoemulsions produced from gum arabic were more stable to heating, pH, and salt. Nanoemulsions produced from both emulsifiers were generally more stable than free vitamin E.

The high acidic environment and enzyme activity of the stomach and duodenum could represent a threat to most of bioactive compounds including proteins, lipids, carbohydrates, and vitamins, which are sensitive to these conditions. Encapsulation of these bioactive compounds allow them to resist low pH conditions, and be easily included in food products [13]. Bioactive compounds such as flavonoids and vitamins can be encapsulated in polymeric nanoparticles to protect and transport them to their target sites [20]. Encapsulating functional ingredients within the nanoemulsion droplets helps in slowing down of chemical degradation processes through modifying of the interfacial layer properties surrounding them. Curcumin is the most active and least stable bioactive component of turmeric (*Curcuma longa*) plant. Encapsulation of curcumin in medium chain triglyceride oil droplets of nanoemulsion made it relatively resistant to pepsin digestion, and stable to pasteurization and at different ionic strength (0.1-1 M) and pH ranging from 3.0 to 7.0 [21].

There is a growing interest of using inorganic nanoparticles in antimicrobial food packaging process because of their strong antibacterial activity, and high stability in extreme conditions [22]. Nanolaminates and nanocomposite can also be used in food packaging and extending food shelf-life because they provide a barrier from extreme mechanical and thermal shock [13]. The incorporation of inorganic particles into bio-polymeric matrix improves the physical characters of the virgin polymers, and enhance rate of biodegradation bio-polymeric matrix [23]. Addition of nanoscale fillers including clay, chitosan, chitin, and silicate nanoplatelets into the polymer matrix enhances its thermal characters, and makes it stronger, lighter, and resistant to fire [24,25].

Chopra and colleagues found that nisin loaded chitosan/carageenan nanocapsules showed better antibacterial effect on *Pseudomonas aeruginosa* MTCC 424, *Micrococcus luteus* MTCC (Microbial Type Culture Collection) 1809, and *Salmonella enterica* MTCC 1253 and *Enterobactor aerogenes* MTCC2823 *in vitro* as well as in tomato juice for prolonged periods as compared to the components evaluated separately [26].

Applications of nanosensors in food microbiology are but not limited to pathogen detection, and quantitative analysis of food constituents [10,27]. Nanosensors act as indicators that detect slight changes in environmental conditions such as temperature or humidity in storage units, microbial contamination, or spoilage of food.

Tan and colleagues developed a Polydimethylsiloxane (PDMS) microfluidic immunosensor integrated with specific antibody immobilized alumina nanoporous membrane for rapid detection of foodborne pathogens *Escherichia coli* O157: H7 and *Staphylococcus aureus* with electrochemical impedance spectrum [28]. Nanotechnology could be applied for the detection of toxins [29], pathogens [30], and pesticides [31] in food quality process.

Carbon nanotubes act as scaffolds for immobilization of biomolecules at their surface, and have extraordinary chemical, physical, optical, and electrical features, making them one of the best suited materials for the transduction of signals associated with the recognition of metabolites, analytes, or disease biomarkers [32]. Carbon nanotubes biosensors have also been successfully used for the detection of toxins, pathogens, and other degraded substances in beverages and food**.** Toxin antibodies attached to single-walled carbon nanotubes led to a detectable change in conductivity when bound to waterborne toxins, making it simple, rapid, and sensitive method for detection of waterborne toxins [33].

Novel detection technique based on 16S rRNA gold nanoprobe-nucleic acid sequence-based amplification was developed to detect most important serovars of the *Salmonella* genus: *Salmonella enteritidis* and *Salmonella typhimurium* [34] with sensitivity around 5 CFUs *Salmonella* per amplification tube. This represents a specific, rapid, simple, and sensitive nanodetection of major serovars of the *Salmonella* genus. The combination between surface enhanced Raman scattering nanoparticles and a novel homogeneous immunoassay allows sensitive detection of pathogens in complex samples such as food, with real-time monitoring of signal and there will be no need to extensive sample preparation steps. Weidemaier and colleagues used surface enhanced Raman scattering system to detect *E. coli, Salmonella,* or *Listeria* in several food products such as chocolate milk, tuna salad, hot dogs, deli turkey, orange juice, raw ground beef, raw ground poultry, and spinach [35].

Applications of microbial nanotechnology in clinical microbiology

Merging technology with science: Nanotechnology and clinical microbiology

Nanotechnology and clinical microbiology disciplines have enriched the fields of both technology and science individually. However, the emergence of some secondary health problems raised the demand for interdisciplinary development to integrate nanotechnology with clinical microbiology. This amalgamation between both disciplines could provide innovative solutions to combat health related problems in a rational manner. Interestingly, there is a two-direction relationship between both of them; first, the use of nanotechnological tools for serving the clinical microbiology. Second, the proper utilization of microorganisms in the biosynthesis of medically important nanoparticles [36]. Furthermore, nanoparticles have a wide range of applications related to clinical microbiology. They have a major role in detection, diagnosis and cure of many infectious diseases as well as in the development of nanovaccines [37].

Applications of nanotechnological tools in clinical microbiology

Various nanoscopic carriers have been developed for the delivery of promising diagnostic elements. These nanoscopic vectors include; liposomes, bio-based nanoparticles, micelles, polymeric nanoparticles, and dendrimers (Figure 2). Furthermore, different therapeutic compounds such as proteins, drugs, siRNA, and genes, formulated using the above mentioned nanovectors to be circulated towards their targets in the diseased tissues in such a smarter way [36].

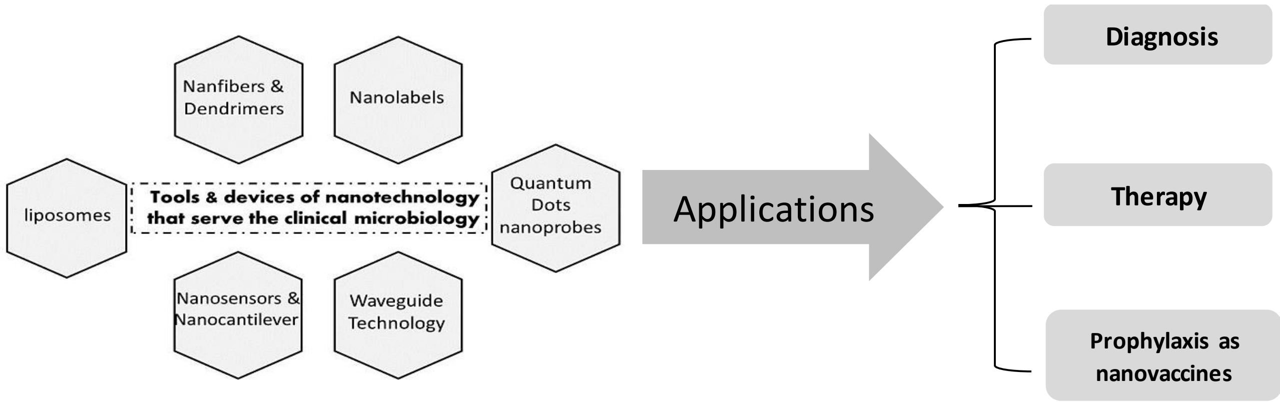

Figure 2: Schematic illustration showing different tools and devices of bio-nanotechnology serve clinical microbiology.

Nanotechnology plays an important role in the biochip design due to its ability to do diagnosis at the level of single cells as well as molecules. Accordingly, nanoparticles that are covalently attached to biomolecules such as nucleic acid and antibodies, have been used as nanoprobes. Hence, a highly sensitive, rapid and direct method has been provided for viral detection using the functionalized nanoparticles. Fluorescent silica nanoparticles is another system that was developed for detection of infections caused by *Salmonella typhimurium*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* complex [37-39].

Waveguide technology is a widely and rapidly used nanomedicinal tool for drug delivery, clinical diagnostics (biosensor), biomedical devices and detection of biomass [40]. For example, detection of Herps Simplex Virus type 1 (HSV-1) depends on using herps antibodies to coat one of the waveguide channels. Additionally, this method can detect viral concentrations ranging from 10^3 to 10^7 particles/mL. It is worth mention that such type of sensors is applicable to any virus including; Severe Acute Respiratory Syndrome (SARS) corona virus, Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) [41].

Another aspect of development is the quantum dots, which consist of flourescent semiconducting nanocrystals characterized by broad excitation but narrow emission spectra with reasonable stability when subjected to light. These nanolables served in the study of Respiratory Syncytial Virus (RSV) pathogenesis when conjugated with oligoRNA probes or antibody. Specific detection of *Serratia marcescens* lipopolysaccharide was facilitated through the use of a biosensor composed of Cadmium telluride (CdTe) quantum dots linked with Concanavalin A [42].

Nanocantilevers detectors are successfully used for early detection of dangerous pathogens as well as the air-borne viral particles. These biosensors are made of silicon and resemble tiny diving boards. They have the ability to vibrate at various frequencies when pathogens stick to them [43].

Dendrimers are known to be branched molecules with specific size and high level of molecular uniformity. They are able to encapsulate hydrophobic as well as hydrophilic molecules [44]. Previous studies developed systems composed of a lipid-dendrimer hybrid nanoparticle which was very effective in the vancomycin delivery for treatment of infections caused by methicillin-resistant *S. aureus* [45,46].

Another approach focusing on the bright future of nanotechnology is the appearance of innovative hybrid technologies. Such as intertwining of biology with nanotechnology that can manipulate genetic materials. For example, screening approaches based nanotechnology that utilizes silicon nanowire combined with siRNA followed by transcriptional profiling along time could be promising for determining the immune response perturbations [47].

Gold and silver nanoparticles are promising nanotools that widely used for delivery of biomolecules, particularly thiol-containing polymers, such as nucleic acids and antibodies. Conjugation with these probes has been used in drug and gene delivery. Furthermore, it was also applied in resonance scattering confocal microscopy [48]. Liposomes are membrane nanoparticles composed of phospholipid bilayer with an aqueous interior. They act as a vehicle carrying either of hydrophobic or hydrophilic drugs for treatment of infectious diseases. They are accumulated within tissues via targeting ligands linked to their surfaces [49]. For example, cholera or botulinum food-borne toxins were detected using rapid liposome-based systems [50,51]. A cationic liposomes enclosing lipopeptide-based vaccine (nanovaccine) was developed for prophylaxis against group A streptococci and it can be administered through intranasal passage [52].

Green synthesis of nanoparticles by microorganisms

A great development has been done in the nanotechnology field regarding synthesis, characterization and mode of action of nanomaterials by either physical or chemical means. Although the later methods were effective and less time consuming, the produced oxides and metal nanoparticles resulted in ecotoxological effects upon environmental release. To overcome these issues, there is a must for "biosynthesis" or "Green Synthesis" of nanoparticles by microorganisms. This approach paid the attention of scientists over the later few years because the resultant nanoparticles showed unique properties, biocompatibility, wider applications, cost-effective production methodologies as well as the environmental sustainability. Moreover, green synthesis of nanoparticles is considered to be an eco-friendly technique [53]. Additionally, diverse natural biological resources are used in the biosynthesis of these nanoparticles such as plants [54], algae [55] Fungi [56], Actinomycetes [57], bacteria [58], viruses [59] , or even the microbial secondary metabolites [60].

Because of the organic origin and the safe properties of green nanoparticles, they are superior compared to their chemical counterparts. Previous study used *Arthrospira* sp. polysaccharide for the green synthesis of silver nanoparticles which were characterized by their safety and high antimicrobial activity against *Pseudomonas aeruginosa* pathogens [60]. The confirmed safety was due to capping with exopolysaccharides via chelation. This was very effective approach to protect tissues against silver nanoparticles toxicity. Furthermore, microbial nanoparticles are known to have expanded applications in the field of clinical microbiology. Beside the high antimicrobial activities [60] , they can be used as biosensors [61], fluid detoxifiers [57], gene and drug delivery [61], and in pathogen diagnosis [62].

Conclusion

In summary, microbial nanotechnology holds a great potential in industrial applications including pollutant sensing, pollutant degradation from industrial wastewater, and removal of heavy metal ions. Nanotechnology has also great applications in food microbiology including food processing, food packaging, food safety, foodborne pathogen detection, and shelf-life extension of food products. The amalgamation between nanotechnology and clinical microbiology could provide innovative solutions in combating health related problems in a rational manner through the use of nanotechnological tools for serving the clinical microbiology or the proper utilization of microorganisms in the biosynthesis of medically important nanoparticles.

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