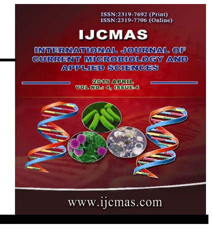

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 4 (2015) pp. 846-853**
http://www.ijcmas.com

Review Article

Applications of Nanotechnology in Food Microbiology

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Keywords

Nanotechnology, Nanobiology, Nanosensors, Nanoparticles, Antimicrobial activity,

A B S T R A C T

Nanotechnology is very interesting for scientists as an invisible world science. This article answers basic questions about using of nanotechnology in food sector and summarizes applications of nanotechnology in food microbiology field. Food safety applications such as antimicrobial activity of nano-particles, nano-sensors for microbial detection and food packaging nano-materials were discussed. But nanotechnology applications need some precautions to avoid its toxicological and negative effects for human and environment.

Introduction

Life secrets lie in invisible world which excites imagine to study. Nowadays, nanotechnology became public terminology for applications of nano-size (nm = 10^{-9} meter) in all sciences. Nano-particles already present in nature such as living cell structures (proteins, DNA, polysaccharides, membranes, etc.) and natural structures such as casein micelles or starch, the folding of globular proteins and protein aggregates are examples of self-assembly structures that create stable entities, but these questions need answer: How can the nano-size change properties of the same active material and how can it be mimicked to get additional benefits for application in life? Moreover is it true or false?

Most of the nanoparticles were traditionally used belong to the group of colloids (i.e. emulsions, micelles, mono- and bi-layers). One of the first colloidal gold dispersions was prepared by Michael Faraday in the middle of the 18th century. The particles were attracted to each other through Van der Waals forces, which give them colloidal stability. In colloidal particles, steric stabilization is achieved by adsorbing polymers and surfactants on the surface. Nanoparticles could be further stabilized by coating them with molecules that can form chemical bonds (Fendler, 2001). Owing to the greater surface area of nanoparticles per mass unit, they are expected to be more biologically active than larger sized particles of the same chemical composition (Chau *et al.*, 2007). Moreover, Egger *et al.* (2009) deduced that, silver nitrate resulted in inhibition that was approximately 10 times more effective than the inhibition observed with the nanocomposite. This can be explained by the fact that in aqueous systems silver nitrate dissolves completely and the silver is completely available in its biologically active ionic form. Thus, although silver nitrate is more effective in applications where high Ag^+ concentrations are required immediately, the effect is only short lived. In contrast, the nanocomposite powder allows slow and controlled release of Ag^+, resulting in long-term antimicrobial activity. This should be a clear advantage in any long-term antimicrobial applications (e.g., contact surfaces, fibers, plastics, medical devices, food-manufacturing equipment, cutting boards, etc.).

Nanotechnology focuses on the characterization, fabrication, and manipulation of biological and non-biological structures smaller than 100 nm. Structures on this scale have been shown to have unique and novel functional properties (Weiss *et al.*, 2006). Applications of nanotechnology within the food industry are rather limited. However, achievements and discoveries in nanotechnology are beginning to impact the food industry; this affects important aspects from food safety to the molecular synthesis of new food products and ingredients (Chen *et al.*, 2006). For food applications, nanotechnology can be applied by two different approaches, either 'top down' or 'bottom up'. The top-down approach is achieved basically by means of a physical processing of the food materials, such as grinding and milling. For example, dry-milling technology can be used to obtain wheat flour of fine size that has a high water-binding capacity (Degant and Schwechten, 2002). Bottom-up techniques build or grow larger structures atom by atom or molecule by molecule. These techniques include chemical synthesis, self-assembly and positional assembly (Sanguansri and Augustin. 2006).

In addition to the scientific and technical advances needed to continue the application of nanotechnology to foods, regulatory considerations (including safety/toxicology and environmental impact), economics, and consumer acceptance of nanotechnology will ultimately dictate its success in food applications (Weiss *et al.*, 2006). Although potential beneficial effects of nanotechnologies are generally well described, the potential toxicological effects and negative impacts of nanoparticles have so far received little attention. The high speed of introduction of nanotechnology-based consumer products observed nowadays urges the need to generate a better understanding about the potential negative impacts that may have on biological systems (Bouwmeester *et al.*, 2009).

This article provides an overview of some applications of nanotechnology in food microbiology field such as food safety programs, antimicrobial activity, sensors for microbial detection and food packaging materials. Also, the article describes the negative and toxicological effects of nanotechnology applications in food sector.

Nanotechnology in food microbiology

Nanotechnology has potential applications in all aspects of food chain including storage, quality monitoring, food processing, and food packaging. Nanotechnology applications in the food industry range from intelligent packaging to creation of on-demand interactive food that allows consumers to modify food, depending on the nutritional needs and tastes (Neethirajan and

Jayas, 2011). Food microbiologists are interested in safety and quality assurance programs to produce safe and high-quality food products which have zero defects and free of pathogens. The main applications of nanotechnology in food safety programs are antimicrobial effect of nanoparticles and nanosensors for detection of pathogens and contaminating microorganisms.

Antimicrobial effect of nanoparticles

The inhibition of microbial growth due to surface contact with the silver-silica nanocomposite-containing polystyrene demonstrated that materials functionalized with the silver nanocomposite have excellent antimicrobial properties (Egger *et al.*, 2009). Cationic peptides nanoparticles form α-helices or β-sheet-like structures that can insert into and subsequently disintegrate negatively charged bacterial cell surfaces. These nanoparticles formed by self-assembly of an amphiphilic peptide have strong antimicrobial properties against a range of bacteria, yeasts and fungi. The nanoparticles show a high therapeutic index against *Staphylococcus aureus* infection in mice (Liu *et al.*, 2009).

Metal oxide nanoparticles, especially TiO_2 and Ag_2O nanoparticles, have demonstrated significant antibacterial activity. They can also be effective against eukaryotic infectious agents (Allahverdiyev *et al.*, 2014). Silver nanoparticles can be prepared by simple green synthesis method using *Plectranthus amboinicus* leaf extract which acts as both reducing and capping agents. Morphological studies show the formation of nearly spherical nanoparticles. The synthesized Ag nanoparticles exhibited better antimicrobial property towards *Escherichia coli* and *Penicillium* spp. than other tested microorganisms using disc diffusion method (Ajitha *et al.*, 2014).

Nanocapsulation can be used to apply antimicrobial activity by nanotechnology. Donsi *et al.* (2010) found that, encapsulation of essential oils into nanometric delivery systems for incorporation into fruit juices enhance their antimicrobial activity while minimizing the impact on the quality attributes of the final product. Also, Ravichandran *et al.* (2011) reported that, Encapsulation of benzoic acid (1,100 µg/mL) in polylactic-co-glycolic acid nanoparticles inhibited *Listeria monocytogenes, Salmonella typhimurium* and *Escherichia coli* in raw and cooked chicken meat systems.

Nanoparticle delivery of benzoic acid was effective against *S. typhimurium* and *L. monocytogenes* (1.0 and 1.6 log CFU/g reduction of *S. typhimurium* and 1.1 and 3.2 log CFU/g reduction of *L. monocytogenes* compared with 1.2 log CFU/g without nanoparticles on the days 9 and 14 of storage, respectively). These findings demonstrate the efficacy of phenolics as natural and safer compounds on pathogen reduction delivered by nanoparticles and their potential for commercial food safety applications.

Nanocapsulation improves the rate of inhibition compared with conventional delivery and retains the antimicrobial efficacy for a longer time. Moreover, Chopra *et al.* (2014) evaluated the antibacterial activity of Nisin loaded chitosan/carageenan nanocapsules, results indicated that encapsulated nanocapsules showed better antibacterial effect on microbe's (*Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella enteric*, and *Enterobactor aerogenes*) *in vitro* as well as in tomato juice for prolonged periods (6 months) as compared to the components evaluated separately.

Nanosensors for pathogens and contamination detection

Development of synthetic tree-shaped DNA being tagged with color-coded probes, as a nanobarcode device, enables the identification of food pathogens (Li *et al*., 2004). Horner *et al*. (2006) have developed an analytical technology called reflective interferometry, using nanotechnology which provides specific, rapid, and label-free optical detection of biomolecules in complex mixtures. This new platform technology has provided food quality assurance by detecting *Escherichia coli* in food samples by measuring and detecting light scattering by cell. This sensor works on the principle that a protein of a known and characterized bacterium set on a silicon chip can bind with any other *E. coli* bacteria present in the food sample. This binding will result in a nano-sized light scattering detectable by analysis of digital images. Food spoilages can be detected with so-called nanosensors, for example, an array of thousands of nanoparticles designed to fluoresce in different colors on contact with food pathogens to reduce the time for pathogen detection from days to hours or even minutes (Bhattacharya *et al.*, 2007). Nanocantilevers consist of tiny pieces of silicon-based materials that have the capability of recognizing proteins and detecting pathogenic bacteria and viruses (Kumar, 2006). Pathogen detection is based on their ability to vibrate at various frequencies in dependence on the biomass of the pathogenic organisms (Jain, 2008).

In the food sector, one of the most important problems is the time-consuming and laborious process of food quality-control analysis. Innovative devices and techniques are being developed that can facilitate the preparation of food samples and their precise and inexpensive analysis. From this point of view, the development of nanosensors to detect microorganisms and contaminants is a particularly promising application of food nanotechnology (Sozer and Kokini, 2008). Nanosensors can provide quality assurance by tracking microbes, toxins, and contaminants throughout food processing chain through data capture for automatic control functions and documentation. Nanotechnology also enables to implement low cost nanosensors in food packaging to monitor the quality of food during various stages of the logistic process to guarantee product quality up until consumption (Neethirajan and Jayas, 2011).

Nano-bio-sensing could provide ideal molecular detection approaches for food-borne pathogens such as salmonellosis-causing agents. So, novel detection technique was developed based on 16S rRNA gold nanoprobe-nucleic acid sequence-based amplification (NASBA), finally, the sensitivity of the developed method was determined to be around 5CFUs *Salmonella* per amplification tube (Mollasalehi and Yazdanparast, 2013). Surface Enhanced Raman Scattering (SERS) nanoparticles are combined with a novel homogeneous immunoassay to allow sensitive detection of pathogens in complex samples such as food without the need for wash steps or extensive sample preparation. SERS-labeled immunoassay reagents are present in the cultural enrichment vessel and the signal is monitored real-time through the wall of the vessel while culture is ongoing. This continuous monitoring of pathogen load throughout the enrichment process enables rapid, hands-free detection of food pathogens, results showing the detection of *E. coli*, *Salmonella*, or *Listeria* in several food products (Weidemaier *et al.*, 2015).

Nanotechnology in food packaging

Nano food packaging materials may extend food life, improve food safety, alert consumers that food is contaminated or spoiled, repair tears in packaging, and even release preservatives to extend the life of food in the package by increasing the barrier properties (Sekhon, 2010). Food packaging nano-materials collect applications of nanosensors for microbial detection and antimicrobial activity of nanoparticles. Intelligent food packaging, incorporating nanosensors, could even provide consumers with information on the state of the food inside. Food packages are embedded with nanoparticles that alert consumers when a product is no longer safe to eat. In fact, nanotechnology is going to change the fabrication of the entire packaging industry (Sekhon, 2010).

Antimicrobial nanoparticles that have been synthesized and tested for applications in antimicrobial packaging and food storage boxes include silver oxide nanoparticles (Sondi and Salopek-Sondi, 2004), zinc oxide, and magnesium oxide nanoparticles (Jones *et al*. 2008) and nisin particles produced from the fermentation of a bacteria (Gadang *et al*. 2008). Commercial nanocomposite food packaging type nano-silver containers were characterised using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). The presence of nanoparticles consistent with the incorporation of 1% nano-silver (Ag) and 0.1% titanium dioxide (TiO_2) nanoparticle into polymeric materials formed into food containers was confirmed, results indicated that both nano-materials used in this type of packaging appear to be embedded in a layered configuration within the bulk polymer (Metak and Ajaal, 2013). Thus, Kanmani and Rhim (2014) reported that commonly used antimicrobial nanocomposite materials for food packaging include metal ions (silver, copper, gold, platinum), metal oxide (titanium dioxide, zinc oxide, magnesium oxide), organically modified nanoclay, natural biopolymers (chitosan), natural antimicrobial agents (nisin, thymol, carvacrol, isothiocyanate, antibiotics).

Toxicological and negative effects of nanoparticles

The application of nanotechnology in food has, therefore, led to concerns that ingestion of nano-sized ingredients and additives through food and drinks may pose certain hazards to consumer health. Such concerns have arisen from a growing body of scientific evidence which indicates that free engineered nanoparticles can cross cellular barriers and that exposure to some forms can lead to increased production of oxyradicals and, consequently, oxidative damage to the cell (Li *et al*. 2003; Donaldson *et al*. 2004; Oberdorster, 2004; Geiser *et al*. 2005). Nanoparticles are for instance incorporated to increase the barrier properties of packaging materials (e.g., silicate nanoparticles, nanocomposites, and nano-silver, magnesium and zinc-oxide). When the nanoparticles are applied into the food packaging materials, direct contact with food is only possible following migration of the nanoparticles. The migration of metals from biodegradable starch/clay nanocomposite films used in packaging materials for its gas barrier properties to vegetable samples was shown to be minimal (Avella *et al*., 2005). Moreover, Impellitteri *et al.* (2009) found that after exposure of Ag nanoparticles to the hypochlorite/detergent solution, a significant portion (more than 50%) of the sock nanoparticles were converted, in situ, to AgCl and suggested that an oxidation step is necessary for the elemental Ag nanoparticles to transform into AgCl. In addition, the efficacy of Ag, as an antimicrobial agent in fabrics, may be limited, or even negated, after washing in solutions containing oxidizers. In another study, silver migration was observed for all samples of three commercial nanosilver plastic food containers, with the total silver migration values ranging between 1.66 and 31.46 ng/cm^2 (Echegoyen and Nerín, 2013).

In conclusion, nanotechnology is a promising applicable science in food microbiology field. Although nanoparticles are present in nature but it can be mimicked in applicable forms to add new features to nano-materials. For food applications, nanotechnology can be applied by two different approaches, either 'bottom up' or 'top down'. Structures on nano-scale have been shown to have unique and novel functional properties. Greater surface area of nanoparticles per mass unit is expected to be more biologically active than larger sized particles of the same chemical composition. In contrast nanocomposite has lower effective activity than ions, molecules or solutions of the same compounds, but it has another advantages and benefits in applications for long-term activity. Nanotechnology has great advantages for food safety applications such as antimicrobial activity of nanoparticles, nanosensors for microbial detection and food packaging nano-materials. But nanotechnology applications need some precautions to avoid its toxicological and negative effects for human and environment.

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