New insights into the role of nanotechnology in microbial food safety

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Received: 13 March 2020 / Accepted: 21 August 2020 / Published online: 11 September 2020
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Abstract

Today, the role of nanotechnology in human life is undeniable as a broad range of industries, particularly food and medicine sectors, have been dramatically influenced. Nanomaterials can contribute to food safety by forming new nano-sized ingredients with modified physicochemical characteristics. Nanotechnologies can inhibit the growth of food spoilage microorganisms by recruiting novel and unique agents that are involved in removal of microbes from foods or prevent adhesion of microbial cells to food surfaces. Hence, nanotechnology could be considered as a high-potential tool in food packaging, safety, and preservation. Moreover, the prevention of biofilm formation by disturbing the attachment of bacteria to the food surface is another useful nanotechnological approach. Recently, nanoparticle-based biosensors have been designed and developed to detect the food-borne pathogens and hazardous substances through complicated mechanisms. During the past half-century, many methods such as freeze-drying and spray drying have been employed for increasing the viability in food industries; however, the other novel approaches such as encapsulation methods have also been developed. Admittedly, some beneficial bacteria such as probiotics bring diverse benefits for human health if only they are in a sufficient number and viability in the food products and gastrointestinal tract (GI). Encapsulation of these valuable microbial strains by nanoparticles improves the survival of probiotics under harsh conditions such as extreme levels of temperature, pH, and salinity during the processing of food products and within the GIT tract. The survival and effectiveness of encapsulated microorganisms depends on different factors including function of cell wall components in bacteria and type of coating materials. This review aims to broadly explore the potential of different aspects of nanotechnology in food industry, especially for packaging, preservation, safety, and viability.

Keywords Nanotechnology · Nutraceuticals · Encapsulation · Probiotics · Food safety · Viability

Abbreviations

NEMS Nanoelectromechanical systems
GIT Gastrointestinal tract
CFU Colony-forming unit
EFSA European food safety authority

FDA Food and drug administration
MDR Multiple drug-resistant
LAB Lactic acid bacteria
WHO World Health Organization
pH Power of hydrogen
SFD Spray-freeze drying
SPR Surface plasmon resonance
LOD Limit of detection
SNPs Silica nanoparticles
AFM Atomic force microscopy
R&D Research and development
DNA Deoxyribonucleic acid
QCM Quartz crystal microbalance

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Introduction

Nanotechnology involves the employment, manipulation, and preparation of systems, apparatus, and materials at the nanoscale (Hatami et al. 2019; Ghorbanpour and Hadian 2017). Nanoparticles, in principal, contain all materials that have unique characteristics and size of smaller than 100 nm compared with their macro-scale (bulk) counterparts, owing to their innovative physicochemical attributes such as solubility, solution thermodynamics, and color as well as high surface to volume ratio (Singh et al. 2017). On the other hand, these unique properties create chances to enhance the sensory attributes of food including color, flavor, aroma, and texture of the product. Additionally, nanotechnology has a great potential to enhance the mechanisms of defense for a wide range of foods. Smart packaging using nanosensors provides reliable, rapid, and sensitive method for identification of harmful chemicals and pesticides, and microbial contamination. Nanoencapsulation systems in food industries may improve the processing stage of the food via enabling the delivery and controlled release (within specific time and zone) of the materials/compounds that exist in different foods (Murthy et al. 2018; Nasr 2015).

In this review, the possible ways to guarantee nanomaterials safety and preparation are explained. Also, this review focuses on various applications of nanotechnology in food industries

such as nanosensors used for detection of food-borne pathogens and chemicals during the processing, packaging, transporting; and control of rheological properties such as aroma and taste, as well as increasing the bioavailability of food molecules into body, and prevention of biofilm formation. Moreover, the adverse impacts of nanomaterials on human health and the environment and limitations of this technology in the food industry are discussed. Finally, this review focuses on the techniques of encapsulation to enhance the viability of probiotics bacteria in lumen and food products.

The role of nanotechnology in food industry

Detection of harmful substances in food

Nanotechnology applications in food industry are schematically presented in Fig. 1. The devices known as nanosensors are of a significant role in food factories as they have great potential to quantify and detect low concentrations of organic compounds, pathogens, and other chemicals. Also, these devices can indicate fast response, high sensitivity,

as well as recovery and integrate arrays on a large scale (Kant 2018). For example, nanosensors are used for detection of organophosphates (originating from pesticides) in water and fruits (Zamora-Sequeira et al. 2019). High sensitivity (low detection limits) and excellent selectivity are some of the recognized benefits of nanosensors. Using quantum spots in the identification of the pesticide 2,4-dichlorophenoxyacetic is an example of semiconductor applications (Rico-Yuste and Carrasco 2019). Generally, quantum spots are used for nanocrystal sensors that can be employed to detect pesticides with moderate sensation (Durán and Marcato 2013). Interestingly, Kraft Food Company provides some sorts of nanocrystal sensors that are directly used in the packaging sector of factories. When the food-borne pathogens produce gas due to their activities leading to food spoilage, these highly sensitive devices detect the produced gases, as a result, the packaged food would be placed away from the packaging line. The color changes in strips inserted on the sensors are a visual signal to detect whether the food is fresh or not, immediately alerting the operators (Durán and Marcato 2013).

Nanosensors are used for the detection of mycotoxins and pathogens in foods. It seems that using conventional methods in the detection of pathogenic microorganisms in food has no sufficient performance, however, newly established methods like nanosensors have resolved this problem by rapid detection of pathogenic strains and the identification of their released toxins in all stages of the food production (Pradhan et al. 2015; Prasad et al. 2017). Today, fluorescent nanosensors, through making different colors, have revolutionized the packaging of food industries. Several apparatuses are invented in identification of many chemicals and bacterial toxins in food packages by antibodies and nanowires (McClements 2015).

A group of nanosensors called nanoelectromechanical systems (NEMS) have previously been recruited in food companies, which can, specifically, detect hazardous materials in food. Because NEMS have advanced transducers for specific detection of biochemical signals and chemicals, therefore, they are used in the quality control sector (Nurfatihah and Siddiquee 2019). There are many merits regarding the utilization of nanosensors in the food technology category, for instance, low costs, and portable instrumentation with quick responses. Nano-cantilever is the most recent type of nanosensors which has emerged as the fundamental components of the micro (nano)-electromechanical systems such as nanomechanical-based mass sensors (Jackman et al. 2017). The main core of these nanosensors is composed of substances derived from small pieces of silicon that are able to detect microbial pathogens and toxic proteins in food. While the specific biomolecular interactions happen between a target in solution and receptor immobilized on the surface of a nanocantilever, a mechanical bending of

Fig. 1 A schematic model that shows different applications of nanotechnology in food industries

the nanocantilever arises as a result of a change in surface stress, mass, optical angle, and frequency, which converts biochemical interactions into a concentration-dependent nano-mechanical response of the nanocantilevers (Soukoulis and Bohn 2018).

Control of flavor characteristics

Undoubtedly, taste is a significant characteristic in any foods and plays a crucial role in consumption, sensory properties, and the quality of food produces. The quality of food significantly relies on its rheological properties such as stability of the flavors and aromas during production and the storage (Estevinho and Rocha 2017). Seemingly, it is difficult to control the flavors and their stabilization, mostly in the storage phase and processes of final products (Anema 2019).

Therefore, to limit flavor loss or degradation during storage and production, it would be better to provide controlled release of encapsulated flavor before application in food, resulting in improving its chemical stability.

Encapsulation with a protective carrier preserves the foods against interactions occurring between flavors and oxidation, as well as reactions triggered by light (Yu et al. 2018). The most popular carriers are chitosan, proteins (e.g., gelatin and whey proteins), biopolymers or carbohydrates (e.g., starch, dextrose, and maltodextrins), gums (e.g., carrageenan, gum arabic, and alginates) (Estevinho and Rocha 2017). Factors considered for designing an ideal encapsulation system are the carrier (viscosity), and the physicochemical properties of the flavor (solubility).

Generally, nanoencapsulation inserts all types of materials into some nanocarriers, therefore, a suitable function of

the ultimate product is guaranteed like the release of core substances. Recruitment of a proper release system under an accurate control such as a sustained and high taste release system provides the conditions for achieving the desired ratio and time (Estevinho et al. 2013). This system has a slow release in solvated criteria but provides a sufficient release owing to temperature changes, ionic strength, and pH while a food product is indirectly contacted with saliva. The sustained release can be achieved by encapsulating substances inside suitable nanocarriers that retain the physical and chemical stabilities of the food materials under expected conditions. It should be emphasized that encapsulation by nanocarriers causes sustainable tastes release during the shelf-life storage, maintaining the flavor quality. Multiple complex factors affect the release mechanisms such as environment, method of preparation, and type of carriers used to encapsulate the flavors. Besides, during the release stages, diffusion, degradation, melting, and osmosis steps are also crucial factors. The release of flavor materials may be influenced by communications between nanocarriers and core materials.

Improving the bioavailability of food materials

Generally, preservation of the bioavailability of food materials and bioactive molecules is the most significant step in production of functional food. Bioavailability refers to the proportion of the nutrient that is digested, absorbed, and metabolized through normal pathways (Esfanjani et al. 2018). Moreover, bioavailability is the main aspect of food that directly affects the body through the blood circulatory system. Before this step, food passes through gastrointestinal tracts with harsh conditions such as extreme levels of temperature and pH and array of digestive enzymes, therefore, the food products should tolerate these conditions to maintain their bioavailability in the body. Food protection against extreme conditions can be enhanced by nanotechnology in food industries. To improve absorption and stability of food bioactive compounds by epithelium cells, it is essential to boost their bioavailability (Chai et al. 2018). In recent years, nanocarriers have been designed to increase the bioavailability of activated mixtures by target delivery systems. Based on their solubility in water, bioactive compounds are classified into hydrophilic and lipophilic groups. Many of the bioactive mixtures are highly lipophilic molecules such as flavonoids, oil-soluble vitamins, polyunsaturated lipids, carotenoids, phytosterols, and curcuminoids. Due to poor absorption in the gastrointestinal fluids, lipophilic activated mixtures show low bioavailability within the GIT (McClements 2015). For resistance, owing to the high acidity in the duodenum and stomach, bioactive compounds are mainly encapsulated (Bao et al. 2019).

Biologically active materials and molecules cannot be employed in the design of food delivery systems in the body due to their potential effects on the human system. It has been indicated that there are differences for each specific compound in terms of polarity, molecular weight, and solubility. Therefore, various encapsulation methods need to meet the specific molecular and physicochemical requirements for a specific bioactive compound. While targeted delivery systems are designed, there is a significant requirement including the encapsulation efficiency of main materials into carriers. The efficiency is mostly associated with specific products that serve as carriers and the type of the molecules to be encapsulated (Bhatia 2016). The top priority is to choose the systems that can incorporate without any changes in food attributes including taste and textures. In the next step, encapsulation efficiency should be considered as an important factor.

Food products have different physicochemical attributes and sensory properties such as texture, flavor, and appearance. While some types of food products like spreads and yogurts are solids or visually opaque semi-solids, the others are visually clear low-viscosity liquids like enriched water (McClements 2015). As a significant factor, delivery systems for a particular bioactive compound should be incorporated without having adverse effects on quality of the final food product (Gasa-Falcon et al. 2020). Nanocarriers supply a developed surface and improve the bioavailability and solubility of the encapsulated mixtures. A decrease in particle size increases the biological activity of the compounds, delivery efficiency, and solubility owing to increased surface area per unit volume (Pisoschi et al. 2018).

Safety points in food nanotechnology

Recently, many industries have been dramatically affected by nanotechnology; as a result, the market of products containing nanoparticles has experienced a considerable growth. Along with different merits of nanotechnology in food science like fast reproduction, the other issues such as ethical, regulatory, and policy points as well as public and environmental safety issues have also been raised. Nanomaterials can show substantially various biological and physicochemical characteristics compared to their respective bulk counterparts, and these unknown characteristics create unpredictable risks (Sajid et al. 2015). Despite numerous studies in this area, the consequences of direct contact of oral intake and nanomaterials in humans remain unclear (He and Hwang 2016).

The destiny of nanocarriers in the gastrointestinal tract (GIT) varies depending on the conditions of GIT and their vulnerability to hydrolysis by digestive enzymes (McClements and Xiao 2017).

Usually, the redundant application of emulsifiers and organic solvents cause many risks because of their toxicity for the arrangement of nanocarriers (Elbagory 2019). Emulsifiers and solvents are categorized as hazardous, with safe doses prepared by the European Food Safety Authority (EFSA), and global organizations like WHO, and FDA (McClements and Rao 2011). These organic solvents should be removed by a process called evaporation but this method can bring undesirable residual solvents that cannot be removed in the final material, resulting in adverse consequences. The safety points associated with nanomaterials and relevant risks are not fully investigated and need further risk assessment (Fadeel et al. 2018). Both indirect and direct impacts of nanoscale materials on the human body should be investigated and the nanomaterials destiny in the aspect of biology after digestion, possible interactions with biological systems, and their behavior within GIT should be determined. Besides, it is essential to expand the existing regulatory rules in order to keep people safe from the side effects related to nanotechnology (Ezhilarasi et al. 2013). For processed foods, modern food legislations with the collaboration of many administrations regulate the rules associated with consumer health. Thus, nanomaterial-based methods used in food industries play an important role (Benard Oloo and Oniang'o 2018). Despite the current lack of risk management and specific regulations for nanotechnology, research in this field has witnessed important advances in terms of regulatory affairs. With information transparency, new specific nanotechnology regulations will guarantee the safe utilization of nanomaterials in the food industries (McClements and Xiao 2017).

Application of antimicrobial nano-dispersed systems

Nano-sized materials can play an important role in any stage of food processing such as transporting, packaging, and labeling. Some experts argue that nanotechnology is a significant platform for construction and preparation of the substances involved in food processing. To prepare nano-structured materials, composites, and emulsions in food industries, nanoparticles are the pioneer group of materials. During the packaging process, nanomaterials display strong abilities for microbiological control of the final food products.

Preservation

Mass production in food preservation is a major challenge. Abuse of antibiotics causes the appearance of multiple drug-resistant (MDR) infectious organisms. Lately, nanotechnology has provided an innovative mechanism toward multiple

MDR organisms specifically through the use of nanomaterials with antimicrobial activity (Lalley et al. 2014). In other words, nanomaterials present various impacts to the functions of microbial cells. Instead of focusing on particular biochemical processes (e.g. those of conventional antibiotics), nanomaterials have the great potential to interrupt compound cellular processes. This complex approach constitutes a major challenge for microorganisms to increase in emerging resistance (Aruguete et al. 2013). Today, there are many commercial nanosilver products that claim to contain powerful nano-Ag ingredients (Fahimirad et al. 2019), including “kills bacteria in a short time as 30 min”, “permanently antimicrobial and antifungal”, “25–50% quicker compared to the other silver forms”, “eliminate 99% of bacteria and fungi”, and “kills approximately 650 types of harmful virus and germs” (Aabdallah et al. 2019). Retrospectively, silver has a marvelous history and our human ancestors used it as an antimicrobial factor, e.g. using silver containers to maintain drinking water, food, wine, and beverage (Zahra et al. 2019). In the past, silver was used as the sterilization and antimicrobial agent for water on NASA space shuttles and the Russian space station (Steele et al. 2018). Relative low cost and the broad-spectrum antimicrobial activity make silver a proper alternative sterilizing agent in developing countries.

Unfortunately, the interactions and influence of nanoparticles on biological systems like human body are relatively unclear. Nanoparticles may penetrate different tissue and cells by inhalation, oral, and dermal routes and spread to various organs by the circulatory system (Fig. 2). Shape, size and surface characteristics of nanoparticles are the key factors determining the cellular uptake, targeting specific tissues, and intracellular fate. It has been acknowledged that once the nanoparticles are absorbed via the human and eukaryote cells, the interaction of proteins with other biomolecules takes place in the extracellular fluids (Brandelli 2020). Other specific features of the nanomaterials including the administration route, dose, and exposure time are also the key factors related to toxicity of nanomaterials to many organisms. Both in vitro and in vivo investigations on various classes of nanoparticles make a better understanding of the influence and interactions of nanoscale materials with biological systems.

In the past decade, FDA adjusted the rules related to food additives as it allows direct utilization of silver nitrate as a sterilizer for production of water bottles at concentrations not to exceed 17 µg/kg (FDA 2009). Also, in medical science, bacteria that are resistant to antibiotics such as certain strains of *Staphylococcus aureus* (causing human infections) are of a high clinical concern (Chang et al. 2013). Silver nanoparticles can make holes into bacteria biofilms. Antibacterial characteristics of AgNPs have been widely explored in many theories. One theory argues that the contact of

Fig. 2 A schematic model for the absorption of nanoparticles by higher organisms. Through inhalation, oral, and dermal routes nanoparticles might be presented in the human and animal bodies. This figure reveals that different nanoparticles are coated by molecules like

protein in the extracellular fluid. The main regulatory protein between nanoparticles and cells called corona (Brandelli 2020). Cellular internalization takes place through passive transport or active method so-called receptor-mediated

surface and AgNPs alters the surface properties of bacterial cells through degradation of several lipopolysaccharide and collection inside membrane space (Slavin et al. 2017). Another theory declares that the released Ag^+ ions damage bacterial DNA. The Ag^+ ions, that have antibacterial characteristics, enter the cell through penetrating the bacterial cell wall. Copper nanoparticles (CuNPs), due to their high surface area to volume ratio, and antimicrobial capacity by various strategies such as blocking biochemical pathways, membrane disruption, DNA damage, and complex formation with proteins, have the toxicity potential to kill a wide range of microorganisms (Baptista et al. 2018; Yaqub et al. 2020). Although, CuNPs can be highly susceptible to oxygen and become oxide, it is possible to protect these nanoparticles safe from oxidation agents by a protein or carbohydrates matrix. In contrast, some scientists assume that many ionic metal nanoparticles that have oxidation toward oxygen, such as copper oxide, can be counted as practical bactericidal agents when they are mixed with unusual crystals, therefore, they have crystal morphologies, and extremely high surface areas (Wani and Lone 2019). Arguably, TiO_2 activated by UV radiation has the highest prevalence of the utilization photocatalyst as it has high potential to destroy any kind of

pathogenic microorganism including both Gram-negative and Gram-positive bacteria (Huang et al. 2017). Because of their ability to form spores, some Gram-positive bacteria are less sensitive than others (Ugwu et al. 2019). Lately, in many studies scientists have revealed that TiO_2 nanoparticles are capable of killing some viruses like *Hepatitis B*, *Poliovirus 1*, and *Herpes simplex virus* through the formation of free radicals (Rudramurthy et al. 2016). This nanoparticle was shown to reduce the in vivo adverse effects in rats including genetic instability and DNA damage, thus, their strict toxicity assays and precautionous approaches are urged.

Packaging

Regarding the application of nanoscience for beverages and food industries, it seems that research and development (R&D) and packaging sectors are highly benefiting from the nanotechnology. Food packaging has the most contribution to the middle-term expectations of nano-activated food (Coles and Frewer 2013). In the food packaging sector, nanosensors have the potential to alert the factories operators about a product's light, pH, and oxidation records, or detect specific indicators of pathogenic metabolism. This

method can provide more information for operators to realize the approximate time of food spoilage or remove the need for expiry dates in some incidences (López et al. 2015). The combination of active substances and food packaging materials is a practical method to control surface microbial contamination in food. As mentioned earlier, some nanomaterials show antimicrobial effects. To have active and protective packaging materials, it is essential that the materials involved have a physical contact with the food surface (Mohanty and Swain 2017). In other words, these active food-nanomaterial contacts may enhance the food safety and quality, prolong the product shelf-life, and finally decrease the food wastes (Theapparat et al. 2019).

Ultimately, those types of nanomaterials associated with complex polymers can remove the present problem of food packaging such as protection against pathogenic microorganisms, dust, dirt, and light as well as water vapor and or natural substances (Dasgupta et al. 2015). There are several materials with filling properties including substances at nanoscales such as cyclodextrins, silica nanoparticles, clay, graphene, chitosan nanoparticles, and starch nanocrystals. All these filler materials are environmentally friendly and have good techno-functional properties (Ranadheera et al. 2017). Also, some of these materials are biodegradable in the environment. Metal-based nanomaterials, e.g. those containing silver and gold, are highly important in food packaging section, presenting strong antimicrobial characteristics (Hoseinzadeh et al. 2017). These materials may display a positive impact on shelf-life of vegetables and fruits by inhibiting senescence. Occasionally, side-effects are reported while AgNPs are used at doses allowed by the FDA (Issa, 2017). The antimicrobial effectiveness of AgNPs has been investigated against several bacteria, associated with food safety. The performed studies revealed that when AgNPs are mixed with polymers, their antimicrobial properties increase remarkably as they were shown to kill silver-resistant *E. coli* (Pereda et al. 2018).

Prevention of biofilm production mechanism

The process of biofilm formation in bacteria is complicated and has several steps. In the first level, the bacterial free-floating characteristic attach to the surface by van der Waals forces that usually is not strong and reversible adhesion (Peralta et al. 2015). This adhesion can be more firm and longer if the bacterial colonies are not instantly separated from the surface.

Usually, the initial colonies pave the way of other bacteria by supplying further various adhesion zones and then making the matrix that may capture the biofilm (Bata-Vidács et al. 2013). Many bacteria species have no ability to attach to a surface on their own, however, they have the potential to anchor themselves to earlier colonies directly or the matrix.

When colonization has started, the biofilm grows by a cell division. Finally, dispersion is the last stage of biofilm formation. The last phase of biofilm formation involves a wide range of changes in cell appearances and shapes (Dzianach et al. 2019).

To control or prevent biofilms, it is crucial to recruit appropriate biocidal agents. The initial colonization deeply relies on the chemical stability, surface properties, and composition of materials. Biofilms play an important role in protecting microbes like *Listeria monocytogenes*, as epidemic strains of this microorganism have crucially higher attachment potential than that of infrequent strains (Jones and Bennett 2017).

To achieve a better attitude toward the attachment stage in biofilm formation, it requires more investigation about cell wall characteristics. Jones and Bennett (2017) explained that neither surface hydrophobicity nor charge was highly effective in the attachment process on the surface. The studies revealed the potential of *L. monocytogenes* that this strain can increase biofilm formation through the accumulation of a large number of extracellular carbohydrates. Hydrophobicity, electron acceptor capability measurements, and atomic force microscopy (AFM) were employed to investigate the potential of *L. monocytogenes* in forming biofilms on the surface of culinary accessories and assess their viability (Fink 2019). It has also been revealed that approximately 100% (the highest percentage) of cells that can attach on polypropylene surfaces show the long viability. However, 18.5% of bacteria with cultivation ability (the lowest percentage) were detected on a synthetic white silestone composite including antibacterial agent composed of triclosan and 94% quartz. Because only adhered cells that remained viable are responsible for post-process contamination, therefore, cell viability is also of high importance.

Lately, enzyme-based listericidal nanocomposites have been introduced. Three effortless paths have been reported for surface incorporation of the listerial endolysin Ply500 include; affinity binding to edible cross-linked starch nanoparticles via the construction of a maltose-binding protein fusion, covalent attachment onto FDA approved silica nanoparticles (SNPs), and incorporation of SNP-Ply500 conjugates into a thin polyhydroxy ethyl methacrylate film (Solanki et al. 2013). Biofilm formation may be prevented by killing the microbes adhered to the surface and through the application of specific covering materials.

The principle involves problem in biofilms removal are their stability, adherence and resistance abilities in the food. Another problem is related to the high-resistance of bacteria to many antibiotics when they colonized into the biofilm because they have ability to escape from antibacterial agents (Gebreyohannes et al. 2019). To overcome this problem usually nanomaterials with antimicrobial characteristics can be considered as an alternative way to kill the bacteria that are

hidden into the biofilm. For example, nanoparticles consider multiple targets including the exchange of ions, disrupting the bacterial outer membrane, and pathways exchange for cell death. Gram-negative bacteria are particularly difficult to kill once they become drug-resistant and constitute biofilms, however, in a study conducted by Lam et al. (2016), they could design star-shaped antimicrobial nanoparticles made of amino acids. These molecules destroyed many common Gram-negative pathogens in culture medium, biofilms, and removed infections in mice caused by *Acinetobacter baumannii*, which is resistant to a wide range of antibiotics. When it cultured with sublethal concentrations of the nanoparticles for three weeks, *A. baumannii* did not grow resistant over 600 generations.

Microbes detection and analysis in food by the specific sensors

Different nanomaterials have been studied to know whether they can be used in biosensors in order to enhance analytical features like the limit of detection (LOD), miniaturization or reusability, and sensitivity. These nanostructures include nano-rods, nano-particles, thin films, nanotubes, and nanofibres (Jeevanandam et al. 2018). Besides, nanostructured sensors play a key role in the enhancement of analytical attributes by macro-quantum tunnel impact, as well as the quantum size and surface effects. In nanotechnology diverse types of biosensors are being used for bacterial analysis and specific targets including the development of bacterial inhibition tests, identification of bacteria, and formation of biofilms. Rapid and sensitive detection methods are vital to ensure food safety because the majority of food-borne pathogens can produce exotoxins even in low doses and density (Yeni et al. 2014). Biomolecules fusion with nanostructures act as the foundation of nano-biorecognition. Numerous solutions namely complementary DNA sequence recognition, antibody-antigen, adhesin-receptor, and antibiotic have been examined for unique identification between biofunctionalized nanomaterials and bacteria (Singh et al. 2014). Usually, the unification of nanomaterials acts bio-functionally for the identification of pathogenic bacteria that leads to increased accuracy identification of microbes in water, beverages, and food products, as well as rapid biofilms formation and nearly real-time pathogen detection (Doyle et al. 2019).

Recognition of bacterial biofilm by different sensors

For the development of anti-biofilm substances and surface treatments or properties of biofilm formation, it is highly essential to detect the physicochemical factors that activate the sensors (Wolska et al. 2015). Numerous methods are

available to investigate the formation of biofilms. To study the effect of nano-layers on the microbial adhesion to form the biofilm on various substances like metals or glass AFM and quartz crystal microbalance, traditional microbiological methods, and electrochemical measurements were also used (Wilson et al. 2017). During scanning electron microscopy (SEM), traditional plating, combination of biochemical methods and microscopy, surface microscopy, and epifluorescence microscopy, the application of nanotechnology is becoming more prevalent like total internal reflection aqueous fluorescence microscopy, quartz crystal microbalance (QCM), atomic force microscopy (AFM), and total internal reflection microscopy. *E. coli* strains have been a practical specimen for the investigation of biofilm.

It has been observed for many sorts of cells that while a cell suspension is cultured onto a surface, cells will primarily attach and then undergo recognition that leads to a spreading and flattening of the cell body (Kalogeris et al. 2012). This suggests that a small and then growing flat zone is being formed at the primary point of contact. It was conducted that just the morphology of cells altered, and their number remained unchanged. Changes in the measured signal induce by spreading and flattening of the cells on the sensor surface in a short time. The sensory method recognizes microbial biofilm formation using a biocompatible surface coating. Also, QCM was used as an extremely sensitive device for the inhibitory effect of biocides and monitoring the formation of biofilm. Komaromy et al. (2012) successfully recruited e-beam lithography to make arrays with silicon or gold oxide nanostructures in the form of lines and dots of various sizes and with unusual spacing. To evaluate the propensity of microbial cells to adhere to these nanostructure arrays, these planar reference surfaces as well as new materials were incubated with suspensions of *S. aureus* and *E. coli* (Lai et al. 2015). The application of nanostructured arrays for probing of microbes-surface interaction might present insight for antibacterial properties and testing special materials.

Biosensors to detect specific bacteria strain

The selective difference of actual cell concentrations and bacterial strains is of great significance in all parts of microbial research like food safety and environmental science, toxicity monitoring, and clinical diagnostics (Gonchar et al. 2017). Applying immobilized antibodies as selective capture agents, cells or bacterial substances bind selectively to the immuno-activated sensor surface. Moreover, whole the surface of the immuno-sensor for microbes, organic micro-contaminants, and proteins is sensitized with an antigen or its relevant protein conjugate, the microbial analyte material in the specimen prevents binding of the antibodies to the immuno-activated surface. Another definition of sensitivity

improvement is, while the antiserum and specimen are pre-incubated before being injected onto the sensor surface, and detection is performed as a binding prevention test, lead to a further diminish in the LOD. Finally, the signal measured by the immunosensor can be strengthened by nanoparticles coupled to the antibody (Cattò and Cappitelli 2019).

The most investigated and developed methods for the selective difference are associated with *E. coli* strains, mainly *E. coli* O157: H7. This strain is responsible for food-borne disease by its secreted effective toxin called verotoxin (Kieckens et al. 2015). This protein binds to receptors in the human brain, kidney, and gut cells, resulted in the death of the influenced cells (Ali et al. 2014). Various types of immunosensors are reported for selective differences of *E. coli* cells. Tan et al. (2011) reported a polydimethylsiloxane microfluidic immunosensor merged with a particular antibody immobilized on an alumina nanoporous membrane for immediate detection of food-borne pathogens *S. aureus* and *E. coli* O157: H7 with electrochemical impedance sample. Subramanian (2006) developed a polyethylene glycol on a surface plasmon resonance (SPR) immunosensor using polyclonal antibodies or purified monoclonal on an activated sensor chip against *E. coli* O157: H7. Antibodies target bacteria were immobilized on the nanoporous alumina membranes. The impedance sample was recorded for the identification of bacteria at varying ranges from 1 Hz to 100 kHz. Joung et al. (2013), based on the impedimetric immunosensor indicated a nanoporous membrane- for label-free monitoring of bacterial pathogens in milk like *E. coli* O157: H7.

Owing to antibody-pathogen interactions, the ionic impedance of electrolytes through alumina nanopores was analyzed by normalized impedance change and watched by impedance spectra. An electrochemical-OWLS method was recruited directly for microbiological measurements using polarization potential in the measured cell to facilitate the adsorption of *E. coli*. An important difference was observed between heat-treated cells and sensor signals of living, so that the rate of living and immobilized cells and the mass of the immobilized cells were determined (Szendrő et al. 2010).

One kind of biosensors called SPR-based biosensor was applied for the rapid detection of *Campylobacter jejuni* in the specimen of broiler chicken. The sensitivity and specificity of antibodies against *C. jejuni* were examined with *Campylobacter* and non-*Campylobacter* bacterial strains. *Salmonella typhimurium* as a common food-borne pathogen in terms of processing of poultry by an SPR immunosensor was determined and showed promising perspectives to identify the presence of *S. typhimurium* at 1×10^6 CFU/mL (Jiang et al. 2015; Umesha and Manukumar 2018). Xi et al. developed a label-free impedimetric biosensor based on the affinity of the lectin with glycosyl complex on the cellular surface for detection of bacteria (Brosel-Oliu et al. 2017). Two plant lectins including *Ricinus communis* and

concanavalin A agglutinin were deposited by an electrostatic interaction on electrodes. Therefore, created lectin-polyelectrolyte film surface supplied a proper biomimetic interface for the specific stick of cells including mammalian cell line (HeLa), Gram-positive bacteria (*Bacillus subtilis*), and yeast (*Saccharomyces cerevisiae*).

Different coating methods used to encapsulate probiotic

The origin of dairy products containing probiotics can be dated back to ancient times. Consumption of probiotics in foodstuff is not a new challenge particularly in terms of fermented food. Generally, probiotics refer to live yeast and bacteria that bring numerous benefits for their hosts when they are on a sufficient scale in the body (Rahmati 2017). These valuable strains are applied in the anticipation, management, and treatment of a wide range of diseases and disorders.

In the milk fermentation process, different probiotics strains play an important role including *Bifidobacterium*, *Lactococcus*, and *Lactobacillus* owing to beneficial impacts on human health. Probiotics must survive sufficient against extreme conditions in the body and they can be easily replicated in the gut and reveal their health benefits to the host such as intestinal system maintenance, immune system stimulation, cholesterol, blood pressure regulation, and anticancer effects. The health features of the probiotic would be revealed when they are consumed alive and sufficiently 10^6 – 10^7 CFU/g to reach the colon (Rahmati 2018a). Thus, the stability of probiotics strains against the environmental circumstances of the product and the digestive system is particularly significant. Probiotics in the food factories are being developed quickly due to their health properties. Scientists have developed a new method for enhancing the stability of probiotics which employs microencapsulation technology (Rahmati 2018b). In other words, microencapsulation defined as a process in which microorganism mainly bacteria is captured and coated by hydro-colloidal material physiochemically. This technique protects them from extreme conditions like antimicrobial agents that inhibit cell damages, and other conditions such as cold shock, heat shock, high acidity and salt concentrations (caused by spray drying), as well as oxygen for obligatory anaerobic bacteria (Dulf et al. 2013). In the microencapsulation technique, bacteria are surrounded in a special coating for protection of bacterial cells. Microencapsulation has many applications in food technology. Numerous compounds are being used to coat probiotics, which the most common are alginate, waxes, milk protein, xanthan gelatin, chitosan, starch, cellulose, and carrageenan (Handford et al. 2014). These

coatings are subject to particular conditions such as pH, the pressure to release their contents and melting temperature. During the last years, many studies have been performed and almost all of them have proven the microencapsulation is one of the feasible and effective ways to increase the viability of beneficial bacteria like probiotics in the food product and human lumen. Recently, Rahmati (2020) employed both chitosan and EU S100 for double coating of probiotic strains and realized that when two materials have been used the viability of bacteria increases dramatically in yogurt and gastrointestinal tract (Fig. 3).

Microencapsulation occurs in a variety of ways, the most important of them about probiotics are emulsion, drying, and extrusion through, sublimation, fluid bed-ding, and spraying. Further ways to increase the survival of probiotics are dry ice. The risk of bacterial infection using this method deeply depends on changes in temperature, as well as on the phase. In this method, the drying process may damage cell membrane of the bacteria (Capozzi et al. 2015). This method recruits edible films to coat the food, which increases the protection of probiotics. Finally, the dry spray is another method, which costs far below dry ice. This method is effective for some species of *Lactobacillus* and *Bifidobacterium*, however, it is not practical for most species.

Emulsion and extrusion

Both techniques are two basic principles of encapsulation of probiotic bacteria, which have also been called two-phase system and droplet methods, respectively. The extrusion technique is low price and convenient that acts moderately, decreases the damage of cells, and relatively increases the viability of probiotics. The biocompatibility and flexibility are two major advantages to this method. In contrast, because of the slow formation of the microbeads, this method is not practical for large-scale production of beads typically between 2–5 mm in diameter (the diameter ranges of spherical beads made by emulsification techniques are from 30 µm to 2 mm). In other words, the capsule sizes vary based on different factors involved in the extrusion method, in which the viscosity of sodium alginate solution is the most important agent (Ching et al. 2017). High viscosity caused by a higher concentration of sodium alginate makes large particle sizes. Low-temperature makes the extrusion technique as the appropriate candidate for encapsulation of different enzymes and microorganisms. This method takes advantage of a complex matrix made of oils, flour and starch (Krempel et al. 2019). Moreover, this procedure is less expensive than other similar methods because the main platform of this method for emulsion is oils extracted from vegetables and crop plants such as sunflower, soy, corn and millet (Quintero et al. 2017). Initially, blended mixture of

Fig. 3 Images including free coated (a), single chitosan coated (b), double-coated bacteria (c), alginate beads extracted from GI condition in the period of 8, 16 and 32 days after storage (Rahmati 2020)

encapsulation materials suspended in probiotic bacteria and oil containing Tween 80. Afterward, the produced product turns into smaller pieces by adding chloride calcium and prepared capsules using a centrifuge. Genipin cross-linked κ -carrageenan, gelatin, and sodium alginate with KCl as the emulsion breaker have the potential to be applied for probiotics coating in emulsion method. The presence of larger beads with a size of about 1,000 μm can create rough texture in coated structures. The viability of probiotic cells and their metabolic rate are affected by the diameter of the beads and sensory properties of the final product. Viscosity concentration and kind of emulsifier are two the most affecting factors that control the size and form of the ultimate microbeads (Rahmati 2019).

Freeze drying and spray drying

The recruitment of drying techniques by freeze and spray as well as their fundamental parameters are essential to provide high viability levels (Moayyedi et al. 2018). Both of these techniques can be used on a large-scale in microencapsulation of probiotics bacteria, however, both techniques expose the bacteria to extreme environmental conditions. Semyonov et al. (2010) assessed the manipulation of spray freeze drying (SFD) to make coated *Lactobacillus paracasei* that can survive longer than usual form as the viability of coated strains increases remarkably by this technique (> 60%) (Liu et al. 2019). As a result, SFD may be used to produce dry microcapsules of probiotics with high viability. Also, they used the number of carbohydrates including trehalose, dextrin, and mannose to assess the impact of created carbohydrate matrix on the viability of bacteria. Results revealed that the molecular weight and concentration of carbohydrates are two key parameters that influence survival of the probiotic bacteria. Generally, drying by spray is categorized as a method for the production of probiotics on a large-scale while basic affecting factors including air pressure, outlet-air temperature, and the type of atomization processes have been highly controlled (Barbosa and Teixeira 2017; Santos et al. 2018). In the freeze-drying technique, several parameters including the amount of freezing, temperature of liquid nitrogen, initial cell concentration, the content of protective compounds (e.g., carbohydrates), pH of the medium, historical documentation of biomass like environmental stresses, and harvesting period must be monitored (Comunian et al. 2013). Schoug et al. (2006) realized that the viability of *Lactobacillus coryniformis* Si3 after a freezing period strongly depends on freezing time, concentration of carbohydrates (e.g. sucrose) in the environment, and other involving factors including the number of bacterial cells (Krasaekoopt 2017). In the freeze-drying method, the first step is to decrease the crucial freezing temperature, where the product would be frozen. The freezing process is followed by a primary

drying, wherein shelf temperature usually increased, the chamber pressure is lowered, and unbound H_2O_2 molecules removed by sublimation. In the second step, it removes the bound water molecules by desorption as the product temperature is progressively reaching the ambient temperature (Rahmati 2020).

Regarding all the advantages and disadvantages of different techniques for microorganism's microencapsulation, it seems that drying by spray is the most suitable method for preparation of dried microcapsules with controlled small size. Owing to the many various reasons such as higher stability, organoleptic properties of food, limited impacts on sensorial, easier handling, and storage of cultures, spray drying is suitable for incorporating immobilized probiotic in food products (Rahmati 2019). To protect the activated materials, microencapsulation through spray drying is not performed for cell immobilization. The reason why this technique is not prevalent is a high rate of death caused from thermal inactivation and bacterial dehydration. During spray drying, one of the mandatory conditions is the presence of high air temperature that facilitates water evaporation for bacterial cultivation. Picot and Lacroix (2004) suggested rapid, low-cost, and easy scaled-up microencapsulation methods to increase the stability of probiotics using encapsulated milk fat droplets containing powder particles of freeze-dried bacteria in a continuous two-step process (Maleki et al. 2015).

The limitations of nanotechnology in food industry

Undoubtedly, like other technologies, nanotechnology has its specific limitations and challenges faced to the researchers on food science. These barriers are remarkably found in two sections, environment and health. By increasing the nanotechnology in the food industry especially food packaging the recycling process after food consumption has been an important issue. A significant proportion of nanomaterial-polymer packaging is likely to be transferred into the water sources and terrestrial ecosystems. Therefore, a large number of nanoparticles are being released in the environment due to their degradation by microbial, chemical, and physical factors (Wyser et al. 2016). Up to now, there are no specific protocols/guidelines for companies dealing with their industrial wastes, whether it should be recycled, incinerated, or land-filled. Nevertheless, recently the scientists have tried to produce biodegradable plastics packaging nanomaterials that are lighter in weight and thermally more stable with improved barrier protection (Naseer et al. 2018). Post-production transportation of nanomaterials and nanomaterial-polymer resins is likely to be carried out in sealed containers. Hence, risk of exposure or release would only be expected in

the case of an accident for instance where a sealed container is breached or nanomaterials containing polymers are combusted in an uncontrolled environment (Grieger et al. 2016). Another aspect of limitation is associated with health. Nanomaterials can bring many problems when they are present in the circulatory systems. Macromolecules mainly proteins and amino acids interact with nanomaterials inside the cells, causing many diseases or even death when the concentration of nanoparticles is high in food products. Thus, a serious limitation in the food industry has been raised as many people ask themselves is it a right approach keeping to use of this technology in our lives or not.

Conclusions

The impacts of nanotechnology on human life are quite obvious as this technology endeavors to improve the overall quality of life. A large number of industries like medicine, food, pharmaceutical, and textile have taken advantages of nanotechnology. In food and relevant industries, the contribution of this benefit has been more than others. For example, improvement of the food quality, safety, packaging as well as increasing the shelf life of the products is just some tangible application of this technology. Nutritional supplements including probiotics tablets have witnessed developments in terms of the bacterial viability. Even the astronautic industry has recruited the acquired progress in food industries as all water and wastewater facilities have been equipped with nanoparticles that remove any pathogens from the surface of the food and environment and ventilations. Although, encapsulation of the nutraceuticals improves their stability and bioavailability, nanotechnology has its adverse and toxic effects on the human/animal health because nanoparticles may be easily entered inside the cells. Therefore, it demands more investigations in this area to decrease the detrimental side effects of nanoparticles when interacts with living cells. Prospective studies should focus on this area because food has firmly tied with the public and individual health.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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