

METAL/METAL OXIDE NANOPARTICLES FOR ENHANCING THE ANTIMICROBIAL ACTIVITY OF FOOD PACKAGING AND REDUCING FOOD-BORNE DISEASE

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Abstract

One of the main challenges of creating food packaging is preventing the products from deteriorating, but still keeping their quality during handling and storage. One factor that decreases shelf life is a lack of antimicrobial packaging films. These normally prevent microbial contamination, but without them might be decreases shelf life of food products. The best packaging films have great mechanical properties and also the permeability of water vapor or oxygen, for example. Because consumers often prefer products that are high quality and fresh, they buy food without additives. This creates issues for manufacturers that need the additives to maintain freshness. In this review, the development of innovative packaging materials using nanotechnology was addressed. Polysaccharides such as chitosan, carboxymethyl cellulose, and starch are biodegradable and nontoxic, so they do not pose environmental threats; however, they have poor antimicrobial activity, mechanical properties, and low water resistance. Therefore, nanomaterials can be employed to improve antimicrobial activity, thermal, mechanical, and gas barrier properties of food packaging. Bionanocomposites technologies are novel, high-performance, lightweight, and ecofriendly materials that can replace traditional nonbiodegradable plastic packaging.

Keywords: Utilization of Nanotechnology in Food Packaging; Antimicrobial Mechanisms; Polymer nanocomposites in Food Packaging; Antimicrobial Inorganic Nanostructures in Food Packaging Applications

Introduction

Several foodborne illnesses caused by a variety of agents such as bacteria, viruses, and fungus continue to be the most serious, global food safety issues. Approximately 600 million people were afflicted by foodborne diseases in 2015 according to estimates from the World Health Organization (WHO), with 420,000 deaths worldwide [1,2]. Indeed, according to the Centers for Disease Control and Prevention (CDCP), bacterial contamination of food results in 3,000 fatalities and 48 million illnesses annually in the United States alone. Bacteria responsible for mortality and foodborne disease in humans include *Toxoplasma gondii*, *Staphylococcus aureus*, *Campylobacter* species, *Listeria monocytogenes*, and *Salmonella* species [3,4]. Raw agricultural crops can be infected by foodborne pathogens [5,6] with increased disease transmission through contaminated food products and foodborne diseases becoming more prevalent as the number of international commerce transactions continues to increase. Hence, there is a need for increased global efforts to decrease the risk of foodborne diseases, with food packaging a particularly important focal area for accomplishing this objective [7].

Packaging has become an important waste product, particularly that made of non-biodegradable polymers and as a major component of municipal solid waste, this has led to an increase in environmental concerns. As a highly visible form of litter, discarded packaging material also presents an enormous waste management problem. Polyethylene (PE) is a petroleum-based polymer frequently utilized in packaging materials [8,9] but it is difficult to biodegrade, thus contributing to pollution. Thus, there have been attempts to develop biodegradable polymers derived from renewable resources in recent years [10-12] promoted by a growing global environmental awareness. Microorganisms, such as bacteria and fungus are often responsible for the breakdown of biodegradable polymers deposited in bioactive environments (e.g., landfills), which is facilitated by enzymatic catalytic processes. Non-enzymatic methods, such as chemical hydrolysis, may also be used to break down polymer chains, with the end products typically containing CO₂, CH₄, water, biomass, and other natural substances, which are important for the balance of greenhouse gases and other environmental effects associated with biodegraded polymers [13]. Despite this, biodegradable packaging materials serve an essential role in the preservation of environmental health. Although bio-based materials have advantages over traditional food packaging materials in terms of barrier and mechanical characteristics, there are certain drawbacks to using them, including poor barrier and mechanical capabilities which often result in a shorter product shelflife [4].

However, outbreaks of microbiological, viral, and bacterial illnesses still occur but foodborne diseases have been significantly reduced in recent years due to technical advances in 'modern food packing'. Nanoparticles used in a conventional food packaging system have a variety of biological and biochemical functions, mechanically enhancing the antibacterial properties of packaging materials and thermally strengthening the polymeric texture of the packaging film. They can also improve the mechanical and thermal properties of the packaging film. Nanosized metal oxides (NMOs) are potential materials for contemporary food packaging and are naturally antibacterial, which means they may protect food items from environmental influences. They also have the added benefit of inhibiting microbial development on food surfaces. The increased surface-area-to-volume ratio of NMOs, along with their antibacterial properties, make them particularly well suited for use in contemporary packaging [14].

Overview of the utilization of nanotechnology in food packaging

Nanotechnology is promising to overcome the current issues in food security and food sustainability. Richard Feynman was the first to propose the idea of nanotechnology in 1959, that is, the capacity to work on a scale of about 1–100 nm is defined as the ability to comprehend, build, describe and utilize material structures, devices, and systems that have novel characteristics resulting from their nanostructures [15]. Due to the reduced particle size, the resultant material displays physical and chemical characteristics that are substantially different from those of macroscale materials containing the same molecular components.

Over the last decade, the field of nanotechnology research has exploded, and there are now a plethora of companies specializing in the fabrication of new forms of nanosized matter, with potential applications in medical therapeutics and diagnostics, energy production, molecular computing, and structural materials [16]. Nanotechnology has been identified as a potential application in virtually every segment of the food industry, from agriculture (e.g., pesticide, fertilizer, or vaccine delivery; animal and plant pathogen detection; and targeted genetic engineering) to food processing (e.g., encapsulation of flavor or odor

enhancers; food texture or quality improvement; new gelation or viscosity agents) to food packaging (e.g., pathogen, gas, or abuse sensors) to food distribution (e.g., nutraceuticals with higher stability and bioavailability). The packaging industry is unquestionably the most active area of nanoscience research and development, with the global nano-enabled food and beverage packaging market valued at 4.13 billion US dollars in 2008 and is expected to grow to 7.3 billion by 2014, representing an annual growth rate of 11.65% over the next five years [16].

The desired packing material must have high gas and moisture permeability, as well as strength and biodegradability [17]. Compared to conventional packaging methods, nano-based “smart” and “active” food packagings offer several advantages ranging from a better packaging material with improved mechanical strength, barrier properties, antimicrobial films, to nanosensing for pathogen detection and alerting consumers to the safe state of their food [18]. It is possible to enhance food packaging via the use of nanocomposites as active materials in packaging and material coatings [19]. [20,21] investigated the antimicrobial properties of organic compounds such as essential oils and organic acids as well as bacteriocins, as well as the use of these compounds as antimicrobial packaging in polymeric matrixes. However, since these compounds are very sensitive to high temperatures and pressures, they cannot be used in the vast majority of food processing processes. Inorganic nanoparticles offer a high level of antibacterial activity at low concentrations with increased stability, so have been investigated for their application in antimicrobial food packaging. Active packaging, that is, antimicrobial packaging that comes into touch with the food product or headspace within to prevent or delay the microbial growth that may be present on food surfaces [22]. In addition to silver, copper, and chitosan, metal oxide nanoparticles such as titanium oxide and zinc oxide have also been found to possess antibacterial properties [8,23].

Antimicrobial mechanisms of metal/metal oxide nanoparticles

A popular and effective antibacterial agent, antimicrobial nanoparticles have been utilized in the food and pharmaceutical sectors for several years. Due to their small size, they have a higher surface-area-to-volume ratio than normal systems, offering better functions such as solubility, adsorption, controlled-release, stability, and bioavailability than normal systems, thus they are excellent antimicrobial agents.

Two types of nanoparticles, food-grade nanoparticles and metal Nano metals exert their antibacterial effects via free metal ion toxicity resulting from the dissolution of metals from the surfaces of nanoparticles or oxidative stress caused by the generation of reactive oxygen species (ROS) on the surfaces of the nanoparticles [25,26].

Cell membrane damage by electrostatic interaction

Both bacteria and spores have a negative charge on their surfaces because of the carboxylic acid groups in the proteins when the pH is within the physiological range. Electrostatic attraction occurs due to the charge difference between bacterial membranes and MeO NPs, causing MeO NPs to collect on the cell surface, eventually allowing them to enter the interior of the bacterial cell. The coordination of membrane polymers with cationic MeO NPs kills the microorganisms. Gram-negative bacteria have a higher negative charge than gram-positive bacteria [27], thus the electrostatic contact is stronger in gram-negative strains. LPS in the outer leaflet of the lipid bilayer in gram-negative bacteria has a higher charge per unit surface area than

other phospholipids, therefore the gram-negative bacteria are strongly negatively charged [28]. Positively charged MeO NPs form a strong connection with membranes causing the disorganization of cell walls, which in turn, ruptures the membrane increasing permeability, thereby drawing in more MeO NPs into the microbial system while simultaneously allowing the contents of the cell to leak into the surrounding environment. MeO NPs pass through the membrane because of their small size compared to the micrometer-sized bacteria, accumulating inside the cell to reduce the proton-motive force, causing the chemiosmotic potential of the membranes to be disrupted and proton leakage to be initiated [29].

Production of ROS and oxidative stress

Once the MeO NPs have entered the bacteria, they cause the production of ROS [superoxide anion ($O_2^\bullet$), hydroxyl radicals ($OH^\bullet$), hydrogen peroxide (H_2O_2), and organic hydroperoxides nanoparticles, have been widely utilized for antimicrobial purposes. Furthermore, surface functionalization has been developed to modify the antibacterial activity of metal nanoparticles, with research ongoing to develop effective approaches for functionalizing food-grade nanocarriers/nanoparticles such as nanoliposomes, solid lipid nanoparticles, and nanoemulsions for use in food formulations and food packaging for food safety purposes [24]. that are toxic to bacteria, causing damage to almost all organic biomolecules (amino acids, carbohydrates, lipids, nucleic acids, protein, etc.) and ultimately leading to microbial death. It is believed that three factors contribute to the production of ROS, active redox cycling, pro-oxidant functional groups on the surface of MeO NPs, and cell-particle interactions [30]. found that the ROS produced by MeO NPs have a primary inhibitory effect on respiratory enzymes [32]. $O_2^\bullet$ causes damage to iron-sulfur (Fe-S) clusters in the electron transport chain, resulting in the release of additional ferrous ions, therefore, a decrease in ATP synthesis. On treatment with MeO NPs, these electrostatic interactions cause morphological changes in bacterial cells, resulting in deformation and damage to the bacterial cell membrane. The Fenton reaction oxidizes these ferrous ions to produce additional $OH^\bullet$ and the resulting damage to DNA, proteins, and lipids [33]. DNA and proteins in microorganisms are damaged by H_2O_2 , a powerful oxidant that is deadly to cells [34]. When ZnO NPs were exposed to fungus, ROS showed anticandidal activity via cytotoxicity and apoptotic cell death [35]. It has been shown that ZnO NPs may suppress fungal growth by interfering with cell activity and the production of fungal hyphae [36].

Disturbance in metal/metal ion homeostasis

Microbial life is dependent on the maintenance of metal ion homeostasis, which controls metabolic processes by aiding the activities of coenzymes, cofactors, and catalysts [37]. When bacteria accumulate an excessive amount of metal or metal ions, metabolic processes are disrupted as the metal ions attach to DNA and cause it to lose its helical structure by forming cross-links between and within the DNA strands. Metal ions released by the MeO NPs have a positive charge, resulting in electrostatic interactions between the metal ions and the surrounding environment. LPS is negatively charged and the metal ions neutralize this charge, increasing the permeability of the outer membrane. Due to the disorganization of the membranes, bacterial growth is inhibited, and increased permeability contributes to the buildup of MeO NPs inside cells. ZnO NPs and silver (Ag) NPs disrupt the porins and LPS in the outer membrane. Evidence from the treatment of *E. coli* with TiO_2 and CdO NPs has shown that strong binding of MeO NPs to the outer

membrane limits active transport, as well as the activities of dehydrogenase and periplasmic enzymes, respectively [38].

Protein and enzyme dysfunction

Metal ions catalyze the oxidation of amino acid side chains to form carbonyls attached to proteins, with the carbonylation inside the protein molecule acting as a signal when there is oxidative protein damage. In the case of enzymes, this protein carbonylation will result in the loss of catalytic activity, eventually destroying the enzyme. In addition, these ions react with the –SH groups of many proteins and enzymes rendering them inactive. Metals such as silver have the potential to function as a weak acid and have a proclivity to react with soft bases such as sulfur and phosphorus, which are the primary components of proteins and genetic material, respectively. Metals discharged into the environment may interact with these soft bases and damage DNA, ultimately resulting in cell death [39]. Furthermore, MeO-NPs are effective in inactivating bacterial Fe–S dehydratases, and Cu-depleted Fe–S dehydratases in *E. coli* resulted in the activation of bacteriostasis [40,41] found that the metalloid oxyanion tellurium (IV) produced ROS and oxidized Fe–S clusters.

Photokilling

Photokilling occurs when MeO NPs come in contact with microorganisms in the presence of light but only transition MeOs can be photosensitized [42]. It is expected that the impact of light would result in the photochemical modification of the cell membrane, decreased Ca^{2+} permeability and superoxide dismutase activity, damage to proteins and DNA, as well as aberrant cell division. The production of $\text{O}_2\bullet$ is the main cause for the creation and release of electrons as a result of light exposure. These electrons are captured by MeO NPs, resulting in the production of additional ROS. The photochemical reactions displayed by Fe_3O_4 NPs are effective in suppressing the development of nosocomial bacteria [43], and in the presence of TiO_2 , 100% mortality of microorganisms occurred after 50 minutes of exposure to UV radiation [44].

Genotoxicity and signal transduction inhibition

MeO NPs interact with nucleic acids, especially genomic and plasmid DNA, due to their electrical characteristics [45]. By interfering with the replication of chromosomal and plasmid DNA, MeO NPs inhibit microbial cell division *in vitro*. H_2O_2 induces DNA damage in aerobic microorganisms via the formation of iron oxide nanoparticles (IO NPs) [46]. Metal ions attach to the 30S ribosomal subunit preventing the ribosome complex from moving and the translation of proteins from mRNA. from taking place. Metal ions only occur in a small number of instances. In bacteria, MeO NPs affect signal transduction by dephosphorylating the phosphotyrosine residues, thereby inhibiting signal transduction and ultimately obstructing bacterial growth [47,48].

polymer nanocomposites in food packaging

Nanocomposite food packaging has been established in the market, with many more expected to be introduced, hence having the potential to contribute a significant proportion of food packaging in the future [49]. The beverage packaging industry has received the most attention, and the tremendous benefits that

nanotechnology can provide to enhance food packaging is the driving force behind this unprecedented bacterial capabilities, and resilience to heat and cold of food packaging materials. The usage of nanocomposites for food packaging dates back to the 1990s, with montmorillonite clay serving as a nano component in a variety of polymers such as polyethylene, nylon, polyvinyl chloride, and starch. The proportion of nanoclays ranges from 1–5% by weight, and the nano components employed should have a width of less than 1 nm on one dimension. The high aspect ratios (the ratio of the length to the thickness) of many of these materials may be achieved by utilizing lateral dimensions that are as small as a few micrometers in length. When nanocomposites are integrated into packaging, their large surface area is responsible for the unique characteristics that are imparted by the materials [50].

Polymer nanocomposites have the potential to be utilized as food packaging materials, and they may be divided into four types depending on their intended application [51], amorphous polymer nanocomposites, crystalline polymer nanocomposites, and amorphous polymer nanocomposites.

Antimicrobial and antioxidant nanofillers (e.g., silver, zinc oxide, magnesium oxide) are used in active packaging systems to inhibit or retard microbial growth and food spoilage. These active packaging systems can extend the shelf life of the product, improve food quality and safety, ultimately reducing food waste [52].

Intelligent packaging

The integration of nanosensors into food packaging materials enhances the detection and monitoring of the food condition throughout storage and transit for safety and quality assurance and biosecurity purposes, communicating information to the consumer based on its ability to monitor, trace, or record external or internal changes in the product environment.

Improved packaging Polymers with nanofillers, i.e. polymer nanocomposite, to improve the packaging properties, such as barrier properties against oxygen, carbon dioxide, volatiles and flavor, temperature control, moisture stability, and ultraviolet blocking properties, have received much attention due to the potential to increase the shelf life of fresh and processed food packed in a modified atmosphere. Polymer nanocomposite can improve the quality of fresh, frozen, and processed meat, poultry, and seafood products by retarding moisture loss, reducing lipid oxidation and discoloration, and enhancing the product's appearance.

Degradable or compostable biopolymers: biopolymers are notorious for their low mechanical strength, poor gas barrier properties, reduced thermal stability, and low melt viscosity. The incorporation of nanofillers to produce biopolymer nanocomposite materials can overcome these problems, improving the chemical and physical properties of the biopolymer. Biopolymer nanocomposites can be used to extend the shelf life of fresh products such as fruits and vegetables by controlling respiratory exchange [53,54].

Bionanocomposites for food packaging application

Bionanocomposites materials provide an opportunity for the use of new, high-performance, lightweight, and environmentally friendly composite materials, which may be used instead of non-biodegradable plastic packaging materials. Due to their biodegradability and non-toxicity, biopolymers such as the

polysaccharides chitosan (CS), carboxymethyl cellulose (CMC), starch, and cellophane may be utilized to address environmental problems. However, polysaccharides have certain drawbacks, such as weak mechanical characteristics and low water resistance, thus nanoparticles are utilized to enhance the thermal, mechanical, and gas barrier characteristics of materials while maintaining their biodegradable and non-toxic qualities. The most advantageous nanomaterials include layered silicate nanoclays such as montmorillonite (MMT) and kaolinite, zinc oxide (ZnO NPs), titanium dioxide (TiO₂ NPs), and silver nanoparticles (Ag NPs). The enhancement of the barrier characteristics of films against the passage of oxygen, carbon dioxide, and flavor compounds is important in the packaging industry. A wide variety of nanomaterials are suitable for providing smart and/or intelligent properties for food packaging materials as demonstrated by oxygen scavenging capability, antimicrobial activity, and an indication of the level of exposure to various harmful features, such as low oxygen levels or insufficient temperatures. The compatibility of nanomaterials with polymer matrixes and the uniform distribution of nanoparticles throughout the polymer matrixes are challenging aspects in the production of bionanocomposites [55]. Bionanocomposites can be categorized based on the matrix utilized, size, shape, and the origin of reinforcements, for example, layered structures, elongated particles, or particulates [4].

Functional properties of bionanocomposite films

There have been many studies on the use of nanostructured materials in food packaging materials e.g. [56,57]. The nanomaterials are divided into three categories: montmorillonite nanoclay (e.g., unmodified and organically modified MMT), natural biopolymeric (e.g., chitosan, cellulose, starch nanoparticles), and inorganic (e.g., metals Ag, Cu) and metallic oxides (e.g. TiO₂, ZnO) [58].

Montmorillonite nanoclay

Montmorillonite (MMT), hectorite, and saponite are layered silicates most often used in nanocomposites that are mixed with polymeric materials to create nanostructures [59]. Microcrystalline talc (MMT) nanoparticles comprised of silica sheets stacked in plate form have a large surface area (700–800 m²/g) and a thickness of about 1 nm, primarily serve as a means of improving the mechanical and physical characteristics of composite materials [60,61]. Given the biological and pharmacological characteristics of montmorillonite (Ms), nanocomposites based on silicate layers, in particular montmorillonite (Ms), have recently received increased interest [62]. Interestingly, montmorillonite is one of the most extensively studied clay minerals owing to its abundant natural supply and low cost. It is one of the most commonly used clays in nanocomposite materials due to its unique properties, which include a high cation exchange capacity (CEC), swelling capacity, good adsorption ability, and a large specific surface area [63]. However, it functions poorly in an organic environment requiring the introduction of organic molecules (cationic surfactants) into the interlayer space through ion exchange to ensure compatibility between the polymer and sodium montmorillonite clay during the preparation of nanocomposite materials [64-66]. investigated the impact of thermoplastic starch, carvacrol essential oil (EO), and montmorillonite (MMT) sheets on bacterial growth showing significant antimicrobial activities against *E. coli* as a consequence of the synergistic impact of MMT and EO. This was attributed to the instability and partial disintegration of the bacterial cell membrane disrupting the permeability, respiration, and electron transport of *E. coli* cells. The TPS-15

hybrid sample exhibited the lowest crystallinity, allowing for more oil penetration through the film while also increasing the bacterial inhibitory effect and functioning as a biocidal agent, thus this hybrid chemical is an excellent choice for starch antimicrobial films for fresh foods such as fruits and vegetables.

Natural biopolymeric nanostructured materials

Cellulose is a renewable material that may be utilized to create cellulose nanocrystal (CNC), which is then used to improve the characteristics of films. Examples of this include the incorporation of CNC (5% w/w) into chitosan nanocomposite films, which significantly enhanced the film TS by 132% while simultaneously decreasing the EB and WVP by 36% and 19%, respectively [68]. As a result of the addition of CNC derived from sugarcane bagasse to WPI nanocomposite films, the nanocomposite films were more hydrophilic with greater water activity and surface tension (WS) but lower water contact angle values. In contrast, the addition of CNCs (2–8% weight) significantly enhanced TS and EM by over a factor of 117, while decreasing WVP by a factor of 17 with no change in the oxygen permeability of WPI-based CNC films [69]. Furthermore, increasing the amount of CNC (1–7% of the total weight) in nanocomposite suspensions increased the shear viscosity, ultimately improving the TS (28–81%) and reducing the air permeability of the chitosan/guar gum/CNC nanocomposite films by 54% [70].

Bionanocomposite films produced from fish gelatin and chitosan nanoparticles for food packaging were successfully developed by [71], who were recognized for their excellent work in increasing the shelf life of food items. It is also a desirable component for film production owing to the low cost, non-toxic nature, biodegradability, biocompatibility, stabilizing, and gel-producing characteristics, as well as the qualities of thickening and thickening agents [72]. When using nano-fibrillated cellulose to reinforce the properties of pullulan films, [73] discovered that pullulan-based nanocomposite films demonstrated significant improvements in thermal stability, mechanical properties, and tensile strength compared to unfilled pullulan films. Additionally, the films were homogenous and transparent [74].

Antimicrobial inorganic nanostructures in food packaging applications

Due to the advances in nanotechnology, there has been an increase in the production of novel antibacterial chemicals, which due to their structure and size, exhibit enhanced and new biological properties [75]. Furthermore, inorganic metal oxides provide an essential benefit by delivering key components of minerals into the body. This is a significant advantage because of the formation of ROS, inorganic metal oxides such as ZnO, MgO, and CaO have shown considerably higher antibacterial activity than organic metal oxides [76]. Nanocrystalline metal oxides are very important because they may be generated with a higher surface area than conventional metal oxides, thus are suitable for biological applications. Antibacterial inorganic compounds offer many advantages over antibacterial organic agents, including lower toxicity, greater specificity, and more selectivity. Different firms are becoming expert in the manufacturing of new nanosized substances in the field of nanotechnology, which has applications in structural materials, molecular computing, energy production, medical diagnostics, and therapeutics. Nanotechnology is a rapidly developing field with a plethora of discoveries and innovations [4].

Antimicrobial activity of zinc oxide NPs

Zirconium oxide (ZnO) is an inorganic substance extensively utilized in a variety of applications including the pharmaceutical and cosmetic industries, food and beverage, rubber, commodity chemicals, painting, ceramics, and glass, as well as glass manufacturing. As a food additive, ZnO is presently classified as safe by the US FDA, which is appropriate since zinc is an important trace element. ZnO nanoparticles have shown antibacterial capabilities and have the potential to be used in food preservation. It has been proposed to integrate ZnO nanoparticles into polymeric matrixes to impart antibacterial activity to the packaging material while also improving some packaging characteristics [77].

According to [78], *S. typhimurium* treated with ZnO nanoparticles exhibited a substantial reduction in viability, dropping from 7.97 log CFU/mL to 1.825 log CFU/mL after 4 hours of incubation. There was also a reduction in *S. typhimurium* viability after 2 hours of incubation from 7.97 log CFU/mL to 4.84 log CFU/mL, suggesting that the nanoparticles were rapid action, effective bactericidals. In contrast, the ZnO nanoparticles were less effective against *S. aureus*, with bactericidal effects observed after 4 hours of incubation, as shown by a reduction in the bacterial count of more than 3 log CFU/mL. *S. typhimurium* was irradiated after 8 hours of incubation with the nanoparticles, while the total bacterial elimination of *S. aureus* was observed after 12 hours of incubation.

ZnO reduces the viability of bacteria but the mechanism of antibacterial action has not been fully elucidated. One hypothesis is that ZnO acts via the production of hydrogen peroxide (H_2O_2). An additional mechanism for the antibacterial action of ZnO NPs is the buildup of particles on the bacterial surface as a consequence of electrostatic forces [79]. As other potential causes of cell harm, ROS production on the surface of the particles, zinc ion release from the particles, membrane malfunction, and NPs internalization may all be taken into consideration [80].

[81] showed that the addition of ZnO NPs to *E. coli*, *P. aeruginosa*, and *S. aureus* enhanced ROS generation, while [82] demonstrated that the treatment of *S. epidermidis* resulted in increased intracellular ROS production. A similar rise in the fluorescence intensity of DCF in *P. aeruginosa* was observed by [83] when the concentration of ZnO NPs treatment was increased to 100 g/mL for 24 hours in the presence of aeruginosa [84].

Antimicrobial activity of silver NPs

Regarding antibacterial characteristics, silver nanoparticles (AgNPs) are efficient against a broad range of harmful microorganisms, including bacteria, yeasts, fungus, and viruses [85-87]. Nanosized silver particles are stable at high temperatures and have a significant surface-to-volume ratio making them ideal for use in electronics [88]. In addition, because of their antibacterial and filling characteristics, Ag NPs may be used in food packaging materials such as paper and plastic. Following the inclusion of Ag NPs, a cellulose-based nanocomposite demonstrated not only improved mechanical strength and water vapor barrier properties but also apparent antibacterial activities against *E. coli* and *S. aureus* [89]. Moreover, a packaging method impregnated with Ag NPs successfully prevented the development of spoilage bacteria and preserved the nutritional content and freshness of vegetables for a longer time [90]. The bactericide effects against *Salmonella holeraesuis*, *Listeria innocua*, *Bacillus cereus*, *E. coli*, *P. aeruginosa*, and *S. aureus* were due to enhanced toxic influence on the bacterial cells [91]. The adjustment of the

physiochemical parameters of Ag NPs also leads to improved binding capacity with phosphorous and sulfur functionalized bacterial proteins for cell death, which increases the effectiveness of the nanoparticles

Antimicrobial activity of copper NPs

Cu NPs inhibit the development of microorganisms in a manner that is mostly dependent on the particle size and concentration of nanoparticles in the solution. Cu NPs are effective against gram +ve and gram ve bacteria, as well as having excellent stability and antifungal activity, by passing through bacterial cell membranes

Antimicrobial activity of TiO₂ NPS and subsequently destroying their essential enzymes

Copper

and copper oxide nanoparticles attached to thin films of silica may

Due to their unique characteristics, including high chemical stability, high photocatalytic performance, strong antibacterial activity, and biocompatibility, TiO₂ nanoparticles have received much interest in recent years [94]. These nanoparticles are extensively utilized in hybrid structured materials designed to be dirt-repellent but due to the prohibited band-gap of 3.2 eV, TiO₂ is only active in the ultraviolet (UV) area [95,96], thus is poorly antibacterial in the presence of visible light [97]. The titanium nanoparticles must react with -OH and O₂ to produce oxygen and hydroxyl free radicals, which are adsorbed on the surface of the titanium nanoparticles [98]. Due to the increased surface area of the nanoparticles, the area of contact with harmful bacteria increases, making them more appropriate as an antibacterial agent than previously thought. Also, due to their small size, the particles readily penetrate bacterial surfaces, causing damage to the bacteria [99,100].

Antimicrobial activity of silica

Silicon dioxide nanoparticles (nano-SiO₂) are food additives that are stable, non-toxic, and safe to use. They have been extensively utilized in the food processing and preservation industries for many years [101]. Furthermore, because of the large number of silanol groups present on the surface of nano-SiO₂, it has a hydrophilic character [102]. SiO₂ NPs have been investigated as a touch surface food material in food packaging [103]. Enhanced silicate nanoparticles were developed in Germany and found to be effective in delaying the appearance of gases such as bacteria and viruses. It was also discovered that nanoparticles made significant contributions to the binding of nanoparticles to decrease cell viability, membrane interference, and cell membranes. The positively charged nanoparticles interact with the negatively charged cell surface of bacteria to promote flocculation, an event not observed during the emergence of microscale particles with charge and matching chemistry be used as an antibacterial agent. Copper oxide nanoparticles have been shown to have higher toxicity against *E. coli* isolates, and nanoscale copper particles have been shown to have delayed germicidal action in photocatalytic applications [106]. Despite the fact that many particles were found to possess antibacterial properties, CuO exhibited much more efficacy compared to the other metal oxides [107].

Regulatory aspects

The behavior of nanomaterials varies significantly from that of bigger bulk materials. There are also

differences in the conventions and processes that must be followed while working with nanomaterials, and as of yet, very little is known about them. This lack of information applies to both the understanding and management of relationships, characteristics, goals, hazards, and other phenomena on the nanoscale, which is a significant limitation. These, in turn, govern the beneficial use, the negative potential, the transition of nanomaterials, and ultimately, where they end up in nature and how they impact the environment and the ecosystem. As a result, it is very difficult to begin by providing recommendations since they must be founded on scientific data and regulations have not yet been established. Furthermore, each nanomaterial is unique in nature, making a case-by-case assessment of each type of nanomaterial necessary to ensure safety [4].

Currently, there is no legal definition of a nanomaterial in the United States since the FDA has not developed explicit rules regulating the use of nanomaterials in food packaging or foods but has instead given advice to the industry. As stated in its “Guidance for Industry,” the FDA defines a nanomaterial as one with a size in the range of 1 nm to 1 μ m. The FDA also states that just because a material has generally recognized as safe (GRAS) status does not mean it is safe if it is at the nanoscale level, a case-by-case evaluation may be required in some instances [108,109]. The FDA has also listed titanium dioxide and silicon dioxide in its inventory of food contact chemicals that have been shown to be safe for particular uses [110,111]. Furthermore, ZnO is a GRAS chemical [112].

Regarding nanotechnology for food applications, the European Union has put in place the most comprehensive set of rules in the world. The European Food Safety Authority (EFSA) addressed the necessity for a comprehensive evaluation of such materials in food applications in its “Guidance on the risk assessment of the use of nanoscience and nanotechnologies in the food and feed chain” [113]. This paper offers advice on the physicochemical characterization needs of nanomaterials, as well as testing methods for determining hazards associated with them. Nonetheless, the absence of appropriate and validated testing for all applications and characteristics is recognized, thus decision trees are provided to assist industry in making the best decisions possible to guarantee the safety of their goods.

According to the European Commission’s 2011/696/ EU recommendation, a nanomaterial is defined as follows: “Nanomaterial” is defined as a natural, incidental, or manufactured material containing particles, either in an unbound state or as an aggregate or as an agglomerate, and where one or more external dimensions in the size range 1 nm – 100 nm are present for 50% or more of the particles in the number size distribution.” It may be necessary to substitute the 50% number size distribution criterion with a threshold between 1 and 50% in certain situations and when required by environmental, health, safety, or competitiveness considerations [114,115].

Conclusion

Nanotechnology is promising to overcome the current issues in food security and food sustainability. Layered silicate nanoclays such as montmorillonite (MMT), zinc oxide (ZnO-NPs), titanium dioxide (TiO₂-NPs), and silver nanoparticles (Ag-NPs) have been used as nanofillers for biopolymers matrices. They have demonstrated successful antimicrobial activity against pathogens commonly found in food by generating reactive oxygen species (ROS) and reducing membrane integrity. It is paramount for industries to choose

the packaging materials that are most suitable for specific food products based on the advantages and disadvantages they have, and aim to provide the best characteristics for both shelf life and the environment. Bionanocomposite technology can achieve many advantages for food packaging including physical, chemical and biological characteristics. We conclude that bionanocomposite films could be used effectively and feasibly to develop antimicrobial and environmental-friendly food packaging materials.

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