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4 Prevention and Control of Biofilms in the Food Industry and Bio-Nanotechnology Approaches

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4.1 INTRODUCTION

4.1.1 What is nanotechnology?

The origin of the history and creation of the field of nanotechnology is attributed to the concepts first discussed by renowned physicist Richard Feynman in his 1959 talk “There’s Plenty of Room at the Bottom” (Drexler 1986) in which he discussed the idea of achieving molecular synthesis through the direct manipulation of atoms. The term *nanotechnology*, first coined in 1974 by Japanese scientist Norio Taniguchi, originally described thin film deposition and ion beam milling where the patterning could be controlled on the order of nanometers (Drexler 1986, 1992). Today, nanotechnology describes the engineering and design of functional systems for the manipulation of the size and shape of matter on the atomic scale (Neethirajan and Jayas 2011). Nanotechnology works on the nanoscale (i.e., \(10^{-9}\) m), with dimensions from 1 to 100 nm being generally accepted as the limited working space (Drexler 1986). Nanotechnology is considered an interdisciplinary field encompassing physics, chemistry, and biology (Drexler 1992; Ferreira *et al.* 2010; Neethirajan and Jayas 2011). The properties of physical, chemical, and biological systems at the nanoscale can be substantially different from their macro-scale counterparts. As particles or compounds are reduced to the nanoscale, surface to volume ratios increase drastically (Sekhon 2010; Neethirajan and Jayas 2011; Roco and Bainbridge 2013). This leads to new compound properties with increased reactivity as well as unique mechanical and electrical properties, all of which can be exploited to deter biofilm growth.

This chapter focuses on the applications of nanotechnology for biofilm prevention and control in the food industry. The occurrence of biofilms in the food manufacturing industries leads not only to reduced shelf life of products, but also to economic losses and transmission of pathogens for food safety concerns (Kim *et al.* 2006; Rode *et al.* 2007; Winkelstroter *et al.* 2013). Nanotechnology provides the ability to create new materials and devices with vast applications in a variety of industries, such as medical,
environmental, electronic, and military (Ferreira et al. 2010). Nanotechnology is also a hotly debated issue in the scientific community with many scientific debates being held about the future implications and repercussions of nanotechnology (Roco et al. 2011). However, with the exponential increase in nanotechnology patents (Figure 4.1) and implementation since the 1990s, many concerns have been raised about its environmental impact and toxicity (Abbot and Maynard 2010; Chen et al. 2008b, 2013; Cushing et al. 2012; Dang et al. 2010; Magnuson et al. 2011; Xu et al. 2010).

### 4.1.2 Common foodborne microorganisms and pathogens

Most microbial flora found in foods is considered "good" and actually benefit food production and flavor. The yeast Saccharomyces cerevisiae is used in the production of both bread and beer, while lactic acid bacteria (Lactobacillus spp., Lactococcus spp., Pediococcus spp., etc.) are used in sauerkraut and dairy products (Jay et al. 2005). Bacteria, eukaryotes, and viruses can all be found in food. The most common foodborne bacterial pathogens include Escherichia coli O157:H7, Campylobacter jejuni, Salmonella enterica, Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes, Shigella spp., and Yersinia spp. E. coli O157:H7 serotype is of great concern due to its enterohemorrhagic capabilities (Jay et al. 2005). L. monocytogenes is also of great concern and gained particular notoriety after the 2008 outbreak at Maple Leaf Foods in Canada (meat processor and distributor) (Montville et al. 2012). Other less common bacterial food pathogens include
*Pseudomonas aeruginosa, Bacillus cereus, Vibrio cholera* (the causative agent of cholera), and *Staphylococcus aureus*, to name a few (Montville et al. 2012).

Most gastrointestinal disease and discomfort caused by ingesting these microbes is not due to their direct action, but by their production of exotoxins (known as enterotoxins; exotoxins targeting the gastrointestinal tract), which may be secreted or exist as toxic components of the lipopolysaccharide layer (Madigan et al. 2012). Both secreted and non-secreted exotoxins are unique in that they can still cause illness in individuals affected even after microbial death. Exotoxin effects may appear rapidly within 1–6 h as seen with *Staph. aureus* gastrointestinal infections and can lead to vomiting and diarrhea (the most common symptoms from exotoxin poisoning) (Montville et al. 2012). Production of exotoxins by microbes occurs in the food before ingestion, with most production occurring in cooked or processed foods improperly maintained or stored. Some exotoxins can be extremely poisonous, such as botulinum toxin produced by the anaerobic microorganism *Clostridium botulinum*. Botulinum toxin is an extremely powerful paralytic toxin (1 ng/kg of body weight) and it is considered a biological weapon (Jay et al. 2005; Madigan et al. 2012; Montville et al. 2012).

The infectious dose, another key aspect regarding foodborne pathogens, can be described as the quantity (usually described in number of viable microbiota) that must be ingested in order to give rise to symptoms of a foodborne illness. A variety of factors such as the consumer’s age and health, as well as the characteristics of the infecting microbe, can affect the infectious dose required to illicit symptoms. For example, >10⁵ *Salmonella* cells are required to establish infection, whereas as little as 500 cells of *C. jejuni* and <10 cells of *E. coli* O157:H7 are needed for infection (Madigan et al. 2012; Montville et al. 2012).

Foodborne pathogens are not limited to bacteria, but can also include eukaryotic organisms and viruses. A common example of a eukaryotic foodborne pathogen is *Trichinella spiralis*: a parasitic worm responsible for the disease trichinosis, caused by ingesting raw or undercooked pork (Montville et al. 2012). Enteric viruses contribute to a substantial number of illnesses around the world, and include hepatitis A, norovirus, rotavirus, and astrovirus, to name a few (Montville et al. 2012). Going forward, the mention of the term microbe or microorganism will refer specifically to bacteria.

### 4.1.3 Biofilm development

It is now commonly accepted that biofilms are the predominant mode of growth for pathogenic food microbes (Abee et al. 2011; Mitik-Dineva et al. 2009; Van Houdt and Michiels 2010). Biofilms consist of complex bacterial communities, commonly polymicrobial, encased within an extracellular polymeric substrate (EPS). The EPS is mainly comprised of polysaccharides among other compounds such as proteins, single amino acids, and nucleic acids (Van Houdt and Michiels 2010; Yang et al. 2011). Biofilms are also structurally complex, forming porous systems allowing for
gas and nutrient diffusion to the inner-most residing microbes. Biofilms can form on solid and liquid surfaces and are typically recalcitrant to conventional methods of removal and disinfection (Ferreira et al. 2010; Van Houdt and Michiels 2010; Yang et al. 2011). Solid surfaces may include pipes, cutting blades, conveyor belts, and mixing bowls, whereas liquid surfaces include surfaces formed between an atmosphere and a liquid food product (e.g., milk, sauces, beer, etc.). The process of biofilm establishment, growth, and maintenance can be described in five steps (Figure 4.2): (1) adhesion, (2) colonization, (3) accumulation, (4) macrocolony and EPS formation, and (5) detachment and dispersal (Shi and Zhu 2009; Yang et al. 2011). Initial adhesion events are considered a random occurrence. If microbial cells are able to adhere to a solid surface, they then will begin to divide and accumulate on the surface. This point of biofilm formation is reversible in that the microbial cell can detach itself and move to another site (using flagella, pili, Brownian motion, or all of these for motility), if the environment, such as the chemical environment (i.e., pH) or the redox potential of the surface, is non-ideal (Van Houdt and Michiels 2010). In an ideal environment, attachment will occur, and the small microbial community will begin to secrete EPS and become irreversibly attached (Srey et al. 2013). Microbial attachment, in most cases, readily occurs on surfaces that are rough, porous (even at the nanoscale), hydrophobic and nonpolar (Detry et al. 2010; Mitik-Dineva et al. 2008; Whitehead and Verran 2006). Attachment also occurs rapidly on surfaces that are “conditioned” with a nutrient source from surrounding food activities (flow of milk, slicing of meat, etc.) (Srey et al. 2013; Van Houdt and Michiels 2010). The entrapment of other microorganisms (rods, cocci, filamentous bacteria, or diatoms, etc.), through EPS production, helps form a complex community that will grow with continued nutrient supplementation (Van Houdt and Michiels 2010). At its most

Figure 4.2 Biofilm formation stages on general substratum promoting microbial attachment. The steps of biofilm formation are shown and includes: (1) adhesion (reversible-step), (2) colonization (irreversible-step), (3) accumulation, (4) macrocolony formation and EPS, and (5) detachment. Sauer (2003). Reproduced with permission of BioMed Central.
Van Houdt and Michiels (2010). Furthermore, it has been shown that commonly used disinfectants, consisting of alkali-based or acid-based cleaners, are less effective when food particulate matter is present on surfaces. These cleaners are only adequate in the removal of biofilms, and are only effective under certain processing parameters, including appropriate cleaner formulation, concentration, duration of application, and temperature; all of which have been shown to significantly affect the overall effectiveness of cleaners in biofilm removal (Simoes et al. 2010). Other processes such as mechanical force (i.e., brushing, scrubbing, scraping, and spraying) are important in the removal of biofilm from surfaces. Mechanical force can vary considerably depending on the biofilm removal method (if done by hand and by various employees), and may also lead to the production of microbial and food aerosols and subsequently, additional methods may need to be employed to prevent further microbial spread. This is one reason why many areas in the food industry have moved away from the use of high pressure sprays in cleaning (Van Houdt and Michiels, 2010).

4.2.1 Chemical disinfectants

Chemical disinfectants are the most commonly used method for biofilm removal. Currently, a wide range of chemical disinfectants are used in the food industry and can be divided into three main groups according to their modes of action: (1) oxidizing agents (chlorine-based compounds, hydrogen peroxide, ozone, and peracetic acid (PAA)); (2) surface-active compounds (quaternary ammonium compounds (QACs) and acidic anionic compounds); and (3) iodophores (Fraise et al. 2013; Van Houdt and Michiels 2010). The efficacy of disinfectants is influenced by factors such as pH, temperature, duration of contact time, and interaction with organic residuals (Simoes et al. 2010). To remove organic residuals, disinfecting detergents are commonly combined with enzymes in an attempt to synergistically enhance disinfection. The increased resistance of biofilms to biocides, mainly due to the interfering/protective effect of the EPS, explains why chemical disinfectants are found to be most effective against active planktonic cells, and are not necessarily effective against mature biofilms (Detry et al. 2010; Fraise et al. 2013). Among oxidizing agents, it has been shown that peroxide-based disinfectants are more effective than their chlorine-based counterparts at degrading and dispersing polymicrobial biofilm of *P. aeruginosa* and *L. monocytogenes* on stainless steel (Van Houdt and Michiels 2010). This difference is even more profound in the presence of organic food compounds. PAA was not shown to be effective against polymicrobial biofilms consisting of *Pseudomonas fluorescens*, *L. monocytogenes*, and *Yersinia enterocolitica* when grown on both rubber and Teflon surfaces (Mosteller and Bishop 1993). It has also been shown that surface-active QACs are more effective at killing microbes in biofilms compared to oxidizing agents (Van Houdt and Michiels 2010). Recently, electrolyzed oxidizing (EO) water has been used as a novel chlorine-based cleaning compound
in food systems with specific application in the cleaning and sanitization of milking systems (Zhang et al. 2011). EO water has been shown to have 50% stronger oxidizing power than free chlorine at the same content (Zhang et al. 2011).

Recently, the application of ozone as an alternative sanitation technique has gained interest in the food industry (Fraise et al. 2013). Ozone is a tri-oxygen molecule with strong oxidizing properties (52% stronger than chlorine) (Guzel-Seydim et al. 2004). The strong oxidation properties of ozone make it an effective candidate at killing a wider-spectrum of microorganisms, compared to chlorine and other disinfectants (Ferreira et al. 2010; Fraise et al. 2013). However, more research is needed in order to acquire information on the efficacy of ozone on food pathogens adhered to different material surfaces. Also, more research is required to determine the effects of various process parameters (e.g., temperature, pH, duration of contact, etc.) on the efficacy of ozone as a disinfectant (Guzel-Seydim et al. 2004).

Chemicals that directly interfere with bacterial quorum sensing, another key component in the maturation and persistence of biofilms (Glinel et al. 2012; Yang et al. 2011) are being developed. Research is also being conducted on the use of naturally occurring biocides (e.g., nisin and lysozyme) with either a wider spectrum of action or a more specific mode of action against particular pathogenic and spoilage microorganisms (Van Houdt and Michiels 2010). It will ultimately come down to case-by-case studies to evaluate the efficacies of these compounds under different process parameters and microbial communities. Safety evaluations of these compounds for human consumption will also have to be examined. The most active and effective disinfectants against planktonic or pure microbial cultures are not necessarily the most effective against the polymicrobial biofilms challenging the food industry. Nevertheless, among the chemical disinfectants described here, chlorine is the most actively used as chlorine-based disinfectants require the least preparation time, are easy to apply, and are the most cost-efficient (Van Houdt and Michiels 2010).

4.2.2 Physical methods

Physical treatment methods, characterized as thermal and non-thermal, have been extensively studied as alternatives to the use of chemical disinfectants in the food industry, and applied to the sanitation of surfaces (Birmpa et al. 2013; Fraise et al. 2013; Montville et al. 2012; Pereira and Vicente 2010). The acceptability of the physical disinfection methods against foodborne bacterial biofilms by the federal regulatory agencies and consumers is important. The acceptance rate is of greater concern because if the technology is perceived negatively (heavily influenced by media outlets) at the consumer level, then the technology will never make it into the marketplace. This is also applicable to nanotechnology in the food industry, and has been seen with the use of gamma-radiation as a method for sanitizing food (Montville et al. 2012). Overall, processes must be scientifically tested and shown to be as, or more effective than previously used methods in order for initial acceptance. New processes must also not
significantly change food quality or texture. Physical methods can be combined with chemical disinfectant methods in many cases to improve overall sanitation (Fraise et al. 2013). An example of this is the use of ultraviolet (UV) light (non-thermal physical method) and ozone as alternatives to heat for pasteurization, which has always been considered a thermal process. Compared to chemical disinfectants, physical disinfecting methods may be more robust and long lasting; however, the costs of implementation are much higher and have stricter regulatory guidelines (Van Houdt and Michiels 2010).

4.2.3 Thermal disinfection

Heat has always been the most widely used method for microbial disinfection. For food microbiologists, understanding a microorganism’s heat resistance is an important characteristic. The processes of pasteurization and canning are the classic examples of thermal disinfection methods. Pasteurization, named after French microbiologist and physiologist Louis Pasteur, consists of a relatively mild heat treatment whose purpose is to kill non-spore-forming pathogenic microbes (Jay et al. 2005; Madigan et al. 2012; Montville et al. 2012). The heat from thermal disinfection helps to inactivate microbial enzymes and kill microorganisms. The benefits to reducing both pathogenic and spoilage microbial loads, aside from the prevention of disease, is that pasteurized foods take longer to spoil and because of this have extended shelf lives. Refrigeration of pasteurized foods can further extend shelf life by delaying the growth of any surviving microorganisms.

Although effective to an extent, pasteurization does not completely sterilize food. Sterilization can be defined as methods that kill all microbial cells (Montville et al. 2012). In the food industry, the goal of sterilization is to pathogens free foods, while concurrently improving shelf stability. Commercial sterilization is not concerned with absolute sterility. Products may still contain viable spores; however, if these cannot germinate (due to low pH of the food or through decreased water availability) the food product is still considered commercially sterile (Jay et al. 2005). For example, C. botulinum spores may be present in high-acid food, but they cannot germinate and proliferate; therefore, they are not considered as a hazard. Commercial sterilization is achieved using small pressure cookers or large retorts, commonly used in the canning industry.

Foods can be processed using heat both before and after packaging, the most commonly, after packaging. This is done in the process of canning (the process of food sterilization in hermetically (airtight) sealed containers). Canning was first invented by Nicolas Appert in the early 1800s as a response to a competition set by Napoleon Bonaparte for the development of a food preservation method that could endure the extended periods of time his troops were deployed (Madigan et al. 2012). Canned foods have since remained a staple of soldiers around the world with C-rations in World War II and as MREs (meals ready to eat) today, for the soldiers in Afghanistan and Iraq.
Packaging may also be disinfected by thermal processes before the addition of food, known as “aseptic packaging”. This technique is widely used in the preparation of foods such as fruit juices, dairy products, sauces, and some soups whose quality may suffer from extended periods of heating. The heating method used here is known as high-temperature, short-time (HTST), which consists of rapid heating to a temperature of 140°C, holding the packaging or food at this temperature for several seconds, followed by rapid cooling. HTST is renowned for maintaining food quality while extending shelf life, and also preserves nutrients and sensory attributes better than other conventional processing methods that may subject food to lower temperature for longer periods. Juice boxes and a variety of condiments are made using HTST technology (Jay et al. 2005).

Two types of heat (i.e., wet and dry), are commonly used in thermal disinfection and both kill microorganisms through different mechanisms. Wet heat kills microorganisms by denaturing their proteins, enzymes, and nucleic acids, and is more lethal than dry heat as it is more effective against spores (Montville et al. 2012). Dry heat kills microorganisms slowly through dehydation and oxidization, and also requires higher temperatures and extended durations to cause the same lethal effects as wet heat (Montville et al. 2012).

4.2.4 Non-thermal disinfection

Non-thermal physical disinfection methods are newer than thermal disinfection methods (Pereira and Vicente 2010). While thermal disinfection methods are effective at killing both pathogenic and spoilage microbes, they often lead to reductions in food flavor, color, quality, and texture (especially with solid foods). Food quality is important to consumers and any changes in the sensory characteristics or organoleptic profile of foods is considered negative. Commercial food consistency is the basis for brand loyalty and repeat purchases by consumers. Non-thermal disinfection processes therefore are advantageous as they do not alter the color, nutrient content, and texture of foods being processed. Non-thermal processes cause less damage to the food, have shorter processing times, and help to retain “fresh” characteristics; all great strengths of non-thermal processing. Most of these processes can be carried out at room temperatures or at cooler conditions and can require less energy than thermal processes while achieving microbial inactivation levels similar to pasteurization. Several types of non-thermal methods exist, including high-pressure processing, UV light, electromagnetic radiation, and ultrasound. Before continuing, it should be noted that some non-thermal processes are not well received by the public, mainly due to lack of knowledge and understanding of the processes’ fundamental working principles.

4.2.5 High-pressure processing

High-pressure processing (HPP), one of the most important food processing procedures of the last 50 years, is considered the starting point of the developmental period leading to the rapid growth in non-thermal processing techniques (Pereira
and Vicente 2010). HPP may also be referred to as high-hydrostatic-pressure processing or ultrahigh-pressure processing (Montville et al. 2012). In HPP, foods are packaged in airtight vacuum-sealed pouches (laminate material), which are subsequently subjected to high pressures up to 700 MPa. HPP treated foods require fewer additives and do not undergo flavor changes, since the process does not induce chemical changes, which can occur in thermal processes (Pereira and Vicente 2010). Pressure is conducted through water, and for the best possible results as much air as possible should be removed from pouches. Also, foods with high moisture contents survive HPP better. For example, “airy” foods, such as marshmallows, tend to become compressed and show macromolecular changes.

HPP kills microorganisms by applying pressures of 300–700 MPa, considered the inactivation range for viruses and vegetative cells (Pereira and Vicente 2010; Montville et al. 2012). Under these pressures, physical damage is done to cells with changes occurring in protein structure and large-scale cell lysing (Pereira and Vicente 2010). HPP is commonly used to sterilize pre-packaged sauces and ready-to-eat (RTE) deli meats, and protects deli meats from L. monocytogenes and norovirus (Montville et al. 2012). Different microorganisms are inactivated at different rates. It has been shown that increasing pressures using HPP leads to increased microbial death; however, increasing pressure duration has not been shown to correlate with enhanced inactivation (Pereira and Vicente 2010). HPP can be combined with heat in a process known as pressure-assisted thermal sterilization, which enhances spore inactivation (Pereira and Vicente 2010).

4.2.6 Ultraviolet light

UV light has been used in the water sanitation industry as a final stage method for disinfecting water, and has also been applied to the food industry as another non-thermal disinfection method. Specifically, UV light at 254 nm (known as UV-C-type light, range = 100–280 nm) is used for disinfection (Fraise et al. 2013). UV light can be used to sterilize both liquid and solid food items and is fairly easy to use. It is also beneficial as it leaves no chemical residues on or in food (Fraise et al. 2013).

UV light sterilization is limited by penetration, which is limited with light intensity. Although UV light can easily penetrate water, penetration is limited when used on opaque solutions such as fruit juices. As such, UV light is best for the surface disinfection of solid foods or the sterilization of clear liquids (Montville et al. 2012). To circumvent the limited penetration capabilities, liquids may be flowed in thin streams under UV light to achieve sterilization, which is done with many fruit juices to achieve HACCP standards of 5-log reductions in E. coli O157:H7 (Montville et al. 2012). UV light has become a popular method for sterilizing unpasteurized apple ciders as it is able to retain the desired “fresh” pressed/squeezed flavor (Fraise et al. 2013).

In order for the successful killing or inactivation of microorganisms, a reliable lamp with good intensity, set at the proper wavelength is required. UV light is able to inactivate microorganisms through photophysical and photochemical processes
(Fraise et al. 2013). UV light affects nucleic acids within cells, specifically pyrimidine base pairs (thymine and cytosine in DNA, cytosine and uracil in RNA) (Madigan et al. 2012). These base pairs can absorb UV light due to aromatic ring(s) in their structures, leading to the generation of thymine dimers within the DNA that act as a physical roadblock to DNA polymerase III (DNA pol III), one of the crucial proteins involved in DNA replication. Upon initial contact with a thymine dimer, DNA pol III falls off the template DNA strand and is unable to move past this section of nucleotides (Madigan et al. 2012). This leads to incomplete DNA synthesis during cell division and the eventual death of the cell.

While thymine dimers are the main photoprodut of UV radiation, other products can be generated. These include cyclobutyl pyrimidine dimers, DNA-protein crosslinks, and single-strand breaks (Madigan et al. 2012). These products are also toxic to the cell and rapidly result in cell inactivation and death. The accumulation and abundance of these byproducts varies with the wavelength of UV light used and the specific DNA sequence (as some are more prone to mutations than others, such as AT rich sequences). If longer UV wavelengths are used (330–480 nm (UV-A)) this may lead to the activation of DNA photolyase (Fraise et al. 2013; Madigan et al. 2012; Montville et al. 2012). This is an enzyme found in almost all organisms (bacteria, fungi, protozoans, plants, mammals, etc.) that belongs to the flavoprotein group, and acts as an initial DNA repair mechanism. Specifically, DNA photolyase works by breaking thymine dimers caused by UV damage, which are later handled by other DNA repair mechanisms. Its activation under UV-A type light may lead to undesired microbial re-activation (Montville et al. 2012). It should be noted that DNA repair mechanisms efficiency can vary greatly between microorganisms and that DNA photolyase is not always used to repair DNA damage from UV light (i.e., SOS mechanism) (FDA 2013; Montville et al. 2012).

Pulsed UV light is another viable option for microbial disinfection. With pulsed UV light, high energy UV light (20,000 times more energy than sunlight) is pulsed at a rate of 1–20 pulses per second with the width between pulses ranging from 300 nanoseconds to 1 milliseconds (Jun and Irudayaraj 2009). The increased energy associated with pulsed UV light makes it more effective than continuous UV light as a food and surface disinfectant (4–6 times more effective than continuous UV light at microbial inactivation (Jun and Irudayaraj 2009).

4.2.7 Electromagnetic radiation

Electromagnetic radiation in the form of oscillating magnetic fields (OMFs) is a novel processing method for microbial inactivation in the food industry. As with other non-thermal methods, this process is beneficial as it is able to preserve food quality, while destroying microorganisms with short treatment times and no significant temperature increase (FDA 2013). A magnetic field is a type of force field that surrounds electrical current circuits as a result of the movement of electrical charges. In this case, food is sent through OMFs in either its natural or packaged
Overall, we have examined some of the traditional methods for microbial inactivation and biofilm prevention, including chemical and physical techniques. Chemical methods are by far among the most common and cost-effective methods for biofilm prevention. Among these, chlorine-based chemicals are most commonly used due to their ease of formulation and application. Higher variability exists with chemical disinfectants as their efficacy can be highly affected by factors such as duration of exposure, mechanical force (method of application), and concentration. Physical methods provide longer lasting solutions to both microbial inactivation and biofilm prevention on processing surfaces and in foods. These can be divided into thermal and non-thermal processes, each with their own benefits and drawbacks. Public perception plays a very important role in the acceptance and widespread implementation of any of these disinfection methods. To be effective, disinfecting methods must inactivate or prevent microbial growth and at the same time not hinder the quality and organoleptic profile of the food or beverage.

4.3 BIO-NANOTEchnology APPROACHES TO BIOFILm PREVENTION

Until now we have introduced the basics of nanotechnology. We briefly covered its history while quickly mentioning its use as a relatively new technology in the food industry. We then discussed some good and bad microbes present in foods, the basics of biofilm formation, and traditional methods of microbial disinfection and biofilm removal. Next, we will delve into the world of nanotechnology and discuss its applications in the food industry. We will do this by examining what is considered to be its most extensive and widely used application for biofilm prevention: the alteration of food contact surfaces by physical and chemical enhancements (Table 4.1).

4.3.1 Food contact surface alterations for biofilm prevention

The development and progress of nanobiotechnology and nanofabrication techniques for the design and fabrication of novel antibacterial (and antifungil) surfaces as a component of biomaterial research remains a high priority in the scientific and engineering communities (Kuan et al. 2012). Because microorganisms are among the oldest life-forms on earth, having survived billions of years of existence, they have developed many versatile and adaptive mechanisms for the colonization of surfaces. Colonization of certain surfaces (such as those in the food industry) is known to affect the function of specific interfaces (i.e., cutting blades, piping, grinders, etc.). In order to substantially reduce or eliminate bacterial attachment (the first step in biofilm formation), efforts are being focused, quite intensively, on the fabrication of new surfaces and the improvement of existing antibacterial surfaces (Chaudhry et al. 2008; Engel et al. 2012; Hasan et al. 2013; Llorens et al. 2012). This can be done, for example, by applying surface coatings, or by modifying the surface architecture.
<table>
<thead>
<tr>
<th>Chemical/material</th>
<th>Bacterial strains affected and mode of action</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver compounds</td>
<td>Gram +ve and Gram −ve bacteria; silver ions readily interact with negatively charged oxygen, nitrogen, and sulfur atoms in carboxyl, amino and thiol functional groups, respectively, in proteins and DNA. Silver ions also inactivate cellular respiratory cycles and TCA (tricarboxylic acid) cycle enzymes. They also produce hydroxyl radicals, which cause further damage through disruption of intracellular pH</td>
<td>Araujo et al. 2012; Dogan et al. 2009; Egger et al. 2009; Dror-Ehre et al. 2010; Lee et al. 2011; Fonseca de Faria et al. 2013; Martínez-Abad et al. 2012a, b; Martínez-Gutierrez et al. 2013; Naik and Kowshik 2013; Saulou et al. 2012; Tran et al. 2013; Vasiliev et al. 2010; Zampino et al. 2011</td>
</tr>
<tr>
<td>Gold, ZnO, and TiO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Gram +ve and Gram −ve bacteria; No antimicrobial properties in the dark. However, become antimicrobial under light</td>
<td>Euwananont et al. 2008; Gamage and Zhang 2010; Gladis et al. 2010; Perni et al. 2011; Sokmen et al. 2011; Yaghoubi et al. 2010</td>
</tr>
<tr>
<td>AMPs</td>
<td>Gram +ve and Gram −ve bacteria; Cationic character allows them to disrupt microbial membranes leading to disruption of electrochemical gradients and protein activity</td>
<td>Fantner et al. 2010; Glinel et al. 2012; Hasan et al. 2013; Nguyen et al. 2011; Onaizi and Leong 2011; Vreuls et al. 2010a, b</td>
</tr>
<tr>
<td>N,N-dimethyl-2-morpholinone</td>
<td>Gram +ve and Gram −ve bacteria; Acts as an antibiofouling agent under wet conditions and a bactericidal agent under dry conditions through hydroxylation of its CB ring</td>
<td>Cao et al. 2012</td>
</tr>
<tr>
<td>QACs [e.g., poly(vinyl-N-hexyl/pyridinium)</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Lin et al. 2003; Siedenbiedel and Tiller 2012; Tiller et al. 2001</td>
</tr>
<tr>
<td>Poly(methacrylate)</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Siedenbiedel and Tiller 2012</td>
</tr>
<tr>
<td>Poly(hexamethylene biguanidinium hydrochloride)</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Siedenbiedel and Tiller 2012</td>
</tr>
<tr>
<td>Polynorbornenes</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Munoz-Bonilla and Fernandez-Garcia 2012; Siedenbiedel and Tiller 2012; Timofeeva and Kleshcheva 2011</td>
</tr>
<tr>
<td>Poly(phenylene ethylene)</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Munoz-Bonilla and Fernandez-Garcia 2012; Siedenbiedel and Tiller 2012; Timofeeva and Kleshcheva 2011</td>
</tr>
<tr>
<td>Carbon nanotubes</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Tiraferri et al. 2011</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>Gram +ve and Gram −ve bacteria; destabilize microbial membranes through surface charge disruption</td>
<td>Shah et al. 2012</td>
</tr>
</tbody>
</table>
In this section, we will attempt to review a variety of surface modification techniques and surface fabrication techniques commonly used in the design of antibacterial surfaces. Antimicrobial surfaces can be further subdivided into antifouling or bactericidal (Hasan et al. 2013). Categorization depends on the effects surfaces have on biological systems with which they come into contact. Surfaces can be further characterized by the type of surface coating or chemical modifications to which they have been subjected including surface functionalization, polymerization, derivatization, or physical modification (change in the surface topography) (Hasan et al. 2013). Natural as well as man-made options are available for surface modification at the nanoscale. Recently, a new generation of antibacterial surfaces that mimic the surface topography of natural items, such as the wing structure of the cicada (*Psaltoda claripennis*) and the leaf structure of the taro plant (*Colocasia esculenta*) have been developed and these will be discussed shortly.

Antibacterial surfaces, classified as antifouling or bactericidal, may work by repelling or resisting initial bacterial attachment to a surface by exhibiting antifouling effects, or by inactivating cells and causing cell death (bactericidal). Antifouling surfaces may cause microbial death, but can resist or prevent microbial attachment through the generation of unfavorable surface chemistry or topography with respect to the microorganism (Barish and Goddard 2013; Lyon et al. 2008; Yang et al. 2011). Bactericidal surfaces, on the other hand, work by disrupting cells upon contact, leading to cell death (Glinel et al. 2012; Hasan et al. 2013). In some cases, antibacterial surfaces may show both antifouling and bactericidal properties. An example of this is, surfaces coated with polymers of cationic N, N-dimethyl-2-morpholinone (CB ring, Figure 4.3) (Cao et al. 2012). This polymer has the ability of inactivating bacteria in dry environments (bactericidal) and preventing bacterial attachment in wet environments (through the zwitterionic carboxybetaine ring, which becomes hydroxylated under moist conditions, CB-OH) (Cao et al. 2012). This hydrolysis step is reversible for N, N-dimethyl-2-morpholinone, making it a valuable antimicrobial polymer surface coating.

### 4.3.2 Naturally inspired antibacterial surfaces

Nature provides a nearly inexhaustible source of inspiration for engineers and scientists in the development of new materials through biomimicry. As with microbes, natural surfaces have been developed by nature over millions of years of evolution, with some surfaces having evolved to prevent or resist bacterial attachment and colonization (biofilm development). Many of these surfaces have multiple functions with plant leaves, gecko feet, insect wings, and spider silk being the most well studied examples (Liu and Jiang 2011). These may not all have relevance in the food industry, but some do. Surfaces that have characteristics of low-adhesion, superhydrophobicity, and self-cleaning are excellent candidates as antibacterial surfaces. Indeed, natural surfaces and biomimicked surfaces such as shark skin, insect wings, and lotus leaves, exhibit antimicrobial properties, preventing algal spores and bacterial cells from attaching to their
surface (Chung et al. 2007; Fadeeva et al. 2011). The wings of the cicada have shown to be a great inspiration as a natural bactericidal agent especially against *P. aeruginosa* cells (Figure 4.4) (Ivanova et al. 2012; Pogodin et al. 2013). Bactericidal effects are exclusively due to the surface nanostructure rather than surface chemical effects. It has been shown that coating these wings with 10 nm of gold improves chemical bactericidal action without affecting the physical bactericidal activity of the surface (Ivanova et al. 2012). Development of surfaces with the cicada wing surface nanotopography structure shows that *P. aeruginosa* cells are able to attach (making it a non-antibiofouling surface) and are mechanically ruptured shortly afterwards (Pogodin et al. 2013). The efficacy of this surface depends on the adsorption behavior of the bacterial outer membrane (Ivanova et al. 2012; Pogodin et al. 2013). As cells attach to the surface they absorb onto nanopillar structures, which causes the cell membrane to stretch, becoming distorted. As *P. aeruginosa* cells absorb further onto the surface, the stretching of the bacterial outer membrane reaches its limits, eventually rupturing, causing cell lysis (Ivanova et al. 2012; Pogodin et al. 2013).

The coating of surfaces with antimicrobial peptides (AMPs) is another nanotechnology designed approach for creating antibacterial surfaces, which prevents biofilm development (Glinel et al. 2012; Nguyen et al. 2011; Vreuls et al. 2010a, b). CM15 is
Figure 4.4 Cicada wing nanopillar arrangement and structure and its effect on Pseudomonas aeruginosa. (a) Cicada (Psaltoda claripennis). (b) Scanning micrograph of nanopillars on cicada wing (200 nm height, 70 nm diameter, 170 nm spacing). (c) Interaction of P. aeruginosa on the wing surface. (d) Cell viability test using propidium iodide (colors of non-viable cells are red). (e) Atomic force microscope image (5 x 5 μm) showing bacterial cells affected by nanopillars. (f) P. aeruginosa cell affected by nanopillar surface even after gold sputtering (10 nm thick). (g) Representation of cellular attachment onto the cicada wing nanopillars. (h) Illustration of the apparent rupture of the cell wall in the region suspended between the nanopillars. Hasan et al. (2013). Reproduced with permission of Elsevier.
a well-known synthetic peptide used in AMP coatings because of its antimicrobial properties (Fantner et al. 2010). This peptide mimics cecropin A, a naturally occurring antimicrobial peptide found in the hemolymph of the moth it is named after (Hyalophora cecropia) (Fantner et al. 2010). This AMP causes bacterial cell lysis by inhibiting proline uptake (leading to leaky membranes) (Fantner et al. 2010; Nyguen et al. 2011). Aside from their application as surface coatings, AMPs are being considered as new antibiotic compounds in the medical industry as there is less microbial resistance built-up towards them (Onaizi and Leong 2011). AMPs are effective at low concentrations and as they are relatively new, most of the microorganisms are not resistant (Onaizi and Leong 2011). AMPs are cationic, making the initial interaction with the bacterial outer membrane electrostatic in nature (Hasan et al. 2013). As such, AMPs interact strongly with negatively charged microbial membrane. Few complexities exist in implementing AMPs as surface coatings for prevention of microbial attachment as they have low minimum inhibitory concentrations and are released at low rates from surfaces, which make them durable and tough (Hasan et al. 2013).

4.3.3 Artificial antibacterial surfaces

Artificial antibacterial surfaces are generated from both traditional and advanced surface modification techniques, compromising a range of polymer and nanoparticle based surfaces. These include surfaces that exhibit antifouling properties, bactericidal properties, or both. Silver-based compounds are the most popular and the most researched (Araujo et al. 2012; Dogan et al. 2009; Dror-Ehre et al. 2010; Egger et al. 2009; Fonseca de Faria et al. 2013; Gutierrez et al. 2013; Lee et al. 2011; Martinez-Abad et al. 2012a, b; Martinez-Naik and Kowshik 2013; Saulou et al. 2012; Tran et al. 2013; Vasilev et al. 2010; Zampino et al. 2011). Bactericidal surfaces therefore are mainly comprised of silver-doped, silver-coated, silver-containing polymers, silver nanoparticles, or thin silver films. Gold, titanium dioxide (TiO₂), and ZnO have also become popular bactericidal compounds due to their photodynamic capabilities, which prevent biofilm growth through rapid heating (after exposure to UV or laser light) (Euvananont et al. 2008; Gamage and Zhang 2010; Gladis et al. 2010; Perni et al. 2011; Sokmen et al. 2011; Yaghoubi et al. 2010). The benefit of photodynamic therapies is that they efficiently inactivate microorganisms without selecting for mutant resistant strains (Gamage and Zhang 2010; Perni et al. 2011). Other antibacterial compounds include nitric oxides and metal oxides such as magnesium and zinc oxides (Hasan et al. 2013).

The bactericidal nature of many nanoparticles arises from their electrostatic characteristics and strong oxidizing powers. Many of these compounds are able to generate reactive oxygen species upon interaction with bacteria. Nanoparticles released from the surface are found to cause damage to the outer cell membrane, inactivating enzymes and nucleic acids (Hasan et al. 2013). It is known that silver ions readily interact with negatively charged oxygen, nitrogen, and sulfur atoms in carboxyl, amino and thiol functional groups, respectively, in proteins and DNA (Knetsch and Kool 2011; Schierholz et al. 1998). Silver ions also inactivate cellular
respiratory cycles and TCA (tricarboxylic acid) cycle enzymes (Gordon et al. 2010). They also produce hydroxyl radicals, which cause further damage through disruption of intracellular pH. The minimum concentration of silver required for bactericidal effects is 0.1 ppb, whereas in eukaryotic cells, cytotoxicity is observed at 10 ppm (Kumar and Munstedt 2005; Schierholz et al. 1998). Therefore, it is possible to carefully design compounds that are only toxic to bacteria. Nanoparticles have been reported to be effective against a wide variety of Gram-positive and Gram-negative organisms including *P. aeruginosa*, *S. epidermidis*, *Staph. aureus*, *E. coli*, and *Klebsiella pneumonia* (Fullenkamp et al. 2012; Kelly et al. 2009).

### 4.3.4 Surface modifications

Many surface modification techniques exist for the fabrication of antibacterial surfaces to prevent biofilm formation. Surfaces can undergo either chemical or physical treatments with surface functionalization, derivatization, and polymerization involving chemical modifications, and surface re-structuring involving physicochemical modifications (Bazaka et al. 2011a, b; Fadeeva et al. 2011; Hochbaum and Aizenberg 2010; Munoz-Bonilla and Fernandez-Garcia 2012; Tiller et al. 2001; Tiraferri et al. 2011).

Surface polymerization involves surfaces that have been modified through the polymerization of antimicrobial agents onto the surface and can occur through different means (i.e., covalent bonding or radical transfer) (Tiller et al. 2001; Lee et al. 2004; Yang et al. 2011). Surfaces containing coatings of polycations of quaternary ammonium salts have been shown to possess strong bactericidal properties (Tiller et al. 2001). Additionally, surfaces of polyethylene, polypropylene, glass, and poly(ethylene terephthalate), coated with covalently attached poly(vinyl-N-hexylpyridinium) (a hydrophobic quaternary ammonium salt) have demonstrated antimicrobial effects (Lin et al. 2003; Tiller et al. 2001; Siedenburg and Tiller 2012). Application of quaternary ammonium groups is commonly carried out through atom transfer radical polymerization (ATRP) (Lee et al. 2004). This method is highly controllable, modifiable, and has shown to provide permanent antimicrobial effects to surfaces. The quaternary ammonium salt coatings exhibit low rates of leaching and are very durable. The commercial applications of this polymerization manufacturing method is still in development stage and requires more investigation and research before it can be applied on a larger scale.

Other surface modification techniques include antimicrobial agent immobilization via physicochemical adsorption. Antibacterial agents comprise various antibacterial polymers, enzymes, and peptides, with each exhibiting different types of antibacterial effects (Munoz-Bonilla and Fernandez-Garcia 2012; Nguyen et al. 2011; Siedenburg and Tiller 2012). Polymer molecules can include poly(methacrylate) or poly(hexamethylene biguanidinium hydrochloride) to name a few (Siedenburg and Tiller 2012). Polycationic polymers are also known to adversely affect bacteria through surface charge disruption, leading to cell lysis and death. Antimicrobial
activities. Problems, however, exist in silver ions leaching from surfaces, the effects of which will be discussed towards the end of this chapter. Hydroxyapatite (HA) coatings provide surfaces, such as titanium, with antimicrobial properties (Shah et al. 2012). HA was never heavily implemented in the food industry due to its mechanical weakness and its application problems (difficult to apply uniformly in density and thickness) (Lazarinis et al. 2011). HA compounds have also been shown to perform poorly in long-term stability trials (Lazarinis et al. 2011). QACs, unlike silver-ions, which are gradually released from surfaces, possess longer-lasting antibacterial capabilities and work through a contact-based mechanism (Murata et al. 2007; Van Houdt and Michiels 2010). Despite this advantage over silver coatings, increased microbial resistance to QACs has been observed (Takenaka et al. 2007).

Combining two or more antibacterial surface coating agents can be done to overcome individual disadvantages and garner synergistic benefits. For example, the combination of silver and QACs has been shown to have better antimicrobial abilities than either compound used individually (Li et al. 2006). The slow release of silver ions combined with the contact-killing abilities of QACs makes for a very effective antibacterial surface coating (Li et al. 2006). Similar combinations have also been manufactured and include silver-doped silica films, titanium-doped iron, silver-doped inorganic/organic hybrids, silver-doped phenyltriethoxysilane, silver-doped titanium, and silver-doped HA coatings (Marini et al. 2007; Yin and Wang 2011). It should be noted that aside from microbial resistance, surface coatings can suffer from disadvantages such as application issues (not well arranged or controllable on the nanoscale level in some cases), leaching, and long-term durability (Madkour and Tew 2008; Wahlig and Dingeldein 1980; Warnes and Keevil 2011).

4.3.6 Physical surface modifications (topography alterations)

Micro- and nano-scopic surface topographical features in food-processing contact surfaces play a crucial role in controlling bacterial attachment and biofilm development (Mitik-Dineva et al. 2008; Whitehead and Verran 2006). Studies in this area of nanotechnology, examining the effects of surface roughness (Ra) and porosity are few, with more research needed (Anselme et al. 2010; Bazaka et al. 2011a; Chung et al. 2007; Truong et al. 2010; Whitehead et al. 2005). Currently, it has been observed that rod bacteria (i.e., P. aeruginosa) are able to attach to surfaces with Ra values close to their longitudinal dimensions (Figure 4.5), while cocci bacteria (i.e., Staph. aureus) can still readily attach to surfaces with Ra values on the order of a few nanometers to subnanometers (Whitehead and Verran 2006). It was previously believed that microbes would not be able to attach to nanoscopically smooth surfaces (Truong et al. 2010). This was shown to be incorrect; however, microbial attachment is significantly decreased compared to surfaces with known Ra values (Truong et al. 2010).
4.3.7 Biocompatibility of antibiofouling and bactericidal surfaces

A variety of QACs (e.g., poly(4-vinylpyridine)) are known to be cytotoxic to mammalian cells (Buffet-Bataillon et al. 2012; Li et al. 1998). However, when combined with biocompatible monomers such as hydroxyethyl methacrylate and polyethylene glycol methyl ether methacrylate through the process of copolymerization, poly(4-vinylpyridine) has been shown to be biocompatible with red blood cells, while still maintaining its antimicrobial effect (Stratton et al. 2009; Timofeeva and Kleshcheva 2011). Further research is required to examine the effects of this compound on different cell lines. The cytotoxic implications of nanoparticles and AMPs have also been investigated. At low concentrations, silver ions and TiO₂ have been shown to be non-toxic; however, at higher concentrations they can cause cell death and affect cell membrane structure, mitochondrial function, and alter genetic material (Ahamed et al. 2010; Hajipour et al. 2012). Thus, the controlled release of nanoparticles is critical. AMPs, even in high concentrations, have not been shown to cause any cell cytotoxicity when tested against blood cells and human osteosarcoma (Gao et al. 2011). AMPs do not activate platelets or complement proteins in humans and therefore no immune response is activated (Gao et al. 2011). The health implications of various nanoparticles and bioactive compounds will be discussed in more detail towards the end of this chapter.

4.4 NANOBIOSENSORS FOR THE DETECTION OF FOOD ANALYTES AND PATHOGENIC MICROORGANISMS

Fresh fruits, vegetables, and meats that have become spoiled exhibit characteristic odors, colors, and unpalatable flavors that are easily discernible. However, packaged foods may not immediately reveal these characteristics and consumers must therefore rely on best-before and sell-by dates. These are determined by food producers, where dates of expiry are based on pre-determined and idealized assumptions on
how the food is stored in-house and during transportation. Nanoscale particles, due to their unique chemical, redox, electrical, and optical properties, can provide solutions to more accurately determine if food has become spoiled or reached unacceptable levels of microbial contamination (Duncan 2011). Nanomaterials can thus be designed to detect the presence of characteristic gases, aromas, contaminants, and pathogens in foods that have spoiled. The ability to accurately detect a variety of chemical and physical attributes associated with microbial contamination and spoilage is not only useful in quality control, but is also useful in improving food safety and reducing food-borne illness. It also ensures that consumers know that the foods they are purchasing are at their peak freshness or ripeness. Companies such as Ripesense (www.ripesense.com) are already marketing nanotechnology products in order to help customers in determining if foods are fit to eat. Most chemical tests involving nanotechnology for the detection of food analytes in quality control are still in their early stages of research and development.

4.4.1 Detection of organic molecules associated with food microbial-spoilage/biofilm contamination

Aside from the potential benefits afforded to consumers and food manufacturers/ producers, the incorporation of nanotechnology in the development of biosensors has the potential to revolutionize the speed and accuracy of contaminants and adulterant detections in foods compared to previous and currently used methods recommended by regulatory agencies. Many nanobiosensor assays are based on color changes that occur in metal nanoparticle solutions (Duncan 2011). An example of this involves the use of cyanuric acid functionalized gold nanoparticles for the detection of melamine (Ai et al. 2009). The cyanuric acid group selectively binds melamine (an unwanted toxic contaminant commonly found as an adulterant in pet foods and infant nutrient formulas) (Ai et al. 2009). The aggregation of any melamine by cyanuric acid gold nanoparticles causes a color change from red to blue depending on melamine (analyte) concentration. This method can accurately detect melamine concentrations as low as 2.5 ppb in milk and pet foods, and is visible to the naked eye (Ai et al. 2009). Another similar approach uses gold nanoparticles and chemical reductants (that bind strongly to melamine, and reduce the gold nanoparticles only when bound to melamine) added to the food solution in sequential order (Cao et al. 2010). If melamine is present in the food sample, it will readily bind to the reductant, which will, in turn, reduce the gold nanoparticles (added secondly) leading to a color shift from red to blue in the sample, indicating the presence of melamine (Cao et al. 2010). The detection of melamine in milk has also been conducted using a combination of crown-ether-modified thiols and gold nanoparticles (Kuang et al. 2011). This colorimetric detection method can detect melamine in solution at 6 ppb (Kuang et al. 2011).

Other nanobiosensor assays for organic molecule contaminants involve fluorescence instead of color changes. A sensor based assay, which uses an enhanced
fluorescence linked immuno-sorbent assay (EFLISA) as a detection methodology, can be used in the detection of the food protein gliadin, the primary food protein that can lead to inflammation in patients with celiac disease (Staiano et al. 2009). This detection system utilizes silver metal-enhanced fluorescence from rhodamine-labeled anti-gliadin antibodies. Gliadin bound antibodies are able to fluoresce after coming in contact with silver island films. This technique could be easily utilized and adapted to analyze the gluten content in gluten-free labeled foods, or for the detection of other protein-based contaminants (Staiano et al. 2009). Other fluorescence-based methods that have been developed include one for cyanide detection in drinking water at concentrations as low as 2 nM through the fluorescent quenching of gold nanoclusters, and a water pesticide contamination detection technique involving nanoscale liposome-based detectors (Liu et al. 2010; Vamvakaki and Chaniotakis 2007). Other bacterial toxins, such as botulinum toxin (serotype A), have been detected at picomolar (pM) concentrations using antibody-labeled luminescent quantum dots (Goldman et al. 2004; Warner et al. 2009). This could find useful applications in food safety in the prevention of bioterrorist attacks. Other easy-to-read methods (as described with melamine) using metal nanoparticles for the detection of small molecules, metal ions, and proteins could be devised for the detection of other food contaminants, allergens, and adulterants (Chen et al. 2008a; Hong et al. 2009; Li et al. 2009; Liu and Lu 2004a, b; Wang et al. 2008a, b; Wu et al. 2008; Xue et al. 2008; Zhang et al. 2008).

Detection of pathogenic biomarkers using electrochemical methods is also popular in the field of nanobiosensor development, in regards to the food industry. Compared to the colorimetric or fluorimetric based optical techniques, electrochemical techniques may be of more use as they are not affected by light scattering or absorption that may occur from other food components (Duncan 2011). Most electrochemical sensors function by monitoring changes in material conductivity from target analyte binding (e.g., analytes may bind to specific antibodies attached to carbon nanotubes on a surface) (Duncan 2011). Microcystin-LR (MCLR) is a toxin produced by cyanobacteria that can be found contaminating water sources (which can lead to food contamination through washing). Anti-MCLR antibody coated carbon nanotube surfaces can be used to detect MCLR at concentrations of 0.6 nM (Wang et al. 2009a). This high resolution and sensitivity of the sensor meets World Health Organization (WHO) guidelines and helps to improve sampling time compared to standard enzyme-linked immunoabsorbent assays (ELISA) for MCLR detection (Wang et al. 2009a). Gold nanoparticles and glucose-sensitive enzymes can also be used to detect glucose concentrations in beverages (Ozdemir et al. 2010). A reusable piezoelectric gold nanoparticle immunosensor has recently been developed to detect the presence of aflatoxin in milk samples at concentrations of 0.1 ng/ml (Jin et al. 2009). Other electrochemical nanosensors include a cerium oxide chitosan nanocomposite for the detection of the fungal contaminant, ochratoxin-A; silicon nanowire transistors for the detection of Staphylococcal enterotoxin-B; and carbon nanotubes for the detection of cholera-toxin and food
colorant (Sudan I) in sauces (Kaushik et al. 2009; Mishra et al. 2008; Morris 2011; Viswanathan et al. 2006). In the last example, the presence of specific food colorants leads to changes in the oxidation peaks of carbon nanotubes, which can be detected and quantified (Mo et al. 2010; Zhang et al. 2010).

4.4.2 Nanosensors for the detection of microbes

Most biological detection methods are based on immunological assays (e.g., ELISA), which can be expensive and time consuming (Duncan 2011). Nanomaterial sensors (Table 4.2) use strategies similar to the conventional assays in terms of selective antibody-antigen interactions. The unique optical and electrical properties of nanomaterials make the nanosensors significant in terms of the speed, selectivity, and sensitivity. Because food is chemically complex, problems can arise in light scattering and sample opacity. Therefore, there are only a few light-based methods for microbial detection. One such approach involved the use of two-photon Rayleigh scattering in combination with antibody-conjugated gold nanoparticles for the selective detection of E. coli (Singh et al. 2009). It is important that methods developed are sufficiently able to separate target microbial pathogens from foods themselves in order to minimize signal-to-noise ratios. A detection method, known as immunomagnetic separation (IMS), works in this manner (Fluit et al. 1993). In IMS, magnetically conjugated antibodies (e.g., Fe₃O₄ conjugated E. coli or L. monocytogenes selective antibody) can be used to separate pathogens from food matrices (possibly from a biofilm in the future) prior to detection (Varshney et al. 2005; Yang et al. 2007). The high surface-to-volume ratios of these magnetic nanoparticles allows for high microbial capturing/coating efficiency (Duncan 2011). Microbes can then be separated from foods through the application of magnetic fields to the substrate and detected and characterized using real-time PCR (rt-PCR) analysis (Yang et al. 2007). The microbial concentration of Mycobacterium avium spp. paratuberculosis in spoiled milk has been determined using magnetic nanomaterials (Kaittanis et al. 2007). This was done by observing the effects of magnetic particle agglomeration on the spin-spin relaxation times of nearby water protons (Kaittanis et al. 2007). One group of researchers used sugar molecules attached to iron oxide nanoparticles for the capture of E. coli, instead of conventional antibiotics (which are relatively expensive), and were able to recover 88% of E. coli in the sample (El-Boubbou et al. 2007). E. coli was subsequently detected using fluorescence staining (El-Boubbou et al. 2007). Other groups have used magnetic particles in combination with gold nanorods (Wang and Irudayaraj 2008, 2010). The magnetic particles facilitate separation while the gold nanorods are used for microbial detection under near-infrared light. Gold nanorods have length-dependent absorptive properties as well as efficient light-to-heat conversion (Wang and Irudayaraj 2008, 2010).

Nanomaterials can be useful in microbial detection even after IMS. As an example of this, it would be the separation of E. coli using IMS and then its detection using
materials, making them very attractive. As such, polymers are the most frequently used packaging in the food industry and include a variety of polyolefin compounds (e.g., polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS), etc.). There still exist major drawbacks with plastics, most notably high gas permeability (Yam 2009). Other bio-derived plastics, such as polysaccharide (starch) based polymers, polyactic acid, polyhydroxyalkanoates, and polycaprolactones, have been developed due to their biodegradability. However, the strengths of many of these compounds are severely affected by moisture (Chiellini 2008). Also, these plastics have oxygen transmission rates (OTRs) that are only as good as or poorer than standard petroleum-derived plastics (Chiellini 2008).

To incorporate multiple desired properties into a single packaging material, multilayer films or polymer blends can be created. For example, in the application where a high oxygen barrier is required over a large range of humidifiers, ethylene-vinyl alcohol (PVA) sandwiched between two layers of polyethylene (hydrophobic polymer) has been shown to provide excellent barrier properties compared to any of the polymer monolayers on their own (Kollen and Gray 1991; Yam 2009; Zhang et al. 1998). More controllable properties can be obtained using polymer blends (Yam 2009). However, multilayer films or polymer blends are limited in their use due to higher production and material costs. The incorporation of additional additives and adhesives also complicates material regulation by federal agencies. Also, blends and multilayer films may have different, unknown, and difficult recycling requirements. This is the main reason why pure polymers are still used in packaging, with pushes being made to improve their mechanical and barrier properties. This is especially true with bio-based materials. However, pure polymers can only be modified so much before one must look towards blends, multilayer films, and composites to improve barrier and mechanical properties of packaging material. Polymer properties can be improved using nanotechnology to create smart antimicrobial packaging. The addition of materials such as clays, metals (silver, gold, zinc, titanium), and natural antimicrobials, in which one or more of the dimensions of the additive is <100 nm, can lead to improved polymer properties and antimicrobial activity (Table 4.3). Nanomaterial packaging materials are classified into three categories: improved, active, and intelligent packaging (Silvestre et al. 2011). In “improved” polymer nanopackaging, the addition of nanomaterial improves polymer properties including flexibility, gas barriers, and temperature and moisture stability. Nanoparticles in “active” polymer nanopackaging may not necessarily lead to improved polymer properties, but directly interact with the food and environment to improve food stability and shelf life through increased antimicrobial activity. Nanoparticles in “intelligent” polymer nanopackaging act as biosensors to monitor the condition of packaged product. This can be done through color changes in the packaging, which help to ensure food integrity. An example of a system using intelligent polymer nanopackaging involves the use of photoactivated ink indicators, utilizing TiO₂ nanoparticles and methylene blue dye to detect oxygen in vacuum packaged foods (Duncan 2011).
### Table 4.3 Nanomaterials for antimicrobial nanopackaging.

<table>
<thead>
<tr>
<th>Antimicrobial in nanopackaging</th>
<th>Bacterial strains affected and mode of action</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactic, malic, and acetic acids</td>
<td>L. monocytogenes, E. coli O157:H7; reduces environmental pH leading to destruction of cell osmoregulation and electrochemical gradients</td>
<td>Campos et al. 2011</td>
</tr>
<tr>
<td>Sorbates (e.g., potassium sorbate, sorbic acid)</td>
<td>L. monocytogenes, E. coli O157:H7, Zygosaccharomyces bailii, various yeasts and molds; destabilizes microbial membranes and inhibits microbial replication</td>
<td>Campos et al. 2011</td>
</tr>
<tr>
<td>Chitosan</td>
<td>L. monocytogenes, S. aureus, P. aeruginosa, E. coli, Bacillus cereus, Aspergillus niger; interacts with negatively charged microbial membranes, increases ion permeability, destroys cell osmoregulation and electrochemical gradients</td>
<td>Campos et al. 2011</td>
</tr>
<tr>
<td>Nisin</td>
<td>L. monocytogenes, E. coli, B. cereus, L. innocua, Micrococcus luteus; becomes incorporated in growing peptidoglycan layers, forms pores in cell wall/membrane</td>
<td>Imran et al. 2010</td>
</tr>
<tr>
<td>Thymol and Carvacrol</td>
<td>S. aureus, L. innocua, E. coli, S. cerevisiae, A. niger; dissolve in the hydrophobic domain of the microbial cytoplasmic membrane, destabilize cell membranes</td>
<td>Campos et al. 2011; Guarda et al. 2011; Rhim et al. 2013</td>
</tr>
<tr>
<td>Lactoperoxidase</td>
<td>Gram +ve and Gram –ve bacteria</td>
<td>Campos et al. 2011</td>
</tr>
<tr>
<td>Silver compounds</td>
<td>Gram +ve and –ve microorganisms; Cause microbial death through protein deactivation, DNA disruption, destabilizes membranes charges</td>
<td>Althues et al. 2007; An et al. 2008; Appendini and Hotchkiss 2002; Berrang et al. 2010; Blaker et al. 2004, Chu et al. 2000; Damm et al. 2008; Duncan 2011; Egger et al. 2009; Gottesman et al. 2011; Guggenbichler et al. 1999; Kampmann et al. 2008; Kounou and Kaneka 2007; Kumar et al. 2005; Loertzer et al. 2006; Quintavalla and Vicini 2002; Radheshkumar and Munstedt 2006; Rhim et al. 2006; Saint et al. 1998; Wright et al. 1998; Yoksan and Chirochanchai 2010</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>L. monocytogenes, Vibrio parahaemolyticus, Salmonella choleraesuis; disrupts microbial membrane charges, photothermal killing, protein and nucleic acid deactivation</td>
<td>Duncan 2011</td>
</tr>
</tbody>
</table>
4.5.1 Clay and silicate nanocomposites

The popularity of nano-clays in food packaging derives from their low cost and high stability. The most typical clay used is montmorillonite (MMT). MMT is soft phyllosilicate clay comprised of highly anisotropic platelets separated by water, with single platelets being ~1 nm thick and several micrometers in its lateral dimensions (Duncan 2011). Each individual platelet contains another layer of aluminum or magnesium hydroxide octahedral, which is sandwiched between two layers of silicon oxide tetrahedral. The surfaces of MMT platelets are negatively charged, and in nature attract calcium, magnesium, and sodium ions that accumulate under appropriate conditions. The high surface area of clay platelets, when combined in polymer matrices, leads to improved strength and barrier properties (Arora and Padua 2010). The intercalation of clays in polymer matrices also varies from fully exfoliated structures (in which platelets are separated by large quantities of polymer) to minimally exfoliated (greater barrier properties) (De Azeredo 2009; Ray and Okamoto 2003). In a recent study by Sothisvit et al. (2010), it was found that the addition of MMT in whey protein isolate/oregano composite films led to increased package opacity, decreased tensile strength, increased percent elongation, and decreased water vapor permeability with increasing MMT concentrations. Increasing MMT concentrations in this film led to increased antimicrobial activity against *L. monocytogenes*, with no noticeable effect against *E. coli* O157:H7 (Sothisvit et al. 2010).

4.5.2 Incorporation of silver nanoparticles in packaging materials

Aside from its widespread and broad-spectrum bactericidal abilities, perhaps the largest advantage of silver nanoparticles is their ability to be easily incorporated into a variety of plastics (Duncan 2011). Silver nanoparticles have been incorporated into plastics in the food industry, with applications ranging from cutting boards, food storage containers, to refrigerator liners (Appendini and Hotchkiss 2002; Berrang et al. 2010; Kampmann et al. 2008; Kounous and Kaneka 2007; Quintavalla and Vicini 2002). The incorporation of silver nanoparticles in plastics has also found extensive applications in the medical industry to prevent microbial growth. Devices such as urinary catheters, sutures, bandages, and cardiovascular implants represent some products in which silver-impregnated plastics are used (Blaker et al. 2004; Chu et al. 2000; Guggenbichler et al. 1999; Loertzer et al. 2006; Ricco 2006; Saint et al. 1998; Wright et al. 1998).

Silver adds an antimicrobial property when incorporated in packaging materials. This is especially effective with the controlled release of silver nanoparticles from packaging, which is critical for maintaining the antimicrobial nature of packaging materials making silver nanocomposites attractive materials for use in packaging. Their incorporation in packaging has been shown to lead to increased food stability and extended shelf life (Althues et al. 2007).
films also showed increased elastic modulus, decreased percentage elongation (more so for 100 nm silver particles), and decreased water vapor permeability compared to virgin HPMC films (De Moura et al. 2012). HPMC films incorporated with non-silver nanoparticle compounds such as nisin have shown antimicrobial activity against L. monocytogenes (Imran et al. 2012). The incorporation of other non-silver nanoparticles, such as ZnO nanoparticles and pediocin, into methylcellulose (MC) films have shown to increase the antimicrobial activity of MC against L. monocytogenes and E. coli (Espitia et al. 2013). Silver nanoparticles incorporated into gelatin films (another biopolymer) were also shown to be effective against multiple foodborne pathogens including: E. coli O157:H7, L. monocytogenes, S. Typhimurium, Staph. aureus, and B. cereus (Kanmani and Rhim 2014). Gelatin film mechanical properties remained largely unhindered after the addition of silver (only showing decreases in water vapor permeability and tensile strength) (Kanmani and Rhim 2014).

Silver nanoparticle PNCs have been tested with real foods and have shown to prolong the shelf life of many fruits and vegetables. Fayaz et al. (2009) coated sterilized carrots and pears with alginate silver nanoparticle solutions. These coatings were found to be edible and led to decreased water loss and retention of color and flavor after 10 days at 27°C (Fayaz et al. 2009). In a similar study, asparagus spears were coated with edible silver nanoparticle-polyvinylpyrrolidone films and were found to have shelf lives of 25 days when stored at 2°C (An et al. 2008). Another edible film based on silver nanoparticles in glycogen has also been reported (Duncan 2011). Yet another study showed that the incorporation of a P105 coating (TiO₂ and 10 nm nanosilver mixture) to low-density PE helped in significantly reducing the growth of Lactobacillus plantarum in orange juice stored at 4°C (Emamifar et al. 2011). Cellulose pads impregnated with silver nanoparticles were shown to reduce microbial loads in beef cuts stored in MAP (Duncan 2011). This was also shown using freshly cut melon slices (Duncan 2011). It should be noted that edible food packaging films incorporating antibiotics instead of silver nanoparticles have been shown to be effective at reducing microbial loads (Cerqueira et al. 2013). Silver impregnated polymer packaging materials have also been shown to reduce fruit ripening (Duncan 2011). Silver in these packaging materials catalyzes the destruction of ethylene gas released from fruits, slowing ripening, and extending shelf life (Duncan 2011). As with antifouling and bactericidal coatings (described previously), much work needs to be done to understand the full effects of these nanocomposites on human health and cell cytotoxicity.

4.5.3 Incorporation of naturally occurring antimicrobial compounds in edible food packaging

Naturally occurring antimicrobial compounds are widely abundant and are generally recognized as safe (GRAS) for ingestion. Many of these compounds can be used in the formulation of edible packages to further inhibit spoilage and pathogenic microorganisms.

Commonly used organic acids in food packaging include lactic, malic, and acetic acids. These acids are widely used in the preservation of many food products such
Interest in the research and development of nanomaterials in packaging has increased exponentially in the last 10 years and has outpaced research and development focused on understanding the toxicological effects of these nanocompounds. More nanomaterials are being developed every day than can be physically tested by scientists to determine their safety. To conclude this chapter we will discuss adaptation of nanotechnology in food industries, and the public perceptions and environmental concerns surrounding nanotechnology in the food industry.

4.6 REGULATORY ISSUES INVOLVING NANOTECHNOLOGY

Regulatory bodies such as the FDA and the Environmental Protection Agency (EPA) have recently begun to deal with new emerging nanotechnologies in food industries. Because much of the technology is so new, neither agency has any guidelines to deal with the use or regulation of nanotechnology in foods. Currently, nanoparticles or foods that contain them are not subject to any special regulations regarding their handling and safety. Lack of regulation has led to the formation of community action groups pushing for effective regulatory arrangements. However, the success of such groups has been very little.

The lack of nanotechnology regulation stems from the idea that regulating such a new technology would hinder its progress and advancement. Another reason for lack regulation is because it is difficult to determine which regulatory body is ultimately responsible. Also, it is difficult to establish proper material safety data sheets (MSDS) for materials distinguishing their differences in bulk or in the nanoscale.

4.7 CONCERNS AND PUBLIC PERCEPTIONS ON NANOTECHNOLOGIES IN THE FOOD INDUSTRY

Potential risks to human health and the environment exist with almost any technology. The application of nanotechnology in the food industry may present potential risks as it is a fairly novel technology with few risk assessment reports having been done (Cushen et al. 2012). Also, because it is so novel, no long-term effects have yet to be accurately determined. With research breakthrough occurring every year in nanotechnology, risk assessment is needed, along with the establishment of a common recognized terminology in the field of research. It has been proposed that an integrated system of research is needed in order to understand the full breadth of effects of nanotechnology on human health (Cushen et al. 2012). This is needed as a preventative measure to avoid adverse health effects and a proactive measure to minimize said health effects (Cushen et al. 2012). The ideal research team would consist of risk assessors working alongside food toxicologists and technologists to provide the maximum amount of direct contact and, hopefully, the earliest possible
prevention of risk (Cushen et al. 2012). For nanotechnologies in the food industry to be used to their full potential, they must be accepted by consumers. Thus, clear communication is necessary to explain the benefits of nanotechnologies over existing technologies, while acknowledging the risks. It should be made clear that in this case the benefits significantly outweigh the risks and that the risks are acceptable.

### 4.7.1 Routes of exposure

Exposure to nanotechnologies in the food industry can occur through three main routes: dermal contact, ingestion, and inhalation (Cushen et al. 2012). Exposure of nanomaterials through any of these routes may be intentional or unintentional. Unintentional exposure may represent a failure in a process that is intended to prevent such exposure. Intentional exposure through dermal contact and inhalation are less of a concern in the food industry. This is mainly a concern in cosmetic industries (e.g., sunscreens containing nanomaterials that are intended for direct skin contact). Food industries are most concerned with exposure via ingestion as it has been shown that the mammalian gut is able to absorb a variety of nanomaterials (Cushen et al. 2012).

### 4.7.2 Toxicological effects

Differences in legislation between countries and organizations on the safety of nanotechnology, in particular regarding testing of product safety, means that not all products may qualify as safe in some countries, and not safe in others. Also, with the advent of the Internet, nanotechnology-derived foods may be sold between countries freely. This has created a difficult situation to manage with regards to the development of a standard set of safety tests. Toxicology research and risk assessments in the food industry, with regards to nanotechnology, are near non-existent, with few providing any valuable insight (Cushen et al. 2012). Conventional toxicity testing approaches are a good starting point; however, modifications must be made to account for the differences between conventional materials and nanomaterials. Research has shown that some nanoparticles exhibit inflammation, oxidative stress, and early tumor formation in tissues (Cushen et al. 2012). In order to determine exact toxicity profiles, specifics about the nanomaterial in question must be known, such as the size, shape, solubility, reactivity, and other physiochemical attributes of the nanoparticle. Since toxicological properties can vary among nanomaterials, risk assessment must be done on a case-by-case basis.

The safety evaluation of most new materials begins with an overview of the physical and chemical properties of the material (Cushen et al. 2012). This ensures the appropriate handling and storage of novel nanomaterials during fabrication, processing, use, and disposal. Next, the toxicity of the new material is carried out through a barrage of acute toxicity tests, chronic toxicity tests, oral toxicity, dermal toxicity, and mutagenicity tests. These tests are carried out in vitro, using cell
cultures, or in vivo, using mice or rats (smaller creatures may also be used such as Drosophila melanogaster or Caenorhabditis elegans). It has been shown that some materials only show toxic effects at the nanoscale and not at the macroscale. In one study, single-walled carbon nanotubes inhibited cell proliferation in human embryonic kidney cells (Cushen et al. 2012). Subsequently, growth and turnover rates for these cells decreased significantly.

Evaluating nanotechnology migration rates from food packaging or processing surfaces provides a useful reference for further toxicological studies, as they are reproducible, reliable, and well established in many cases. This acts as an area for preliminary risk assessment. Since novel materials have the potential to act in novel ways, they may also present novel toxicity effects.

### 4.7.3 Public perception

Public perception is crucial for any technology to be commercially successful. This is particularly true for technologies surrounding the consumption of foods and beverages. Recent studies have shown that participants were more hesitant to purchase nanotechnology-related foods or foods packaged using nanotechnology compared to "regular" foods (Cushen et al. 2012). This result was expected as it has been found that public knowledge on nanotechnology in the US is lacking (Cushen et al. 2012). Results also show that people are optimistic about nanotechnology in future applications, but this seems odd as nanotechnologies are already being applied in the food industry at various stages and in various forms. In another study, 153 participants were asked to choose whether they would purchase tomatoes with a nanocoating preventing the loss of oxygen and moisture, or bread with nanoencapsulated omega-3 fatty acids, or fruit juice with vitamin A encapsulated in starch nanocapsules. Results showed that people overwhelmingly said they would rather purchase the tomatoes, as the packaging was perceived to be safer and more beneficial than the latter nanotechnology-engineered foods (Cushen et al. 2012). This supports the hypothesis that foods containing nanoingredients are perceived as less acceptable by the public than nanotechnologies not contained within foods.

### 4.8 CONCLUSIONS AND FUTURE TRENDS

The applications of nanotechnology in the prevention of biofilms in the food processing industries are significant. We started by discussing the history of nanotechnology, the role of traditional disinfection methods (both chemical and physical), the alteration of food contact surfaces (physical and chemical) for microbial and biofilm prevention, the use of biosensors for the detection of microbial exotoxins and analytes associated with food spoilage, the prevention of microbial growth and spoilage through smart packaging technologies, and lastly we discussed concerns and public perceptions regarding nanotechnology in the food industry. Nanotechnology
is here to stay, with more and more patents being developed every day. With the ever increasing array of new nanotechnologies, there needs to be equal exploration into the safety and toxicological effects these technologies may have. It is up to the scientific community to develop new methods with which to examine all possible toxicological areas.

Nanotechnology has only recently been applied to food and agricultural systems on a commercial scale (starting in the twenty-first century). Nanotechnology has the potential to impact many aspects of these industries by improving food security, decreasing disease transmission, and providing new tools for scientists to detect pathogens and create new materials. Strategies for applying nanotechnology to the food industry are quite different from traditional areas of research focus (e.g., chemistry, electronics, etc.). Food and agricultural industries are multi-technological and involve a wide array of raw materials, safety requirements, and federal regulations. Nanotechnology, therefore, has many possible applications ranging from the development of new materials, products, methods, and instruments for improved food security. Some nanotechnologies may not be appropriate for food industries due to high industrial scale implementation costs, impracticality, and health concerns. However, with continued basic and applied research funding in the field of nanotechnology, the sky is the limit when it comes to what can be accomplished at the nanoscale.

REFERENCES


