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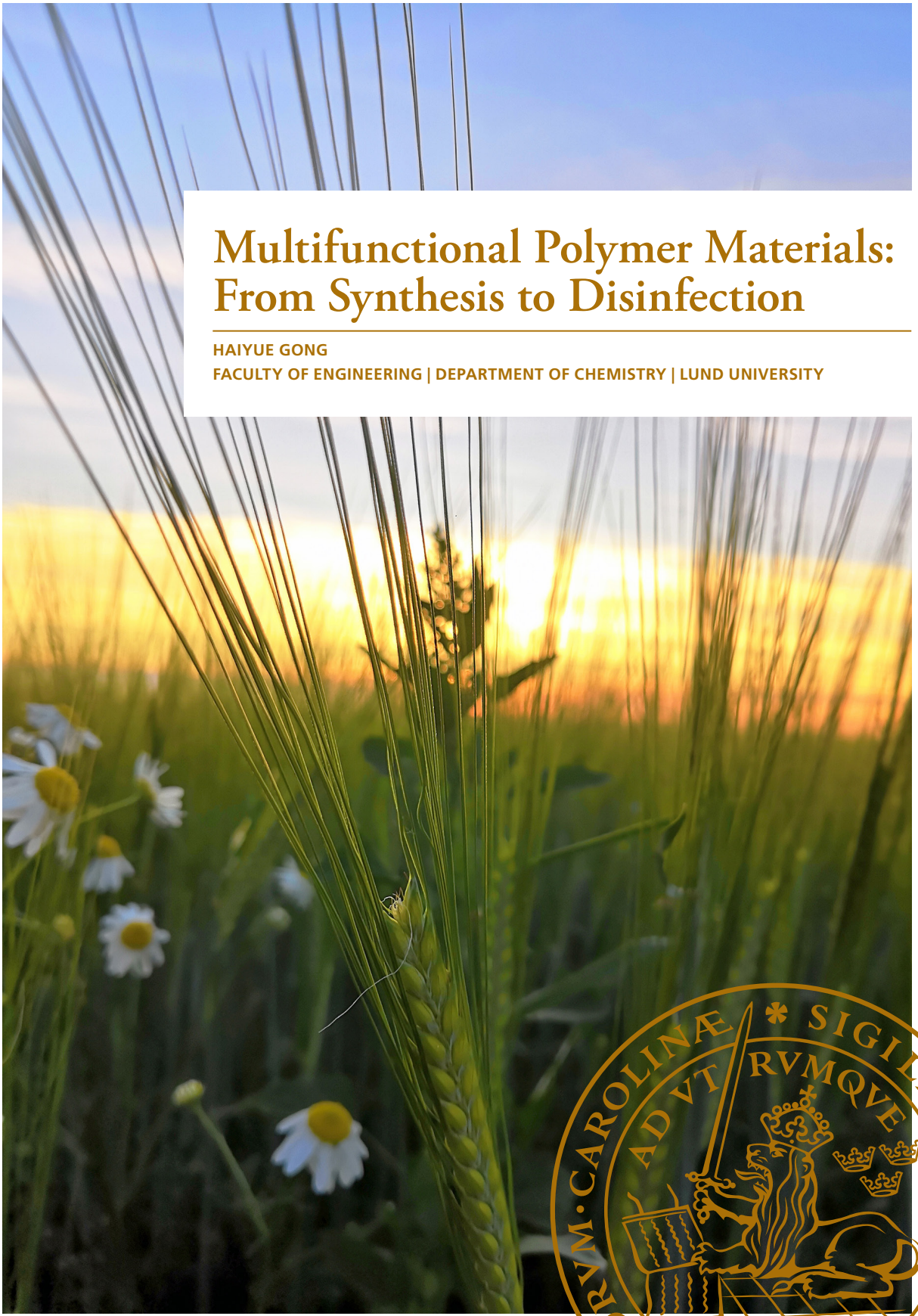

Multifunctional Polymer Materials: From Synthesis to Disinfection

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Multifunctional Polymer Materials: From Synthesis to Disinfection

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Polymer materials have wide applications in many industries, such as the food, pharmacy, construction, textile, and cosmetics industries. For the past few years, polymer materials have drawn the attention of scientists and engineers as a good disinfectant due to their advanced manufacturing methods, large surface areas, good stability, and low cost. More importantly, polymer materials can be functionalized with various chemical groups to increase their affinity towards microorganisms and broaden their applications. In this thesis, four types of multifunctional polymer materials were synthesized to investigate their disinfection ability on bacterial cells.

By using molecular imprinting technology, a small molecule-chloramphenicol-imprinted polymer material of nanometer size was prepared via precipitation polymerization, and large bacteria-imprinted polymer materials of micrometer size were synthesized via surface imprinting-Pickering emulsion polymerization. Both materials had highly specific binding to the targeted template and could be used as adsorbents. In precipitation polymerization, 3-(acrylamido)phenylboronic acid was added to introduce boronic acid on the material surface. In neutral and basic aqueous solutions, boronic acid groups formed reversible boronate ester bonds with the cis-diol groups of the polysaccharides on bacterial surfaces. The release of chloramphenicol led to a high antibiotic concentration around the bacterial cells, which killed the cells. In Pickering emulsion polymerization, positively charged vinyl-functionalized polyethylenimine self-assembled with negatively charged bacterial cells and acted as a stabilizer for the emulsion. Therefore, bacteria-recognition sites based on the bacteria's physical property formed on the surface of polymer beads after crosslinking polymerization. Ag^+ was released from the preloaded hydrophobic Ag nanoparticles in the polymer beads to deactivate the bound bacterial cells.

To realize multifunctional materials for antibacterial applications, nanometer sized polymer materials were prepared with glycidyl methacrylate by precipitation polymerization and microemulsion polymerization. The epoxide groups were opened by polyethylenimine, which was further used to stabilize Ag nanoparticles. The final material self-assembled with bacterial cells via electrostatic interactions. The amino groups and Ag nanoparticles endowed the composite material with disinfection ability. The molecular spectra of bacteria could also be acquired via surface-enhanced Raman scattering from the surface Ag nanoparticles.

In addition to spherical polymer materials, temperature tunable deactivation polymers were also synthesized with (methacryloyloxy)ethyl]trimethylammonium by atom transfer radical polymerization, which was initiated by an initiator containing a boronic acid group. By further modification of the terminal alkyl bromide, a fluorescent molecule, fluorescein 5(6)-isothiocyanate, was added to the polymer chain. The obtained polymers self-assembled with bacterial cells via reversible boronate ester bonds and electrostatic interactions. At 40 ℃, the polymers showed effective deactivation of bacterial cells via a synergistic effect. At 20 ℃, the polymers displayed lower or no toxicity to bacterial cells and could be used to label bacterial cells in flow cytometry and fluorescence imaging.

Key words: Molecularly imprinted polymer, antibacterial, Ag nanoparticles, antibiotic, boronic acid, polyethylenimine, quaternary ammonium

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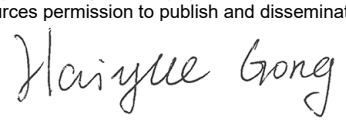
Signature  Date 2021-02-15

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To my family

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Popular science summary

What are microorganisms? What is the relation between human beings and microorganisms?

Microorganisms are tiny organisms that live in every corner of the planet and cannot be seen directly by our naked eye. They were the first life form to develop on Earth approximately 3.5 billion years ago. Although humans have lived on Earth for approximately 300,000 years, the first discussion of microorganisms occurred in the 1st century BC book *On Agriculture* by Marcus Terentius Varro. The scientific study of microorganisms began with observation under a microscope in the 1670s by Antonie van Leeuwenhoek. The number of microorganism types is still growing, as they are still being discovered today. The majority of them are harmless to us and have been used in many fields. For example, lactic acid bacteria in the bowel help us digest food; some microorganisms are used to produce yogurt and wine by fermentation; and some microorganisms are used to produce biobased fuel, many commercial and industrial chemicals, polymers, enzymes and other bioactive molecules.

Although only a minimal ratio of microorganisms is harmful, for instance, less than 1% of all bacteria are responsible for diseases, they could cause severe diseases in plants, animals, and humans. For example, fungi could cause athlete foot, ringworm and thrush; protozoa could cause malaria, sleeping sickness and dysentery; bacteria could cause cholera, tuberculosis, septicemia and anthrax; and viruses could cause the flu, the common cold, measles, mumps, smallpox, cowpox, chicken pox and rabies. Therefore, antimicrobial or disinfection methods have been developed and used to kill or inhibit them for 2000 years. However, some traditional disinfection methods are limited in their usage. For instance, heating cannot be used on precious equipment or heat-sensitive products; radiation is not suitable for turbid systems; and some chemical disinfectants have a single function and can produce hazardous byproducts, resistance and other disadvantages. Therefore, seeking a highly efficient, multifunctional and low-cost disinfection method has always been an important topic for researchers.

In this thesis, polymer materials with different size distributions were synthesized using different methods and designed with various chemical groups and surface structures to increase their affinity to bacterial cells and enhance their antibacterial ability. I demonstrated the possibility of using these polymer materials as effective disinfectants for antibacterial activity. Additionally, these polymer materials showed other potential applications, such as removing chloramphenicol as an adsorbent, collecting and detecting bacterial strains with Raman techniques, and imaging bacterial cells with fluorescent techniques or providing a method for reducing the leakage of heavy metals in aquatic environments, while the antibacterial effect of Ag nanoparticles can still be successfully exploited. I believe that polymer materials are good disinfectant materials and can help extend other applications.

List of papers

This thesis is based on the following scientific papers, which will be referred to in the text by their Roman numerals. The publications and manuscripts are appended at the end of the thesis.

Paper I **Haiyue Gong**, Weifeng Liu, Magnus Carlquist, and Lei Ye, *Boronic acid modified polymer nanoparticles for enhanced bacterial deactivation*. ChemBioChem, 2019, 20, 2991-2995.

Paper II **Haiyue Gong**, Solmaz Hajizadeh, Weifeng Liu and Lei Ye, *Imprinted polymer beads loaded with silver nanoparticles for anti-bacterial applications*. Submitted.

Paper III **Haiyue Gong**, Ka Zhang, Cedric Dicko, Leif Bülow, and Lei Ye, *Ag−polymer nanocomposites for capture, detection, and destruction of bacteria*. ACS Applied Nano Materials, 2019, 2, 1655-63.

Paper IV **Haiyue Gong**, Magnus Carlquist, Hongwei Zheng and Lei Ye, *A multi-functional polymer platform towards labelling, imaging and deactivating bacteria*. Submitted.

Papers not included in the thesis

Paper I **Haiyue Gong**, Solmaz Hajizadeh, Lingdong Jiang, Huiting Ma and Lei Ye, *Dynamic assembly of molecularly imprinted polymer nanoparticles*. Journal of colloid and interface science, 2018, 509, 463-71.

Paper II Chen Liu, **Haiyue Gong**, Weifeng Liu, Bin Lu and Lei Ye, *Separation and recycling of functional nanoparticles using reversible boronate ester and boroxine bonds.* Industrial & Engineering Chemistry Research, 2019, 58, 4695-703.

Paper III Pengfei Guo, **Haiyue Gong**, Hongwei Zheng, Mingli Chen, Jianhua Wang and Lei Ye, *Iron-chelated thermoresponsive polymer brushes on bismuth titanate nanosheets for metal affinity separation of phosphoproteins*. Colloids and Surfaces B: Biointerfaces, 2020, 196, 111282.

Paper IV Hongwei Zheng, **Haiyue Gong**, Limin Cao, Hong Lin, Lei Ye, *Photoconjugation of Temperature- and pH-Responsive Polymer with Silica Nanoparticles for Separation and Enrichment of Bacteria*. Colloids and Surfaces B: Biointerfaces, 2021, 197, 111433.

Paper V Hongwei Zheng, Solmaz Hajizadeh, **Haiyue Gong**, Hong Lin and Lei Ye, *Preparation of boronic acid-functionalized cryogels using modular and clickable building blocks for bacteria separation.* Journal of Agricultural and Food Chemistry, 2021, 69, 135–145.

My contribution to the papers

Paper I I designed the majority of the experiments, performed all the experiments and all the data analyses, wrote the first version of the manuscript, and revised it with coauthors.

Paper II I designed the experiments, performed the majority of the experiments and all the data analyses, wrote the first version of the manuscript, and revised it with coauthors.

Paper III I designed the experiments with the help of coauthors, performed all the experiments and all the data analyses, wrote the first version of the manuscript, and revised it with coauthors.

Paper IV I designed the experiments, performed all the experiments and majority of the data analyses, wrote the first version of the manuscript, and revised it with coauthors.

Abbreviations

Ag	Silver
ATRP	Atom transfer radical polymerization
BA	Boronic acid
BIBs	Bacteria-imprinted beads
E. coli	*Escherichia coli*
FITC	Fluorescein 5(6)-isothiocyanate
GMA	Glycidyl methacrylate
H_2O_2	Hydrogen peroxide
L. pneumophila	*Legionella pneumophila*
LB	Lysogeny broth
LEDs	Light-emitting diodes
MIPs	Molecularly imprinted polymers
MRSA	Methicillin-resistant *Staphylococcus aureus*
NIPAm	N-isopropylacrylamide
PBS	Phosphate-buffered saline
PEI	Polyethylenimine
QACs	Quaternary ammonium compounds
S. epidermidis	*Staphylococcus epidermidis*
UV	Ultraviolet

1 Introduction

1.1 Background

Organisms can be divided into three categories: animals, plants, and microorganisms. Microorganisms are microscopic organisms that exist in their single-celled form or in a colony of cells and can be divided into bacteria, algae, fungi, protozoa and viruses. They live almost everywhere, even in extreme environments, such as deserts, rocks, and the deep sea. Microorganisms are closely related to our daily lives, from our skin to the plate, going through all equipment, and are widely used and studied in many fields [1]. However, some microorganisms have threatened our lives. According to a study, microorganisms that cause plant diseases can cause 10% of crop failures every year [2]. In addition to their influence on agriculture, microorganisms are a major threat to plants, animals and human health, especially since the emergence of antibiotic-resistant species and the difficulties in developing new antibiotics [3]. Enteric microorganisms (such as bacteria, viruses and protozoa) that can be transmitted by the fecal-oral route are the most common reason for waterborne diseases [4]. In contrast to waterborne diseases, *Salmonella*, *Listeria monocytogenes* and enterohemorrhagic *Escherichia coli* (*E. coli*) are three typical foodborne microorganisms that cause a million cases of diseases every year [5]. Pathogen contamination is a lasting worldwide challenge in many areas. Their detection and deactivation as soon as possible are necessary and play an important role in forestalling the explosion of pathogenic diseases [6]. Although some methods, such as polymerase chain reactions and enzyme-linked immunosorbent assays, have been used for identifying bacteria, they are time consuming, expensive, complex and sometimes produce false-positive results [7-9]. Hence, new methods that can fulfil the detection, identification, isolation and deactivation of microorganisms are becoming increasingly important.

Different kinds of traditional and conventional methods have been used for disinfection, such as different physical processes and chemical processes. However, some chemical disinfectants and undesired side products that accumulate are toxic, which also causes antimicrobial resistance [10]. To overcome these limitations, polymer materials have been applied in disinfection. The macromolecular properties of polymer materials diminish the risk of small molecular disinfectants (minimize the residual disinfectants), improve their stability, exhibit long-term activity, are nonvolatile, do not permeate through the skin and offer alternative mechanisms to

disinfect resistant microorganisms [10, 11]. Another significant feature of polymer materials is their large surface area-to-volume ratio, especially for nanoparticles. This large surface area paves the way for future research in surface-based sciences. Therefore, polymer materials with advanced properties need to be developed to replace traditional disinfection methods, especially for antimicrobial-resistant microorganisms.

1.2 Aim and scope of the thesis

The aim of this thesis was to design and develop new approaches to synthesize polymer materials that can be used in disinfection, especially for bacteria. To reach this goal, synthetic polymer materials should enable isolation, deactivation or detection of microorganisms. In Paper I, a drug delivery model of antibiotic-imprinted polymer nanomaterials with boronic acid (BA) groups was synthesized using precipitation polymerization. Under neutral conditions, BA groups can bind with cis-diol groups on the microorganism surface, and the release of the bound antibiotic leads to a higher concentration of antibiotic around the bacterial cells and better disinfection. In Paper II, microorganism-imprinted polymer materials were synthesized using Pickering emulsion polymerization. The imprinted polymer materials have a specific binding ability determined by different bacterial shapes. The hydrophobic Ag nanoparticles embedded in the polymer materials provided disinfection to the bound microorganisms. In Paper III, a polymer nanomaterial with a positive charge and surface Ag nanoparticles was synthesized. The positive charge from polyethylenimine (PEI) can not only isolate microorganisms from water but also disinfect bacteria and stabilize Ag nanoparticles. The Ag nanoparticles enhanced the polymer nanomaterial's disinfection ability and introduced surface-enhanced Raman scattering, allowing for the detection of the bound microorganisms. In Paper IV, a polymer containing quaternary ammonium compounds (QACs) and a terminal fluorescent molecule was synthesized with a novel BA initiator. The polymers bind to bacteria by two different mechanisms: reversible boronate ester bonds and electrostatic interactions. By changing the temperature, the polymers acted as disinfectants at higher temperatures and acted as fluorescent probes for imaging bacteria at room temperature.

2 Microorganisms

Microorganisms live with us throughout our life. On the one hand, they play a critical role in human life, from fermenting food to treating wastewater and producing fuel, enzymes, chemicals, polymers and other bioactive compounds, and they even comprise our bodies' microbiota [12-14]. On the other hand, they are the pathogens responsible for many infectious diseases. Some of the microorganisms we regularly hear about are *Salmonella*, *E. coli*, *yeast*, methicillin-resistant *Staphylococcus aureus* (MRSA), *malaria*, bird flu and coronavirus. Based on their structures, they are divided into different categories, such as bacteria, algae, fungi, protozoa, and viruses [15]. Among them, viruses are not considered living organisms.

2.1 Bacteria

Bacteria are the most well-known microorganisms, and they are a type of prokaryote, which are single-celled organisms without nuclear membranes [16]. Therefore, their genetic material exists in the cells as a long, folded thread with no specific location. Individual bacterial cells with widths normally ranging from 0.5 to 5 μm have various shapes, including spheres, rods, spirals and comma shapes [17]. Bacteria can be further divided into two different species based on their cell wall reaction to Gram staining: Gram-positive bacteria and Gram-negative bacteria [18]. Bacteria with a thick cell wall combined with numerous peptidoglycan layers are Gram-positive bacterial cells [Figure 1a, 19]. In contrast, bacteria with a thin cell wall combined with a certain amount of peptidoglycan layers enclosed by an outer membrane are Gram-negative bacterial cells [Figure 1a, 19]. A typical bacterial structure is shown in Figure 1b. Many bacteria can cause diseases. *Salmonella* is a typical bacterium that causes infections such as food poisoning [20]. *E. coli* causes gastrointestinal distress [21]. Mycobacterium tuberculosis bacteria are the cause of tuberculosis, which is a highly contagious disease [22]. As an antibiotic-resistant bacterium, MRSA is fatal, especially in people who have compromised immune systems [23].

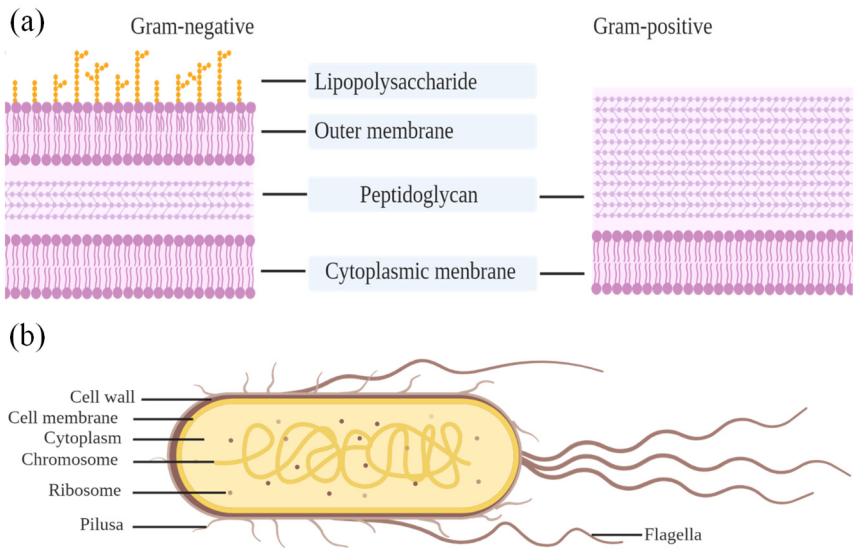

Figure 1. Structure and contents of the bacterial cell wall (a) and a typical bacterial cell (b). Created with BioRender.com.

2.2 Other types of microorganisms

Algae, unlike bacteria, are eukaryotic cells containing a nucleus. Algae, unlike other microorganisms, contain chloroplasts, a photosynthetic structure that can consume carbon dioxide and generate oxygen and energy, similar to plants [24]. A typical algal structure is shown in Figure 2a. In addition to photosynthesis, algae also play an important role as a source of biofuels [25], food and even pharmaceutical and industrial products [26]. However, the rapid growth of algae can also induce danger to our life, as they can act as toxins that can cause a series of diseases (amnesic shellfish poisoning, ciguatera fish poisoning, diarrhetic shellfish poisoning and so on) [27, 28].

Fungi have well-defined nuclei and organelles, are a type of eukaryote and are much larger than bacteria. Yeast is one of the most well-known fungi, and a typical cell structure is shown in Figure 2b. Some types of fungi also pose a threat to our life; for example, coccidioidomycosis, also called valley fever, is an infection caused by the fungi *Coccidioides immitis* and *Coccidioides posadasii* [29].

Protozoa are single-celled microorganisms with membrane-bound organelles and nuclei that are characteristic of eukaryotes [30]. They have different shapes and a wide size range from 1 μm to 2 mm. Figure 2c shows the structure of a typical paramecium. Not all of them are friendly to human beings. Malaria, leishmaniasis,

African sleeping sickness (African trypanosomiasis) and Chagas disease (American trypanosomiasis) are all diseases caused by different protozoan parasites [31].

Compared with the microorganisms mentioned above, viruses are not considered living microorganisms, as they only have nucleic acids (DNA or RNA) and a protein coating [Figure 2d, 32]. Therefore, viruses cannot live alone, and they need to parasitize other host cells to replicate [32]. Not all viruses are innocent. Some pathogenic viruses invade our body and can be detected by our immune system [33]. When the immune system starts to work, it leaves us with a symptom of a common cold or influenza. Coronavirus disease (such as severe acute respiratory syndrome and coronavirus disease 2019) is an infectious disease that can lead to influenza symptoms such as those caused by the newly discovered coronavirus. However, some viruses can cause permanent and irreversible damage to cells, such as human immunodeficiency viruses [34].

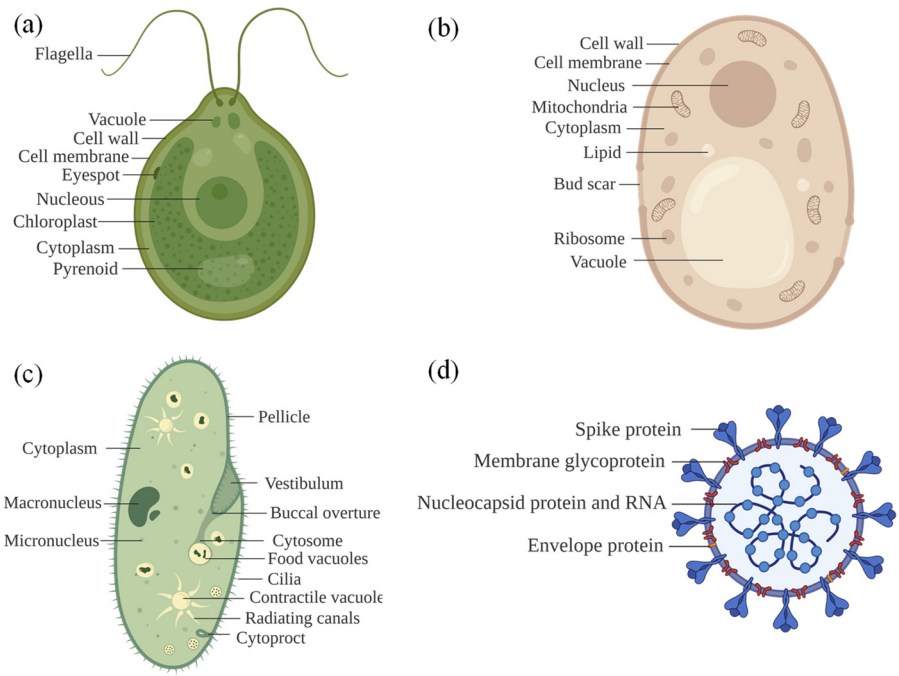

Figure 2. Structure and contents of a typical algal cell, *Chlamydomonas* (a), *yeast* (b), *paramecium* (c) and coronavirus (d). Created with BioRender.com.

3 Disinfection

Disinfection is a process of the destruction or removal of microorganisms and is an effective method to reduce outbreaks of water- and food-borne diseases [35]. Until now, different methods with different disinfection mechanisms have been used to meet the needs of providing safe drinking water and food worldwide. Disinfection methods can be divided into four categories: physical processes, chemical processes, biological processes and photocatalytic disinfection, as shown in Figure 3 [36-38]. Physical processes include thermal disinfection and nonthermal disinfection. Chemical processes include processes using metal ions, oxidizing agents, nonoxidizing agents and antibiotics. Biological processes include processes using metabolites of microorganisms, bacteriophages and lysozymes. Photocatalytic disinfection uses some semiconductor materials. Each method has its own antimicrobial mechanisms, advantages and disadvantages. The following is a brief introduction to some normally used disinfection methods.

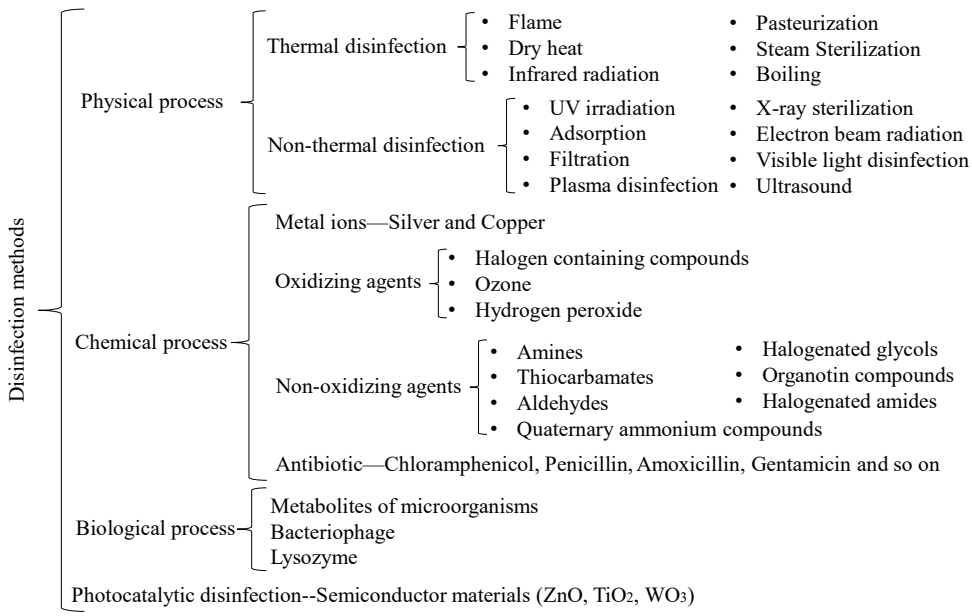

Figure 3. Disinfection methods.

3.1 Thermal disinfection

Thermal disinfection is the most widely used method. It uses heating to damage proteins and fatty acids, denature enzymes and expand fluids within the cell to kill microorganisms [39, 40]. By studying the survival ability of *Legionella pneumophila* (*L. pneumophila*), Rogers et al. found that the maximum accumulation of bacteria occurred on plastics at 40 ℃; at 20–50 ℃, they were able to survive; but at 60 ℃, they were all killed by heating [41]. Muraca et al. found that *L. pneumophila* can be eliminated at 50–60 ℃ in 3 h [42]. Lin et al. [43] concluded that the time required to obtain a 90% reduction in *Legionella* at 60 ℃ was less than 5 min. All these studies show that heating is an effective disinfection method, especially when the temperature is above 60 ℃. Thermal disinfection has many advantages. One advantage of using thermal disinfection is that no hazardous chemicals are used or no hazardous residues are produced during the process [44]. Therefore, thermal disinfection is not hazardous to people or the environment. In addition, thermal disinfection also has other advantages, such as efficient deactivation in a short time, simple manipulation, and penetration inside materials [44, 45]. However, it also has some disadvantages. For example, it may destroy some heat-sensitive instruments and equipment and may also cause burns [45].

3.2 UV irradiation

Ultraviolet (UV) radiation is a type of electromagnetic radiation with a wavelength range from 10 nm to 400 nm and has a bactericidal effect between 240 and 290 nm [46]. Benefitting from the development of semiconductors, UV light-emitting diodes (UV-LEDs) have emerged as a new source for the generation of UV radiation in recent years. UV-LEDs can be divided into three categories: UVC (200–295 nm), UVB (295–325 nm), and UVA (325–390 nm) [47]. The bactericidal effects of UVC and UVB are that the transcription and replication of genetic material are inhibited by the adsorption of UV photons [48, 49]. UVA destroys the cell membrane and other cellular components by producing active substances hazardous to proteins (hydroxyl and oxygen radicals) [50]. However, cellular damage caused by UVA is irreparable, and genomic damage caused by UVC and UVB is repairable through DNA repair mechanisms [51-53]. By using UV irradiation, the majority of microorganisms can be killed in seconds. Compared with thermal disinfection, no chemicals are added and no hazardous residues are produced during the process, and the taste of food and beverages remains the same after UV irradiation [37, 54]. In addition to these advantages, UV irradiation is fast, applicable at low temperature, low cost, etc. [44]. However, cell damage can be repaired by the mechanisms mentioned above. In addition, UV light cannot penetrate a turbid sample to obtain

effective disinfection [54]. These samples may need to be pretreated with other methods, such as filtration.

3.3 Adsorption and filtration

The adsorption and filtration technique is one of the most commonly used methods to remove bacteria because it is cheap and simple [55]. Adsorption and filtration are simple technologies suitable for treating many different samples and have high efficiency and fast speed, with a variety of commercial products/instruments being available [56, 57]. The adsorption and filtration mechanism is mainly based on the surface properties of microorganisms. During growth, microorganisms secrete different kinds of extracellular polymeric substances or organic substances that consist of polysaccharides, proteins, nucleic acids and lipids [58]. Based on the chemical and physical properties of microorganisms, the adsorption and filtration mechanisms can be divided into the following categories: size exclusion, electrostatic and hydrophobic interactions, covalent bonding, recognition by imprinted cavities (Paper II) and polymer-polymer interactions [58, 59]. Based on the different mechanisms, the isolation of bacteria can be fulfilled. After a period of time, the adsorption and filtration materials need to be replaced or regenerated because of saturation and clogging. Thus, this method has a high cost. Additionally, the requirement of chemical derivatization (to improve adsorption and filtration ability) may produce hazardous residues and cause environmental problems.

3.4 Silver

Silver (Ag) has been widely used in keeping food and cleaning water in families since ancient times throughout different civilizations [60]. With the development of science, Ag nanoparticles have emerged to replace bulk silver in some fields and act as biocidal materials [61]. Currently, because of their low manufacturing costs and wide applications, Ag nanoparticles are one of the most commonly used nanoparticles. The antibacterial mechanisms caused by the adsorption of Ag^+ ions onto the negatively charged bacterial cell wall can be illustrated from the following aspects: Ag^+ ions bind with enzymes involved in the respiratory chain reaction [62] and transport proteins [63] via their affinity to thiol groups in cysteine residues [62, 64, 65], leading to eventual cell lysis and death [66]. In addition, Ag^+ could also interact with DNA and inhibit DNA replication [67]. Currently, Ag nanoparticles are regarded as an effective disinfectant in a wide range of commercial products, including clothes, cleaners, hand wash for wound dressings and medical items. By combining them with other disinfectants, such as hydrogen peroxide (H_2O_2), the

bactericidal effects of the Ag-H_2O_2 complex are 1000 times higher than those achieved with adding their effects alone together [68]. If combined with other equipment (such as surface-enhanced Raman scattering equipment), Ag nanoparticles can enable additional applications (Paper III). However, Ag nanoparticles also have some drawbacks. They do not have the same effect on all microorganisms. After disinfection, the remaining Ag needs to be treated before discharge; otherwise, it will contaminate the environment and cause disease to humans by accumulation [69]. Meanwhile, the detection and monitoring of Ag at a low concentration level are not easy and are costly.

3.5 Ozone

Ozone is an efficient, fast and broad-spectrum disinfectant that can be used for the treatment of drinking water and food. Compared with traditional agents, such as chlorine, ozone can react up to 3000 times faster than chlorine with organic matter and disrupt microbial cell membranes and bacterial spore coatings [70, 71]. As a very strong oxidant, ozone induces antibacterial mechanisms by oxidizing cell wall and cell membrane constituents (proteins, enzymes, fatty acids, and polysaccharides), cytoplasmic enzymes and genetic material (DNA and RNA) to destroy the metabolic and reproductive capabilities of bacteria [72-76]. Ozone has the highest oxidation-reduction potential of all disinfectants and needs a shorter time and lower dosage to achieve high efficiency. It can eliminate color, control taste and odor and deal with turbid systems [38, 77, 78]. Because of its high oxidation-reduction potential, ozone is extremely volatile, may react with other compounds and must be produced onsite [38]. During ozonation, some hazardous byproducts (mutagenic and carcinogenic materials) may be produced [79-81].

3.6 Amines

Amines can be divided into three categories according to the number of substituents on one nitrogen atom: primary (1°) amines with an alkyl or aromatic group and two hydrogens, secondary (2°) amines with two alkyl or aromatic groups and one hydrogen and tertiary (3°) amines with three alkyl or aromatic groups [82]. They all have applications in disinfection [Paper III, 83, 84]. The antibacterial mechanism is that positively charged amines interact with negatively charged bacterial cell membranes, combine with lipids through hydrogen bonding and electrostatic interactions, increase membrane permeability, disrupt bilayer structures and eventually lead to lysis and broad-spectrum antimicrobial activity [85-90]. Some amine disinfectants are derived from natural materials, and they are nontoxic,

biologically compatible and biodegradable. The properties of disinfectants can be further modified to widen their applications (Paper III). Nevertheless, their killing effect may be restricted by some conditions, for example, protonated amine groups, and the solubility of chitosan depends on pH values [91].

3.7 Quaternary ammonium compounds

QACs are permanently charged and have four alkyl or aromatic substituents linked to one nitrogen atom. Since the late 1930s, QACs have been one of the most widely used disinfectants due to their low toxicity, cationic structure and contact killing-mediated antimicrobial properties [92-95]. In addition to binding to the bacterial cell surfaces, disrupting the membrane potential and pH gradient and causing lysis of the cells, QACs can also interact with endocellular composites and even bind to genetic materials [96, 97]. The greatest advantages of QACs are their high efficacy, low price and broad-spectrum antimicrobial ability (Paper IV). QACs are nonhazardous, commercialized and available in condensed form and can be diluted for use [95, 98]. In addition to these advantages, they can also prevent bacterial regrowth and are scentless, tasteless, nonirritating and noncorrosive to clothing, metal and other surfaces [99-101]. Thus, QACs are widely applied in our life. Although they are safe to use after dilution, skin and oral mucosa can be burned by concentrated QACs [102]. Organic materials and hard water could affect their efficacies [103].

3.8 Antibiotics

Antibiotics are antimicrobial substances that are secreted by microorganisms to fight against other species [104]. Even though they have been used since ancient civilizations, John Parkinson was the first person to document the use of molds to treat infections in 1640 [105]. With Alexander Fleming's discovery of the first antibiotic, penicillin, in 1928, penicillin was commercially available in the 1940s [106]. Since then, antibiotics have been widely used as medicine to kill or inhibit the growth of bacteria [107]. Based on different antibacterial mechanisms, antibiotics are classified into three categories according to their inhibition of protein synthesis (Paper I), nucleic acid synthesis and cell wall synthesis [Figure 4, 108]. As an effective broad-spectrum disinfectant, antibiotics can kill microorganisms and cure infections without harming normal body cells. Unfortunately, antibiotics can also kill useful bacteria in our body and cause some side effects [109]. Today, the biggest problem of using antibiotics is antibiotic resistance, which is caused by the

abuse of antibiotics. The situation worsens when one considers the difficulty of finding new antibiotics in recent years [110, 111].

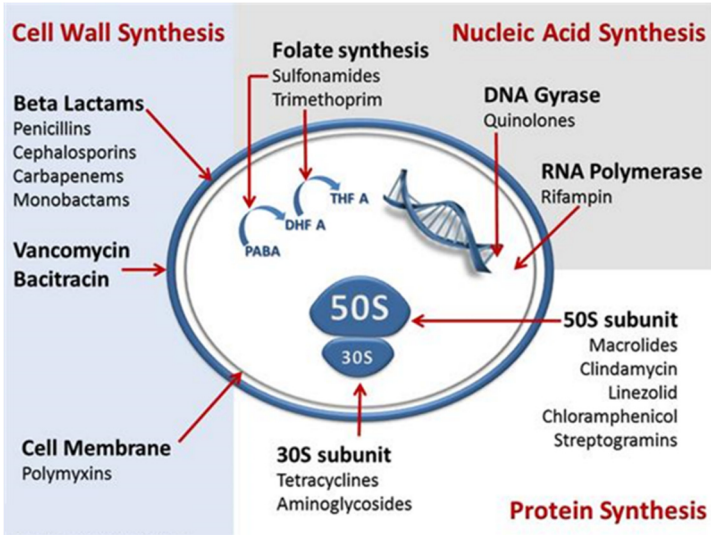

Figure 4. Mechanism of action of antibiotics. Reproduced with permission from reference 108. Copyright 2017 Journal of Anesthesiology Clinical Pharmacology.

4 Polymer materials

Polymer materials have very broad applications, have improved our society dramatically and are still a research hotspot. Compared with other kinds of antibacterial materials, polymer materials have some extremely desirable features, such as high strength, tenacity, resistance to chemicals, light weight, good antimicrobial efficacy and accessibility at low cost [10]. These properties make polymer materials perfect candidates as multifunctional materials in many fields, such as health care, biomedical devices, agriculture, water purification, and the food and textile industry [112].

4.1 Molecularly imprinted polymer materials

Molecularly imprinted polymer (MIP) materials are cross-linked materials with binding cavities that are complementary to the template in terms of physical (size and shape) and chemical functionalities [113, 114]. The molecular imprinting technique is inspired by biological recognition systems such as antigens and antibodies [115]. To imprint templates, different molecular interactions are used, such as covalent bonds [116] and noncovalent bonds [117]. Covalent bonds are based on the chemical reaction between functional monomers and templates [115]. Noncovalent bonds are based on weak interactions, such as hydrogen bonding (Paper I and Paper III), ionic interactions, van der Waals forces, π-π stacking and metal coordination between functional monomers and templates. Noncovalent molecular imprinting is the most widely used method to prepare MIPs [115]. In addition to covalent bonds and hydrogen bonding, hydrophobic and electrostatic interactions can also be used to form template binding cavities in water [Paper II, 118]. Based on different imprinting methodologies, different techniques have been developed to prepare MIP materials, and they can be divided into three types: bulk imprinting, epitope imprinting and surface imprinting [114]. In bulk imprinting, templates are imprinted as a whole in the polymer matrix, which is generally suitable for small molecules [Paper I and Paper III, 119]. In epitope imprinting, only a small part of the target (macromolecule-like proteins) is imprinted as a representative of the whole molecule [120]. Surface imprinting forms template cavities on the surface of polymer materials [121], which are normally used for biomacromolecules (proteins [122], microorganisms [Paper II, 123] and cells [124]). Surface imprinting

includes the following categories: soft lithography [125], template immobilization [126], grafting [127] and emulsion polymerization [Paper II, 123].

Molecular imprinting technology, as a promising technique for producing polymer materials with obvious specific binding, high sensitivity and long-term-stable template binding cavities [114], has many applications, such as affinity separation [128], immunoassays [129], (bio)chemical sensing [130], solid-phase extraction [131], directed synthesis and catalysis [132, 134], controlled drug release [134], and cell and tissue imaging [135], and can even be used as potentially useful drugs [136].

4.2 Methods to prepare polymer materials

Polymer particles can be divided into two categories: spheres and capsules [137]. Two main strategies that have been used for the preparation of polymer particles are the "top-down" approach and the "bottom-up" approach [138]. In the top-down approach, polymer particles are produced on dispersed prepolymers, while in the bottom-up approach, polymer materials are synthesized by the polymerization of monomers. Different methods used for producing polymer particles are illustrated in Figure 5 [138-141]. The "top-down" method includes the emulsion evaporation method [141, 142], emulsion diffusion method [143], coacervation [144], precipitation [145] and interfacial deposition [146], all of which start with preformed polymers. Emulsion polymerization [Paper II, 147, 148], interfacial polymerization [149], interfacial polycondensation [150], molecular inclusion [151] and precipitation polymerization [Paper I, Paper III, 119] using monomers to start the synthesis of polymer particles are "bottom-up" processes. In addition, top-down and bottom-up methods use the same types of synthetic polymers/monomers, stabilizers, organic solvents and so on [138]. In addition to polymer particles, other polymer materials with different chemical and physical properties have also been widely used, such as linear polymers [Paper IV, 152, 153], three-dimensional metal-organic framework polymers [154], reticulated polymers [155], and star polymers [156].

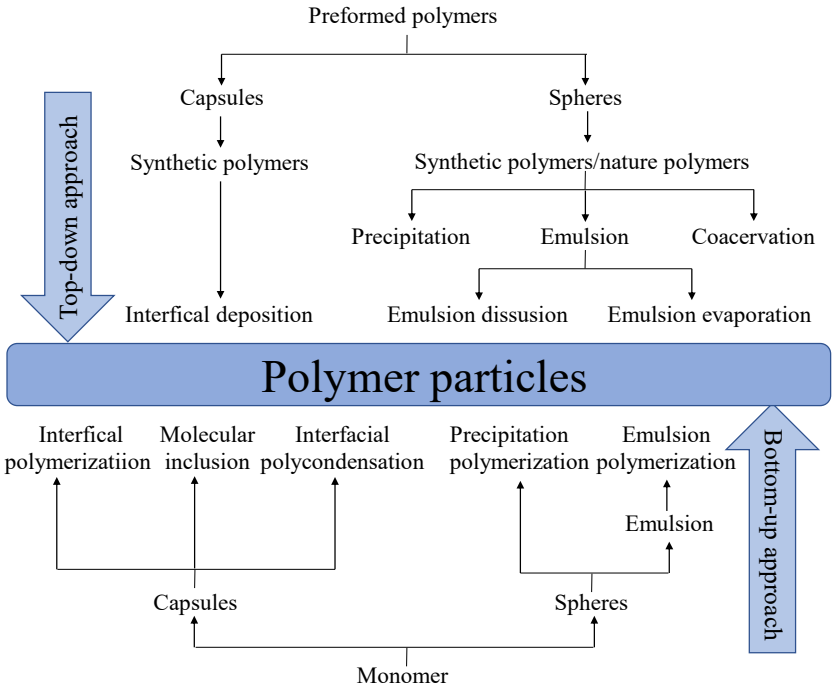

Figure 5. Top-down and bottom-up approaches for synthesizing polymer particles. Adapted and modified from references 138-141.

There are many factors that influence the selection of a suitable method for synthesizing polymer materials, such as the types of solvents and other chemicals and materials that participate in the reaction, the properties (physical and chemical) and application areas of polymer materials [138]. The following are some approaches that have been used in this thesis for the preparation of different polymer materials with different shapes, sizes, surface properties, and applications.

4.2.1 Precipitation polymerization

Precipitation polymerization is a unique particle-forming polymerization method that generates microspheres with clean and smooth surfaces and controllable particle sizes [157-161]. Precipitation polymerization starts with a homogeneous solution of monomer, initiator and crosslinker in a solvent and ends up with insoluble polymer particles [162]. Figure 6 shows the polymerization process, where in the second step, the reaction system experiences phase separation to form polymer chains that nucleate to form unstable nuclei. Finally, the unstable nuclei aggregate to form polymer particles. Although this polymerization method has been

used for a long time, its first application to synthesize MIPs was proposed by Ye et al. in 1999 [163]. This method has some unique advantages, such as being easy to use, not requiring other additives, being capable of using a high content of crosslinkers, and being compatible with polar aprotic solvents to enable noncovalent bonds between templates and functional monomers. The method has been widely used for the preparation of noncovalent MIPs [164]. In the process of the preparation of MIP materials, in addition to the polymerization process, the templates form noncovalent bonds with functional monomers, and thus, imprinted cavities are formed under the influence of the templates.

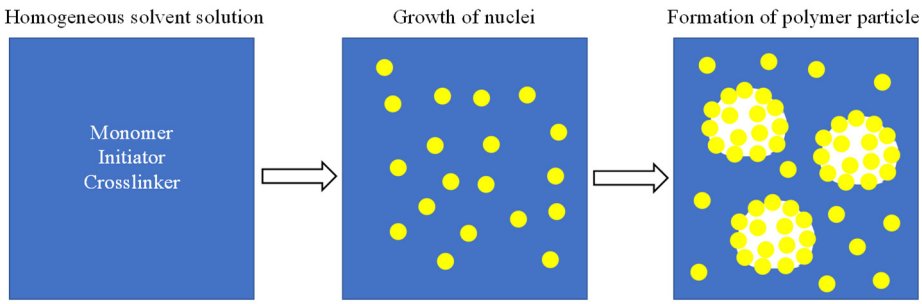

Figure 6. Schematic description of the stages of precipitation polymerization.

In Papers I and III, precipitation polymerization was used to synthesize the polymer particles through one-step polymerization. All the chemicals were dissolved in the solvent. The templates (Paper I: chloramphenicol; Paper III: propranolol) formed noncovalent bonds (hydrogen bonds) in organic solvents with functional monomers that contain carboxyl groups. After polymerization, nanosized polymer particles precipitated from the solvent and were purified to remove the template by washing, centrifugation or filtration. In this approach, the polymer particles had a narrow size distribution and were suitable for drug-delivery systems (Paper I), as well as for in situ modification to produce core-shell polymer particles (Paper III). After removing the template, imprinted cavities were formed. The imprinted polymer particles underwent specific binding to the templates in the presence of organic solvents.

4.2.2 Emulsion polymerization

Emulsion polymerization is a form of heterogeneous free radical chain polymerization, where hydrophobic polymer particles are formed in an aqueous dispersion medium [165]. It is one of the fastest and most widely used methods for the preparation of polymer particles and has been used on an industrial scale to synthesize different kinds of products used in coatings, bulk polymers, paints, binders and so on [166-169]. An emulsion contains stabilizers, a dispersed phase

and a continuous phase. In emulsion polymerization, the emulsion is composed of monomer-solubilized micelles, monomer droplets and initiators. Polymerization can be divided into three steps: 1. the particle nucleation period, whereby particle nucleation occurs until all the micelles disappear; 2. the particle growth period, whereby polymerization continues with the increase in the size of polymer particles until monomer droplets disappear; and 3. the particle end period, whereby the amount of monomers inside each polymerizing droplet decreases until the end of polymerization [170]. Compared with other methods, emulsion polymerization is considered to be a more sustainable and environmentally friendly way to produce a wide range of polymer particles. Emulsion polymerization meets the majority of the 12 principles of green chemistry [171]: the weight, size and properties of polymer particles are under greater control with a high conversion, which reduces the amount of unreacted chemicals (principle 1: prevent waste); using water as the reaction solvent minimizes the risk of using organic solvents (used for processes such as evaporation) and reduces the energy costs (principle 3: less hazardous synthesis; 4: benign chemicals and products; and 5: safer solvents and reaction conditions.); the use of environmentally friendly products decreases the hazard risk to the environment (principles 4: benign chemicals and products; 7: renewable feedstocks; and 10: degradable chemicals and products).); in solution polymerization, the use of small amounts of a free radical initiator overcomes the need for excess stoichiometric reagents (principle 9: use of catalysts), and lower viscosity profiles lead to enhanced heat transfer and therefore lower energy requirements (principle 11: apply real-time analysis to prevent pollution) [172, 173].

Pickering emulsions, which are stabilized by colloidal solid particles, were first described by Pickering in 1907 [174]. To date, many different solid particles have been used to stabilize Pickering emulsions, such as SiO_2, TiO_2, polystyrene, proteins and bacteria [175-179]. Benefitting from Pickering emulsion and emulsion polymerization, Pickering emulsion polymerization is a novel method used to synthesize bacterial recognition polymer particles by interfacial bacterial imprinting, as shown in Figure 7 [123]. Bacterial cells form a network structure with modified chitosan to stabilize a Pickering emulsion. After polymerization of the oil phase and removal of the bacterial cells, binding cavities formed on the emulsion bead surface.

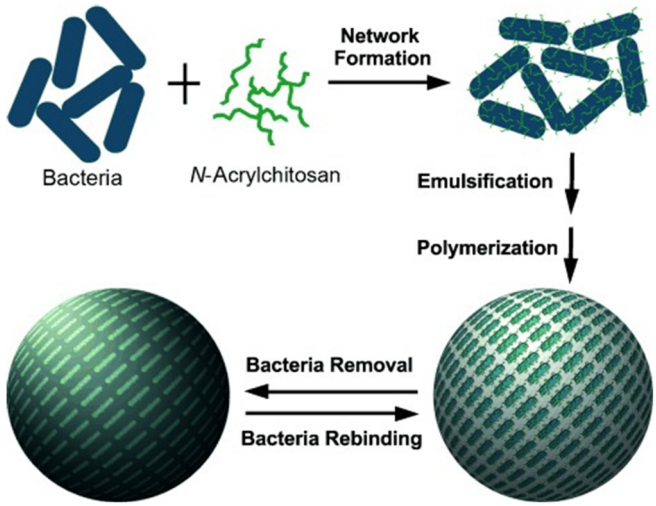

Figure 7. Interfacial bacterial imprinting by Pickering emulsion polymerization. Reproduced with permission from reference 123. Copyright 2014 John Wiley and Sons.

To prepare a biomacromolecule-bacteria-imprinted material that is still feasible in the water phase, in Paper II, Pickering emulsion polymerization was used. Glycidyl methacrylate (GMA)-modified PEI polymers were used to form a self-assembled network with bacterial cells. This network was further used as a stabilizer to stabilize Pickering emulsions by hydrophobic and electrostatic interactions. After polymerization of the oil phase, bacterial imprinted cavities were formed on the surface of the material. These cavities had specific binding to the template cells. Unlike the work of Shen et al. reported in reference 123, hydrophobic Ag nanoparticles were added to the oil phase. The slow release of Ag^{+} in the emulsion beads endowed the polymer beads with a bacterial killing effect.

4.2.3 Microemulsion polymerization

Compared with emulsions, microemulsions are systems that contain water, oil and surfactant and form a thermodynamically stable dispersion. Microemulsions do not have the problem of early phase separation [180, 181], but they require a high loading of surfactant, which is inconvenient [182]. Since the first report of microemulsion polymerization by Stofer and Bone in 1980 [183], microemulsion polymerization has emerged as an alternative method to synthesize polymer particles with smaller particle sizes than those achieved by emulsion polymerization [184]. The microemulsion polymerization process is shown in Figure 8. First, with the addition of a large amount of surfactant, a microemulsion or micelle is formed spontaneously or with mild magnetic stirring (to improve the kinetics of

microemulsification) [185]. Microemulsions or micelles in aqueous solution form hydrophilic "head" regions (blue part) when in contact with water, and hydrophobic single-tailed regions (red part) gather in the micelle center. The micellization phenomenon is a spontaneous process as a result of a balance between entropy and enthalpy [186] and occurs when the concentration of surfactant is greater than the critical micelle concentration and the temperature of the system is greater than the critical micelle temperature to form thermodynamically equilibrated spherical surfactant aggregates [181]. After the formation of micelles, monomers and redox initiators are added, and polymerization occurs in the microemulsion or micelle.

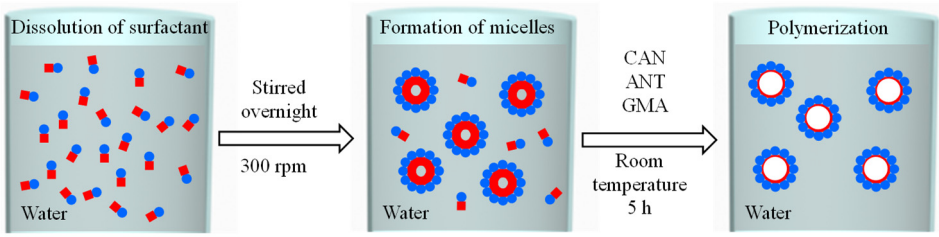

Figure 8. Schematic description of the stages of microemulsion polymerization. Octyltrimethylammonium bromide (OTAB), glycidyl methacrylate (GMA), nitrilotriacetic acid (NTA), and cerium ammonium nitrate (CAN).

Microemulsion polymerization was the second method reported in this thesis that occurs in the water phase. In paper III, octyltrimethylammonium bromide was the surfactant that formed micelles. GMA was the monomer. Nitrilotriacetic acid and cerium ammonium nitrate were redox initiators that started polymerization. In this approach, the polymer particles formed a stable nanosize-diameter dispersion with a low polydispersity index.

4.2.4 Atom transfer radical polymerization

Since its discovery by Krzysztof Matyjaszewski and Jin-Shan Wang and by Mitsuo Sawamoto in 1995 [187, 188], atom transfer radical polymerization (ATRP) has provided a straightforward method to synthesize polymers not only with the desired molecular weight and various architectures but also with a narrow molecular weight distribution [189]. The wide use of the ATRP reaction also benefits from the variety of monomers, initiators, catalysts, ligands and solvents that can be used to produce various polymer materials, which can interact with other materials, especially some biological materials [190]. The mechanism of ATRP is shown in Figure 9. At equilibrium, the initiator (R_n–X, X = Br or Cl) is the dormant species that reacts with a reduced transition metal complex ($Cu^{I}X/L$, the activator, a catalyst complex with a ligand) to initiate the reaction, generating a growing radical (R_n•) and an oxidized transition metal complex (X-$Cu^{II}X/L$, the deactivator) [191]. Before the

deactivation of the propagating radicals, the intermittently formed radicals (R_n•) propagate to extend the polymer.

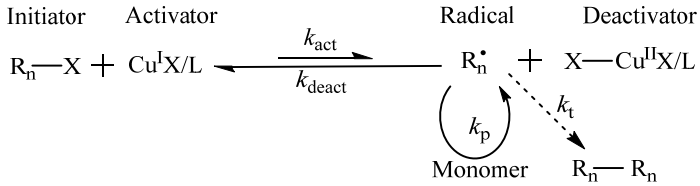

Figure 9. Mechanism of ATRP.

ATRP was used to synthesize a multifunctional polymer in water in this thesis. In Paper IV, N-isopropylacrylamide (NIPAm) and [2-(methacryloyloxy)ethyl]trimethylammonium chloride were used as monomers. $Cu^{I}Br$ was used as the catalyst. (3-(2-Bromoacetamido)phenyl)boronic acid, a novel initiator containing a BA group, was used to initiate the ATRP reaction in water. In this approach, further modification of the polymer products can be fulfilled using different methods, for example, prolonging the polymer chains by subsequent ATRP or derivatizing the side chains or the terminal alkyl bromide (Paper IV).

5 Disinfectant polymer materials

5.1 Chloramphenicol-imprinted BA polymer materials

Antibiotics are a powerful weapon for killing bacteria or inhibiting their growth [108]. They are widely used with poor control, which leads to the occurrence of bacteria that are resistant to antibiotics [192]. Unfortunately, no new antibiotics have been discovered since the 1990s (as shown in Figure 10). Antibiotic resistance has become a more acute problem [193]. To prevent outbreaks and the fast growth of antibiotic-resistant bacteria, it is crucial to avoid the widespread use of antibiotics and to ensure that the antibiotics used are delivered directly to deactivate target microbial pathogens (Paper I).

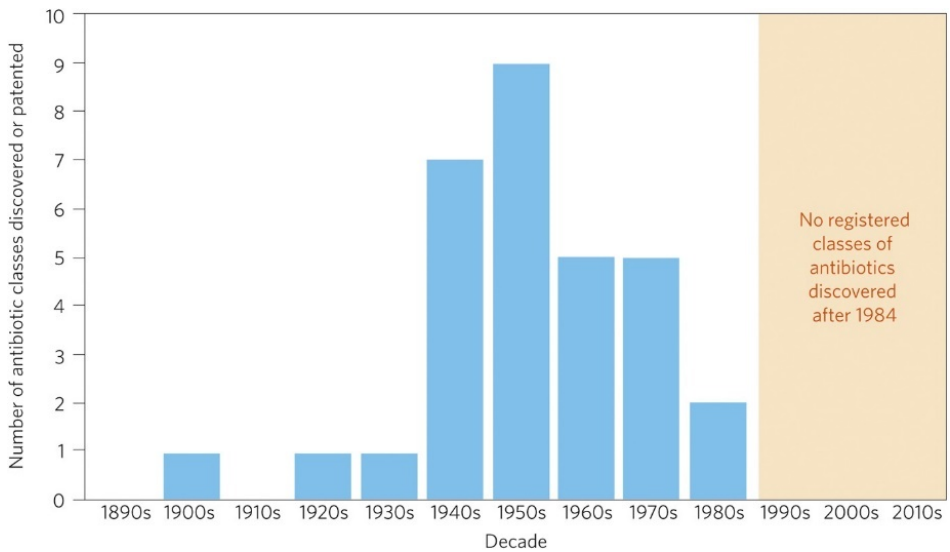

Figure 10. Discovery of novel antibiotic classes (groups of drugs with similar chemical structures). Reproduced with permission from reference 193. Copyright 2016 Springer Nature.

In Paper I, a new approach was developed to improve the inhibitory effect of available antibiotics. In addition to facilitating antibacterial efficiency, BA-modified polymer materials could also be used as cleaning materials to separate residual antibiotics from the environment. Chloramphenicol-imprinted BA particles

were synthesized using precipitation polymerization. Chloramphenicol formed a noncovalent bond (hydrogen bond) with methacrylic acid during the molecular imprinting reaction. After polymerization, chloramphenicol was removed, and a temperature-responsive polymer particle containing binding sites specific to chloramphenicol was obtained. The BA groups were located on the particle surface.

The specific binding of chloramphenicol by different polymer materials was studied in acetonitrile and phosphate-buffered saline (PBS) buffer (0.1 M, pH 6.8), individually. In acetonitrile, MIP showed a high binding efficiency (84%) to chloramphenicol due to the binding cavities and hydrogen bonds. In PBS buffer, the loading of chloramphenicol on MIP was 37% due to the reaction between BA groups and the 1,3 cis-diol structures. Therefore, after loading chloramphenicol in acetonitrile, by changing the solvent to PBS buffer, the binding mechanism changed from hydrogen bonding to revisable covalent bonds, which caused the release of chloramphenicol from the cavity. Meanwhile, the BA groups on the polymer surface reacted with the 1,2 cis-diol groups on the bacterial surface, which enhanced the release of chloramphenicol [194], forming a higher concentration of chloramphenicol around the bacteria (Figure 11a). The MIP itself had almost no bacteria-killing effect (Figure 11b and 11c). Compared with chloramphenicol itself, the antibiotic-loaded MIP had a higher antibacterial effect, and this effect was better at 20 ℃ than at 37 ℃ (Figure 11b and 11c). This temperature effect was caused by the thermoresponsive property of polyNIPAm. The hydrodynamic diameters of MIP at 20 ℃ and 37 ℃ were found to be 529 ± 5.3 nm and 374 ± 31 nm, respectively. The denser structure of the polymer at higher temperature made the loaded chloramphenicol more difficult to release.

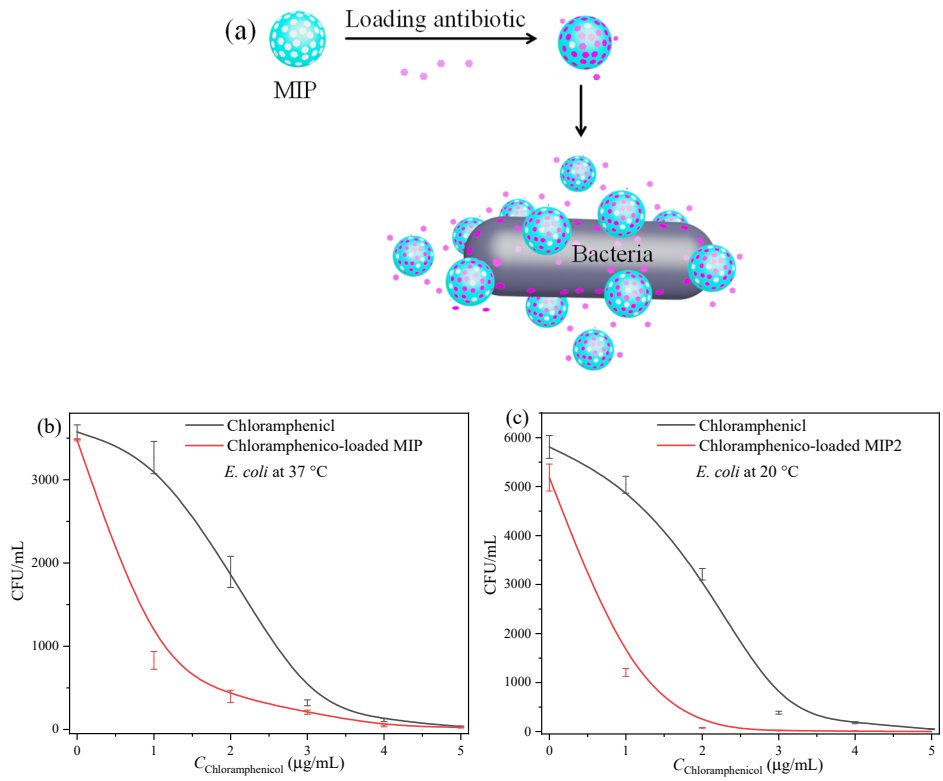

Figure 11. Schematic of the disinfectant mechanism of chloramphenicol-imprinted BA particles (a). Antibacterial effect of chloramphenicol and chloramphenicol-loaded MIPs on *E. coli* at 37 ℃ (b) and 20 ℃ (c). CFU mL^{-1} is the number of colonies forming units per mL after 10^6 dilution.

5.2 Bacteria-imprinted polymer beads embedded with Ag

Compared with antibiotics, Ag is considered to be an optimal candidate for the inactivation of pathogenic bacteria because Ag has strong and broad-spectrum antimicrobial characteristics without causing multidrug resistance [195]. In Paper II, a new antibacterial polymer material was developed to selectively capture and destroy bacteria based on their physical characteristics. As shown in Figure 12, positively charged GMA-modified PEI polymers self-assembled with negatively charged bacterial cells. Hydrophobic Ag nanoparticles were suspended in an oil phase containing crosslinkers and initiators. By mixing the two phases to establish a stable Pickering emulsion and polymerizing the oil phase, bacteria-imprinted polymer beads (BIBs) were obtained.

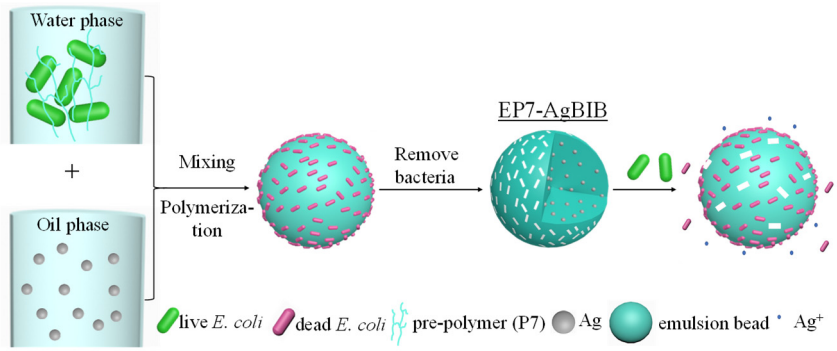

Figure 12. Schematic of the preparation of bacteria-imprinting polymer beads embedded with Ag (EP7-AgBIB).

The adsorption of *E. coli* (Figure 13a) and *Staphylococcus epidermidis* (*S. epidermidis*, Figure 13b) on different AgBIBs in PBS buffer was investigated. While the Ag nanoparticles embedded in the polymers exhibited nonselective bacterial killing, the two bacteria-imprinted polymers EP7-AgBIB and SP7-AgBIB had preferential binding to their corresponding template cells, demonstrating a clear selectivity between rod-shaped and spherical bacteria. The binding selectivity was mainly caused by the bacteria-imprinted sites. Figure 13c and 13d show the inhibition of *E. coli* and *S. epidermidis* growth in lysogeny broth (LB) medium by the Ag-loaded BIBs. Without Ag nanoparticles, EP7-BIB itself had a very weak inhibition effect on *E. coli* growth, but SP7-BIB obviously inhibited the growth of *S. epidermidis*. After loading Ag nanoparticles, the *E. coli*-imprinted polymer EP7-AgBIB strongly inhibited bacterial growth in the first 4 h, and the *S. epidermidis*-imprinted SP7-AgBIB exhibited the strongest inhibitory effect on bacterial growth in the first 17 h. The antibacterial activity of EP7-AgBIB and SP7-AgBIB was attributed mainly to the Ag nanoparticles embedded in the polymer beads. As EP7-AgBIB was able to bind *E. coli* cells and SP7-AgBIB was able to bind *S. epidermidis* cells, the Ag^+ ions were released from the polymer beads more effectively, deactivating the bound bacterial cells. These results indicate that it is feasible to realize selective bacterial destruction using bacteria-imprinted polymer materials loaded with general-purpose antibacterial reagents (e.g., Ag nanoparticles).

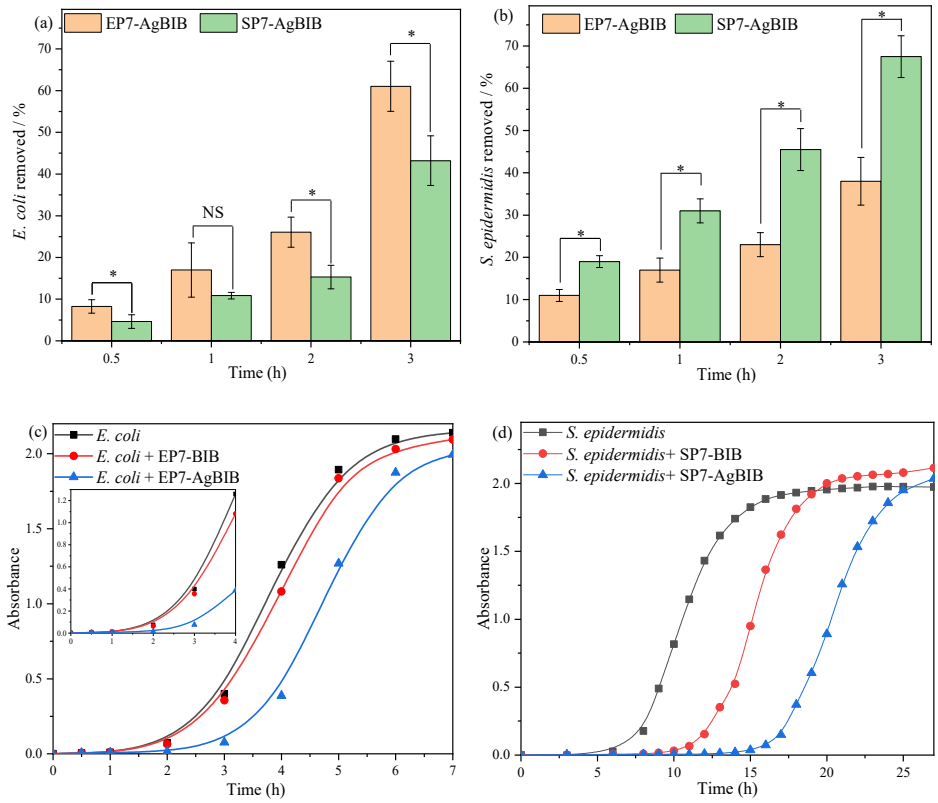

Figure 13. Removal of *E. coli* (a) and *S. epidermidis* (b) on different BIBs in PBS buffer. Bacteria (optical density at 600 nm = 0.1) and BIB particles (a and b: 10 mg/mL) were gently stirred at 6 ℃ for 3 h before the unbound cells were quantified. *E. coli* (c) and *S. epidermidis* (d) growth curves measured in LB media containing different bacteria-imprinted polymers (5 mg/mL). The bacteria were cultured at 37 ℃ and with 160 rpm. EP7-BIB refers to emulsion beads imprinted with *E. coli*. SP7-BIB refers to emulsion beads imprinted with *S. epidermidis*. EP7-AgBIB refers to emulsion beads imprinted with *E. coli* containing Ag nanoparticles. SP7-AgBIB refers to emulsion beads imprinted with *S. epidermidis* containing Ag nanoparticles. One-way analysis of variance (ANOVA) was applied to estimate the statistically significant differences at the 0.05 level ($P < 0.05$, *). NS stands for no significant difference.

5.3 PEI-modified polymer materials with surface Ag

In addition to capturing and deactivating bacteria, in Paper III, additional functionality was introduced to a multifunctional polymer to enable the rapid capture, detection, and destruction of bacteria.

Polymer materials with epoxide groups were first prepared as scaffolds. One approach was to use a linear polymer synthesized via microemulsion polymerization using GMA as a monomer. The other approach was to use crosslinked polymer particles synthesized via precipitation polymerization by using GMA as one of the monomers to allow postpolymerization modification. Using crosslinked polymer particles (cPGMA) as an example, amine groups were introduced via the reaction

between PEI and the epoxide groups on the polymer surface (Figure 14). The obtained core-shell polymer particles were used as a scaffold to synthesize and stabilize Ag nanoparticles [196]. The final material was named Ag-PEI-cPGMA. The antibacterial effect of this cPGMA polymer was studied on *E. coli* (Figure 14). The addition of the Ag-PEI-cPGMA polymer to *E. coli* cells showed that the polymer had a strong ability to quickly capture and destroy *E. coli* cells due to the presence of positively charged amino groups on the PEI shell and the Ag nanoparticles present on the polymer surface. Normally, the Raman signal of *E. coli* itself is very weak and cannot be detected. However, after binding to Ag-PEI-cPGMA polymer particles, the Raman signal of *E. coli* became significantly amplified due to surface-enhanced Raman scattering, and several Raman bands originating from *E. coli* cells became detectable.

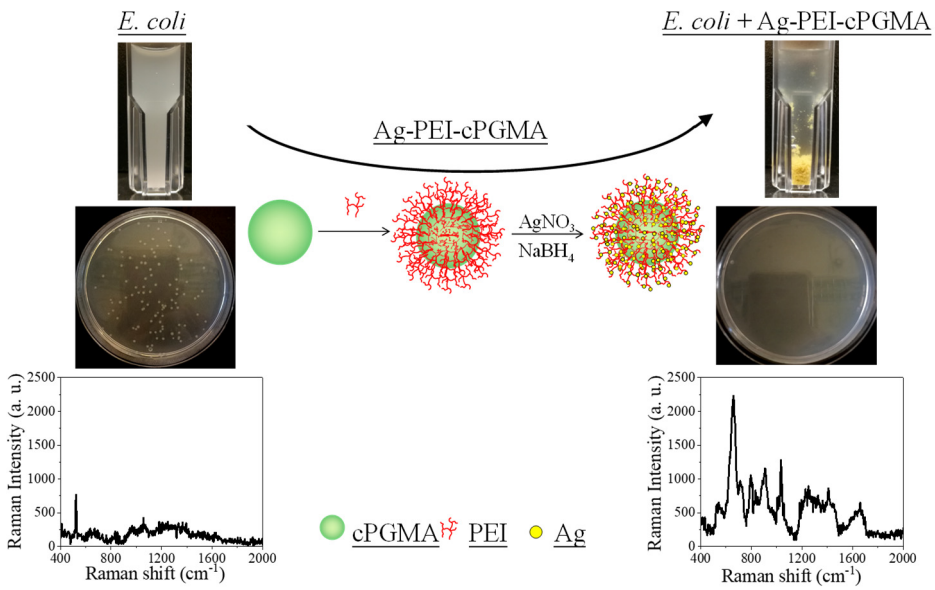

Figure 14. Multifunctional Ag-polymer nanocomposite Ag-PEI-cPGMA used for the capture, detection, and destruction of bacteria.

5.4 Fluorescent QACs copolymers

In Paper IV, a multifunctional polymer was synthesized to realize fast labeling and imaging and to inhibit bacterial growth with temperature-tunable activity.

First, a novel ATRP initiator was designed to introduce a BA group to an alkyl bromide initiator. Then, this initiator was used to synthesize water-soluble polymers containing QACs by the ATRP reaction. Fluorescein 5(6)-isothiocyanate (FITC)

was introduced into the polymers in three steps, as shown in Figure 15. First, the terminal bromine was replaced by an azide group. Then, an amino group was connected to the chain end by a click reaction. Finally, FITC reacted with the amino end. In this way, a fluorescent polymer with multiple functions was obtained and named BA-R-FITC. The bacterial killing effect of BA-R-FITC polymers was studied. As shown in Figure 15, at 20 °C, compared with the control experiment, BA-R-FITC displayed a weak antibacterial effect on *E. coli*. At 40 °C, BA-R-FITC had a strong bacterial killing effect due to the synergistic effect between high temperature and QACs [197, 198]. The fast labeling and imaging functions of BA-R-FITC polymers on bacterial cells were studied by fluorescence microscopy, as shown in Figure 15. Compared with *E. coli* itself, after mixing with BA-R-FITC polymers, the bacterial cells became easily detectable under a fluorescence microscope. Furthermore, the fast labeling and imaging functions of BA-R-FITC polymers for bacterial cells were also studied using flow cytometry, as shown in Figure 15. During the experiments, bacterial cells alone were used as control samples. Due to the endogenous fluorophores (such as porphyrins, collagens, and flavins) in bacterial cells, the control samples also generated a low fluorescence intensity [199]. However, after the addition of the BA-R-FITC polymer, the bacterial cells displayed significantly enhanced fluorescence. With a high fluorescence quantum yield and photostability, the polymers can thereby serve as an excellent fluorescence marker to enable the detection of live bacterial cells using a flow cytometer. Therefore, at 40 °C, BA-R-FITC had the potential to be used as an efficient antibacterial material, whereas at 20 °C, BA-R-FITC had the potential to be used as a fluorescence probe for labeling and imaging bacterial cells.

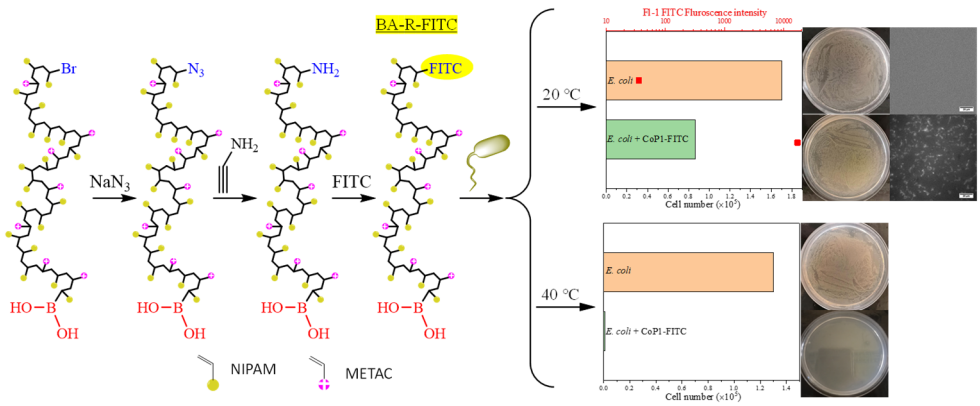

Figure 15. Reaction scheme and applications of BA-R-FITC polymers at different temperatures. FITC fluorescence intensity is shown as the logarithm on the y-axis.

6 Conclusions and future outlooks

Multifunctional polymer materials have drawn much attention in disinfection applications. They provide a facile, efficient and sustainable method for microorganism inactivation. In this thesis, different polymer materials were synthesized by different methods with various disinfection mechanisms and other potential applications.

In Paper I, antibiotic-imprinted polymer materials were used to improve the efficiency of antibacterial agents for the first time. The obtained polymer material had a high loading capacity in organic solvent, a comparable lower loading capacity in PBS buffer and could dynamically bind bacteria via BA groups. Therefore, the release of preloaded chloramphenicol enhanced the deactivation of the target microorganism. These results clearly indicate that antibiotic-imprinted polymer materials are a good disinfectant and provide a means of removing overused antibiotics from the environment. Future studies could aim to obtain drug-delivery model polymer materials that have improved specific binding to antibiotics in water.

In Paper II, instead of small molecules, microorganisms were used as templates to prepare imprinted polymer materials. A new multifunctional polymer material was developed to enable specific binding based on the different shapes of the target bacteria and effective killing of the bound bacteria via the hydrophobic Ag nanoparticles embedded in the polymer particles. The bacteria-imprinted sites played a key role in specific binding, which depended on the shape of the bacterial cells. The Ag nanoparticles embedded in the polymer beads enhanced bacterial inactivation through released Ag^{+}. This work also demonstrated a potential method to reduce the leakage of heavy metals in aquatic environments. In the future, it would be interesting to synthesize imprinted cavities using bacteria-mimetics as templates that have the same shape as target bacterial cells to gain a better understanding of the imprinting mechanism.

In Paper III, multifunctional polymer nanomaterials containing surface-bound Ag nanoparticles were synthesized. The surface Ag nanoparticles not only improved the antibacterial activity but also provided a method for the direct detection of bacteria through their characteristic Raman signal. Therefore, the multifunctional polymer nanoparticles enabled the simultaneous collection, destruction, and identification of pathogenic bacteria. If combined with other functional

nanoparticles, such as quantum dots, this type of polymer material may allow more sensitive bacterial detection to be realized.

In Paper IV, a new polymer platform structure was proposed for the multifunctionalities of antibacterial materials. For bacterial inhibition, a temperature-tunable deactivation ability was demonstrated. At 40 °C, the polymer could be used as an effective disinfectant due to the synergistic effect between temperature and QACs; at 20 °C, the polymer could be used as a fluorescence probe for bacterial imaging. In the future, different polymer structures could be synthesized using the modular synthetic approach, and more fluorescent molecules can be added to increase the sensitivity of the analysis.

Taken together, the new polymer materials described in this thesis have different antibacterial abilities and potential for other applications and could be used as disinfectants for different purposes. Despite the rapid development of antimicrobial materials, there are many unresolved important questions about the technological, biological, and economic aspects of antimicrobial materials and the related microbial treatment methods. These challenges should be addressed in future research.

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