# Mechanism and application of the anti-bacterial nanomaterials

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**Abstract.** The abuse of antibiotics has brought a series of medical problems including the increase of bacterial drug-resistance. Given the current conditions researchers tend to develop a new field of medicines to gradually replace conventional anti-bacterial agents. Nanomaterials-based anti-bacterial agents have drawn tremendous interest because of a range of advantages over traditional anti-bacterial agents including higher efficiency, low provocation of drug-resistance, lower cost and other specific properties given by the nanoscale, making it a research topic in great demand. At present, numerous categories of anti-bacterial agents have been developed and completed with various properties, and along with the process many hypothesis and mechanisms have been suggested to serve as conferences to guide further research and creation. This research aims at summarizing mechanisms of current typical nanomaterials-based anti-bacterial agents including anti-bacterial peptide, carbon-based nanomaterials (CNMs) and metal nanomaterials while also providing related application fields in order to give a primary and wide acknowledge.

**Keywords:** Anti-bacterial; nanomaterials; mechanism; application; antimicrobial agent.

## 1. Introduction

In the history people have never stopped the battle against the microbes. About two thousand and five hundred years, the ancient Chinese people primarily realized that a kind of "mildew" could be used for wound cure which was currently known as "blue mould". Until 1928 the bacteriologist Alexander Fleming discovering and refining the penicillin people had never mastered an efficient anti-bacterial method. However, currently people have found various kinds of antibiotics including colistin and halicin, simultaneously antibiotics have been widely applied in medicines and related fields such as cosmetics and injury cure, applying antibiotics does bring many benefits such as rapid killing of most bacteria and thus shows fast improvement of illness. Antibiotics is also an efficient method to prevent infection and deterioration of wounds. But what was also brought is the sequence of the abusing of the antibiotics such as the drug resistance of the bacteria and antibiotics pollution. The generation of super bacteria, one of the subsequences of abusing antibiotics, may cause more potential death in the future for the extreme drug-immune property of the super bacteria. China is a typical area for antibiotics abusing. Some organization even predicted that in the future 20 years, approximately 80 thousand patients would die from no suitable antibiotics.

Given the current situation that the development of applying the antibiotics to fight against the bacteria may face bottleneck, researchers have gradually turned to the nanomaterial field to search for strategies. Due to the special scale effect, nanomaterials could provide a series of advantages over non-nanomaterials including higher penetration, wider adjustability, lower drug-resistance provocation and etc. Researchers have developed various sorts of anti-bacterial nanomaterials, which could be simply divided into two main categories: the inorganic and organic streams. The inorganic stream mainly locates in metal and metal oxide nanomaterials and nanozymes [1], which is not a biomolecule literally but a complex of metallic. Some researchers reported black phosphorus (BP) and other semiconductors materials present antibacterial properties [2]. The second category is the organic stream containing antimicrobial peptide (AMP), carbon-based nanomaterials (CNMs) such as fullerene and carbon nanotube (CNT) and also biodegradable and non-biodegradable polymeric nanocomposite [3]. Literatures and studies related to nano anti-bacterial agents have drawn increasing interest and new-type anti-bacterial agents are being developed continuously. However, literatures focused on mechanism and application field still lacks, which is indispensable for offering a comprehensive cognition for researchers and those who expect to enter this field. This research collected and arranged mechanisms and application fields of current mostly acknowledged nano anti-bacterial agents and would provide an overall background of related anti-bacterial agents.

The author also hopes to summarize the related mechanisms of both streams of nanomaterials for the fact that comprehending the mechanism is also indispensable to get familiar with this field. Mechanisms about the anti-bacteria effect of the metal and metal oxide nanomaterials have been classified practically, but the counterpart of organic materials seems to be quite more multiple and related literature lacks. In order to give a clearer understanding, some representative applications of the nanomaterials would be mentioned and detailed.

## 2. Current main streams of anti-bacterial materials

### 2.1. The organic stream

#### 2.1.1. Antimicrobial peptide.

Antimicrobial peptide (AMP), also called peptide is a sort of alkaline peptide with the molecular weight ranging from 2000 to 7000 and consisting of 20 to 60 amino acid residues. The antimicrobial peptide could be divided into two types according to its functions: Cathelicidin and Defensin. Naturally antimicrobial peptides could derive from six approaches: four ways from animals and plant antimicrobial peptide and bacterial antimicrobial peptide. Of note is the fact that AMP exists high biotoxicity, low bio accessibility, vulnerability to protease degradation and other disadvantages which may limit its function. Thus, commonly in order to achieve the most content results single antimicrobial peptide is not usually utilized in remedy or therapy, instead, polymers, nanoparticles, nanotube and dendritic molecules are added to serve as carriers to deliver these peptides. Also, antimicrobial peptide could be taken to cooperate with other nanomaterials to enhance the anti-bacterial effect. For instance, a literature reported a biodegradable colloidal nano-network is presented to protect bioactive peptides against enzymatic degradation and also help their deliveries [4]. the researchers encapsulate a sort of antimicrobial peptide named PA-13 electrostatically into oppositely charged nanoparticles created by chitosan and dextran sulfate, which made the encapsulated peptide effective to kill *Pseudomonas aeruginosa* during in vitro culture, while the undealt peptide showed no significant antibacterial effect.

#### 2.1.2. CNMs.

The CNMs refer to a wide series of materials which are mainly elementary carbon substances. These molecules could be classified in terms of their dimension. The zero-dimensional species contains fullerene, nanodiamond (ND), carbon dots (CDs), and graphene quantum dots (GQDs); the one-dimensional species chiefly include carbon nanotube (CNT); the two-dimensional species include graphene and its ramifications, with recently reported graphitic carbon nitride (g-CN) [5]. these substances all show fine biocompatibility and environment-friendly properties, and some literature pointed out through modifying the lateral size, shape, number of layers and surface charge the antibacterial activity of CNMs could be different. Mechanisms about the anti-bacterial effect of CNM have been widely researched and will be explained lately.

#### 2.1.3. Polymeric nanocomposites.

Actually, the polymeric nanocomposites could not be directly classified as a sort of organic nanomaterial for the fact that it is a composite probably consisting both inorganic effective components and organic components. But here the polymeric materials mainly focused refer to the polymeric matrix or polymeric matrices. The application of antimicrobial polymer could solve the residual toxicity, short-term activity of conventional antimicrobial agents [3]. They could also enhance the biocompatibility and absorption of antibacterial agents such as metal oxide nanoparticles. One literature reported that Cu nanoparticle-coated cellulose shows excellent antibacterial properties against *S. aureus* and *E. coli* [6]. However, such technology currently is mainly applied in prevention of colonization in material surface and environmental anti-bacteria, and the utilization in the medicine field is waiting for exploration.

### 2.2. Inorganic stream

#### 2.2.1. Metal and metal oxide nanoparticles.

The metal and metal oxide nanoparticles (MNPs) are one of the hottest antibacterial fields nowadays, and related mechanisms have been enough clear. Great attention has been paid to metal and metal oxide nanomaterials especially due to the fact that MNPs could provoke significantly weak drug-resistance for bacteria. Also, with recently development of green synthesis conducted by plants MNPs are playing a more and more important role in antibacterial field. Many metals and metal oxides have been proved to present antibacterial effect, including Ag, Au, $Al_2O_3$, Bi, Ce, Cu (CuO, $Cu_2O$), $Fe_2O_3$, MgO, $TiO_2$ and ZnO [7]. Many researchers suggested that MNPs are especially efficient to eliminate Gram-negative bacteria but show limited effect against Gram-positive bacteria, as shown in Figure 1. But as mentioned above, cooperating with other material may bring more outstanding sequence. Among the metals listed before one of the hottest metals is Ag, and many products and formulations containing nano-Ag have been commercial. However, the use of nano-Ag still remains controversial due to it may simultaneously cause damage to the human body.

#### 2.2.2. Nanozymes.

Nanozymes is a sort of nanomaterial which presents properties of natural enzymes. It exists both physical properties and enzyme-like activity and serves as a highly stable, low-cost substitute for natural enzymes. So far over 300 nanomaterial could be classified as nanozymes [8] and a new filed is even thus forming. Like the common enzymes, nanozymes could catalyze the bio-reactions conforming the same dynamical disciplines of enzymatic reaction. Of note is that this sort of enzyme-like substances does not need extra catalysis-functional groups [1]. $Fe_3O_4$ MNPs were proofed to show catalase-like activity by Yan, and chromogenic reaction was found in the substrates which show same phenomenon in terms of catalase.

## 3. The mechanism of anti-bacterial effect in terms of nanomaterials

### 3.1. Mechanism of organic substances' antibacterial effect

As stated above, it is hard to classify clear and general categories of mechanism in terms of organic substances partially because that different species may have different target and process. They may attack various parts of microbes such as cytomembrane and cell nucleus. Here mechanisms are detailed according to the species.

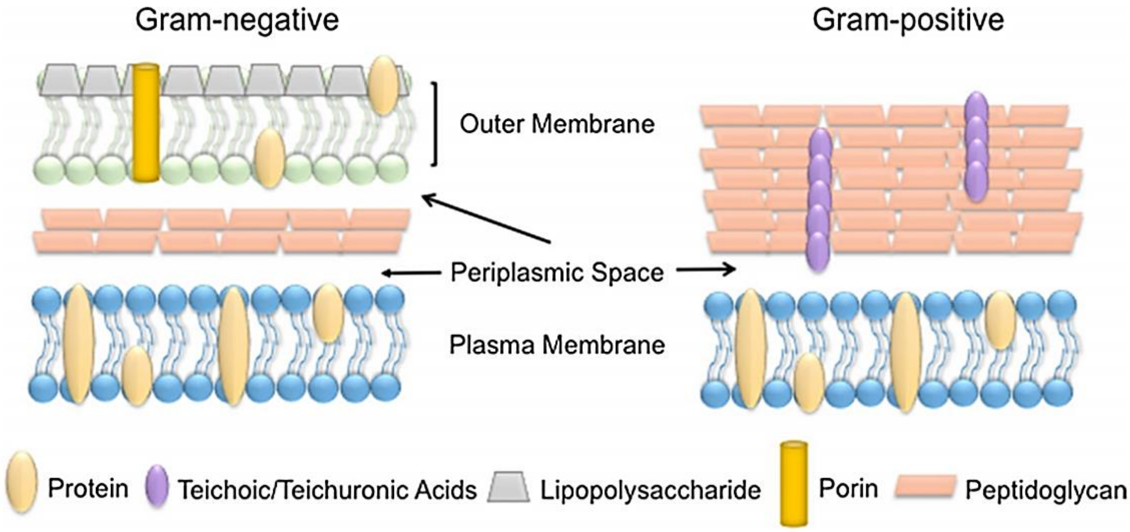


**Figure 1.** The structure of bacterial cytoderms [7].

### 3.1.1. Mechanism of antimicrobial peptide.

The mechanism of antimicrobial peptide could be briefly divided into two steps: the interaction with bio membrane and attack towards the intracellular components. Usually, the former step is paid more attention and related hypothesis also abound, so here the article mainly introduces some information about the transport or destroy mechanism of the bio membrane. However, of note is that rigidly speaking the complete mechanisms of the both steps still suffer controversies, and many debates such as whether specific receptors exist, whether other cooperative factors exist and other questions still remain unclear and require further exploration.

The primary step, namely the interaction with bio membrane includes two aspects: interaction with cytoderm and cytomembrane. Most bacteria could be divided into two categories: Gram-positive strain and Gram-negative strain mainly based on their cytoderm structure. Gram-positive bacteria contain a thicker layer of peptidoglycan than Gram-negative bacteria, but Gram-negative bacteria have an extra outer layer consisting of lipopolysaccharide. No matter for which strain of bacteria the peptidoglycan is one of the main components which contributes a lot to the homeostasis and integrity of the cell. The lipid II is the precursor for the synthesis of peptidoglycan [9], and some antimicrobial peptide such as bacitracin and vancomycin could selectively bind with lipid II so that to inhibit the synthesis of the component of the cytoderm [10]. Moreover, AMPs were also shown to be enable to destroy the formed cytoderm. One literature demonstrated that a sort of derivative peptide named RWRWRW-$NH_2$ could impede the respiration and delocalize the cytoderm protein MurG and thus makes the cytoderm malfunctioned [11].

The second sort of interaction with the bio membrane is the influence caused against cytomembrane. To build connection with the cytomembrane antimicrobial peptide needs to primarily get close to the surface of the cytomembrane. The electrostatic force plays an important role in the building of the connection. Due to the fact that cytomembrane of both Gram-positive and Gram-negative bacteria contains teichoic acid and lipopolysaccharides, which results in the distribution of negative charge in the surface of the cytomembrane. AMPs tend to be attracted by the microbes naturally for most of them are positively-charged. Few factors will generate effect on the strength of the connect such as the peptide molecular structure and its molecular weight, commonly with the increase of the molecular weight the connection is supposed to also increase [12]. Once the AMPs get connected to the surface of the cells, the next stages would happen successively. About the detailed mechanism of the interaction with the cytomembrane exist many models and hypothesis, such as barrel-stave model, toroidal-pore model, carpet model and detergent-like model [13]. These models could be divided into two streams: transmembrane pore models and non-membrane pore models [14], as shown in Figure 2. The former models refer to AMPs creating pores in the surface of the cytomembrane and thus destroying the membrane while the latter models refer to AMPs totally re-moulding the cytomembrane.

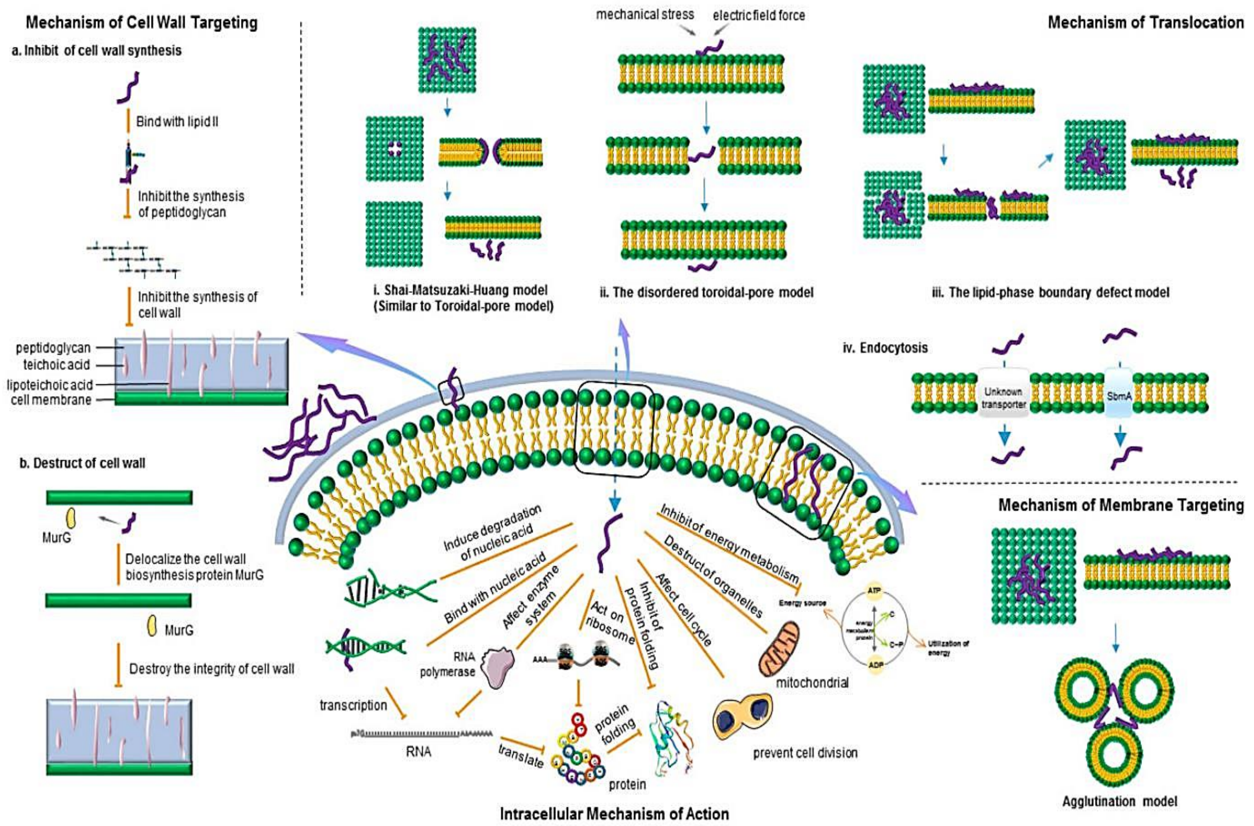


**Figure 2.** An overall view of mechanisms of anti-bacterial peptide [14].

Here one mechanism will be detailed in each stream. The barrel-stave model suggests that initially the peptide molecules could not insert into the cytomembrane smoothly in low concentration or their inserting behavior would be limited to certain degree. Till the threshold concentration is arrived, the peptide molecules then form oligomers with others and by which these new-generated substances may undergo the change of the solution namely slightly becoming more hydrophobic, and thus they could insert more deeply into the bilayer of the cytomembrane. When the peptide finally connects the both hydrophilic sides of the bilayer, a hole thus generated which means simultaneously the structure of the cytomembrane is also destroyed. Then the peptide could more freely enter the cells and continue the intracellular processes [15].

The carpet model suggests that primarily the AMPs gather in the surface of the cells and arrange parallel on the cytomembrane in the outer layer. The name comes from the distribution situation of the AMP on the surface of the cytomembrane which just alike a carpet. With the concentration arrives the critical value, the AMP molecules rotate and thus destroy the direction of phospholipid molecules, remarkably boosting the membrane fluidity, which resulting in the cell membrane splitting into separate parts and forming micelles. This process is also named as detergent-like model for the similar features of the detergent effect [16].

Recently in order to make better use of the AMPs, an increasing number of nanoscale drug delivery systems (nano-DDS) have been developed. Nano-DDS could bring many advantages compared to the free peptides. A literature summarized several points about the features of the nano-DDS used in the transport of the bacteriocin, which is one of the main streams of AMP[17]: (1) nano-DDS can improve AMP pharmacokinetics properties such as the solubility, charge and stability and thus improve the activity, shelf-life and even bioavailability of the medicine carried; (2) nano-DDS can improve bacteriocin effectiveness against bacterial resistance mechanisms such as helping the peptides fight against the degradative enzymes contained in microbes which may deactivate the medicine. (3) nano-DDS can deliver bacteriocins more efficiently to the diseased tissue sites either intracellularly or extracellularly. The literature also analyzed the related literature focusing on one certain nano-DDS and gave the statistics about the percentage of each substance: the liposome is the hottest nano-DDS following the chitosan nanoparticles, nanofibers and carbohydrate nanoparticles [17].

### 3.1.2. Mechanism of CNMs.

A plenty of mechanisms hypothesis have been suggested to try to explain the anti-bacteria or bactericidal effect of different types of CNMs [5]. Some of them are dimension-related and others are not. Below several mature and widely acknowledged mechanisms theories will be detailed.

The first is physical destruction. Maybe sound a little uncommon, but CNMs does be proved to enable to damage the bio membrane through physical process. This phenomenon is especially evident for 1D CNMs and 2D CNMs, while not so pure and persuasive for 0 D CNMs partially due to their effect is related to the change of structure of the bacterial proteins. In brief the 1D CNMs acts like "dart" which could "stick" the bio membrane of the microbes. This action could be proved by measuring the intracellular components lacking from the cell inside. Also, some researchers used pristine single-walled nanotube to conduct experiments in order to exclude the possibility of other process causing the same subsequence [18]. Some related SEM images could also proof the mechanism. 2D CNMs such as graphene oxide nanowalls could act like a "blade" or "knife" to "cut" the bio membranes of cells. By changing the orientation of 2D CNMs sheets namely turning more edges vertically towards the microbes the anti-bacterial effect even gets enhanced [19]. Furthermore, Gram-positive bacteria are more vulnerable to the contact with such 2D CNMs for their lackage of the outer extra membrane compared to Gram-negative bacteria. The volume or size of 2D CNMs could influence the efficiency of anti-bacteria, and the relationship presents like "the smaller, the better". Besides, the shaking frequency will also affect the action of the CNMs, as envisaged, the higher frequency provided, the more chances will be for the CNMs to collide with the cells and thus damage the bio membranes.

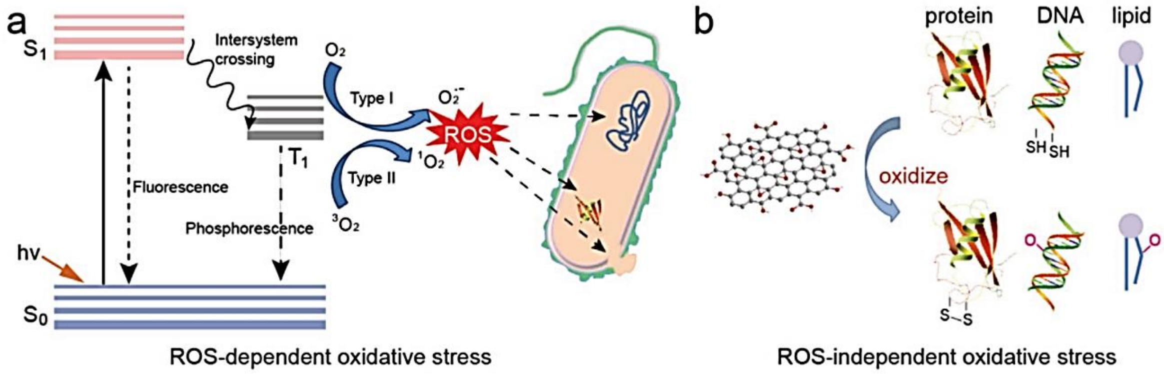


**Figure 3.** The mechanism of oxidative stress [5].

The second is Oxidative stress. Causing oxidative stress to the homeostasis of the microbes is another main mechanism for CNMs, as shown in Figure 3. CNMs could conduct oxidative stress to bacteria by producing reactive oxygen species (ROS) or not, which are two different mechanisms the article is going to detail. The former mechanism, named as ROS-related mechanism will initially undergo several photochemical steps. When CNMs are excited by the light, one electron located in the highest occupied molecular orbital (HOMO) will transfer to the lowest unoccupied molecular orbital (LUMO), after that, the molecule will proceed an intersystem crossing step to form a triplet excited state called T1. This moment, two approaches exist for the triplet excited state species to react with the $O_2$ to give ROS. The approach one is that one electron transfers from CNM to $O_2$ to generate superoxide anion $O_2^{-}\cdot$. And the approach two is that only energy is past from CNM to $O_2$ to form singlet molecular oxygen $^1O_2$, which owns higher energy than common oxygen molecule with also more harm to the organisms [20]. Moreover, although light plays an important role in the generation of ROS, when the light is inadequate or there is no light at all, this process could also happen. This time, the CNM serves as a channel or tool to help the electron transfer from the biological electron donors to $O_2$, resulting in damaging the intracellular components such as lipids and proteins.

Moreover, if the CNMs contain oxygen atom, for instance the graphene oxides, they can directly oxidize the substitute. Graphene quantum dots, graphene oxide and reduced graphene oxide have all been reported to exert such mechanism. On the other hand, although most of the time the ROS-related mechanism may play a dominant role in the anti-bacteria effect of CNMs, ROS-unrelated mechanism has also been observed and confirmed. Upon observing the bacteriocidal effect of $nC_{60}$, researchers found that neither oxygen nor light was provided and the ROS production was also hard to detected, which implying that a ROS-unrelated mechanism existing. One study has proved that nC60 could directly react with the cytomembrane and interrupt the electron transport chain of the respiration effect process. More similar situations could be presented in some graphene-based materials. For graphene oxide and reduced graphene oxide's treatment, little ROS species could be detected proving the existence of this mechanism. To be more detailed, these CNMs could impose oxidative stress towards GSH, which is an important amino acid for creatures. When cooperated with Cu film and Ge film, these CNMs could also show high anti-bacterial or bacteriocidal effect partially due to the fact that the films could serve as the electron receptor facilitating the electron transport process of the CNMs. CNMs could also accomplish anti-bacterial or bacteriocidal effect through photo-thermal process, which is another well-proved mechanism. When irritated by light, CNMs could back to ground state through non-radiative pathway and thus produce heat alternatively, which is named photo-thermal effect. This phenomenon was firstly observed in CNTs for its strong near-infrared absorption and high quantum efficiency for the conversion from light to heat effect [21].

The third mechanism is lipid extraction mechanism. Literally, some CNMs could penetrate into the bio membrane and extract its phospholipids out and thus achieve the destruction of the bio membrane. This mechanism has been proved in graphene oxide and graphene nanosheets. In the process of the extraction of the lipid, Van der Waals force and hydrophobic interaction works in order.

The last one mechanism is the isolation mechanism, which refers to some CNMs with large surface area could wrap the microbes and enact as a "prison" to refrain the bacteria from getting the nutrients outside the CNMs. This phenomenon was observed mainly in graphene nanosheets and other long SWCNT aggregates [22].

### 3.1.3. Mechanism of polymeric nanocomposites.

Polymer nanocomposites are drawing increasing interest in many fields including medicine field, cosmetics field and etc. The aim of utilizing polymer into nanomaterials is various, such as changing the solubility of nanomaterials and controlling its release. Nowadays the field which gets most researches is applying polymer to cooperate with metal or metal oxide nanomaterials, also, collaborating with peptide, CNMs is more and more studied by researchers.

Incorporating nanomaterials with polymer could confer the effective components many advantages. Also, polymer could bring more design flexibility, which is an excellent point making the nanomaterials suitable to tackle with wider requests. Most polymers are organic molecules, means that through adding functional chemical groups and other ways the properties could be manipulated in accordance to the detailed conditions. Currently some popular polymeric ingredients including alginate, chitosan and polylactic acid.

The effect polymer could bring compared to bold nanomaterials could be roughly divided into two sorts. The first sort is protection-controlling effect. For some nanomaterials such as anti-bacterial peptide as mentioned before, many internal environment factors could influence their efficiency and performance, which means a kind of substance is necessary to apply to cover or isolate the medicines in order to avoid advanced and invalid consumption. The substance must simultaneously request biodegradability for the release of the medicine. A potent antimicrobial peptide, named as PA-13 here would be taken as example. PA-13 is developed to deal with antimicrobial resistance. However, PA-13, is especially vulnerable to enzymatic degradation and the D-isomer is of very low efficiency [4], the group used chitosan and dextran sulfate is encapsulate the antimicrobial peptide to form an antimicrobial nano-network and thus prevent the PA-13 from directly contacting the degradable enzymes. Based on fluorescamine analysis the group reported the loading efficiency of PA-13 had reached approximately 77%. And the transplantation of PA-13 only resulted in a tiny increase of the hydrodynamic diameter and dispersity. Moreover, the followed experiments proved that the nanocomposites did not affect the metabolism of human organs meaning the nano-network is safe and not cytotoxic. As envisaged, even less than 1% of loaded peptide was released after 24 hours of applying, indicating the requested ambition was achieved. Given the fact that both PA-13 and chitosan show anti-bacterial effect, so there is no worry about the later release of the PA-13. On the other hand, for some nanomaterials which many provoke drastic side-affect, it is always difficult to control its dosage for such problem would be faced: low dosage will not maintain effective dose long without the assistance of the polymer matrix while higher dosage will put the patient at the risk of side-effect. So here the polymer could be utilized to control the release of the medicine, making the internal dose keep at an effective and safe degree also with long lasting time.

The other effect could be named as modification-enhancing effect. Conventional intravenous drug transport such as injection would transfer the medicine to both healthy issue and infectious site which needs cure, resulting in the low-efficiency and more side-effect subsequences. Also, some substances are limited by hydrophobicity and low-solution, or low bio-absorption and other problems which lead to low-efficiency of drugs. Here using polymeric additives could significantly change the related biologic and pharmacodynamic properties and improve their performance. One literature showed Cu nanoparticle-coated cellulose film exhibit strong anti-bacterial effect against *S. aureus* and *E. coli* [23]. The literature claimed that the rapid bacteriocidal effect was not only due to the release of the Cu ions, but also with contact killing. This may be attributed to the collaboration with the cellulose which serves as the material of the film. Furthermore, other metal and metal oxide nanoparticles have also been tried to cooperate with polymer to enhance their performance such as Ag NPs and ZnO NPs.

### 3.2. Mechanism of inorganic substances` antibacterial effect

#### 3.2.1. Mechanism of metal and metal oxide nanoparticles.

As one of the hottest fields of anti-bacteria, metal and metal oxide nanoparticles is draw increasing attention of researches and companies. More and more metals and their oxides are being proved anti-bacterial or bacteriocidal. As mentioned in introduction, the drug-resistance has become one of the most severe problems abusing antibiotics brings, which made the metal NPs gain enough importance due to the fact that metal NPs will not provoke drug-resistance partially because they kill bacteria through some most fundamental mechanisms which most of the mutations happened in bacteria will totally not help.

To build connection to bacteria and then implement anti-bacteria to target, like other medicine, metal NPs must get close to the surface physically. In solution metal atoms will show a trend to give electrons and enter the aqueous system to form hydrated ions, this phenomenon is especially typical in the surface of NPs. Then, as described before, both Gram-negative and Gram-positive bacteria`s surface are negatively charged, meaning that metal ions will naturally tend to appeal to microbes due to electrostastic force. Moreover, some studies showed that more factors, including Van der Waals force, hydrophobic interaction and receptor-ligand effect will all contribute to the connection building. However, only getting close to the bacteria bio membranes does not mean efficient penetration into the cell inside. Some studies reported, a kind of special bacteria showed metal NPs resistance especially for heavy metal species which originally show outstanding anti-bacterial effect [24]. The researchers suggested that these bacteria may exist in places which are heavily polluted by heavy metals and their compounds. They may generate unique immune mechanism via these approaches: (1) modifying the peptidoglycan layer; (2) conducting bio membrane repair; (3) implement ion sequestration by metabolism or proteins [7]. For this reason, collaborating metal NPs with other organic transport systems such as liposome, polymeric composites may achieve significant effect.

Theories about the anti-bacterial effect of metal NPs have been systematic and mature, three main mechanisms are oxidative stress, metal ions release and non-oxidative damage [25, 26, 27]. Below will introduce each in details.

The free radical, is a kind of chemical group which contains single electron and thus shows extraordinary chemical activity. According to the researches from a range of countries, although the categories of free radicals could be many, the strains which could generate malignant damage to cells mainly locate on five categories: the superoxide radical, the hydrogen peroxide, the hydroxyl radical, the singlet oxygen and the lipid peroxidase.

For aerobic bacteria, they also take oxygen to meet their energy needs just like human cells. Among all the oxygen absorbed, approximately 98% of the molecules are used to oxidize the organic substances or nutrient substances such as glucose. The other 2% of the oxygen would convert into oxygen free radical. In the normal conditions, these oxygen free radicals would not cause noticeable damage to the cells for in cells also exist some anti-oxidation substances such as vitamin E and vitamin C. these reductive components would neutralize the oxygen free radicals and prevent the cells from the harm of the free radicals, thus maintaining the homeostasis of free radical. However, metal NPs could act through single electron transfer methods or other ways to give single electrons, and then the electrons attack some substances such as oxygen and combine with the covalent bonds by which generating the free radicals. The free radicals would cause catastrophic sequences to the cells. The free radicals will influence the metabolism processes, making the cytochrome oxidase lose the ability to reduce the oxygen into the water, and thus the oxygen atom will conversely lose one electron and become free radical after other process which make it neutralized again. The oxygen free radical will affect the structure and functions of cytomembrane, changing the permeability of the biomembranes and thus destroying the homeostasis of the cells. Free radicals will also destroy the chondriosomes and thus block the energy source of the cells. Moreover, the attack towards the lysosomes could cause the cells autolysis. The extremely oxidative free radicals will also attack DNA and cause mutation and lack of genetic segments. Researches show that silver NPs could form hydroxyl radicals, the Au, ZnO and MgO NPs could generate peroxide, and $TiO_2$ could form both [28].

The second sort of anti-bacteria effect is due to the ions release of the metal NPs. The metallic ions generated could defunctionalize many important chemical groups such as mercapto, carboxyl and amino groups and proteins or amino acids. When the metallic ions attack functional proteins or amino acids, they could influence the bioprocess or metabolism process of the cells, which could surely lead to the death of the microbes. On the other hand, when the ions interact with the structural proteins such as some proteins embedded in the cytomembrane which take charge of the influx and efllux of the intracellular substances, they could affect the cellular structure and thus disturb the intracellular homeostasis and environment. However, the ions release effect of the metal NPs seems to be only an auxiliary effect of the overall anti-bacteria effect. Because if following the evident the pure metallic ion solution should be more effective than the solution of NP for it presents higher solubility and purity. However, some researches show that the metallic ion solution only incur limited anti-bacteria activity [29]. In fact, it cannot simply substitute the NP solution by the ion solution because what really matters may be the amount or concentration of the ion which truly enters the cell. In the solution the pure ions may undergo various kinds of chemical process such as hydrolysis, aggregation appealing other ligands and etc. However, for the metal NPs the situation is somewhat different because the NP could enter the cell through endocytosis or channel protein if the size allows, and thus the essential ion concentration could even be higher than the pure metal ion solution. However, to give a more persuasive answer to this question more evidence and studies are needed. The anti-bacteria effect of NPs is also determined by the category of the metal. Taking the difference between Cu and Ag for example. In solution conditions, Cu NPs could release approximately as 253 times number of ions as Ag does and also show higher anti-bacteria effect than Ag [30], however, it is widely acknowledged that Ag NPs present better anti-bacteria effect than Cu NPs. If it makes sure that the same number of NPs are provided for both metals, it could easily draw a conclusion that Ag presents higher anti-bacteria effect than Cu does [30]. The anti-bacteria effect could not be simply compared by the trend of ion release, but also according to the chemical or biochemical properties of the metals. For creatures, Cu is a kind of essential element which is required for enzyme construction while Ag is harmful for cells for it is a sort of heavy metal. Our cells could actually bear higher concentration of Cu ions than that of Ag, which implying that cells possess certain mechanism which could utilize that kind of metal ion, either consuming them or controlling the concentration or number itself. For instance, when $Cu^{2+}$ binds with cysteine, it will be reduced in $Cu^{+}$ with a concomitant production of cystine, which is the oxidized dimer of cysteine, then after other process the $Cu^{2+}$ will be returned, and this process will surely reduce the toxicity of Cu ions [31].

Besides the existed two kinds of mechanisms mentioned above, there also lies a third kind of mechanism. Some sorts of metal or metal oxides may not present anti-bacteria effect by themselves or in other words, more mechanisms besides the mentioned two kinds contribute to their anti-bacterial effect, but they could collaborate with the given immune system of human or animals to achieve anti-bacteria effect. Some NPs could cooperate with the macrophages and neutrophils to accomplish the anti-bacteria effect. Macrophage and neutrophil are two sorts of immune cells in human body which own the ability to phagocytose the antigens or microbes. Some kinds of metal NPs, when coated by plasma proteins or other proteins which could interact with the receptors in the surface of the immune cells mentioned above, could enter the immune cells. Then the immune cells will be killed by the NPs and thus organize the extracellular traps, which have the anti-bacterial functions. The generation of extracellular traps is also a kind of sterilization mechanism [32]. More special mechanisms may exist as well and could be classified into this category.

### 3.2.2. Mechanism of nanozymes.

As stated, nanozymes just function like natural enzymes in human body, and their catalysis mechanism also comply or be similar to that of natural enzymes. One tool to evaluate the activity of nanozymes is Michaelis-Menten catalytic kinetic function, where kcat and KM are used to describe the property of nanozymes as well.

According to the reactions nanozymes catalyze, nanozymes could be classified into different groups: hydrolase, peroxidase, superoxide dismutase, oxidase, catalase and other groups. There nanozymes are mostly based on one or one series of substrate.

Hydrolase works similarly to phosphodiesterase (PDE), which catalyzes the hydrolysis of phosphodiester bonds through cleavage of phosphate bonds of cyclic 3,5 adenosine monophosphate and cyclic 3,5 guanosine monophosphate. One group anchored triazacyclononane units on the surface of gold NPs to stable $Zn^{2+}$ ions so that to simulate PDE's function, and they tested the efficiency of that kind of nanozyme by the substrate of 2-hydroxypropyl p-nitro-phenyl phosphate [33]. The result showed the activity of nanozyme was significantly improved compared to $Zn^{2+}$ chelated triazacycoinonane.

Peroxidase mainly catalyze the oxidation of substrates with the collaboration of peroxides including $H_2O_2$. One renowned nanozyme of this strain is $Fe_3O_4$, which was firstly demonstrated by Yan and his group. The $Fe_3O_4$ magnetic nanoparticles could catalyze DAB, OPD and other molecules like horseradish peroxidase [34]. In terms of action of this nanozyme, researchers have suggested "Ping Pong mechanism" referring that one product is generated and released completely before another substrate's reaction beginning.

Other nanozymes are all based on mimicking one particular biomolecule or existed enzyme, knowing the original mechanism will also help to understand the nanozyme's process. Recently, some groups are even trying to develop nanozymes with a multi-substrate mechanism. Jiang and his colleagues studied many nanozymes with multi-substrate which includes metal-based nanozyme, $Cu_2O$ nanozyme, nanoceria, melanin nanoparticles and Prussian blue NPs. One literature also suggested several methods to enhance the performance of nanozymes including increasing the roughness of the surface to promote the bacteria-combination ability, applying cascade reaction to reduce the toxicity and utilizing the light to regulate the activity of nanozymes.

## 4. The application of anti-bacterial nanomaterials

Anti-bacterial nanomaterials have been applied into a wide range of fields due to its biocompatibility, environment-friendly, high-efficiency, low cost and green-source, as shown in Figure 4. One of the most outstanding properties nanomaterials possess over other anti-bacterial agents is that they will not provoke magnificent drug-resistance, which makes them become a completely new generation of anti-bacterial agent. Below some current applications will be detailed.


**Figure 4.** Partial application fields of nano anti-bacterial agents.

### 4.1. Against hospital acquired infections (HAIs)

HAIs refer to all infections happened in hospital, including all hospital staff and patients getting infected in and after the period of being in hospital. HAIs caused tremendous damage to both the economics and public health care. A survey conducted by WHO reported that averagely 8.7% peoples suffered by nosocomial infection and approximately 1.4 million people have been influenced [35]. Moreover, recently the advent of bacterial drug-resistance has given rise to the HAIs because traditional antibiotics could no longer work as efficient as before. A large number of anti-bacterial agents have utilized in the fight against HAI, including peptide, metal nanoparticles and other categories. Among these agents, Ag nanoparticles have been paid immense interest. Ag nanoparticles gain plenty of advantages such as high-efficiency and etc. Of note is that researchers have developed methods that use biological route to synthesis the Ag nanoparticles, making the Ag nanoparticles even get lower cost and less cytotoxic with the enclosure with the biomass. Ag nanoparticles could be added to other materials to perform better. A literature reported combining the Ag NPs and allicin to prevent skin infection [35], which achieved lower Minimum inhibitory concentration (MIC) and Minimum bacterial concentration (MBC).

As infectant, anti-bacterial materials could be used in many fields containing cosmetics industry, medicine industry, environment disinfection, hospital application, agriculture, dental care and etc. However, current trend of developing NMPs is not only focused on exploring more categories of them, but also modifying existed anti-bacterial agent and promoting the synthesis route. Take the Ag NPs for example, despite many advantages, Ag NPs could not avoid the high-toxicity and other disadvantages. Therefore, it is also a potential researching direction for companies and institutes to pay enough attention to design more approaches to reduce the toxicity, optimize the synthesis routes or discover more sources.

### 4.2. Wound dressing

Physical wounds, especially chronic wounds impose more risks to the patients getting infected and sometimes even give extra burden to therapy protocol. Developing an advanced and biocompatible wound dressing material thus become especially crucial for health care. Nanomaterials have also been widely spread into wound dressing field for its higher porosity and more permeable to water and air.

One literature suggested a new-type material named nanofibrous dressing material, which is more porous, more permeable to both water, oxygen and inside metabolic wastes compared to traditional micro fibrous and mesh ones [36]. Furthermore, the nanofibers also make the dressing gain small interstices and higher surface are, which is beneficial to enhancing homeostasis and prevent bacterial infection and tissue ingrowth. Due to the higher surface area, nanofibrous wound dressing allows efficient drug load which is essential to wound healing.

Among a range of wound dressing materials, biopolymeric nanofibers show many advantages over other material such as better bioactivity and biodegradability. The substance used for wound dressing construction which gets most study is polysaccharides. One literature summarized common biopolymers suggested for wound dressing including collagen, gelatin, chitosan and alginate. The literature also stressed cellulose, as a naturally existed substance with wide source, it is especially valid for dealing chronic wounds. Researches showed that cellulose could stimulate granulation and epithelialization process so that accelerate the healing of wounds.

### 4.3. Food package

Food spoilage is one of the social problems which cause both economic loss and health threat, and it is also one of the reasons for food poisoning. The main factor causes food spoilage is the infection of bacteria and fungi. Researchers have developed various kinds of methods to avoid the food package for instance using additives. However, currently consumers show an increasing trend to require healthier or more natural additives, imposing challenge to traditional additives. Nanoencapsulation system [37] provided a completely new horizon for food producers to take advantage of additives more efficiently and healthily. With the application of nanoencapsulation, adverse connection with food including unexpected chemical reaction could be prevented, and the reservation of additives could be prolonged as well. Moreover, it could manipulate the additives to gather in sites where bacteria and fungi are more possible to locate. Also, as mentioned, cooperation with nanoencapsulation could change or improve the absorption and permeability of additives, bringing higher sterilization ability and less side effect. These nanoencapsulations include nanoemulsions, nanoparticles, nanoliposomes and nanofibers. Marra and his colleagues added ZnO nanoparticles to PLA films and thus improved their barrier property and mechanical properties, with also anti-bacteria. The experimental results presented that PLA contained 5% of ZnO nanoparticles showed lower penetration for oxygen and carbon dioxide, and outstanding anti-bacterial activity for *E. coli* [37].

### 4.4. Textile application

Textile anti-bacteria is another growing field which requires the nano-antibacterial technology. When textile, or clothing contacts human body, due to the large surface which guarantees oxygen, water, heat and nutrients from human skin, it easily becomes an ideal environment for the reproduction and growth of bacteria, which will adversely influence the reservation of textile and health of the human body. Although a series of anti-bacterial agent have been developed to tackle with the above phenomenon, many conventional anti-bacterial agents are blamed for side effects including high-toxicity and low skin-comfort-level [38]. On the other hand, inorganic nanomaterials have gained increasing interest of researchers and organizations, as shown in Figure 5. Most of the inorganic materials are metal NPs and metal oxides such as titanium dioxide nanoparticles, silver nanoparticles and gold nanoparticles and etc. Recently, some organic nanomaterials have also been utilized to collaborate with those metal nanomaterials in order to modify their biocompatibility and safety, and these ingredients include nanoliposome and cyclodextrin and nanofibers.


**Figure 5.** Partial inorganic based nano anti-bacterial agents [39].

## 5. Conclusion

This research summarized partial mostly acknowledged hypothesis of the mechanisms and currently mostly applied fields of the nano anti-bacterial agents including organic stream and inorganic stream. And in order to support the opinions, some representative studies and research examples have been provided. The readers should not be limited to the current application fields due to the fact that the mechanism research for anti-bacterial agents would be developed and synthesized, and thus the application field would be broadened as well.

The combination of nanotechnology and anti-bacterial materials has suggested a new direction for the development of medicines and other related subjects. And it showed a possibility to take place of the conventional drugs due to its various advantages such as higher efficiency, lower cost and lower possibility to provoke drug-resistance. Of note is that with the maturation of the field, more and more creatives opinions are being mentioned. Undoubtedly, developing more categories of new-type nano anti-bacterial agents will keep being focused, such as many natural antimicrobial peptides derived from animals and plants still face limitations could not being produced in industrial scale. Ideas from other aspects should also be paid attention. These include utilizing current nano anti-bacterial of more efficiency (for example, combine them with polymeric substances of matrix); applying nano anti-bacterial agents into more fields; illuminating the mechanisms of nano anti-bacterial agents and dealing with the potential risks of using nano anti-bacterial agents. This is a new field growing quickly and flourishingly, but will in the future it will face the results of abusing the nano anti-bacterial agents as well?

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