Surface Functionalization of Gold Nanoparticles for Physical Antibiotics Coping with Antibiotic-Resistant Bacteria

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Abstract: Surface functionalization of gold nanoparticles is theoretically investigated in order to develop physical antibiotics coping with antibiotic-resistant bacteria. The core concept of the physical antibiotics is based on the design strategy utilizing molecular elements in peptidoglycan for surface functionalization of gold nanoparticles. These gold nanoparticles will couple to peptidoglycan easily during the proliferation or growth of the bacteria, leading to the destruction of cell walls. Additionally, physical fields (electromagnetic wave, X-ray or ultrasound) bring enhanced suppression of the proliferation. This strategy allows us to have wide variety of antibiotics designs and the suppression against the outbreak of antibiotic-resistant bacteria.

Keywords: Gold nanoparticles, antibiotics, antibiotic-resistant bacteria, peptidoglycan, cell wall, electromagnetic wave, X-ray, ultrasound.

1. INTRODUCTION

Recently nanotechnology is widely recognized as leading innovations in various fields such as advanced electronic devices, material science and medical science. Especially, nanoparticles will bring many important applications in future. For example, photovoltaic applications using silver based QD (quantum dot) [1], intracellular tracking technology [2] and cancer treatments [3] using gold based nanoparticles are promising.

In this paper surface functionalization of gold nanoparticles is theoretically investigated towards advanced and powerful structure of physical antibiotics. The concept of physical antibiotics using electrically conductive nanoparticles appeared in 2005 [4] as a challenge to antibiotic-resistant bacteria. The main feature is that the high-temperature of nanoparticles under AC magnetic fields will drill into cell walls of the bacteria and consequently destroy the bacteria.

Conventional antibiotics like penicillin [5] and vancomycin [6] are powerful medicine to cure fatal diseases caused by bacteria. However, there are remaining issues such as their harmful side effects and outbreak of multiple antibiotic-resistant bacteria such as MRSA (Methicillin-resistant Staphylococcus aureus) and vancomycin-resistant bacteria. Recently a new antibiotic (teixobactin) was developed by taking advantage of weakness in the defenses of pathogens [7]. However it will take some time to evaluate the time scale for the suppression of emerging antibiotic-resistant bacteria.

The core concept of the physical antibiotics in this paper is based on the design strategy utilizing molecular elements in peptidoglycan for surface functionalization of gold nanoparticles. These gold nanoparticles will couple to peptidoglycan easily during the proliferation or growth of the bacteria under the effect of autolytic enzyme [8] and synthesizing enzyme as shown in Figure **1**. Conventional antibiotics such as penicillin and vancomycin inhibit the peptidoglycan formation process of the targeted bacteria. On the contrary, the physical antibiotics in this paper utilize the established peptidoglycan formation process of the bacteria in order to introduce gold nanoparticles. This novel strategy (symbiosis with bacteria) allows us to have wide variety of antibiotics designs and the suppression against outbreak of antibiotic-resistant bacteria. The detailed reasons for the two advantages will be explained in Section 2.

The introduced gold nanoparticles in peptidoglycan will disturb crystalline process of peptidoglycan by forming amorphous region around them, which will reduce the capacity to resist pressure from inside of cell walls. Usually peptidoglycan has a crystalline structure that is resistant against high osmotic pressure inside the cell, which may reach 20 atmospheres in the case of gram-positive bacteria [5]. So the nanopartcles will act as destruction triggering points of cell walls. In addition, gold nanoparticles can be utilized as energy sources of physical energy under the fields of electromagnetic wave, X-ray or ultrasound to damage cell walls or chromosomes, leading to the suppression

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of bacteria growth and proliferation. The detailed mechanism will be discussed in Section 3.

As is described in the reference [2], it is assumed that the size distribution of the gold nanoparticles is between 10nm to 15 nm. It is recommendable that the size of nanoparticles should be less than the thickness of peptidoglycan in order to disturb the crystalline structure from the inside of cell walls. The size of the nanoparticles is an important design factor depending on the applications (photovoltaic application [1]: around 10 nm, gene analysis [9]: around 5 nm).

A possible synthesis method for surface functionalized gold nanoparticles is by using chemical reactions between gold nanoparticles and thiol (sulfur) with molecules relating to peptidoglycan. In the case of surface functionalization with high polymers, block or graft polymerization are applicable.

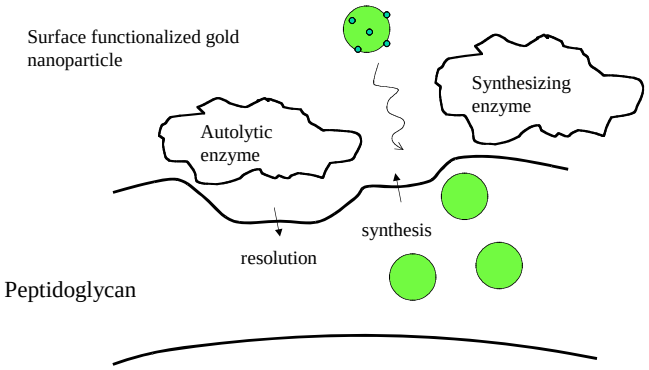

Figure 1: Schematic representation of coupling between gold nanoparticles (10-15 nm) and peptidoglycan (20nm – 80nm) during the proliferation or growth.

2. DESIGN STRATEGY FOR PHYSICAL ANTIBIOTICS

The peptidoglycan consists of structural units shown in Figure **2**, and the synthesis of the peptidoglycan is summarized in the following consecutive five stages [5].

1. A peptide unit is built on NAM (N-acetyl-muramic acid) while the sugar is attached to uridine diphosphate.

2. The NAM-peptide unit is transferred to a carrier lipid.

3. NAG (N-acetyl-glucosamine) and the pentaglycine bridge are added to the NAM-peptide unit while it is attached to the carrier lipid.

4. The disaccharide peptide unit is transferred from the carrier lipid to a growing polysaccharide chain.

5. Different polysaccharide strands are cross-linked by a transpeptidation reaction involving the pentaglycine bridges.

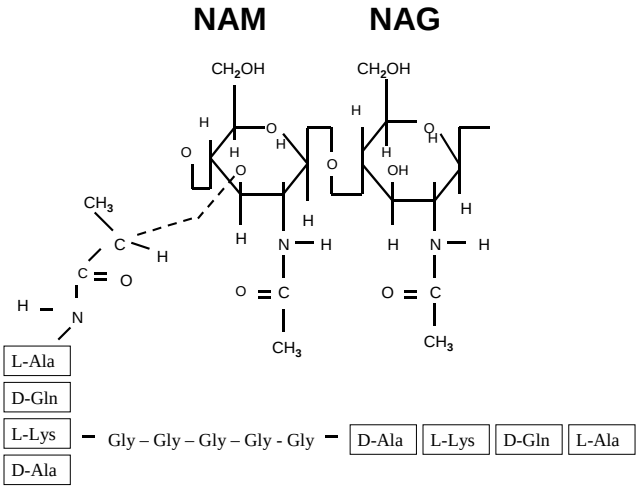

Figure 2: Basic structural unit of peptidoglycan of *Staphylococcus aureus.* As peptidoglycan has the periodical crystalline structures, it is strong and resistant against high osmotic pressure of the bacteria cell.

The above synthesis can be expressed in the simplified biochemical equation as follows:

$$\sum \mathrm{pg} + \sum \mathrm{m(inside)} \rightarrow \sum \mathrm{pg} + \sum \mathrm{m(outside)} + \sum \mathrm{e(+)} \rightarrow \mathrm{PG\ (cell\ wall)}, \quad (1)$$

where Σpg is group of molecular elements in peptidoglycan, $\sum \mathrm{e(+)}$ is synthesizing enzymes such as glycopeptide transpeptidase, Σm (inside) is the group of molecules existing inside of the cell such as ATP (Adenosine triphosphate) and UDP (Uridine diphosphate), Σm (outside) is the group of molecules existing outside of the cell such as diphosphate-carrier lipid, and PG is the cell wall (three dimensional structure of peptidoglycan).

It is possible to design the structure of the physical antibiotics based on the following equation, i.e.

$$\mathrm{Au-S-(Molecules\ for\ surface\ functionalization)} \in [\sum \mathrm{pg} + (\sum \mathrm{pg}) * (\sum \mathrm{m(outside)})], \quad (2)$$

where S is sulfur, $\in$ is the symbol for expressing possible choice from the group [ ] and * is the chemical coupling between molecules. Sulfur is appropriate for making conjugation between gold nanoparticles and organic molecules.

The reason for establishing design strategy by the equation (2) is that the surface functionalization expressed by [Σpg + (Σpg) * (Σm (outside))] will bring the smooth and strong chemical coupling between gold nanoparticles and growing peptidoglycan layers in the metabolism process. Furthermore this approach brings the two advantages. They are the variety of antibiotics designs and also the suppression against emerging of antibiotic-resistant bacteria. The first advantage is due to the large number of possible combination expressed in the equation (2). The examples are explained in Section 4. The second advantage of the physical antibiotics is brought by the prediction that the emerging probability of antibiotic-resistant bacteria is small, as they utilize the stable and established synthesizing process of forming peptidoglycan in the bacteria. Even if the outbreak of antibiotic-resistant bacteria happens, it is easy to design new antibiotics based on the equation (2).

3. THEORETICAL CONSIDERATION ON THE MECHANISMS OF THE PHYSICAL ANTIBIOTICS

3.1. Reducing the Strength of Cell Walls Against Osmotic Pressure

Although the normal peptidoglycan layers have strong crystalline structure, the periphery of the gold nanoparticles will form non-crystalline (amorphous) structure shown in Figure **3** schematically. The comparison between the crystalline and amorphous region in terms of strength against high pressure is predictable based on the following equation [10]:

$$K = 3k_B T / b^2, \quad (3)$$

where K is the entropy spring constant for the polymer, k_B is Boltmann constant, T is absolute temperature and b is the macroscopic size of segment. The “b” is defined as normalized distance factor of Gaussian distribution for segment edge vector which relates to the randomness of the polymer. As “b” in amorphous region is much larger than that of crystalline region, K hence the strength of polymers in amorphous region is much smaller compared to the normal peptidoglycan region. Consequently, gold nanoparticles will trigger the destruction of cell walls, leading to the extinction of the bacteria.

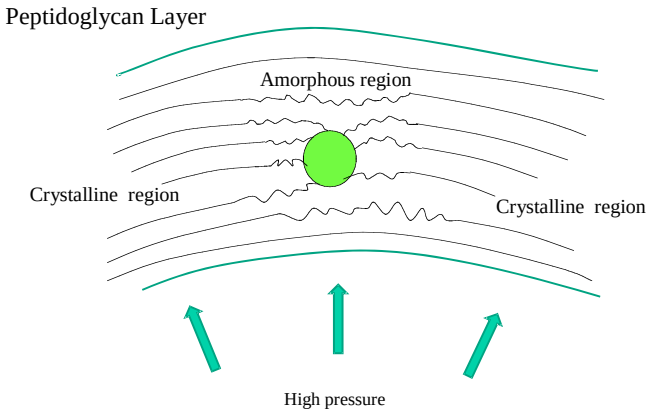

Figure 3: Gold nanoparticles are destruction points in cell walls, as the periphery forms amorphous region.

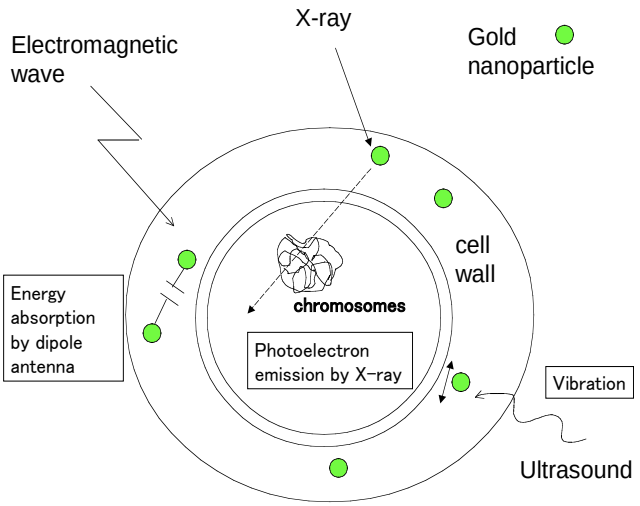

Figure 4: Schematic representation of the three mechanisms for suppressing proliferation of the bacteria. They are temperature rise of cell wall by the absorption of electromagnetic wave in the dipole antennas, damage of chromosomes by the emission of photoelectrons and the structural damage of cell wall by the ultrasound vibration.

3.2. Utilization of Physical Field to Suppress the Proliferation of the Bacteria

Besides the structural degradation of peptidoglycan, there are three effects by exposing gold nanoparticles under physical fields schematically shown in Figure **4** [11]. The fields are electromagnetic fields, X-ray radiation and ultrasound, and the mechanism of bacteria destruction is explained as follows.

Under the electromagnetic fields, the nanoparicles introduced in cell walls act as dipole antennas that will absorb electromagnetic wave efficiently. This phenomenon will cause temperature increase of nanoparticles, which will damage cell walls of bacteria.

Gold nanoparticles will emit high energy electrons by photoelectric effect under X-ray exposure. These photoelectrons will bring about damage of chromosomes in bacteria, as the energy dissipation of photoelectron by interaction with DNA is very high.

By applying ultrasound, the nanoparticles in cell walls will have large vibration force as the density of gold is very large compared to the surrounding peptidoglycan. Consequently the gold nanoparticles emit friction heat and acceleration forces leading to the destruction of cell walls.

4. EXAMPLES OF SURFACE FUNCTIONALIZATION FOR PHYSICAL ANTIBIOTICS

In this section, four examples of the physical antibiotics are shown based on the equation (2).

Example 1

The gold nanoparticle having glycine shown in Figure **5** can be coupled with D-alanine in the growing pepetidoglycan with the help of glycopeptide transpeptidase. As there are many glycine molecules on the surface of gold nanoparticles, the normal process of crystalline peptidoglycan growth will be disturbed and the amorphous region around them will be formed, which will reduce the capacity to resist pressure from inside of cell walls.

Au – S – Gly

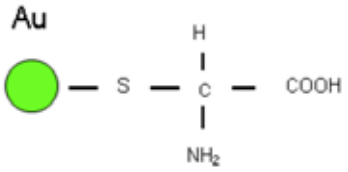

Figure 5: Surface functionalization of Σpg type: Au-S-Glycine.

Example 2

Similarly, the gold nanoparticle having D-alanyne shown in Figure **6** can be coupled with glycine in the growing pepetidoglycan with the help of glycopeptide transpeptidase.

Au – S - D-Ala-D-Ala

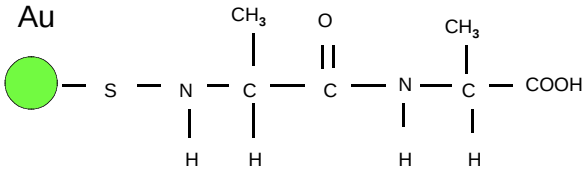

Figure 6: Surface functionalization of Σpg type : Au-S-D-Alanine-D-Alanine.

Example 3

This surface functionalization shown in Figure **7** is effective for vancomycin-resistant bacteria based on the analysis of peptidoglycan of the bacteria [6]. As this structure has the lactic acid part, the gold nanoparticle can couple with the peptidoglycan of vancomycin-resistant bacteria.

Au – S - D-Ala - Lact

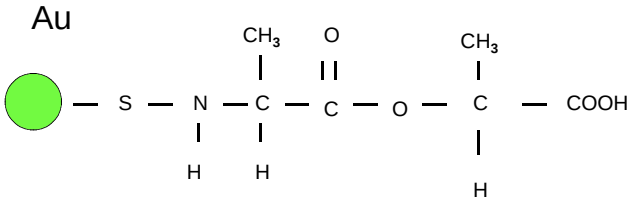

Figure 7: Surface functionalization of Σpg type: Au-S-D-Alanine-Lactic Acid.

Example 4

In the surface functionalization as shown in Figure **8**, the activated carbon C1 of NAM in this structure will couple to the activated carbon C4 of NAG part of the growing peptidoglycan utilizing the release energy of diphosphate. So that the normal process of crystalline peptidoglycan growth will be disturbed and the amorphous region around them will be formed, which will reduce the capacity to resist the pressure from inside of cell walls.

Au-NAM-diphosphate

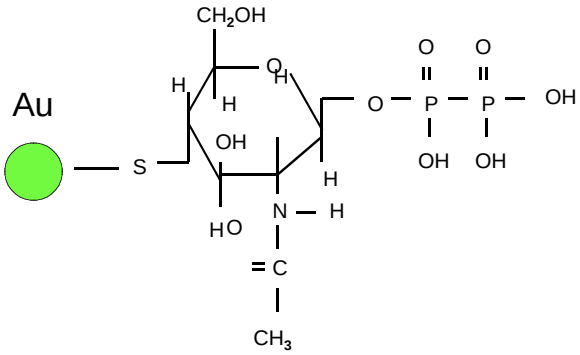

Figure 8: Surface functionalization of (Σpg) * (Σm(outside)) type: Au-S-NAM-diphosphate.

5. CONCLUSIONS

The major conclusions resulting from theoretical consideration in this paper are as follows.

Surface functionalization of gold nanoparticles by utilizing molecular elements in peptidoglycan will open new physical antibiotics. The structure brings the variety of antibiotics designs and also the suppression against outbreak of antibiotic-resistant bacteria. The first advantage is due to the large number of combination expressed in the equation (2). The second advantage of the physical antibiotics is brought by the prediction that the gold nanoparticles with surface functionalization based on equation (2) tend to be introduced into cell walls smoothly without blocking the fundamental metabolism of the bacteria.

It is recommendable that the size of nanoparticles should be less than the thickness of peptidoglycan in order to disturb the crystalline structure from the inside of cell walls.

As the gold has large electrical conductivity, mass (6086 kDa for gold particle with diameter of 10 nm) and X-ray absorption coefficient (7.256 cm^2/g at the photon energy of 50keV) compared to the organic elements [11], we can utilize large interaction between the gold nanoparticles and the physical fields of electromagnetic wave, ultrasound and X-ray for suppressing growth or proliferation of the bacteria.

In order to deepen and develop the theoretical considerations in this paper, further studies and technologies are especially needed as for the following points.

- Coupling experiments between surface functionalized gold nanoparticles and living bacteria.
- Computer simulation for chemical reaction between growing peptidoglycan and surface functionalized gold nanoparticles
- Theoretical analysis and experimental investigation for bacteria having thin peptidoglycan layers such as gram-negative bacteria and acid-fast bacillus.
- The dependable and reproducible techniques for the surface functionalization of gold nanoparticles and accurate measurement methods for the surface condition of gold nanoparticles.

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