Surface Functionalization of Gold Nanoparticles for Antiviral Medicines by Simulating the Surface Structure of Host Cells

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Abstract: Surface functionalized gold nanoparticles that mimic the surfaces of host cells are proposed for antiviral medicines and the strategic design is theoretically described. As for the interaction between the gold nanoparticles and the viruses, the former acts as the adhesive balls for viruses. This will lead to gathering viruses and forming clusters or chains of them, bringing about four-stage antiviral mechanisms. The mechanisms are the interference against budding, reduction of diffusion velocity, inhibition of entry into host cells and the damage to RNA/DNA by X-ray exposure.

Keywords: Influenza, virus, NANA (N-Acetylneuraminic acid), endocytosis, HA (hemagglutinin), NA (neuraminidase).

1. INTRODUCTION

There are many diseases caused by viruses, such as influenza, AIDS (Acquired Immune Deficiency Syndrome), measles, hepatitis, Ebola hemorrhagic fever and MERS (Middle East respiratory syndrome). So far, various medicines were developed to cope with these virus-related diseases. However, we are always facing emerging new types of viruses which may cause fatal diseases.

In order to develop effective antiviral medicines in a comparatively short time scale based on simple guiding principle, surface functionalization of gold nanoparticles by simulating the surface structure of host cells is proposed in this paper. As for the surface functionalization of gold nanoparticles, there are interesting applications in the field of cell biology, such as intracellular tracking [1], cancer treatment [2] and antibiotics [3]. Gold is focused as an example for nanoparticle materials in this paper, however $AgInSe_2$ is also promising material [4].

Although the originality of this paper is not based on our own experimental data, we found theoretical breakthrough for designing novel antiviral medicines by combining advanced nanotechnology and recent outcomes in virology.

The strategy of the antiviral gold nanoparticle is initiated by understanding of the usual infection process of viruses into host cells. The basic structure of antiviral gold nanoparticles has surfaces that mimic the surfaces of host cells. The detailed surface structure will be presented in Section 2. As for the interaction between the gold nanoparticles and the viruses, the former acts as the adhesive balls for viruses in order to gather viruses and form clusters or chains of them. Based on the novelty of this adhesive scheme, the advantage of the antiviral gold nanoparticles arises from the four-stage effective interactions with viruses, as is shown in Figure **1**.

The brief explanation as for the four stages is given as follows:

Stage 1: Interference against budding

As the antiviral gold nanoparticles act as adhesive balls for viruses, it is possible to block the smooth budding by forming clusters or chains of viruses.

Stage 2: Reduction of diffusion velocity

As the diffusion velocity of clusters or chains of viruses are smaller than that of single virus, the infection transfer among host cells will be delayed.

Stage 3: Inhibition of entry into host cells

From the point of viral entry energy into host cells, it is very hard for the clusters or chains of viruses to enter into host cells.

Stage 4: Damage to RNA/DNA by X-ray exposure

Under the exposure of X-ray, the DNA or RNA of viruses attached by gold nanoparticles will be damaged.

The detailed explanation will be given in Section 3.

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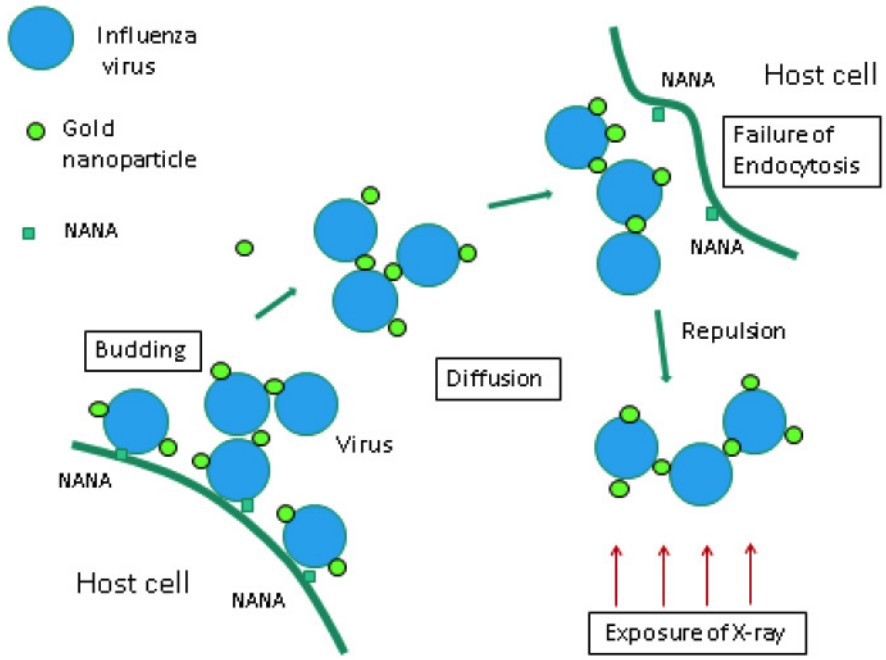

Figure 1: Schematic representation for four-stage (budding, diffusion, endocytosis, and X-ray exposure) strategy against influenza viruses. NANA (N-Acetylneuraminic acid: $C_{11}H_{19}NO_9$) represents the receptor of host cells.

2. DESIGN STRATEGY FOR ANTIVIRAL MEDICINES

In this section, the design strategy for antiviral drugs using surface functionalization gold nanoparticles is described theoretically.

The core concept of the surface functionalization can be expressed by the following symbolic equation:

(Au) - [Conjugation] - [Transcellular] assist- [Cell membrane] hydrophobic- [Receptor] authentic/mimic (1)

where

[Receptor] authentic/mimic represents the receptor structure itself or molecules which are similar to the receptors of the host cells,

[Cell membrane] hydrophobic represents the part of hydrocarbon chain in the membrane,

[Transcellular] assist represents molecules which will enhance the transport through cell membranes, and

[Conjugation] represents conjugating atoms or molecules such as thiol type -S- [1] or amid coupling -NHCO- type [5].

The detailed explanation as for the background of equation (1) is given as follows.

[Receptor] authentic / mimic represents molecules which are similar to the receptors of host cells. For example, the receptors of HIV and influenza virus are CD4 and NANA(N-Acetylneuraminic acid: $C_{11}H_{19}NO_9$) respectively. In this paper we will focus on the case of influenza A type viruses.

In the first stage of influenza virus infection, the envelope protein hemagglutinin (HA) will firstly couple with the receptors (NANA) on the surface of host cells. Therefore, the surface functionalization of NANA will play the key role of initial adhesion between viruses and gold nanoparticles. Although the influenza viruses have another envelope protein NA (neuraminidase) which will try to remove the NANA, it will be possible for gold nanoparticles to sustain the function of adhesion balls, based on the following consideration.

As the surface functionalization structure of molecules ([Transcellular] assist - [Cell membrane] hydrophobic - [Receptor:NANA] authentic/mimic) are distributed three dimensionally on the surface of gold nanoparticles and the number of NA is usually smaller than that of HA per a virus [6], it is rather difficult for the NA on the surface of the virus to remove all of the NANA existing on the gold nanoparticles.

In order to make the gold nanoparticles function as effective adhesion balls, the optimization for the size of gold nanoparticles is very important. Although the

effectiveness of adhesion depends on the number of surface functionalization molecules, coupling probability between viruses with the help of gold nanoparticles will increase when the diameter of the gold nanoparticle (D) is more than the twice length of HA (L_{HA}), as is shown in Figure **2**.

$$D > 2\,L_{HA} \quad (2)$$

The requirement of equation (2) is explained as follows.

If the diameter is less than the twice length of HA, the remaining adhesive surface area of a nanoparticle firstly coupled by a virus will be almost shadowed from the sight of the second coming virus. As the L_{HA} is about 13.5nm [7], D should be larger than 27nm.

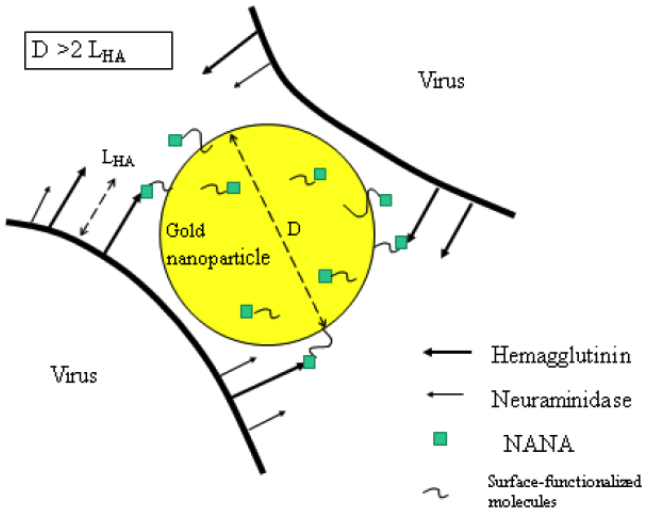

Figure 2: Schematic representation for the coupling between two viruses by a surface-functionalized gold nanoparticle. The NANA molecules on the gold nanoparticle play an important role for the adhesion to the hemagglutinin of the viruses. If the diameter D is less than the twice length of HA, the remaining adhesive surface area of a nanoparticle firstly coupled by a virus will be almost shadowed from the sight of the second coming virus. For the next coupling the condition of equation (2) is necessary.

[Cell membrane] hydrophobic represents molecules which are similar to cell membrane. The structure --(CH_2)n-- is an example. In the process of entry of viruses into host cells, conformation of HA will take place and then couple with cell membrane through fusion peptide [8]. Consequently, the coupling between gold nanoparticles and viruses will be strengthened by this process. Another merit of this part is enhancing the flexibility in direction of molecular branch due to sigma coupling of (CH_2)n type, which will lead to the increase of coupling probability between gold nanoparticles and viruses.

[Transcellular] assist is an essential part for oral medicines, as it is necessary to make nanoparticles pass through the cells in intestinal walls easily. In our case the molecules of [Transcellular] assist will function for the purpose. A promising example is PEG structure in the research of cellular tracking [1]. Additionally, the hydrophobic part of [Cell membrane] hydrophobic, such as lipid molecules will also enhance the transcellular effect in intestinal walls.

3. ANTIVIRAL MECHANISM IN FOUR STAGES

Four antiviral mechanisms are summarized as follows.

Stage 1 (Interference against budding)

As the antiviral gold nanoparticles act as adhesive balls for viruses, it is possible to block the smooth budding by forming clusters or chains of viruses, as follows. Initially, the gold nanoparticles having many NANA will couple to HA on newly born viruses, while NA on the viruses will try to remove the NANA molecules on the gold nanoparticles in addition to the NANA attached to the viruses. So that the removing process against increased NANA (both on the surfaces of gold nanoparticles and viruses) by NA on the surface of viruses will be overloaded and largely delayed or blocked, leading to the formation of clusters or chains of viruses.

Stage 2 (Reduction of diffusion velocity)

As the diffusion velocity of clusters or chains of viruses are smaller than that of single virus, the infection transfer among host cells will be delayed. The approximate expression for diffusion coefficient is given as follows [9]:

$$D = k\,T/6\,\pi\,\eta\,R \quad (3)$$

where k is Boltzmann constant, T is absolute temperature, η is viscosity constant of the surrounding medium and R is the virtual radius of the aggregated zone of the gold nanoparticles.

Stage 3 (Inhibition of entry into host cells)

From the point of viral entry energy into host cells, it is very hard for aggregation (clusters or chains) of viruses to enter into host cells from the following two reasons.

Firstly, as the surfaces of aggregated viruses are covered by many gold nanoparticles, the spikes of HA

are partly shadowed and are not so effective to enter into the host cells by coupling with NANA on them.

Secondly, in the process of endocytosis, the threshold energy in order to form endosome will increase based on the analysis of flexure elastic energy in the reference [10]. The large cluster or chains of viruses require much entry energy, because the larger transformation for cell membrane is inevitable,

Stage 4 (Damage to RNA/DNA by X-ray exposure)

Application of X-ray is useful for increasing antiviral effects. Under the exposure of X-ray, gold nanoparticles around viruses will emit high energy electrons by photoelectric effect [2, 3, 11]. These photoelectrons will bring about damage of DNA or RNA in the viruses, as the energy dissipation of photoelectrons by interacting with DNA or RNA is very high.

4. AN EXAMPLE OF SURFACE FUNCTIONALIZATION OF GOLD NANOPARTICLES AGAINST INFLUENZA VIRUSES

An example of surface functionalization for influenza antiviral gold nanoparticles is shown in Figure **3**. The linkage structure is gold-S-PEG-Lipid hydrocarbon-NANA.

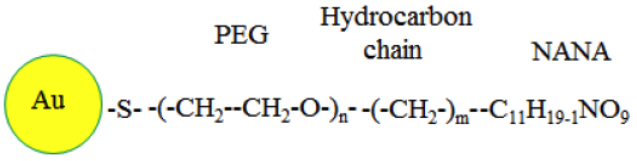

Figure 3: An example of the surface functionalization of gold nanoparticles for influenza antiviral medicines.

In the correspondence of equation (1), S is [Conjugation], PEG is [Transcellular] assist, hydrocarbon chain is [Cell membrane] hydrophobic and NANA is [Receptor] authentic, respectively.

The linkage between NANA and hydrocarbon is able to be processed through six possible sites of OH-terminals of NANA. As the reliability and reproducibility in the synthesis is important, the advanced methods such as block polymerization or graft polymerization can be applied.

5. CONCLUSIONS

Surface functionalized gold nanoparticles that mimic the surfaces of host cells are proposed for developing antiviral medicines and the design strategy is described theoretically.

As for the interaction between the gold nanoparticles and the viruses, the former acts as the adhesive balls for viruses. In order to make the gold nanoparticles function as effective adhesion balls, it is necessary that the diameter of the gold nanoparticle (D) is more than the twice length of HA (L_{HA}) that is $D > 2\ L_{HA}$. In the case of influenza viruses, D should be larger than 27nm. This will lead to gathering viruses and forming clusters or chains of them, bringing about four-stage antiviral effectiveness. The outstanding features of our antiviral medicines are summarized as follows:

1. Comparatively short development time based on simple guiding principle:

In the design of the antiviral medicines there is no need for finding inhibitors against specific proteins relating to the viruses. Consequently the development time will be comparatively short by following the simple guiding principle described in this paper.

2. Four stage effectiveness:

As the gold nanoparticles for antiviral medicines designed in this paper work in the four stages (budding, diffusion, entry and X-ray exposure), medical effectiveness will be high.

3. Realization for oral drugs:

The parts of [Transcellular] assist and [Cell membrane] hydrophobic (lipid) will help the gold nanoparticles pass through intestinal walls.

The following steps are needed towards practical use of the antiviral medicine in this paper.

1. Collaborative research between the fields of advanced nanotechnology and virology
2. *In-vitro* experiments for coupling between viruses and gold nanoparticles in order to observe the aggregation phenomena using microscope
3. Computer simulations for coupling between viruses and gold nanoparticles
4. Clinical treatments using the antiviral medicines in hospitals

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