Surface Functionalization of Gold Nanoparticles for Simultaneous Suppression of Cancer Stem Cells and Cancer Cells

Yujiro Naruse*

Japan Science and Technology Agency, K's Gobancho 7, Chiyoda-ku, Tokyo 102-0076, Japan

Abstract: Surface functionalization of gold nanoparticles is designed for simultaneous suppression of cancer stem cells and cancer cells. The fundamental mechanism of the simultaneous suppression is based on the blocking against DNA polymerase, DNA helicase and RNA polymerase by positively charged gold nanoparticles introduced into nucleuses. In addition, applications of physical fields (ultrasound, X-ray or electromagnetic wave) will enhance the suppression effects of the cancer cells by interactions between gold nanoparticles and physical fields. It is predicted theoretically that the surface functionalization of gold nanoparticle is promising for radical treatments of cancers.

Keywords: Gold nanoparticles, cancer cells, cancer stem cells, DNA, chromosome, histones, DNA helicase, DNA polymerase, RNA polymerase.

1. INTRODUCTION

Cancer treatments are progressing remarkably based on basic research on the proliferation mechanism of cancer cells. However recently, due to the increased knowledge about cancer stem cells, new approaches for advanced cancer treatments are needed [1]. Although the cancer stem cells do not have frequent cell divisions, they play an important role in redevelopment and metastasis. The cancer stem cells are very tough in terms of resistance against conventional anti-cancer drugs.

In this paper, a novel design of surface functionalization of gold nanoparticles is proposed in order to make them to reach inside of nucleus, and then suppress the functions of cancer cells and cancer stem cells simultaneously.

Three sequential steps are necessary for gold nanoparticles to reach the final destination (inside of cell nucleuses), as shown in Figure **1**. The first step is gathering gold nanoparticles towards cancer region by angiogenesis, the second step is passage through cell membranes and the third step is entering into nucleuses. The detailed method for guiding nanoparticles is explained in section 2. This is followed by the principles of suppressing cancer cells and cancer stem cells in section 3.

2. DESIGN STRATEGY FOR DELIVERING GOLD NANOPARTICLES INTO NUCLEUSES

As is described in reference [2], it is assumed that the size distribution is between 10nm to 30 nm using the following process. Gold nanoparticles were synthesized by reduction of gold chroride with freshly prepared sodium citrate and allowed to boil under reflux conditions. In order to deliver nanoparticles to the nucleuses, the following surface functionalization is designed. The molecular structures of 1, 2 and 3 in Figure **2** are designed in order to make gold nanoparticles to reach cancer region, pass through cell membranes and finally enter into nucleuses respectively.

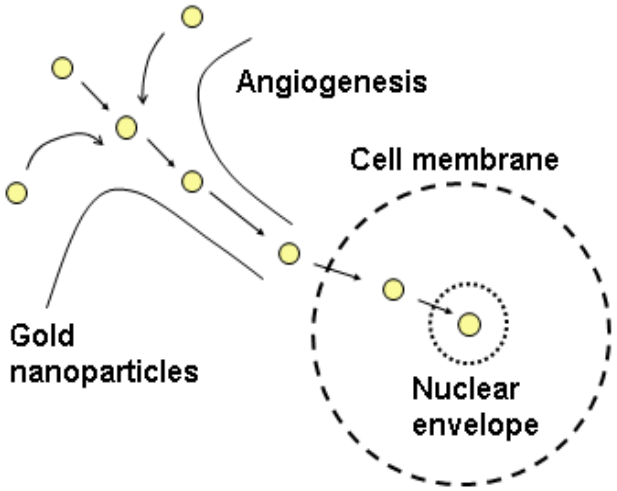

Figure 1: Three sequential steps for gold nanoparticles to reach the cell nucleuses: The first step is gathering gold nanoparticles towards cancer region, the second step is passing through cell membranes and the third step is entering into nucleuses.

There are three types of surface structures (series, parallel and branch) in Figure **2**. There may exist an optimum type from the point of effectiveness of cancer treatment and fabrication process. Although further studies are needed in this respect, brief comparison of the three types is discussed in section 4.

*Address correspondence to this author at the Japan Science and Technology Agency, K's Gobancho 7, Chiyoda-ku, Tokyo 102-0076, Japan; Tel: +81-3-3512-3523; Fax: +81-3-3222-2063; E-mail: y2naruse@jst.go.jp

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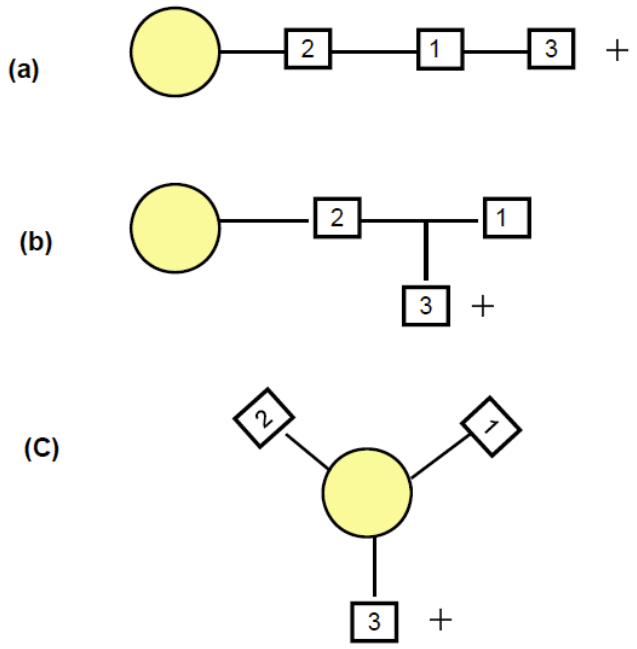

Figure 2: Three types of surface functionalization: (**a**) series type, (**b**) branch type, (**c**) parallel type: The molecular structures of 1, 2 and 3 are for gathering gold nanoparticles towards cancer region, passage through cell membranes and entering into nucleuses, respectively.

The examples of proposed surface structure for 1, 2 and 3 are glucose, PEG (poly ethylene glycol) and arginine type structures.

The glucose is chosen based on the phenomena that the cancer cells tend to live in glycolysis with active transport associated with accumulating high concentration of molecules that the cells need, such as ions, glucose and amino-acids [3]. The PET (Positron Emission Tomography) molecular probes utilize the similar characteristics of cancer cells. In addition, EPR (enhanced permeability and retention) effect which will occur in blood vessels around the cancer tissues can be utilized for surface functionalized gold nanoparticles with diameter size of 20 to 30 nm [4].

The PEG (poly ethylene glycol) is chosen from the experimental results of reference 2 which have revealed the gold nanoparticles with PEG spacer can pass through cell membrane and gather in the perinuclear region [2].

The arginine structure is chosen from the following research outcomes [5]. The nuclear pores have been induced to widen to permit the passage of materials with NLS (nuclear localization signals). In many nuclear proteins they consist of one or two short sequences that are rich in the positively charged amino acids lysine and arginine.

Incidentally, the time transient concentration of gold nanoparticles is estimated from cytosol to chromosomes (DNA) *via* nucleus. It is important to evaluate the effectiveness of suppression of cancer cells from the point of concentration of nanoparticles. Assuming that all of the gold nanoparticles entered into cytosol will be absorbed by chromosomes, the density ratio between cytosol, nucleus and chromosome are calculated to be 1: 6: 48, as shown in Figure **3**.

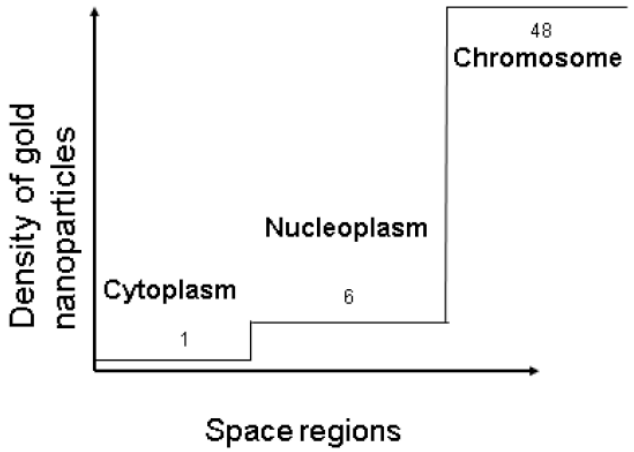

Figure 3: Comparison between the time transient densities of gold nanoparticles in cytoplasm, nucleoplasm and chromosomes. The ratio is calculated as about 1:6:48.

This calculation is based on the following assumption and parameters.

◊ The shapes of cells and nucleus are assumed to be sphere and column, respectively.

◊ All of the nanoparticles entered into cytoplasm will enter into nucleoplasm and finally all of them were absorbed into chromosomes.

◊ The size of cells (V_1), nucleus (V_2) and chromosomes (V_3) are calculated as follows.

$$V_1 = 4\pi r_1^3 / 3 \quad (1)$$

$$V_2 = 4\pi r_2^3 / 3 \quad (2)$$

$$V_3 = \pi r_3^2 \, 2L \quad (3)$$

where the typical parameters are given as follows.

Radius of cell	r_1	20μm
Radius of nucleus	r_2	10μm
Radius of chromosome cross section	r_3	350nm

Total length of chromosomes (sister chromatids) 2L 229μm

Table 1: Classification of cancer treatment PPB and MPB effects on cancer stem cells and cancer cells with and without physically excited fields. PPB effect represents suppression of protein production from DNA, and MPB effect represents suppression of cell divisions of cancer cells. ◎Very Effective ○Effective △Not so effective

	Cancer cells	Cancer stem cells
Without physical fields	○PPB effect ○MPB effect	○PPB effect △MPB effect
With physical fields	◎Enhancement of PPB effect (Electromagnetic wave, X-ray) ◎Enhancement of MPB effect (Ultrasound)	◎Enhancement of PPB effect (Electromagnetic wave, X-ray) △Enhancement of MPB effect (Ultrasound)

(The data are based on the picture of page 199 in Reference [6]).

Consequently, the higher concentration of gold nanoparticles can be expected in nucleuses.

3. THE PRINCIPLES OF CANCER TREATMENTS BY GOLD NANOPARTICLES

As the surface functionalized gold nanoparticles have a variety of possible ways to suppress cancer stem cells and cancer cells, we have summarized the possible effects (PPB: Protein Production Block and MPB: Multiplication Process Block) in Table **1**. This table has two by two sections for classifying the effectiveness for cancer stem cells (frequency of cell division is low) and cancer cells (frequency of cell division is high), in the case of with or without physical fields.

3.1. PPB (Protein Production Block) Effects without Physical Fields

As for the PPB-effect (suppression of protein production) without physical fields, the essential mechanism is competing coupling between gold nanoparticles and RNA polymerase to DNA strands. The starting point of protein production process is reducing the binding tension of DNA with histones and then the replication of DNA information to m-RNA begins. In this process RNA polymerase plays an important role in promoting the replication. However if the gold nanoparticles are around the copying site, there will be PPB effects due to coupling between DNA and positively charged nanoparticles as shown in Figure **4**.

3.2. MPB (Multiplication Process Block) Effects without Physical Fields

There are two kinds of MPB effects explained in Figures **5** and **6**.

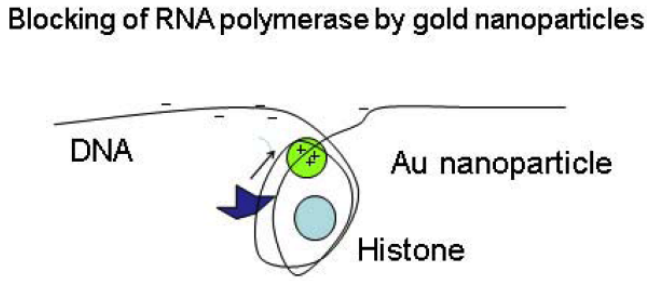

Figure 4: Schematic representation for PPB by positively charged gold nanoparticles. It is difficult to copy the protein information from DNA to m-RNA as the RNA polymerase is blocked by gold nanoparticles.

The first MPB effect (without physical field) appears during duplication of DNA shown in Figure **5**. In this process the DNA polymerase and DNA helicase play important roles. However positively charged gold nanoparticles around this region will stick to DNA strands and obstruct the movements of DNA polymerase and helicase. This will block normal DNA duplication process as shown in Figure **5**.

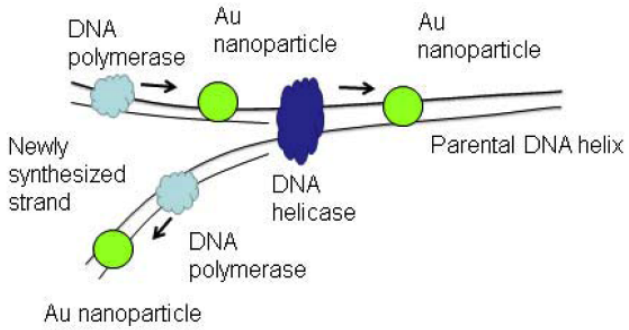

Figure 5: The movement of DNA polymerase and DNA helicase is blocked by positively charged gold nanoparticles sticking to DNA strands. This will bring MPB effect.

The second MPB effect (without physical fields) happens from prophase to metaphase of cell division cycle. As is shown in Figure **6**, positively charged gold nanoparticles plays similar roles as histones in the

DNA condensation process. So that the excessive number of positively charged gold nanoparticles will remain between DNA strands which will hinder the accurate alignment of chromosomes at metaphase plate and free separation of chromatids at cell division. This is because of the attraction forces between positively charged gold nanoparticles and negatively charged chromosomes.

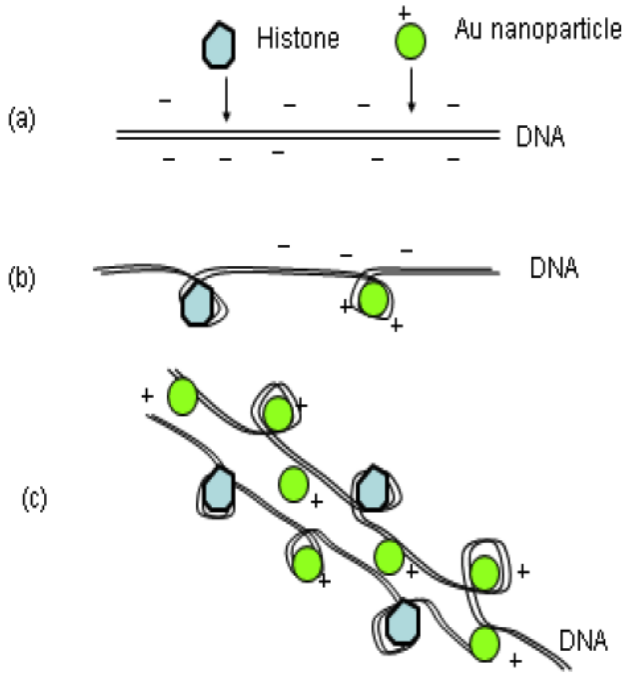

Figure 6: As the positively charged gold nanoparticles behave like histones, they will be involved and condensed into doubled sister chromosomes in metaphase of cell division process. (**a**)→ (**b**)→(**c**) So that they will hinder stable separation of chromosomes and lead to MPB effects.

There is also a possibility that PPB effect will enhance MPB effect due to suppression of necessary proteins or enzymes for cell division process.

3.3. Enhancement of PPB Effects and MPB Effects under Physical Fields

PPB effects with physical fields are explained as follows [7]. In the condensation process of chromosomes, the distances between gold nanoparticles will be shortened, and eventually virtual gold wires will emerge. Under the exposure of electromagnetic waves, the gold wires composed of gold nanoparticles will act as antennas in chromosomes, which will absorb electromagnetic energy and increase the temperature of chromosomes. The temperature rise will bring about the transformation of DNA structure and interrupt the protein production process. Gold nanoparticles exposed by X-rays in chromosomes emit high energy electrons through photoelectric effect, which will bring about a possibility that the high energy electrons will break the DNA structure. In addition, the photoelectric effect will increase the positive charge of gold nanoparticles after the emission of electrons (negative charge) from them. This effect is similar to the charged phenomena induced by the interaction between light and metal electrodes [8]. So that the coupling between gold nanoparticles (positively charged) and DNA (negatively charged) will be increased and enhance the PPB effect, as is shown in Figure **4**.

MPB effects introduced by ultrasound are based on the following principle. Due to the mass increase of chromosomes, ultrasound enhances the vibration of

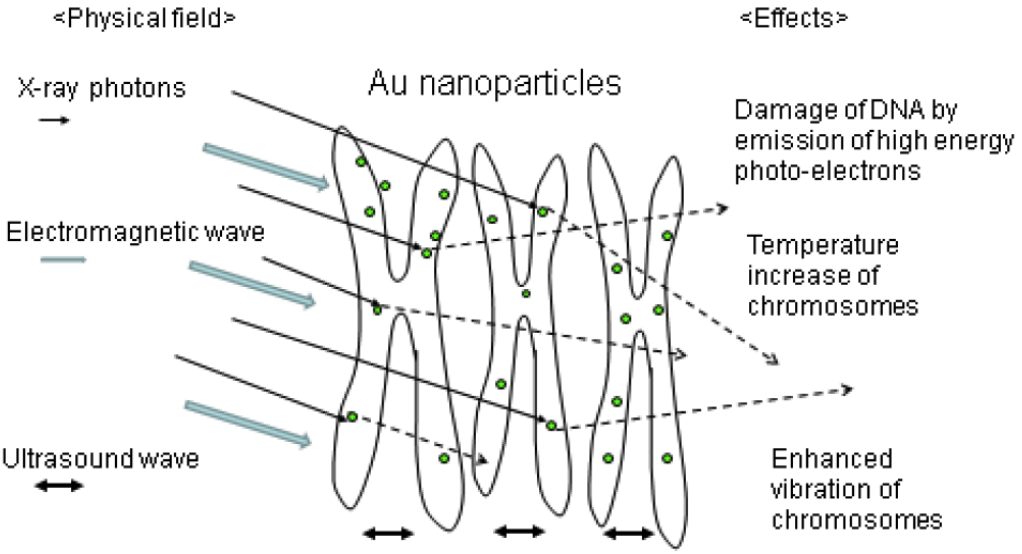

Figure 7: Enhancement of PPB and MPB effects by using X-rays, electromagnetic waves or ultrasounds.

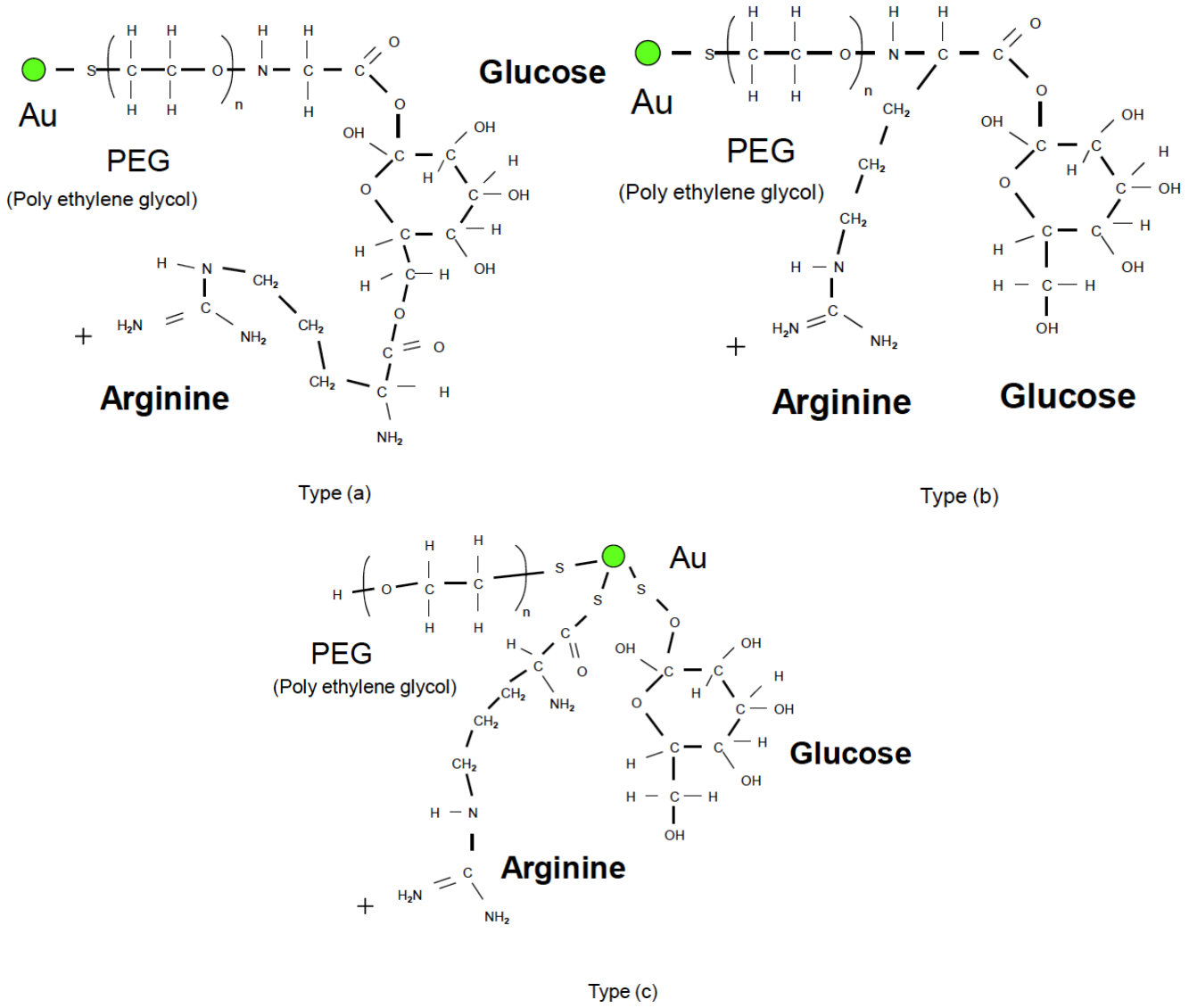

Figure 8: Examples of surface functionalized gold nanoparticles for simultaneous suppression of cancer stem cells and cancer cells. Types (**a**), (**b**) and (**c**) correspond to (**a**), (**b**) and (**c**) in Fig. **2**, respectively.

chromosomes in metaphase of mitosis, which will disturb the alignment of chromosomes on metaphase plates and will suppress cell divisions [9].

4. EXAMPLES OF SURFACE FUNCTIONALIZATION FOR GOLD NANOPARTICLES

Examples of surface structures of gold nanoparticles are shown in Figure **8**. In this figure, (a), (b) and (c) correspond to (a), (b) and (c) in Figure **2**, respectively. Possible synthesis methods for (a), (b) and (c) are block polymerization, graft polymerization and blend polymerization, respectively. From the point of surface functionalization process, it seems that types (a) and (b) are better than type (c) in terms of reproducibility. However, it seems that type (c) is better than types (a) and (b) in terms of effectiveness on cancer cells, because PEG, glucose and arginine are equally positioned on the top surface of gold nanoparticles.

5. CONCLUSIONS

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

- Surface functionalization of gold nanoparticles by three kinds of molecules (typically, glucose, PEG and arginine, as an example) will open new cancer treatments which are simultaneously effective for cancer stem cells and cancer cells.
- As for the PPB (Protein Production Block) effect without physical fields, the essential mechanism

is competing coupling between gold nanoparticles and RNA polymerase to DNA strands. As the gold nanoparticles are around the copying site, there will be PPB effects due to coupling between DNA and positively charged nanoparticles.

- As for the MPB (Multiplication Process Block) effects without physical fields, there are two kinds of blocking effects. The first effect appears during duplications of DNA. In the process, positively charged gold nanoparticles will block DNA polymerase and DNA helicase activity by sticking to DNA strand. Secondly MPB effect happens from prophase to metaphase of cell division cycle by disturbing alignment of chromosomes at metaphase plate.

- PPB and MPB effects will be enhanced by applying physical fields such as electromagnetic waves, X-rays or ultrasound.

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

- Experiments of introducing surface functionalized gold nanoparticles into living cells.

- Comparison of the effectiveness of cancer treatments depending on the three types in Figure **2**.

REFERENCES

[1] Takeishi S, Matsumoto A, Onoyama I, Naka K, Hirao A, Nakayama KI. Ablation of Fbxw7 eliminates leukemia-initiating cells by preventing quiescence. Cancer Cell 2013; 23: 347-61. http://dx.doi.org/10.1016/j.ccr.2013.01.026

[2] Shenoy D, Fu W, Li J, *et al*. Surface functionalization of gold nanoparticles using hetero-bifunctional poly(ethylene glycol)spacer for intracellular tracking and delivery. Int J Nanomedicine 2006; 1: 51-7. http://dx.doi.org/10.2147/nano.2006.1.1.51

[3] Bruce A, *et al*. Molecular biology of the cell, 4th ed. Gerland Science. New York, USA 2002; p. 621.

[4] Maeda H. Tumor-selective delivery of macromolecular drugs *via* EPR effect: Background and future prospects. Bioconjug Chem 2010; 21: 797-802. http://dx.doi.org/10.1021/bc100070g

[5] Bruce A, *et al*. Molecular biology of the cell, 4th ed. Gerland Science. New York, USA 2002; p. 672.

[6] Bruce A, *et al*. Molecular biology of the cell, 4th ed. Gerland Science, New York, USA 2002; p. 199.

[7] Naruse Y. Theoretical analysis on the possible interruption of cell division by applying physical excitation to gold nanoparticles introduced in chromosomes. J Memb Sepa Technol 2013; 2: 120-24.

[8] Williams R. Becquerel photovoltaic effect in binary compounds. J Chem Phys 1960; 32: 1505-14. http://dx.doi.org/10.1063/1.1730950

[9] Naruse Y. Mechanical vibration model for chromosomes in metaphase of mitosis and possible application to the interruption of cell division. Biosystems 2002; 66: 55-63. http://dx.doi.org/10.1016/S0303-2647(02)00033-3

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