Bacteria-assisted Production System for Functional Nano- and Microstructures made with Programmable Viruses

Hayato Goto[1], Shotaro Kadoya[1], Masaki Michihata[1] and Satoru Takahashi[1,#]

1 Department of Precision Engineering, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan
Corresponding Author / Email: takahashi@nanolab.t.u-tokyo.ac.jp, TEL: +81-3-5841-6451, FAX: +81-3-5841-8553

KEYWORDS: Nanostructure, Virus, M13 bacteriophage, Bacteria, Escherichia coli, Production system

In biological systems, efficient assembly of nanoscale structures is realized thanks to biomolecules which specifically recognize and bind to certain target. In recent years, by mimicking those biological mechanisms in laboratory, some assembly techniques including DNA origami have been developed for systematic production of functional nano- and microstructures. Here we newly propose a bacteria-assisted production system using viruses as programmable and replicable components of nano- and microstructures. As viruses, we utilize M13 bacteriophages because their coat protein can be genetically programmed to display peptides which bind to specific target including both organic and inorganic particles or bulk materials. The production process consists of 2 stages: component manufacturing and assembly. In the component manufacturing, viruses are genetically edited to bind to selected target and replicated through infection to host bacteria, E. coli. In the assembly process, the virus is attached to its binding target, whose position is controlled by optical tweezers. The product of our production system can have various materials and structures, and it is expected to have various application fields such as drug delivery. In this report, as a first step of the validation of assembly process, the optical trapping of nanoparticle is demonstrated.

1. Introduction

In recent years, there are many attempts to incorporate biological systems into nano- and microscale manufacturing. The most actively and successfully studied example is the creation of nanostructure using DNA [1-3]. DNA is a biomolecule that can be edited with nanoscale precision by gene editing techniques. It is possible to program a site on DNA binding to another specific site on DNA by utilizing base complementarity. Meanwhile, other biological systems including bacteria, viruses, and various biomolecules are also have the potential to contribute to nano- and micromanufacturing technology [4, 5].

In this study, we focus on viruses. Generally, viruses gain opportunities for infection by binding specifically to specific sites on the host cell, called receptor, and multiply by borrowing the host cell's protein synthesis system. In that process, viruses self-assemble their well-defined bodies from synthesized proteins. These biological functions of viruses, specific binding and self-assembly, are available for the construction of nano- and microstructures. Comparing viruses and DNA in terms of nano- and microstructure component, viruses are inferior to DNA in processing precision but the size of structures that can be stably produced is larger than that of DNA. One reason for this is that, considering the hierarchical structure of biological systems, viruses are positioned one level above DNA.

Some attempts have been made to utilize viruses for nano- and micromanufacturing. The representative work is the one conducted by Angela Belcher and her group, who focus on M13 bacteriophage. Fig. 1 is an illustration of M13 bacteriophage. M13 bacteriophage is a filamentous virus with about 7 nm in diameter and 760-1950 nm in length. The body consists of 5 types of protein, and the important thing is that M13 bacteriophages can be genetically programmed to display peptides which bind to specific target materials. Since the specific binding of M13 bacteriophage to semiconductor surface is

demonstrated in 2000 [6], the possibility of using M13 bacteriophages to produce nano- and microstructures has been explored. For example, Belcher's group successfully synthesized some nanostructures such as nanoring made with a few bacteriophages or nanowires by using bacteriophage as a template [7, 8]. However, it is still necessary to sophisticate and organize the manufacturing process for stable and repeatable production of nano- and microstructures.

In our project, we aim to organize and control the whole manufacturing process of nano- and microstructures made with viruses as a single production system. Virus editing and assembly are packaged as individual processes within a single production line, which allows more systematic production of the functional nano- and microstructures such as carrier of drug delivery system (DDS). In this paper, we propose a production system, in which the optical tweezer is incorporated, and as a first step of the realization of proposed system, the experimental study about optical trapping of nanoparticle is demonstrated.

2. Bacteria-assisted production system for functional nano- and microstructures made with programmable viruses

Fig. 2 shows the schematic of the production system we propose. The system consists of 2 stages: component manufacturing and assembly.

In the component manufacturing, viruses are genetically edited to bind to selected target and replicated through infection to host bacteria, E. coli. Although there is constraint that the peptides displayed on M13 bacteriophages must not interrupt the virus multiplication process, previous studies demonstrate that the binding target of edited M13 bacteriophage can be both organic and inorganic materials, and both particles and bulk [9, 10]. This diversity of the biding target guarantees a high degree of freedom of nano- and microstructures to be produced. Also, the multiplication of edited viruses, which takes about 20 minutes to create 100-1,000 copies for example, progresses automatically once the original edited virus is obtained. In other words, it is possible to produce a large amount of well-defined nanoscale component at low cost by utilizing the superiority of virus as biological replication system.

In the assembly process, viruses are attached to their binding targets and final product is assembled. Because the M13 bacteriophage specifically binds to its target, the assembly can be conducted without taking care of incorrect binding. In our strategy, the binding targets are spatially fixed by optical tweezer and the viruses are attached to their biding target without precise control of their position and posture. Thus, the virus-receptor binding specificity in nature is exploited in assembly process of proposed production system.

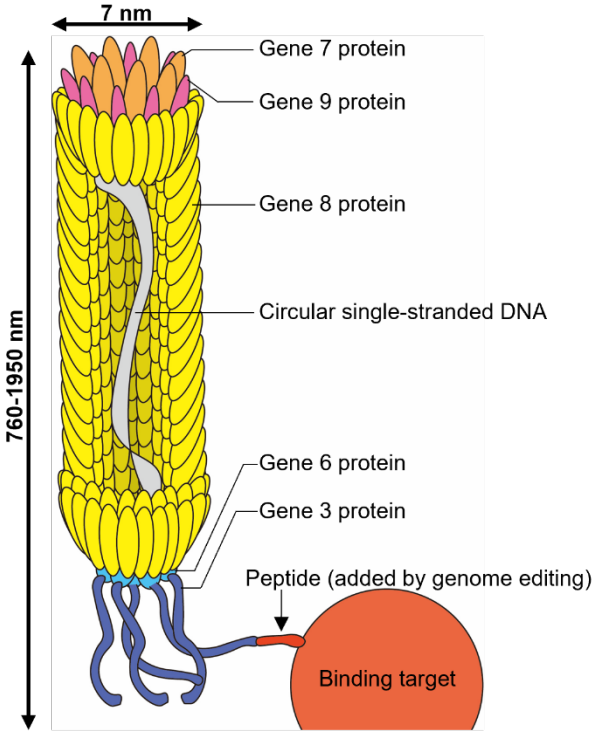

Fig. 1 Illustration of M13 bacteriophage and its binding to specific target selected by the displayed peptide.

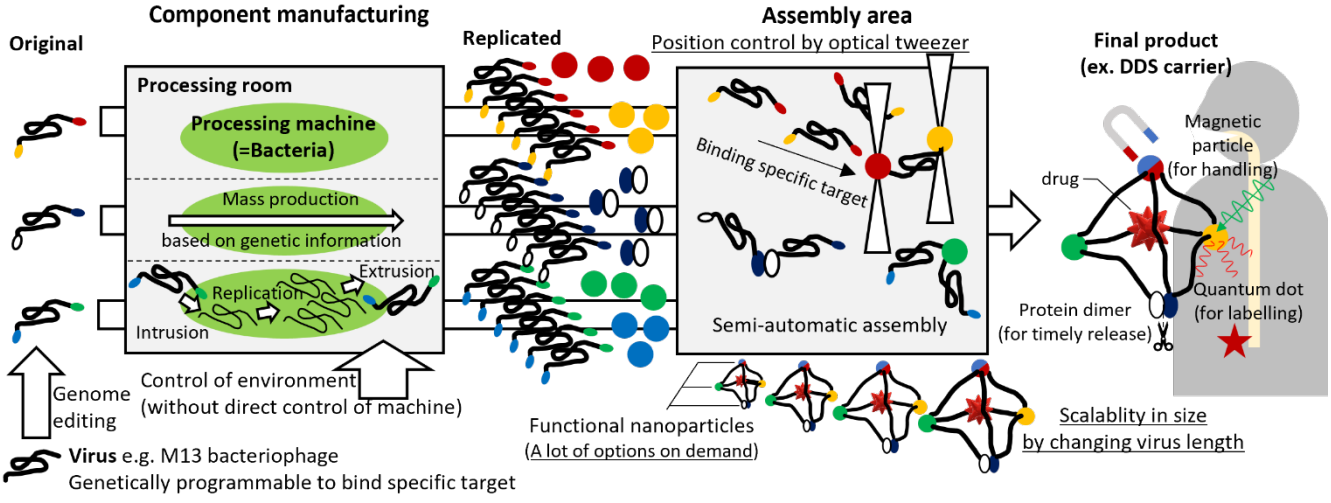

Fig. 2 Schematic of bacteria-assisted production system for functional nano- and microstructures made with programmable viruses.

The product of our production system can have various materials and structures and is scalable in size by changing the length of bacteriophage. It is expected to have various application fields such as drug delivery. For example, Fig. 2 shows the production process of multifunctional drug carrier which is created by connecting some different functional nanoparticles via M13 bacteriophages.

It would be good to mention to necessity of optical trapping in this system. Ideally, nanostructures are expected to be automatically constructed from component viruses and nanoparticles, given a sufficient time for the viruses and nanoparticles to meet by chance after random Brownian motion. Because virus-nanoparticle combinations are fixed due to binding specificity. However, it often takes a long time for the nanoscale components to diffuse and encounter purely by Brownian motion. It is known that the average displacement by Brownian motion is proportional to square root of time. For example, if it takes 1 second to diffuse 1 μm, it takes 1 hour to diffuse 60 μm. Also, considering implementation as a device, a means of transportation from the storage to the assembly area is necessary. Therefore, it is desirable to exploit some manipulation method for position control of the components. Optical tweezer is expected to be one of suitable methods. Optical tweezer realizes non-contact manipulation of nanoparticles. In addition, it may be possible to collectively trap nanoparticles in geometric configuration within a single beam spot by utilizing light-matter interaction such as plasmon polariton coupling (Fig. 3). It allows assembly process to be more efficient.

3. Fundamental experiment for collective trapping of nanoparticles into geometric configuration

For the first step of implementation of the production system, we aim to realize the binding of genetically edited M13 bacteriophage and its binding target with optical tweezer. In this paper, the fundamental experiment for collective trapping of nanoparticles into geometric configuration is conducted.

3.1. Method

Fig. 4 shows the optical system for nanoparticle trapping. The trapping laser with a wavelength of 976 nm is focused on the sample through oil immersion objective lens (Olympus, UPLXAPO 60X NA1.42). The focusing position is controlled by Galvano mirror. The LED light is illuminated for observation, and microscopic images are captured by the two cameras. Since the focal planes of the two objective lenses are aligned, the area near the focus of the trapping laser is observed. Sample cell is prepared as shown in the upper right inset of Fig. 4. The paraffin films create a space with a height of about 0.1 mm between the slide glass and the cover glass, and the suspension of nanoparticle is injected into that space.

Gold nanoparticles with a diameter of 100 nm (Sigma-Aldrich, 742031-25ML) are prepared as trapping targets because gold nanoparticles are widely used not only as binding targets of M13 bacteriophages but also as labels for biological imaging. Gold

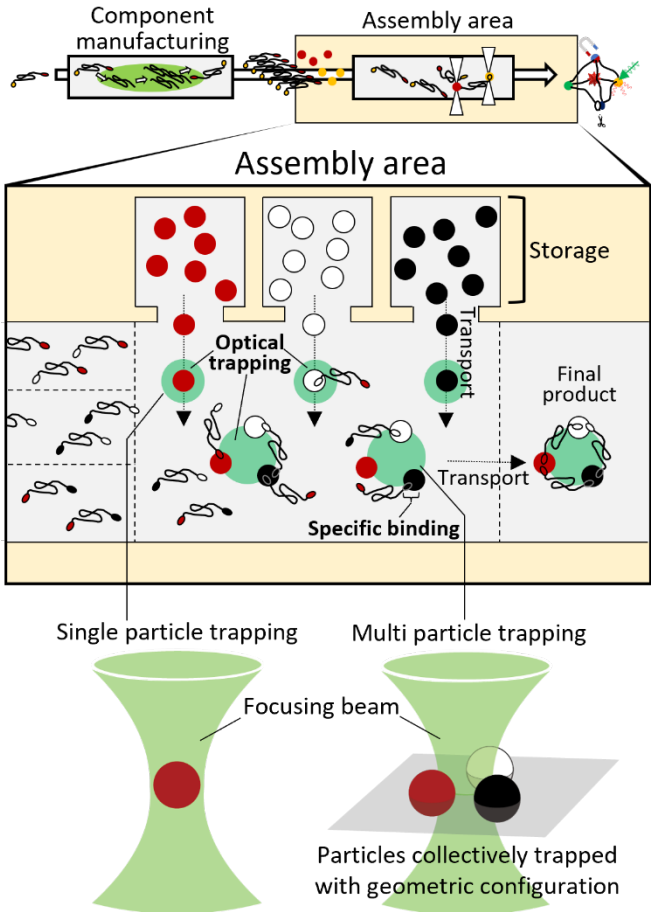

Fig. 3 Details of assembly process assisted by optical tweezer.

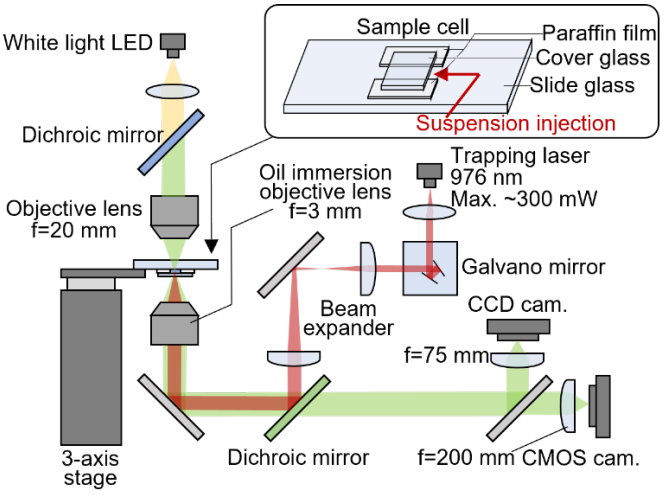

Fig. 4 Schematic of the optical system for nanoparticle trapping.

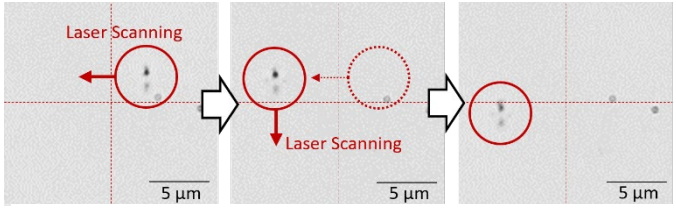

Fig. 5 Result of trapping experiment of gold nanoparticles. A group of particles is trapped and follows the movement of the laser.

nanoparticles are dispersed in citrate buffer with a concentration of 3.8×10^8 particles/mL.

3.2. Result and discussion

Without irradiation of the trapping laser, the particles in Brownian motion are observed. When the trapping laser is turned on, multiple particles are trapped at once and follow the focal position moved by Galvano mirror (Fig. 5). The observation along the optical axis suggests that the trapping position is not near the wall but in the middle of the cell. This means that the gradient force worked effectively enough against the scattering force. As a result, the collective retention and migration of gold nanoparticles are successfully achieved.

Also, it is observed that gold nanoparticles are collectively trapped with fixed geometric configuration such as line and triangle (Fig. 6). Interestingly, the trapped particles usually did not aggregate at a single position, but separately aggregated at a few different positions equally spaced. The mechanism of this phenomenon is not clear, but it can be thought that the interaction between the light and plasma oscillations in gold nanoparticles contributes. Considering the operation in the proposed production system, this phenomenon could be used to separately hold the nanoparticles in a geometrical configuration and contributes to assembly efficiency.

The result validates the fundamental applicability of the trapping system to assembly of nanoparticles and viruses. In the future, more investigation is needed to confirm the applicable range of this trapping system with respect to nanoparticle size and material.

4. Conclusions

We proposed the bacteria-assisted production system for functional nano- and microstructures made with programmable viruses. The proposed system incorporates the advantages of viruses as living systems in terms of replicability and binding specificity. Replicability of viruses is considered to allow for mass production of well-defined nanocomponents without precise control of the workpiece or processing machine. Due to binding specificity, the assembly process progresses quasi-autonomously with the aid of optical tweezers, and the final product can be obtained at low cost. Various materials can be selected as binding targets for the virus, including organic materials, metals, and semiconductors. We believe that a more systematic production of functional nano- and microstructures will be possible with the proposed system.

In this paper, as a first step of the validation of assembly process, the experiment for collective trapping of nanoparticle into geometric configuration is conducted with optical tweezer. As a result, gold nanoparticles with 100 nm diameter are successively retained and relocated. In addition, it is confirmed that gold nanoparticles are spontaneously positioned in geometric configuration due to light-matter interaction. In the future, we will investigate the applicable range of this trapping system with respect to nanoparticle size and material. In parallel, we will proceed to binding experiments of viruses and nanoparticles with the aid of optical tweezers.

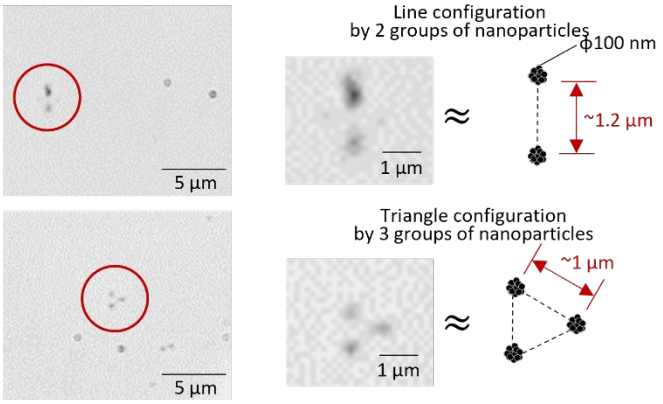

Fig. 6 Geometric configuration of trapped gold nanoparticles. Particle groups are separately fixed at 2 or 3 different sites and form line or triangle configuration.

ACKNOWLEDGEMENT

This work was partially supported by JST, CREST grant number JPMJCR2322.

REFERENCES

1. Rothemund, P. W. K., “Folding DNA to create nanoscale shapes and patterns,” Nature., Vol. 440, No. 7082, pp. 297-302, 2006.
2. Furukawa, H., Endo, T., Yanagiba, Y. and Hatsuzawa, T., “Design and Fabrication of Nanostructures Based on DNA Ring–Protein Complex,” Jpn. J. Appl. Phys., Vol. 47, No. 6R, pp. 4810, 2008.
3. Hayashi, T., Takaya, Y., Miyoshi, T. and Shibata, T., “Self Addressing of Micro Parts for constructing MOEMS Devices using DNA Autonomous Joint,” Proc. of Jpn. Soc. Precision Eng., Vol. 2007s, pp. 303-304, 2007.
4. Uno, Y., Kaneeda, T., Yokomizo, S. and Yoshimura, T., “Fundamental Study on Electric Field Assisted Biomachining,” J. Jpn. Soc. Precision Eng., Vol. 62, No. 4, pp. 540-543, 1996.
5. Zhang, W., Xu, C., Yin, G., Zhang, X., Wang, Q and Li, F., “Encapsulation of Inorganic Nanomaterials inside Virus-Based Nanoparticles for Bioimaging,” Nanotheranostics, Vol. 1, No. 4, pp. 358-368, 2007.
6. Whaley, S., English, D., Hu, E., Barbara, P. and Belcher, A., “Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly,” Nature, Vol. 405, No. 6787, pp. 665-668, 2000.
7. Nam, K., Peelle, B., Lee, S. and Belcher, A., “Genetically Driven Assembly of Nanorings Based on the M13 Virus,” Nano Letters, Vol. 4, No. 1, pp. 23-27, 2004.
8. Records, W., Yoon, Y., Ohmura, J., Chanut, N. and Belcher, A., “Virus-templated Pt-Ni(OH)$_2$ nanonetworks for enhanced electrocatalytic reduction of water,” Nano Energy, Vol. 58, pp. 167-174, 2019.
9. Flynn, C., Lee, S,. Peelle, B. and Belcher, A., “Viruses as vehicles for growth, organization and assembly of materials,” Acta Materialia, Vol. 51, No. 19, pp. 5867-5880, 2003.
10. Bratkovic, T., “Progress in phage display: evolution of the technique and its applications,” Cell. Mol. Life Sci., Vol. 67, No. 5, pp. 749-767, 2010.