FORMATION OF DNA MICRO-SKELETON STRUCTURES IN WATER-IN-OIL MICRODROPLETS

Masamune Morita[1], Shin-ichiro M. Nomura[2], Satoshi Murata[2], Miho Yanagisawa[*3] and Masahiro Takinoue[*1]

[1]Tokyo Institute of Technology, Japan, [2]Tohoku University, Japan and [3]Tokyo University of Agriculture and Technology, Japan

ABSTRACT

We show a novel DNA microstructure, named "DNA micro-skeleton". This DNA microstructure is constructed by self-assembly of Y-motif DNA inside water-in-oil microdroplets covered with cationic lipids. The DNA micro-skeleton is a porous and hollow microcapsule with a large surface area. Therefore, we believe the structure will have potentials for physical and biomedical use such as artificial cells and molecular robots, which will be realized by the advantage of the soft but robust structure and also its large surface area.

KEYWORDS: DNA nanotechnology, DNA hydrogel, self-assembly, water-in-oil droplets

INTRODUCTION

The production of complex self-organized structures inspired by nature or living systems is one of the major goals of material science. Based on bottom-up DNA nanotechnology, various-sized DNA structures such as nanoscopic DNA origami [1] and macroscopic DNA hydrogels [2] have been created to date. The shapes of nanostructures are controlled by DNA sequence design. The mechanical and physicochemical properties of DNA macroscopic gels are also tuned by DNA sequence design, but the overall shapes of gels are decided just by the shape of a container for gelation of DNA solutions and are difficult to be controlled. DNA microstructures have potentials for future technologies such as artificial cell-like systems, molecular robots [3] and biomedical application. However, concepts are still missing to facilitate the production of DNA microstructures. Here, we report self-assembled DNA microstructures with micrometer-sized patterns.

EXPERIMENTAL

A Y-motif DNA monomer is formed from three kinds of single-stranded DNA (ssDNA) by hybridization of the same-colored sequences [Figure 1A; a-a' (blue), b-b' (pink), and c-c' (green)]. The Y-motif DNA is able to hybridize with each other via self-complementary sticky-ends (Figure 1A; purple), resulting in a formation of a gel-like network of DNA (Figure 1A). The three kinds of ssDNA were solved in a buffer solution containing 20 mM Tris-HCl (pH 8.0), 350 mM NaCl, and 10,000-fold diluted SYBR Gold. The DNA solution (2 μL) was added to mineral oil (50 μL) containing a cationic lipid (DOTAP) and a neutral lipid (DOPC) in a PCR tube. Lipid-stabilized water-in-oil (W/O) microdroplets were obtained by hand tapping (40-50 times). For the preparation of the DNA microstructures, the W/O microdroplets were annealed by decreasing temperature from 95 to 20℃ at cooling rate of 0.01℃/s. The obtained DNA microstructures inside W/O microdroplets were observed by a confocal laser scanning microscope.

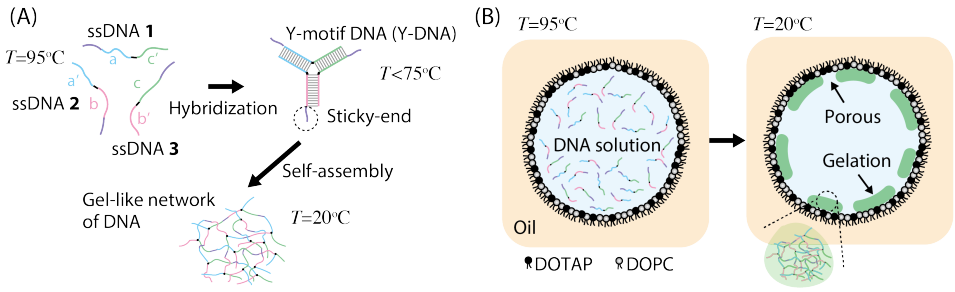

Figure 1: Schematic illustration of DNA microstructure formation. (A) DNA used in this experiments. The Y-motif DNA formed from three kinds of single-stranded DNA. The Y-motif DNA can assemble with one another using the self-complementary sticky-ends. B) DNA microstructure was formed by the assembly of DNA inside the Water-in-oil microdroplet. The oil-water interface has positive charge caused by positively charged lipid DOTAP.

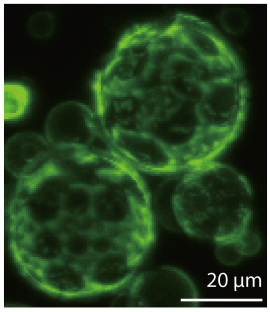

Figure 2: Produced porous and hollow capsular DNA microstructures, named "DNA micro-skeleton".

RESULTS AND DISCUSSION

The DNA microstructures were generated with Y-motif DNA in W/O microdroplets with a cationic lipid interface (Figure 2). The electrostatic interaction between the cationic lipid interface and anionic phosphate backbone of DNA induced gelation of Y-motif DNA on the cationic lipid interface of the W/O microdroplets as a result of annealing of the solution from 95°C to 20°C (Fig.1B). Figure 2 shows the confocal laser scanning microscope images of produced DNA microstructures. We obtained porous and hollow capsular structures with a large surface area, named "DNA micro-skeleton". This microstructure could be collected into an aqueous solution from the W/O microdroplets, which suggests that the structure is robust to hold against mechanical manipulation and thus should have a kind of gel structure.

CONCLUSION

We have developed porous and hollow capsular DNA microstructures with a large surface area, named "DNA micro-skeleton" by self-assembly of Y-motif DNA at the interface of W/O microdroplet. We believe that this DNA microstructure opens the new tool for physical and biomedical application such as artificial cell-like system [4] or soft molecular robots.

ACKNOWLEDGEMENTS

We thank Prof. M. Ichikawa (Kyoto Univ.), Prof. T. Hamada (JAIST), Prof. K. Yoshikawa (Doshisha Univ.), and Prof. K. Okabe (Univ. Tokyo) for fruitful discussions. This work was supported by a Grant-in-Aid for Scientific Research on Innovative Area "Molecular Robotics" (No. 24104002 (M.T.), No. 25104522 (M.Y.), No. 24104004 (S.M.N), No. 24104005 (S.M.)) and a Leading Initiative for Excellent Young Researchers (16812285) (M.M.) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan; and Grant-in-Aid for Scientific Research B (No. 26280097, No. 17H01813) (M.T.), Scientific Research on Innovative Areas (No. 16K12521) (M.T.), Scientific Research S (No. 22220001) (S.M. and M.T.), Young Scientists B (No. 16K21034) (M.M.) and a Research Fellowship for Young Scientists (14J10002) from the Japan Society for the Promotion of Science (JSPS)

REFERENCES

[1] P. W. K. Rothemund, "Folding DNA to create nanoscale shapes and patterns", *Nature*, 440, 297-302, 2006.

[2] S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach, D. Luo, "Enzyme-catalysed assembly of DNA hydrogel", *Nat. Mat.* 5, 797-801, 2006,.

[3] M. Hagiya, A. Konagaya, S. Kobayashi, H. Saito, S. Murata, "Molecular Robots with Sensors and Intelligence," *Acc. Chem. Res.*, 47, 1681-1690, 2014.

[4] C. Kurokawa, K. Fujiwara, M. Morita, I. Kawamata, Y. Kawagishi, A. Sakai, Y. Murayama, S.-i. M. Nomura, S. Murata, *M. Takinoue, *M. Yanagisawa, "DNA cytoskeleton for stabilizing artificial cells". *Proc. Natl. Acad. Sci. U.S.A.* 114, 7228-7233 (2017).

CONTACT

* MT: takinoue@c.titech.ac.jp; MY: myanagi@cc.tuat.ac.jp