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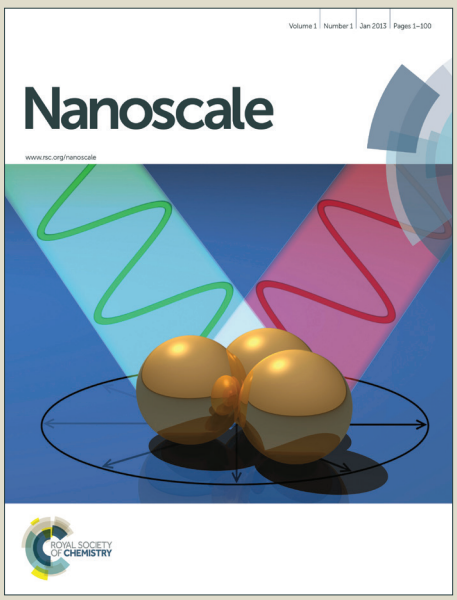

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DNA-templated assembly of viral protein hydrogel

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Xin Xu,[a] Ailin Tao [a*] and Yun Xu [a, b*]

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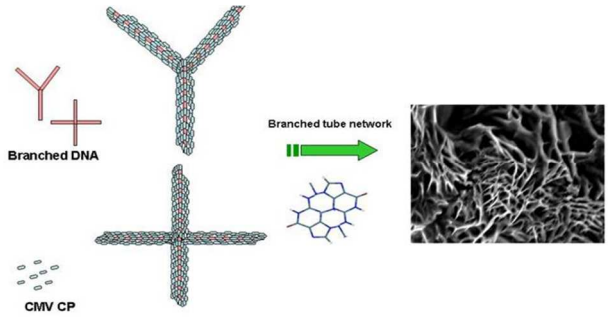
Hydrogels are a promising class of biomaterial that can be easily tailored to produce a native extracellular matrix that exhibits desirable mechanical and chemical properties. Here we report the construction of a hydrogel via the assembly of Cucumber Mosaic Virus (CMV) capsid protein and Y-shaped and cross-shaped DNAs.

Hydrogels are a promising class of biomaterial that can be easily tailored to produce a native extracellular matrix that exhibits desirable mechanical and chemical properties such as high permeability to oxygen, nutrients, and other water-soluble metabolites [1-4]. Directed protein assembly can provide a model to reveal the assembly mechanism and facilitate the construction of nanomaterials as building blocks in a bottom-up approach [5-8]. Recently, the in vitro assembly of viral capsid protein (CP) has attracted more attention for use as a nanotechnology building block because it can be induced to assemble into non-native configurations and easily functionalized by well-established protein engineering methods [9, 10]. Previously, we synthesized linear nanotubes from DNA and CMV capsid protein，confirming the ability of CMV CP to assemble with non-specific DNA in vitro [11]. Here we report the construction of a hydrogel via the assembly of Cucumber Mosaic Virus (CMV) capsid protein and branched DNA (Y-shaped and cross-shaped DNA) (**Scheme 1**).

Branched DNA templates were designed as follows: the initial short arms of Y-DNA, about 10-mer in length, were synthesized using single-stranded oligonucleotides partly complementary to each other with each arm possessing a 5'-overhang thymine (T) [12]. We further extended each of the arms to a reasonable length by ligating a nonspecific 500 base pairs (bp) double-stranded DNA with a 3'-overhang adenine (A) that was amplified by polymerase chain reaction (PCR) using the pBR322 plasmid template (see the Supporting Information). Thus, each arm of the Y-DNA was about 510 bp and 170 nm in length, as observed by Atomic force microscopy (AFM) and shown in Figure.1 The full-length CMV CP gene encoding 218 amino acid residues (GenBank ID AB008777) was PCR-amplified and cloned into the expression vector pET11d (Novagen, USA) (see the Supporting Information). The expression products were purified and the soluble CMV CP was obtained using an improved method. Before being applied to the assembly process, the refolded CP was concentrated through an ultrafiltration system to obtain a final concentration of 50 μg ml^{-1} in Tris buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.5). The CMV CPs was then applied to the assembly process with the ratio of five branched DNA base pair to one protein (5bp:cp) in the buffer mentioned above at room temperature overnight.[11].



Scheme 1. Schematic of hydrogel formation via the crosslinking of the branched tubular network [11] at the direction of branched DNA.

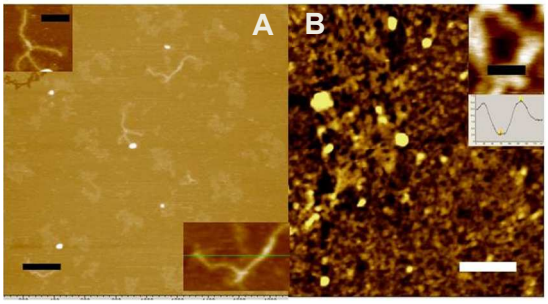


Fig. 1. Atomic force microscopy images of (A) Y-DNA, scale bar 200 nm. Inset: A zoomed image showing the length of the branch (about 200 nm), (B) the dried

gel, scale bar 250 nm. Inset: A zoomed AFM image showing the pore size of the gel (about 100 nm).

A swollen hydrogel produced by CP-branched DNA co-assembly is shown in Figure 2. A protein-specific dye (Coomassie Blue R-250) was used to stain the gel. After extensive washing, the co-assembled gel still appeared blue (Fig. 2A). The hydrogel construction was investigated by a variety of methods including optical microscopy, environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). In the swollen state, the gel showed a large number of scale-like structures.

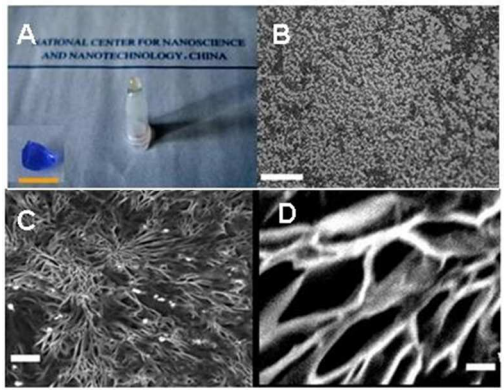


Fig. 2. (A) Hydrogel made from DNA-templated virus CP assembly shown in an inverted vial, the inset shows the gel stained with Coomassie Blue R-250. The scale bar is 5 mm. (B) Optical microscopic image of the hydrogel. The scale bar is 25 μm. (C and D) Surface morphology of DNA-templated protein hydrogel under ESEM. The scale bars are 10 μm (left) and 1 μm (right).

Detailed microscope studies have provided an insight into the self-assembly of the DNA-templated CMV viral capsid protein complexes into hydrogels. Using ESEM, the surface morphology of the neither stained nor metal-coated hydrogel showed spherical and sheet- or strip-shaped gel conformations of different sizes, indicating the stepped configuration stages of the gelation process (Fig. 2, C & D). AFM visualization revealed that the dry hydrogel had nanoscale holes in the network structure (Fig. 1B), suggesting that the assembled tube branches were linked with one another to form the cross-linked, network-structured local hydrogel. Although individual branched DNA may be fairly flexible, when constrained by the templated assembly of a protein, the tube structure network is sufficiently rigid and thus more efficient in forming a hydrogel.

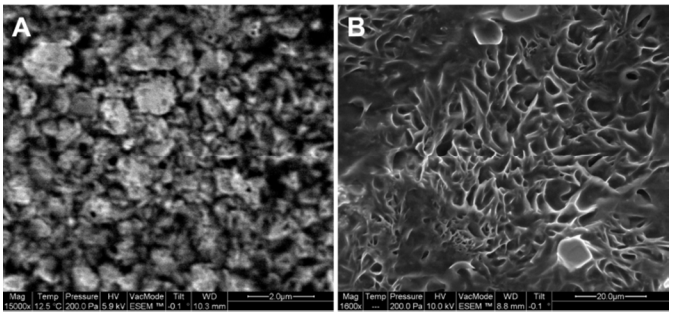


Fig 3. Surface morphology deciphered by ESEM for DNA-templated protein hydrogel formed under the ratio of branched DNA base pair to protein at 2bp:cp (A) and at 5bp:cp (B).

While the CMV CP can assemble into tubular nanostructures templated by heterogeneous linear DNA as we have previously shown [11], branched DNA has the ability to offer a three dimensional scaffold and direct the CMV CP to assemble into a hydrogel. Since this hydrogel is based on the linkage of assembled CMV capsid protein and branched DNA structures, such DNA directed CP assemblies might be seen as crosslinkers.

When the ratio of branched DNA base pair to protein came to 2bp:cp, we still observed the gelation (see Fig 3A). However, the gel was not strong enough to endure the succeeding process of dyeing and cleanout, thus suggesting that the DNA ratio is important to maintain the form of gelation. We have also tried different temperatures for gelation. When the temperature was set at 4°C degree, for example, the gelation could not be fulfilled until two days later. However there was no difference in the gel characteristics comparing with gel formed at room temperature (data not shown).

Compared with other bio-inspired hydrogels such as peptide-based, DNA-polyacrylamide based or pure DNA-based hydrogels [13-23], our DNA-protein co-assembled hydrogel can be easier to both chemically and genetically modify on the protein surface without affecting the overall architecture and could give rise to a rich resource for materials and pharmaceutical applications. Furthermore, CMV has the widest host range of any known plant virus and is distributed worldwide. In this regard, the DNA-templated virus protein assembly could also have potential uses in agriculture. Moreover, it was suggested that the self-assembled molecular hydrogel has some advantages over polymeric hydrogel because covalently crosslinked random coils form the network in polymeric hydrogels while self-assembled nanostructures of amphiphilic molecules form the network in molecular hydrogels [24-26]. Thus, the tailoring of self-assembled nanostructures may provide an optimal microenvironment for biomedical applications.

Conclusions

Branched-DNA templated virus protein assembly represents a new method of synthesizing a three-dimensional DNA/viral structure. By combining established protein engineering skills with DNA template design, this method will also be of benefit to other more complicated bio-nanomaterial preparations. Furthermore, the possibility of designing therapeutic relevant DNA sequences, such as antisense DNA, as well as opportunities for engineering viral capsid protein with specific affinities and therapeutic actions [27], suggests broader opportunities for these strategies in the production of responsive matrices for biomedical applications.

Notes and references

[a] *Guangdong Provincial Key Laboratory of Allergy & Immunology, The State Key Laboratory of Respiratory Disease, The Second Affiliated Hospital, Guangzhou Medical University, China Guangzhou 510260 (P.R. China). E-mail: taoailin@gzmu.edu.cn.*

[b] *Centre For Medical Device Evaluation, CFDA. E-mail: phoebexuyun@gmail.com*

† We thank Professor Dongsheng Liu for his helpful discussion and selfless advice. This work is partly funded by the National Natural Science Foundation of China (20573027, 50625312). **Limited experimental**: Branched DNA, including Y-shaped and cross shaped DNA，about 10-mer in length, was synthesized using single-stranded oligonucleotides partly complementary to each other with each arm possessing a 5'-overhang thymine (T). The chains have been carefully designed to have two half-complimentary sequences so that equal amounts of the DNA strands will hybridize to each other to produce the Y-unit/"+"-unit. The formation of "Y" DNA/"+" DNA was confirmed

by electrophoresis in 1.7% agarose gel (see the Supporting Information). The branched units were then extended to about 510-mer by ligating a nonspecific 500 base pairs (bp) double-stranded DNA with a 3'-overhang adenine (see Supporting Information). The extended branched units were examined with an Agilent 5500 Atomic Force Microscope (AFM). The pure soluble CMV CPs were produced using the following steps: 1) the CP gene was cloned into the expression vector pET11a (Novagen, USA); 2) after expression, the inclusion bodies were separated and purified, then solubilized in a 2 M urea solution by denaturation; 3) the denatured protein solution was then subjected to a refolding procedure where the soluble denatured CP was allowed to refold at a relatively low concentration (see Supporting Information for detailed experimental procedures and conditions). Before beginning the assembly process, the refolded CP was concentrated through an ultrafiltration system to obtain a final concentration of 50 µg /ml in Tris buffer (20 mM Tris-HCl, 150 mM NaCl, pH 7.5). The purified CP was mixed with the branched DNA and incubated in 20 mM Tris-Cl, pH7.5, 150 mM NaCl at room temperature overnight on a gentle rotator. The resulting hydrogel was then examined using various microscopic methods.

Electronic Supplementary Information (ESI) available: See DOI: 10.1039/c000000x/

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