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Original Article

Ultrastructural characterizations of DNA nanotubes using scanning tunneling and atomic force microscopes

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ABSTRACT

The potential applications of scanning tunneling microscopy and atomic force microscopy for the characterizations of DNA nanotubes in nanoscale have been described here. The nanotubes were designed using the Cadnano software according to $M_{13}mp_{18}$ DNA as a scaffold. DNA nanotubes were fabricated using the origami technique assisted with ligase treatment subsequently. Transmission electron microscopy confirmed the morphology of DNA nanotubes. For the topographic characterization of DNA nanotubes, an atomic force microscope was used in comparison to a scanning tunneling microscope. The scanning tunneling microscopy results revealed a high-resolution topography of DNA nanotubes in the constant-current mode; however, more details of the self-assembly in DNA strands in nanotubes were explored by atomic force microscopy with contact mode (or constant height). Our findings suggested that those two microscopes could be candidates for ultrastructural characterizations of DNA nanotubes for obtaining two- and three-dimensional micrographs

1. Introduction

The potential applications of DNA nanotubes in nanotechnology and medicine, particularly as intracellular delivery vehicles, need simplified procedures for fabrication and characterization of DNA nanotubes [1]. These architectures have recently been candidates as peptide-delivery vehicles for enhancing the differentiation of neural stem cells into neurons [2]. DNA nanotubes are filamentous structures formed from double-stranded DNA helix [3]. The high aspect ratio, the long and narrow central channel, and the sidewalls from the DNA material are characteristics of DNA nanotubes [4]. Since the fabrication of DNA nanotubes is an interesting subject in DNA nanobiotechnology, different methods for the fabrication of DNA nanotubes have been developed [5–15]. Recently, we described a simplified method for the fabrication of DNA nanotubes with minimum numbers of staples using an origami technique assisted with a ligation treatment of sticky-ended DNA nanostructures (Fig. 1) [16].

There are limited methods for the microscopic characterization of DNA nanotubes. The most well-known microscope is the transmission electron microscope (TEM) [7,8]; however, the TEM could not demonstrate more details of these DNA nanostructures, especially in topographic mode. Hence, scanning probe microscopes,

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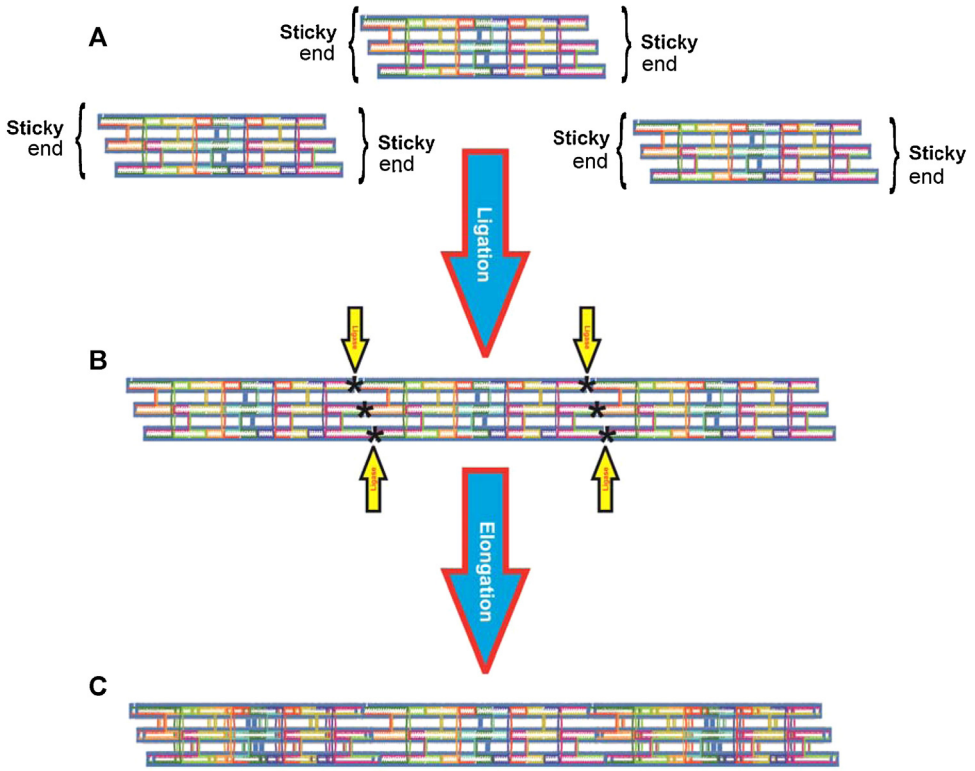

Fig. 1. Schematic of fabrication DNA nanotubes via integration of sticky-ended DNA nanostructures by ligation treatment. (A) Sticky-ended DNA nanostructure; (B) Ligase-treated sticky-ended DNA nanostructures; (C) Fabricated DNA nanotube.

particularly the atomic force microscope (AFM), have commonly been employed for this purpose [17]. For the characterization of DNA nanotubes, the AFM needs specialized cantilever probes that are expensive and need carefulness in practice [18].

However, the scanning tunneling microscope (STM) is another scanning probe microscope that gives more details of DNA nanostructures in topographic mode [19,20]. In addition, the STM could partially explore the electrical characteristics of the nanostructures via the tunneling effect of electrons between the sample and surface molecules [21]. Moreover, the cost of tips for the STM is less than those probes in the AFM. Here, we compared the performance of the AFM and STM for the ultrastructural characterizations of DNA nanotubes. In addition, the critical factors that strongly affect the quality of the DNA-nanotube structural information are introduced.

2. Materials and methods

2.1. Chemicals and instruments

The thermal condition for self-assembly in origami reaction was set using C1000 thermal cycler (Bio-Rad, California, USA). Transmission electron microscopy was done using Philips EM028 TEM, Aachen, Germany. The micrographs were obtained by JPK-AFM (JPK Instruments AG, Berlin, Germany). Mica was prepared from Nanotechnology Systems Corporation, Tehran, Iran. $M_{13}mp_{18}$ phage genome and T4 DNA ligase were purchased from New England Biolabs (Massachusetts, USA). Desired single-stranded oligonucleotides were synthesized and desalted by Sigma–Aldrich Chemie GmbH (Munich, Germany). Quantum Prep Freeze 'N Squeeze DNA gel-extraction spin columns were from Bio-Rad. SYBR Gold nucleic-acid gel stain was purchased from Molecular Probes Inc. (Eugene, Oregon, USA). GeneRuler DNA Ladder Mix was from Thermo Fisher Scientific, Inc. (Waltham, MA, USA).

2.2. Fabrication of DNA nanotubes

Using the Cadnano software with honeycomb style, staple sequences for the folding and fabrication of DNA nanotubes were selected (Table 1). The $M_{13}mp_{18}$ phage genome was used as the scaffolded DNA, and the staples were designed based on their complementarities with the special sites of the scaffold sequence for shaping sticky-ended nanostructures [16].

The origami reaction was prepared by combining 20 nM DNA scaffold ($M_{13}mp_{18}$ single-stranded DNA) and 100 nM of each staple oligonucleotide that were diluted in 1× Tris base, acetic acid, and EDTA buffer (40 mM Tris–acetic acid buffer, pH 8.0, and 12.5 mM magnesium acetate), and then the mixtures were kept at 95 °C for 5 minutes, and then annealed from 95 °C to 20 °C with a constant rate of–1 °C/min in the thermocycler [16]. For the fabrication of DNA nanotubes, the origami products were treated by ligase. The ligation-reaction mix was prepared

Table 1
Staple results obtained from the Cadnano software for self-assembly of desired DNA nanotubes.

No.	Sequence (5′ to 3′)
1	CCAACGTGCAGGTCATTCGTA
2	CACTATTCCGGTTCATGGTCG
3	TTCCAGTTCCCTTAAGCAGGC
4	GAGATAGGGTTGACGCGCGGGGAGAGGCGGT
5	ACGGCCAGTGCCTGTTTCCTG
6	CATGCCTCAAAGGGGCGCTCA
7	GAGGATCAAAGAACGTCGGGA
8	GGCAAAATTGGAACGCTGCAT
9	ATCATGGGCTCACAAATGAGTGAGCTAACTCAC
10	GGTACCGACGAGCCAGTGTAA
11	GAAAATCTTGCCCTCACCAGT
12	AGCGGTCCACGCTGGTTGAGAGACGCCAGG
13	TGTGAAATTGTTATCCTCATAGCAAGCTTG
14	ACAACATAGCTCGAGACTCTA
15	CAGCTGACTGTTTGCGAAATC
16	CTGGCCCTTGCCCCTAAATCAAAAGAATAGCCC
17	AGCCTGGCTTTCCAGTGGACT
18	GAGACGGCGTGCCAAAGAGTC
19	GTGGTTTTCGGCCAAGTGTTG
20	TTGCGTATTGGGGTTGCAGCA
21	ATTAATTGCGTTCGAAAAACCGTCTATCACG
22	CTGCCCGGGTGCCTATTCCAC
23	AACCTGTGCCATAAGGAAGAA
24	CCAACGTGCAGGTCATTCGTA

containing 2 μL of 10× T4 DNA ligase reaction buffer, 10 μL of self-assembled DNA nanotubes, 2 μL of 50% polyethylene glycol, and 1 μL of T4 DNA ligase enzyme. Finally, the ligation mix was incubated at 16 °C for 2 hours [16,22].

2.3. Transmission electron microscopy of DNA nanotubes

The TEM was used to determine the size and morphology of DNA nanotubes. For this purpose, the gel-extracted DNA nanotubes by Quantum Prep Freeze 'N Squeeze DNA gel-extraction spin columns were immobilized by syringe spraying on Agar Scientific (Stansted, Essex CM24 8GF, United Kingdom) holey carbon film with 300-mesh $Cu_{(50)}$.

2.4. Atomic force microscopy of DNA nanotubes

Five microliters of the gel-extracted DNA nanotubes was immobilized on a mica surface for 4 hours at room temperature (25 °C) to be dried. The samples were imaged using contact mode with JPK-AFM, with 150 Hz IGain, 0.0048 PGain, and 1.0 V set point via a JPK NanoWizard control. The cantilever was ACTA-10 probe model (material: silicon, N-type, 0.01–0.025 Ω/cm). Rough data were graphically processed with the JPK Nanoanalyzer software.

2.5. Characterization of DNA nanotubes by STM

The gel-extracted DNA nanotubes were diluted 10^3-folds in Tris base, acetic acid, and EDTA–Mg^{2+} buffer (pH 8.0). Then, 5 μL of diluted sample was immobilized on the highly ordered pyrolytic graphite (HOPG) by drying for 3 hours at room temperature [21,23]. The samples were imaged using topographic mode with STM, with 0.1 nA current set point and 0.2 V sample bias through a platinum–iridium tip. Rough data were first processed by using line adjust, plain adjust, and average filters of the NAMA-STM Nanoanalyzer software (Nanotechnology System Corporation, Tehran, Iran). Then, the coloring process was tested on the obtained micrographs for different levels [21].

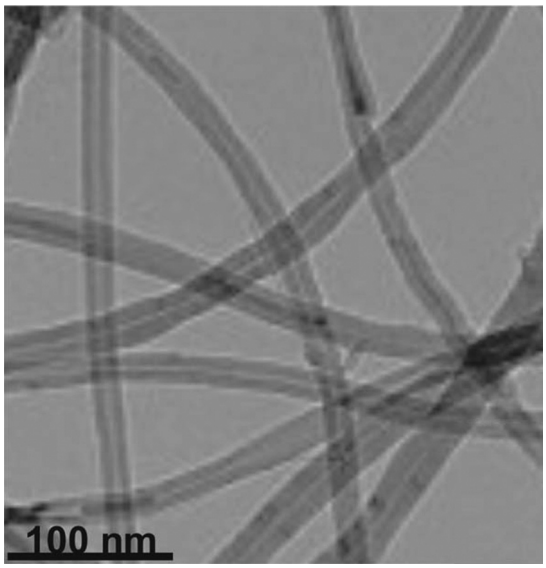

Fig. 2. Transmission-electron-microscope micrograph of DNA nanotubes. The nanotubes have micron lengths with nanoscale widths. The micrograph was obtained with Philips EM028 transmission electron microscope equipped with field-emission gun.

3. Results

3.1. TEM micrograph of DNA nanotubes

The TEM micrograph of DNA nanotubes is shown in Fig. 2. The nanotubes were in filamentous shapes. The nanotubes were in micron sizes in lengths, and their morphologies confirmed their fabrication efficiently.

3.2. AFM micrograph of DNA nanotubes

The AFM was used for the characterization of the produced DNA nanotubes. The atomic force micrograph of the fabricated DNA nanotubes demonstrated the tube-shaped nanostructures of the DNAs (Fig. 3). The presence of these filamentous nanostructures confirmed efficiently the self-assemblies and ligations of DNA nanotubes; however, the ultrastructures of DNA nanotubes have been magnified at the inset.

3.3. STM micrograph of DNA nanotubes

Fig. 4 shows the three-dimensional micrograph of DNA nanotubes by STM with zoom-in ultrastructures. The micrograph (inset) indicates the two-dimensional micrograph of the nanotubes. This micrograph demonstrated highly ordered nanotemplates clearly; moreover, the sticky ends of the primary nanotemplates could be joined successfully together among the elongated DNA nanotubes.

Table 2
Parameters affecting capabilities of atomic force microscope and scanning tunneling microscope in ultrastructural microscopy of DNA nanotubes.

Microscope	Scanning range	Scanning frequency	Scanning mode	Probe	Figuration of micrographs	Scanning phase	Substrate for sampling
AFM	Wide ($50 \times 50\ \mu m$)	Slow	Contact, noncontact, tapping	Silicon cantilever	Two- & three-dimensional	Vacuum, air, liquid	Mica
STM	Limited ($8 \times 8\ \mu m$)	Slow	Constant current, constant height	Pt–Ir tip	Two- & three-dimensional	Vacuum, air	HOPG

AFM = atomic force microscope; HOPG = highly ordered pyrolytic graphite; Pt–Ir = platinum–iridium; STM = scanning tunneling microscope.

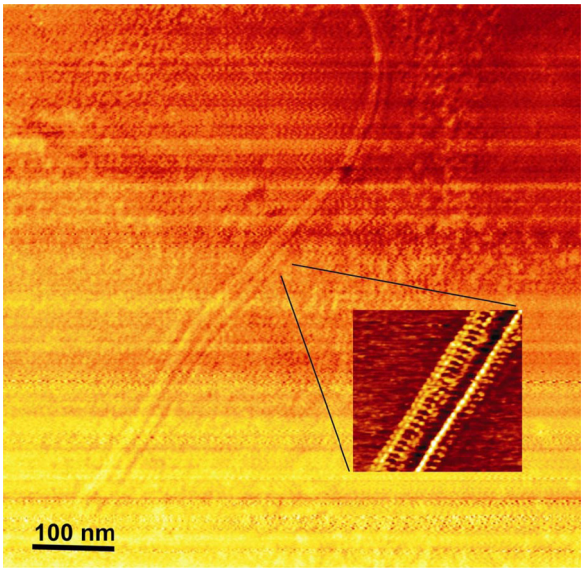

Fig. 3. Atomic force micrograph of a DNA nanotube on mica surface. Two-dimensional micrograph of DNA nanotubes; inset, high-resolution micrograph of DNA nanotube ultrastructures. The image has been obtained and analyzed using nanoatomic force microscopy with 150 Hz IGain, 0.0048 PGain, and 1.0 V set point via a JPK NanoWizard control.

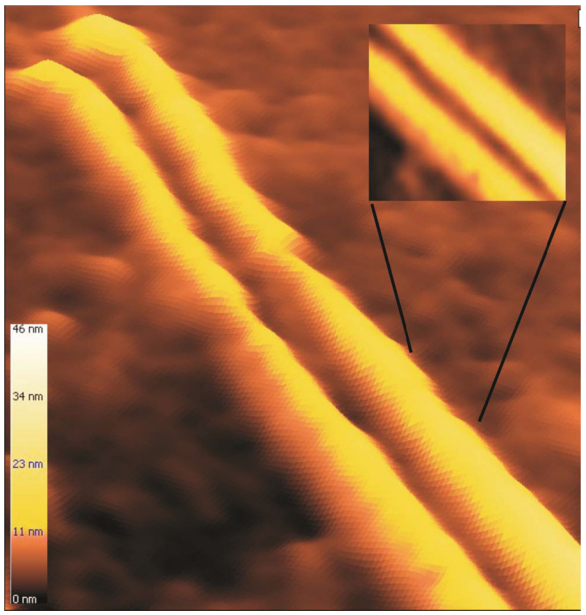

Fig. 4. High resolution of a scanning tunneling microscope micrograph from DNA nanotubes on highly ordered pyrolytic graphite surface. Three-dimensional micrograph of DNA nanotubes with ultrastructures; inset, two-dimensional micrograph of DNA nanotubes with more details of their structures. The image has been obtained by NAMA-STM SS-3L1 (Nanotechnology Systems Corporation). Current set point and sample bias voltage were set at 0.1 nA and 0.2 V, respectively. Rough data were filtered by line, plain adjusts, and average filters of NAMA-STM SS-3L1 Nanoanalyzer software.

The color diagram demonstrates the nearly 46-nm height of nanotubes from the HOPG surface.

4. Discussion

One of the many exciting prospects of DNA nanotechnology lies in the design of DNA nanostructures, which, under the right conditions, are self-assembled into discrete nanotools with novel applications [3]. The nanotubes are promising nanomaterials not only at the nanoscale applications [1,2], but also their fabrication methodologies are important based on their components and designs [4,5]. DNA nanotubes have the advantage of being readily self-assembled in the liquid phase and easily functionalized on their outer surface through modification of the side chains [2]. This allows their physical and chemical characteristics to be tailored for specific medical or other technological applications [1,2]. A challenging aspect of this new nanomaterial, however, is their characterization, since they are sensitive to damage by traditional microscopy techniques [24–26]. Here, we aimed to introduce the capabilities of the AFM and STM for an in-depth analysis of DNA-nanotube ultrastructures with standard dimensions. The ultrastructural characteristics of DNA nanotubes give invaluable information about the correct shaping of these nanostructures, and give topographic data with more precise measurements than those data via the electron microscopes. Among the myriad of characterization methods for studying materials with smaller dimensions, we discuss here the capabilities of AFM and STM for the characterization of self-assembled DNA nanotubes (Table 2).

As demonstrated in Fig. 2, the TEM provided some information about the DNA nanotubes in cross-sectional and longitudinal dimensions; however, it did not provide direct information about their topologies. The technique that was therefore utilized to access this information was the AFM; however, it was important to consider several factors for characterizations by the AFM, such as sample preparation, appropriate tips, and the optimized compression power. For obtaining the optimized height profile, silicon cantilevers with low spring constants were used in soft tapping mode, because the DNA nanotubes were soft and easily compressed. A clear image from the surface can be realized using a low scan rate and amplitude set point during the measurement. Fig. 3 shows an example of an AFM image

of DNA nanotubes deposited on a mica substrate. It was therefore important to carefully identify the single-DNA nanotube in order to obtain accurate aspect profiles.

For a successful STM investigation of the DNA nanotubes, a well-defined sharp tip, evenly dispersed DNA nanotubes on the conductive surface of HOPG, and the optimized operating conditions of the STM were considered. The STM image shown in Fig. 4 was scanned using the constant-current mode with a low scan rate. Fig. 4 clearly indicates that the DNA nanotubes have a helical surface structure. Interestingly, compared to most of the STM investigations described in the literature that were done under an ultrahigh-vacuum condition with constant-current mode [27,28], the detailed helical molecular structure of the DNA nanotubes and measurements between the stacks were obtained using the constant-current mode with a slow scan rate under ambient conditions.

In conclusion, DNA nanotubes have been successfully characterized using the TEM, AFM, and STM. Whereas the TEM was useful for providing images and measurements of the DNA-nanotube diameters, the STM revealed the details of the molecular organization. By combining all of the information, we now have a clearer understanding of the DNA-nanotube ultrastructures, which are essential for designing and fabricating DNA nanotubes for new applications in biomedicine [1,2]. As with many systems though, the new characterization methodologies would certainly be explored in order to understand other features of DNA nanotubes, such as their electrical and mechanical characteristics.

Conflicts of interest

The authors declare no conflicts of interest.

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