Supramolecular Materials Comprising Nucleic Acid Biopolymers

Wirasak Smitthipong,[1,2] *Thorsten Neumann,*[1,3] *Arkadiusz Chworos,*[4] *Luc Jaeger,*[1,3] *Matthew Tirrell**[1,2]

Summary: We have generated a supramolecular self-assembling film by exchanging the counter-ions of the phosphate moieties in nucleic acid with those of cationic amphiphiles as didodecyldimethylammonium bromide (or DDAB). SAXS and WAXS data for all film samples showed similar harmonic peaks suggesting a lamellar multilayer structure with layers of nucleic acids being separated by lipid bilayers of DDAB. AFM height images also showed that double stranded nucleic acid film can form the step or plateau type of structure and shorter nucleic acid film showed shorter step feature. Moreover, the length and the molecular structure of DNA and RNA can be used to manipulate the mechanical properties of these self-assembled films.

Keywords: biomaterials; biopolymers; self-assembly; supramolecular structures

Introduction

Nucleic acids are nature's best example of supramolecular materials. They are programmed at the level of nanoscopic structure to assemble with exquisite precision and reliability.[1,2] Perhaps surprisingly, there have been relatively few attempts to use the abilities of nucleic acid polymers in the formation of macro-scale materials.[3,4] In addition to their programmability and addressability, nucleic acids can also be used to form complexes with oppositely charged surfactants, macromolecules, assemblies and nano-particles. Electrostatic interaction assembly methods employ oppositely charged polyelectrolytes which build self-assembled multilayers made of polyanions with positively charged polycations.[5] Many different routes are being attempted for the use of electrostatic interactions as cationic/anionic polyelectrolyte interaction in large-scale biomaterial properties such as drug delivery,[6] gene delivery,[7] biosensors[8] and it has even been studied in electronics[9] for many years. However, the control of this mechanism is not well-developed and the resulting structures are not well-understood in terms of predictable processing-structure-property relationships.

Therefore, existing biomaterial applications may benefit from a better understanding of polyelectrolyte multilayer films as model systems, especially the structure and property of self-assembly biopolymers based on nucleic acid. We demonstrate herein the formation and structure of self-standing cast films using anionic molecules, such as tRNA (yeast) and DNA (Salmon), with the cationic amphiphile as didodecyldimethylammonium bromide (DDAB). The preparation method of these self-standing films in the present work is based on Okahata's works.[3,4] However, we have improved this method to obtain the better yields of material and to make it easier to manipulate the film. For example,

[1] Materials Research Laboratory, University of California, Santa Barbara, California 93106, USA
E-mail: tirrell@engineering.ucsb.edu
[2] College of Engineering, University of California, Santa Barbara, California 93106, USA
[3] Department of Chemistry and Biochemistry, University of California, Santa Barbara, California 93106, USA
[4] Department of Physics, University of California, Santa Barbara, California 93106, USA

we used 1× TBE buffer solution (0.089 M Tris, 0.089 M Borate and 0.002 M EDTA) instead of water to dissolve nucleic acids which can soluble the whole nucleic acids, we can use another organic solvent as isopropanol to dissolve the complex that can change the film's morphology and we used a glass mold instead of a Teflon or plastic mold for film casting, which enables easier pull-off of the film, especially in case of short chain RNA film.

Preparation of Supramolecular Self-Standing Film

Nucleic acids, tRNA from yeast (15 base pairs, as determined by polyacrylamide gel electrophoresis and HPLC) and DNA from Salmon (2000 base pairs, as determined by agarose gel electrophoresis), were dissolved in 1× TBE buffer solution, and then were mixed with an equivalent aqueous solution of 1.1 mol cationic amphiphile or DDAB. The reaction mixture of complex formation was first stirred at room temperature for 3 hours, and the water-insoluble complexes were then collected by centrifugation and lyophilizing. The complexes obtained were soluble only in organic solvents, such as chloroform, ethanol and isopropanol. The solution was then cast on a glass mould under slow evaporation conditions at room temperature which allowed the formation of the self-assembling transparent films with a thickness of several microns. The obtained films were formed in one-to-one stoichiometry of a phosphate anion and the cationic amphiphile, which was confirmed by elemental analysis. (Figure 1)

Structure of the Film

X-ray diffraction experiments were used to determine the structures of the film. Wide-Angle X-ray Scattering (WAXS) were performed with Cu Kα radiation using custom built X-ray diffractometers with a MAR345 image plate detector and a rotating anode X-ray generator. The sample to detector distance was 235 mm. The integrated diffraction data are plotted as a function of $q = (4\pi/\lambda)\sin(\theta)$, where λ is the wavelength of the beam ($\lambda = 0.154$ nm) and θ is the Bragg angle. WAXS profiles of the

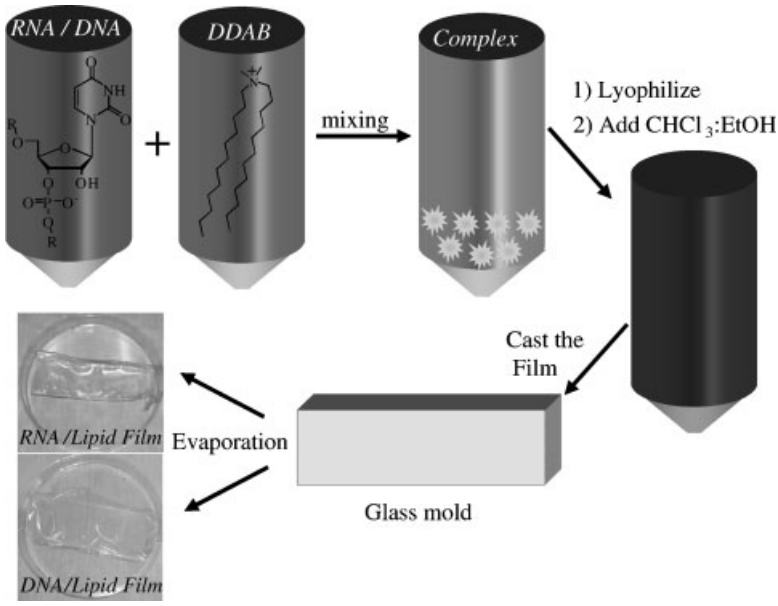

Figure 1.
Preparation method of self-standing films via electrostatic interaction between polyanion (RNA or DNA) and cationic amphiphile (DDAB).

complexes of DNA with the double-chain surfactants like DDAB have been identified as multilamellar structures with alternating bilayers and DNA monolayers.[10,11] However, the films that we investigated were dried samples, whereas in the previous work samples[10,12] were generally in the aqueous environment. Interestingly, the peak width (001) observed for RNA film (Figure 2B) was typically narrower than that of DNA film (Figure 2A), implying a higher degree of local ordering. The broad peak seen in WAXS data from all films ($q \approx 14\text{nm}^{-1}$) confirmed that DDAB chains are in the fluid state, as expected from L_α phase. The WAXS data for both DNA and RNA films showed similar harmonic peaks (00L), suggesting a lamellar multilayer structure with layers of nucleic acids being separated by lipid bilayers of DDAB (Figure 2C).

The electrostatic interaction between the polyanion and the cationic lipid plays an essential role in constructing of the polyelectrolyte lipid complexes in which the cationic lipids are concentrated in the vicinity of the polyelectrolyte.[13] The effect of electrostatic and hydrophobic interactions on the structural formation and the transition of complexes were examined in terms of the charge density, hydrophobicity, backbone flexibility of polyelectrolyte chains, and the surfactant tail length.[14–15] Moreover, the disordered fluid state of DDAB's tails has been detected in the same value ($q \approx 14\ \text{nm}^{-1}$)[12] for all the samples. This result revealed that the hydrophobic interactions among the double hydrophobic tails of DDAB molecules were very important in forming the lipid bilayers in the complexes.[16]

Morphology

The AFM experiments were performed using a Digital Instrument Nanoscope IIIA (model: NS-3) under ambient conditions and using a commercial silicon tip (NSC12). All the sample films were analyzed in the tapping mode. When we compare the images of $5\mu\text{m} \times 5\mu\text{m}$ (Figure 3A to B), we can see that there are step-like structures within the DNA films, but not in the

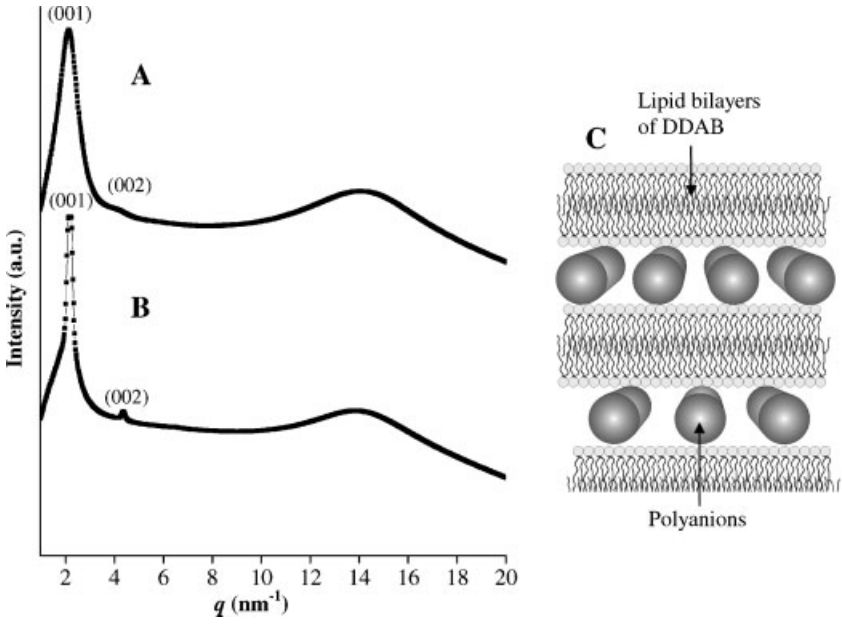

Figure 2.
WAXS scans of polyanionic/DDAB films: (**A**) DNA/DDAB film and (**B**) RNA/DDAB film. Schematic picture of the lamellar multilayer structure of polyanions/DDAB film (**C**).

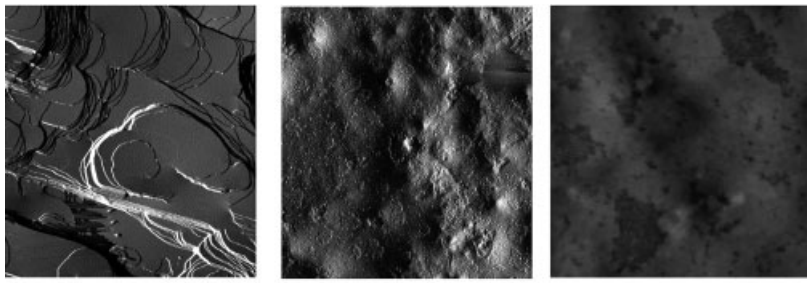

Figure 3.
AFM images 5 μm × 5 μm of (**A**) DNA film (2000 bp) and (**B**) RNA film (15 bp). AFM image 1 μm × 1 μm of (**C**) RNA film (15 bp).

RNA films. This, we expect, due to the fact that DNA (2000 bp) is one hundred times longer than RNA (15 bp). However, if we increased the resolution of the RNA film to a scale of 1 μm (Figure 3C), we see the holes of RNA instead of the steps seen in the DNA film. This allows us to predict that step-like layers will be formed with both nucleic acids, but the lengths of the strands are responsible for the degree of order within the film. Large molecules form more homogeneous layers with wide-spread plateaus, while shorter chains form shrunken plateaus several 100 nm in diameter. This result is in good agreement with the results obtained by the tensile test,[3] in which the length and the molecular structure of DNA and RNA can account for the tensile properties of these self-assembly films.

Mechanical Properties

We also investigated the macro-scale properties via tensile tests of the films. Mechanical properties were characterized using a tensile testing machine (Instron) according to ISO 527-1:1993 (E). The tensile test was carried out on dumbbell specimens using a crosshead speed of 0.5 inch/min. The dumbbell specimens were produced from the supramolecular films having 10–40 μm thickness. Each of films was tested at least three times. Figure 4 presented the stress-strain curve of our nucleic acid films compare to typical polymers as polystyrene[17] and silicone rubber[18]. We found that longer nucleic acid molecules possess a higher tensile strength, in which DNA film (Figure 4B) has an elastic property with strain and also

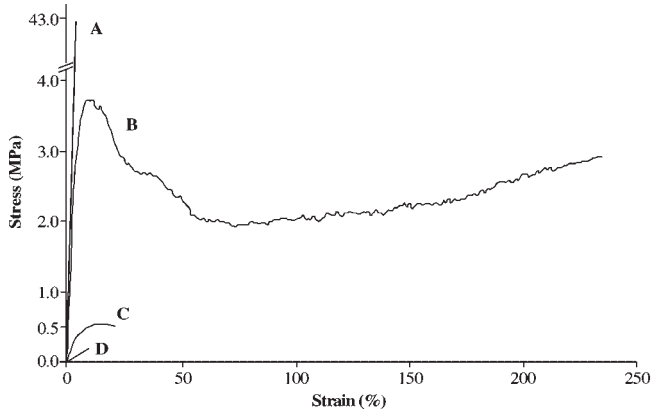

Figure 4.
Stress-strain curve of (**A**) polystyrene, (**B**) DNA film, (**C**) RNA film and (**D**) silicone rubber or PDMS.

has a stronger tensile strength than RNA film (Figure 4C). The mechanical properties of the nucleic acid films are in good agreement with the AFM results of nano-scale property. From the AFM images of films, the step features of DNA film (Figure 3A) can make the film more stretchable due to the sliding of each layer independently, which is why it presents the highest strain at break. However, topology of short RNA film (Figure 3B to C) looks amorphous, which leads to easier breakage. These results illustrate the advantageous correlation of nucleic acid nano-scale structure to the formation and properties of macro-scale films.

Conclusions

Nucleic acids are interesting nano-biomaterials which are able to self-assemble into supramolecular architectures through the formation of specific hydrogen bonds between complementary nucleotide strands. We have succeeded in preparation the self-supporting films incorporating cationic lipids, creating complexes based on electrostatic interaction. X-ray diffraction experiments and AFM results indicate that these films have a lamellar multi-layered structure with layers of nucleic acids being separated by lipid bilayers of DDAB. Interestingly, the tensile properties of the film depend on the molecular structure as well as the length of the nucleic acids. The effect of nucleic acid molecular structure (single and double strands) and molecular weight (different lengths) on the properties of these films is presently being investigated in more details. Moreover, we will focus on the addressability and programmability of the nucleic acid into this supramolecular film, for example, a non-covalent network structure based on complementary poly(homonucleotides) formed by hydrogen bonds which may have an effect on the film's properties and applications, etc. We anticipate that these new bio-materials could have interesting applications as the naturally-occurring functional material, drug delivery material, biosensor, etc.

[1] N. C. Seeman, *Nature* **2003**, *421*, 427.
[2] F. Huo, A. K. R. Lytton-Jean, C. A. Mirkin, *Adv. Mater.* **2006**, *18*, 2304.
[3] Y. Hoshino, S. Tajima, H. Nakayama, Y. Okahata, *Macromol. Rapid Commun.* **2002**, *23*, 253.
[4] Y. Okahata, T. Kawasaki, *Top. Curr. Chem.* **2005**, *260*, 57.
[5] M. Tirrell, *AIChE Journal* **2005**, *51*, 2386.
[6] R. Langer, *Science* **2001**, *293*, 58.
[7] C. R. Safinya, *Curr. Opin. Struct. Biol.* **2001**, *11*, 440.
[8] M. L. Pedano, G. A. Rivas, *Sensors* **2005**, *5*, 424.
[9] G. Hartwich, D. J. Caruana, T. de Lumley-Woodyear, Y. Wu, C. N. Campbell, A. Heller, *J. Am. Chem. Soc.* **1999**, *121*, 10803.
[10] J. O. Radler, I. Koltover, T. Salditt, C. R. Safinya, *Science* **1997**, *275*, 810.
[11] S. Q. Zhou, D. Liang, C. Burger, F. Yeh, B. Chu, *Biomacromolecules* **2004**, *5*, 1256.
[12] G. Subramanian, R. P. Hjelm, T. J. Deming, G. S. Smith, Y. Li, C. R. Safinya, *J. Am. Chem. Soc.* **2000**, *122*, 26.
[13] T. Kawashima, A. Sasaki, S. Sasaki, *Biomacromolecules* **2006**, *7*, 1942.
[14] F. Yeh, E. L. Sokolov, T. Walter, B. Chu, *Langmuir* **1998**, *14*, 4350.
[15] S. Q. Zhou, H. Hu, C. Burger, B. Chu, *Macromolecules* **2001**, *34*, 1772.
[16] M. Tirrell, E. Kokkoli, M. Biesalski, *Surf. Sci.* **2002**, *500*, 61.
[17] S. C. Tjong, S. A. Xu, *J. Appl. Polym. Sci.* **1998**, *68*, 1099.
[18] K. Hosokawa, K. Hanada, R. Maeda, *J. Micromech. Microeng.* **2002**, *12*, 1.

