DNA Structures

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DNA Hydrogel Fiber with Self-Entanglement Prepared by Using an Ionic Liquid**

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DNA hydrogels have a wide range of biomedical applications in tissue engineering and drug-delivery systems.[1,2] There are two ways to create hydrogel structures: one is enzyme-catalyzed assembly of synthetic DNA[3] and the other is by crosslinking natural DNA chemically.[2] For natural DNA, formaldehyde and metal compounds such as arsenic, chromate, and nickel are widely used as crosslinkers.[4] However, these modified DNA hydrogels are unsafe to apply in biological systems because the crosslinkers have potentially adverse side effects, with some being carcinogens.[5] Besides this, these DNA hydrogels are difficult to form into hydrogel fibers by using conventional spinning methods in the absence of chemical crosslinking.[6,7]

In solution, DNA resembles modular proteins such as titin, silk, and polysaccharides.[8] The very flexible linear DNA strands and their noncovalent assemblies can form compacted interwound supercoils in bulk aqueous solution with cationic salts. Alternatively, they can roll into soluble clusters of toroids. In concentrated DNA solutions in poor solvents, rodlike multiple-chain bundling occurs[9] and, simultaneously, single or multiple loops form knots with themselves or with adjacent loops through a nucleation-growth pathway.[10–12] Thus, these condensates are seen primarily as intertwined aggregates of toroids.[10,11]

We were inspired by the spinning processes used by insects (for example, silkworms and spiders) to develop spinning conditions to create the desired DNA hydrogel fibers. It is known that the last process to occur in insect spinning is the formation of a dragline in air.[13] It can be considered that the air effectively removes water through evaporation to produce dense, dried fibers. To replicate this process in wet spinning, we need to ensure that the coagulation solvent does not fill the space created in the spinning droplet by the exiting water. In addition, the diffusion rates of the coagulation solvent and water must be controlled to prevent the formation of a dense skin on the fiber, which could trap water and create a porous structure. If the coagulation solvent also contains crosslinking cations, then the concentrated DNA solution can form hydrogel fibers with intertwined toroidal entanglements. We have found that room-temperature hydrophilic ionic liquids (RTILs) can produce suitable conditions. The feasibility of using RTILs was suggested by our previous work,[14] which showed that 100% of RTILs will absorb water even when bound to a polymer network. Moreover, some RTILs can create low-pH conditions when in contact with water, and such acidic conditions have been used to promote coagulation in the wet spinning of DNA fibers. The cations present in RTILs condense the DNA as a matter of course. In this work, we prepared a DNA hydrogel fiber in a single step by injecting aqueous DNA solution into a coagulation bath of an RTIL.

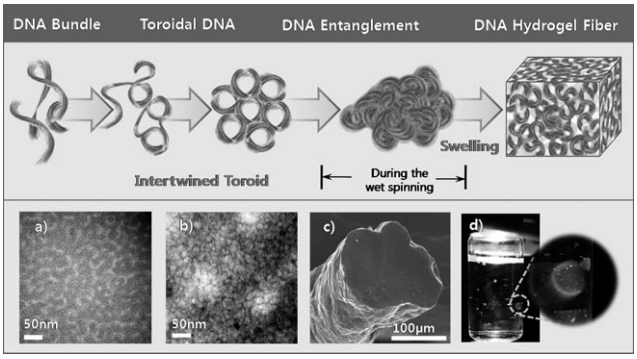

Figure 1. Preparation of the DNA hydrogel fiber: a) DNA condensation in torus-shaped and rodlike morphology; b) toroids circumferentially wrapped around by the RTIL; c) morphology of the DNA hydrogel fiber; d) characterization of the swollen DNA hydrogel.

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This process provided a hydrogel structure without the need for any further chemical modification.

Figure 1 shows a proposed structure of the DNA hydrogel, in which the RTIL was used as both a condensing agent and coagulation solvent. The state of the spinning solution and the morphology of a fiber with a diameter of 200 μm were confirmed by cryotransmission electron microscopy (TEM) and scanning electron microscopy (SEM), respectively. The DNA bundles were transformed into multitoroidal forms and entanglements when the RTIL was added to the concentrated DNA solution (Figure 1 a, b). This indicated that the RTIL was an appropriate condensing agent for wet spinning continuous DNA fibers; as expected, the cations present in the RTIL showed a good affinity for the heteroaromatic rings of DNA. After the wet-spinning process was finished, the condensed DNA successfully formed a continuous hydrogel fiber (Figure 1 c, d) and the RTIL was removed entirely by washing, as confirmed by Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS; see the Supporting Information). Thus, the DNA strands formed a tightly intertwined and entangled state during wet spinning in the RTIL.

The state of the DNA strands in the spinning solution and the hydrogel fiber was characterized by using circular dichroism (CD) and polarized Raman spectroscopy. Figure 2 a shows the CD spectrum of the DNA. The entire DNA CD spectrum showed characteristic features of B-form DNA. These consisted of a positive band around 275 nm, a negative signal with approximately the same intensity at 245 nm, and a maximum in absorbance at the crossover point near 258 nm.[15] When DNA strands are condensed by binding to cations, the amplitudes of positive CD bands are decreased and the amplitudes of negative CD bands are increased.[16,17] Upon addition of the RTIL, the DNA strand in the spinning solution was condensed efficiently because the amplitude of the positive CD band decreased proportionately to the amount of RTIL added. When salt is removed from DNA, there is normally a shift of the wavelength crossover from 255 to 261 nm and an increase in the amplitude of negative CD bands. These features usually indicate the packing state of DNA and DNA denaturation or melting,[17] thereby indicating decreasing rigidity of the DNA strands. However, the amplitude of the negative CD band in Figure 2 a decreased even when the RTIL was added to DNA. This result shows that RTIL treatment can produce the entangled DNA

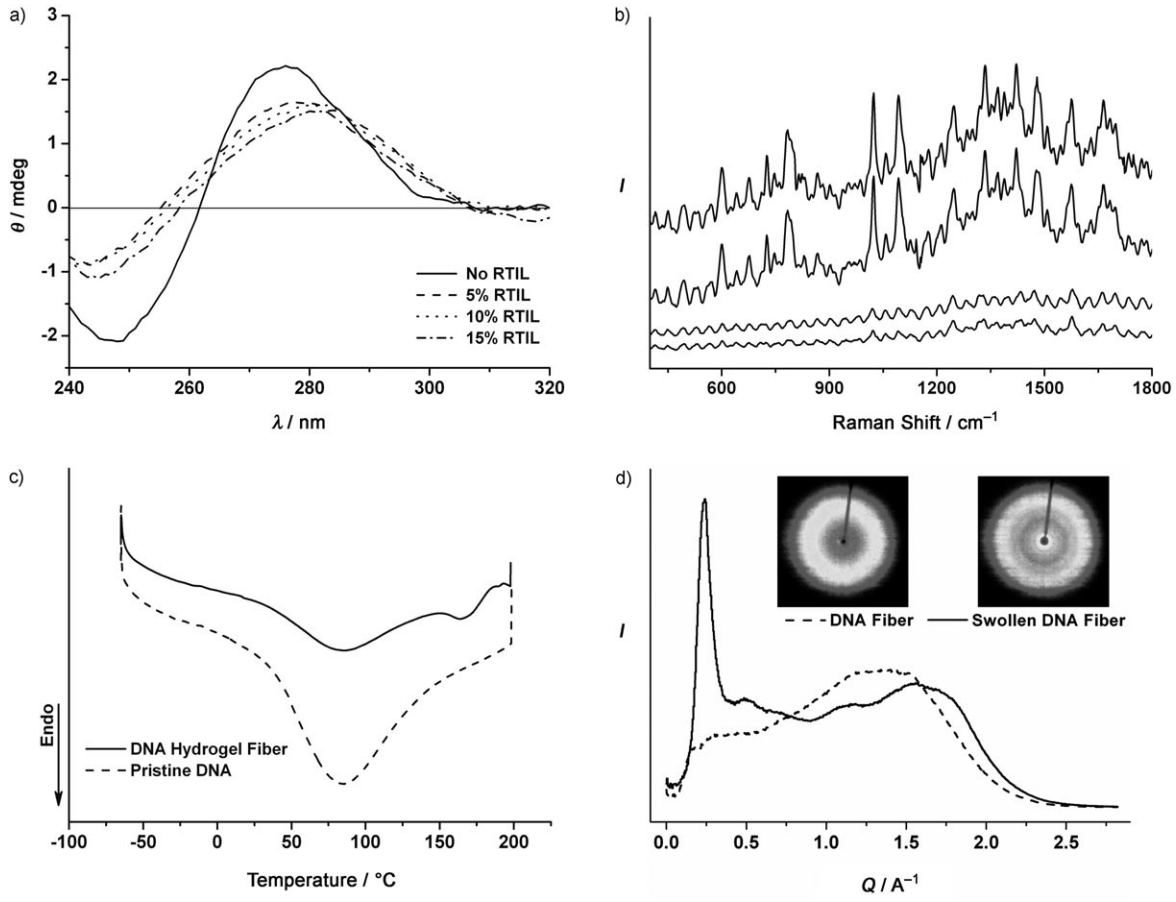

Figure 2. a) Circular dichroism of DNA (0.1 mg mL^{-1}) at different concentrations of the RTIL 1-butyl-3-methylimidazolium tetrafluoroborate, [bmim]BF_4. The curves for RTIL concentrations of 5, 10, and 15% overlap closely and correspond to the native B-form of DNA. $T = 25$ °C. b) Polarized ($I_{\parallel}$, $I_{\perp}$, and $I_{/}$; parallel, perpendicular, and oblique to the axes, respectively) Raman spectra (514.5-nm excitation) for the DNA fiber. These intensities correspond to the relative orientation of the transition moment and to rotary polarization. c) Thermal stability of DNA fibers by differential scanning calorimetry (DSC). The DNA hydrogel fiber had undergone drying. d) X-ray scattering profiles of the DNA hydrogel fiber in a dry and a swollen state.

assembly more efficiently than ordinary salts such as sodium and calcium, because the DNA strands undergo condensation and melting (or denaturation) at the same time, which is not the case with ordinary salts. Thus, the helicity of the DNA condensed by the RTIL is reduced and this is also manifest by a decrease in the amplitude of the negative CD band.[18]

It was expected from the CD spectrum that the ordered transition of DNA strands in the fiber would be changed along with the fiber axis after the wet-spinning process. Polarized Raman spectroscopy is a useful tool to confirm any alteration in the DNA helix or other conformational structures and to test the ordered transition of the DNA strands.[19] Absorbances were measured for the Raman radiation parallel, oblique, and perpendicular to the helical axes and to the fiber axes, $I_{\parallel}$, $I_{/}$, and $I_{\perp}$, respectively. The polarized Raman intensity ratio ($I_{\parallel}/I_{\perp}$) is related to the average α-helix tilt angle with respect to the fiber axis. A disordered structure is indicated by a unity ratio: $I_{\parallel}/I_{\perp} = 1$. As seen in Figure 2b, the intensity ratio was almost 1, a result indicating that the interior structure of the DNA hydrogel fiber consisted of disordered entanglements of DNA strands forming knotted networks. By contrast, the intensity ratio of oriented DNA (see the Supporting Information) was about 0.68, which indicates that each DNA strand could be considered to be ordered according to its fiber axis. As phosphate vibrations are not influenced by the types of bases involved, they play an important role in the structural conformation of DNA, as confirmed by Raman spectroscopy. The band at 815 cm^{-1} caused by stretching vibrations of phosphodiester bonds (O–P–O) in the double-stranded helix[20] is apparent from samples of dried DNA hydrogel fibers. The bands at 835 cm^{-1} and 791 cm^{-1} (O–P–O stretch) are markers of B-form DNA and appeared after the DNA hydrogel fiber was swollen with water. This is because A-DNA forms under nonphysiological conditions when B-DNA is dehydrated (see the Supporting Information). The present findings provide evidence that native DNA was maintained in its B-DNA form after wet spinning.

The effect of temperature upon the native DNA hydrogel fiber was investigated by differential scanning calorimetry (DSC), which measures the heat absorbed during thermal denaturation, as depicted in Figure 2c. In pristine DNA, the melting transition is broad because of the heterogeneous base composition of the DNA fragments. However, a flatter melting transition was observed in the DNA fiber. In the case of polymeric crosslinked networks, the flatter melting endotherm is attributed to disordering of the regions between the crosslinked chains. This disorder indicates that amorphous networks were created by entanglements with intertwined DNA strands in the DNA fiber. This amorphous structure was confirmed by X-ray scattering analysis (Figure 2d). In dried DNA hydrogel fibers, there were no observed peaks that characterized the amorphous form, but the DNA hydrogel fibers had a variously spaced lattice, with values of about 25, 12, 9.6, 5.3, and 4.05 Å as the d spacings, when they were swollen with water. This result is attributed by the work of Parsegian and co-workers[21] to repulsive interactions between DNA double helices that are caused by water bonded to the surface as hydration. Thus, voids are formed by reversion of the DNA strand to an unfolded state from a condensed state following swelling. These results suggest that the DNA hydrogel fibers had a network of random entanglements.

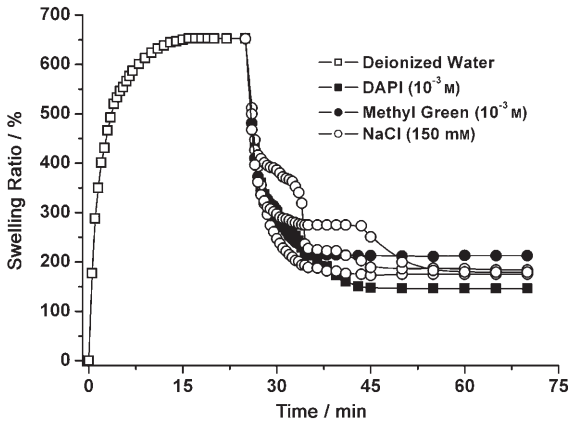

Figure 3. The swelling behavior of the DNA hydrogel fiber. Initially, swelling was confirmed in deionized water from the dry state for 25 min. The ionic effect was exhibited continuously during deswelling by the introduction of specific groove-binding molecules. 4′,6-Diamidino-2-phenylindole (DAPI) is a minor-groove binder, methyl green is a major-groove binder, and Na^+ binds in both grooves. The swelling ratio is calculated as $[(L_s-L_d)/L_d]\times 100\%$, with L_d and L_s being the lengths of the dry and swollen fiber, respectively.

Figure 3 shows that the swelling ratio of DNA hydrogel fibers depended on cation associations. The DNA fiber had a high swelling ratio of over 600% in deionized water compared with the dry state. The equilibrium state was reached in less than 15 min. In the absence of a counterion (for example, in deionized water), the DNA hydrogel fiber showed normal swelling behavior because the nucleic acid phosphodioxy (PO_2^-) groups formed hydrogen bonds with the water molecules. However, we found that the condensing (deswelling) behavior caused by the introduction of ionic species into the surrounding aqueous medium was different, as it was caused by counterion interaction of the cations and phosphate groups. These cations neutralized the charge on the DNA and disrupted the hydrogen bonding of the surrounding DNA (that is, the local electrostatic repulsion between the DNA strands became weaker). Thus, the deswelling ratio of the DNA hydrogel fiber depended on the binding mode of the cations to the DNA according to the steric effects and valence. Generally, there is a tendency for cations to bind to the major DNA groove as this effects more efficient DNA condensation[22] and leads to a large deswelling ratio. However, minor-groove binding was efficient in these DNA fibers. This could be ascribed to the steric effect of methyl green, because the major groove would be distorted by DNA condensation where the major groove was less accessible. For NaCl solutions, the deswelling curve unusually shows a stepwise decrease. This anomalous result can be attributed to the steric effect of Na^+ on the random network structure of the DNA fibers, because Na^+ can be bound in both grooves of the DNA.[23] Consequently, the DNA hydrogel fiber appeared to be composed of randomly intertwined DNA entangle-

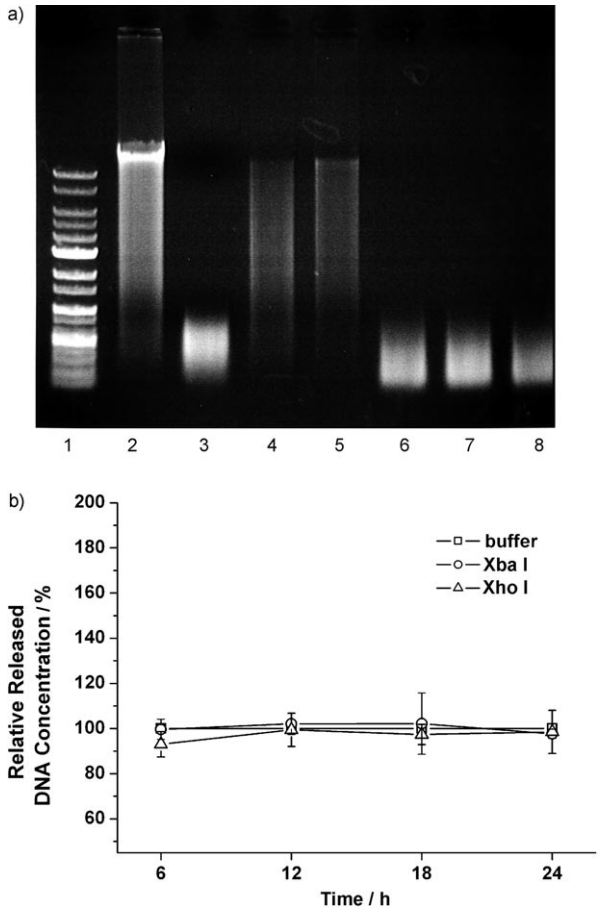

Figure 4. Effects of restriction endonuclease (DNase) digestion on pristine DNA and the DNA hydrogel fiber. a) Gel electrophoresis results. Lane 1: marker; lanes 2 and 3: the pristine DNA solution and the spinning solution, respectively, without DNase; lanes 4 and 5: the pristine DNA digested with XbaI and XhoI, respectively; lane 6: the DNA hydrogel fiber without DNase; lanes 7 and 8: the DNA hydrogel fiber digested with XbaI and XhoI, respectively. b) UV detection of DNA cleaved by the DNases from the DNA hydrogel fiber.

ments squeezed tightly by the ionic liquid. This is in good agreement with the results of the X-ray scattering analysis, which showed that the swollen DNA hydrogel had a variously spaced lattice.

Figure 4 shows that the DNA hydrogel fiber exhibited resistance to restriction endonucleases (DNases), which are enzymes that cleave DNA at specific nucleotide sequences. The sequences recognized are four to six exposed nucleotides long. For example, the DNases XbaI and XhoI recognize the sequences TCTAGA and CTCGAG, respectively. Thus, the DNase can bind to and cleave both strands of the DNA molecules because the same recognition sequence occurs in both strands of the DNA duplex. The pristine DNA without DNase was detected as one major transcript of 2000 kb; it was cleaved efficiently by the DNases, as shown in Figure 4a (lanes 4 and 5). However, the DNA hydrogel fiber was not cleaved and no release was shown in the UV results (Figure 4a, lanes 7 and 8; Figure 4b). This result indicates that the nucleotide sequence of this DNA hydrogel fiber could not be exposed to the DNases because the DNA strands were highly entangled at levels below four to six nucleotides long.

In conclusion, we have developed the first DNA hydrogel fiber without the need for crosslinking agents and high temperatures. The DNA fibers maintained their hydrogel form for about 3 months after soaking in deionized water and over a wide range of pH values from about 1 to 10. The DNA hydrogel consisted of native DNA that formed random entanglements to provide physically crosslinked networks. Such DNA hydrogel fibers showed resistance to digestion by DNases and may be exploited in a variety of biomedical applications, such as drug delivery, tissue engineering, and biocompatible composites.

Experimental Section

DNA from salmon testes (approximately 20000 bp), comprising oriented fibers, was purchased from Sigma–Aldrich (St Louis, MO, USA). The room-temperature ionic liquid, 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim]BF_4) was purchased from the Solvent-Innovation Co. (Köln, Germany). All other chemicals were used without further purification. The DNA was completely dissolved in deionized water at 2 mg mL^{-1}. The DNA solution formed a pregel state when [bmim]BF_4 was added dropwise to a final concentration of about 5% w/w. A narrow jet of the DNA solution was injected though a needle (1 mm inner diameter) at 1.5 mL min^{-1} into a coagulation bath containing [bmim]BF_4/ethanol (9:1 w/w) and rotating at 15 rpm. The coagulation time was about 20 min and the coagulated microfibers were then washed several times with ethanol and deionized water.

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