DNA Hydrogels: New Functional Soft Materials

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ABSTRACT: Deoxyribonucleic acid (DNA) hydrogel is a network of crosslinked DNA strands swollen in aqueous solutions. The crosslinks may be physical or chemical, such as the hydrogen bonds or ethylene glycol units, respectively, connecting the strands belonging to different double-helical DNA molecules. As DNA network strands in the hydrogels exhibit properties similar to those of the individual DNA molecules, such soft materials are a good candidate to make use of the characteristics of DNA such as coil-globule transition, biocompatibility, selective binding, and molecular recognition. Physical DNA hydrogels with an elastic modulus in the order of megapascals can be prepared by subjecting semidilute aqueous solutions of DNA to successive heating–cooling cycles between below and above the melting temperature of DNA. Chemical DNA hydrogels can be prepared by connecting the amino groups on the nucleotide bases through covalent bonds to form a three-dimensional DNA network in aqueous solutions. In this article, we summarize the preparation strategies of DNA hydrogels with a wide range of tunable properties. © 2011 Wiley Periodicals, Inc. J Polym Sci Part B: Polym Phys 49: 551–556, 2011

KEYWORDS: biopolymers; gelation; hydrogels; viscoelastic properties

All living cells contain deoxyribonucleic acid (DNA) molecules serving as the carrier of genetic information in their base sequences. DNA is a biopolymer composed of building blocks called nucleotides consisting of deoxyribose sugar, a phosphate group, and side group amine bases.[1] DNA hydrogel is a human-made network of crosslinked DNA strands swollen in aqueous solutions.[2] Such soft materials with a wide range of tunable properties are a good candidate to make use of the characteristics of DNA such as coil-globule transition, biocompatibility, selective binding, and molecular recognition.[3,4] Because of the unique structure of DNA, chemical compounds having aromatic planar groups are known to intercalate between adjacent base pairs of ds-DNA and result in mutation and endocrine disruption. This fact also suggests that DNA hydrogels can be utilized as an adsorbent specific for toxic materials.

DNA has a double-helical conformation in its native state, which is stable because of the stacking of the amine bases and of the hydrogen bonding between them. When an aqueous solution of DNA is subjected to high temperatures (80–90 °C), the hydrogen bonds holding the two strands together break and the double helix dissociates into two single strands having a random coil conformation.[5] This transition from double stranded (ds) to single stranded (ss) DNA is known as denaturation or melting and can be reversed by slow cooling of dilute DNA solutions. The primary experimental tool for studying thermal denaturation of DNA is the measurement of the UV light absorption at 260 nm. The disruption of base stacking during the dissociation of the double helix decreases the electronic interaction between the bases, so that it becomes easier for an electron to absorb a photon. Fluorescence measurements using ethidium bromide (EtBr) as a fluorescent probe is another tool to monitor DNA denaturation; when EtBr is bound to DNA, its fluorescence increases, and this increase depends on the DNA conformation. Recently, it was shown that the denaturation of DNA can also be monitored using dynamic rheological measurements.[6,7] As ss-DNA is a flexible polymer with a persistence length p of about 1 nm, when compared with semiflexible ds-DNA with $p = 50$ nm,[8–10] the critical overlap concentration c^* of ss-DNA is 80-fold larger than that of ds-DNA. For example, c^* of an aqueous solution of DNA of molecular weight of 2000 base pairs (bp) increases from 0.043 to 3.2 w/v % during denaturation.[6] Hence, solutions of DNA over a wide range of concentration will undergo reversible transitions between semidilute and dilute regimes during the heating–cooling cycle between below and above the melting temperature of DNA. Temperature sweep test of the dynamic moduli of DNA solutions can be used to monitor these transitions.

Figure 1(A) illustrates typical thermal denaturation and renaturation curves of DNA. Here, the elastic modulus G' (symbols) and the loss factor tan δ (curves) of a 3 w/v % DNA solution in 4.0 mM NaBr are shown during the heating–cooling cycle between 25 and 90 °C. ds-DNA from salmon testes with 2000 bp and 41.2% G-C content (melting temperature = 87.5 °C) was used in the experiments.[6,7] G' decreases about two orders of magnitude during the heating

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period and particularly above 70 °C due to the dissociation of ds-DNA strands. UV and fluorescence measurements indeed show complete melting of DNA at 90 °C. The peak appearing in tan δ versus temperature plot at 81 °C is due to the dissipation of energy caused by the dissociated, flexible strands in the solution. Tan δ becomes larger than unity above 70 °C indicating liquid-like response of the system at high temperatures. On cooling back to 25 °C, G' increases again and tan δ decreases below unity due to the reformation of semiflexible double helical fragments building a viscoelastic gel. Experiments show the occurrence of a reversible transition between the semidilute (gel) and dilute (sol) regimes at or below 6 w/v % DNA concentration,[6] indicating that the viscoelastic DNA gels can be melted and reformed by the heating and cooling cycles.

However, a different behavior is observable at higher DNA concentrations [Fig. 1(B)]. In this case, G' remains almost unchanged during the heating period, but it increases at 90 °C and particularly during the cooling period, whereas tan δ remains below unity over the whole range of temperature. At 9.3 w/v % DNA, measurements show that only about 20% ds-DNA fragments melt at 90 °C, whereas 65% of which form again on cooling back to 25 °C. Why ds-DNA remains stable and does not dissociate completely even at 90 °C is due to the high concentration of the DNA counterions. DNA solution (9.3%) is 0.3 M in phosphate groups carrying a net negative charge, or, due to the condition of electroneutrality, 0.3 M in counterions. Such a high counterion concentration in the solution increases the stability of ds-DNA, as was observed before by adding salt to dilute DNA solutions.[11]

Interesting to note that, on cooling back, the final moduli of elasticity of DNA solutions are always larger than their initial values (Fig. 1). Further, both G' and the viscous modulus G''

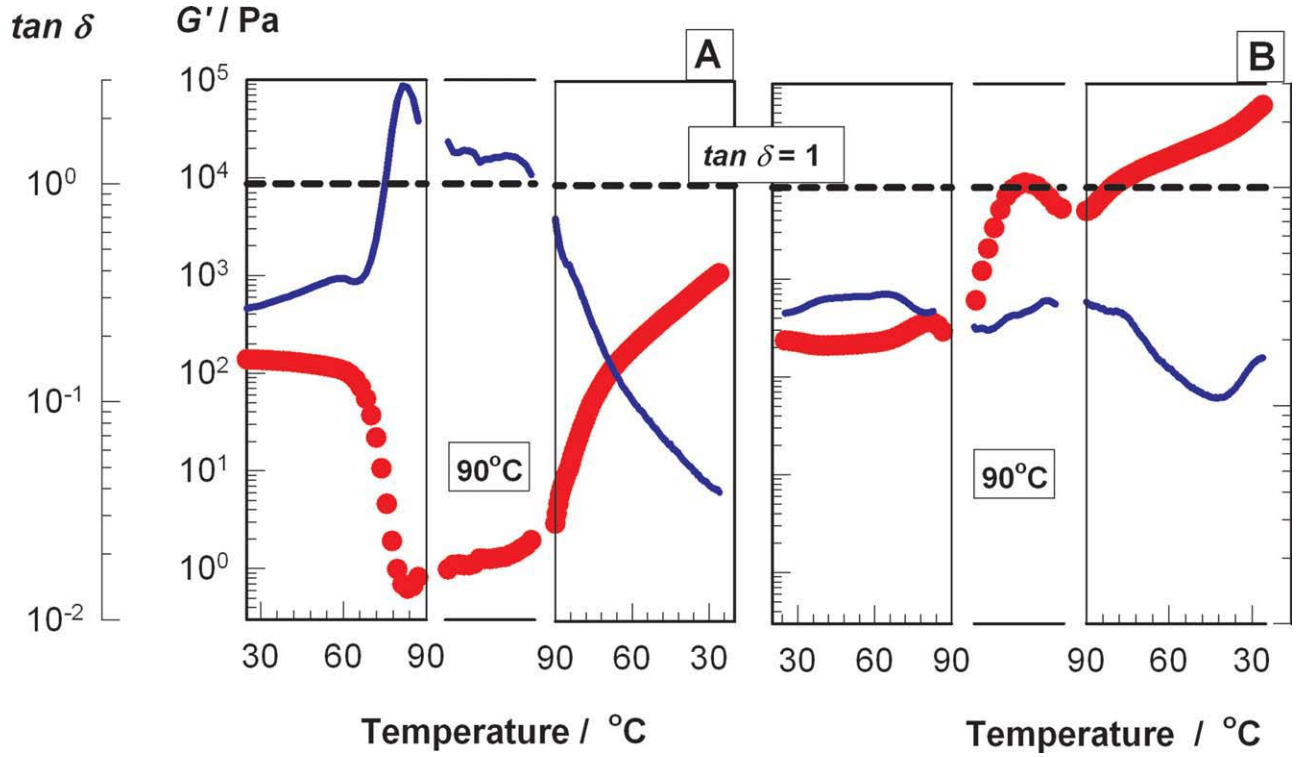

FIGURE 1 The elastic modulus G' (symbols) and the loss factor tan δ (curves) of DNA solutions shown as a function of temperature. The dotted horizontal line represents the condition tan $\delta = 1$. DNA concentration = 3 (A) and 9.3 w/v % (B). Aqueous solutions of DNA in 4.0 mM NaBr were heated between the parallel plates of the rheometer from 25 to 90 °C with a heating rate of 3.25 °C/min, kept at 90 °C for 10 min, and subsequently cooled down to 25 °C with a rate of 1.08 °C/min. $\omega = 1$ Hz. $\gamma_o = 0.01$.

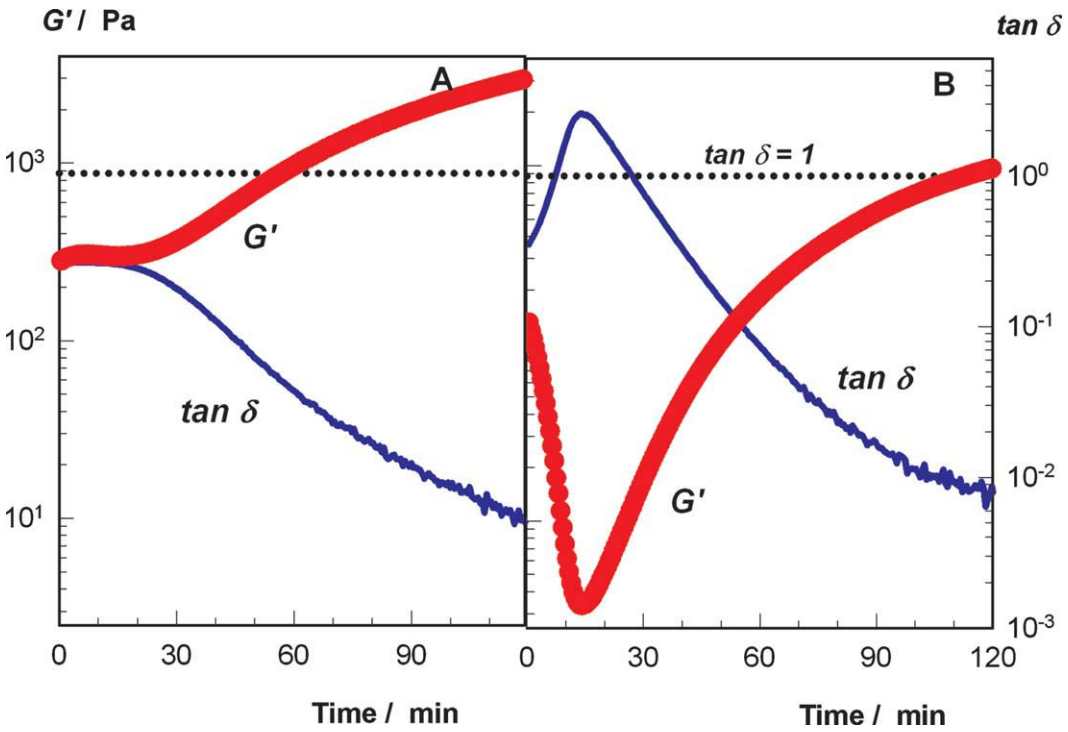

FIGURE 2 Elastic modulus G' and the loss factor tan δ during the crosslinking of ds-DNA at 50 °C in the presence of 10% EGDE. DNA concentration = 9.3 (A) and 6.3% (B). The dotted horizontal line represents the condition tan $\delta = 1$. $\omega = 1$ Hz. $\gamma_o = 0.01$.

of DNA solutions (1–10 w/v %) become frequency independent over the range 10^{-1}–10^{2} rad/s after the heating-cooling cycle, which indicates the dominant viscoelastic relaxations of DNA networks are at lower frequencies. The larger the DNA concentration, the larger is the increase of the modulus due to the heating–cooling cycle. For example, at 9.3% DNA, the elastic modulus increases from 0.3 to 65 kPa while the loss factor decreases from 0.3 to 0.1. Successive heating–cooling cycles of concentrated DNA solutions further increase the elastic modulus and, DNA hydrogels exhibiting moduli of elasticity in the order of megapascals were obtained.[6,7] This highlights a new strategy for the preparation of DNA hydrogels by subjecting concentrated solutions of DNA to heating–cooling cycles. Formation of physical crosslinks is due to the formation of hydrogen bonds between strands belonging to different ds-DNA molecules. Heating of semidilute solutions of ds-DNA above its melting temperature results in the partial or complete dissociation of the double helix into flexible single strand fragments. On cooling back to the room temperature at a slow rate, the dissociated strands cannot reorganize to form the initial double-stranded conformation, so that the hydrogen bonds formed between different strands act as physical junction zones leading to the formation of DNA hydrogels. We have to mention that these additional crosslinks were too weak to withstand swelling pressure. Stress relaxation measurements show that the physical crosslinks formed by the heating–cooling cycle easily dissociate even at strains below 10% and the modulus approaches to that of the initial solutions.[12]

As reported first time by Amiya and Tanaka,[2] DNA hydrogels can also be prepared from aqueous DNA solutions using a chemical crosslinker such as ethylene glycol diglycidyl ether (EGDE) in the presence of TEMED catalyst.[6,7,12–14] EGDE contains epoxide groups on both ends that can react with the amino groups on the nucleotide bases to form a three-dimensional DNA network. Figure 2(A) shows typical gelation profile at 50 °C and at a DNA concentration of 9.3%, where the elastic modulus G' and the loss factor tan δ are shown as a function of the gelation time.[6] The crosslinking of DNA is characterized by an initial lag phase of about 30 min, during which the dynamic moduli remain almost unchanged. The lag phase is followed by a log phase during which G' rapidly increases while tan δ decreases, suggesting the occurrence of the crosslinking reactions between the DNA aggregates formed during the initial reaction period. Fluorescence measurements show that the gels formed at 9.3% DNA concentration consist of mainly ds-DNA network strands.

In contrast, when the crosslinking reactions are carried out at lower DNA concentrations, the gelation profile is characterized by alternate gel–sol and sol–gel transitions resulting in the formation of ss-DNA hydrogels.[7] Figure 2(B) shows a typical gelation profile at 6.3% DNA. During the initial period of the reactions, G' rapidly decreases while the loss factor tan δ increases and becomes unity after 7 min, indicating that the reaction system undergoes a gel–sol transition at this time. G' starts to increase again after crossing a minimum and a sol–gel transition occurs after 27 min. G' keeps

increasing while tan δ decreases at longer times. UV and fluorescence measurements show that the dramatic decrease of the modulus during the initial period is due to the denaturation of DNA strands.[7] At the end of the reactions, the amount of ss-DNA fragments of the gel network is about 90% indicating that the gelation reactions are accompanied by the denaturation of DNA fragments. The rate of denaturation increased with increasing amount of the crosslinker EGDE in the reaction solution. The appearance of alternate gel–sol and sol–gel transitions is due to two antagonistic effects of EGDE-TEMED pair: the one destroying the physical bonds (denaturation) and the other creating chemical bonds (crosslinking). However, at higher DNA concentrations, as ds-DNA conformation is stable due to the high concentration of DNA counterions, ds-DNA hydrogels could be obtained. Thus, the conformation of DNA network strands in hydrogels could be adjusted by the DNA concentration at the gel preparation.

Depending on the crosslinker content, strong to weak hydrogels could be obtained by the solution crosslinking of DNA at 50 °C. At 10 w/w % EGDE with respect to DNA, corresponding to 1.8 moles of epoxide groups per mole of guanine base in the ds-DNA, the elastic modulus G' of DNA hydrogels is more than two orders of magnitude larger than the viscous modulus G'', and both moduli are essentially independent of frequency over the range 10^{-2}–10^{1} rad/s. At high crosslinker contents, the hydrogels are thermally stable and, even at 90 °C, no significant changes in their dynamic moduli were observed.[6] At low crosslinker contents, however, a significant increase in the dynamic moduli is observed both during heating and cooling due to the partial dissociation of the double helix into flexible single strand fragments leading to the formation of entanglements and physical crosslinks. Moreover, although physical DNA hydrogels prepared by the heating-cooling cycle gradually dissolve in water, chemical DNA hydrogels formed using EGDE crosslinker are insoluble in water. DNA hydrogels exhibit typical behavior of polyelectrolyte hydrogels, that is, high swelling ratios in water due to the osmotic pressure of DNA counterions and deswelling in aqueous salt solutions.[7,15–17] At swelling equilibrium in water, DNA concentration in hydrogels is about 0.1%, that is, the strands forming the network chains are in a fourfold extended conformation compared with their conformation after the gel preparation. Similar to the individual DNA molecules exhibiting a discrete transition between elongated coil and a compact globule, DNA hydrogels also exhibit discrete volume phase transition in water–acetone mixtures[2] or in aqueous solutions of polyethylene glycol of molecular weight 20,000 g/mol.[7]

The solution crosslinking reactions of DNA with EGDE crosslinker can also be conducted at subzero temperatures, for example, at −18 °C; in this case, macroporous DNA cryogels are obtained suitable as sorbent for the removal of carcinogenic agents from aqueous solutions.[18] This low temperature gelation technique known as cryogelation is a simple route for the preparation of macroporous gels.[19,20] During the freezing of an aqueous solution of DNA containing the chemical crosslinker EGDE, DNA strands and the crosslinker molecules expel from the ice, and they concentrate within the channels between the ice crystals, so that the crosslinking reactions only take place in these unfrozen liquid channels. After crosslinking and, after thawing of ice, macroporous DNA cryogels are produced whose microstructure is a negative replica of the ice formed. In dry state, the cryogels contain irregular large pores of 10^{1}–10^{2} μm in sizes due to the ice crystals acting as a template during the gelation reactions.[18] Because of the high DNA concentration in the unfrozen domains of the reaction system, the hydrogels are very tough and can be compressed up to about 80% strain without any crack developments.

Biological gels consisting of semiflexible filaments are known to exhibit viscoelastic properties greatly differing from those of synthetic gels of flexible polymers.[21–23] For example, mechanical response of fibrin gel, the major constituent of blood clots, is highly nonlinear and it exhibits an increase in elastic modulus at strain amplitudes above 10%.[24] Gels formed from cytoskeletal and extracellular proteins also stiffen as they are strained, so that they resist large deformations to protect the tissue integrity.[25] Experimental results suggest that the origin of the nonlinear elasticity in biological gels is the close proximity of the contour and persistence lengths of the semiflexible filaments. Thus, the filaments are only slightly coiled between the junction zones so that, even at a modest strain, their end-to-end distance approaches to their contour length L_c. The degree of strain hardening and the strain, at which stiffening becomes significant, depends on the persistence length of the filament. Stiffer filaments such as F-actin or collagen stiffen at a few percent strains while more flexible filaments such as vimentin stiffen only at larger strains, approaching 100%.

Although nonlinear elasticity seems to be general to any network composed of semiflexible filamentous proteins, strain hardening phenomenon in ds-DNA solutions has not been observed. This is attributed to the smaller persistence length of ds-DNA (50 nm), when compared with the strain stiffening proteins (a few micrometers). However, as strain hardening could be facilitated by reducing the contour length L_c of the chain, one may imagine that, by reducing the distance between crosslink points, ds-DNA hydrogels with stiffing properties could be prepared. Indeed, recent stress relaxation experiments conducted on ds-DNA hydrogels show strain hardening behavior of ds-DNA network strands.[12] In Figure 3, the relaxation moduli G_t at a given time scale t are shown as a function of the strain amplitude γ_o. The hydrogels subjected to the measurements were prepared at 5% EGDE and at a DNA concentration of 9.3%; thus, they consist of ds-DNA strands.[12] DNA gel is in the linear regime, that is, the modulus G_t is independent of strain for γ_o below 40%, while it exhibits strain hardening for γ_o between 40 and 250%, before softening at higher strains. The shorter the time scale, the larger the degree of strain hardening, the larger the yield strain γ_c, that is, the strain at which the modulus starts decreasing. At time scales t shorter than 0.10 s, strain hardening was pronounced, and G_t increased up to 100% compared with its value at low strains.

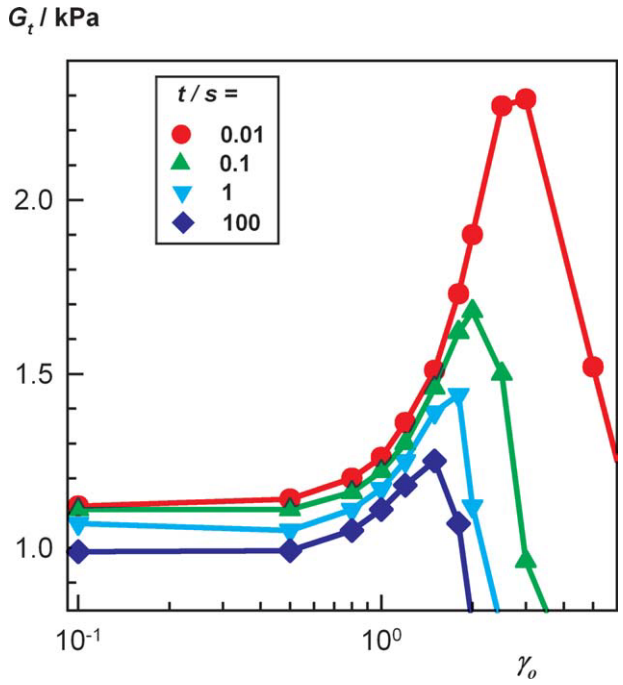

FIGURE 3 The relaxation modulus G_t of chemical DNA hydrogels as a function of the strain amplitude γ_o for various time scales t indicated. The gel was prepared at 5% EGDE and at 9.3% DNA.

Experiments show a clear correlation between the hardening behavior and the elasticity of DNA hydrogels.[12] Like many biological gels, DNA hydrogels formed under various experimental conditions exhibit universal scaled stiffening behavior that can be reproduced by a worm-like chain model taking into account the entropic elasticity of DNA strands.[12,26,27] The degree of hardening increases with decreasing contour length L_c of the network chains, that is, with decreasing distance between the effective crosslink points in the gel network. A maximum degree of hardening appears as L_c approaches to 100 nm,[12] which is the Kuhn length, or twice the persistence length of ds-DNA. As there is only one Kuhn chain for L_c = 100 nm, such a ds-DNA strand should behave, by definition, as a rigid rod and should exhibit infinite modulus. It is interesting to mention that L_c values below 100 nm were also observed in highly crosslinked DNA hydrogels. As a decrease of L_c below the Kuhn length of ds-DNA is not possible, the results suggest local opening of double helical fragments along a Kuhn length due to the action of additional crosslinks. As the Kuhn length of ss-DNA is about 1 nm, such openings may allow decrease of L_c below 100 nm at high crosslink densities.

The preparation strategies, thermoreversible behavior, and nonlinear elasticity of DNA hydrogels summarized in this work provide a basic platform to build smart and functional DNA hydrogels for a diverse range of biological and biomedical applications. Collapse of DNA hydrogels induced by condensing agents may represent a potential tool in gene therapy allowing the condensation and transfer of DNA into cells.[28] New synthetic strategies for the preparation of microgels and nanogels of DNA would develop efficient gene delivery systems. Because of the unique chain structure, DNA molecules are able to bind specific biomolecules and synthetic molecules, leading to a change in the size and structure of DNA strands. DNA hydrogel as a macroscopic assemble of DNA strands is thus a responsive soft material with molecular recognition capability, which is very suitable for nanotechnological applications. DNA hybrid materials formed by combination of DNA and inorganic or organic materials open a new avenue toward the developments of novel drug delivery systems, detection strategies, nanoelectronic structures, and nanomechanical devices.[29] Research on DNA hydrogels will not only offer new application areas but also provide a deeper understanding of how DNA behaves and interacts on the molecular level. In typical mammalian cells, long DNA molecules on the order of 10^9 bp are concentrated in the nuclei, where DNA concentration is around 1 w/v %. As the viscoelastic moduli and the mesh size of such semidilute solutions do not depend on the polymer molecular weight, DNA hydrogels represent a model system for understanding viscoelasticity of DNA at high concentrations. Studies on DNA hydrogels and conformational transitions between their swollen and collapsed states may also elucidate a more comprehensive understanding of the packing of DNA in confined geometries such as cell nuclei.

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