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Macroporous, responsive DNA cryogel beads

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ABSTRACT

Biocompatible soft materials that are macroporous and tough are in demand for a range of applications. Here, we describe the preparation of macroporous DNA cryogel beads by crosslinking DNA in frozen aqueous solution droplets at −18 °C. Ethylene glycol diglycidyl ether was used as the crosslinker and N,N,N′,N′-tetramethylethylenediamine as catalyst. The beads swell in 4.0 mM NaBr 74–212 times their dry weights and exhibit moduli of elasticity around 0.5 kPa. In dry state, they 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. DNA beads can be compressed up to about 80% strain without any crack developments. They also exhibit reversible swelling–deswelling cycles in water and acetone, respectively, undergoing a discrete phase transition in aqueous acetone solutions containing 51% acetone. The ability of the beads for the removal of carcinogenic agents from aqueous solutions was also demonstrated using phenanthrene as a model compound. The sorption capacity of the beads was found to be 420 μg phenanthrene/g DNA.

1. Introduction

All living cells contain deoxyribonucleic acid (DNA) molecules carrying genetic information in their base sequences. In its native form, DNA is a semi-flexible polymer with a double-helical (ds) conformation stabilized by hydrogen bonds between the amine bases [1]. When a DNA solution is subjected to high temperature, the hydrogen bonds holding the two strands together break and the double helix dissociates into two single flexible strands having a random coil conformation [2]. Due to the unique structure of DNA, chemical compounds having aromatic planner groups such as benzopyrine and phenanthrene intercalate between adjacent base pairs of ds-DNA and result in mutation and endocrine disruption [3,4]. This fact also suggests that, after insolubilization of DNA, it can be utilized as an adsorbent for such toxic materials from waters.

Several techniques were developed to prepare water-insoluble DNA compounds for the removal of toxic materials. Yamada et al. prepared water-insoluble and nuclease-resistant DNA films by UV radiation [5], while Yamamoto and co-workers prepared DNA liquid crystal gels by the reaction with metal cations [6,7]. DNA/nanoparticle hybrids [8], DNA-immobilized nonwoven cellulose fabric [9], DNA complexes with lipids [10], cationic surfactants [11,12], DNA immobilized in gels [13,14], semi-interpenetrating networks [15], DNA films [16], and hydrogels containing DNA strands as grafts [17], or as crosslinks [18] are among materials containing water-insoluble DNA. Although such materials can be used as specific sorbent for the removal of carcinogens, they have the general drawback that they are nonporous, and mechanically weak. An efficient sorbent material should have a macroporous structure allowing a fast liquid transport through the continuous macropores [19]. In addition, a fast response against the external stimuli and a good mechanical performance are also requirements for efficient materials.

DNA hydrogel is a network of chemically crosslinked DNA strands swollen in aqueous solutions [20]. 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 [16,18]. DNA hydrogels were prepared starting from branched DNA molecules via ligase-mediated reactions [21]. These hydrogels can also be prepared by crosslinking DNA in semi-dilute solutions using a chemical crosslinker such as ethylene glycol diglycidyl ether (EGDE) in the presence of N,N,N′,N′-tetramethylethylenediamine (TEMED) catalyst [22]. 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 [23]. DNA hydrogels are responsive systems exhibiting drastic volume changes in response to the external stimuli, such as the composition of aqueous solutions of acetone [22,24], or polyethylene glycol [25], concentrations of inorganic salts [26–29], polyamines [27,28], cationic macromolecules [30], or surfactants [27,28,31]. We have recently focused on developing responsive DNA hydrogels with a wide range of tunable properties such as the conformation of the network strands [25], viscoelasticity [32], and nonlinear elasticity (strain hardening) [33]. Gelation reactions conducted at

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50 °C show that a high concentration of DNA such as 9.3 w/v% stabilizes double-stranded (ds) DNA conformation while, at lower concentrations, single stranded (ss) DNA gels were obtained [25]. No gel formation was observed at DNA concentrations as low as 5%. Gels formed from ss- or ds-DNA strands were highly transparent indicating that they are nonporous in dry state.

Preparation of DNA hydrogels with a macroporous structure has, to our knowledge, not been previously reported. Creating an interconnected pore structure within the crosslinked DNA network would result in the formation of fast responsive DNA hydrogels. In the present study, we describe the preparation of macroporous DNA hydrogels in the form of millimeter-sized beads suitable as specific adsorbent for carcinogenic compounds. Our strategy to prepare such gel beads is to conduct the crosslinking reactions of ds-DNA (about 2000 base pairs long) using EGDE crosslinker within the droplets of frozen DNA solutions at −18 °C. This low temperature gelation technique known as cryogelation is a simple route for the preparation of macroporous gels [34–40]. During the freezing of an aqueous polymer solution containing a chemical crosslinker, the polymer chains and the crosslinker molecules expelled from the ice 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, a macroporous material (cryogel) is produced whose microstructure is a negative replica of the ice formed. As will be seen below, the crosslinking DNA in aqueous frozen solution droplets at −18 °C produces spherical, macroporous, tough cryogel particles with fast-responsivity. The characteristics of polyelectrolyte hydrogels such as the volume phase transition can be observed in the cryogel beads in a very short period of time and in a reversible manner. Here, we also show that macroporous DNA beads can effectively be used in the adsorption processes of carcinogenic compounds such as dilute aqueous solutions of phenanthrene.

2. Experimental

2.1. Materials

Cryogels were made from deoxyribonucleic acid sodium salt from salmon testes (DNA, Sigma). According to the manufacturer, the% G–C content of the DNA used is 41.2%, and the melting temperature is reported to be 87.5 °C in 0.15 M sodium chloride plus 0.015 M sodium citrate. The molecular weight determined by ultracentrifugation is 1.3×10^6 g/mol, which corresponds to approximately 2000 base pairs. The crosslinker ethylene glycol diglycidyl ether (EGDE, 50%, technical grade, Fluka), N,N,N′,N′-tetramethylethylenediamine (TEMED, Merck), phenanthrene (Fluka), and sodium bromide (NaBr, Merck) were used as received. Stock solutions of EGDE and TEMED were prepared by dissolving 2.61 mL EGDE and 0.568 mL TEMED in 10 and 20 mL 4.0 mM NaBr, respectively. DNA and TEMED concentrations in the gelation solutions were expressed as DNA% and TEMED%, respectively, which are the mass of DNA and the volume of TEMED in 100 g reaction solution. The crosslinker (EGDE) content of the reaction solution was expressed as EGDE%, the mass of EGDE added per 100 g of DNA.

2.2. Gelation reactions

The crosslinking reaction of DNA was carried out at −18 °C in the presence of 50% EGDE. The pH of the reaction solution was set to 11.0 by the addition of 0.44% TEMED [25]. DNA was first dissolved in 4.0 mM NaBr at 35 °C for 1 day. After addition of EGDE and stirring for 1 h, TEMED was included into the reaction solution. Note that the solutions containing more than 1% DNA were too viscous and could not be dropped into the continuous phase to obtain spherical beads. Therefore, they were heated to 50 °C and held at this temperature for 10 min to partially denature DNA and thus, to decrease the viscosity of the solutions. Two techniques were used for the preparation of macroporous DNA cryogel beads:

Technique A: DNA solution containing EGDE and TEMED was added dropwise into an excess of liquid nitrogen to create small frozen droplets. As each droplet touches the liquid nitrogen, nitrogen starts to boil while the droplet spins on the liquid surface until it falls down into the liquid nitrogen. After obtaining frozen droplets, they were transferred into paraffin oil as the continuous phase at −18 °C and the reactions were conducted for 3 days.

Technique B: DNA solution was directly dropped into paraffin oil at −7 °C and after complete addition, the temperature of the oil phase was decreased to −18 °C and the reactions were conducted for 3 days at this temperature. Note that droplets of DNA solutions in paraffin oil cannot be obtained if the temperature was initially set to −18 °C due to the high viscosity of the oil. Preliminary experiments conducted under different experimental conditions showed that −7 °C is the optimum temperature for the addition of the droplets into the oil phase.

After 3 days, the gel beads were removed from the oil phase, washed several times with acetone. Thereafter, they were placed in an excess of 4.0 mM NaBr solution and the solution was replaced many times.

DNA cryogels were also prepared in the form of cylinders of about 4.5 mm diameter. The reaction solution containing DNA, EGDE, and TEMED prepared as described above was transferred into plastic syringes. Half of the syringes were immediately frozen in liquid nitrogen to prevent denaturation of DNA due to the effect of EGDE-TEMED pair [25]. The other half of the syringes were heated in an oven at 50 °C for 20 min to completely melt DNA, following quenching in liquid nitrogen to fix ss-DNA conformation. Indeed, hyperchromicity measurements (see below) showed 90 ± 10% denaturation of ds-DNA in these samples All syringes were then transferred into a freezer at −18 °C to conduct the gelation reactions for 3 days. In this way, cryogels consisting of mainly ss-DNA and ds-DNA strands were prepared.

For comparison, hydrogels of DNA were also prepared by conducting the gelation reactions at 50 °C both in plastic syringes and between the parallel plates of the rheometer (Gemini 150 Rheometer system, Bohlin Instruments) equipped with a Peltier device for temperature control. The upper plate (diameter 40 mm) was set at a distance of 500 μm before the onset of the reactions. During all rheological measurements, a solvent trap was used to minimize the evaporation. Further, the outside of the upper plate was covered with a thin layer of low-viscosity silicone oil to prevent evaporation of solvent. A frequency of ω = 1 Hz and a deformation amplitude γ = 0.01 were selected to ensure that the oscillatory deformation is within the linear regime.

2.3. Hyperchromicity measurements

For the hyperchromicity measurements, gelation reactions were conducted, as described above, except that the frozen droplets or the reaction solutions were transferred into empty glass vials at −18 °C instead of paraffin oil. After 5–10 min of the reaction time, samples were taken and after thawing, they were diluted to a concentration of 26 mg/L with 4.0 mM NaBr. The degree of denaturation was estimated from the optical absorbance at 260 nm measured with a T80 UV–visible spectrophotometer. The results were presented as the normalized absorbance A_{rel} with respect to that measured from starting DNA solution. Because melting of

DNA strands leads to a rise of the normalized absorbance A_{rel} up to 1.4 [41], the fraction of ss-DNA fragments in DNA (ss-DNA%) was estimated as ss-DNA% = 250(A_{rel} − 1).

2.4. Swelling measurements

Swelling measurements were conducted on individual gel beads equilibrium swollen in 4.0 mM NaBr (pH = 6.0). The beads were placed separately in glass vials containing an excess of 4.0 mM NaBr at 21 °C. The swelling equilibrium was tested by monitoring the diameter of the gel beads using an image analyzing system consisting of a microscope (XSZ single Zoom microscope), a CDD digital camera (TK 1381 EG) and a PC with the data analyzing system Image-Pro Plus. The swelling equilibrium was also tested by weighing the gel beads. Thereafter, the equilibrium swollen gel beads were first added into acetone (poor solvent) and then dried in vacuum at room temperature. The equilibrium volume and the equilibrium weight swelling ratios of the beads, q_v and q_w, respectively, were calculated as:

$$q_v = (D/D_{dry})^3 \quad (1)$$

$$q_w = m/m_{dry} \quad (2)$$

where D and D_{dry} are the diameters of the equilibrium swollen and dry beads, respectively, m and m_{dry} are the weights of beads after equilibrium swelling and after drying, respectively. Note that the swelling measurements were conducted on at least six individual beads prepared under the same experimental condition and the results were averaged.

For the measurement of the deswelling rates of gel beads, the equilibrium swollen gel beads in NaBr solution were immersed in acetone at 21 °C. The volume changes of beads were measured in situ by following the diameter of the samples under microscope using the image analyzing system. For the measurement of the swelling rates of beads, the collapsed gel beads in acetone were transferred into 4.0 mM NaBr at 21 °C. The diameter changes of the beads were also monitored as described above. The results were given as the relative volume swelling ratio $V_{rel} = (D_t/D)^3$ where D_t is the gel diameter at time t. Swelling tests of the gels in aqueous acetone solutions of various compositions were also carried out as described above except that both the diameter and the mass of the beads were monitored as a function of acetone content.

For the determination of the swelling ratios of the cryogels in the form of cylinders, the gels taken from the syringes were cut into samples of about 5 mm in length and they were placed in an excess of 4.0 mM aqueous NaBr solution. In order to reach swelling equilibrium, the samples were immersed in solution for at least 4 weeks replacing the solution many times. After weighing the samples, they were freeze-dried and the swelling ratio q_w was calculated using Eq. (2). The gel fraction, that is, the mass of cross-linked DNA obtained from one gram of DNA was calculated from the masses of dry, extracted DNA network and from the DNA mass in the feed.

2.5. Elasticity tests

Uniaxial compression measurements were performed on individual gel beads in their swollen states. All the mechanical measurements were conducted in a thermostated room of 21 ± 0.5 °C. The stress–strain isotherms were measured by using an apparatus previously described [42,43]. Briefly, a swollen gel bead of 10–14 mm in diameter was placed on a digital balance (Precisa 320 XB – 220A, readability and reproducibility = 0.1 mg). A load was transmitted vertically to the gel through a rod fitted with a PTFE end-plate. The force F acting on the gel was calculated from the reading of the balance m as $F = mg$, where g is the gravitational acceleration. The resulting deformation ΔD was measured using a digital comparator (IDC type Digimatic Indicator 543-262, Mitutoyo Co.), which was sensitive to displacements of 10^{-3} mm. The force and the resulting deformation were recorded after 20 s of relaxation. The measurements were conducted up to about 20% compression. The weight loss of single beads during the measurement due to solvent evaporation or due to the applied force was found to be negligible.

We have to mention that the interpretation of the compression test data of spherical gel particles is complicated due to the significant variation of the contact area between the wall (PTFE end-plate) and the originally spherical gel during deformation. For a sphere with a constant volume during deformation, Hertz derived the following equation for small deformation ranges [44–50].

$$F = \frac{4}{3} G D^{0.5} \Delta D^{1.5} \quad (3)$$

where G is the elastic modulus, and $\Delta D = D - D'$, D and D' are the initial (swollen) undeformed and deformed diameters of gel sample, respectively. The Hertz theory (Eq. (3)) considers the contact deformation of elastic spheres under normal loads in the absence of adhesion and friction. According to Eq. (3), $(3/4)F/D^{0.5}$ vs $\Delta D^{1.5}$ plots should be linear for a given bead, with a slope equals to its elastic modulus. Indeed, linear plots were obtained for all the beads studied in the range of deformation ratios above 5%. In Fig. 1, $(3/4)FD^{-0.5}$ is plotted against $\Delta D^{1.5}$ for swollen DNA gel beads formed at 3% and 5% DNA concentrations. The variations in the stress–strain curves depending on the bead diameter were in the range of experimental error.

2.6. Texture determination and porosity of beads

For the texture determination of dried gel beads, scanning electron microscopy (SEM) studies were carried out at various magnifications between 50 and 2000 times (Jeol JSM 6335F Field Emission SEM). Prior to the measurements, network samples were sputter-coated with gold for 3 min using Sputter-coater S150 B Edwards instrument. The pore volume V_p of the gel beads was esti-

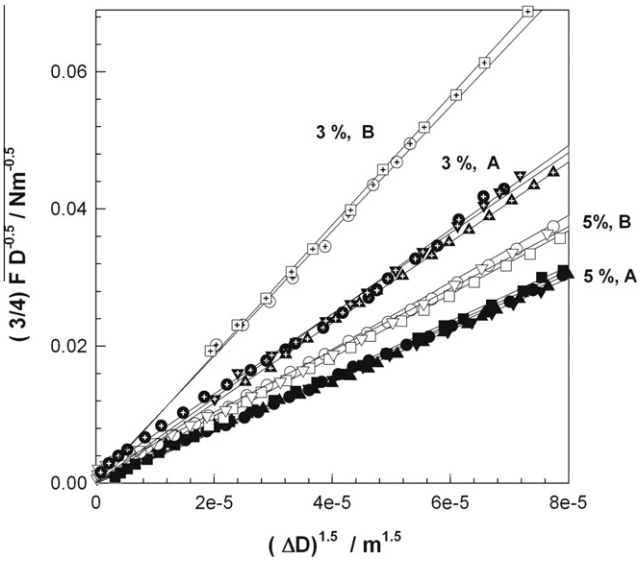

Fig. 1. Typical stress–strain data of DNA gel beads as $(3/4)\ F/D^{0.5}$ vs $\Delta D^{1.5}$ plots according to Eq. (3). DNA concentrations and the technique used in the gel preparation are indicated.

mated through uptake of methanol of the swollen gel beads. The gel beads swollen in 4.0 mM NaCl were transferred into methanol and methanol was refreshed several times until the mass of the beads remains unchanged. Since methanol is a nonsolvent, it only enters into the pores of the DNA network. Thus, V_p (mL pores in one gram of dry DNA network) was estimated as

$$V_p = (m_M - m_{dry})/(d_M m_{dry}) \quad (4)$$

where m_M is the mass of the bead immersed in methanol and d_M is the density of methanol (0.792 g/mL).

2.7. Adsorption of carcinogens to DNA cryogel beads

For the determination of the adsorption capacity of DNA beads for carcinogens, phenanthrene was used as a model polycyclic aromatic hydrocarbon. Taking into account its solubility range in water (1.2 μg/mL), concentration of the test solutions was around 1 μg/mL. Stock solutions were prepared by dissolving 0.050 g of phenanthrene in 50 mL acetone. Test solutions were prepared by diluting 1 mL of the stock solution with water to 1L. Phenanthrene removal tests were performed by placing 25–80 g of gel beads swollen in 4.0 mM NaBr into 200 mL test solution and shaking for 5 h at 21 °C and 170 rpm. Since phenanthrene is known to degrade through the process of photooxidation, the test solutions were covered with dark sheets. Additionally, control solutions were also prepared and checked for their phenanthrene contents throughout the experiments. Samples were taken at certain time intervals and the measurements of phenanthrene concentrations were performed using fluorescence spectrometer (Perkin Elmer LS 55) at excitation and emission wavelengths of 209 and 369 nm. FLWinlab software was used for calculations.

3. Results and discussion

Gelation reactions were carried out at −18 °C in frozen aqueous solutions of DNA using EGDE as a crosslinker and TEMED as a catalyst. Previous work on DNA gelation at 50 °C showed that the gels exhibit a maximum modulus of elasticity at pH = 11.0, corresponding to 0.44% TEMED [25,32]. This value of pH was fixed in the present study. Preliminary experiments conducted at a DNA concentration of 3% showed that gel formation at −18 °C requires at least of 50% EGDE (mass of EGDE per 100 g of DNA). Since the molecular weights of EGDE and the nucleotide repeat unit of DNA are 174.2 and 324.5 g/mol, respectively, and G-C content of the DNA used is 41.2%, 50% EGDE corresponds to 9 mol of epoxide groups added per mole of guanine base in the ds-DNA. This excess amount of the epoxide groups required for gelation indicates existence of intramolecular crosslinking reactions. In the following, the crosslinker content was set to 50% while the concentration of ds-DNA in the reaction solution was varied between 1% and 5%.

At the usual crosslinking temperature of DNA, namely at 50 °C [25,32], no gel formation was observed under these reaction conditions (0.44% TEMED and 50% EGDE). Rheological tests showed rapid decrease of the dynamic moduli of the reaction solution with time, while hyperchromicity measurements indicated partial dissociation of the double helix into flexible single strand fragments. For example, Fig. 2A–C shows the elastic modulus G' (filled symbols) and the viscous modulus G'' (open symbols) during the crosslinking of ds-DNA at 50 °C. The solid and dashed horizontal lines represent G' and G'' of the initial DNA solutions before the addition of EGDE and TEMED. Both moduli rapidly decrease and, at 3% and 5% DNA, they again increase at longer times. However, after 3 h of reaction time, elastic moduli are below their initial values and, no water-insoluble DNA gels were obtained. Fig. 2D shows the fraction of ss-DNA fragments (ss-DNA%) as a function of the reaction time at a DNA concentration of 3%. Even at 25 °C, ss-DNA% rapidly increases from 5% to 80% and, after heating to 50 °C; it becomes 90%. Further, almost complete melting of DNA was observed at 1% concentration while at 5%, the fraction of ss-DNA fragments increased to 63 ± 8%. Thus, instead of gelation, denaturation occurs during the crosslinking of DNA at 50 °C and at or below 5% DNA concentration.

However, DNA gels could be obtained by conducting the crosslinking reactions in frozen solutions at −18 °C. Two techniques were used for the preparation of DNA gel beads: According to the first technique (technique A), the aqueous solution containing DNA, EGDE and TEMED was dropped into liquid nitrogen to create small frozen organic droplets at −196 °C. Then, the frozen droplets were transferred into paraffin oil at −18 °C as the continuous phase and the reactions were carried out for 3 days. Images shown in Fig. 3A–C were taken from frozen solution droplets in liquid nitrogen (A), from DNA beads just after preparation (B), and after equilibrium swelling in 4.0 mM NaBr. Freezing of DNA solution in liquid nitrogen results in the formation of uniform frozen solution droplets; the spherical shape of the frozen droplets remained unchanged after the crosslinking reactions as well as after swelling of the crosslinked DNA particles in aqueous solutions. The occurrence of the gelation reactions below the freezing point of water is due to the cryoconcentration of DNA in unfrozen domains of the apparently frozen reaction system (Fig. 3D). It was shown recently that frozen hydrogel samples at temperatures between −10 and −24 °C contain 6% non-freezable water bound to the polymer chains [51]. Thus, as water freezes, DNA strands, EGDE,

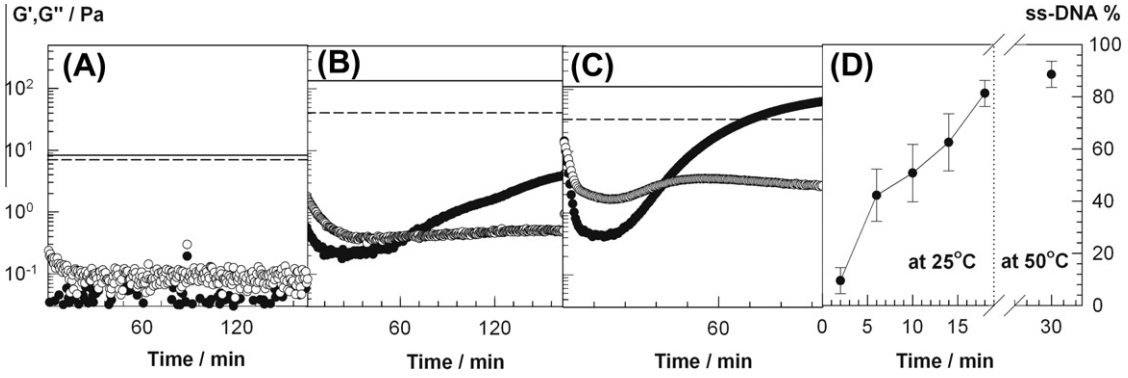

Fig. 2. (A–C) Elastic modulus G' (filled symbols) and the viscous modulus G'' (open symbols) during the crosslinking of ds-DNA at 50 °C. EGDE = 50%. TEMED = 0.44%. DNA = 1 (A), 3 (B), and 5%. (C) The solid and dashed horizontal lines represent G' and G'' of the initial DNA solutions before the addition of EGDE and TEMED. ω = 1 Hz. γ = 0.01. (D) The fraction of ss-DNA fragments (ss-DNA%) shown as a function of the reaction time. The temperature is 25 and 50 °C before and after the dotted vertical line, respectively. DNA = 3%. TEMED = 0.44%. EGDE = 50%.

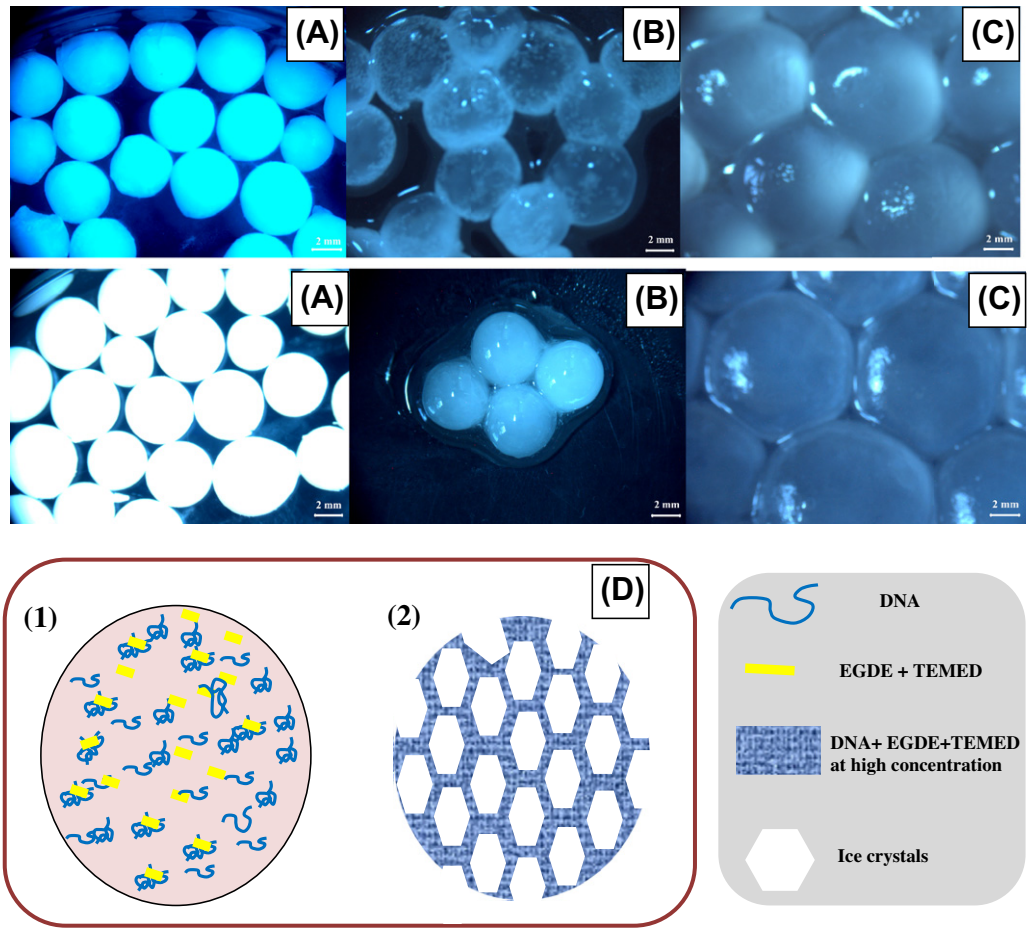

Fig. 3. (A–C) Optical microscopy images of frozen DNA solution droplets in liquid nitrogen (A), after crosslinking reaction in paraffin oil at −18 °C (B), and after equilibrium swelling in 4.0 mM NaBr (C). DNA = 3 (first row) and 5% (second row). EGDE = 50%. TEMED = 0.44%. All the scaling bars are 2 mm. (D) Scheme of a solution droplet before (1) and after freezing at −18 °C (2).

Table 1
Properties of DNA gel beads formed at −18 °C. EGDE = 50%. TEMED = 0.44%. ss-DNA% = the amount of ss-DNA fragments in DNA network chains. q_w, q_v = weight and volume swelling ratios, respectively. V_p = total volume of the pores. G = modulus of swollen beads. Standard deviations are indicated in parenthesis.

DNA%	Technique	ss-DNA%	q_w	q_v	V_p (mL/g)	G (kPa)
3	A	85 (10)	74 (14)	50 (15)	5.4 (1.7)	0.6 (0.1)
5	A	63 (8)	133 (10)	122 (9)	7.4 (2.9)	0.4 (0.1)
3	B	79 (10)	121 (20)	63 (17)	8.3 (0.9)	0.7 (0.2)
5	B	12 (10)	212 (45)	147 (27)	10 (2.5)	0.5 (0.1)

and TEMED are excluded from the ice structure and they accumulate in the unfrozen microregions, making the DNA concentration in these regions high enough to conduct the crosslinking reactions at −18 °C. The second technique for the preparation of DNA beads (technique B) involves dropwise addition of the aqueous DNA solution containing EGDE and TEMED into paraffin oil as the continuous phase and crosslinking at −18 °C for 3 days. Similar beads as shown in Fig. 3A–C were also obtained by technique B. Both techniques provide formation of DNA beads of diameters 2–3 mm in dry or collapsed states, and 8–14 mm in swollen states. Note that the size of the beads could be adjusted by changing the tip diameter (D_{tip}) of the pipettes from which the aqueous solution was dropped into liquid nitrogen or into paraffin oil. In the present work, D_{tip} was taken as constant at 2 mm.

Swelling, porosity, and elasticity characteristics of DNA beads as well as the conformation of DNA network strands in terms of ss-DNA% are collected in Table 1. All the measurements were conducted on individual gel beads and the results were averaged. The

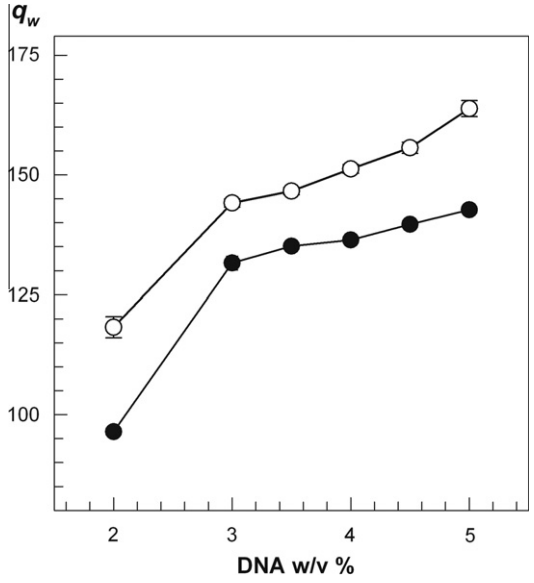

Fig. 4. Equilibrium weight swelling ratio q_w of ss-DNA (open symbols) and ds-DNA cryogels (filled symbols) in the form of cylinders plotted against DNA concentration at the gel preparation. EGDE = 50%. TEMED = 0.44%.

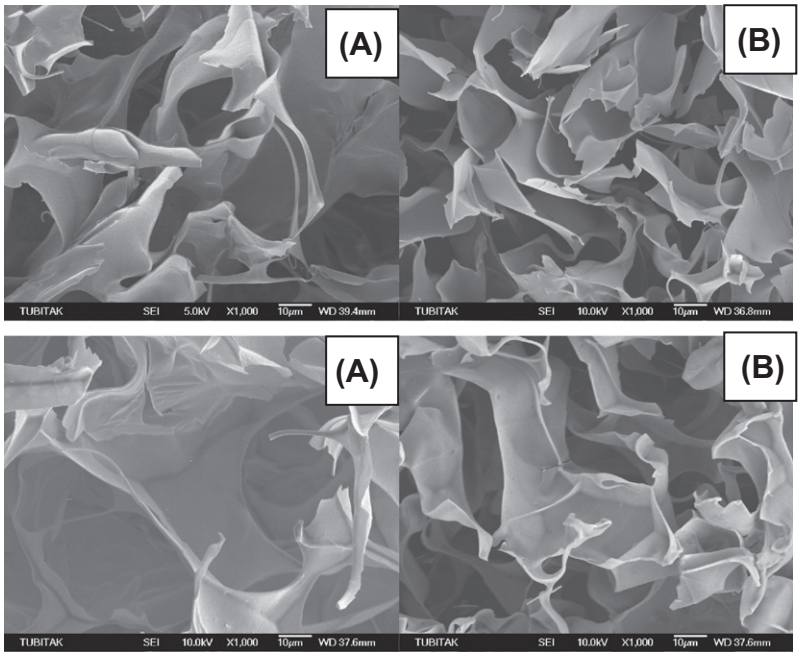

Fig. 5. SEM of DNA networks formed by technique A (upper images) and B (bottom images). DNA concentration at the gel preparation = 3 (A) and 5% (B). EGDE = 50%. TEMED = 0.44%. The scaling bars are 10 μm. Magnification = 1000×.

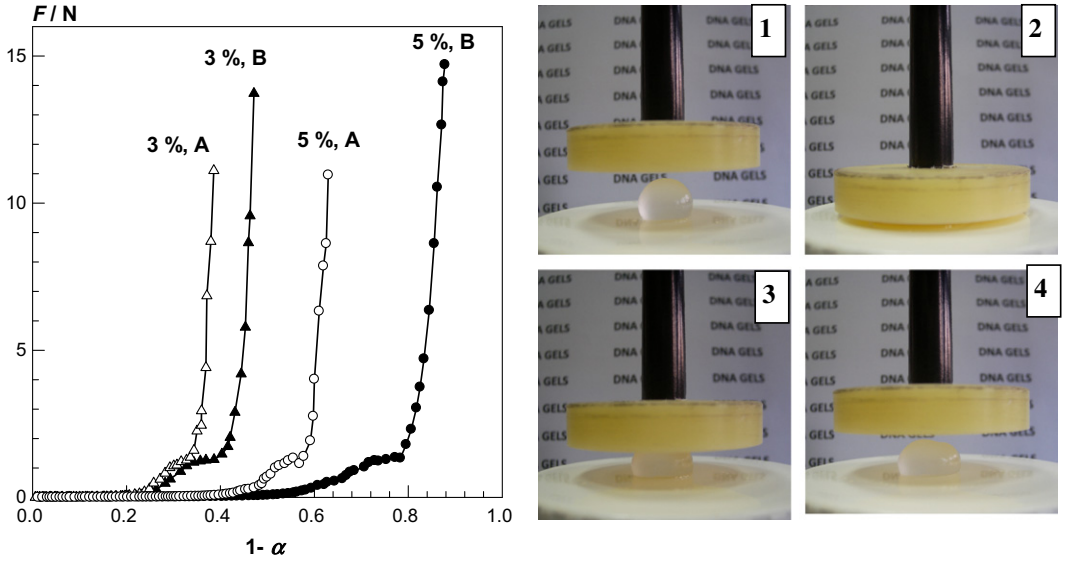

Fig. 6. Stress–strain data of DNA gel beads as the dependence of force F on the fractional deformation $1 - \alpha$ of the gel bead. Photographs show a swollen DNA gel bead formed at −18 °C by technique B during the compression test. After compression, addition of 4.0 mM NaBr converts the gel bead back to its initial state. DNA concentration = 5%.

gel beads formed at 1% DNA were too weak to obtain reproducible results. Due to the preheating period of the reaction solutions, DNA network strands consist of 12–85% ss-DNA fragments. The fraction of ss-DNA fragments decreases with increasing DNA concentration due to the simultaneous increase of the concentration of DNA counterions (Na^+), which stabilize ds-DNA conformation, as observed before by the addition of salts to dilute DNA solutions [52]. Indeed, the degree of denaturation of ds-DNA in water at 90 °C was found to be 100% and 18% at 1% and 9% DNA concentrations, respectively. Table 1 also shows that the beads swell in 4.0 mM NaBr 74–212 times their dry weights. Increasing DNA concentration at the gel preparation also increases their weight swelling ratios while the modulus of elasticity G of the swollen beads remains around 0.5 kPa. We have to mention that the large standard deviations in the measured values given in Table 1 are due to the bead-to-bead variation of the gel properties.

Due to the different degree of denaturation of the DNA network strands in the gel beads, a definitive conclusion regarding the effect of the chain conformation and DNA concentration on the gel swelling cannot be drawn. Moreover, the gel fraction, that is, the amount of crosslinked DNA obtained from one gram of DNA cannot be determined. This is due to the presence of the continuous oil phase during the formation of DNA beads, which hindered the determination of the bead mass at the state of gel preparation. Therefore, experiments were repeated under the same experimental conditions but in plastic syringes at −18 °C. As described in the experimental part, ds-DNA and ss-DNA cryogels in the form of cylinders were obtained. The gel fraction was found to be unity between 2%

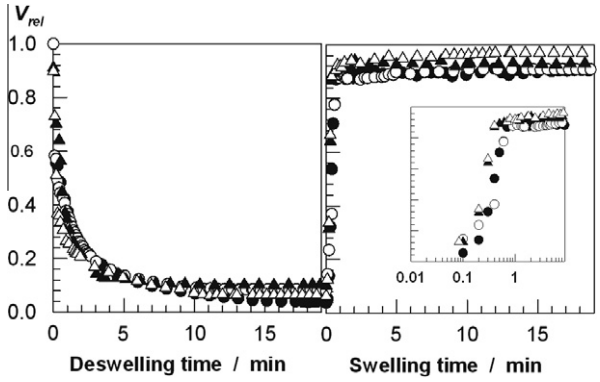

Fig. 7. The normalized volume V_{rel} of DNA gel beads shown as a function of the time of deswelling in acetone and re-swelling in aqueous 4.0 mM NaBr. DNA = 3%. Technique used: A (open symbols), and B (filled symbols). EGDE = 50%. TEMED = 0.44%. The first and second deswelling–reswelling cycles are shown by circles and triangles, respectively. The inset shows a semi-logarithmic plot of the swelling data.

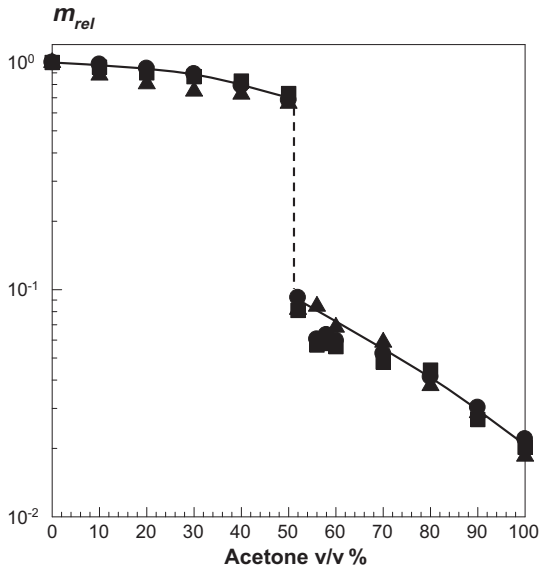

Fig. 8. Variation of the swelling ratio mr_{el} of DNA gel beads with the acetone content of the external acetone/water mixture. Swelling temperature = 21 °C. EGDE = 50%. TEMED = 0.44%. DNA = 3%, Method B (▲). DNA = 5%, Method A (■) and B (●). The dashed vertical line illustrates the phase transition region.

and 5% DNA indicating that DNA incorporates completely into the network structure. Fig. 4 shows the equilibrium weight swelling ratio q_w of ds-DNA (filled symbols) and ss-DNA cryogels (open symbols) plotted against DNA concentration. In contrast to the large standard deviations in q_w values of the cryogel beads (Table 1), most of the error bars in the figure are smaller than the symbols. In accord with the results obtained using cryogel beads, the higher the DNA concentration, the larger is the swelling capacity of the cryogels. Thus, the results suggest decreasing crosslinking efficiency of EGDE as the concentration of DNA in the reaction solution is increased. This unexpected behavior could be related to diffusion restrictions of DNA strands in nonfrozen phase [53], the extent of which increase with increasing DNA concentration. Another interesting finding is that ss-DNA cryogels swell more than ds-DNA cryogels indicating the effect of DNA conformation on the gel swelling. Since ss-DNA is a flexible polymer with a persistence length p of about 1 nm, as compared to semiflexible ds-DNA with $p = 50$ nm [54], the critical overlap concentration c^* of

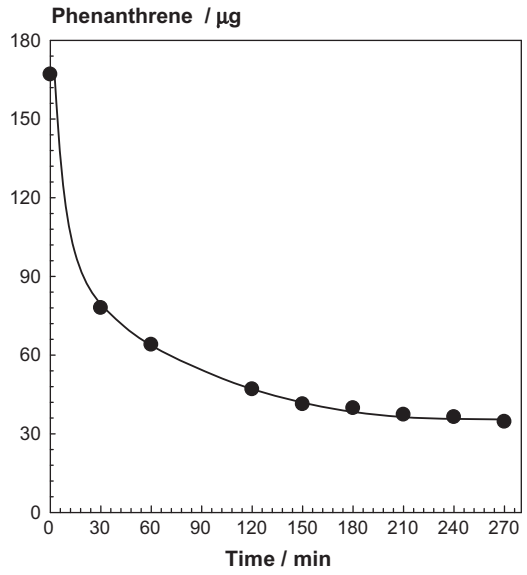

Fig. 9. The amount of phenanthrene in the external solution shown as a function of contact time with DNA gel beads formed by technique B. DNA = 5%. EGDE = 50%. TEMED = 0.44%. 0.34 g beads were used for 200 mL of a phenanthrene solution of initial concentration 0.835 µg/mL.

ss-DNA is 80-fold larger than that of ds-DNA [25]. For example, c^* of an aqueous solution of DNA of molecular weight of 2000 base pairs (bp), as used in the present experiments, increases from 0.043 to 3.2 w/v% during denaturation [25]. Thus, for a given DNA concentration, ds-DNA strands are more overlapped than ss-DNA strands in the reaction solution, which favors the occurrence of intermolecular EGDE crosslinks and leads to the formation of gels exhibiting a lesser degree of swelling.

Table 1 also shows that the total volume of the pores in the DNA beads estimated from the uptake of methanol is 5–10 mL/g. To visualize the pores in DNA gels, the network samples were investigated by scanning electron microscopy (SEM). Fig. 5 shows SEM images of the samples prepared by techniques A (upper panel) and B (bottom panel). DNA networks have a porous structure consisting of irregular large pores of sizes 10^1–10^2 µm. As schematically shown in Fig. 3D, the formation of a macroporous structure in DNA gels is due to the presence of ice domains in the gelation system. The crosslinking reactions of DNA only proceed in the unfrozen liquid channel around the ice crystals so that, after melting of ice, a porous material is produced whose pore walls consist of crosslinked DNA strands of high concentration.

The mechanical properties of DNA gels were investigated in their swollen states in 4.0 mM NaBr by the compression tests. Linear stress–strain curves were obtained by application of Hertz equation to the force–deformation data of the gel beads (Fig. 1). An important point was that the beads can be compressed up to about 80% strain without any crack development. This behavior is illustrated in Fig. 6 where the force F acting on the gel bead is plotted against the fractional deformation (diameter change/initial diameter, $1 - \alpha$). The mechanical stability of the beads increased with increasing DNA concentration and, the beads formed by technique B were more stable than those formed by technique A. Photographs in Fig. 6 demonstrate how a DNA gel bead prepared at 5% DNA according to technique B sustains a high compression. The bead remains mechanically stable up to about 80% compression. During the compression step and as the bead is squeezed under the piston, it releases its water so that it can be compressed to high strains. After the release of the load and, after addition of aqueous NaBr, the bead immediately recovers its original shape, as seen from the image denoted by 4 in Fig. 6.

Fig. 7 shows the response rate of DNA beads prepared at 3% DNA concentration, where the normalized gel volume V_{rel} (volume of gel at time t/equilibrium swollen volume in water) is plotted as a function of the time t of deswelling in acetone and re-swelling in water. In order to check the durability of the gel beads against the volume changes, this swelling-deswelling cycle was repeated twice. It is seen that, in contrast to the very long equilibration time of the conventional DNA gels [30], the beads attain their equilibrium collapsed states within 10 min. Further, the re-swelling period of the beads occurs much faster; they return back to their initial equilibrium swollen state within less than 1 min. All these cycles were reversible and, no substantial differences were observed between the beads prepared at various concentrations and by use of both techniques. The fast responsivity of the gel beads is due to their large pore volumes, which provide easy penetration of the solvent molecules within the DNA network structure.

Since DNA is a polyelectrolyte, single DNA molecules as well as the gels derived from DNA undergo volume phase transition in response to changes in the external stimuli, as typically observed in synthetic ionic hydrogels [22]. The fast responsivity of the macroporous DNA gel beads offer the advantage that the phase transition phenomena in DNA can be observed over a short period of time and in a reversible manner. We investigated the phase transition in DNA beads in water–acetone mixtures induced by a change in solvent composition. For this purpose, the mass of a single DNA bead was monitored as a function of the solvent composition. Each bead which is originally in 4.0 mM NaBr was first immersed in an excess solution containing 10 v/v% acetone and after 20 min, the bead was transferred into the next concentrated solution. This procedure was conducted in up and down directions between 0% and 100% acetone and, could be completed within 1 day. Note that due to the non-uniformity of the diameter of the beads in collapsed state, the measurements were conducted by gravimetry. The results are presented in Fig. 8 where the relative mass m_{rel} of the bead is plotted against the acetone concentration. At low acetone concentrations the beads are swollen, at high concentrations the beads are collapsed. A discrete phase transition was observed at 51 ± 1% acetone, during which the bead mass changes about eight times. In this concentration range marked with the vertical dashed line in the figure, only swollen or collapsed states could be observed but never an intermediate state.

It is interesting to examine the abilities of DNA beads for the adsorption of carcinogenic agents. As a demonstration, the beads prepared at 5% DNA by technique B with 88% ds-DNA were immersed in 200 mL of an aqueous solution of phenanthrene of concentrations between 0.8 and 1 μg/mL, which is close to its solubility limit (1.2 μg/mL). Since phenanthrene molecules are non-ionic carcinogens, they are adsorbed only by intercalation into the DNA double helices. After 2 h of contact time, the concentration of phenanthrene could be reduced by 70%. Fig. 9 shows the amount of phenanthrene in the external solution plotted against the contact time with DNA beads. The amount of phenanthrene in the solution rapidly decreases in the first 30 min but then approaches a limiting value. The maximum sorption capacity of the beads after 4 h was found to be 420 μg phenanthrene/g dry bead with 10% standard deviation. It is seen that, even in such dilute solutions, phenanthrene can be effectively removed using the DNA beads. Experiments conducted using DNA gels in the form of rods consisting of 100% ds-DNA gave the same sorption capacity for phenanthrene.

4. Conclusions

We described the preparation of macroporous DNA hydrogel beads by crosslinking DNA using EGDE as a crosslinker in frozen aqueous solution droplets at −18 °C. DNA beads of diameters 2–3 mm in dry or collapsed states, and 8–14 mm in swollen state were obtained at a DNA concentration between 3% and 5%. The beads swell in 4.0 mM NaBr 74–212 times their dry weights and exhibit moduli of elasticity around 0.5 kPa. In dry state, they 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. DNA beads can be compressed up to about 80% strain without any crack developments. They also exhibit completely reversible swelling–deswelling cycles in water and acetone, respectively, i.e., they return to their original volume after a short period of time. The gel beads deswell in aqueous acetone solutions of increasing acetone content undergoing a discrete phase transition in solutions containing 51% acetone. The ability of the beads for the removal of carcinogenic agents from aqueous solutions was also demonstrated using phenanthrene as a model compound. The sorption capacity of the beads was found to be 420 μg phenanthrene/g DNA.

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