We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



2

Development of DNA Based Active Macro–Materials for Biology and Medicine: A Review

Frank Xue Jiang[1], Bernard Yurke[2], Devendra Verma[3], Michelle Previtera[3], Rene Schloss[3] and Noshir A. Langrana[3]

[1]*Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL*

[2]*Departments of Materials Science and Engineering and Electrical and Computer Engineering, Boise State University, Boise, Idaho*

[3]*Departments of Biomedical Engineering and Mechanical and Aerospace Engineering, Rutgers, The State University of New Jersey, Piscataway, New Jersey USA*

1. Introduction

DNA was first discovered as the carrier of genetic information for the majority of the known living organisms, encoding the secret of life. Its delicate design based upon double helical structure and base pairing offers a stable and reliable media for storing hereditary codes, laying the foundation for the central dogma (Watson et al. 2003). The impact of this molecule is far reaching into scientific community and our society, as manifested in many fields, for instance, forensics (Budowle et al. 2003), besides medicine.

To date, a great deal of research effort has been directed towards understanding DNA's role in maintenance and expression of genome, and in the application of this understanding to biology and medicine, which is partly fueled by the market needs (e.g., DNA sequencer equipment market alone is expected to reach $450 million by 2010 (Saeks 2007)). For reviews on the development in this area, especially using DNA or RNA per se as therapeutic reagents in applications such as gene therapies, one is referred to a large number of reports (Blagbrough & Zara 2009, Cao et al. 2010, Patil et al. 2005, Ritter 2009). While this remains the center of the attention with the emergence of new subjects of knowledge including genetics and genomics, recent decades have witnessed increased interest in using DNA as structural components or guiding tools (LaBean & Li 2007) in developing novel materials thanks to DNA's many unique features. Among these features are its molecular recognition with only four bases (specificity and simplicity), stable structure held by stacking H-bonds and other weak forces and interactions (stability), and the ease in breaking of base-pairs and thus separating strands allowing modification different than covalent-bond based structures (reversibility and flexibility).

These attributes of DNA give rise to many favorable properties of DNA based macro-materials that are having and will have a wide range of applications. In synthesizing and constructing these DNA based structures, DNA has been used to provide template (e.g., (Aldaye et al. 2008, Niemeyer 2000)), serve as building block (e.g., (Ball 2005)), function as

versatile linkages in the network (e.g., (Lin et al. 2004b, Um et al. 2006b)), and aid in the fabrication of the nano-, micro-, and macro-materials (e.g., (Alemdaroglu et al. 2008)). This is also of interest to the community of synthetic chemistry (Alemdaroglu & Herrmann 2007). The scope of the current and potential applications of DNA based materials ranges from DNA based electronics (Berashevich & Chakraborty 2008) and computing (Deaton et al. 1998) to novel material design (Dong Liu et al. 2007, Um et al. 2006a). The similar interest in using other three major types of macromolecules, namely, protein, lipids, carbonhydrates, as structural component for synthetic materials is also increasing (Ball 2005). For reviews in this regard particularly those on DNA based nanomaterials, readers are referred to the latest and comprehensive reviews by Seeman (Seeman 2007), Lu (Lu & Liu 2006, Lu & Liu 2007) and others (Alemdaroglu et al. 2008, Ball 2005, Condon 2006, Mrksich 2005, Niemeyer 2000). The focus of this review is the macroscopic materials designed, synthesized, and applied based on or inspired by DNA and the application of these materials specifically for biology and medicine.

Changes in the nanoscale structures can trigger macroscopic changes in the materials (Schneider & Strongin 2009). For these macro-materials, incorporation of DNA into the structural design confers a number of possibilities that would otherwise not be feasible. For instance, DNA imparts temperature dependent mechanical properties to structures crosslinked by them (Lin et al. 2004b), and unique aptamer interactions make possible phase transition at room temperature (Yang et al. 2008). For these materials, variation at nano-scale DNA structures can lead to sometimes dramatic changes in the bulk material properties, exemplifying 'little trigger' for 'big changes'. Among these DNA based macro-materials, of particular interest are a class of polymeric hydrogel materials, with the ever-increasing significance and promises along with the rapid development in the area of tissue engineering and biomaterials (Jiang et al. 2008b, Jiang et al. 2010c, Lin et al. 2006, Lin 2005, Lin et al. 2004b, Luo 2003, Um et al. 2006b). Additionally, mimicking *in vivo* tissue remodeling and property dynamics is of great importance in the reconstruction of the physiological conditions for cell growth and tissue repair, and DNA based macro-materials help contribute to address the issue thanks to modifications and alterations of the DNA based structures (Jiang et al. 2010b, Jiang et al. 2010c). Therefore, the review first sought to identify the key properties that are directly related to the design and synthesis of DNA based macro-materials and further recognizes the unique properties that result from incorporation of DNA in the structures of macroscopic materials. We then classified these DNA based macro-materials based upon the structural designs (i.e., DNA only, DNA as backbone, and DNA as crosslinker), and surveyed the current studies and potential application for each category of the materials from the literature. To aid in the further development of DNA based macro-materials, we summarized the key design parameters, considerations and major challenges. Lastly, we presented a conjecture on the potential directions.

2. Properties of DNA and DNA based structures

2.1 Properties of DNA

The properties of DNA can be classified in mainly three different levels: the sequence, the structure, and the folding pathway (Condon 2006). The composition or the sequence of DNA based on only four nucleotides, namely adenine (A), thymine (T), guanine (G), and cytosine (C), lays the basis for the primary structure, which also largely determines the secondary and tertiary structures of DNA. The complementarity underlying Watson-Crick

base-pairing of A:T and G:C and the stacking forces leads to the classical double helical structure as well as other forms of secondary DNA structures (e.g., A and Z form of DNA). Watson-Crick base-paring is intricately orchestrated by a number of week forces, including hydrogen bonding, π-stacking, electrostatic forces, and hydrophobic effect (Aldaye et al. 2008). It also offers foundation for molecular recognition of DNA. Double stranded DNA is capable of self-folding into complex structures, enabling it to locomote and respond to the environment (Condon 2006). These three levels of structures give rise to some interesting and useful features or attributes.

2.1.1 Chemical properties

DNA is a water-soluble macromolecule, and synthetic DNA displayed good biocompatibility (Um et al. 2006b). It is generally stable under physiological conditions, but can be hydrolyzed by acid and alkali when pH changes. At the same time, DNA is a highly charged polymer mostly due to the phosphate group in the nucleotides. The flexibility of single-stranded DNA (ssDNA) and the relatively weak bonding between base pairs of duplex DNA allows interacting DNA strands to seek thermodynamically favored configurations, making possible programmed self assembly of complex structures (SantaLucia & Hicks 2004). G:C base-pair is more stable than A:C one due to the stronger hydrogen bond present, thus GC content markedly influence DNA properties.

2.1.2 Cleavage

DNA can be cleaved primarily based on three ways: hydrolysis, photochemistry and oxidative reactions (Biggins et al. 2006). In the natural living systems, hydrolysis is the primary mechanism. These mechanisms allow for different approaches in degradation of the DNA based materials or ways of protecting these materials from attacks. For example, in designing DNA only or DNA crosslinked macro-materials, the sequence of the DNA can be chosen in a way that it would be protected under physiological conditions while degraded upon pathological cues (e.g., bio-metal concentration (Jain et al. 1996)) releasing encapsulated therapeutic agents.

2.1.3 Enzymatic modification

DNA is susceptible to modification including cleavage and chemical deletion or addition by a large number of enzymes. Nature has created a rather delicate and precise machinery to manipulate, such as cutting, ligating, unwinding, folding, synthesizing, initiating, modifying, and deleting, DNA in vivo(Braun & Keren 2004, Watson et al. 2003). Many of the enzymes involved in the process have been identified, namely, restriction enzymes, ligase, helicase, gyrase, polymerase, primase, proofreading exonuclease, and a host of other enzymes.

2.1.4 Physical properties

Double stranded DNA is semi-flexible and can possess high rigidity. Upon based pairing, and DNA strands can be straightened, which underlying the design of a nano-actuator (Simmel & Yurke 2001) (**Figure 1**). Previous studies on DNA mechanics suggest that DNA strands can be considered as rigid rods with tensile modulus of hundreds of MPa (Smith et al. 1996) when the force applied is below certain threshold, and this leads to a smaller possibility of stretching DNA longitudinally.. Meanwhile, the energy to bend a DNA strand

is inversely correlated to its length (Bustamante et al. 2003). It is emphasized here that as the earlier work pointed out, the physical properties of DNA are closely tied to its biological functions (Vologodskii & Cozzarelli 1994).

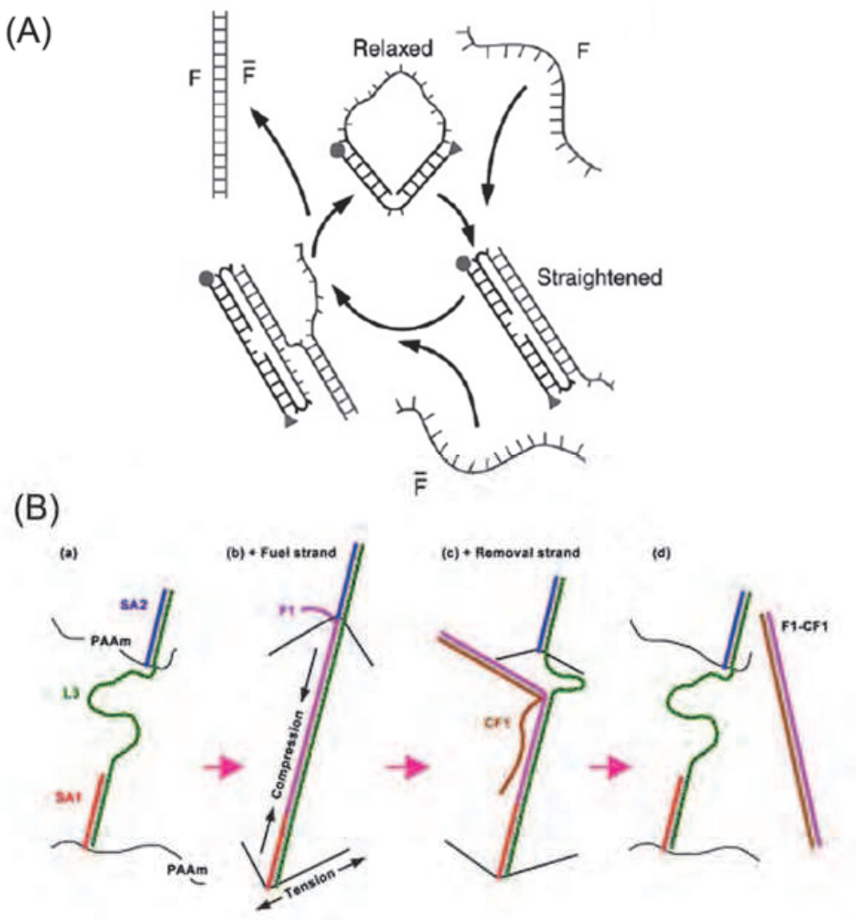


Fig. 1. Schematic of a DNA- based actuator and application to a DNA crosslinked macro-material. (**A**) In the nano-scale structure, at 'Relaxed' state, double-stranded (ds-) DNA is held by a single stranded (ss-) DNA, forming a loop structure. Upon the delivery of fuel strand (***F***) which base-pair with the ssDNA portion of the loop straightens the loop. With the introduction of the complementary of ***F***, or $\bar{F}$,the fuel strand is displaced, resulting in restoration of the relaxed stated of the loop. Extracted from (Simmel & Yurke 2001), with publisher's permission. (**B**) Incorporation of this nano-structure to the formation of a polymer gel. (Ba) Initially, both the crosslinks and polymer chains are slack. (Bb) As the fuel strand hybridizes with the motor domain, the crosslink stiffens and compressive forces are generated, in turn tensing the polymer chains. (Bc) The removal strand hybridizes with the fuel strand at the toehold region. (Bd) Once the removal and fuel strands are fully hybridized, the gel reverts to its initial state. Extracted from (Lin et al. 2006), with publisher's permission.

2.1.5 Denaturing/Re-annealing

Upon being heated above its melting temperature (T_m), duplex DNA will separate into complimentary strands of ssDNA since the hydrogen bonds between the two strands and other stabilizing forces in the duplex can not withstand the separating forces. Once this occurs, DNA is called being degraded, denatured, or melted, and this process can be reversed if the melted DNA is cooled slowly, for which the term 're-association', 're-naturation', or 're-annealing' is used (Bart Haegeman 2008, Dhillon et al. 1980, Li et al. 2001, Smith et al. 1975). Four major factors dictate the rate of re-association: temperature, salt concentration, DNA concentration, and the length of DNA strand (Li et al. 2001). The optimal re-association temperature is approximately 20°C below melting temperature, and the presence of adequate amount of cations is necessary for re-annealing (Li et al. 2001). In this regard, the lack of proper re-associate conditions leads to the non-reversible change in the materials properties, such as that of EGDE crosslinked DNA gels (Topuz & Okay 2008).

Besides the aforementioned properties that are most relevant to the derivation of DNA based materials for biological application, DNA also possesses a multitude of other properties, including electronic and magnetic ones, that are attractive for applications including DNA based molecular electronics (Berashevich & Chakraborty 2008).

2.1.6 Self-assembly in DNA hydrogels

The remarkable molecular recognition capabilities of DNA make it a promising candidate for development of materials with highly complex structures (Chhabra et al. 2010, Um et al. 2006a). Xing at el. reported synthesis of pure DNA hydrogels, based on self-assembled DNA building blocks with more than two branches. These DNA hydrogels showed thermal and enzymatic responsive properties (Xing et al. 2011). DNA can also be covalently grafted onto synthetic polymers and serve as a cross-linker (Alemdaroglu & Herrmann 2007). The recognition of complementary DNA strands leads to cross-linking of polymer chains and causes hydrogel formation. Zhang et al. reported DNA hydrogels based on *N*-(fluorenyl-9-methoxycarbonyl)-D-Ala-D-Ala as the cross-linker, which exhibited gel-sol transition upon binding to its ligand (Zhang et al. 2003). Kang et al. developed a photo-responsive DNA-cross-linked hydrogel that exhibited sol-gel transition on exposure to different wavelengths of light. Specifically, photosensitive azobenzene moieties were incorporated into DNA strands, such that their hybridization to complementary DNAs responded differently to different wavelengths of light (Kang et al. 2011). They also showed the capability of such photo-responsive gels by controlling encapsulation and release of multiple drugs. Jiang et al. designed and developed DNA-polyacrylamide hydrogels based biomaterials, which exhibited the ability to increase and decrease its stiffness in-situ, depending on the DNA cross-linker (Jiang et al. 2008a, Jiang et al. 2010a, Jiang et al. 2010c).

2.2 Properties of DNA based macromaterials

DNA has also proven to be a useful material to give bulk materials added functionality. This is exemplified in the introduction of DNA nanostructures to the design of DNA based macromaterials (**Figure 1**) While we will discuss the classification of the DNA based macro-material in depth in the next section, here we survey the new and added functionality that are reported.

2.2.1 Adhesivity

The adhesive properties or the DNA based macromaterials are of significance when they are to be used for applications such as tissue repairs or wound healing where most cells in contact are anchorage-dependent. In a DNA crosslinked hydrogel material, it has been established that with the varying length of DNA crosslinker and different crosslink density, the surface ligand density is not noticeably modified (Jiang et al. 2008b).

2.2.2 Swelling

DNA based macromaterials particularly hydrogel, similar to other hydrogels, can swell in the aqueous conditions, which will be encountered in the in vivo applications. In a DNA-only gel system, it has been observed that in de-ionized water the gel can swell to over 6 times by volume (fiber length) (Lee et al. 2008). Up to one fold increase in weight has been observed for a DNA gel where dsDNA or ssDNA interacts with a cationic surfactant, CTAB (cetyltrimetrylammonium bromide), after swelling (Moran et al. 2007), which is in contrast to the case where proteins (e.g., lysozyme) replace CTAB. For a DNA crosslinked hydrogel, the observed swelling ratio reaches up to 4 times in volume (unpublished data).

2.2.3 Pore structure

In designing bio-scaffolds for tissue engineering applications, the size and range of the pores in the hydrogels is one of the most critical issues. Early investigation has primarily determined the range of pore size from ~20 um to 100 um suitable for cell growth and functioning (Chevalier et al. 2008), while in the drug delivery applications, pore size affects the size of the drug the delivery vehicle is capable of carrying and releasing (Lin & Metters 2006). Um and colleagues devised a hydrogel material based purely on DNA strands for cell encapsulation and reported survival and growth cell of CHO cells inside the hydrogel days after the culture. Aiming at the potential drug delivery applications, Liedl and coworkers examined a DNA crosslinked hydrogel and inferred the pore size from experimental investigation by using quantum dots (QDs) (Liedl et al. 2007). Interestingly, although the pore size of this hydrogel was found to be ~100 um, nanoparticles of 10 nm range can still be trapped. In this hydrogel, the pore structure depends on the length of the crosslinker, the nature of the polymer and interactions between the two. It is noted that in addition to the pore size/distribution and porosity that are generally of concern, pore interconnectivity, shape and uniformity are also of great significance in certain applications (Li et al. 2003).

2.2.4 Sol-gel transition

For DNA based macromaterials, particularly polymeric hydrogel material, **g**elation point exists between the solid and gel phases. For DNA crosslinked hydrogel, it is a function of crosslinking density, monomer concentration, and crosslinker length (Jiang et al. 2008a, Wei et al. 2008). At a pre-determined crosslinker length and monomer concentration, raising crosslinking density results in sol-gel transition, as reflected in high viscosity (Lin et al. 2004b) (**Figure 2**) also observed in other studies (Li et al. 2005). For DNA gels based on crosslinked DNA network (e.g., by EGDE) discontinuous phase transition has been reported (Amiya & Tanaka 1987, Topuz & Okay 2008).

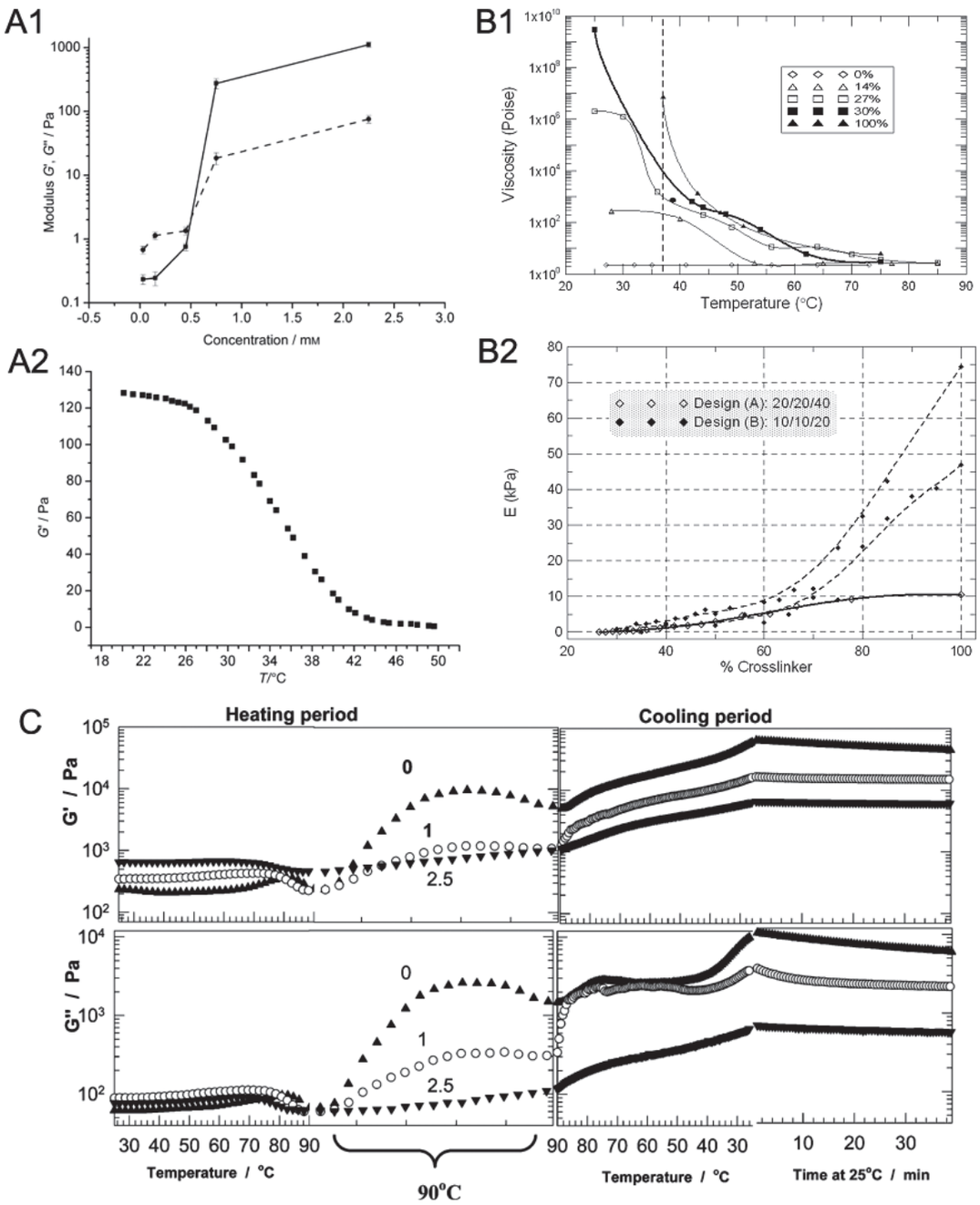


Fig. 2. Mechanical properties of DNA based macro-materials. (**A**) Rhelogy of DNA gels prepared from Y-shaped DNA at pH5.0. Dependence of storage (G', solid line) and loss (G'', dashed line) modulus on the concentration of the Y-shaped DNA unit (**A1**) at 25°C and on the temperature at the concentration of 0.60 mM of Y-shaped DNA unit (**A2**). Extracted from (Cheng et al. 2009). (**B**) Changes in viscosity of DNA gels of Design B with respect to the temperature at various levels of crosslinking (only show 0%, 14%, 27%, 30% and 100% for clarity) (**B1**) and changes in mechanical stiffness (modulus, E) with respect to the level of crosslinking for a DNA-crosslinked hydrogels of two designs (A) and (B) (Jiang et al. 2008b)

(**B2**). Open symbols and solid line represent stiffnesses of DNA gels of Design A (20/20/40 for length of SA1/SA2/L2) (extracted from (Lin et al. 2004b)) and solid symbols and dotted line represent stiffnesses of DNA gels of Design B (10/10/20). Note that at 37°C, the DNA gels are effectively solid. (C) Rhelogy of DNA solutions or gels with EGDE crosslinking (▲0%; **O** 1.0%; ▼2.5%) during heating-cooling cycles. ω= 1 Hz, strain is 0.01. Extracted from (Topuz & Okay 2008). Both images with publisher's permission.

2.2.5 Reversibility

As pointed in the previous section, DNA can be thermally degraded, and naturally bulk material based on DNA could experience property change along with DNA denaturing. Upon slow cooling and other proper conditions, DNA can re-anneal restoring the macro-material. For DNA crosslinked hydrogel, it is also possible to realize the reversible property change by introducing carefully design DNA strand bypassing the need of applying environmental stimuli such as light, pressure or temperature (Jiang et al. 2010b, Liedl et al. 2007, Lin et al. 2006). The key to this feature is branch migration based strand displacement (Lin et al. 2006) where a sticky end at the periphery of the DNA strands is necessary. Typically, the hybridization reaction occurs between two complementary DNA strands, and is affected by temperature and strand length. The process has a low rate constant several orders of magnitude less than the hybridization reaction (Reynaldo et al. 2000), and by designing a toehold, the process can increase dramatically (Yurke & Mills 2003). Yurke and Mills have determined that for a toehold length of eight bases, the exchange rate increases by six orders of magnitude (Yurke & Mills 2003). Branch migration takes place when a single-stranded DNA (ssDNA) competitively hybridizes with one strand of the DNA duplex starting at the sticky ends (or 'toehold'), and extends the hybridization until that strand is displaced entirely from the original DNA duplex (Watson et al. 2003). (**Figure 1B**) Essentially, since this strand has more complementary base pairs with the targeted ssDNA than do the side chains, generation of the doubled-stranded product is energetically favorable (Yurke & Mills 2003).

Consequently, the absence/presence of sticky ends offers off/on switch for the reversibility of gelation or possibility of structural modification with crosslinking density change. This special feature has fueled the interest in its drug delivery application (Liedl et al. 2007, Wei et al. 2008).

2.2.6 Mechanical properties

Mechanical properties including moduli have been investigated for various DNA based macromaterials (**Figure 2**). DNA crosslinked hydrogels display temperature and crosslinking density dependent viscosity, mechanical property and gelation point (**Figure 2B**) (Jiang et al. 2008a, Lin et al. 2004a). Chippada and colleagues developed formulation based on non-spherical inclusions, and made possible the probe of heterogeneity (variation with respect to. location) and anisotropy (difference with respect to direction) in the materials commonly seen in biological tissues (Chippada et al. 2009a, Chippada et al. 2009b). Other investigator used rheology and other techniques in mechanical characterization (Topuz & Okay 2008).

Increase in crosslinking density, microscopically straightens the single-stranded DNA side chain, and stiffens the micro-structure. Macroscopically, it is reflected in the increase in mechanical stiffness. The rigid dsDNA provides resistance also to compression,

contributing to the creation of artificial tensegrity (Ghosh & Ingber 2007, Ingber 2006, Liu et al. 2004).

2.3 Approaches in characterization of DNA based macro-materials

Owning to the unique features from DNA, special considerations have to be taken in characterization of the DNA based macro-materials, which poses challenges and stimulated novel ways of probing.

2.3.1 DNA incorporation

Incorporation of the delivered DNA can be assessed indirectly by probing the residual DNA concentration where direct assessment is difficult, if not impossible (Jiang et al. 2010c). In this approach, a DNA strand with non-specific sequence was also included as a negative control to show that the only DNA strands with specific sequence can base-pair with the available DNA side chains on the polymer, and were truly incorporated into the network rather than pure diffusion. Additionally, in measuring DNA concentration, the differential in UV absorbance between ds- and ss-DNA can be used for the detection of crosslinking or de-crosslinking (see, for example, (Cheng et al. 2009, Topuz & Okay 2008)).

2.3.2 Mechanical properties

To investigate mechanical properties of the DNA based materials, a number of methods has been developed (Chippada et al. 2009a, Lin et al. 2004b, Topuz & Okay 2008). Lin and colleagues developed an inclusion based formulation to address the issue of limited availability of samples, sample preparation and intrusiveness associated with conventional testing apparatus (e.g. Instron, or dynamic mechanical analysis (Um et al. 2006b)) for these materials (Lin 2005, Lin et al. 2004b). Recently, along this line of work, nanoscale rods were deployed and new formation has been developed to assess the inhomogeneity and anisotropy of the hydrogel materials (Chippada et al. 2009a). Mechanical properties including stiffness can be used to infer the structure of the DNA based macro-structures. For instance, for a DNA crosslinked polyacrylamide hydrogel, the crosslinking density of DNA crosslinked hydrogel has been correlated to its mechanical stiffness for a specific crosslinker design, thus the choice of crosslinking density can be made aiming at specific mechanical stiffness range (Jiang et al. 2008b, Lin et al. 2004b).. Moreover, drastic change in the viscosity or rheology has been used as indicator as watershed between sol and gel-states

2.3.3 State of DNA strands

Fluorophore attached DNA strands have been previously deployed to examined the dynamics of DNA base-pairing. The mechanism behind this approach is that the distance change between two dyes, or fluorophore/quencher, can be probed by various techniques including FRET (Fluorescence resonance energy transfer), which indicates the state (e.g., bent or straightened) of the DNA strands (Simmel & Yurke 2001). Atomic force microscopy (AFM) is a powerful tool capable of resolving nano-scale features, and has been used to probe the DNA based structures (e.g., (Liu et al. 2004)) with limitations in resolution (a few nm) (Um et al. 2006b). Optical properties can also be used to monitor the state change (e.g., DNA binding to cations, DNA packing or denaturation) based on drug-hydrogel interactions by using circular dichroism (CD) along with other techniques such as polarized Raman spectroscopy(Lee et al. 2008, Tang et al. 2009).

3. Current DNA based macro-materials and applications in biology and medicine

Seeman and colleagues pioneered the work employing DNA as a structural material in creating nanodevices (Seeman 1981, Seeman 1982), and reported designs of nano-scale structures such as rings(Mao et al. 1997), cubes(Chen & Seeman 1991), and octahedral (Zhang & Seeman 1994). More investigators joined the effort stimulating the emergence of structural DNA nanotechnology (Douglas et al. 2009, Rothemund 2006, Seeman 2007, Yurke et al. 2000), particularly aptamers, DNAzymes, and molecular beacon (Condon 2006, Lu & Liu 2006, Wang et al. 2009).

Of particular interest is the fact that a number of the designed structures inspired by DNA offer a large variety of design parameters (e.g., sequence and DNA-protein interactions) to the nanotechnology engineers. When they are incorporated as part of the macrostructures such as a hydrogel network, by changing the design parameters at the nanoscale DNA structures, dramatic physical and chemical properties changes can be achieved at macro-level. Some of these properties and functionalities are highly desirable in biology and medicine. Moreover, due to the unique properties of DNA, *in situ* modifications of nano-level structures become possible, which often result in the dynamic properties of macro-level materials thus supply dynamic cues in biological applications. Furthermore, in realizing these changes, a great number of physical, chemical and biological stimuli can be employed together (Lu & Liu 2007), offering augmented flexibility in designs.

Owing to DNA's water solubility and the resemblance to the physiological environment, DNA based macro-materials particularly hydrogels is stimulating ever-increasing interest. Hydrogels are a class of hydrophilic polymers that possess both solid- and liquid-like properties, and they typically consist of an insoluble network of crosslinked polymer chains immersed in solvent. They have attracted great interest and have become ever-increasingly popular for many applications, including biomedical ones. Due to its hydrated nature, a hydrogel can better mimic the properties of the natural tissues and neural micro-environment that cells reside in. This has fueled the interest and development of hydrogels-based tissue engineering scaffolds. In addition, hydrogels generally respond to the environmental factors such as temperature and pH, and thus are among the candidates for the development of drug delivery systems. Based on the types of crosslinker, hydrogels can be categorized into two classes; gels with covalent junctions and gels with physical junctions (weak forces, physical entanglement or others), or more simply stated, chemical and physical gels. Natural polymers are synthesized by living organisms mostly through enzymatic processes, while synthetic polymers generally involve either condensation or addition approaches. Crosslinking yields a polymer network where polymer chains are inter-connected.

Along this line, a number of DNA based hydrogel materials have been devised and characterized, among which are those consist solely of DNA strands (Cheng et al. 2009, Mason et al. 1998, Um et al. 2006b), those with polymer backbone and DNA crosslinkers (Lin et al. 2004b, Nagahara & Matsuda 1996), and those with DNA as polymer backbone connected via physical or chemical bonds (Topuz & Okay 2008), where DNA 'nanoswitch' impart the hydrogel materials desired functionality and properties (**Figure 3**).

3.1 DNA based macro-materials

The majority of the DNA based macromaterials fall in the following three categories (**Table 1**) and examples of these materials are shown in **Figure 3**.

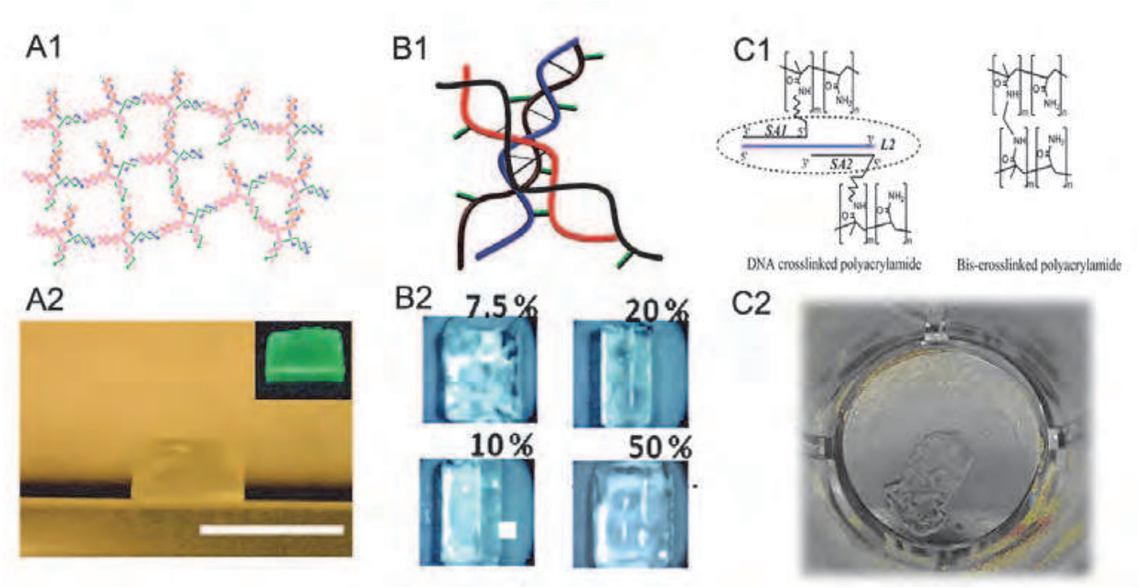


Fig. 3. Examples of DNA based macromaterials. (**A**) A DNA-only hydrogel: the schematic of the DNA structures (A1) and a prepared DNA gel (A2). Extracted from (Um et al. 2006b). (**B**) A EGDE crosslinked DNA gel (DNA as backbone): the schematic of the crosslinked structure (B1) with short thick black stick indicating EGDE bond and thin black stick indicating hydrogen bond (extracted from (Topuz & Okay 2008)) and resulting gels with varying EGDE content (B2) (extracted from (Topuz & Okay 2009)). (**C**) DNA crosslinked polyacrylamide hydrogel: the schematic showing the difference between DNA- and bis-crosslinked hydrogels (C1) (extracted from (Jiang et al. 2008b)) and a prepared gel (C2) in a well of a 24-well plate. All images with permission from publishers.

3.1.1 DNA-only network

The aqueous solution of DNA strands (~ 2,000 bps in length) can be viscous at high concentration before the critical overlap concentration is reached. Beyond this critical concentration, a weak gel can be formed due to the overlapping and entanglement of the DNA strands (Mason et al. 1998, Topuz & Okay 2008). Since the gelation point is reached based upon physical interactions rather than chemical bonds, this hydrogel is termed 'physical gel' (Mason et al. 1998, Topuz & Okay 2008). Though this approach has its advantages in the availability of the natural long double stranded DNA, the lack of controllability and stability limits their further application. Moreover, based on electrostatic interactions, by introducing hydrophilic ionic liquids, DNA hydrogel fibers have also been made (Lee et al. 2008). In this gel system, DNA strands compact into supercoils and bundle up forming aggregates, and give rise to new material properties such as stability and resistance to DNase digestion.

Luo group at Cornell University developed a hydrogel based entirely on DNA strand base pairing (**Figure 3A**). The synthesis involves two major steps: first, branched three- or four-armed 'X', 'Y', and 'T'-shaped DNA structures were synthesized from single-stranded DNA with partial complementarity based on DNA self-assembly; the sequence of the DNA strands were chosen and sticky ends were included such that it is available for enzymatic action; next, ligase, an enzyme capable of ligating DNA strands were deployed to connect

the building blocks produced from the first step, thus forming a crosslinked DNA polymer network. The resulting hydrogel has been shown to have swelling and mechanical properties that are dependent on the initial concentration and the forms of DNA building blocks, and biodegradability determined also by the building blocks (Um et al. 2006b). The potential of applying this hydrogel for drug delivery application has also been demonstrated. Very recently, this group also reported that by incorporating linear plasmids into polymer network, this hydrogel is capable of generating natural proteins under cell-free conditions (Park et al. 2009).

By using the similar Y-shaped DNA building blocks, but a different mechanism to connect these building blocks, Cheng group also put forth a hydrogel design based entirely on DNA nanostructures (Cheng et al. 2009). Different than the approach by Luo and colleagues, enzymes are not needed in the synthesis. Rather, the sequence of DNA strands are designed that it contains C-rich domain to take advantage of the triple hydrogen bond formation which results in a crosslinker between two DNA building blocks. Because the formation of such crosslinkers is pH dependent, the resulting macroscopic hydrogel can be formed only at suitable pH and hence responsive to pH changes. This feature allows for a new scheme for drug delivery based on pH, which hold promises in cancer therapies particularly those associated with local pH changes. It is worthwhile noting that in these studies, relatively short synthetic single-stranded DNA is required and that the quantity of the samples is still limited (~20 μL) primarily due to the limited availability and cost in synthesis.

3.1.2 DNA as backbone

While DNA strands can be connected via enzymatic actions or base interactions where no other chemical entity is involved, they also can be crosslinked by other molecules via either physical or chemical interactions. In these materials, DNA constitutes the polymer backbone. As an example, physical DNA gels have been developed based on the interactions between DNA strands and sulfonium precursor of poly-phenylenevinylene (SP-PPV) (Tang et al. 2009). Positively charged SP-PPV resulting from polymerization at alkaline solution contributes to the hydrogel formation based on DNA/SP-PPV hybrids due to electrostatic interactions. This gel system has demonstrated interesting stability and resistance to heat or DNase attack, and the presence of DNA strands in gel network imparts the material unique biological properties. As a proof of concept, its optical properties have been shown to assist in monitoring drug delivery as illustrated in the recovery of fluorescence upon release of drugs (Tang et al. 2009). Further application of this system awaits the investigation on whether DNA will be shielded from enzymatic digestion under physiological conditions or diffuse out of gel network (Tang et al. 2009).

Besides physical crosslinking, DNA backbone can also be chemically crosslinked. Topuz and Okay (Topuz & Okay 2008) used ethylene glycol diglycidyl ether (EGDE) for this purpose (**Figure 3B**), since epoxide group of EGDE can react with amino group in the bases of two DNA strands, although the two bases can also be from the single strand. They discovered novel thermal properties. At low crosslinking density, dynamic moduli are altered in a non-reversible way when gels are subjected to heating and cooling, and this leads to a hydrogel with Young's modulus in the mega-Pascal (MPa) range. The increased physical entanglement upon heating and hydrogen bond formation at cooling were identified as the cause, although it is not clear whether controlling the kinetics of the DNA re-annealing could affect the process. Horkay and Basser examined the effect of ion strength and

concentration on osmotic and mechanical properties of these DNA gels (Horkay & Basser 2004). DNA gel particles based on interactions between DNA and CTAB, a cationic surfactant, or lysozyme were developed by Moran and colleagues. In this physical gel, the electrostatic forces help stabilize the gel network (Moran et al. 2007).

3.1.3 DNA as crosslinker

DNA has long been used to provide bases for assembling microscopic structures into macroscopic objects by functioning as crosslinkers, and to give bulk materials added functionality (Lin et al. 2004b, Nagahara & Matsuda 1996, Neher & Gerland 2005). For instance, Mirkin and colleagues reported a method to organize colloidal gold nanoparticles and form aggregates (Mirkin et al. 1996) based on DNA crosslinking. The motivation in using DNA as linking reagents rather than the main building blocks or polymer backbone lies partly on the fact that in those cases large quantities of synthetic DNA are currently prohibitively expensive (Jiang et al. 2008a, Lin et al. 2004a, Mangalam et al. 2009), and that it is challenging to characterize these structures(Storhoff & Mirkin 1999).

By using DNA hybridization instead of covalent bonding to form crosslinks between polymer strands, hydrogel polymers have been given a temperature-dependent rigidity and thermal reversibility in crosslinking and gelation(Lin et al. 2004b, Nagahara & Matsuda 1996), and a number of new possibilities including *in situ* property change (Jiang et al. 2010c). In an early work (Nagahara & Matsuda 1996), poly(N,N-dimethylacrylamide-co-N-acryloxyloxysuccinimide) was reacted with 5'-amino-modified 10-mer oligonucleotides (oligoA or oligoT) to form polymer chains with short DNA side branches (**Figure 4**). Two different crosslinked structures (**Figure 4A**) were produced: in one of them oligoA branches from one solution of polymer chains hybridized with oligoT branches from the second solution, and in the other of them two oligoT branches hybridized with a third 20-mer OligoA strand. Gelation of the polymers as well as thermo-reversibility of crosslinking at elevated temperatures was demonstrated.

While the simple sequences used in (Nagahara & Matsuda 1996) preclude the formation of secondary structures (e.g., the hairpin structure), the possibility of off-alignment binding between two complementary sequences is high. Although perfect alignment of oligoA and oligoT strands is energetically favorable, misalignment by only a few bases may occur with little penalty. Such misalignments may result in mechanically weakened, kinked crosslinks. The probability of off-alignment binding between complementary DNA strands can be reduced by designing base sequences. Towards this end, by incorporating Acrydite™ modified oligonucleotides in PAM gels, Lin and colleagues (Lin et al. 2004b) illustrated and characterized reversible gelation and achieved a range of stiffness from a few hundred Pa to 10 kPa by varying crosslinker DNA density. They showed that sequence optimization is an effective method of enhancing the stability of DNA crosslinks (**Figure 4B**). In these gels, Acrydite™ modified oligonucleotides co-polymerize with acrylamide monomers to form polymer long chains with DNA side chains of specific length and sequence designated as SA1 and SA2. 'Crosslinker' oligonucleotides (L2) with a 'toehold' assume the functions of a crosslinker by hybridizing with SA1 and SA2 at the same time. By carefully designing another single-stranded DNA (ssDNA), also called "removal" DNA that is complementary to L2, one is able to reverse crosslinking process (**Figure 5C**). With this gel system, the pore structure upon reversible crosslinking was explored, giving rise to the potential application for controlled drug delivery (Liedl et al. 2007).

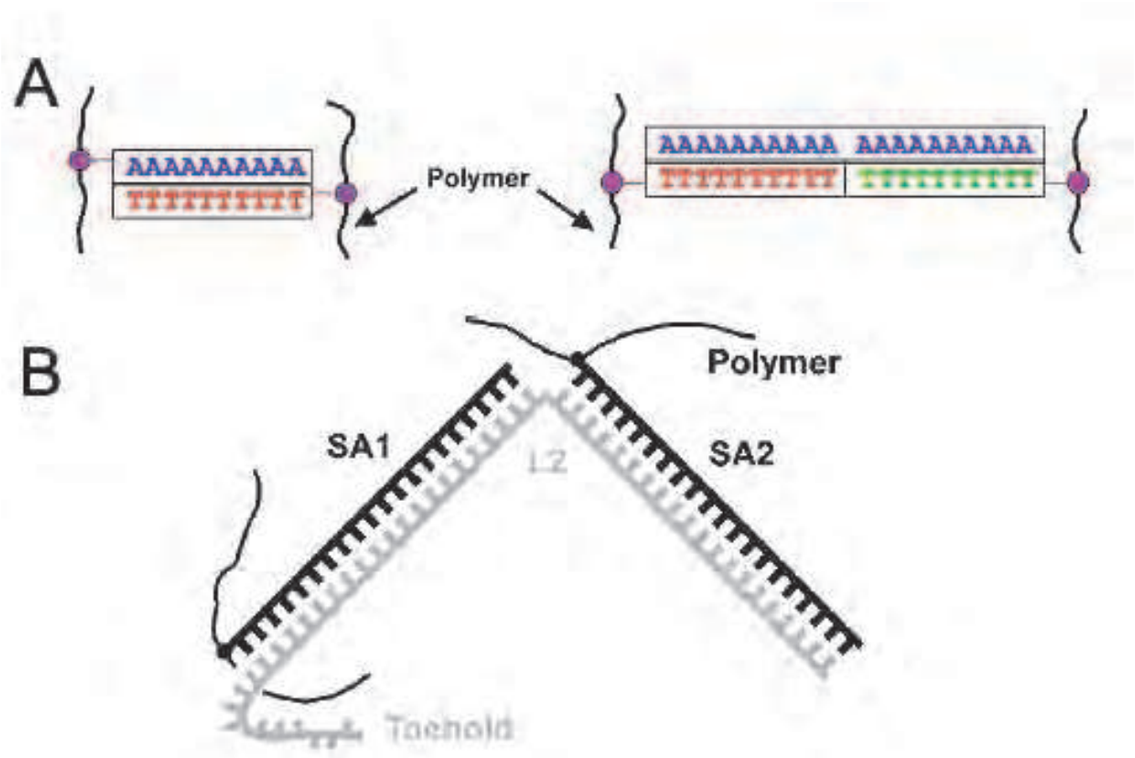


Fig. 4. Design of DNA crosslinked hydrogel. (**A**) The two DNA-crosslinked structures by Nagahara and Matsuda (Nagahara & Matsuda 1996). Oligonucleotides and their sequences are represented by the boxes and oligonucleotides-polymer connections by the dots. Extracted from (Nagahara & Matsuda 1996), with publisher's permission. (**B**) The design by Lin et al. (Lin et al. 2004b). SA1, SA2, and L2 represent DNA side chains and a crosslinker complementary to both DNA side chains. A toehold can be added for reversibility. Extracted from (Lin et al. 2006), with publisher's permission.

Replacing the covalently bound bis-crosslinks with paired DNA strands results in a gel possessing a number of potentially useful properties, such as thermal reversibility with a tunable melting temperature, reversibility of gelation without heating and without the need of initiator-catalyst system for re-gelation(Lin 2005, Lin et al. 2004b). More interestingly, by modifying the DNA crosslinking (i.e., oligonucleotide length or concentration), the mechanical properties of the gels can be engineered to take on particular values. Specifically, via delivery of more crosslinks, the DNA association/dissociation ratio could increase, resulting in a stiffened gel; in contrast, gels could be softened by lowering the crosslink density with removal DNA strands (designated as CL2, **Figure 5C**) that are complementary to L2. CL2 strands competitively base-pair with L2 strands and remove them from the gel network. The ease with which the mechanical properties of DNA crosslinked gels can be changed suggests that they would be useful in tissue engineering applications. This has generated interests in using DNA as crosslinking agent for various applications (Alemdaroglu & Herrmann 2007, Liedl et al. 2007, Murakami & Maeda 2005, Roberts et al. 2007, Wei et al. 2008, Yang et al. 2008). In addition, DNA has also been reported to crosslink organic network such as cellulose (Mangalam et al. 2009).

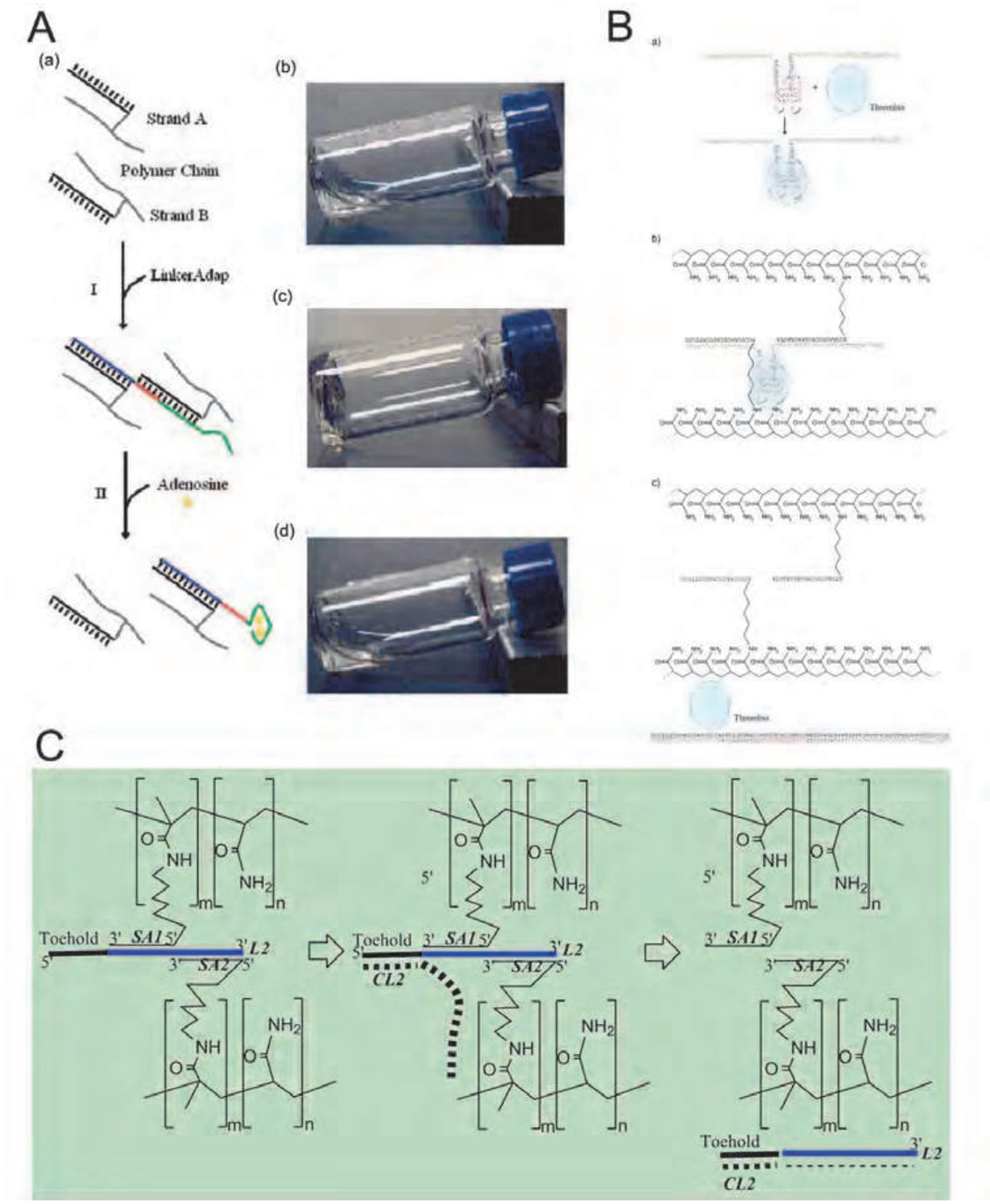


Fig. 5. Induce dynamic changes in DNA crosslinked hydrogels. (**A**) Addition of DNA crosslinks the hydrogel and upon delivery of adenosine which competitively binds to the crosslinker DNA, resulting in the reverse of gelation (a) as demonstrated in the transition from solution (b) to gel (c) and then back to solution (d). Extracted from (Yang et al. 2008) with publisher's permission. (**B**) DNA hydrogel capable of capturing and releasing thrombin based on thrombin-aptamer interactions. The end structure of DNA side chain A has high affinity to thrombin to form thrombin-aptamer complex (Ba), which can be capture

via DNA hybridization (Bb). Upon delivery of the ssDNA complementary to strand A, thrombin is released. Extracted from (Wei et al. 2008) with publisher's permission. (**C**) For a DNA crosslinked hydrogel (Jiang et al. 2010b), delivery of the 'removal' DNA strand complementary to the crosslinker DNA leads to de-crosslinking.

Dynamic materials can be used to manipulate cell behavior. In studies performed by Langrana and colleagues, DNA-crosslinked hydrogels (DNA hydrogels) were used as the underlying substrate to study the effects of dynamic mechanical cues on fibroblast behavior (Jiang et al. 2010c, Previtera et al. 2011). The DNA hydrogels have the ability to temporally change stiffness (Jiang et al. 2008a, Jiang et al. 2010c, Lin 2005, Lin et al. 2004a, Lin et al. 2005, Previtera et al. 2011). Upon a decrease or increase in DNA hydrogel stiffness, expansion or contraction forces are generated, respectively. The two properties cannot be decoupled (data unpublished). When grown on these dynamic hydrogels, fibroblast morphology is noticeably different compared to static hydrogels, which do not change in stiffness and thus do not generate forces (Jiang et al. 2010c, Previtera et al. 2011). GFP fibroblast became larger and more circular, compared to static conditions, when grown on DNA hydrogels that became softer and expanded (Previtera et al. 2011). Therefore, as the underlying substrate expands and softens, the GFP fibroblasts expand and become rounder morphology. This is in contrast to GFP fibroblast grown on dynamic hydrogels with increasing stiffness and contraction forces (Jiang et al. 2010c). These GFP fibroblasts became smaller and/or longer when compared to static hydrogels. However, these results depended on magnitude of hydrogel stiffness change (Jiang et al. 2010c).

3.2 Potential application of DNA based macro-materials

Three main areas of application are being explored by using these DNA based macromaterials (**Table 1**).

3.2.1 Biosensor| Actuator| Bioelectronics

Hydrogels synthesized from DNA nanostructures hold promises as biosensor (Cheng et al. 2009, Lin et al. 2004b), Simmel and Yurke designed a DNA-based actuator capable of switching between two physical states, which can potentially be used as motor to drive the nano-robot (**Figure 1**) (Simmel & Yurke 2001). This approach, together with others (Knoblauch & Peters 2004), can be adopted in hydrogel formation, giving rise to novel materials with changing properties upon 'fuel strand' delivery. Besides the potential uses of DNA based macromaterials in sensors and actuators, DNA's electronic properties and molecular recognition, feasibility of DNA manipulation at nano-scale, and the trend of miniaturization are driving the synergy between DNA and electronics. Braun and Keren (Braun & Keren 2004) put forth a scheme of constructing DNA based transistors, in which DNA is metallized and serves as a template for electronic circuit, which exemplifies DNA's impressive capability of information storage and molecular recognition mechanism. Incorporation of grafted oligonucleotides also leads to novel materials with high optical resolution, and can be potentially used in biosensing (Tierney & Stokke 2009) (**Figure 6A**).

3.2.2 Drug delivery vehicle

In response to various environmental factors, DNA may alter its secondary and tertiary structures, resulting in alterations in the bulk materials that are built upon them. Aiming at drug delivery application for cancer therapy, a great deal of effort has been made in

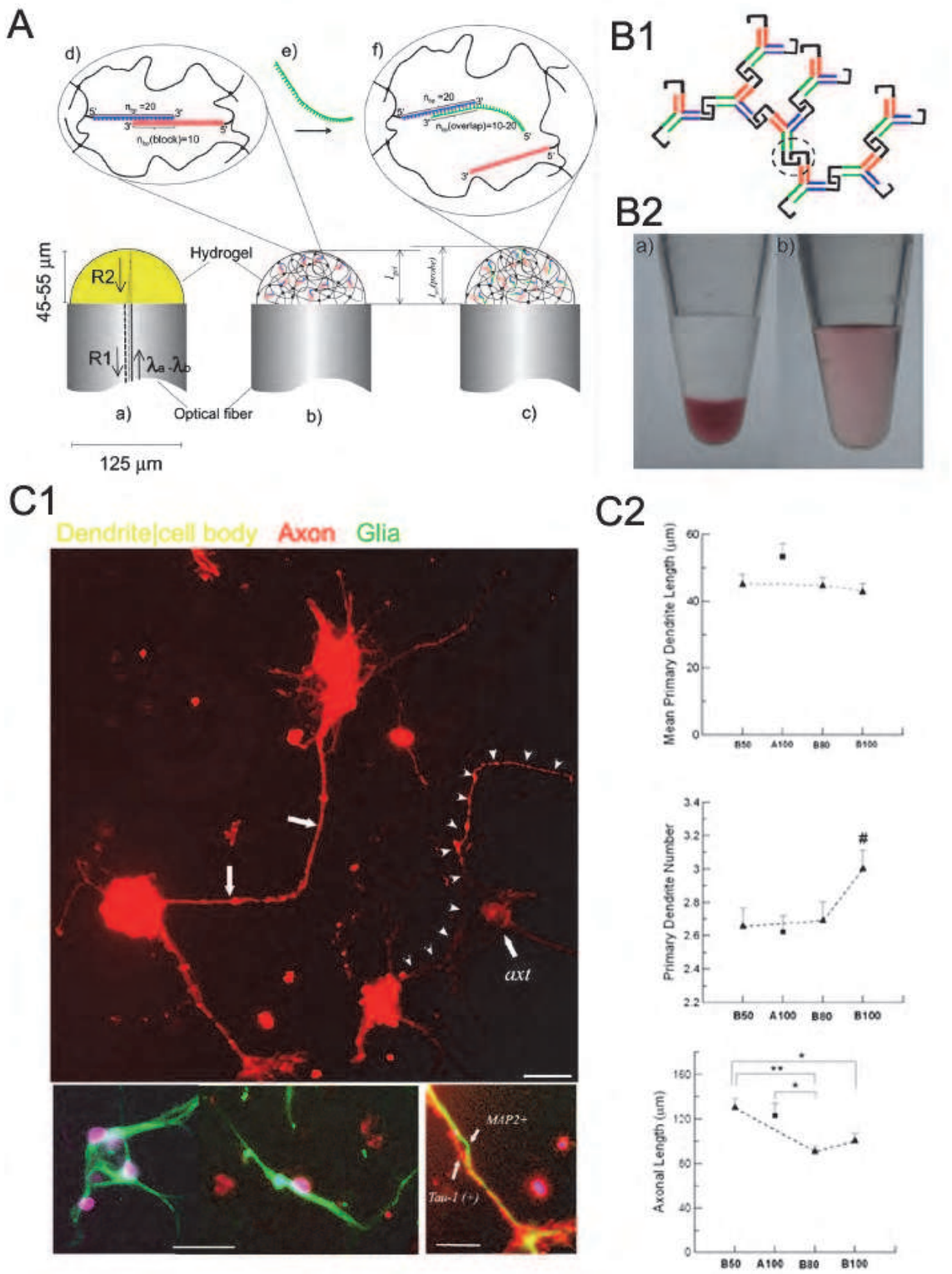


Fig. 6. Examples of application of DNA based macromaterials. (**A**) Schematic of hemispherical bio-sensitive hydrogel attached to the end of an optical fiber to determine

changes in the optical length for biosensing applications. Extracted from (Tierney & Stokke 2009). (**B**) DNA-only hydrogels based on branched Y-shaped DNA unit. Black i motif with cytosine-rich regions crosslinks adjacent Y units (B1). The DNA gel prepared from this design exhibited responsiveness to pH, in low pH where gold naoparticle (AuNP) was trapped in the gel (a) and at high pH gel dissociation leading to AuNP release (b) Extracted from (Cheng et al. 2009). (**C**) Neurite outgrowth on a DNA crosslinked hydrogel. Overlay of higher power images of MAP2 and Tau-1 stain reveals that axons and dendrites could reside closely in parallel with each other (C1). Red: Tau-1 immunostaining; Green: MAP2 immunostaining; Blue: GFAP immunostaining; Purple: DAPI staining. Scale bar is 50 □m. Comparison of neurite outgrowth, including mean primary dendrite length, primary dendrite number, and axonal length per neuron, on DNA gels of two designs. Extracted from (Jiang et al. 2008b). All images with publisher's permission.

designing responsive DNA gels. Among all the cues is pH due to the fact that certain cancer types are associated with local acidity (Gerweck & Seetharaman 1996). DNA motifs sensitive to changes in H+ concentration has been incorporated in the DNA based hydrogel to realize pH responsiveness. A DNA hydrogel in which gel-'drug' interactions are pH dependent was also proposed (Tang et al. 2009) (**Figure 6B**) along with others gels (Roberts et al. 2007) (**Table 2**). In this design, the electrostatic interactions that retain drugs in the gel network can be reduced resulting in subsequent drug release (Tang et al. 2009). Besides pH, temperature may be another environmental trigger for drug release, particularly for those diseases with local temperature change (e.g., (Hildebrandt-Eriksen et al. 2002, Letchworth & Carmichael 1984)). Thermal responsiveness of the DNA hydrogel has been designed based on the temperature-dependent hybridization, sol-gel transition or physical properties (Costa et al. 2007, Lin et al. 2004b, Topuz & Okay 2008). Ion strength or concentration has also been explored to initiate drug release using DNA based macromaterials (Costa et al. 2006., Horkay & Basser 2004).

These hydrogels responsive to environmental factors hold promises in facilitating targeted delivery of therapeutic reagents, while their application has inherent limitation. First, their application is limited to where such environmental alterations exist; and second, their controllability is limited due to undesired environmental changes that may occur; third, their applicability is limited when temporal control in delivery is desired. Looking to expand the scope of application, some investigators attempted to develop dynamic DNA gel system without the need of environmental factors. DNA strand per se is naturally an ideal candidate. Lin and colleagues demonstrated possibility of triggering de-gelation by delivering ssDNA (Lin et al. 2006), and a similar scheme was adopted by Wei et al.. in designing a DNA gel capable of releasing proteins based on aptamer-thrombin interactions (Wei et al. 2008). Aiming at the same application relying on DNA aptamer-protein interactions, a latest study explored a hydrogel system capable of sustained protein release (Soontornworajit et al.). Diffusion profile and relationship between cargo size and pore size of this system were studied, and it was found that the nano-scale particles can be trapped even their size is smaller than the average pore size of the hydrogel network (Liedl et al. 2007). In addition to DNA strands, by using the similar system, adenosine has also been shown as the trigger for changes based on its interactions with aptamers (Yang et al. 2008). A recent work reported the enzyme triggered release of DNA in a polymer network with grafted DNA duplex (Venkatesh et al. 2009). This system is based on the conventional crosslinking but contains Acrydite modified DNA recognized by specific enzymes.

Additionally, DNA gels have been shown to be an ideal candidate for cell capsulation (Um et al. 2006b), potentially, serving as *in vivo* protein factory for protein synthesis and delivery (Park et al. 2009). Examples of the studies using DNA-only, DNA-as-backbone, and DNA crosslinked macromaterials on potential drug or gene delivery applications and the kinetics of release are in **Figure 7**.

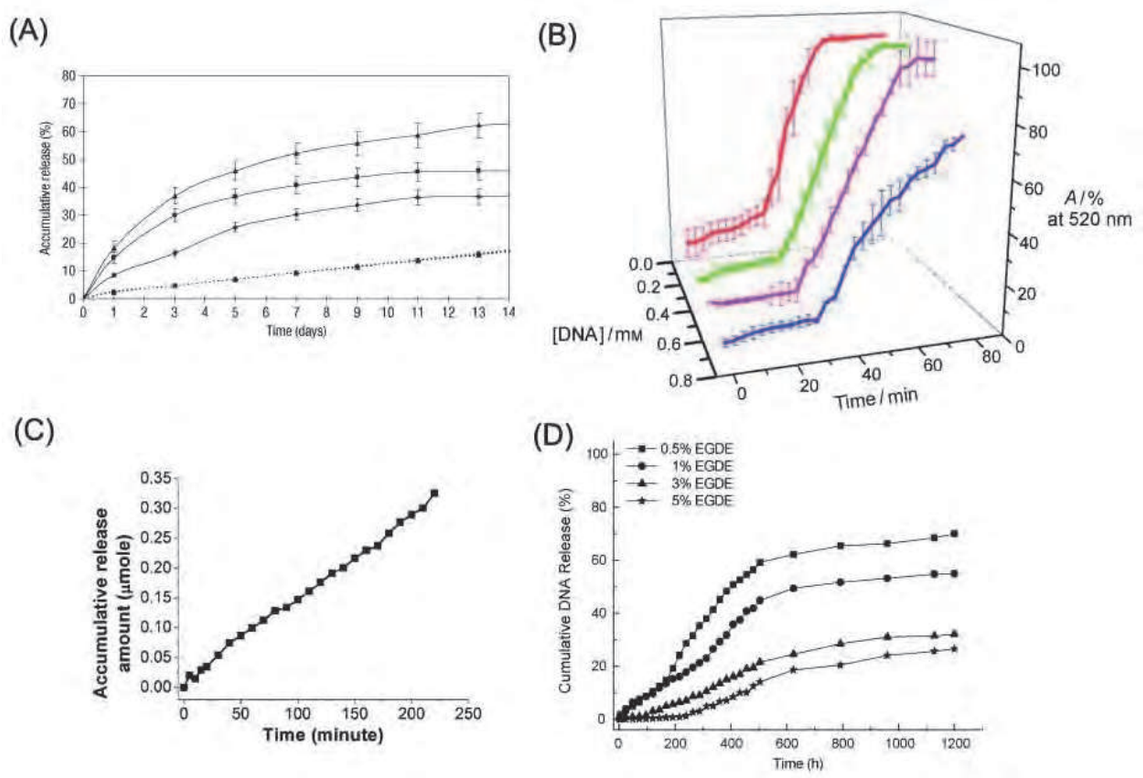


Fig. 7. Kinetics in the release of therapeutic agent using DNA based macromaterials. (**A**) Release of insulin (solid line) and CPT (camptothecin, dotted line) from a DNA-only gel. From top down, the lines indicate Y-, T-, X-, and T- DNA gels. Extracted from (Um et al. 2006b). (**B**) Release of gold nanoparticles from an aptamer-crosslinked hydrogel at interaction with cocaine. Extracted from (Zhu et al. 2010). (**C**) Release of antihypertensive nicardipine hydrochloride containing one nitro group from a physical DNA gel based on DNA and SP-PPV interactions. Extracted from (Tang et al. 2009) with publisher's permission (D) Cumulative release of DNA from a EGDE crosslinked DNA (as backbone) gel with various crosslinking density under sunlight. Extracted from (Costa et al. 2010). All images with publisher's permission.

3.2.3 Biomaterials/Tissue engineering

As mentioned in the previous discussion, hydrogel materials has been gaining increasing popularity due to its hydrated state mimicking natural tissues (Janmey et al. 2009, Nemir & West 2009, Uibo et al. 2009). Following this direction, one line of interest in applying DNA based macro-materials is to study cell-ECM interactions, an analog of tissue-biomaterials interplay. A DNA only gel system has been proved to possess cyto-biocompatibility by

encapsulating CHO cells (Um et al. 2006b). Replacing the traditional bis-acrylamide crosslinker in a popular bis-gel system (Wang & Pelham 1998), DNA crosslinker of 20-50 nt long was used for the study of the effect of substrate stiffness on neurite outgrowth (Jiang et al. 2008b) (**Figure 6C**). In this system, difference in rigidity was created by varying length of the crosslinker, crosslinking density, or monomer concentration, among which crosslinking density can be modified via DNA strand delivery in situ. The potential of using these DNA crosslinked gels in tissue engineering application is promising (Chan & Mooney 2008, Ghosh & Ingber 2007).

The added advantages by using DNA based macromaterials were further demonstrated recently in subjecting cells to dynamic stiffness of the substrates (Jiang et al. 2010b, Jiang et al. 2010c). These studies were motivated by the fact that the micro-environment that cells reside in within natural tissues is dynamic and undergoes constant synthesis and degradation in both normal and pathological conditions (Lahann & Langer 2005, Mrksich 2005). Moreover, aging, development, external assault, and pathological processes can also lead to the alternations in the extracellular matrix (ECM) (Georges et al. 2007, Ingber 2002, Silver et al. 2003). In addition, at the tissue-implant interface, cells can actively modify surface of the implants, altering the stiffness of microenvironment of their own or other cells (Marquez et al. 2006). The changing stiffness could potentially make it possible to achieve optimal growth of a specific cell property (Jiang et al. 2008b) or direct stem cell differentiation (Engler et al. 2006) at different time points. These facts make it very desirable for the biomemetic materials to have the capability of undergoing controlled remodeling with respect to time. Previously, a limited number of attempts have yielded exciting findings (Chen et al. 2005, Lahann & Langer 2005, Mrksich 2005), in which dynamic changes were induced largely through application of environmental factors (e.g., temperature, pH, and electric field). However, the utility of these approaches in clinical setting could be problematic. With the unique hydrogen bond based crosslinking, DNA based and crosslinked materials, therefore, demonstrate time-dependent properties as reflected in swelling and mechanical modulus, and offer a feasible way of dynamically altering the macro-scale structure mimicking the *in vivo* conditions (**Figure 8**). Indeed, the initial results have indicated that encapsulated cells are viable in a DNA-only hydrogel, and in a DNA crosslinked hydrogel both mechano-sensitive cell types (e.g., fibroblast) (**Figure 9**) and neuron whose mechano-responses are being appreciated just recently respond to the changing stiffnesses, and the responses are specific to range and rate of changes and cell type (Jiang et al. 2010a, Jiang et al. 2010c). A summary of DNA based macromaterials with dynamic and responsive properties is presented in **Table 2**.

4. Design considerations in DNA based macro-materials

Different than other materials, DNA based macro-materials necessitate some unique considerations due to involvement of DNA nano-materials.

4.1 Stability

As pointed out in the last section, DNA strand can respond to a variety of environmental factors such as temperature, pH, and ion concentration and non-environmental factors such as exogenous DNA or enzyme. While it allows design of smart responsive materials, it also poses difficulties in maintaining the integrity of structures. Divalent or multi-valent cations

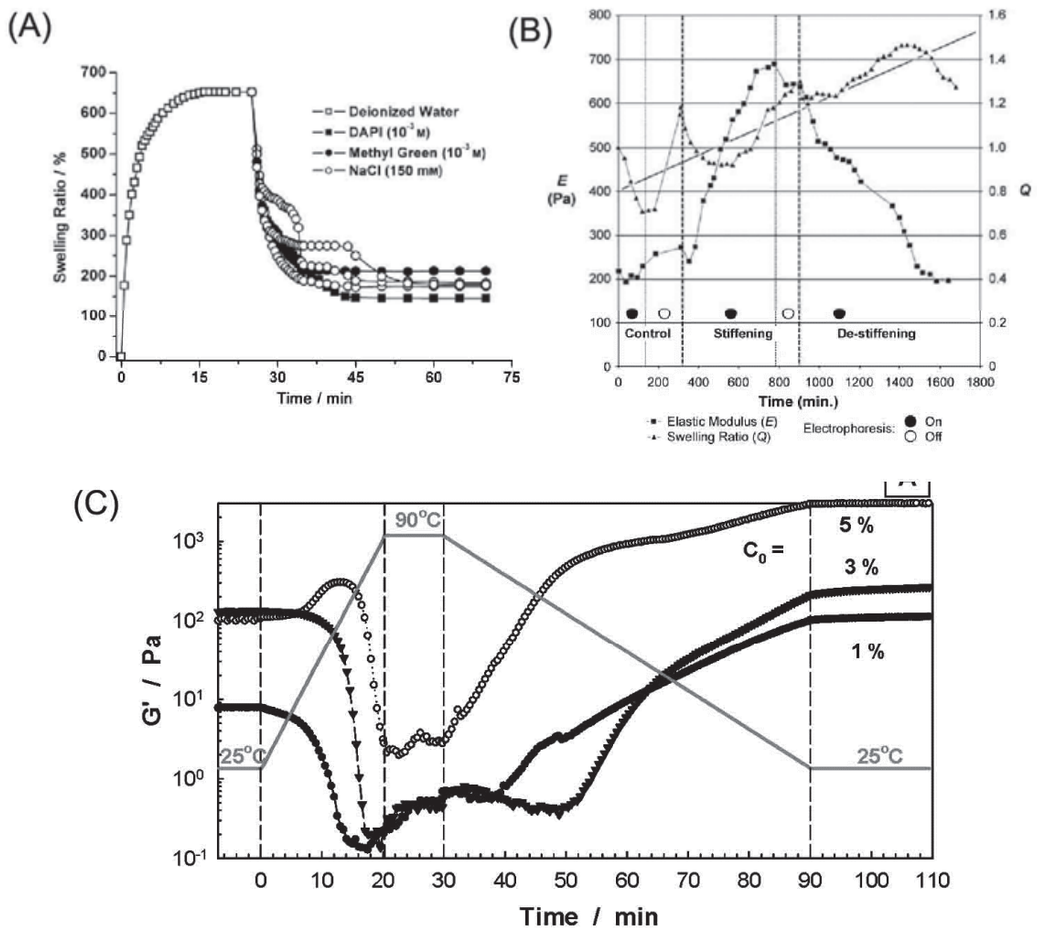


Fig. 8. Time history studies of DNA based macromaterials. (**A**) Swelling/Deswelling profile of a DNA-only hydrogel fiber. Swelling last till 25 min time point when different groove-binding molecules were introduced. Extracted from (Lee et al. 2008). (**B**) Time history plots of the elastic modulus and swelling ratio of a DNA crosslinked hydrogel. Both quantities were first measured throughout the control phase during which no DNA strands were added, first under the influence of electrophoretic power and then without it. Fuel strands were migrated into the gel during the stiffening phase and removal strands were introduced during the de-stiffening phase. Extracted from (Lin et al. 2006). (**C**) Changes in elastic modulus G′ and temperature of DNA (as backbone) gel with time at different DNA content. Extracted from (Orakdogen et al. 2010). All images with publisher's permission.

such as magnesium have been shown critical in both dsDNA stability and re-annealing. Thus using ion-containing buffer would be a better choice than deionized water in maintaining gel structure and integrity. Interestingly, the gel collapse has been observed for a EDGA crosslinked DNA gel system, where the form of dsDNA or ssDNA, DNA content, and co-solutes in the medium contribute to the kinetics (Costa et al. 2007). The thermal stability has been investigated in a number of studies. For a DNA only gel system, gels based on ssDNA were less stable than those made from dsDNA perhaps due to the

synergistic effect of multiple strands, possibly due to distinct linear charge density, strand flexibility and hydrophobicity (Costa et al. 2007). DNA crosslinked polymeric hydrogel exhibited thermal reversibility and sol-gel transition, which is correlated to the thermal stability of the DNA base-pairing. As a result, in these gels DNA sequence has to be designed for desired melting temperature (Tm) by adjusting length of the strand, GC content, and/or thermal dynamics(Cheng et al. 2009, Lin et al. 2004a). It is noted that the critical temperature for the DNA based bulk material may be different from that of the involved DNA strands (Sun et al. 2005, Topuz & Okay 2008).

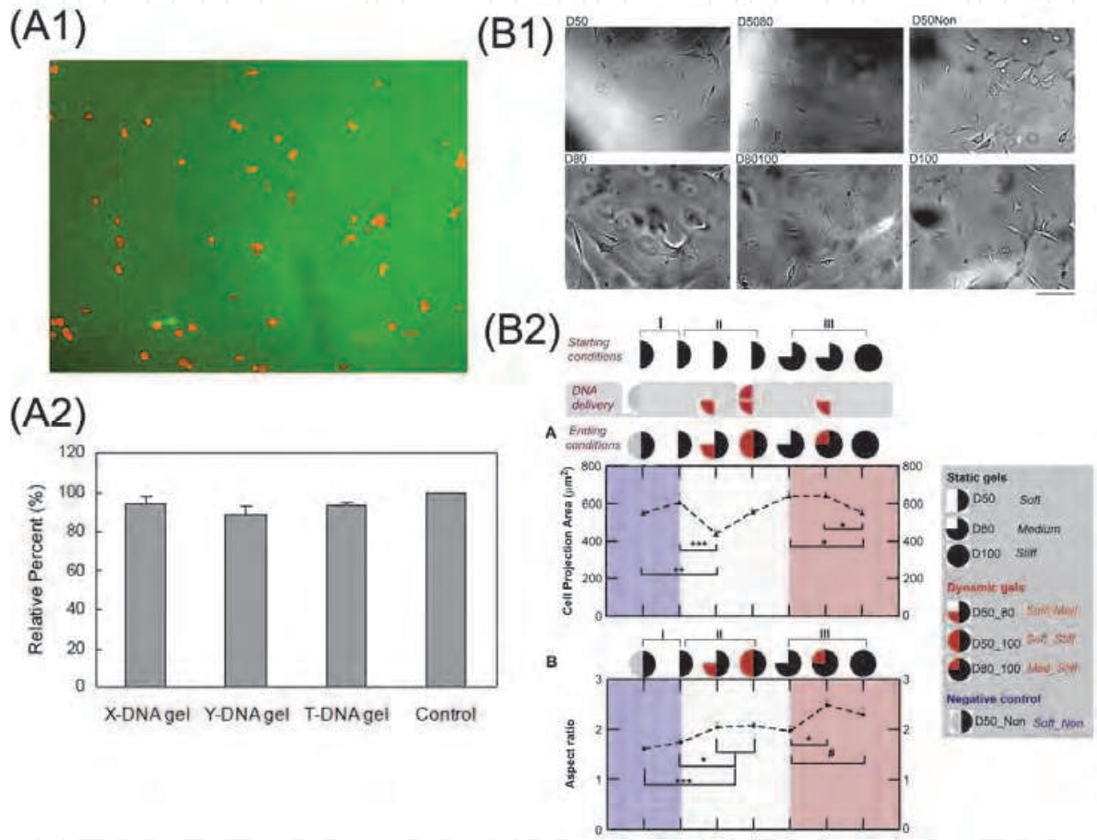


Fig. 9. Study of cellular behavior using DNA based macromaterials. (**A**) CHO cells in a DNA-only gel. Stained CHO cell encapsulated inside the gel (A1) and the majority of the cells were viable (A2). Extracted from (Um et al. 2006b) with publisher's permission. (**B**) L929 fibroblasts growth on dynamic substrates based on a DNA crosslinked hydrogel. (Upper panel) Typical morphology of L929 fibroblasts grown on DNA crosslinked hydrogels at Day 4, two days following DNA delivery. Scale bar is 100 mm. (Lower panel) Projection area and aspect ratio of L929 fibroblasts on DNA crosslinked hydrogels on Day 4. Extracted from (Jiang et al. 2010c) with publisher's permission.

Gels consisting of physically entangled DNA strands display resistance to DNase digestion (Lee et al. 2008). The hybrid between DNA strands and other polymer (such as SP-PPV (Tang et al. 2009)) also possess resistance to enzyme or heat. Thus, physical interactions between DNA strands and composite between DNA and other polymer may provide shield against enzymatic action.

4.2 Sequence design

Different to the majority of the materials based on DNA as backbones and some DNA-only gels where natural DNA (e.g., from salmon) was used, DNA crosslinked materials and DNA-only gels with designed DNA building blocks carry synthetic DNA. The sequence can be designed allowing added features. As we have discussed in the properties of DNA, the primary structure, i.e., the sequence or the order of nucleotides, of DNA primarily determines its secondary and tertiary structures, thus it is of significance to design sequence which gives the desired bulk material properties. Meanwhile, although two complementary DNA strands achieve their minimum energy state when they hybridize in the perfectly aligned configuration, DNA hybridization does not occur without error (Deaton et al. 1998). In addition, undesired interactions can occur between two strands as well as within a single strand. Such interactions typically involve the binding of complementary regions comprising only a small number of base pairs and include the formation of secondary structures such as the hairpin loop. In designing DNA sequences, it is desirable to decrease the number of possible mismatched hybridizations in order to maximize the efficiency of hybridization.

Design of a pair of equal-length sequences is essentially an optimization problem with the minimization of undesirable (e.g., off-alignment) interactions as the objective function. For instance, in the work by Lin et al. (Lin et al. 2004b), DNA sequence was generated by incorporating into the algorithm the following considerations: minimization of undesired interactions among strands and potential secondary structures, thermodynamic stability of the hybridized sequence pairs (e.g., GC content, terminal sequences, and hairpin structures (Lin et al. 2004b, SantaLucia & Hicks 2004)), and initiation of branch migration (e.g., length of sticky ends) (Deaton et al. 1998, Felsenfeld & Miles 2003). C-rich domain can be incorporated where it is desirable to have pH responsiveness (Cheng et al. 2009). It is noted, however, that due to the limitation on the current technology, and synthetic single-stranded DNA can have length up to 100 nt. The biological applications of these DNA crosslinked structures require additional caution. Examples include the ending sequence of the DNA strands, and the melting temperature needed to maintain the integrity of the DNA base structures.

4.3 Interaction between DNA and other entities

In the application of DNA based macro-materials for biology and medicine, DNA may potentially interact with an array of biologic entities such as protein, small molecules, other biopolymers, and endogenous DNA. The potential immunogenicity is also of concern. For dynamic DNA based macro-materials, the interactions between stimuli and DNA are also of interest. As an example, under physiological ion concentration, it was found that exchange between mono-(e.g., Na+) and bi-(e.g., Ca2+)valent cations affects volume, osmotic, and mechanical properties of a DNA gel consisting of DNA strands of ~2,000 bp. To avoid the unwanted biological effect, such as delivery DNA serving as anti-sense DNA, in the design of crosslinker DNA sequence, candidate sequences were screened by using a basic local alignment search tool (BLAST) algorithm which checks against the sequence in the genome of a specific specie and tissue type. To the same gel system, interactions between DNA aptamer and adenosine was explored as a way to initiate de-gelation (Yang et al. 2008), thus care needs to be exerted where such interactions are to be minimized in the presence of natural adenosine. Additionally, DNA strands can react with proteins and lipids (Liu et al. 2007). For example, DNA strands were reported to affect fibril formation of collagen matrix, and cation lipids (Liu et al. 2007). DNA-antibody interactions is another potential consideration in the design of DNA based macromaterials (Di Pietro et al. 2003). Of

particular concern in the drug delivery applications are the drug-DNA interactions (Chaires & Waring 2001, Lu & Liu 2007).

4.4 Application of stimuli

Introduction of stimuli such as pH, ion concentration, or temperature may appear straightforward, while it is potentially a concern for the delivery of large molecule such as ssDNA strands as cues. The kinetics and efficiency of delivery may be determined by the pore size of the structures, biochemical conditions, and interactions between DNA and other entities (e.g., soluble factors, inorganic compounds,) in the local microenvironment. To this end, more effective and delivery of ssDNA may be required in the clinical application. The thermal responses of the certain DNA gels merit attention due to the complexity in the changes of the material properties observed. For example, the alterations in materials properties induced by DNA denaturation and physical entanglement of resulting ssDNA may not be apparent (Topuz & Okay 2008).

4.5 Crosslinker parameters

In DNA as backbone gel system with EGDE as crosslinker, better stability but low dynamic moduli have been correlated to higher crosslinker content (Topuz & Okay 2008). Common design parameter for DNA based macro-material using synthetic DNA as crosslinker include DNA length and concentration and relative ratio of DNA and other components in the composite. Increased DNA concentration, or crosslinking density, causes materials to reach sol-gel transition and elevated mechanical stiffness beyond critical crosslinking density (Lin et al. 2004b). DNA length may be another design parameter, although its effect on bulk material properties was not noticeable when the length is in the 10 to 20 nt range (Jiang et al. 2008b).
Lastly but not the least, one of the major hurdles of research and development of DNA based active materials using synthetic DNA is the relative high cost and limited availability of the synthetic forms of DNA (Jiang et al. 2008b, Lin et al. 2004b, Mangalam et al. 2009). Thus, this field of research awaits the development from other areas including synthetic chemistry and molecular biology to address this issue, and the trend has been towards the positive direction (Carlson 2009).

5. Outlook and potential directions

The progress outlined above has laid a solid foundation for the further development of the DNA based macro-material and for the further application of these materials in biology and medicine.

5.1 Inspiration from DNA nano-materials

The rapid development of DNA based nano-materials offers vast pool of ideas and hints, based on which novel macro-materials can be designed. For instance, from an ion-concentration based DNA-actuator (Fahlman et al. 2003), one could device a macromaterial based on formation of intermolecular guanine quartets. Another example is that DNA sliding, if tailored through the choice of base sequence in a periodic manner, may be useful in imparting unique properties to the resulting materials (Neher & Gerland 2005). Moreover, new stimuli for DNA nanostructures can be used for macromaterials. For instance, some DNA strands have been shown to interact with biometals (Goritz & Kramer 2005), thus the macromaterials constructed based on these DNA strands may have novel properties at the

presence of physiological conditions. Other stimuli including light (Ogura et al. 2009), antibodies (Wiegel et al. 1987), proteins (Xie et al. 2007) and micelle (Ding et al. 2007) used in nanotechonlogy could be explored as the trigger for dynamic DNA based macro-materials. Along this line, DNA interstrand crosslinking from radical precursor independent of O2 (Greenberg 2005) may be of interest.

5.2 Refinement of the current designs

For DNA only system, multiple designs of the DNA building blocks can be incorporated for graded (with respect to time or location) control of the material properties. By combining physical and chemical crosslinking, gels with DNA backbones may achieve properties not seen in either system. Refinement of the DNA crosslinked hydrogel includes multi-step control by introducing multiple DNA crosslinker in a single system allowing multiple-step in increasing or decreasing crosslinking density. It also includes adding responsiveness to multiple cues by inclusion of DNA crosslinkers that are sensitive to stimuli including pH, temperature, and exogenous DNA strands. Responsiveness of these materials to different stimuli may be combined for the benefits of versatility and wider range of applications and control.

5.3 Force generation

It is possible to induce volume change of DNA based macromaterials as a way of generating forces in all three categories of DNA gel system (i.e., DNA-only, DNA as backbone, and DNA as crosslinker) (e.g., (Amiya & Tanaka 1987, Horkay & Basser 2004, Jiang et al. 2010c, Um et al. 2006b)) if the materials are implanted at injury site (e.g., spinal cord injury). This has been implicated to be useful in a myriad of applications including 'towed' (stretched) axonal regeneration (Bray 1984) in neural tissue engineering.

5.4 Dynamic porous scaffold

The porosity of the DNA-only gel system may be adjusted with DNA content and design for specific applications such as drug delivery or tissue engineering. For the DNA crosslinked macromaterials, the porosity and pore structure can be altered with the choice of crosslinking density, monomer concentration and monomer nature. For example, in constructing Acrydite-DNA crosslinked polymers (Jiang et al. 2008b, Lin et al. 2004b), the reactive end of the Acrydite-modified oligonucleotides contains vinyl group, thus besides polyacrylamide, poly-hydroxyethyl methacrylate (pHEMA), poly-hydroxy-propyl-methacrylamide(pHPMA), polymethyl methacrylate (pMMA), and copolymers (e.g. pHEMA-co-MMA and pHEMA-co-AEMA) are also candidates for DNA crosslinking (**Table 3**). These polymers are among the most studied non-biodegradable polymers for tissue engineering applications, including spinal cord injury research (Duconseille et al. 1998, Flynn et al. 2003, Lesny et al. 2002, Novikova et al. 2003) owing in part to their inhere biocompatibility (Ratner & Bryant 2004) and suitable pore size and porosity They have been engineered to carry neuro-trophic factors and present communicating porous structures (e.g., (Bakshi et al. 2004)), and to facilitate necrosis reduction, vasculature formation and axonal outgrowth across the graft-tissue interface (Dalton et al. 2002, Lesny et al. 2002, Yu & Shoichet 2005).

5.5 Controlled delivery

Previous work indicates that biomaterials based scaffold can provide enhanced gene delivery efficiency (De Laporte & Shea 2007). In the DNA crosslinked gel network,

possibilities exist that by designing DNA sequence specific for an enzymatic action, the gel work can facilitate controlled release of the therapeutic reagent that is trapped in the gels (**Figure 9**). Venkatesh et al. illustrated that such enzymatic mechanism can be used for the delivery of DNA, though the release is not based on the change in the macro-material, but rather the by-product of restriction enzyme action (Venkatesh et al. 2009). Pore size and porosity of the gel network ought to design to facilitate such aim (Liedl et al. 2007).

5.6 DNA base-pairing

Although DNA's capability of binding complementary strands with high affinity is remarkable, it is not with limitations, as manifested in the errors in base-pairing and the hybridization kinetics (Condon 2006). While Nature has come up with elegant and complex machinery for error checking and correction in organisms, it remains a challenge in the synthetic DNA and it is much desirable to have such capability as well in the synthesis of DNA based active materials (Aldaye et al. 2008).

5.7 DNA modifications

It is promising to use DNA based materials as carrier for various protein-based therapeutics. For instance, biotin-labeled DNA (Kuzuya et al. 2009) can be incorporated in the gel network to attach streptavidin offering a means for protein separation, purification and potentially delivery. In this design, the 5′ end of the strands of DNA is biotinylated with a biotin-triethyleneglycol (TEG) residual. The effect of the environmental conditions and other factors on the biotin-streptavidin interactions could potentially be implemented as releasing mechanism. Realization of these promises hinges on the deep understanding of the DNA structure and properties and the interactions between DNA and others entities.

6. Concluding remark

Using DNA as a structural component has extended its functionality and significance from its critical biological roles, and has yielded DNA based macromaterials with DNA only, using DNA as backbone, and crosslinked by DNA. These DNA based macromaterials have benefited a great deal from the unique properties possessed by this molecule, and gained added functionalities and features such as thermal reversibility, sol-gel transition dependent on crosslinking density, and tunable mechanical stiffness. Currently, the application of these materials to the areas of bio-sensor/actuator, bioelectronics, drug delivery, and bioscaffold and tissue engineering is under investigation. There are a number of design considerations and parameters that are important to the success of applying these materials, which include stability, DNA sequence design, interactions between DNA and other molecules, and application of the stimuli in the materials. A wide range of applications await further development of these materials, particularly in the area of biology and medicine.

7. Acknowledgements

We want to express our appreciation to our collaborators in the previous projects, and apologize to those investigators whose work we were not able to cite appropriately due to space limitations.

	Gel design		Properties/Features of the structures	Potential application	Ref
	Backbone	**Crosslinker**			
DNA only	DNA, branched structure (X, Y, T-shaped DNA)	DNA (Structure formation based on ligase-mediated reactions)	Tunable stiffness based on degree of branching Controllable swelling Biodegradable	Cell encapsulation Drug delivery Cell-free protein production	(Park et al 2009, Um et al 2006b)
	Y shaped DNA strands	Interlocking motif based on protonation of C-rich domain	pH dependent stability; Fast responses to stimuli Sol-gel transition based upon DNA concentration	pH-sensitive drug-delivery system Tissue Engineering	(Cheng et al 2009)
	Purified sodium DNA from calf thymus (~ 13,000 bp)	Physical entanglement between DNA strands	Entanglement occurs when reaching critical overlap concentration; Viscoelastic moduli dependence on DNA concentration	Understanding of biological processes such as mitosis	(Mason et al 1998)
	Salmon DNA (20,000 bp)	Random physical entanglement between DNA strands	Stability for at least 3 months Resistance to DNase digestion	Drug delivery Tissue Engineering	(Lee et al 2008)
DNA as backbone	Salmon DNA (2000 bp) 9.3 % (w/w) in 4.0 mM sodium bromide solution at pH) 10.	ethylene glycol diglycidyl ether (EGDE). (epoxide groups on both ends react with amino group of base in nucleic acid)	At high cross-linker contents, no significant changes in the dynamic moduli were observed Physical gels exhibiting an elastic modulus in the order of MPa Strain hardening	Drug delivery Biosensor	(Orakdogen et al 2010, Topuz & Okay 2008, Topuz & Okay 2009)
	Salmon DNA (2000 bp) 3 % (w/w)	EGDE	At low CaCl2 concentration, the gel volume gradually decreases as the CaCl2 concentration increases Exchange between mono- and di-valent cations causes volume change	Understanding of DNA-ion interactions under physiological conditions	(Horkay & Basser 2004)
	Salmon DNA	electrostatic forces between DNA and poly(phenylenevinylene) (PPV) (Physical gel)	Swelling up to a hundred times, and Swelling degree decreases with increasing feed molar ratios of monomer to DNA and reaches a plateau Gel remains stable to heat, ultrasound, or DNase digestion	Drug delivery; Monitoring drug release	(Tang et al 2009)
	Salmon DNA: 2000 bp 9 % (w/w)	EGDE (at different crosslinking density)	Shear-thinning behavior	Drug delivery; To study DNA–co-solute interactions;	(Costa et al 2006, Costa et al 2007, Costa et al 2006., Costa et al 2010)
	Salmon DNA and cationic hydro-xyethyl celluloses based polymers	electrostatic forces between DNA and cations	Stochiometry, rheology shows a non-monotonic behavior with respect to charge ratio	Drug delivery	(Costa et al 2006, Costa et al 2006., Costa et al 2010)
	Salmon DNA: 1000 bp	electrostatic forces between DNA and cationic surfactant or protein (e.g., lysozyme)	Different mechanisms for the interactions between ssDNA or dsDNA and cationic surfactant or protein Sustained DNA release	Gene delivery	(Moran et al 2007)

Table 1. Continued

	Gel design		Properties/Features of the structures	Potential application	Ref
	Backbone	**Crosslinker**			
DNA as crosslinker	Cellulose nanocrystals (CNXLs)	20 nt and 78 nt DNA	DNA grafting for ordered assembly of CNXLs Biocompatibility	Scaffold design for tissue engineering	(Mangalam et al 2009)
	polyacrylamide	Thrombin associated ssDNA aptamer	Capture and release of thrombin based on its interaction with aptamer	Drug delivery vehicle Protein detection biosensor	(Wei et al 2008)
	polyacrylamide	Bis and complementary DNA side chains	Optical property change due to alterations in DNA crosslinking	Biosensor	(Tierney & Stokke 2009)
	polyacrylamide	The third DNA strand complementary to DNA side chain	Controlled drug release Quantum dots being trapped even when the average pore size is larger.	Drug delivery of nanoscale agents	(Liedl et al 2007)
	Polyacrylamide	Aptamer	Adenosine induced gel dissociation Fast response	Drug delivery	(Yang et al 2008)
	Polyacrylamide	Aptamer	Color change upon aptamer-target interactions	Visual detection platform Drug delivery	(Zhu et al 2010)
	polyacrylamide	The third DNA strand complementary to DNA side chain	Temperature dependent gelation point; Tunable mechanical stiffness dependent on crosslinking density Reversible gelation via delivery of 'removal' DNA	Drug delivery Scaffold design for tissue engineering Substrate for study of cell-ECM interactions	(Jiang et al 2008b, Jiang et al 2010b, Jiang et al 2010c, Lin et al 2006, Lin et al 2004b)

*Note: bp: base-pair for double-stranded DNA; nt: nucleotide for single-stranded DNA

Table 1. Summary of DNA based hydrogel materials.

Gel structure	Stimuli	Reversible?	Characterization	Potential Application	Ref
Y shaped building block and interlocking forces	pH	Y	Rheological properties AuNP release DNA structures using PAGE Gel structures using SEM	Drug delivery Bio-sensing	(Cheng et al 2009)
DNA strands and SP-PPV, and electrostatic force between the two	pH	TBD	Swelling degree Resistance to DNase	Drug delivery Monitoring drug release	(Tang et al 2009)
EGDE crosslinked long DNA strand	Temperature	N	Dynamic rheological measurements	Drug delivery Biosensor	(Topuz & Okay 2008)
DNA gel formation based on branched DNA nano-structure	nuclease	TBD (e.g., with nuclease)	AFM examination of pore structure DNA gel mass	Cell encapsulation and delivery	(Um et al 2006b)
DNA (20 bp) crosslinked polyacrylamide	ssDNA	Y	Indirect measurement Diffusion (Quantum dots tracing)	Drug delivery for nanoscale agents	(Liedl et al 2007)
DNA (10 and 20 bp) crosslinked polyacrylamide	ssDNA	Y	Gel mechanical properties (stiffness/ viscosity) Concentration of residual DNA outside gel network	Tissue engineering substrate and scaffold stem cell expansion platform Delivery vehicle	(Jiang et al 2010c, Lin et al 2006)
Thrombin associated aptamer as crosslinker	ssDNA	Y	DNA gel electrophoresis Viscosity	Drug delivery Controlled release	(Wei et al 2008)
DNA (10 and 20 bp) crosslinked polyacrylamide	Adenosine	Y	Absorbance of gold nanoparticles in gels	Drug delivery (target specific)	(Yang et al 2008)
DNA crosslinked polyacrylamide	Target interacting with aptamer	Y	Gel color change	Visual detection Drug delivery	(Zhu et al 2010)

Note: AuNP: Gold nano-particle; ssDNA: single-stranded DNA. PAGE: polyacrylamide gel electrophoresis. SEM: scanning electron microscopy. TBD: To be determined

Table 2. Summary of DNA base materials with dynamic and responsive properties.

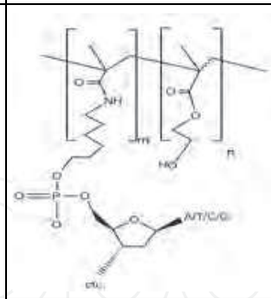
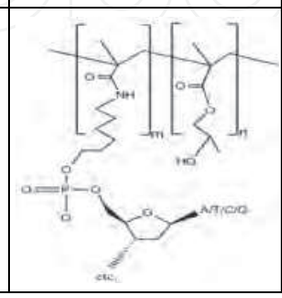
DNA X-linked polymer	Gel preparation	Chemical structure	Notes
pHEMA, poly-(hydroxyethyl methacrylate) (Bakshi et al. 2004, Carone & Hasenwinkel 2006, Flynn et al. 2003)	Crosslink: L2 (replacing EGDMA) HEMA (Aldrich, St. Louis, MO) (20%-60%) Initiator-catalyst (APS-TEMED)		Pore size: 10 μm to 20 μm
pHPMA, poly-(hydroxypropyl-methacrylamide) (Duconseille et al. 1998, St'astny et al. 2002)	Crosslink: L2 (replacing DMHA) HPMA monomer Initiator (AIBN)		Pore size: 10 μm to 50 μm

Table 3. DNA crosslinked vinyl polymers such as pHEMA and pHPMA.

8. Abbreviation

dsDNA: double-stranded DNA; ssDNA: single-stranded DNA; ECM: extracellular matrix; bp: basepair; nt: nucleotide.

9. Reference

Aldaye FA, Palmer AL, Sleiman HF. 2008. Assembling materials with DNA as the guide. *Science* 321: 1795-9

Alemdaroglu FE, Alemdaroglu NC, Langguth P, Herrmann A. 2008. DNA Block Copolymer Micelles - A Combinatorial Tool for Cancer Nanotechnology. *Advanced Materials* 20: 899-902

Alemdaroglu FE, Herrmann A. 2007. DNA meets synthetic polymers--highly versatile hybrid materials. *Org Biomol Chem* 5: 1311-20

Amiya T, Tanaka T. 1987. Phase Transitions in Cross-Linked Gels of Natural Polymers. *Macromolecules* 20: 1162-64

Bakshi A, Fisher O, Dagci T, Himes BT, Fischer I, Lowman A. 2004. Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury. *J Neurosurg Spine* 1: 322-9

Ball P. 2005. Synthetic biology for nanotechnology. *Nanotechnology* 16: R1-R8

Bart Haegeman DV, Jean-Jacques Godon and Jérôme Hamelin. 2008. DNA reassociation kinetics and diversity indices: richness is not rich enough. *Oikos*

Berashevich J, Chakraborty T. 2008. How the surrounding water changes the electronic and magnetic properties of DNA. *J Phys Chem B* 112: 14083-9

Biggins JB, Prudent JR, Marshall DJ, Thorson JS. 2006. A continuous assay for DNA cleavage using molecular break lights. *Methods Mol Biol* 335: 83-92

Blagbrough IS, Zara C. 2009. Animal models for target diseases in gene therapy—Using DNA and siRNA delivery strategies. *Pharmaceutical research* 26: 1-18

Braun E, Keren K. 2004. From DNA to transistors. *Advances in Physics* 53: 441 — 96

Bray D. 1984. Axonal growth in response to experimentally applied mechanical tension. *Dev Biol* 102: 379-89

Budowle B, Allard MW, Wilson MR, Chakraborty R. 2003. Forensics and mitochondrial DNA: applications, debates, and foundations. *Annu Rev Genomics Hum Genet* 4: 119-41

Bustamante C, Bryant Z, Smith SB. 2003. Ten years of tension: single-molecule DNA mechanics. *Nature* 421: 423-7

Cao S, Cripps A, Wei MQ. 2010. New strategies for cancer gene therapy: Progress and opportunities. *Clinical and Experimental Pharmacology and Physiology* 37: 108-14

Carlson R. 2009. The changing economics of DNA synthesis. *Nat Biotechnol* 27: 1091-4

Carone TW, Hasenwinkel JM. 2006. Mechanical and morphological characterization of homogeneous and bilayered poly(2-hydroxyethyl methacrylate) scaffolds for use in CNS nerve regeneration. *J Biomed Mater Res B Appl Biomater*

Chaires J, Waring M. 2001. *Drug-nucleic acid interactions*. Academic press.

Chan G, Mooney DJ. 2008. New materials for tissue engineering: towards greater control over the biological response. *Trends in biotechnology* 26: 382-92

Chen CS, Jiang X, Whitesides GM. 2005. Microengineering the Environment of Mammalian Cells in Culture. *MRS Bulletin* 30: 194-201

Chen JH, Seeman NC. 1991. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature* 350: 631-3

Cheng E, Xing Y, Chen P, Yang Y, Sun Y, et al. 2009. A pH-triggered, fast-responding DNA hydrogel. *Angew Chem Int Ed Engl* 48: 7660-3

Chevalier E, Chulia D, Pouget C, Viana M. 2008. Fabrication of porous substrates: a review of processes using pore forming agents in the biomaterial field. *J Pharm Sci* 97: 1135-54

Chhabra R, Sharma J, Liu Y, Rinker S, Yan H. 2010. DNA Self-assembly for Nanomedicine. *Advanced Drug Delivery Reviews* 62: 617-25

Chippada U, Langrana N, Yurke B. 2009a. Complete mechanical characterization of soft media using nonspherical rods. *J Appl Phys* 106: 63528

Chippada U, Yurke B, Georges PC, Langrana NA. 2009b. A nonintrusive method of measuring the local mechanical properties of soft hydrogels using magnetic microneedles. *J Biomech Eng* 131: 021014

Condon A. 2006. Designed DNA molecules: principles and applications of molecular nanotechnology. *Nat Rev Genet* 7: 565-75

Costa D, Hansson P, Schneider S, GraÃ§a Miguel M, Lindman Br. 2006. Interaction between Covalent DNA Gels and a Cationic Surfactant. *Biomacromolecules* 7: 1090-95

Costa D, Miguel MG, Lindman B. 2007. Responsive polymer gels: double-stranded versus single-stranded DNA. *J Phys Chem B* 111: 10886-96

Costa D, Santos Sd, Antunes FE, Miguel MG, Lindman B. 2006. Some novel aspects of DNA physical and chemical gels. *ARKIVOC* 4: 161-72

Costa D, Valente AJM, Pais AACC, Miguel MG, Lindman B. 2010. Cross-linked DNA gels: Disruption and release properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 354: 28-33

Dalton PD, Flynn L, Shoichet MS. 2002. Manufacture of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel tubes for use as nerve guidance channels. *Biomaterials* 23: 3843-51

De Laporte L, Shea LD. 2007. Matrices and scaffolds for DNA delivery in tissue engineering. *Adv Drug Deliv Rev* 59: 292-307

Deaton R, Garzon M, Murphy RC, Rose JA, Franceschetti DR, Stevens SE. 1998. Reliability and Efficiency of a DNA-Based Computation. *Physical Review Letters* 80: 417

Dhillon SS, Rake AV, Miksche JP. 1980. Reassociation Kinetics and Cytophotometric Characterization of Peanut (Arachis hypogaea L.) DNA. *Plant Physiol* 65: 1121-27

Di Pietro SM, Centeno JM, Cerutti ML, Lodeiro MF, Ferreiro DU, et al. 2003. Specific antibody-DNA interaction: a novel strategy for tight DNA recognition. *Biochemistry* 42: 6218-27

Ding K, Alemdaroglu FE, Borsch M, Berger R, Herrmann A. 2007. Engineering the structural properties of DNA block copolymer micelles by molecular recognition. *Angew Chem Int Ed Engl* 46: 1172-5

Dong Liu X, Yamada M, Matsunaga M, Nishi N. 2007. Functional Materials Derived from DNA. *Functional Materials and Biomaterials*: 149-78

Douglas SM, Dietz H, Liedl T, Hogberg B, Graf F, Shih WM. 2009. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature* 459: 414-8

Duconseille E, Woerly S, Kelche C, Will B, Cassel JC. 1998. Polymeric hydrogels placed into a fimbria-fornix lesion cavity promote fiber (re)growth: a morphological study in the rat. *Restor Neurol Neurosci* 13: 193-203

Engler AJ, Sen S, Sweeney HL, Discher DE. 2006. Matrix elasticity directs stem cell lineage specification. *Cell* 126: 677-89

Fahlman RP, Hsing M, Sporer-Tuhten CS, Sen D. 2003. Duplex Pinching: A Structural Switch Suitable for Contractile DNA Nanoconstructions. *Nano Letters* 3: 1073-78

Felsenfeld G, Miles HT. 2003. The Physical and Chemical Properties of Nucleic Acids. *Annual Review of Biochemistry* 36: 407-48

Flynn L, Dalton PD, Shoichet MS. 2003. Fiber templating of poly(2-hydroxyethyl methacrylate) for neural tissue engineering. *Biomaterials* 24: 4265-72

Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, et al. 2007. Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am J Physiol Gastrointest Liver Physiol* 293: G1147-54

Gerweck LE, Seetharaman K. 1996. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer Res* 56: 1194-8

Ghosh K, Ingber DE. 2007. Micromechanical control of cell and tissue development: implications for tissue engineering. *Advanced Drug Delivery Reviews* 59: 1306-18

Goritz M, Kramer R. 2005. Allosteric control of oligonucleotide hybridization by metal-induced cyclization. *J Am Chem Soc* 127: 18016-7

Greenberg MM. 2005. DNA interstrand cross-links from modified nucleotides: mechanism and application. *Nucleic Acids Symp Ser (Oxf)*: 57-8

Hildebrandt-Eriksen ES, Christensen T, Diemer NH. 2002. Mild focal cerebral ischemia in the rat. The effect of local temperature on infarct size. *Neurol Res* 24: 781-8

Horkay F, Basser PJ. 2004. Osmotic observations on chemically cross-linked DNA gels in physiological salt solutions. *Biomacromolecules* 5: 232-7

Ingber DE. 2002. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. *Circ Res* 91: 877-87

Ingber DE. 2006. Cellular mechanotransduction: putting all the pieces together again. *The FASEB journal* 20: 811

Jain A, Alvi NK, Parish JH, Hadi SM. 1996. Oxygen is not required for degradation of DNA by glutathione and Cu(II). *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 357: 83-88

Janmey PA, Winer JP, Murray ME, Wen Q. 2009. The hard life of soft cells. *Cell Motil Cytoskeleton* 66: 597-605

Jiang FX, Yurke B, Firestein BL, Langrana NA. 2008a. Neurite outgrowth on a DNA crosslinked hydrogel with tunable stiffnesses. *Annals of Biomedical Engineering* 36: 1565-79

Jiang FX, Yurke B, Firestein BL, Langrana NA. 2008b. Neurite outgrowth on a DNA crosslinked hydrogel with tunable stiffnesses. *Ann Biomed Eng* 36: 1565-79

Jiang FX, Yurke B, Schloss RS, Firestein BL, Langrana NA. 2010a. Effect of Dynamic Stiffness of the Substrates on Neurite Outgrowth by Using a DNA-Crosslinked Hydrogel. *Tissue Engineering Part A* 16: 1873-89

Jiang FX, Yurke B, Schloss RS, Firestein BL, Langrana NA. 2010b. Effect of dynamic stiffness of the substrates on neurite outgrowth by using a DNA-crosslinked hydrogel. *Tissue Eng Part A* (In press)

Jiang FX, Yurke B, Schloss RS, Firestein BL, Langrana NA. 2010c. The relationship between fibroblast growth and the dynamic stiffnesses of a DNA crosslinked hydrogel. *Biomaterials* 31: 1199-212

Kang H, Liu H, Zhang X, Yan J, Zhu Z, et al. 2011. Photoresponsive DNA-Cross-Linked Hydrogels for Controllable Release and Cancer Therapy. *Langmuir*

Knoblauch M, Peters W. 2004. Biomimetic actuators: where technology and cell biology merge. *Cellular and molecular life sciences* 61: 2497-509

Kuzuya A, Kimura M, Numajiri K, Koshi N, Ohnishi T, et al. 2009. Precisely programmed and robust 2D streptavidin nanoarrays by using periodical nanometer-scale wells embedded in DNA origami assembly. *Chembiochem* 10: 1811-5

LaBean TH, Li H. 2007. Constructing novel materials with DNA. *Nano Today* 2: 26-35

Lahann J, Langer R. 2005. Smart Materials with Dynamically Controllable Surfaces. *MRS Bulletin* 30: 185-88

Lee CK, Shin SR, Lee SH, Jeon JH, So I, et al. 2008. DNA hydrogel fiber with self-entanglement prepared by using an ionic liquid. *Angew Chem Int Ed Engl* 47: 2470-4

Lesny P, De Croos J, Pradny M, Vacik J, Michalek J, et al. 2002. Polymer hydrogels usable for nervous tissue repair. *J Chem Neuroanat* 23: 243-7

Letchworth GJ, Carmichael LE. 1984. Local tissue temperature: a critical factor in the pathogenesis of bovid herpesvirus 2. *Infect Immun* 43: 1072-9

Li R, McCoy BJ, Diemer RB. 2005. Cluster aggregation and fragmentation kinetics model for gelation. *J Colloid Interface Sci* 291: 375-87

Li S, De Wijn JR, Li J, Layrolle P, De Groot K. 2003. Macroporous biphasic calcium phosphate scaffold with high permeability/porosity ratio. *Tissue Eng* 9: 535-48

Li Y, White J, Stokes D, Sayler G, Sepaniak M. 2001. Capillary electrophoresis as a method to study DNA reassociation. *Biotechnol Prog* 17: 348-54

Liedl T, Dietz H, Yurke B, Simmel F. 2007. Controlled trapping and release of quantum dots in a DNA-switchable hydrogel. *Small* 3: 1688-93

Lin CC, Metters AT. 2006. Hydrogels in controlled release formulations: network design and mathematical modeling. *Adv Drug Deliv Rev* 58: 1379-408

Lin D, Langrana N, Yurke B. 2005. Inducing reversible stiffness changes in DNA-crosslinked gels. *Journal of Materials Research* 20: 1456-64

Lin DC. 2005. *Design and properties of a new dna-crosslinked polymer hydrogel*. Rutgers University, Piscataway, NJ

Lin DC, Yurke B, Langrana NA. 2004a. Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel. *Journal of Biomechanical Engineering* 126: 104

Lin DC, Yurke B, Langrana NA. 2004b. Mechanical properties of a reversible, DNA-crosslinked polyacrylamide hydrogel. *J Biomech Eng* 126: 104-10

Liu D, Wang M, Deng Z, Walulu R, Mao C. 2004. Tensegrity: construction of rigid DNA triangles with flexible four-arm DNA junctions. *J Am Chem Soc* 126: 2324-5

Liu XD, Yamada M, Matsunaga M, Nishi N. 2007. Functional Materials Derived from DNA In *Functional Materials and Biomaterials*, pp. 149-78

Lu Y, Liu J. 2006. Functional DNA nanotechnology: emerging applications of DNAzymes and aptamers. *Curr Opin Biotechnol* 17: 580-8

Lu Y, Liu J. 2007. Smart nanomaterials inspired by biology: dynamic assembly of error-free nanomaterials in response to multiple chemical and biological stimuli. *Acc Chem Res* 40: 315-23

Luo D. 2003. The road from biology to materials. *Materials Today* 6: 38-43

Mangalam AP, Simonsen J, Benight AS. 2009. Cellulose/DNA Hybrid Nanomaterials. *Biomacromolecules*

Mao C, Sun W, Seeman NC. 1997. Assembly of Borromean rings from DNA. *Nature* 386: 137-8

Marquez JP, Genin GM, Pryse KM, Elson EL. 2006. Cellular and matrix contributions to tissue construct stiffness increase with cellular concentration. *Ann Biomed Eng* 34: 1475-82

Mason TG, Dhople A, Wirtz D. 1998. Linear Viscoelastic Moduli of Concentrated DNA Solutions. *Macromolecules* 31: 3600-03

Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ. 1996. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* 382: 607-9

Moran MC, Miguel MG, Lindman B. 2007. DNA gel particles: particle preparation and release characteristics. *Langmuir* 23: 6478-81

Mrksich M. 2005. Dynamic Substrates for Cell Biology. *MRS Bulletin* 30: 180-84

Murakami Y, Maeda M. 2005. DNA-responsive hydrogels that can shrink or swell. *Biomacromolecules* 6: 2927-9

Nagahara S, Matsuda T. 1996. Hydrogel formation via hybridization of oligonucleotides derivatized in water-soluble vinyl polymers. *Polym Gels Networks* 4: 111-27

Neher RA, Gerland U. 2005. DNA as a programmable viscoelastic nanoelement. *Biophys J* 89: 3846-55

Nemir S, West JL. 2009. Synthetic Materials in the Study of Cell Response to Substrate Rigidity. *Ann Biomed Eng*

Niemeyer CM. 2000. Self-assembled nanostructures based on DNA: towards the development of nanobiotechnology. *Curr Opin Chem Biol* 4: 609-18

Novikova LN, Novikov LN, Kellerth JO. 2003. Biopolymers and biodegradable smart implants for tissue regeneration after spinal cord injury. *Curr Opin Neurol* 16: 711-5

Ogura Y, Nishimura T, Tanida J. 2009. Self-Contained Photonically-Controlled DNA Tweezers. *Applied Physics Express* 2: 025004

Orakdogen N, Erman B, Okay O. 2010. Evidence of Strain Hardening in DNA Gels. *Macromolecules* 43: 1530–38

Park N, Um SH, Funabashi H, Xu J, Luo D. 2009. A cell-free protein-producing gel. *Nat Mater* 8: 432-7

Patil SD, Rhodes DG, Burgess DJ. 2005. DNA-based therapeutics and DNA delivery systems: a comprehensive review. *The AAPS Journal* 7: 61-77

Previtera ML, Trout K, Chippada U, Schloss R, Langrana NA. 2011. *Fibroblast behavior on tunable gels with decreasing elasticity.* Presented at ASME 2011 Summer Bioengineering Conference.

Ratner BD, Bryant SJ. 2004. BIOMATERIALS: Where We Have Been and Where We are Going. *Annual Review of Biomedical Engineering* 6: 41-75

Reynaldo LP, Vologodskii AV, Neri BP, Lyamichev VI. 2000. The kinetics of oligonucleotide replacements. *J Mol Biol* 297: 511-20

Ritter T. 2009. Gene therapy in transplantation: Toward clinical trials. *Current Opinion in Molecular Therapeutics* 11: 504-12

Roberts MC, Hanson MC, Massey AP, Karren EA, Kiser PF. 2007. Dynamically Restructuring Hydrogel Networks Formed with Reversible Covalent Crosslinks. *Advanced Materials* 19: 2503-07

Rothemund PW. 2006. Folding DNA to create nanoscale shapes and patterns. *Nature* 440: 297-302

Saeks J. 2007. DNA Sequencing Equipment and Services Markets, Kalorama Information, New York, NY

SantaLucia J, Jr., Hicks D. 2004. The thermodynamics of DNA structural motifs. *Annu Rev Biophys Biomol Struct* 33: 415-40

Schneider HJ, Strongin RM. 2009. Supramolecular interactions in chemomechanical polymers. *Acc Chem Res* 42: 1489-500

Seeman NC. 1981. *Nucleic Acid Junctions: Building Blocks for Genetic Engineering in Three Dimensions*. pp. 269-277. New York: Adenine Press.

Seeman NC. 1982. Nucleic acid junctions and lattices. *J Theor Biol* 99: 237-47

Seeman NC. 2007. An overview of structural DNA nanotechnology. *Mol Biotechnol* 37: 246-57

Silver FH, DeVore D, Siperko LM. 2003. Invited Review: Role of mechanophysiology in aging of ECM: effects of changes in mechanochemical transduction. *J Appl Physiol* 95: 2134-41

Simmel FC, Yurke B. 2001. Using DNA to construct and power a nanoactuator. *Physical Review E* 63: 041913

Smith MJ, Britten RJ, Davidson EH. 1975. Studies on nucleic acid reassociation kinetics: reactivity of single-stranded tails in DNA-DNA renaturation. *Proc Natl Acad Sci U S A* 72: 4805-9

Smith SB, Cui Y, Bustamante C. 1996. Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science* 271: 795-9

Soontornworajit B, Zhou J, Shaw MT, Fan TH, Wang Y. 2010. Hydrogel functionalization with DNA aptamers for sustained PDGF-BB release. *Chem Commun (Camb)* 46: 1857-9

St'astny M, Plocova D, Etrych T, Kovar M, Ulbrich K, Rihova B. 2002. HPMA-hydrogels containing cytostatic drugs. Kinetics of the drug release and in vivo efficacy. *J Control Release* 81: 101-11

Storhoff JJ, Mirkin CA. 1999. Programmed Materials Synthesis with DNA. *Chemical Reviews* 99: 1849-62

Sun M, Pejanovic S, Mijovic J. 2005. Dynamics of Deoxyribonucleic Acid Solutions As Studied by Dielectric Relaxation Spectroscopy and Dynamic Mechanical Spectroscopy. *Macromolecules* 38: 9854-64

Tang H, Duan X, Feng X, Liu L, Wang S, et al. 2009. Fluorescent DNA-poly(phenylenevinylene) hybrid hydrogels for monitoring drug release. *Chem Commun (Camb)*: 641-3

Tierney S, Stokke BT. 2009. Development of an oligonucleotide functionalized hydrogel integrated on a high resolution interferometric readout platform as a label-free macromolecule sensing device. *Biomacromolecules* 10: 1619-26

Topuz F, Okay O. 2008. Rheological Behavior of Responsive DNA Hydrogels. *Macromolecules* 41: 8847-54

Topuz F, Okay O. 2009. Formation of Hydrogels by Simultaneous Denaturation and Cross-Linking of DNA. *Biomacromolecules* 10: 2652-61

Uibo R, Laidmae I, Sawyer ES, Flanagan LA, Georges PC, et al. 2009. Soft materials to treat central nervous system injuries: evaluation of the suitability of non-mammalian fibrin gels. *Biochim Biophys Acta* 1793: 924-30

Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D. 2006a. Enzyme-catalysed assembly of DNA hydrogel. *Nature Materials* 5: 797-801

Um SH, Lee JB, Park N, Kwon SY, Umbach CC, Luo D. 2006b. Enzyme-catalysed assembly of DNA hydrogel. *Nat Mater* 5: 797-801

Venkatesh S, Wower J, Byrne ME. 2009. Nucleic acid therapeutic carriers with on-demand triggered release. *Bioconjug Chem* 20: 1773-82

Vologodskii AV, Cozzarelli NR. 1994. Conformational and thermodynamic properties of supercoiled DNA. *Annu Rev Biophys Biomol Struct* 23: 609-43

Wang K, Tang Z, Yang CJ, Kim Y, Fang X, et al. 2009. Molecular engineering of DNA: molecular beacons. *Angew Chem Int Ed Engl* 48: 856-70

Wang YL, Pelham RJ, Jr. 1998. Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells. *Methods Enzymol* 298: 489-96

Watson JD, Baker TA, Bell SP, Gann A, Levine MA, Losick R. 2003. *Molecular Biology of the Gene*. Benjamin Cummings.

Wei B, Cheng I, Luo KQ, Mi Y. 2008. Capture and release of protein by a reversible DNA-induced sol-gel transition system. *Angew Chem Int Ed Engl* 47: 331-3

Wiegel FW, Geurts BJ, Goldstein B. 1987. Crosslinking and gelation between linear polymers: DNA-antibody complexes in systemic lupus erythematosus. *Journal of Physics A: Mathematical and General* 20: 5205-18

Xie J, Fan R, Meng Z. 2007. Protein oxidation and DNA-protein crosslink induced by sulfur dioxide in lungs, livers, and hearts from mice. *Inhal Toxicol* 19: 759-65

Xing Y, Cheng E, Yang Y, Chen P, Zhang T, et al. 2011. DNA HYDROGELS: Self-Assembled DNA Hydrogels with Designable Thermal and Enzymatic Responsiveness. *Advanced Materials* 23: 1116-16

Yang H, Liu H, Kang H, Tan W. 2008. Engineering target-responsive hydrogels based on aptamer-target interactions. *J Am Chem Soc* 130: 6320-1

Yu TT, Shoichet MS. 2005. Guided cell adhesion and outgrowth in peptide-modified channels for neural tissue engineering. *Biomaterials* 26: 1507-14

Yurke B, Mills A. 2003. Using DNA to Power Nanostructures. *Genetic Programming and Evolvable Machines* 4: 111-22

Yurke B, Turberfield AJ, Mills AP, Jr., Simmel FC, Neumann JL. 2000. A DNA-fuelled molecular machine made of DNA. *Nature* 406: 605-8

Zhang Y, Gu H, Yang Z, Xu B. 2003. Supramolecular hydrogels respond to ligand-receptor interaction. *Journal of the American Chemical Society* 125: 13680-81

Zhang Y, Seeman NC. 1994. Construction of a DNA-truncated octahedron. *J Am Chem Soc,* 116: 1661-69

Zhu Z, Wu C, Liu H, Zou Y, Zhang X, et al. 2010. An aptamer cross-linked hydrogel as a colorimetric platform for visual detection. *Angew Chem Int Ed Engl* 49: 1052-6



Biomaterials Science and Engineering

Edited by Prof. Rosario Pignatello

ISBN 978-953-307-609-6
Hard cover, 456 pages
Publisher InTech
Published online 15, September, 2011
Published in print edition September, 2011

These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentials of different synthetic and engineered biomaterials. Contributions were not selected based on a direct market or clinical interest, than on results coming from very fundamental studies which have been mainly gathered for this book. This fact will also allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessarily imposed by industrial or profit concerns. The book collects 22 chapters related to recent researches on new materials, particularly dealing with their potential and different applications in biomedicine and clinics: from tissue engineering to polymeric scaffolds, from bone mimetic products to prostheses, up to strategies to manage their interaction with living cells.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Frank Xue Jiang, Bernard Yurke, Devendra Verma, Michelle Previtera, Rene Schloss and Noshir A. Langrana (2011). Development of DNA Based Active Macro–Materials for Biology and Medicine: A Review, Biomaterials Science and Engineering, Prof. Rosario Pignatello (Ed.), ISBN: 978-953-307-609-6, InTech, Available from: http://www.intechopen.com/books/biomaterials-science-and-engineering/development-of-dna-based-active-macro-materials-for-biology-and-medicine-a-review

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen