Preparation and Characterization of Photocrosslinked DNA-PEG Hybrid Hydrogels For Biomedical Applications

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by

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ABSTRACT

DNA can be utilized as structural components of hydrogels, which are widely used biomaterials in the human body. Recently, our lab developed soft DNA hydrogels, which were entirely constructed from branched DNA structure via enzyme ligation and were capable of encapsulating live cells. Here, we have synthesized and characterized a photocrosslinked DNA-PEG hybrid hydrogel (PEG: poly ethylene glycol). The construction of these hydrogels is distinctly different from enzyme-catalyzed DNA hydrogels in that acylate-functionalized branched DNA was used as structural building units. Also these hydrogels were constructed via a rapid photocrosslinking upon short UV illumination and remote activation process. Our DNA-PEG hybrid hydrogels were characterized by employing IR, gel electrophoresis, swelling measurements, Scanning Electron Microscopy (SEM), mechanical analysis and controlled drug release experiments. Our hydrogels possess the unique advantages of increased mechanical strength and adjustable internal network structure by simply changing the initial concentration of PEG momoners. We envision that the photocrosslinked DNA-PEG hybrid hydrogels can be utilized in a variety of biomedical applications including drug delivery and tissue engineering.

BIOGRAPHICAL SKETCH

Bojeong Kim was born in Daejeon, South Korea on August 13, 1987. In 2010, she received her B.S. in Biological Engineering from Cornell University.

To my family

ACKNOWLEDGMENTS

I would like to thank my research advisor Professor Dan Luo and Dr. Young Hoon Roh. I would especially like to thank Prof Luo for his guidance in research and study throughout my bachelor's and master's study while at Cornell University. It was delightful to interact with him through classes and weekly lab meetings. I would like to thank Dr. Young Hoon Roh for his generous support and guidance throughout the project. Lastly, I give sincere thanks to all members of LuoLab.

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CHAPTER 1. INTRODUCTIONS

1.1. Introduction to Hydrogels

Hydrogels are crosslinked network that are composed of high water contents. Hydrogels are attractive biomaterials that are applied in wide range of biomedical applications due to their capability of swell water but inability to dissolve in water. Due to the fact that hydrogels exhibit high water contents, they resemble natural living tissue-like environment for cells and tissue growth [6]. Hydrogels can be divided into two categories based on their crosslinking nature. Hydrogels can be fabricated by crosslinking either physically with entanglements or chemically with junctions [3].

1.2. DNA Hydrogels

For the last two decades, DNA has been attracted considerable attention to be used as not only a genetic material but also as a generic material. Considering many appealing properties of DNA that makes a DNA a desirable candidate for nanomaterial building blocks [13], recently, a new class of biodegradable polymer network hydrogels, which is entirely constructed from branched DNA molecules, has been developed [4]. In the presence of DNA ligase, DNA hydrogels are crosslinked by covalent linkages between ends of DNA branches [6]. In the past, many studies demonstrated early successes in growing tissues in naturally forming and biodegradable hydrogels prepared from collagen, fibrin or alginate [6]. However, these hydrogels had limitations such that the gelation process, mechanical properties and degradation rates were difficult to control. Um et al. presented many attractive attributes of DNA hydrogels that can

overcome these limitations that are present in other hydrogels. DNA hydrogels are biocompatible and biodegradable. DNA hydrogels have tunable properties in which their pore sizes and shapes are tunable. Also, their gelling processes are under physiological conditions, and thus there is no need for organic solvent or high temperature. Like other hydrogels, DNA hydrogels are capable of encapsulating cells and drugs including proteins *in situ*. DNA hydrogels can even encapsulate live mammalian cells. The mechanical properties of DNA hydrogels are adjustable in fabrication process by using different DNA building blocks. DNA hydrogels can be utilized as a long-term controlled drug release system.

1.3. Polyethylene Glycol (PEG)

Polyethylene Glycol hydrogels are widely used in a variety of biomedical applications due to its unique properties. PEG is available in a variety of Molecular weights. PEG is soluble in both aqueous and many organic solvents. Moreover, PEG is nontoxic and non-immunogenic [7]. PEG does not harm active proteins or cells although they interact with cell membranes. Another favorable property of PEG is that one type of PEG (PEG 3350) is approved by FDA for drug carrier. By possessing favorable properties to be utilized in biomedical applications, PEG is used as food additives, components of pharmaceuticals, components of medical devices, tissue engineering and drug delivery.

1.4. Photocrosslinked DNA-PEG Hybrid Hydrogels

Photocrosslinked DNA-PEG hybrid hydrogels are just like recently developed enzyme-catalyzed DNA hydrogels. However, the construction of these hydrogels is distinctly different from enzyme-catalyzed DNA hydrogels in that acrylate-functionalized branched DNA was used as structural building units. Also these hydrogels are constructed via photopolymerization upon short UV illumination and remote activation process. Compared to the reaction time of enzyme – catalyzed DNA hydrogel, which takes about 2 to 4 hours, the gelling process time for photocrosslinked DNA-PEG hybrid hydrogels is shortened tremendously. By quick photocrosslinking, the gelling process time is within 10 minutes. The high reactivity and the acrylate double bond and hydrophobic interactions in the polymer network gives photocrosslinked DNA-PEG hydrogels a high mechanical strength [1]. Moreover, the material properties such as pore size and shapes are precisely tunable by simply changing the concentration of PEG monomers. Also, since these hydrogels are fabricated upon UV illumination, the activation process of fabricating these hydrogels is remotely controlled by on and off manner. This property can be very appealing in some situations where we need to treat one specific area of tissue. These hydrogels are also capable of encapsulating cells and drugs including proteins. Another advantage of these hydrogels is that there is no need for enzyme in contrast to enzyme-catalyzed hydrogels. Thus, the gelation process does not have to be carried out in physiological condition.

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CHAPTER 2. MATERIALS AND METHODS

2.1. Preparation of DNA-PEG Hydrogels

All chemicals used in this work were purchased from Sigma-Aldrich (St. Louis, Missouri) unless otherwise mentioned. 20mg of PEG monomers were dissolved in 1mL of deionized water to give a final concentration of 20 wt. % monomer.

2.1.1. Conjugation of PEGA (photoreactive groups) onto ssDNA

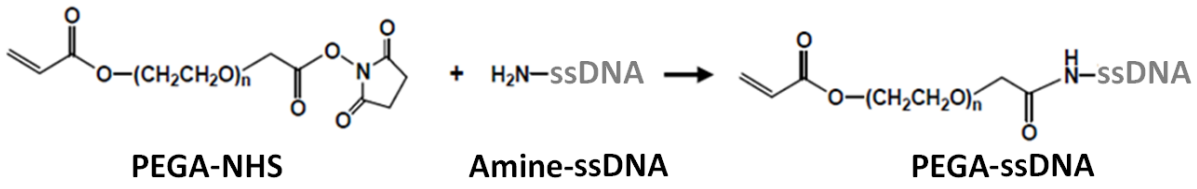

Figure 1. Conjugation of PEGA onto ssDNA

The DNA sequences were designed and synthesized based on previous work [3]. The photoreactive groups were conjugated onto DNA building blocks by adapting the previous published methods [4]. All oligonucleotides were commercially synthesized with standard desalting (Integrated DNA Technologies, Coralville, Iowa). By dissolving oligonucleotides in annealed buffer (10×10^{-3} M Tris, pH 8.0, 1×10^{-3} M EDTA, and 50×10^{-3} M NaCl), the solution with a final concentration of 0.2 mM was made. In order to conjugate PEGA succinimidyl carboxyl methyl ester (Nektar Huntsville, Alabana) and ssDNA, they were mixed in a 5:1 molar ratio, and the reaction mixture was incubated for 4 h at room temperature. Non-reacted reagents were purified by employing High Performance Liquid Chromatography (HPLC). Impurities were removed through an XBridge C18 column equipped with a photodiode array detector for UV detection at 260 nm (Waters Corp., Milford, Massachusetts). A gradient of 0-50% acetonitrile in

0.1 M triethylammonium acetate (TEAA, pH 7.0) at a flow rate of 1.0mL min^{-1} was used. Gel electrophoresis also confirms the synthesis of X-DNA-PEGA conjugates. Gel electrophoresis was conducted at a constant voltage of 90 V for 60 minutes on 3% agarose gel. Acrylate functionalized X-DNA were fabricated according to our previously published methods [1-3].

2.1.2. Preparation of DNA-PEG Hybrid Hydrogels via Photopolymerization

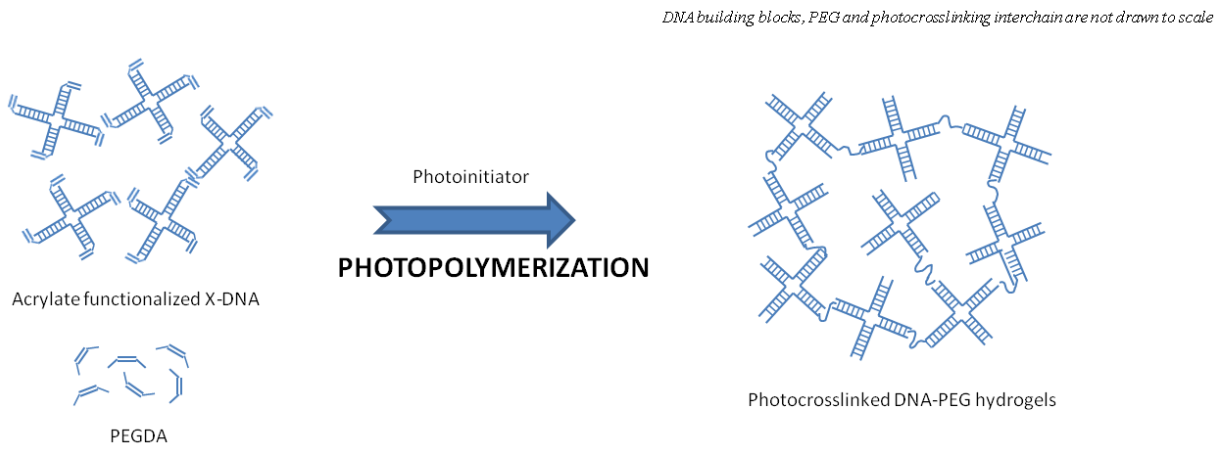

Figure 2. Schematic diagram of fabrication method of Photocrosslinked DNA-PEG hydrogels

The solution mixture of acrylate-functionalized X-DNA and PEGDA solution (10%) were put into 96-well plate, and photopolymerization was carried out for 10 min upon exposure to a UV light source (8 mW cm^{-2}) in the presence of 1-[4-(2-hydroxyethoxy)phenyl]-2-hydroxy-2-phenylpropan-1-one (Ciba Specialty Chemicals, Tarrytown, New York), a photoinitiator, using a XL-1000 UV crosslinker (Spectronic Corp., Westbury, New York).

2.2. Morphological Studies of DNA-PEG Hydrogels

The swollen hydrogel samples were quickly frozen in liquid nitrogen and then were freeze dried for 24 h. Freeze dried samples were placed on silicon wafer and then mounted on aluminum

stabs using the carbon tape. The interior morphological structure of photocrosslinked DNA-PEG hydrogels were analyzed as a function of DNA to PEG ratio using scanning electron microscopy (KECK FE-FEM LEO 1500). The porous layers were characterized with respect to pore size at an electron acceleration voltage of 2-5 keV.

2.3. Mechanical Testing

Dynamic mechanical analysis measurements of photocrosslinked DNA-PEG hydrogels were performed using a TA Instruments DMA Q800 in a compression mode at room temperature. Stress [MPa] and strain [m/m] were recorded automatically by the system. A sample with 6.35 mm in diameter and thickness ranged from 0.21 mm to 5.15 mm was used.

2.4. Swelling Studies of DNA-PEG Hydrogels

The cylindrical hydrogels were freeze dried using FreeZone 4.5 freeze-dry system (Labconco Corp., Kansas City, Missouri) for 24 h. Freeze-dried samples were weighed, and initial weight of each hydrogel was recorded. Hydrogels were swollen in 1000 µl of deionized water at room temperature. At 10 min time intervals, the samples were removed and blotted on piece of filter paper prior to weighing to remove excess surface moisture. The swelling ratio was determined according to the following expression.

$$\text{Swelling Ratio} = \frac{\text{Ws} - \text{Wd}}{\text{Wd}} \text{x } 100\%$$

2.5. Bovine Serum Albumin Release Studies

1.0 mg of BSA was dissolved in each X-DNA/PEG initiator mixture solution in the gelation solutions prior to crosslinking. After preparing gels, each gel was placed in the tube and 300 µl

of deionized water was added to each tube. The release experiment was carried out at room temperature. The protein content of each sample was analyzed with Bio-Rad (Hercules, CA) protein assay kit using the microassay procedure.

2.6. References

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CHAPTER 3. RESULTS AND DISCUSSIONS

3.1. Characterization of DNA-PEG Hydrogels

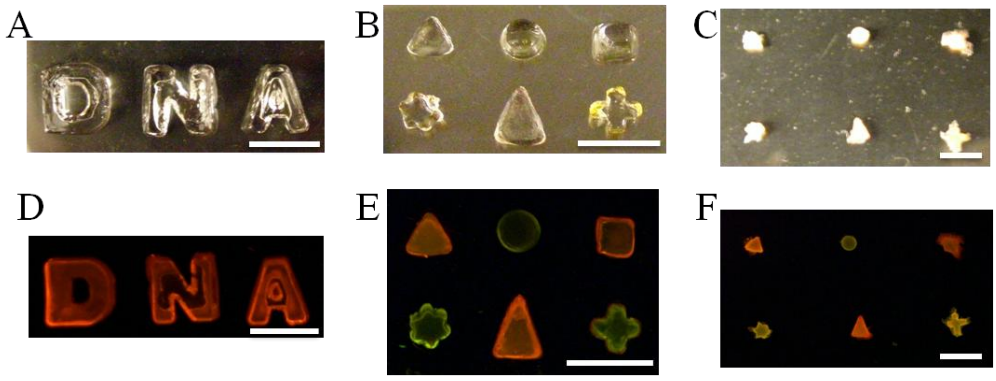

Figure 3. Photocrosslinked DNA-PEG Hybrid Hydrogels
A: DNA-PEG hybrid gels patterned in DNA shape at centimeter scale;
B & **C**: swollen DNA-PEG hydrogels and Dried DNA-PEG hybrid gels, respectively, with different patterns: equilateral triangular, circular, square, cross, isosceles triangular and star (from the top left corner, clockwise)
D, **E** & **F**: stained with gel red and gel green

Figure 3 shows the swollen DNA-PEG hybrid hydrogels patterned into DNA shape and swollen and dried DNA-PEG hybrid hydrogels patterned into other different shapes such as equilateral triangle, circular, square, cross, isosceles triangular and star. Figure 3 shows that DNA-PEG hybrid hydrogels possess a property of retaining their shapes rather than collapsing to films either after hydrating and drying.

3.1.1. Characterization of PEGA-ssDNA conjugates

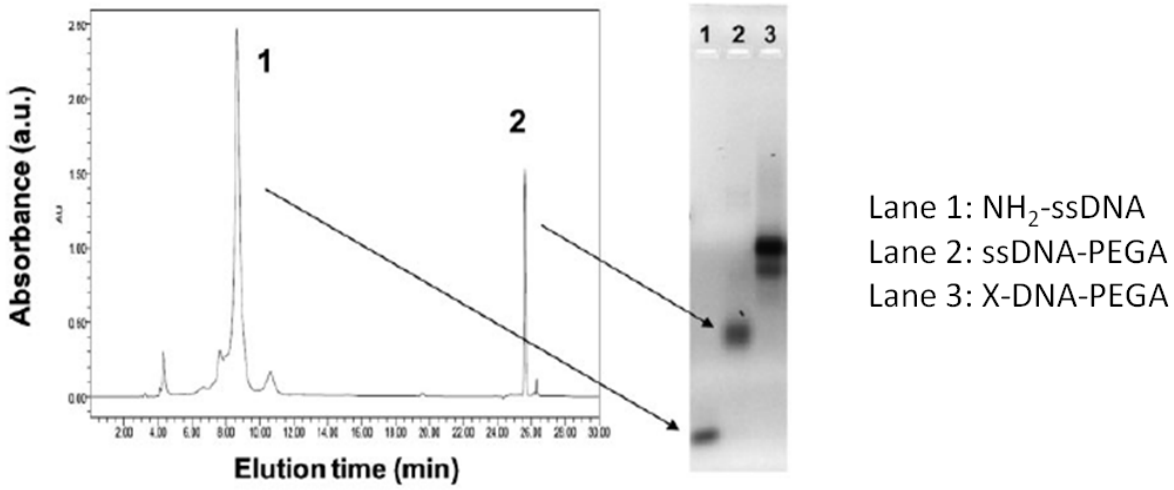

Figure 4. Characterization of photocrosslinkable X-DNA (a) HPLC chromatogram of products formed in the reaction of ssDNA and PEGA (b) Electrophoretic mobility shift of ssDNA and X-DNA building blocks

In order to generate X-DNA-PEGA, we first obtained ssDNA-PEGA conjugates by reacting the amine groups of ssDNA and the activated ester groups of the PEGA molecule. By employing HPLC, the final conjugated product was purified from unreacted reagents. Two distinct peaks in HPLC spectrum indicate the initial amine-modified ssDNA and ssDNA-PEG conjugates. Following by HPLC, gel electrophoresis was conducted to confirm the conjugation of X-DNA and PEGA. Lane 1, 2, 3 from gel electrophoresis results indicate initial amine-modified ssDNA, ssDNA-PEGA conjugates and X-DNA-PEGA conjugates, respectively. From the electrophoresis result, it is observable that the electrophoretic mobility of ssDNA-PEGA was retarded compared to the initial amine-modified ssDNA due to the increased molecular weight. Also, for the same reason, it can be seen that the electrophoretic mobility of X-DNA-PEGA was retarded compared to that of ssDNA-PEGA conjugate.

3.1.2. Characterization of DNA-PEG conjugates

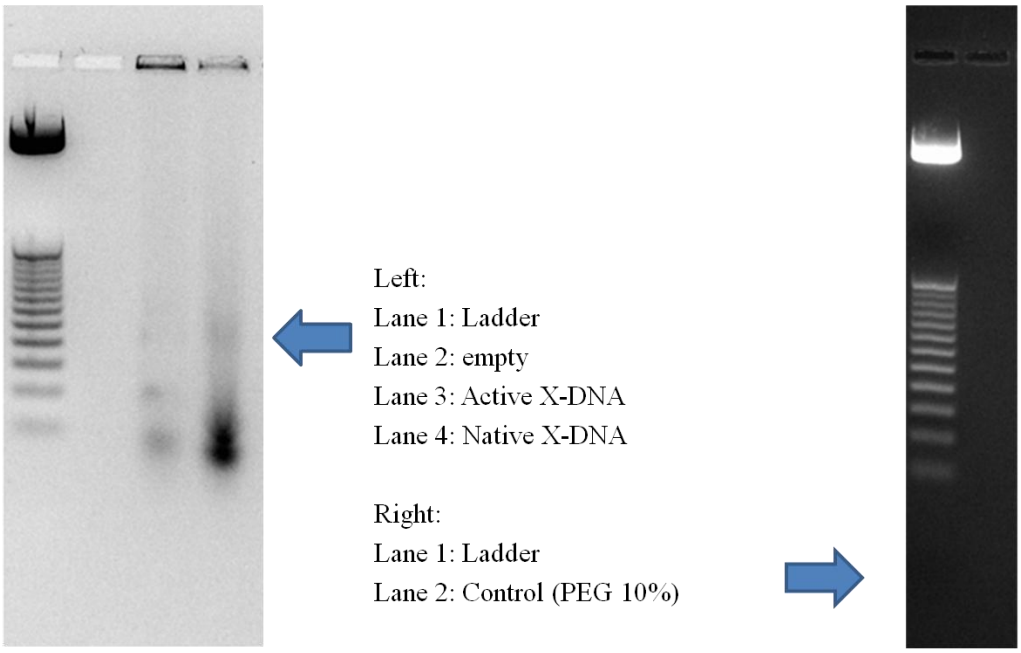

Figure 5. Characterization of DNA-PEG conjugates: electrophoretic mobility shift of active X-DNA and native X-DNA

In order to confirm the successful conjugation of acrylated-functionalized X DNA and PEGDA and thus forming DNA-PEG hydrogel, the gel electrophoresis was conducted. Lane 1, 3, 4 on Figure 5a (left) indicate ladder, active X-DNA and native X-DNA, respectively. The electrophoresis result indicates the successful formation of DNA-PEG hybrid hydrogel. By comparing wells of lane 3 and 4, it can be seen that active X-DNA (lane 3) formed a photocrosslinked hydrogel and thus X-DNA was entrapped in hydrogels instead of being separated (lane 4). Lane 1 and 2 on Figure 5b indicate the ladder and pure PEG hydrogel, respectively.

3.2. Morphological Studies of DNA-PEG Hydrogels

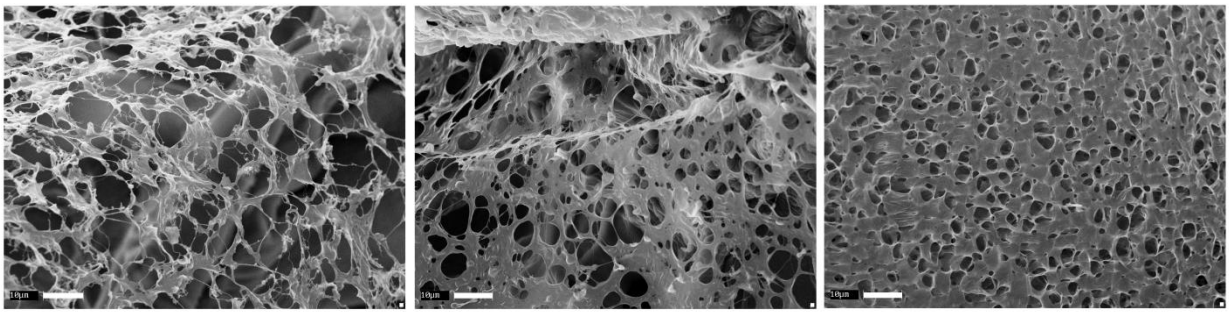

Figure 6. SEM images of DNA-PEG hybrid hydrogels at different DNA contents (DNA w/v.%) (a) Pure PEG hydrogel (0) (b) DNA-PEG hydrogel (0.04%) (c) DNA-PEG hydrogel (0.052%) The scale bars are 10 μm

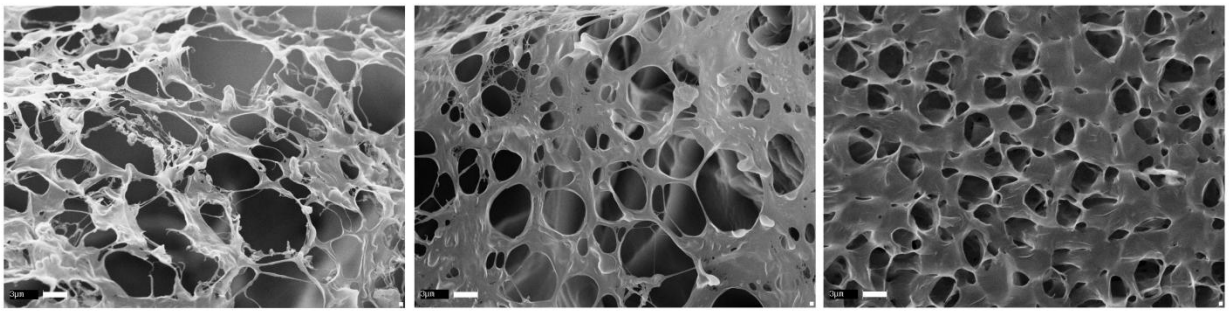

Figure 7. SEM images of DNA-PEG hybrid hydrogels at different DNA contents (DNA w/v.%) (a) Pure PEG hydrogel (0) (b) DNA-PEG hydrogel (0.04%) (c) DNA-PEG hydrogel (0.052%) The scale bars are 3 μm

The morphology of freeze-dried DNA-PEG hydrogels was then investigated using scanning electron microscopy (SEM). Both PEG and DNA-PEG hybrid hydrogels displayed porous network structures. Figure 6 shows that the pore size of these porous structures decreased with an increase in the amount of DNA. Pure PEG hydrogel had the largest pore size while DNA-PEG hybrid hydrogels 2 with the most DNA contents among three hydrogels had the smallest pore size. The morphological data shown in Figure 6 are consistent with the effect of the DNA on swelling ratio. DNA-PEG hybrid hydrogels with the most DNA contents showed the lowest

swelling ratio. The pore sizes seem to be highly dependent on the crosslinking density of monomers. In the DNA-PEG hybrid hydrogel system as the DNA contents increased the crosslinking density of the hybrid hydrogel networks increased due to the increased concentration of acrylate group in the hybrid hydrogel 2. Thus, hybrid hydrogel 2 possess the tighter network structure with thicker walls and smaller pore size distribution.

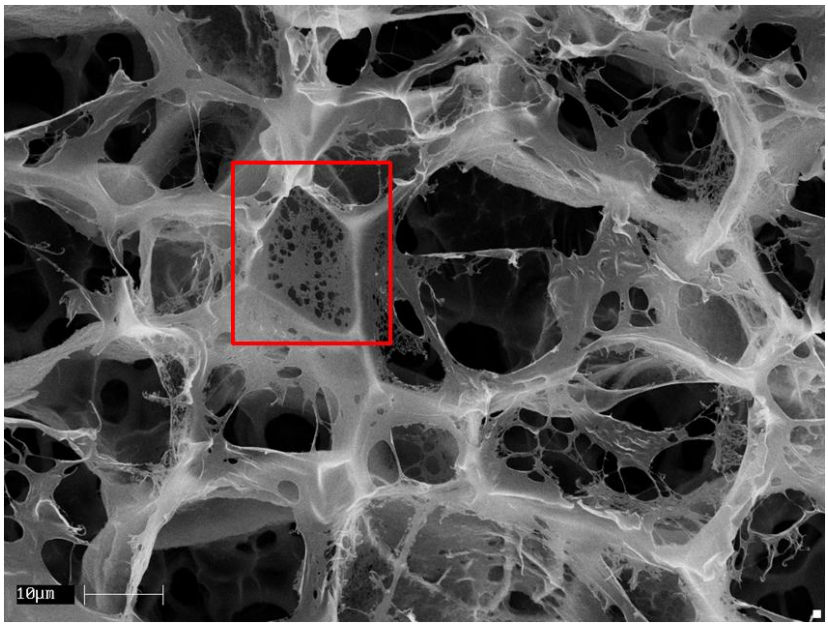

Figure 8. SEM images of DNA-PEG hybrid hydrogel (0.065 w/v.%)

However, Figure 8 shows the DNA-PEG hydrogel 3 with the most DNA contents. It shows both large pores and small pores on the same hydrogel. With the increase content of DNA, heterogeneity in pore sizes can be observable. Figure 8 shows that pore sizes are uniform in the most of the area but smaller in some areas (indicated by the red rectangle).

3.3. Mechanical Studies of DNA-PEG Hydrogels

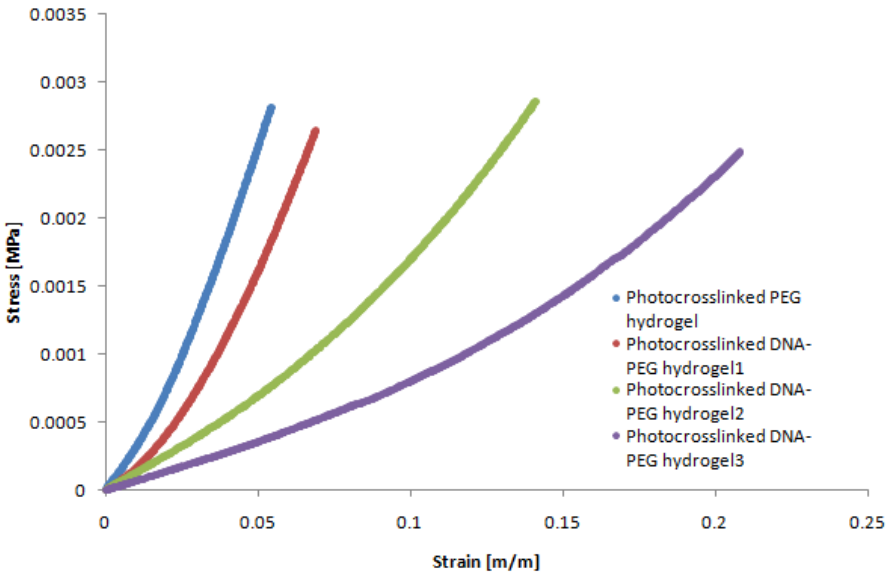

Figure 9. Mechanical Properties of DNA-PEG hydrogels

Figure 9 shows the measured compression stress-strain curves of pure PEG, DNA-PEG hydrogel 1, 2 and 3. The effect of the DNA content on the mechanical property of the DNA-PEG hybrid hydrogels is shown in Figure 9. The data in this figure indicates that the incorporation of DNA content reduces the Young's modulus of hydrogels. The young's modulus of DNA-PEG hydrogel 1, 2 and 3 are calculated to be 0.0156 MPa, 0.009 MPa and 0.0067 MPA, respectively. By comparing these values with Young's Modulus of pure DNA hydrogel, it is apparent that that the DNA-PEG hybrid hydrogels have higher Young's Modulus and thus have improved mechanical properties with the incorporation of PEG monomers. The pure PEG hydrogel shows the highest compressive modulus while the DNA-PEG hybrid hydrogel with the most DNA contents showed the lowest compressive modulus. This mechanical properties data are not in agreement with the morphological data. Unlike other hydrogel systems in which the tighter network structure results the higher compressive modulus [5-6], the results of DNA-PEG hybrid

hydrogels shows opposite trend. Figure 9 shows a group of smaller pores is present in one specific area among larger pores. It is hypothesized that the addition of DNA contents causes the expulsion of water and results in additional localized crosslinking in the hydrogel network. It is deduced that the increase in localized crosslinking in the hydrogel network affects the porous network structure of hydrogel.

3.4. Swelling Studies of DNA-PEG Hydrogels

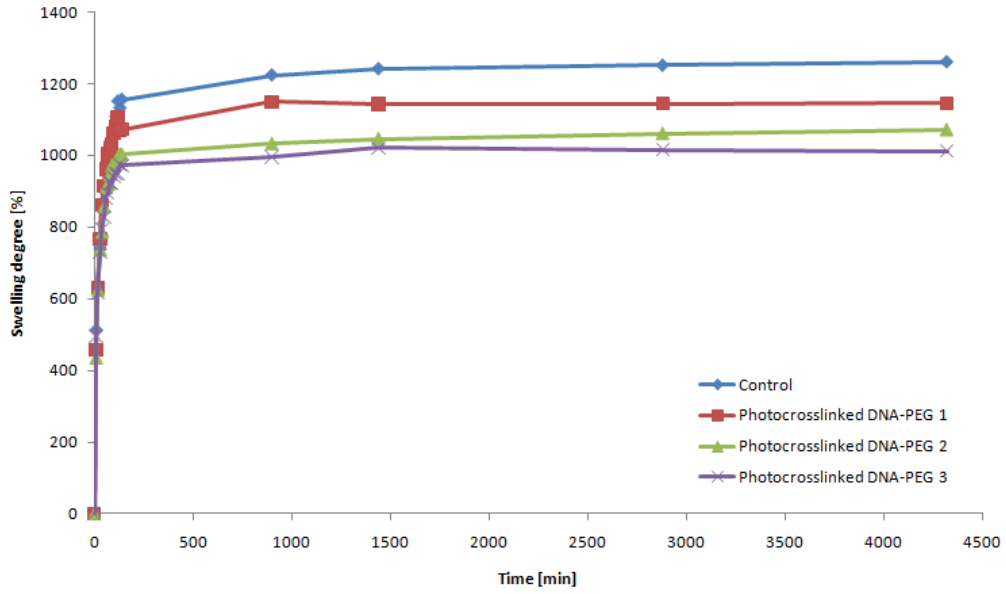

Figure 10. Effect of DNA content on the swelling kinetics of DNA-PEG hydrogel

The swelling kinetics of the DNA-PEG hydrogels was measured over a period of 3 days at room temperature in deionized water. As shown in Figure 10, all of the DNA-PEG hydrogels with different DNA contents showed a high swelling rate. The increase in DNA content in DNA-PEG hydrogels attributed to the decrease in swelling ratio. The swelling ratio of the hybrid hydrogels was higher than that of pure DNA hydrogels.

The swelling ratio has an inverse relationship between with the crosslinking density. As the crosslinking density increased with the increased DNA contents, the water content of DNA-PEG hydrogels decreased. Figure 9 shows that DNA-PEG hydrogel 1 has the fastest swelling kinetics and high water retention capability among hybrid hydrogels due to hydrophilic nature. It is well known that the hydrophilic nature of functional groups and the crosslinking density of hydrogels are two factors that affect the swelling properties of hydrogels. [1] Moreover, the increase of crosslinking density of hydrogels leads to smaller pore size distribution of hydrogel network and

low water retention capability. The concentration of acrylate group in the gel 3 was the highest so it possessed the highest crosslinking density and the lowest water retention capability.

3.5. In vitro Bovine Serum Albumin Release Studies

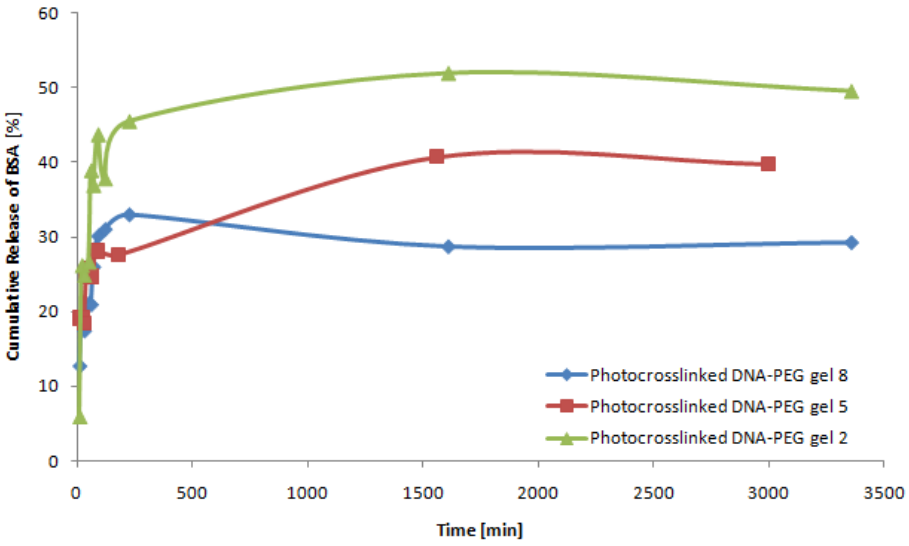

Figure 11. Protein Release test of DNA-PEG hydrogels

In protein release study, Bovine Serum Albumin (BSA) was chosen as a model drug and was incorporated into our DNA-PEG hydrogels to simulate delivery of protein-based drugs. Figure shows the cumulative amounts of BSA released from the DNA-PEG hydrogels at room temperature. The cumulative BSA release % during 5 days was 50% for gel 2, 40% for gel 5 and 30% for gel 8. As the DNA contents increased in DNA-PEG hydrogels, the protein release rate gradually slowed down. Although drug release rate varied on each hydrogel with different DNA contents, all three DNA-PEG hydrogels exhibited an initial burst release of BSA. The protein release through a hydrogel matrix was controlled by swelling behavior, which was simply controlled by adjusting DNA contents on each hydrogel. As the hydrogel swells, the pore size increases and thus allows the drug to diffuse out of the hydrogel more easily. DNA-PEG gel 2 showed the fastest protein release rate.

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CHAPTER 4. CONCLUSIONS

In this study, a unique DNA-PEG hybrid hydrogels were fabricated using UV photopolymerization and were characterized by employing high performance liquid chromatography (HPLC), gel electrophoresis, swelling measurements, Scanning Electron Microscopy (SEM), mechanical analysis and controlled drug release experiments. The interior morphology study by SEM reveals that an increase of DNA content led to tighter hydrogel network structure. The swelling study and protein release study further demonstrate that the swelling capability and drug release rates are mostly determined by the network structure. The mechanical testing reveals that DNA-PEG hybrid hydrogels possess increased mechanical strength compared to DNA hydrogels by the incorporation of PEG monomers. Due to many unique properties these hydrogels possess, we envision that DNA-PEG hybrid hydrogels can be utilized in a variety of biomedical applications including drug delivery and tissue engineering.