FORMATION OF HOLLOW-SPHERE DNA HYDROGELS THROUGH ELECTROSPRAY IONIZATION

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Engineering

Biological and Environmental Engineering

by

Kevin He Li

December 2018

Abstract:

In recent years, hydrogels have found applications in a number of fields. One area where hydrogels have been extremely influential is biomedical applications. Hydrogels can be utilized in cell and drug delivery systems, tissue engineering matrices, and as absorbers of toxins. Hollow-structure hydrogels are especially attractive in drug delivery applications due to their faster diffusion dynamics and the capability to encapsulate greater amounts of drugs. Hydrogels can also be composed of DNA and due to the biological origin of DNA, these hydrogels are biocompatible and biodegradable, an aspect that can be challenging for synthetic hydrogels. In this work, we fabricated hollow-structure DNA hydrogels through electrospray ionization in combination with a bath solution containing bicine and aluminum ions (Al^{3+}). We then optimized the DNA concentration and bath solution to produce hollow-structure hydrogels with minimal shape deformation. Afterwards, we fabricated hollow-structure DNA hydrogels that contained particles of interest and hollow-structure hydrogels composed of predominately carbon nanotubes. Lastly, we attempted to encapsulate particles of interest through the usage of a coaxial needle. The results from this work provide a preliminary investigation into the fabrication of hollow-structure DNA hydrogels and motivation for further study into hollow-structure DNA hydrogels.

1. *Introduction*

A hydrogel is a network of hydrophilic polymer chains that are capable of absorbing water and dissolved solutes to a high degree. When saturated, they are predominately composed of water. Hydrogels have been utilized in a number of applications, such as drug delivery, cell delivery, tissue engineering matrices, and as absorbers of environmental pollutants.[1,2] Hydrogels can also be constructed from DNA and these hydrogels have a variety of advantages. With recent advances in DNA production, sequences are relatively cheap to produce. Studies have identified that DNA hydrogels are capable of both non-biological and biological stimuli response.[3,4] Other studies have shown that DNA hydrogels are capable of producing proteins without requiring living cells.[5] Furthermore, DNA hydrogels have a high degree of biocompatibility and biodegradability due to their biological origin. Therefore, DNA hydrogels are attractive candidates for medical applications. Hydrogels and DNA hydrogels can been cross-linked through a number of methods. This includes cross-linking through UV light, chemical crosslinking, and physical crosslinking. In this work, we mainly focus on chemical crosslinking, which requires a cross-linker. We used aluminum ions (Al^{3+}) as a cross-linker which would cross-link DNA through the Michael Addition reaction. Figure #1 provides the general cross-linking mechanism in which Al^{3+} operates. In this study, we employ electrospray ionization which uses a high voltage source to generate an aerosol from a solution in combination with a bicine bath solution in order to generate hollow-structure DNA hydrogels.

Hydrogels that are hollow in structure are particularly attractive in the field of drug delivery. They are capable of serving as molecule and drug carriers as the outer layer of cross-linked polymer provides protection from degradative reactions, allowing for directed transport to specified areas. In addition to directed transport, hollow-sphere structure has a number of advantages over solid-sphere structure. Since the inside of the hydrogel is hollow or empty space, this allows for a greater amount drug to be encapsulated. In addition, hollow-sphere structures have a faster diffusion profile when compared to solid-sphere structures in aqueous solutions.[6] Since there is less of a barrier between encapsulated particles or chemicals of interest and the outside environment and therefore a shorter diffusion path, there will be a faster diffusion rate from inside the hydrogel to the outside environment. The ability to hold greater amounts encapsulated chemicals such as drugs and faster diffusion properties could lead to increases in treatment efficiency and effectiveness. Therefore, DNA hollow-sphere hydrogels could provide a method to more efficiently transport drugs while posing low toxicity risks to the body. Figure #2 represents the mechanism in which a hollow-structure hydrogel is utilized as a drug carrier. As previously mentioned, recent studies have confirmed the possibility of specific protein production by DNA hydrogels, allowing for the possibility of novel treatments such as in situ protein production therapies.[5]

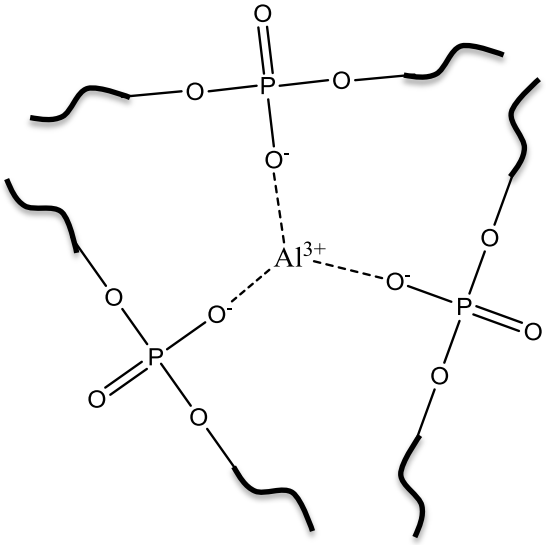


Figure 1: Al^{3+} Cross-linking mechanism.

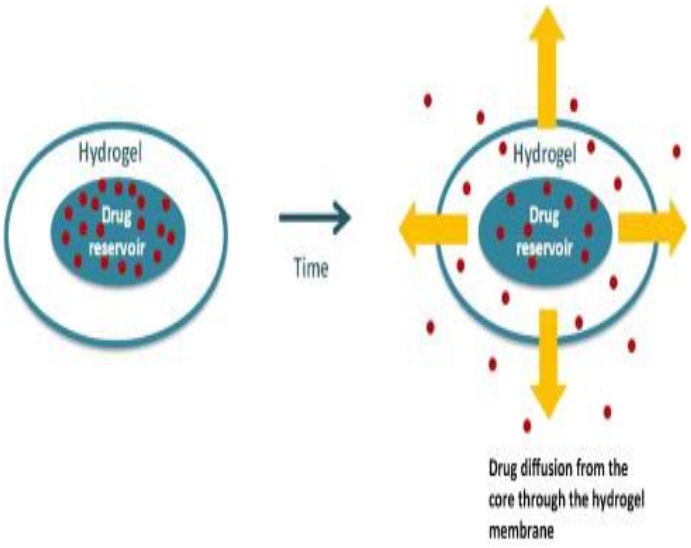


Figure 2: Drug delivery mechanism

2. ***Methods & Materials***

2.1. DNA Solution

DNA solutions that were utilized in this study were formulated in 1 mL containers. These solutions were composed of 1-5% salmon testes DNA dissolved in deionized (DI) water. Various concentrations of sucrose were added to the DNA solutions to modify the viscosity and explore the effects of viscosity on hydrogel formation. Other DNA solutions that were used included mixtures of DNA and iron (III) oxide, gold (Au) nanoparticles, and carbon

nanotubes. These solutions were used as a proof of concept for acceptable hydrogel formation with particles of interest encapsulated inside. All DNA solutions were manually mixed until DNA or other solids were no longer present and a uniform solution was observed. The solutions were then centrifuged to remove pockets of air within the solution.

2.2. Bath Solution

Bath solutions that were utilized in this study were formulated in petri-dishes. Typically, these bath solutions were 8 mL in volume and served as a method of collecting and cross-linking DNA hydrogel particles post electrospray ionization. These bath solutions were a combination of DI water, aluminum ion (Al^{3+}) solution, and bicine. In certain experiments, bicine was substituted with citric acid. Al^{3+} and bicine concentrations were optimized through experimentation.

2.3. Electrospray Ionization

In order to produce the hollow-sphere DNA particles, an electrospray apparatus was used. DNA solutions of interest were placed into syringes with needles of size 100 μm in diameter. The syringes were then placed on the electrospray apparatus which applied a steady downward force. A voltage was applied to the metal tip of the needle through a metal wire fixed to the needle to produce an electrical field. The bath solution was placed on top of a metal holder that was grounded. Particles that were resultant from the electrospray process were caught in the bath solution and cross-linked. Figure #3 provides a simplified visual representation of the electrospray process.

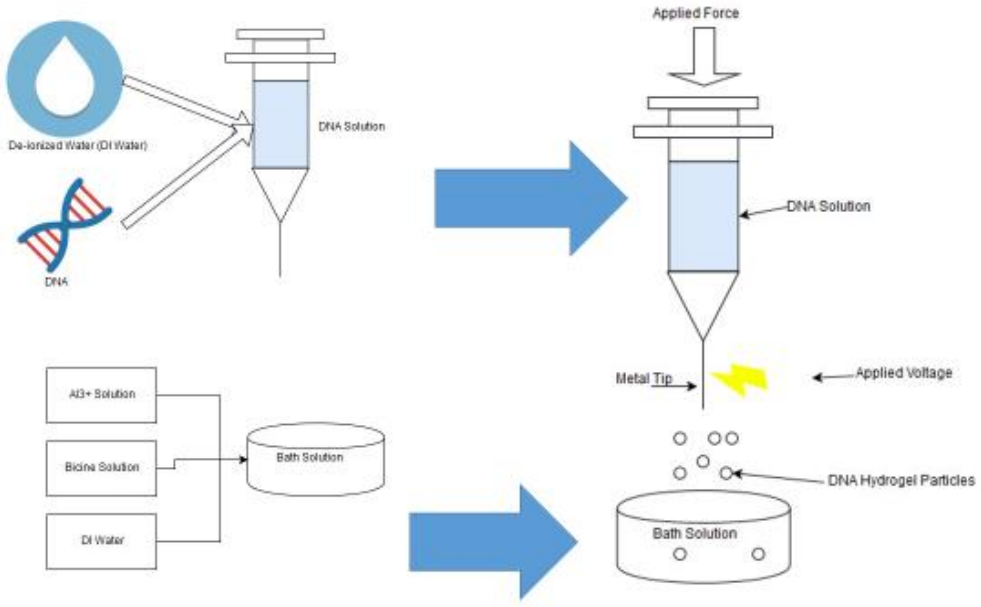


Figure #3: Experimental methods and components. DNA solution was generated from a mixture of salmon testes DNA and DI water. This solution was transferred to a syringe. The bicine bath solution was a combination of bicine, aluminum ions, and DI water. These components were placed on an apparatus that applied constant force and high voltage, generating an aerosol that was caught by the bath solution.

3. ***Results***

3.1. Formation of Hollow-Sphere DNA Hydrogels

Formation of hollow-sphere DNA hydrogels was dependent on three factors, a bath solution which would collect and cross-link the electrosprayed DNA solution, the DNA solution, and the applied voltage. It is hypothesized that bicine within the bath solution is crucial to the formation of hollow-sphere hydrogels. Removal of bicine from the bath solution resulted in dark spots when observing the hydrogels under bright field microscopy (Figure #4). While the origin and determination of these dark spots is not confirmed, it is predicted that a dark spot is indicative of a solid DNA hydrogel. Bicine also plays a crucial role in tempering the acidic effect of Al^{3+} ions. Al^{3+} solutions are typically very acidic with a pH around 3. When bicine is included within the bath solution, the overall pH of the solution is around 6.5, which is a more neutral solution and tolerable with respect to the human body. We have also experimented with bicine within the DNA solution, however the observed DNA hydrogel products showed no significant differences between those fabricated from DNA solutions that did not contain bicine.

In order to optimize the formation of hollow-sphere hydrogels, we made a number of modifications to the bath solution. We characterized optimal conditions as the formation of a hollow-structure hydrogel (absence of a dark spot), lack of wrinkling and folding of the hydrogel wall, and spherical in shape. It should be noted that due to the mechanism by which hollow-hydrogel formation occurs, it is unlikely that perfect spherical structure could be achieved but rather teardrop shaped hydrogels is observed. Former studies have attributed this observation to the impact of the DNA solution with the bath solution.[7] Hydrogel size can be modified through electrospray ionization. Higher voltages used in the electrospray process typically resulted in smaller DNA hydrogel particles. Therefore, we often used a voltage of 10 kV across all experiments as we wished to obtain the smallest particles we could achieve.

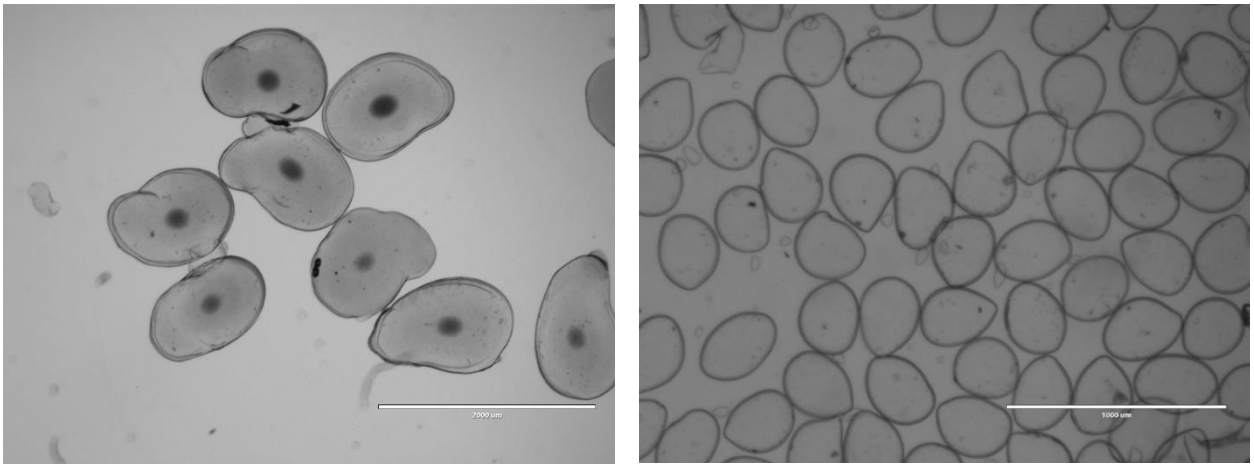
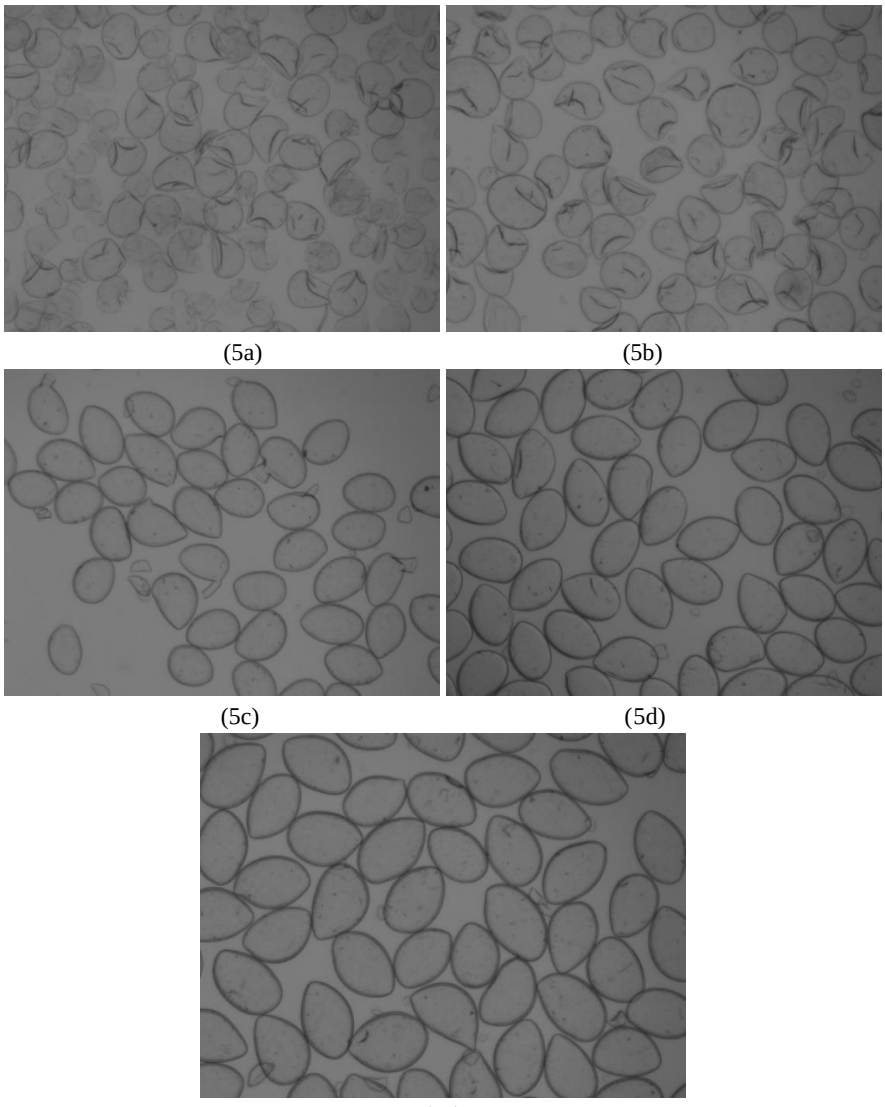


Figure #4: Effect of bicine within the bath solution. Lack of bicine (left) results in dark spots in bright-field microscopy. Inclusion of bicine (right) results in hollow-sphere hydrogels.

3.2. Optimization of DNA Concentration

Variations in the DNA concentration within the DNA solution itself had shown that DNA played a crucial role in hydrogel formation and structure. Increases in DNA concentration resulted in more smoothed hydrogels and less shape deformation. Figure #5 depicts preferential structural qualities with higher DNA concentrations. 1% and 2% DNA show visible crumpling, folding, and structural indents whereas 3%, 4%, and 5% DNA show smoothed hydrogel walls and sphere-like shape. We determined that concentrations of DNA over 3% were viable for the generation of hollow-sphere DNA hydrogels. We ultimately chose 4% DNA as our baseline as we found that this concentration had a lower frequency of minor hydrogel structural imperfections.



(5a) (5b)

(5c) (5d)

(5e)

Figure #5: Figures 5a and 5b represent DNA hydrogels produced by 1% and 2% DNA respectively. These hydrogels show wrinkling, folding, and imperfections in DNA hydrogel shape. Figures 5c, 5d, and 5e represent DNA hydrogels produced by 3%, 4%, and 5% respectively. These hydrogels are suitable candidates for hollow-sphere hydrogels with preferential shape and no observable structural imperfections. All DNA hydrogels were formulated under conditions of 25 mM Al^{3+} and 25 mM bicine.

3.3. Optimization of Bicine Concentration

Bicine is an organic compound that is often used as a buffering agent. It is known as one of Good's buffers. Good's buffers are compounds that can be used as buffers for biological and biochemical research. Bicine was chosen as a component of the bath solution for two main reasons. One being that bicine is capable of mitigating the acidic effect Al^{3+} has, causing the overall pH of the solution to be in a suitable range for biological applications. The other reason is the hollow-structure phenomenon that is observed when bicine is added. Other compounds have been tested for viability within the bath solution such as citric acid. However, these particles were observed to either to stick to other particles and were difficult to separate at low citric acid concentrations or very short lived at higher concentrations. In addition, bicine may play a role in the formation of the structure of the hydrogel as higher concentrations of bicine resulted in more spherical hydrogels (Figure #7).

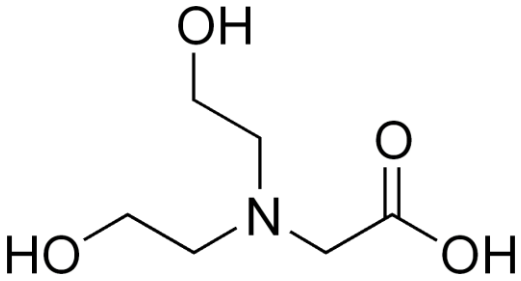
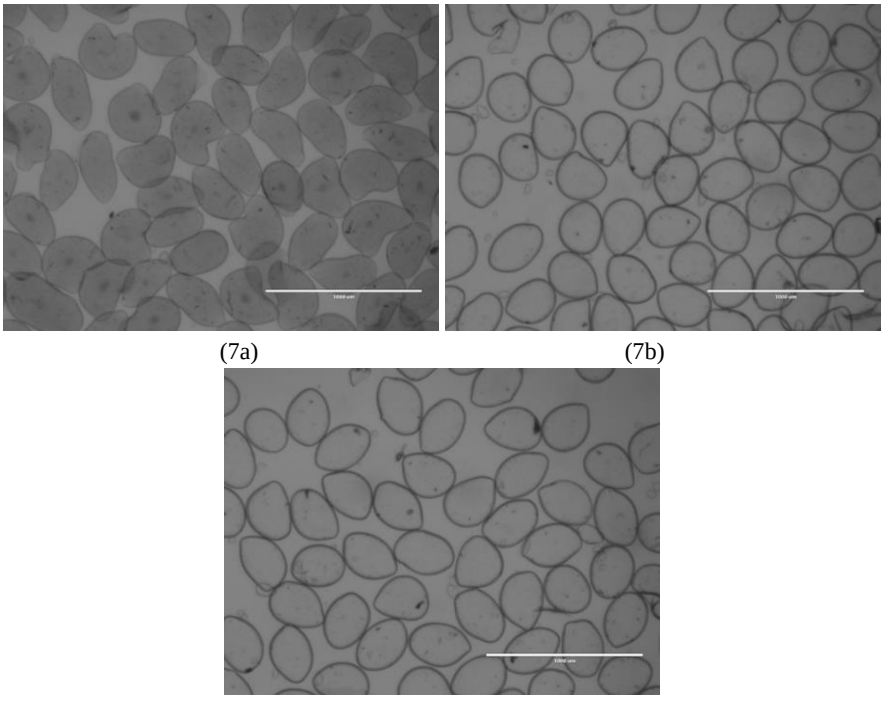


Figure #6: Chemical structure of bicine.



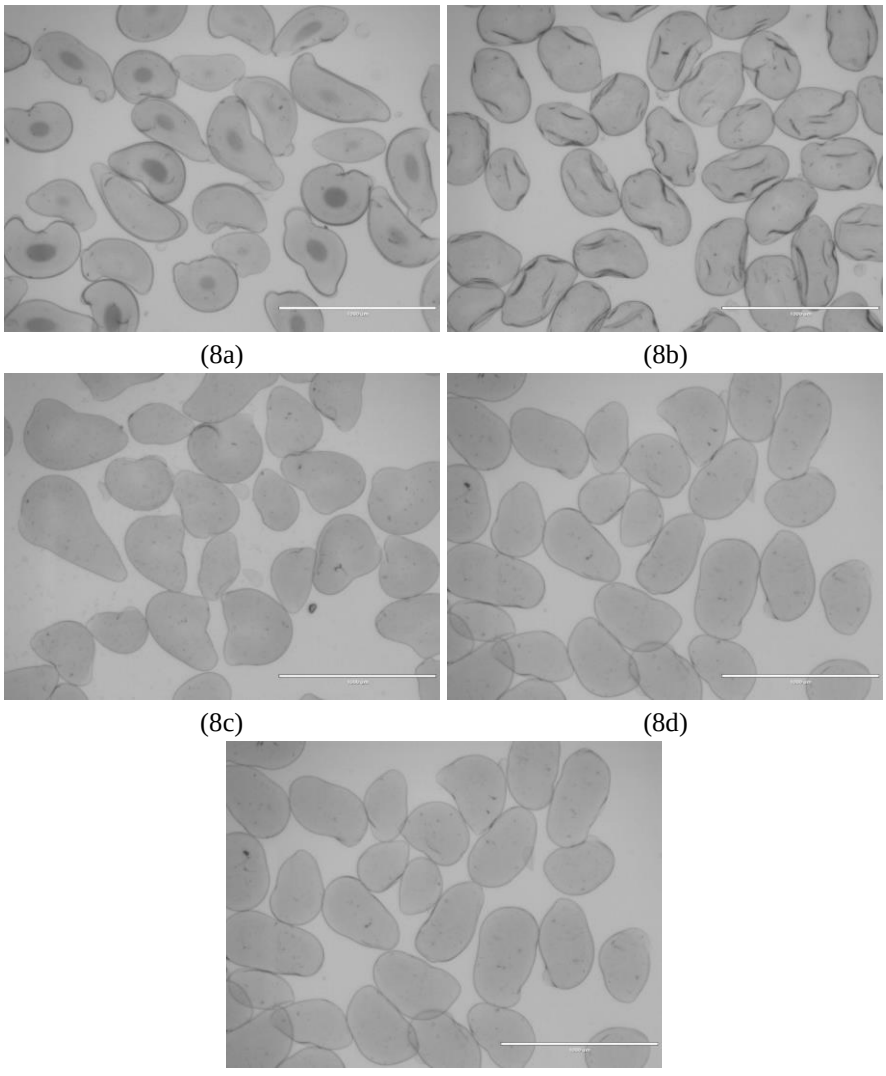
(7a) (7b)

(7c)

Figure #7: Figures 7a-7c represent DNA hydrogels formulated in bath solutions in 10 mM, 50 mM, and 100 mM bicine bath solutions. All solutions contained 25 mM Al^{3+} within the bath solution. Bicine concentrations of 50 mM and 100 mM resulted in favorable hydrogel shape.

3.4. Optimization of Al^{3+} Concentration

Aluminum ions (Al^{3+}) play a crucial role in the formation of the hydrogel. Aluminum ions are essential to the Michael-Addition reaction mechanism that is responsible for the cross-linking of the DNA hydrogel. Higher concentrations of aluminum ions resulted in almost instantaneous cross-linking times. Lower concentrations yielded inability to maintain a spherical structure. Shape deformations at lower concentrations are likely attributed to greater amounts of diffusion when the DNA is not cross-linked fast enough. This is contrasted with higher concentrations of Al^{3+} which had no observable hydrogel wall deformities. Figure #8 depicts the structural differences between hydrogels cross-linked within bath solutions of varying concentrations of Al^{3+}.



(8a) (8b)

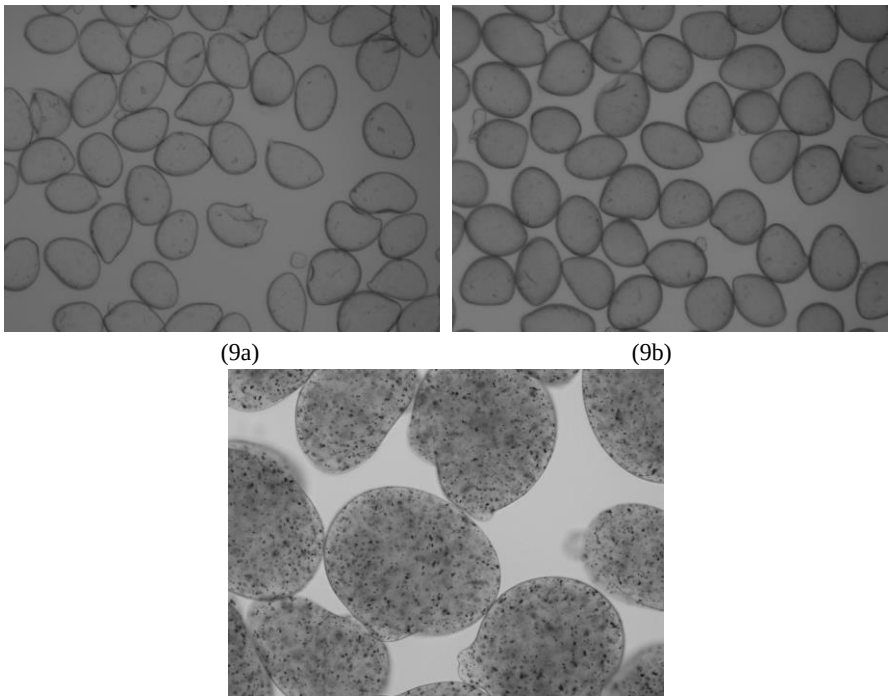
(8c) (8d)

(8e)

Figure #8: Variation of Al^{3+} within the bath solution. Figures 8a-8e represents bath solutions with 25 mM bicine and 10, 50, 100, 150, and 200 mM of Al^{3+} respectively.

3.5. Formation of DNA Hydrogels with Embedded Particles

After determining a bath solution and DNA concentration that was capable of forming DNA hydrogels that contained a hollow-sphere structure, we attempted to create hydrogels that encapsulated other particles of interest. We attempted to fabricate hydrogels with iron (III) oxide (Fe_2O_3), gold nanoparticles (Au nanoparticles), and carbon nanotubes. Results from the electrospray process indicate fabrication of hollow-sphere DNA hydrogel structure with no structural deformities. As expected, the embedded particles were unorganized within the hydrogel due to a lack of an organizational mechanism. Note that Au nanoparticles and carbon nanotubes were smaller than the diffraction limit of a bright-field microscope and were unable to be seen through this method.



(9a) (9b)

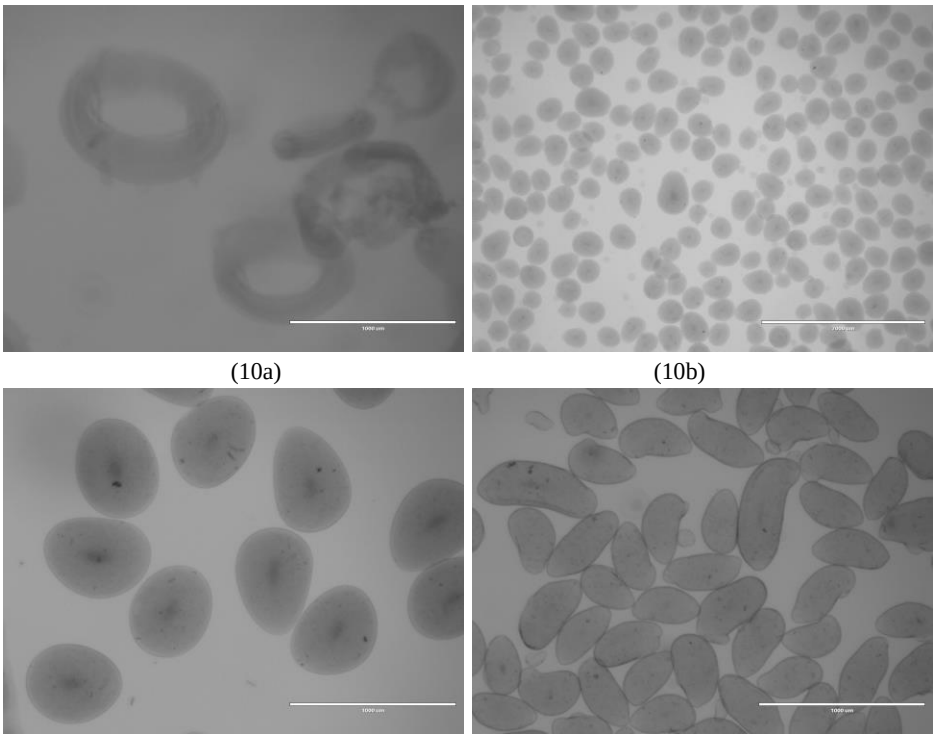
(9c)

Figure #9: Bright field microscopy of DNA hydrogels with encapsulated particles. Figure 9a-9c depicts DNA hydrogels with gold nanoparticles, carbon nanotubes, and iron oxide encapsulated respectively.

3.6. *Formation of Carbon Nanotube Hollow-Structure Gels.*

Carbon nanotubes have a variety of applications ranging from composite materials to microelectronics. This is due to their favorable properties of mechanical strength, thermal conduction, and electrical conduction. Carbon nanotubes are also used in the field of biotechnology where they have served as biosensors and medical devices. They have also been utilized in fluorescent imaging, photo-acoustic imaging, and localized heating within the body as they can be internalized by cells.[8] Due to these biological applications, we explored the possibility of whether the formation of hydrogel particles that were predominately composed of carbon nanotubes was possible. Pure carbon nanotubes were unable to create spherical gel-like structures but rather donut-shaped structures that were

incapable of being submerged within the bath solution. To counteract buoyancy forces, we incorporated sucrose as a viscosity modifier to allow for the carbon nanotube gels to be submerged. Increases in viscosity lead to submergence of particles as well as increased adherence to other particles. In addition, we incorporated a small amount of DNA (0.3%) in attempts to achieve spherical gel structure while maintaining a solution that was predominately composed of carbon nanotubes. Results of the experimentation indicated that formation of carbon nanotube spherical hydrogel structure was possible with minimal structural imperfections. When added to carbon nanotube solutions with 0.3% DNA addition to assist in submerging the particles, sucrose did not appear to cause any significant impact to the structure of the hydrogel.



(10a) (10b)

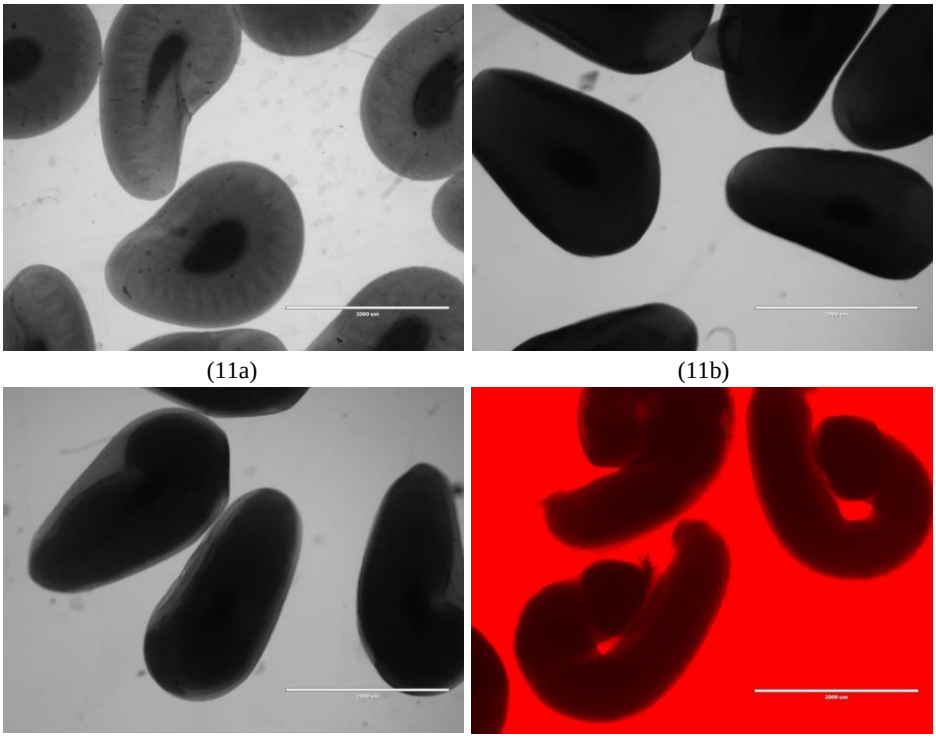
(10c) (10d)

Figure #10: Hydrogels formed from carbon nanotube solution. Figure 10a represents particles formed under no DNA addition. Figures 10b-10d represents hydrogels formed from solutions with 0.3%, 1%, and 4% DNA additions respectively.

3.7. Usage of Coaxial Needle in Hollow-Sphere Hydrogel Formation

In order to achieve DNA hydrogel structures that were capable of containing different solutions within in a distinct compartment, we utilized a coaxial needle. Experimentation with gold nanoparticle solutions, deionized water dyes, and other DNA solutions provided evidence that distinct encapsulation was possible. This was confirmed using bright field microscopy and visual confirmation. When two DNA solutions are used, hydrogels that are formed contained replicable characteristics. Repeatable shapes only appear to occur when the more viscous solution is contained on the inside. We believe that the formation of such shapes is related to the higher viscosity of the inner solution. However, hollow-structure

DNA hydrogel experimentation using coaxial needles are still within the preliminary phase and many of the mechanisms behind the hydrogels formed are not known.



(11a) (11b)

(11c) (11d)

Figure #11: Notable coaxial needle experimental results. Figure 11a depicts successful encapsulation of dyed water. Figures 11b and 11c represents experimentation regarding two DNA solutions of same concentration but different viscosity. Figure 11b depicts a hydrogel with a more viscous DNA solution on the outside whereas the hydrogels on figure 11c have a more viscous DNA solution on the inside. Figure 11d depicts a repeatable "6" shape when a more viscous fluid and more DNA concentrate solution are placed on the inside.

3.8. Usage of Bicine Bath Solution in DNA Membrane Production

DNA membrane fabrication through the use of the bath solution was also explored. By uniformly spreading DNA solution on glass slides and silicon wafer substrates followed by usage of a spin-coater, it was possible to generate DNA membranes of near uniform thickness. Fabrication of DNA membranes were possible with DNA solutions composed of 1-5% DNA, however lower concentrations of DNA lead to more fragile membranes (susceptible to tearing). Higher concentrations of Al^{3+} increased cross-linking speed but also increased difficulty of removing the membrane from the substrate.

4. ***Conclusions & Future Directions***

Within the past decade, there has been an increase in interest in DNA Hydrogels due to their biocompatibility qualities and unique potential applications. With the capability of forming hollow-structure hydrogels through chemical cross-linking in the presence of bicine, we find that DNA hydrogels could be utilized in a number of applications which capitalize on the advantages of the hollow-structure. Traditionally, hollow-sphere hydrogels have found applications in the field of drug and cell delivery due to their faster diffusion mechanisms

and ability to encapsulate larger quantities of drugs and cells. This study provides preliminary results and conclusions related to the formation of DNA hydrogels that are hollow in structure. We found through optimization of Al^{3+}, bicine, and DNA concentrations, it was possible to form hollow-sphere hydrogels that had favorable qualities. In addition, we provided a proof of concept in which we were able to create hydrogels with other particles embedded inside with qualities comparable to those composed of pure DNA. While this study provides a preliminary proof of concept of hollow-structure DNA hydrogels; there are a still numerous mechanisms and directions that can be explored. While we were able to achieve a hollow-structure hydrogel, the detailed role that bicine plays in the formation of said hydrogels is still completely known. Furthermore, encapsulation and embedding of cells, drugs, micro-electronics, or micro-bioreactors has not been explored. Results from electrospray ionization using a coaxial needle are in the primitive stages as well with only a limited number of mechanisms and solution combinations explored. In conclusion, the capability of DNA to form into hollow-sphere hydrogels can have numerous potential applications, however many mechanisms and interactions will first have to be clarified before real-world application of these hydrogels can be implemented.

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