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Project title Printable Zwitterionic Hydrogel

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Printable Zwitterionic Hydrogel

by

Mr. Sant Kunsook

In Partial Fulfillment for the Degree of Bachelor of Science

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Department of Chemistry, Faculty of Science

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By Mr. Sant Kunsook

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Abstract

Zwitterionic hydrogel has gained an increasing attention due to its biocompatibility and anti-biofouling property. Fabrication of hydrogel into three-dimensional (3D) constructs using bioprinting is a powerful toolbox for tissue engineering and biological-related applications since it can overcome the limitation of the traditional scaffold-based methods. Here in this research, bioinks for 3D bioprinting was developed from Pluronic F-127 dimethacrylate (F-127 DMA) which acts as a macro-photocrosslinker and two zwitterionic monomers, 2-methacryloyloxyethyl phosphorylcholine (MPC) and sulfobetaine methacrylate (SBMA). The bioinks having varied composition between F-127 DMA and zwitterionic monomer (MPC or SBMA) were subjected to 3D printing followed by UV-induced crosslinking to yield zwitterionic hydrogels which were characterized for shape integrity and swelling properties. Having the same composition, SBMA-based hydrogels showed higher swelling property and softer than the MPC-based hydrogels. The higher the zwitterionic monomer was, the greater swellability and the weaker mechanical integrity of the gel became. Further systematic investigation on rheological property of the bioinks and mechanical properties of the resulting hydrogels have to be performed before the zwitterionic bioinks can be applied for 3D-bioprinting.

Keywords: 3D printing, bioink, zwitterionic polymer, MPC, SBMA

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Chapter 1

Introduction

1.1 Introduction to the research problem and significance

Nowadays, there is a great demand for the organ replacement and clinical treatment that is related to the regenerative medicine. Due to the high demand, the best solution at that time is to create artificial organ. The artificial organ should be able to function similar to real organ. Despite the solution is the best at that time, the drawback is the precision on the fabrication. The precision and accuracy are considered one of the most crucial components for creating a 3D object. At the same time, the raise of the 3D bioprinting has begun, many researchers have focused more of their work on the 3D bioprinting technology. The development of versatile and effective bio-inks that can accommodate 3D bioprinting and preserve biological functions of incorporated living microorganisms or biomolecules is quite challenging from material perspective. Printability, biocompatibility and antifouling property are equally important in developing bioinks. Zwitterionic polymers are well recognized for their effective antifouling characteristics so that they are widely used as biomaterials and biomedical applications. Despite their great potential, there are only a limited number of reports on zwitterionic-based bioinks for 3D bioprinting. This research aims to develop zwitterionic bioinks from triblock copolymer of poly(ethylene oxide)-poly(propylene oxide)-poly (ethylene oxide) (PEO-PPO-PEO), commercially known as “Pluronic”, a well-known shear-thinning printable matrix in combination with zwitterionic monomers. Fundamental knowledges obtained from this research should be beneficial for 3D bioprinting in the future

1.2 Research objectives

1. To synthesize and characterize dimethacrylate-functionalized pluronic
2. To prepare zwitterionic hydrogel from dimethacrylate-functionalized pluronic and zwitterionic monomers
3. To fabricate zwitterionic hydrogel by 3D printing

1.3 Literature review

1.3.1 3D bioprinting technology

The term of 3D bioprinting and 3D printing has a different definition in terms of the process. 3D bioprinting is a fabrication process that involves the combination of printable material and living microorganisms or biomolecules to form a complex 3-dimensional structure such as artificial tissue. [1] Nowadays, the technology of 3D bioprinting allows the field of tissue engineering and regenerative medicine to advance exponentially. The advantage of this has overcome the limitation of the traditional practice of scaffold-based tissue fabrication. [2] Due to the variety of printable materials, the equipment of the 3D bioprinting must be designed to accommodate the nature of ink. Currently, there are 3 different types of printing, which are ink jet printing, laser-assisted printing, and micro-exclusion-based printing. [3] Ink jet printing technique utilizes the spreading of the precursor droplet to form a 3D structure. The material is dispersed in a solvent, then loaded into the injector. The injection takes place by applying voltage causing liquid to vibrate and drop. After that the droplet is dried though evaporation and leaving the solid precursor material. Despite the technique is adaptable to the living cell, the drawback is that the technique cannot generate a continuous flow, which can limit the precision and accuracy of the printed material [4]. Another technique is the laser-assisted printing, the method works by depositing the precursor solution (ink + living microorganisms or biomolecules) onto the metal surface. Then the machine sends the laser pulse to the metal surface causing the solution to drop on to the printing surface, and form a 3-dimensional structure. [5] The advantage of this method is the high preservation of living cell due to low sheer stress is being apply to the solution. Unfortunately, the technique suffers from drawback such as expensive equipment and difficulty in terms of handing the laser pulse instrument. [6] Lastly is the micro-exclusion-based technique, the technique requires the ink and living microorganisms or biomolecules to be loaded onto the dispenser. The disperser system can either be a pressurizing gas or physical press such as piston. The dispensing system allows the ink to be ejected out into the printing surface. The advantage of this technique is to provide a high cell density in the printed object. While the disadvantage is that it has the lowest cell viability among 3 technique with cell survival rate between 40-80%. [7] This is due to the sheer stress that is applied to the ink and cells, of which magnitude can greatly affect the cell survival rate. [8] Therefore, as a part of this research, it is crucial to understand the fundamental knowledge of 3D bioprinting, which will have an effect later on in this work.

1.3.2 Bioink

One of the most crucial part for performing a 3D printing is the choice of bioink. The term "bioink" is used to describe a 3D printing ink that is applied to the biological specimen such as living microorganisms or biomolecules. [9] Typically, an ideal bioink should possess the following properties. The bioink should be biocompatible. The inks itself should not be harmful to the living specimen once it has been dispersed into the ink solution. The ink should be biodegradable, meaning that it degrades naturally overtime. The ink should mimic a natural environment living microorganisms or biomolecules. [9] Besides the mentioned properties, other properties such as proper mechanical and rheological properties are equally important, as they play a major role of retaining the structure once the object is printed.[10]

Alginate

One of the materials commonly used for bioink in 3D bioprinting is alginate. Alginate is an anionic polysaccharide composing of 2 blocks of (1-4) β-D-mannuronic acid (M Block) and α-L-guluronic acid (G Block) (**Figure 1.1**). [11] The material can be isolated from the cell walls and intracellular space of a brown algae. [12] The material itself have been used in many biomedical applications due to low cytoxicity, good biocompatibility and low cost. [13] The gelation of alginate can occur physically by using a divalent ion form an ionic crossbridge between 2 polysaccharide chains. [14] The gelation process can occur fast, which is one of the reasons for applying this material to a 3D bioprinting. One of the disadvantages of alginate is the nature of the material being bioinert. Without any structural modification, the material will have a low cell-adhesive moiety, which limits the biological application. [15]

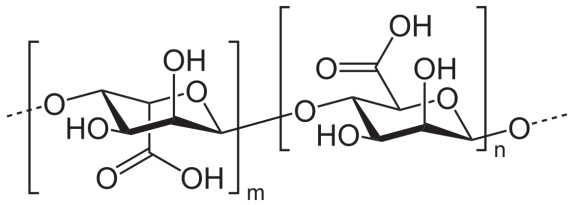


Figure 1.1 Chemical structure of alginic acid

In 3D bioprinting application, Narayanan and coworkers have found a strategic way to overcome the disadvantages of alginate by incorporating other biocompatible polymers into the bioink. In this work, the author managed to encapsulate human adipose derived stem cell by using the combination of alginate and polylactic acid, which the material was printed via micro-exclusion 3D bioprinting technique. The results showed that adding the polylactic acid did not have any significant change to the mechanical properties when compared to the pure alginate gel. This can

be implied that the material is printable. For cell viability test, the results showed that polylactic acid nanofibers has the potential to aid the cell metabolism as well as cell proliferation. [16]

Collagen

Collagen is a fibrous natural protein, which is one of the major components in extracellular matrix. The material is well known for excellent biocompatibility as well as low immunogenicity due to the material is mimicking the extracellular matrix, which makes the material applicable in a biomedical application. In 3D bioprinting technology, collagen had been considered a choice for bioink component. [17] One work of Koch and coworkers involves that usage of collagen a bioink to retain keratinocytes and fibroblast, then the formulated bioink is printed using laser-assisted printing technique in 3D bioprinting. The result of this work shows that the cell in a printed collagen is able to evolve the intercellular adhesion as well as cell communication, which is a sign of tissue development. [18] Kim and coworkers have developed a new collagen bioink by incorporating a crosslinking agent for the purpose of high porous hydrogel for cell culturing. In this work, the material was printed together with the human adipose derived stem cell. The results showed that the printing was able to provide the gel with high porosity and stable in terms of mechanical properties. Also, the new bioink formula enable the cell to proliferate without any harm during printing. [19]

Pluronic F-127

Pluronic F-127 is a trademark name for Poloxamer 407 that is used for many applications including biological applications due to its biocompatibility. [20] It is triblock copolymer of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO_{100}-PPO_{69}-PEO_{100}) (**Figure 1.2)** [21]. Due to the nature of the compound being amphipathic, it contains both hydrophobic part (PPO) and hydrophilic part (PEO). Thus, the polymer chain can behave like a non-ionic surfactant [22]. Another interesting property of this material is the thermal responsive gelation which is a reversible gelation process. In a high temperature condition, the F-127 chains self-assemble to form micelles. The micellization allows the micelles to pack closely, which induces the rigidity and increases the viscosity of the gel until it becomes a solid gel. [23] The ability of self-assembly allows the material to have a property of shear thinning. The viscosity decreases when a shear stress is applied to the material. This property is crucial for cell survivability. [24] Although this criterion is considered perfect for fabricating hydrogel for biological application. There is a limitation towards the pure F-127 hydrogel. The drawbacks of pure F-127 are the low viscosity at 37°C, which is inadequate for clinical application. Also, the cell resident time is low, this can cause a long-term problem during cell proliferation. In order to overcome this problem, Sun and coworkers

have come up with a solution by incorporating the crosslinkable acrylate group to the F-127. [25] This method opens up many possible modification and reaction. One application is forming hydrogel with high mechanical strength through crosslinking by the usage of photoinitiator.

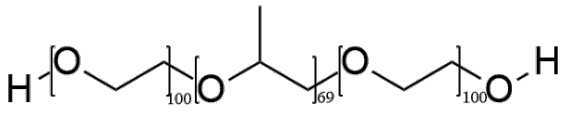


Figure 1.2 Chemical structure of Pluronic® F-127

There are previous works on the usage of Pluronic in a 3D-Bioprinting technology. One work was done by Müller and coworkers, which was to develop the bioink by using Pluronic F-127 for the cell culturing application. Due to the low cell viability of Pluronic, the author had compensated the problem by modifying the nanostructure of the hydrogel. This was done by combining diacrylate Pluronic and normal Pluronic. Then the ink was applied together with bovine chondrocytes in a 3D Bioprinter. After printing and photocrosslinking, the normal Pluronic was washed off the hydrogel. The results showed that the modification of nanostructure of the hydrogel increased the cell viability up to 14 days. In terms of printability, the gel was able to retained its shape and easily handle around using tweezer. [26]

1.3.3 Zwitterionic monomer and antifouling properties

Zwitterionic hydrogel is a type of hydrogel that contains a group with both positive charge and negative charge. In the biological fields, materials that are used for biosensors, drug delivery, etc. have a high tendency to interact with other substance in the body. The worst scenario is some of the biological substance can non-specifically adsorb onto the device. This would deteriorate the device performance. [27] Several solutions have been made to solve this problem. One of the solutions is to apply the antifouling polymer coating such as poly (ethylene glycol) (PEG). However, the terminal hydroxyl groups of PEG can undergo oxidation, and resulting in the aldehyde groups which can react with proteins. Therefore, zwitterionic hydrogel has been used as an alternative antifouling material. The material is able to provide a much better performance. [28] This research is interested in two zwitterionic monomers which are sulfobetaine methacrylate (SBMA) (**Figure 1.2)** and 2-methacryloyloxyethyl phosphorylcholine (MPC) (**Figure 1.3)**.

SBMA is a zwitterionic monomer that contains methacrylate and sulfobetaine. This compound has been reported to have excellent antifouling property, due to the high hydrophilic property. [29, 30] Beside antifouling property, the material also has an excellent biocompatibility. One other usefulness of SBMA is the functionalization of the zwitterionic group, which allows the

group to be modified to enable the selective cell adhesion. This makes the compound very popular among the choice of material for antifouling purpose.

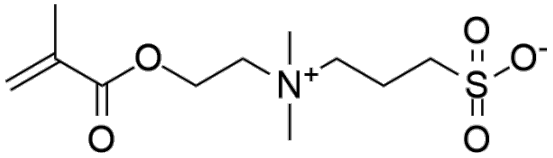


Figure 1.3 Chemical structure of SBMA.

MPC is a another zwitterionic monomer of which design is inspired by phospholipid of cell membrane. [31] The natural membrane consists of a phospholipid bilayer containing an external surface of hydrophilic phosphorylcholine (PC) moieties. MPC polymers have been generally used for biomedical fields owing to their great hydrophilicity, nontoxicity, and antibiofouling behaviors. [32, 33]

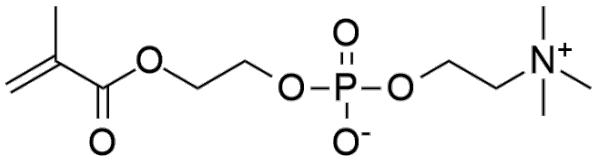


Figure 1.4 Chemical structure of MPC**.**

Chapter 2

Experimental

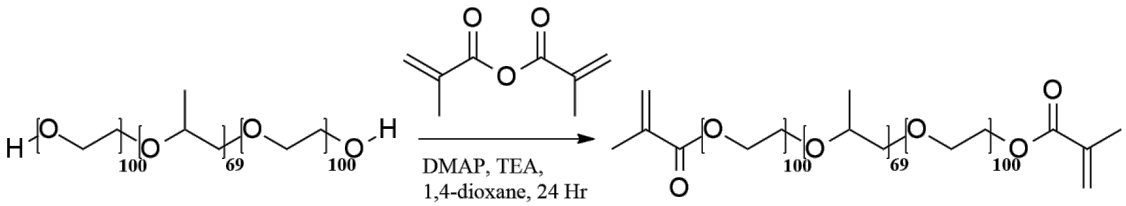
2.1 Materials

Pluronic® F-127 (PEO_{100}-PPO_{65}-PEO_{100}) (M_n = 1.26 x 10^3), 4-(dimethylamino)pyridine (DMAP), methacrylic anhydride (MA), phosphate buffered saline, pH 7.4 (PBS), Irgacure 2959, albumin–fluorescein isothiocyanate conjugate (FITC-BSA) and anhydrous 1,4-dioxane (99.8%) were purchased from Sigma-Aldrich, USA. Both triethylamine and [2-(methacryloxloxy) ethyl] dimethyl-(3-sulfopropyl) ammonium hydroxide (SBMA) was supplied by Tokyo Chemical Industry, Japan. Diethyl ether (analytical grade) was commercially available from Merck and used as received. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was supplied by NOF Co., Ltd., Japan. 3D printing syringes and 25-gauge blunt end needles were purchased from Nordson Medical, USA.

2.2 Instruments

All of the proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at room temperature using JEOL (Japan) operating at 500 MHz using deuterated chloroform as a solvent. Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) analysis was performed using a Bruker Alpha II with 32 scans at a resolution of 4 cm^{-1}. The printed hydrogels were obtained via 3D-Bioplotter® Envisiontec, Gladbeck, Germany. All hydrogels were prepared by ultraviolet (UV) irradiation (365 nm) with UV nail lamp (ZH-818A, 36 W, China).

2.3 Synthesis of F-127 dimethacrylate (F-127 DMA)



Scheme 2.1 Synthetic route of F-127 DMA**.**

F-127 DMA was synthesized according to a modified method from previously published work [34]. Dried powder of Pluronic® F-127 (6.500 g, 0.5158 mmol) was weighed into a round bottom flask and dissolved in 50 mL of 1,4-dioxane. After the reactant was dissolved, 4-

dimethylaminopyridine (130 mg) and triethylamine (1.4 mL) was added to the Pluronic F-127 mixture as a catalyst and the mixture was stirred for 2 h at room temperature. Methacrylic anhydride (465 µL, 3.095 mmol) was added to the solution and kept the reaction going at room temperature. After 24 h, the light-yellow solution was reprecipitated in cold diethyl ether (300 mL), the white precipitate was collected by centrifugation. The reprecipitate step was repeated for 2 more times with less amount of cold diethyl ether (50 mL). Lastly, the white solid product was vacuum-dried overnight to remove excess solvent. The product was characterized by ATR-FTIR spectroscopy and ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectrum of the product was used to calculate percentage of substitution by the following equation.

$$\% \text{ Substitution} = \frac{3}{4}\frac{(\Sigma I_{VP})(N_{PPO})}{I_{ME}} x\ 100\% \qquad \text{eq.2.1}$$

I_{VP} = Integration value of the vinyl proton (δ_1=5.6 ppm, δ_2=6.2ppm)

I_{ME} = Integration value of methyl proton (δ_1=1.2 ppm)

N_{PPO} = Number of repeating units of poly(propylene glycol)

2.4 Swelling experiment

The swelling experiments were performed on the series of printed hydrogel. The hydrated samples were freeze-dried for 24 h to remove water content within the hydrogel. The dried samples were weighed, measured their size, and placed in the microplate. After that, PBS (2 mL) was added to the dried sample and placed in an oven at 37 °C. The samples were incubated for 24 h. The samples were then taken out, reweighed and remeasured for their size. The percentage of swelling can be calculated according to the following equation.

$$\text{Swelling Ratio} = \frac{W_{Post} - W_{Pre}}{W_{Pre}} x\ 100\% \qquad \text{eq.2.2}$$

W_{Post} = Weight of the gel after rehydration

W_{Pre} = Weight of the gel before rehydration

2.5 Preparation of zwitterionic hydrogel

In order to formulate a successful 3D printed hydrogel, the bioink formula must be considered. All bioink formula are listed in **Table 2.1**. To duplicate bioink for different condition, first dried F-127 dimethacrylate was equally weighed into a microwell. Then follow by dropping 250 µL of the stock solution dissolved in PBS composing of Irgacure 2959 photoinitiator and zwitterionic monomer, SBMA or MPC. Next, the bioink samples were cooled at 4 °C overnight to allow the solid F-127 dimethacrylate fully dissolve. Finally, the bioink samples were taken out to the room temperature and radiated with UV lamp (365 nm) for 2 min.

Table 2.1 Bioink formula.

	Control	Mole ratio (F-127 DMA : SBMA)				
SBMA	1:0	1:2	1:4	1:6	1:8	1:10
Irgacure 2959	0.03M					
F-127 DMA (% w/v)	25%					
	Control	Mole ratio (F-127 DMA : MPC)				
MPC	1:0	1:2	1:4	1:6	1:8	1:10
Irgacure 2959	0.03M					
F-127 DMA (% w/v)	25%					

2.6 3D printing of zwitterionic bioink

All bioink formulas were prepared with the same method as mentioned in section 2.5. The bioink was loaded onto the 3D bioprinter syringe affixed to a blunt needle as shown in **Figure 2.1**. Before loading the syringe into the holder of the machine, the syringe was heated until the bioink was slightly warm. This step was performed to raise the ink viscosity, which was considered very crucial for printing. The instrument was operated via Visual Machine software (Version 2.10.130r10), and ink injection pressure was optimized to be running at 2.5 bar with 10 mm/sec speed. The 10×10×5 mm^3 block of the printed object can be seen. The printed gels were then exposed to UV light (365 nm) to crosslink the structure for 2 min.

Figure 2.1 3D-printing syringe with 25-gauge blunt needle.

2.7 Determination of antifouling property of zwitterionic hydrogel

The non-specific protein binding property of the zwitterionic hydrogel was tested on the printed 10x10x5 mm^3 block hydrogel by using fluorescent-labeled bovine serum albumin (FITC-BSA) as a protein model. First, the samples were immersed under PBS, pH 7.4 overnight. After this step, the samples were transferred from PBS solution to FITC-BSA solution (1 mg mL^{-1}). Then the samples were incubated in the oven for 6h at 37 °C. Next, the samples were removed from the oven and rinsed 3 times with PBS, pH 7.4. Lastly, the fluorescence stains of the protein were observed under UV lamp.

Chapter 3

Results and Discussion

3.1 Synthesis and characterization of F-127 DMA

F-127 DMA was successfully synthesized with 78% yield via the reaction between OH-terminated Pluronic and methacrylic anhydride in order to be used as hydrogel crosslinker. The ^{1}H-NMR spectra depicted in **Figure 3.1** revealed the signals of Pluronic F-127 before and after modification. The signals at 1.2, 3.2-3.8, and 4.5 ppm are contributed to characteristic protons of PEO and PPO units. After the modification, the F-127 DMA spectrum displays an incorporation of methacrylate groups at the ends of the polymer. The vinyl protons of the methacrylate groups ($=CH_2$) appeared at 5.6 ppm and 6.1 ppm, and the methyl protons of the substituent ($=CCH_3$) appeared at 1.9 ppm. Moreover, by taking the ^{1}H NMR integration value of the vinyl groups of the modified methacrylate ends and methyl groups of PPO units, the degree of substitution of the product was found to be 93%, suggesting that the functionalization strategy was successful.

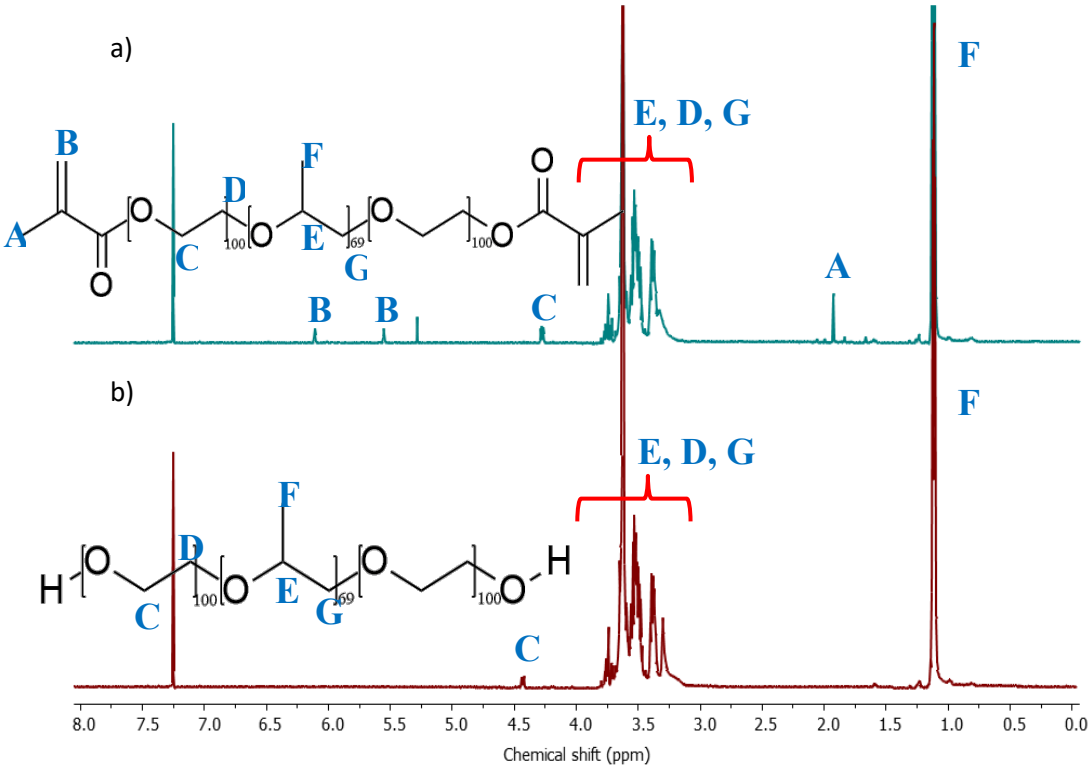


Figure 3.1 ^{1}H-NMR spectra in $CDCl_3$ of (a) F-127 DMA and (b) F-127.

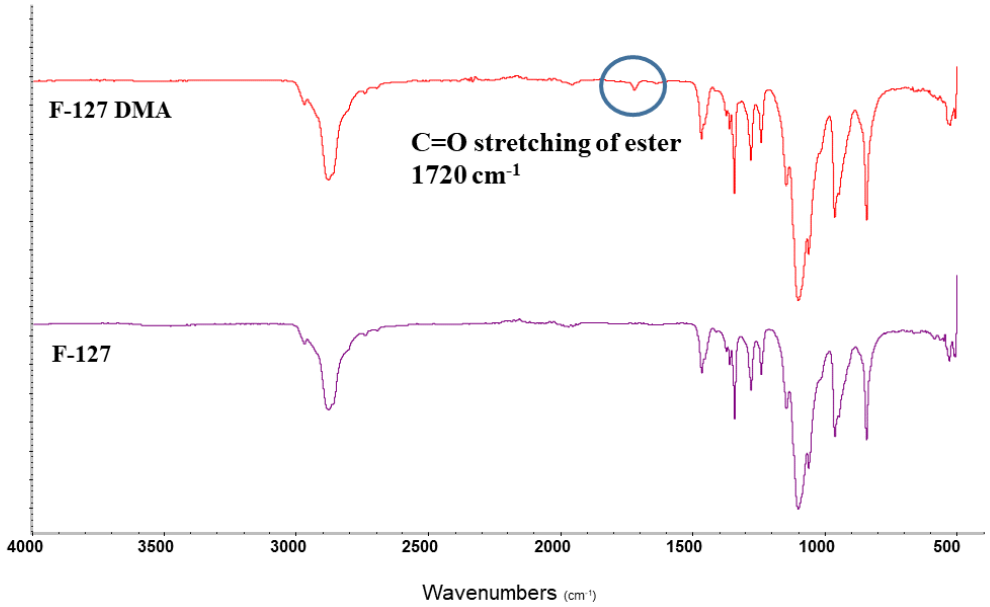


Figure 3.2 ATR-FTIR spectra of F-127 and F-127 DMA.

Another evidence that supports the successful synthesis of F-127 DMA was from FTIR analysis. **Figure 3.2** illustrates the comparison between IR spectra of F-127 and F-127 DMA. The emergence of ester signal (C=O stretching) at 1720 cm^{-1} suggested that there was ester bond formation as a result of the introduced methacrylate moieties into the F-127 structure.

3.2 Preparation of zwitterionic hydrogel

In this part, the main objective is to find a suitable bioink formula that can be applied to the 3D bioprinter. The criteria focuses on the morphology of the printed hydrogel as well as the ability of the hydrogel to retain its shape. **Figure 3.3** shows the images of the hydrogels prepared from different formula. By observing the controlled sample, the pure F-127 DMA gel was able to form a perfect shape of the well and the gel can hold its shape and can be easily handled. This indicates that the F-127 DMA alone was able to form a stable hydrogel network. Upon adding the zwitterionic monomer, the ability to form the hydrogel is slightly deteriorated. The gels showed some cracks and defects especially those having quite high composition of zwitterionic monomers (at ratios of 1:6, 1:8, 1:10). For the SBMA-based hydrogels, the composition of 1:6, 1:8, 1:10 yielded gels with non-uniform shape and almost impossible to be handled. Apparently, the addition of both SBMA and MPC reduced the ability to form hydrogel.

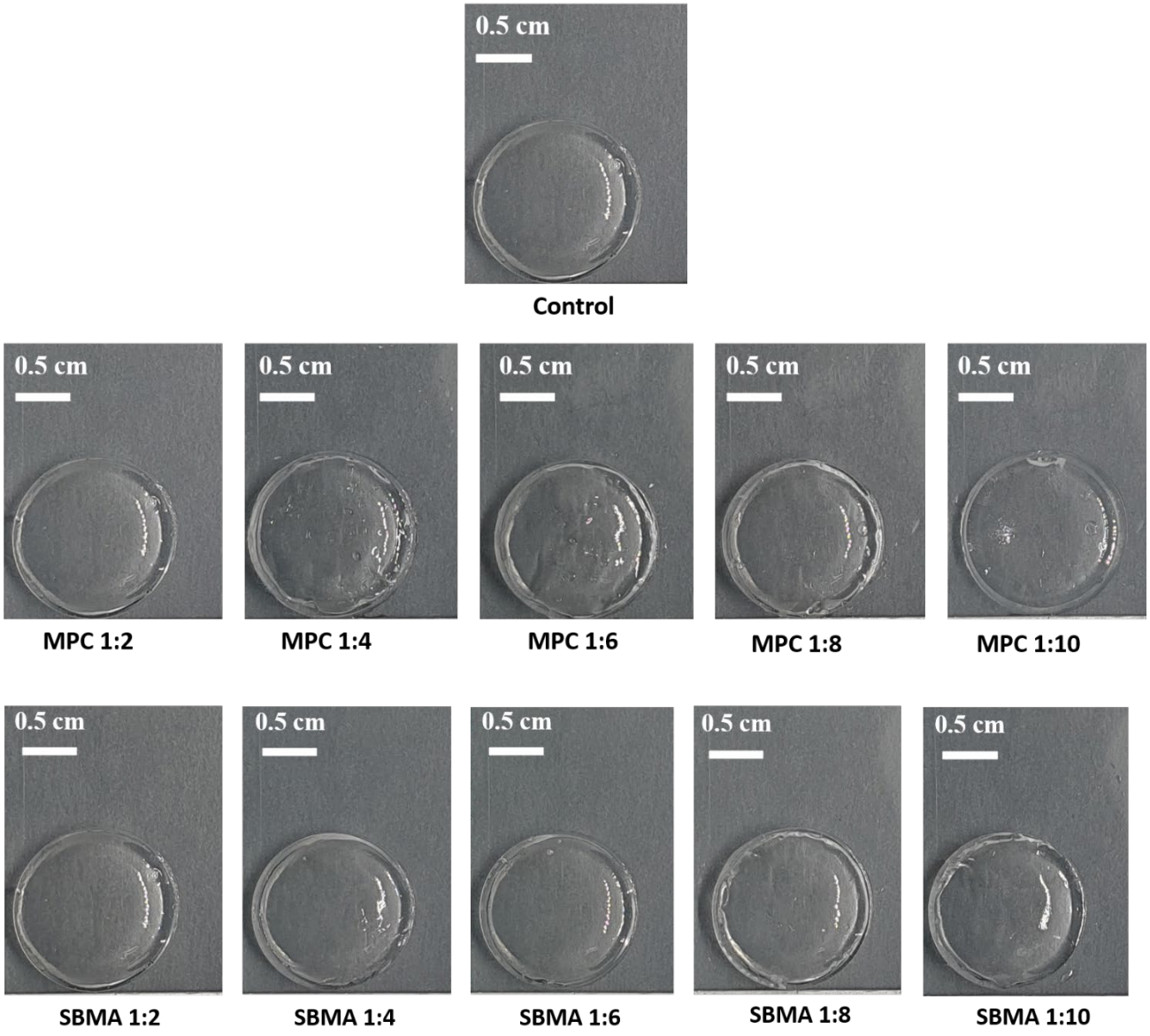


Figure 3.3 Appearances of hydrogels obtained from inks having different formula.

The main goal of this part is to use the formulated inks for 3D-printing. **Figure 3.4** shows the printed hydrogels fabricated from inks having different formula. The results showed a similar trend with those shown in **Figure 3.3**. The printed hydrogels from formula having the ratio of F-127 DMA: SBMA of 1:4 and 1:6 collapsed and cannot maintain their cubic shape. The formula having the ratio of 1:8 and 1:10 were not printable. Unlike the SBMA-based inks, the MPC-based inks of all ratios were printable. The printed hydrogels can retain their shape without collapsing

except that was fabricated from the ink having the composition of F-127 DMA: MPC of 1:10. The hydrogel collapsed as indicated by the pore becoming narrower than the expected dimension.

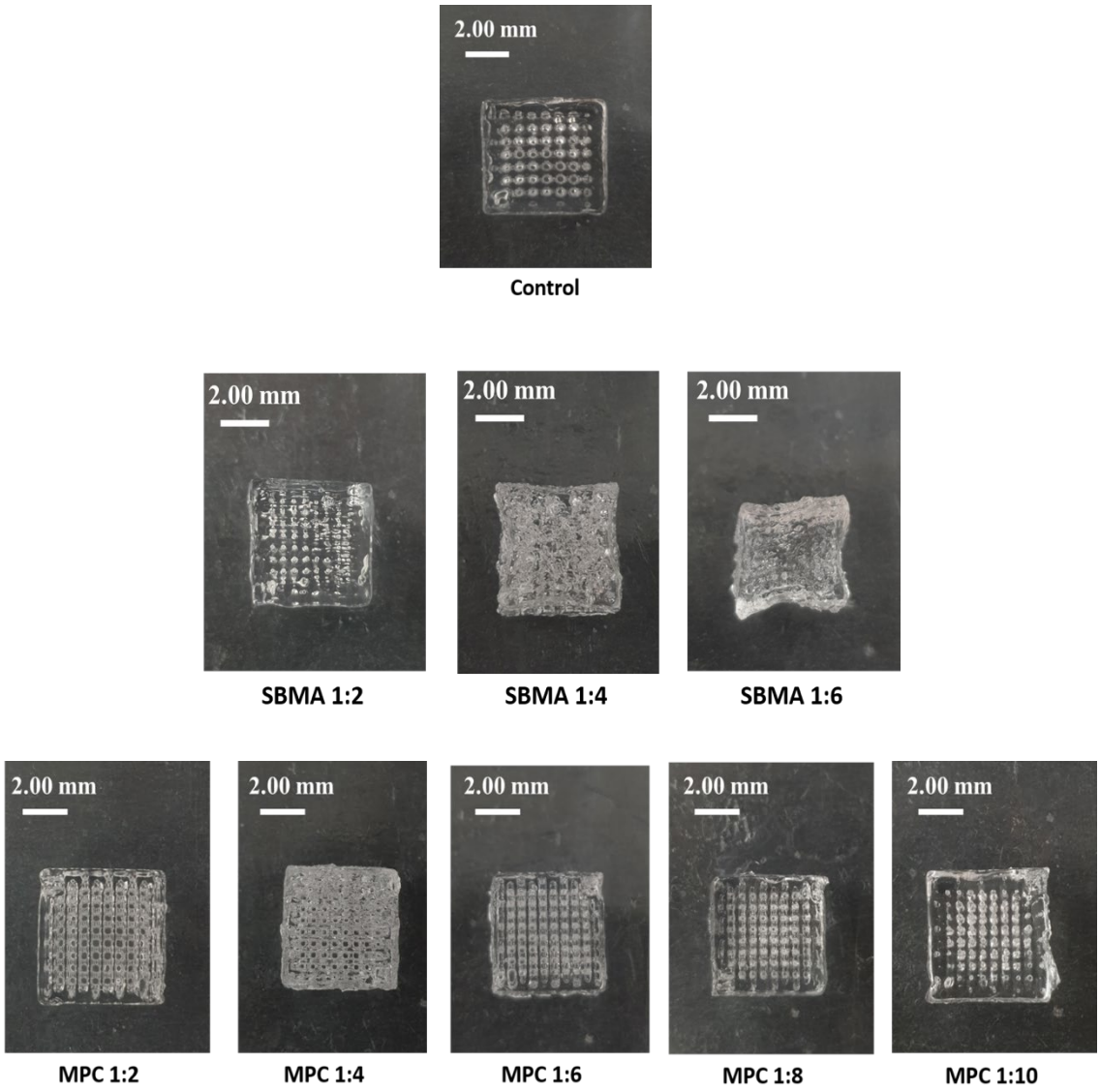


Figure 3.4. Appearances of the 3D printed hydrogels obtained from inks having different formula.

3.3 Swelling property

In order to apply the hydrogel for soft tissue engineering, swelling behavior is one of important factors for cell growth to allow nutrients and air exchange. The swelling test, defined as the fractional increase in the hydrogel weight due to water absorption, was performed as displayed in **Figure 3.5**. The hydrogels fabricated from the inks having the F-127 DMA:SBMA ratios of 1:2 and 1:4 showed similar swelling ratios to the F-127 DMA hydrogel. The swelling ratio of SBMA-based hydrogel fabricated from the F-127 DMA:SBMA ratios of 1:6 increased to nearly 600%. This high swelling property can be used as an evidence to explain the collapse of the printed hydrogel fabricated from the F-127 DMA:SBMA ratios of 1:6 from **Figure 3.4**. This result follows the same trend reported by Sun and Co-workers in that the increasing swelling ratio as a function of SBMA. The hydrophilic property of the SBMA has a potential to increase the osmotic pressure of the hydrogel and allows more water to flow in causing hydrogel to swell better. [25] In contrast, incorporating MPC decreased swelling property of the resulting hydrogels. This is the reason why all MPC-based printed hydrogels fabricated from all formula can well maintain their shapes.

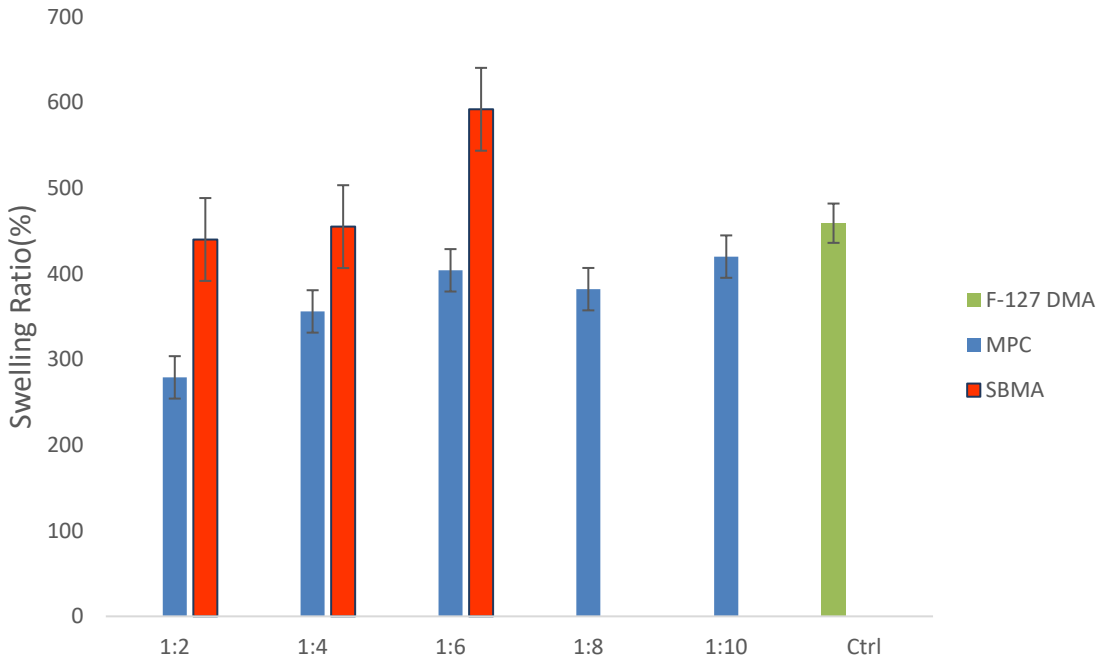


Figure 3.5 Swelling ratio of F-127 DMA hydrogel (Ctrl) and zwitterionic hydrogels obtained from inks having different F-127 DMA : zwitterionic monomer ratio (1:2, 1:4, 1:6, 1:8, and 1:10) at 37 °C in PBS buffer, pH 7.4 (n=3).

3.4 Antifouling property

The properties that are considered one of the most important attributes in creating hydrogel for biological applications is the antifouling property. To evaluate the property, the protein adsorption test was performed. **Figure 3.6** illustrates the series of hydrogel that have been adsorbed by FITC-labeled BSA as the protein model, of which the adsorption capacity was determined from the green fluorescence signal of the FITC-BSA. As a result, it showed that there were negative fluorescence responses from 3 samples. This indicated that no protein was adsorbed onto the hydrogel, which was the true intention of this material. However, the negative fluorescence signal from the control sample could imply that the adding zwitterionic monomer to the hydrogel did not have any significant effect on the antifouling property of the material. In theory, zwitterionic monomer has an ability to resist biological compound adhesion. This means the antifouling property should enhance upon adding SBMA and MPC. Ideally the fluorescence intensity should be lower than F-127 DMA without zwitterionic monomer. There are two possible explanations that can clarify this phenomenon. First is the nature of the F-127 itself, the co-polymer chain contain mainly poly(ethylene glycol) (PEG) block, which has a hydrophilicity property and give a potential to resist biological compound adhesion [28]. Second is the pore structure of the hydrogel, the antifouling protocol required the hydrogel to be submerged under PBS for a certain of time. This could result in a swelling of the hydrogel and causing the pore opening to reduce in size, which could limit the amount of protein passing through the hydrogel pore. However, to clarify this hypothesis the gel morphology analysis is needed.

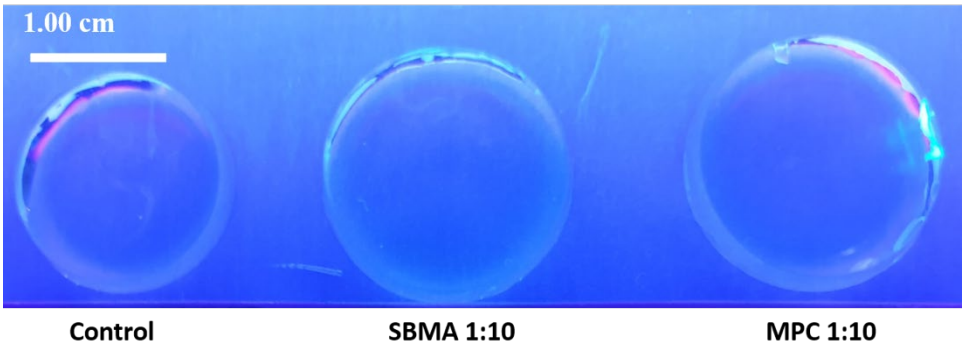


Figure 3.6 Appearances of hydrogels after exposing to FITC-labeled BSA.

Chapter 4

Conclusion

The aim of this project is to be able to successfully fabricate zwitterionic hydrogel through 3D-bioprinting. The tasks in the objective were able to complete well. However, according to the result, several things are need to be adjusted in future work. This study showed that by adding the zwitterionic monomer, MPC in the ratio of 1:2-1:10 into the hydrogel system gave gels with slightly shape deformation, while SBMA in the ratio of 1:2-1:6 yielded gels that cannot maintain their shape, The ratio of 1:6 gave the gel having lost shape complexly. This shows that upon adding zwitterionic into the hydrogel system, the mechanical strength decreases. One reason that can partially support this is the swelling experiment which shows that SBMA has a very high swelling ratio, thus can be implied that more water is flowing into the gel causing the gel to hold too much water and collapse. From the experiences in this project had showed that more parameters are needed to be set out into this work. For example, the temperature must be more precisely control, this is due to the thermal responsive nature of F-127 DMA that can shift the out come of each experiment. Also, more investigations are needed to perform on the result occur in the zwitterionic hydrogel, which can lead to a better solution in future. Another thing that can be performed into this work is adding another hydrogel network and make it a double network hydrogel, which have shown an acceptable mechanical strength and antifouling property[25].

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Biography

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