Novel Biodegradable Polymer based on Low Molecular Weight Hydrolyzing Units

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Abstract

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Polymer scaffolds can be used to restore, maintain, or improve tissues and organs. Poly(2-hydroxyethyl methacrylate), also known as pHEMA, is a commonly used polymer in tissue engineering due to its versatility in application. A major problem is that pHEMA is non-degradable. pHEMA can be made degradable by crosslinkers and a degradable initiator. A previous study from the Ratner lab found that degradation products of pHEMA that are <5kDa are soluble in the environment of the body and can be cleared from the body. Building upon that study, we wanted to synthesize pHEMA with an even lower molecular weight. This paper focuses on synthesizing 2-bromoethyl 3-bromopropanoate to use as a bi-functional initiator for atom transfer radical polymerization (ATRP) because it has an ester group between two organocarbon units and symmetric bromine leaving groups; the initiator was then used to synthesize symmetric, low molecular weight (<2kDa each) pHEMA chains growing from each

Br with ATRP synthesis. Steglich esterification reaction with anhydrous dichloromethane (DCM) as the solvent had the highest yield at 14.6%. Copper bromide was used as the catalyst and the ATRP reaction was ran for 24 hours. Various combinations of ligands, solvents, and temperatures were used to increase the conversion of HEMA monomer to pHEMA. There was 100% conversion for the condition where 1,1,4,7,10,10-hexamethyl-triethylenetetramine (HMTETA) was the ligand, methanol/water (50:50, v/v) was the solvent, and at both room temperature and 50°C. pHEMA was crosslinked with 5% EDGMA using various solvents, temperatures, and stir bar conditions. 2-bromoethyl 3-bromopropanoate is a promising initiator to synthesize degradable, low molecular weight pHEMA hydrogels for cardiac tissue engineering applications.

Introduction

Cardiovascular disease is one of the leading causes of death in the United States[1]. Cardiomyocytes have limited regenerative capabilities and are unable to heal themselves. Instead, the damaged tissue becomes nonfunctional scar tissue that prevents the heart from efficiently pumping blood throughout the body[2]. The current therapies used to address damaged cardiac tissues are heart transplants performed by physicians and blood thinners to minimize the strain on the heart to pump blood[3].

Tissue-engineered scaffolds can be used to restore, maintain, or improve tissues or organs[4]. Scaffolds are versatile engineering tools in tissue engineering and can serve many purposes. Scaffolds can be used to implant artificial vasculature to increase flow of nutrients and gases for cardiac repair[5], to diffuse therapeutic drugs at a tunable rate for localized delivery[6], or to seed with cardiomyocytes and growth factors to facilitate 3D cellular growth and cell differentiation for tissue regeneration[7] to improve functionality of the heart.

There are a variety of natural polymers such as fibrin, collagen, gelatin, alginate, Matrigel, and chitosan in addition to synthetic polymers such as PGA, PLLA, PLGA, PCL, PU, and PEG used for cardiac tissue engineering[8]. Some of the advantages of the natural polymers are their low toxicity, good biocompatibility, water absorption, controlled biodegradation rate, and elasticity while some of the disadvantages includes weak mechanical properties, lack of integrity, and high hydrophilicity. Biocompatible scaffolds decrease the likelihood of a local or systemic immune response from the body to the material. Water absorption is necessary for the water to hydrolyze the scaffold for degradation. Elastic properties of the scaffolds help mimic the contractility of cardiac tissue to minimize mechanical mismatch that would lead to rejection of the tissue. Lack of integrity is a disadvantage for some natural polymers because they can be too

soft to accurately mimic the cardiac tissue. Too hydrophilic means that the scaffold will not be able to provide adequate cell adhesion and cell proliferation. While the aforementioned synthetic polymers have biocompatibility and biodegradability, their disadvantage is the long biodegradation rate. A degradation rate too slow or too fast can impair the development of new tissue[9].

Poly(2-hydroxylethyl methacrylate) (pHEMA), a well-known methacrylate, is biocompatible, hydrophilic, elastomeric, inexpensive, non-degradable and can mimic tissue-like mechanical properties; it has been used in a variety of applications such as contact lenses, drug delivery vehicles, tissue engineering scaffolds, bone tissue regeneration, wound healing, and cancer therapy[10,11]. A disadvantage for our application is the lack of degradation; however, a degradable unit within the backbone chain or in the crosslinker will make the pHEMA scaffold biodegradable. A study that came from the Ratner lab showed that chains of pHEMA that are approximately <5kDa are water-soluble and can be cleared out of the body through renal clearance[12]. The lowest molecular weight of pHEMA synthesized was approximately 6kDa although the theoretical weight for the given condition was 2kDa. We wanted to find an alternative, controlled way to decrease pHEMA to <5kDa.

Atom transfer radical polymerization (ATRP) is a technique discovered and publicized by the Matyjaszewski Polymer Group at Carnegie Mellon University and has been used to synthesize controlled molecular weight pHEMA with low polydispersity[13]. The initiator utilized for the synthesis can greatly alter the activation rate constant of ATRP depending on its degree of substitution, type of transferable atom, and the radical stabilizing group[14]. In addition, temperature, pressure, solvent type, and the catalyst and ligand complex can alter the equilibrium

of ATRP in interdependent ways[15]. The conditions and environment of ATRP is integral to the efficacy of the reaction.

Another study that came from the Ratner lab showed the feasibility of using a haloester with two carbons between the halogen and ester as an initiator to synthesize pHEMA through ARGET ATRP[16]. The degree of substitution is low for this initiator because it is a primary radical with an ester two carbons away. This distance between electron density of the ester and the free radical of the initiator decreases the stability of the initiator, causing low radical concentrations and decreasing termination reactions[14].

We predicted that the initiator, 2-bromoethyl 3-bromopropanoate, shown in Figure 1, would have a low ATRP activation rate that can synthesize a controlled, low molecular weight polymer. The symmetric bromine groups facilitate symmetric pHEMA synthesis. We wanted to synthesize a polymer, shown in Figure 2, that is about 4kDa, with a 2kDa chain on each side of the initiator to ensure that degradation products can be solubilized in the bloodstream and can safely be cleared from the body. The ester linkage in the middle of the initiator is degradable through a hydrolytic and enzymatic reaction.

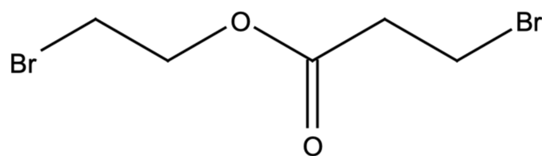


Figure 1. The structure of the desired 2-bromoethyl 3-bromopropanoate initiator for symmetric pHEMA synthesis. The molecule is an ester with bromine groups on either end.

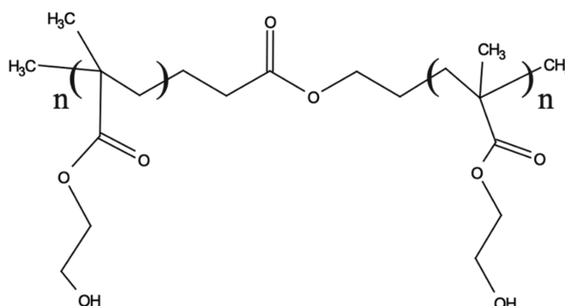


Figure 2. Structure of pHEMA synthesized by the symmetric initiator. The goal is to have 2kDa HEMA chains on either end which approximates to about n=15 repeating units of HEMA.

We synthesized the desired initiator through Steglich esterification. In this type of esterification, 4-Dimethylaminopyridine (DMAP) is used to activate the carboxylic acid and N,N'-dicyclohexylcarbodiimide (DCC), is used to capture formed water[17]. We tested different solvents and mole ratios to increase the yield. Then, we used the initiator to synthesize pHEMA via ATRP. We tested different combinations of solvents, temperatures, and ligands to find conditions that can facilitate polymerization despite the low degree of substitution of the initiator. Finally, we synthesized pHEMA crosslinked with a non-degradable crosslinker and studied the hydrogel degradation profile.

Materials

For the synthesis of initiation reaction, 2-bromoethanol (95%, Sigma Aldrich), 3-bromopropionic acid (97%, Sigma Aldrich), DMAP (98%, Oakwood Chemical), and DCC (99%, Sigma-Aldrich) were used. The reaction schematic is in the Appendix as Figure S1. The three solvents tested were DCM (99.9%, Fisher Chemical), anhydrous DCM (99.8%, Sigma-Aldrich), and THF (99.9%, Sigma-Aldrich). Prepared anhydrous THF by adding THF to a bottle with molecular sieves to remove moisture. The materials used for the filtration and column chromatography were sodium chloride (99%, Fisher Chemical), granular anhydrous sodium

sulfate (99.4%, J.T.Baker), hexane (98.5%, Sigma-Aldrich), ethyl acetate (≥99.7%, Sigma Aldrich), and silica gel (Sigma-Aldrich). The eluted products were checked with TLC silica gel 60 F_{254} (MilliporeSigma) and iodine flakes (99.8%, Fisher Chemical). For NMR analysis chloroform-d (99.8%, Sigma-Aldrich) was used.

Copper (I) bromide (99.999%, Sigma-Aldrich), 2-hydroxyethyl methacrylate monomer – Ophthalmologic grade (HEMA, 99%, Polysciences), ethylene glycol dimethacrylate (EDGMA, 98%, Polysciences) were used for ATRP synthesis reactions. 2,2'Bipyridyl (bpy, ≥99.7%, Sigma Aldrich) and 1,1,4,7,10,10-hexamethyl-triethylenetetramine (HMTETA, 97%, Sigma-Aldrich) were used as the ligands. 1-propanol (99.5%, TCI), 2-butanone (MEK, 99%, Acros Organics), methanol (99.9%, Sigma-Aldrich), and DI water were used as solvents. For NMR analysis of pHEMA methanol-d4 (99.8%, Sigma-Aldrich) were used. Diethyl ether (99.9%, Fischer Chemical) and acetone (99.5%, Fischer Chemical) were used for purification. The degradation study used sodium hydroxide solution N/10 (certified 0.0995-0.1005N, Fisher Chemical).

Hydrogen-1 Nuclear Magnetic Resonance (^{1}H NMR) instrumentation with 300.13MHz with Bruker PABBI probe was used to analyze the samples. The BUCHI-Rotavapor R-205 was used to evaporate the solvents from the product. The 5873 Mass Selective Detector was used to predict the mass and structure of the initiator.

Methods

I. Synthesizing the initiator

Steglich esterification was used to synthesize the initiator and different solvents and mole ratios were used to optimize the yield. The amount of compounds presented in the methods section is reflective of the mole ratio 1:1.5:0.2:1.2 for 3-bromopropionic acid (BPA), 2-

bromoethanol (BE), DMAP, and DCC, respectively. BPA (MW = 153, 0.765g), BE (MW = 125, 0.938g), and DMAP (MW = 122.17, 0.122g) were dissolved in the chosen solvent of anhydrous THF, DCM, or anhydrous DCM (10mL). DCC (MW = 206.33, 1.238g) dissolved in 2mL of solvent was slowly added to the reaction. For anhydrous DCM, a pressure-equalizing addition funnel was used and purged with nitrogen gas for an hour. The reaction was cooled with an ice bath and stopped after 48 hours.

The DCC-urea product was precipitated out as a white powder and separated through filtration. A separatory funnel was used to further separate the impurities from the desired product. DI water and the product dissolved in ethyl acetate were added to the funnel and mixed to create two separate layers. Two drops of water saturated by sodium chloride were added to the funnel to facilitate further emulsion separation. The water layer was removed and anhydrous sodium sulfate was added to the ethyl acetate layer until the residual water was quenched.

Column chromatography with silica gel and hexane/ethyl acetate (90:10, v/v) was used to extract the desired product. TLC plates were used to evaluate the unique compounds being collected. NMR tests were conducted to analyze the sample per unique product on the TLC plate and to find the flask with the desired initiator.

II. ATRP Reactions

Previously methanol/water[18], just methanol[18], and 2-butanone (also known as methyl ethyl ketone or MEK)/1-propanol[13] have been successful at synthesizing pHEMA. The mole ratio of reagents utilized in ATRP synthesis was chosen to maximize activation kinetics[18, 19]. ATRP was used to synthesize pHEMA and also pHEMA hydrogels cross-linked with 5% EDGMA. Test tubes of solvents like methanol, DI water, 1-proponal, 2-butanone (also known as

methyl ethyl ketone or MEK) were purged with nitrogen gas for an hour. For just pHEMA synthesis, the initiator, catalyst, ligand, and HEMA monomer was added at a ratio of 1:1:2.5:100, respectively. The initiator (MW = 260, 10mg), HEMA (MW = 130.14, 0.50g), and stir bar were added to a test tube and purged for 10 minutes. And for the synthesis of pHEMA cross-linked with EDGMA, the initiator, catalyst, ligand, HEMA monomer, and EDGMA was added at a ratio of 1:1:2.5:95:5, respectively. The initiator (MW = 260, 10mg), HEMA (MW = 130.14, 0.48g), EDGMA (MW = 198.20, 38.12mg) and stir bar were added to a test tube and purged for 10 minutes.

The total amount of solvent added to each ATRP test tube was 4mL. Bpy (MW = 156.19 ,15.02mg) and CuBr (MW = 143.35, 5.52mg) or HMTETA (MW = 230.40, 22.15mg) and CuBr (MW = 143.35, 5.52mg) were purged with nitrogen gas for 10 minutes and then added to the solution with the HEMA monomer, initiator, and stir bar.

ATRP reactions for synthesizing pHEMA were ran for 24 hours while those for synthesizing the cross-linked hydrogels were ran for 48 hours. Post reaction, water was evaporated under high-velocity conditions of the fume hood.

Hydrogen-1 Nuclear Magnetic Resonance (^{1}H-NMR) Conversion Analysis. Vinylic hydrogens on the HEMA monomer have a higher chemical shift due to the neighboring alkene group. Another property is the parallel peaks due to the geometry of the molecule. According to previous studies[20, 21], the vinylic hydrogens have chemical shifts of approximately $\delta 5.5$ and $\delta 6$. As the HEMA monomer polymerizes, the alkene group reacts and becomes part of the backbone. Those signature parallel hydrogen peaks disappear. However, according to a previous study[20], pHEMA has a unique signature peak at $\delta 1.5$-2 due to a different set of hydrogens in the backbone. The two peaks at $\delta 5.5$ and $\delta 6$ are integrated separately and compared relative to the

peak at δ1.5-2 with calculation accounting for the ratio of 1 hydrogen, 1 hydrogen, and 3 hydrogens, respectively. This separate integration of each vinylic hydrogen to the unique peak in pHEMA was used to approximate a range of conversion percentages.

PHEMA Purification. Column chromatography with methanol was used to purify linear pHEMA by removing the blue catalyst/ligand complex. The elution should be a clear color. The product was concentrated and added to a beaker with 50mL of diethyl ether to remove impurities and unreacted HEMA monomer[12]. The precipitate was collected and spun in a centrifuge at 3000G for 15seconds.

Hydrogel Degradation Study. For the degradation study, the hydrogel was washed multiple times with acetone/water (90:10, v/v)[12] and left overnight on the orbital shaker at 60 rpm. The hydrogel was submerged in 0.1M NaOH and the mass was taken initially and then after 5 days. The hydrogel was left in the fume hood for 2 hours to dry before mass was measured.

Results and Discussion

I. Synthesizing the initiator

Condition	BPA	BE	DMAP	DCC	Solvent
1	1	1.2	0.2	1.5	Anhydrous THF
2	1	1.2	0.2	1.2	DCM
3	1	1.5	0.2	1.2	DCM
4	1	1.5	0.2	1.2	Anhydrous DCM

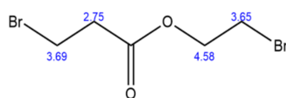
Table 1. Mole ratios and solvents used to synthesize the initiator.

The initiator, 2-bromoethyl 3-bromopropanoate, was synthesized using Steglich esterification with a range of conditions listed in Table 1. The product was assessed using ^{1}H-NMR by using the unique chemical shift of the hydrogen atoms in the compound. The chemical

shift is measured in parts per million (ppm, δ). And the heights of the peaks determine the relative abundance of the hydrogens.

ChemDraw software was used to predict the ^{1}H-NMR structure of the 2-bromoethyl 3-bromopropanoate initiator to utilize as a reference for the experimental product. The predicted NMR (Figure 3a) and NMR from the initiator synthesized with condition 3 from Table 1, (Figure 3b) appear identical to each other. Both have a triplet at approximately δ2.8, a quintuplet at approximately δ3.6, and a triplet at approximately δ 4.5. The slight differences in chemical shift between those two figures can be caused by the approximation of the computational guess and/or the solvent used.

3A.



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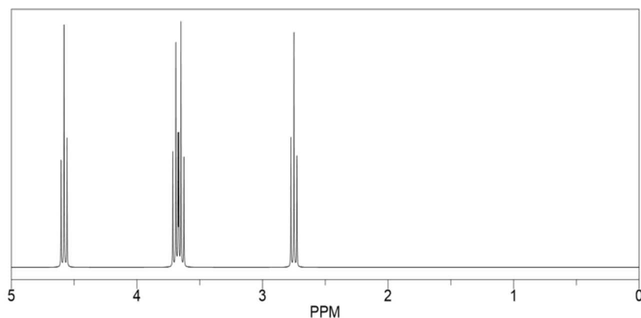

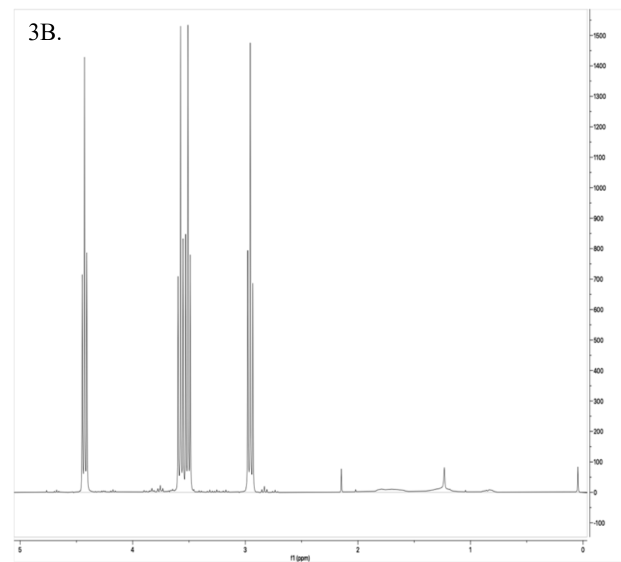

Figure 3. (A) Predicted NMR of 2-bromoethyl 3-bromopropanoate approximated by ChemDraw. (B) NMR of purified initiator from Condition 3 in Table 1. Both have identical peaks at approximately δ 2.8, δ 3.6, and δ 4.5.

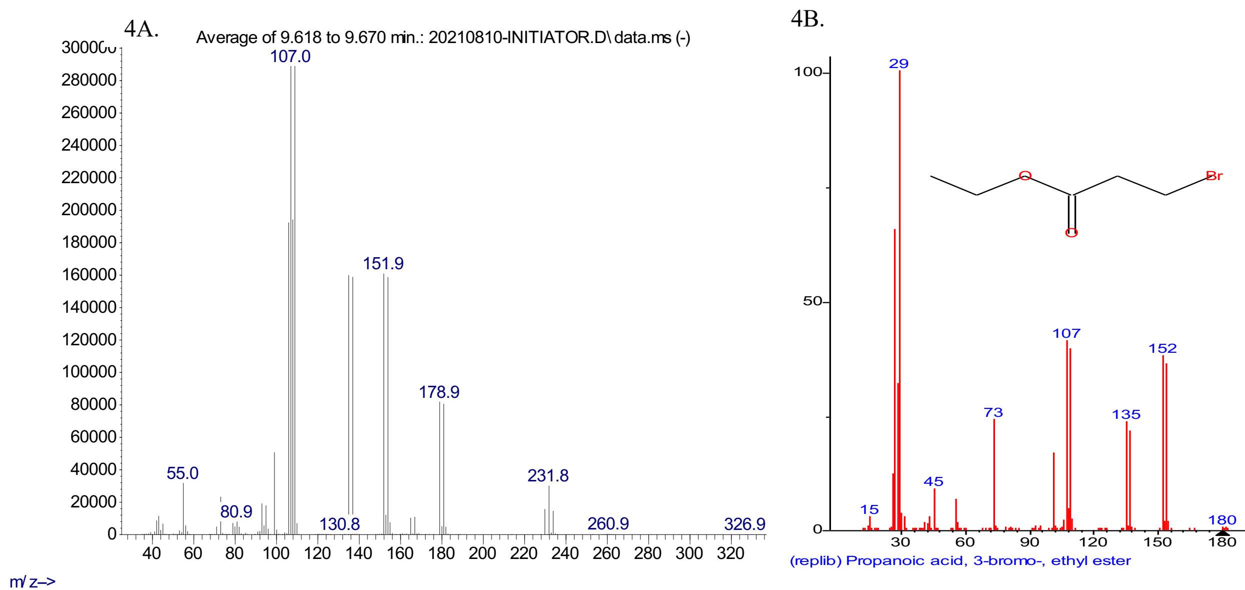

Figure 4. (A) Peaks from the mass spectrometry analysis of the initiator we synthesized. (B) Computer prediction of the structure. The predicted structure, *propanoic acid, 3 bromo-, ethyl ester*, has one less bromine than the desired initiator.

Mass spectrometry was used to analyze the product. The mass to charge ratios (*m/z)* for the ions created from the mass spectrometer and the heights of the peaks that relative intensity were used to analyze the compound. Figure 4a demonstrates the *m/*z peaks of a sample of the 2-bromoethyl 3-bromopropanoate initiator. The symmetric doublets at *m/z* values of 107.0, 130.8, 151.9, and 178.9 show that there is symmetry in the compound In addition, the triplet with identical peaks on either side at the *m/z* value of 231.8 suggests that there are symmetric bromine groups.

Figure 4b is the predicted compound generated by the computational software by analyzing *m/z* values and intensity peaks. The predicted compound from its database is propanoic acid, 3-bromo-, ethyl ester. The predicted molecule is missing a bromine group at the end compared to the 2-bromoethyl 3-bromopropanoate initiator. However, due to that triplet peak at 231.8 *m/z* and the other symmetric peaks in Figure 4a, the synthesize molecule is likely to be

symmetric. The desired initiator might not have been in the database and we were shown the closest prediction. Therefore, we can assume that the initiator was successfully synthesized and purified.

Condition	Amount of purified initiator (mg)	Yield
1	N/A	N/A
2	90	6.9%
3	170	13.1%
4	190	14.6%

Table 2. Presents the amount of purified initiator per the different conditions listed in Table 1. The yield is calculated by purified product divided by theoretical yield (1.3g).

Table 2 shows the percent yield of the initiator for different mole ratios and solvent combinations. The theoretical yield for all of the reactions was 1.3g as the amount of 3-bromopropionic acid was the limiting reagent. Condition 1 is marked N/A because the relevant NMR peaks for the initiator disappeared post-purification. The NMR peaks for the impure initiator sample already had low intensity so the product might have been lost in the column. The NMRs for conditions 2, 3, and 4 were identical and matched the predicted NMR. As illustrated in conditions 2 and 3, increasing the mole ratio of 2-bromoethanol (BE) from 1.2 to 1.5 (by 25%), doubled the amount of initiator synthesized. Despite this increase, the-yield for condition 3 was only 13.08%. Anhydrous DCM had the highest yield at 14.6%. This low yield was unexpected.

Esterification reactions in anhydrous solvents tend to synthesize esters at a high yield. The reason for the low yield can be attributed to unexpected side reactions or undetected moisture in the system. A design of experiments with varying conditions can be used to identify what variable can increase yield of the initiator. While a low yield would increase the cost of synthesis, it is sufficient enough to determine the properties of pHEMA.

II. Synthesizing pHEMA

Condition	Ligand	Solvent	Temperature (°C)
1	Bpy	Methanol/Water	RT
2	Bpy	Methanol/Water	50
3	HMTETA	Methanol/Water	RT
4	HMTETA	Methanol/Water	50
5	Bpy	Methanol	RT
6	Bpy	Methanol	50

Table 3. Presents the different combinations of ligands, solvents, and temperatures used to synthesize pHEMA. RT stands for room temperature.

Table 3 lists the conditions used for ATRP synthesis of pHEMA with the 2-bromoethyl 3-bromopropanoate initiator. All of the reactions were run for 24 hours and used copper (I) bromide (CuBr) as the catalyst.

The initiator synthesized from condition 3 was then used in the ATRP reaction for pHEMA synthesis with varying conditions listed in Table 3. The NMR results were used to analyze conversion from signature HEMA monomer and pHEMA peaks. Figures 5 and 6 demonstrate an increased degree of polymerization as the temperature is increased from room temperature to 50°C while using 2,2'Bipyridyl (bpy) as the ligand and water/methanol (50:50, v/v) as the solvent. The conversion for conditions 1 and 2 were 31.7-32.9% and 78.0-88.5%, respectively.

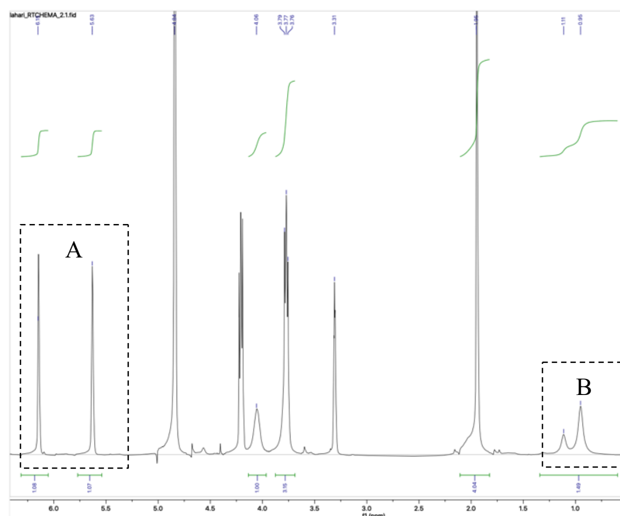

Figure 5. Condition 1 from Table 3. Bpy is the ligand, methanol/water is the solvent, and reaction was ran at RT. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

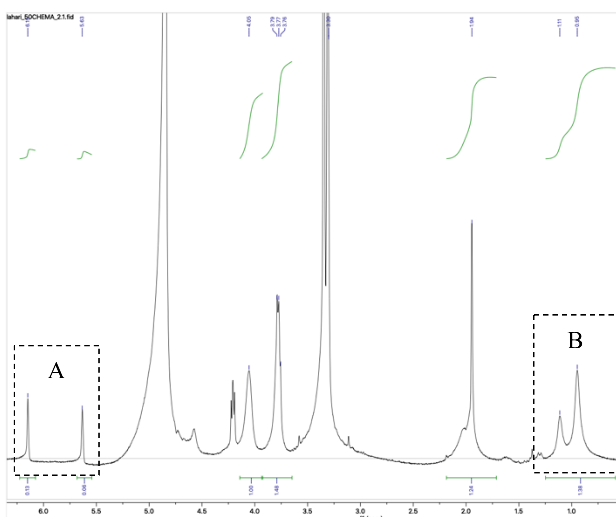

Figure 6. Condition 2 from Table 3. Bpy is the ligand, methanol/water is the solvent, and reaction was ran at 50°C. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

Figures 7 and 8 show that there is a 100% conversation rate for when 1,1,4,7,10,10-hexamethyl-triethylenetetramine (HMTETA) is used as the ligand, water/methanol (50:50, v/v) as the solvent, regardless of the temperature. Between conditions 1 and 2, the only difference is the ligand. The HMTETA ligand increased conversion by a factor of 3-3.2 and 1.1-1.3 for RT

and 50°C, respectively. The relative higher kinetic activation rate constant for HMTETA to bpy corroborates with kinetic constants found in literature for different solvents[22-24].

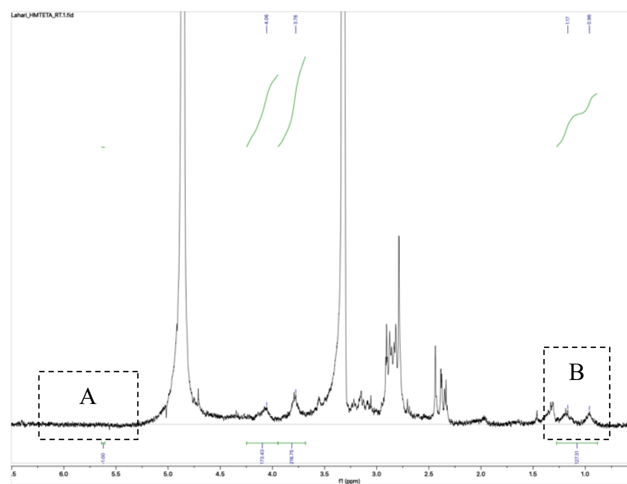

Figure 7. Condition 3 from Table 3. HMTETA is the ligand, methanol/water is the solvent, and reaction was ran at RT. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

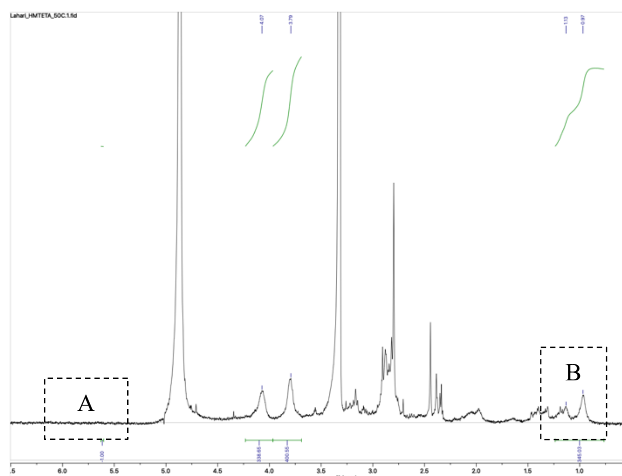

Figure 8. Condition 4 from Table 3. HMTETA is the ligand, methanol/water is the solvent, and reaction was ran at 50°C. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

Figures 9 and 10 show that there is a lower conversion rate for when bpy is used as the ligand and only methanol is used as the solvent, regardless of temperature used. The conversion for conditions 5 and 6 were 2.6-2.9% and 11.5-12.5%, respectively. Compared to conditions 1

and 2, the difference was the lack of water as 50% of the solvent. This suggests that copper bromide catalyst is more active in water and agrees with previous literature that the methanol solvent has lower kinetics[25]. Table 4 shows the integration values per condition and Table 5 summarizes the conversion rates for each condition.

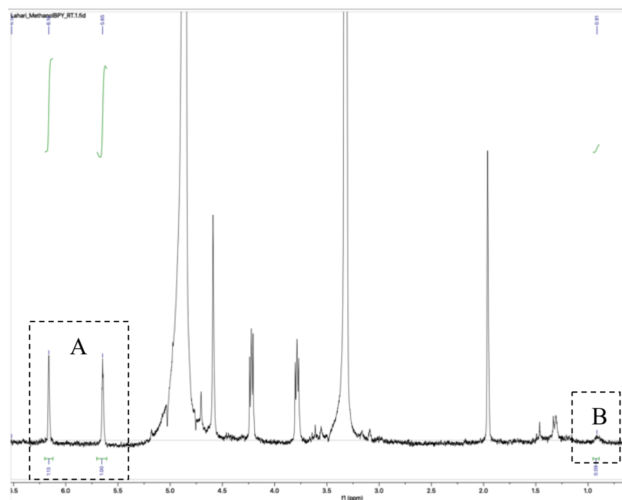

Figure 9. Condition 5 from Table 3. Bpy is the ligand, methanol is the solvent, and reaction was ran at RT. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

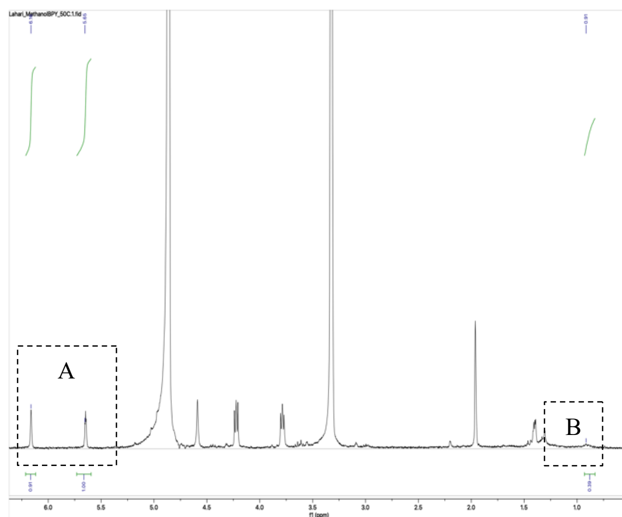

Figure 10. Condition 6 from Table 3. Bpy is the ligand, methanol is the solvent, and reaction was ran at 50°C. The vinylic hydrogen shifts are labeled A and the unique pHEMA shift is labeled B.

	Integral Values from NMR Analysis					
Chemical Shift	Condition 1	Condition 2	Condition 3	Condition 4	Condition 5	Condition 6
6.15	1.06	0.13	1	1	1.13	0.91
5.63	1.07	0.06	1	1	1	1
0.95-1.11	1.49	1.38	127.31	345.03	0.09	0.39

Table 4. The integrals values for each vinylic hydrogen peaks at approximately 6.15 ppm and 5.63 ppm and signature pHEMA peak at about 0.95-1.11 ppm for each condition from Table 3.

Condition	Ligand	Solvent	Temperature (°C)	Conversion (%)
1	Bpy	Methanol/Water	RT	31.7 – 31.9%
2	Bpy	Methanol/Water	50	78.0 – 88.5%
3	HMTETA	Methanol/Water	RT	100%
4	HMTETA	Methanol/Water	50	100%
5	Bpy	Methanol	RT	2.6 – 2.9%
6	Bpy	Methanol	50	11.5 – 12.5%

Table 5. The approximated conversion rates from HEMA monomer to pHEMA through NMR analysis for six different conditions.

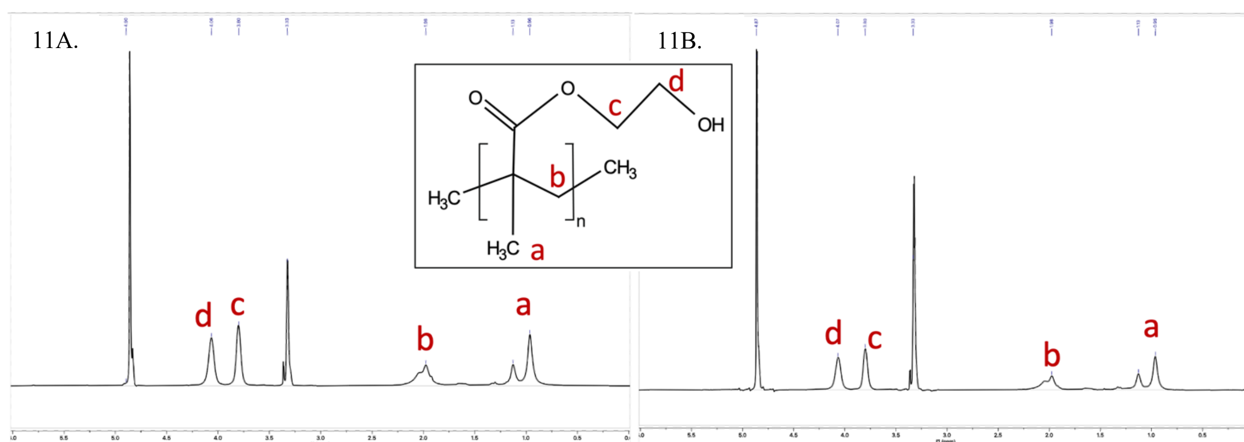

Figure 11. Purified pHEMA from (A) Condition 1 and (B) Condition 2 from Table 3. The NMR is labeled with letters denoting the set of hydrogens being representing in the pHEMA molecule by each chemical shift.

The pHEMA synthesized from conditions 1 and 2 were purified and analyzed with NMR, seen in Figure 11. The unlabeled peaks are from the methanol-d4 solvent used to dissolve the

polymer. The peaks were at δ0.96 and 1.13, δ1.98, δ3.80 and δ4.06 for condition 1 for a, b, c, d, respectively. The only difference is that peak d is at δ4.07 for condition 2. Compared to Figures 5 and 6, there is a total disappearance in the peaks for vinylic hydrogens.

III. Synthesizing pHEMA hydrogel cross-linked with 5% EDGMA

Condition	Solvent	Temperature (°C)	Stir
1	MEK /1-propanol	RT	Yes
2	MEK /1-propanol	70	Yes
3	Methanol/Water	RT	No
4	Methanol/Water	50	No

Table 6. Presents the different combinations of solvents, temperatures, and stir conditions used to synthesize pHEMA hydrogel crosslinked with 5% EDGMA. RT stands for room temperature.

The 2-bromoethyl 3-bromopropanoate initiator was used to synthesize pHEMA crosslinked with 5% ethylene glycol dimethacrylate (EDGMA), a non-degradable crosslinker. Table 6 lists the conditions used for the ATRP synthesis. Each reaction was ran 48 hours and CuBr was used as the catalyst.

To conclude that the product was a polymer, methanol was added to determine solubility. The presence of dissolution suggests that the product is a polymer because pHEMA hydrogels are able to maintain integrity.

Conditions 1 and 3 from Table 6 formed a polymer. Condition 2 formed a very soft product that did not dissolve in the presence of methanol. The product maintained separate clumps that did not mix with each other. When the clumps were manually mixed, it wasn’t viscous as a polymer would be but rather showed slight tear. The product that formed was not a polymer and not entirely a hydrogel. Without adding the stir bar, a hydrogel might have formed. Condition 4 formed a strong hydrogel. Running the reaction for 48 hours is possibly not the only

explanation for the mechanical properties of Condition 4. There could have been other side reactions that led to further cross-linking to increase the strength of the hydrogel.

Condition 2 used the solvents MEK/1-propanol (70:30, v/v) at a temperature of 70°C along with a stir bar. Condition 4 used the solvents methanol/water (50:50, v/v) at a temperature of 50°C without a stir bar. As the temperature was 20°C higher for condition 2, the soft properties could have been caused by stir bar mechanically breaking bonds.

Sample	Mass on day 1 (mg)	Mass on day 5 (mg)
1	68.6	68.5
2	370.1	366.4
3	42.9	43.8

Table 7. Presents the degradation of three samples of the hard hydrogel synthesized from condition 4 from Table 6 in 0.1M NaOH solution. Mass was taken on day 1 and day 5.

A degradation study was conducted on the product from condition 4. Table 7 summarizes the result. There was a decrease of 0.1mg, decrease of 3.7mg, and an increase of 0.9mg for samples 1, 2, and 3, respectively. There was no significant degradation of the three samples. The linear pHEMA bonds crosslinked with EDGMA are undegradable without the presence of a degradable initiator. The initiator might not have been easily penetrated by the 0.1M NaOH for hydrolysis due to the strength of the crosslinking reactions. Some HEMA polymers could have been self-polymerized, decreasing the amount of degradable molecules in the backbone.

Conclusion

Steglich esterification successfully synthesized the initiator although anhydrous DCM and DCM had a similar yield of 2-bromoethyl 3-bromopropanoate. The percent yield was lower

than expected. The initiator had differential success in polymerizing HEMA monomers via ATRP depending on the conditions. There was total conversion using HMTETA as the ligand and methanol/water as the solvent at both RT and 50°C and decent conversion using bpy as the ligand, methanol/water as the solvent at 50°C. Synthesizing pHEMA cross-linked with 5% EDGMA created a polymer, polymer and hydrogel mix, and hydrogel depending on the solvents and stir bar conditions. The variable mechanics can be used to mimic different types of tissues. There was no degradation seen after 5 days, demonstrating that having only a degradable backbone is not enough to hydrolyze the pHEMA hydrogel synthesized with the 2-bromoethyl 3-bromopropanoate initiator.

Future Directions

Large-scale industrial synthesis of this initiator would waste a bulk of reactants, reagents, and money because of the smaller percent yield. Fischer esterification with a sulfuric acid catalyst can be tested to try to increase the yield of 2-bromoethyl 3-bromopropanoate. Another idea would be to increase the reaction time from 48 hours to 96 hours to see if it makes a difference. To ensure that the low yield is not caused by moisture in the currently used set up, it would be worthwhile to see what byproducts water can make in this system and then quantify it with NMR analysis.

The assumption is that the polymer synthesized has a low molecular weight. The next step would be to find the weight of the polymer synthesized with different conditions through gel permeation chromatography (GPC) to find the weight distribution. Ideally the dispersity index would be close to 1. By stopping reactions at different times and varying monomer to initiator

ratios[12, 14] and then using GPC to find molecular weight, the methodology can be finetuned to synthesize a polymer with the ideal molecular weight.

The degradation experiment needs to be repeated with a degradable crosslinker such as methacryloyl chloride[12]. A degradation profile study should be performed in a lipase environment to mimic the enzymatic reactions that can also break the ester bonds. A cytotoxicity study needs to be done on the hydrogel and its degradation products via the MTT assay to make sure the material is not toxic in the body. A swelling ratio and Youngs' Modulus study should be done on the hydrogel over the length of the degradation experiment.

2-bromoethyl 3-bromopropanoate is a promising initiator to synthesize degradable, low molecular weight pHEMA hydrogels for cardiac tissue engineering applications.

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Appendix

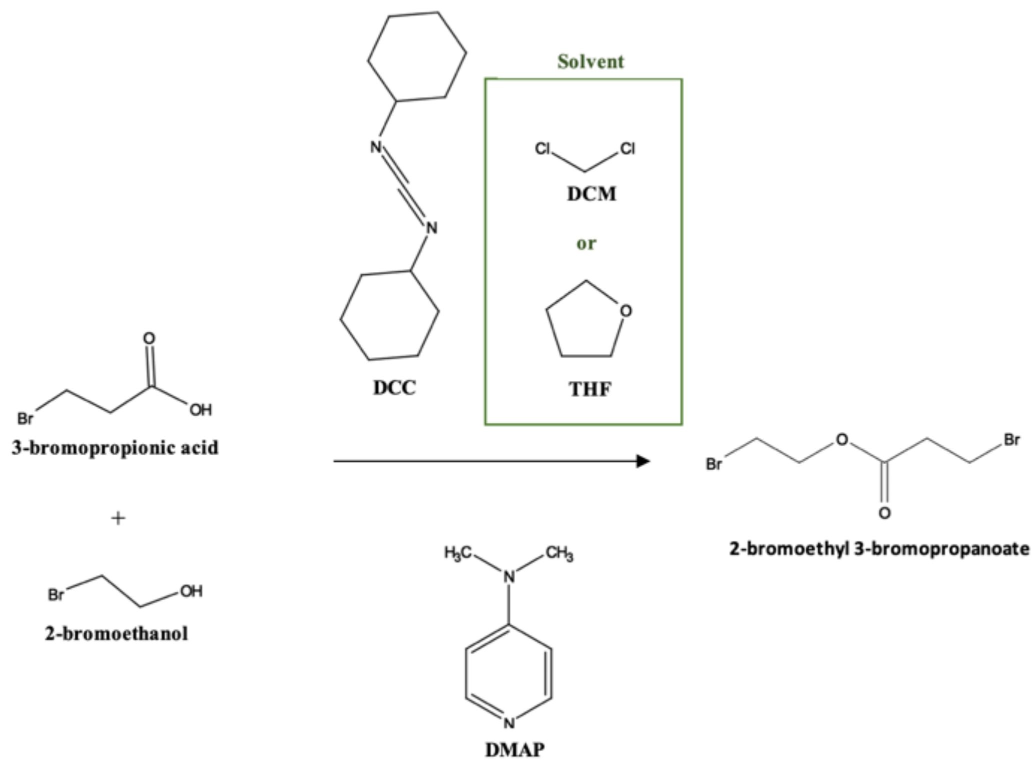

Figure S1. Reaction schematic of the reagents used to synthesize 2-bromoethyl 3-propanoate.