Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):168-172

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and viability of hyperbranched polylysine polycaprolactone star polymers as organic scaffolds for tissue engineering

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ABSTRACT

Due to their strength, flexibility, biocompatibility and degradation properties, organic polymers films are promising materials for the replacement of bone grafts. Previous work has revealed that the dendritic polylysine-co-polycaprolactone star polymer is flexible, strong and biocompatible. In this study, a hyperbranched version of the polylysine-co-polycaprolactone star polymer was tested for its ability to make composite films with hydroxyapatite (HA) and undergo proteolytic enzymatic degradation. The hyperbranched polymer was proposed to facilitate the synthesis as well as increase degradation rates compared to the dendritic based co-polymer by leaving free amine recognition sites in the lysine units. Utilizing a one-pot slow addition to core hyperbranching method produced a hyperbranched polymer analogous to the dendritic polylysine studied previously, but in a significantly shorter time. This hyperbranched polymer was then utilized as a core to initiate the ring opening polymerization of caprolactone to yield star co-polymers of various average molecule weights. The original dendritic moiety and the new star co-polymers were then tested for enzymatic degradation using both trypsin and elastase. The hyperbranched polymer composites demonstrated some increased enzymatic degradation versus the dendritic moiety during the eight week period tested. However, the size of the polycaprolactone (PCL) chains appears to be a significant contributor to its degradation rate.

Keywords: Hyperbranching, star polymers, polylysine-co-polycaprolactone, tissue engineering, enzymatic degradation

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INTRODUCTION

Organic polymers are promising molecules in the field of biomaterials research. Polymers are used in the biomedical setting in multiple capacities, but in particular, polymers are being utilized to form organic scaffolding materials in artificial tissues[2, 3].These scaffolds are designed to support healing and to ultimately be replaced by natural tissue over time. In order for this to be accomplished, the scaffolding materials must be easy to use, provide appropriate strength and flexibility for the particular application, be biocompatible and ultimately be biodegradable. Previous work with hydroxyapatite composites synthesized using polylysine-co-polycaprolactone star polymers with dendritic polylysine cores showed promising results, providing materials that were strong, flexible and biocompatible[1]. However, the synthesis of the dendrimer core was slow (4 to 14 days depending on the generation of dendrimer synthesized) and when the final dendritic star polymer was tested for enzymatic degradation in this study, no enzymatic activity was observed.

In an attempt to improve the rate of polymer degradation and simplify the overall synthetic route, the present study explored the use of a hyperbranched molecule. A one-pot slow monomer addition method for preparing hyperbranched polylysine was employed to produce a highly branched polymer analogous to the dendritic polylysine. This method was previously shown to yield hyperbranched polylysine in one day[4, 5].It is believed this

star polymer core molecule would then initiate ring-opening polymerization of caprolactone in the same manner as the previously prepared polylysine dendrimer[1]. Both the dendrimer and the hyperbranched polymer were synthesized on a 5000 average molecular weight monomethoxy poly(ethylene glycol) (mPEG). This mPEG tail provided an internal standard in order to calculate the average molecular weight of the final star co-polymer solely utilizing ^{1}H NMR. Once synthesized, this study explored the enzymatic degradation of the new polymer. It is proposed that the lack of free amine end groups in the dendritic lysine polymer and the densely packed structure may have led to the slow rates of enzymatic degradation seen in the dendritic moiety. The hyperbranched star polymer, which is likely less dense and potentially contains more readily accessible amine end groups, should facilitate enzymatic degradation.

EXPERIMENTAL SECTION

Methods

All chemicals were from commercially available sources and used as received. ^{1}H NMR data was acquired using a 400MHz Bruker Advance Spectrophotometer with $CDCl_3$ utilized as the solvent.

Synthesis of monomethoxy poly(ethylene glycol) hyperbranched polylysine:

To 19 mL of DMSO, 5.0 g of N-hydroxysuccinimide lysine monomer (**1**) was added. The solution was gently heated to aid in dissolution prior to placing the solution in a syringe. In a separate 3-neck round bottom flask, 1.52 g of mPEG glycine core, 5 mg of dimethylaminopyridine, and 14 mL of DMSO were added and stirred. Once the solids were dissolved, 11.5 mL of N,N-diisopropylethylamine was added and the flask was purged with nitrogen gas. The monomer solution was then added to the round bottom reaction flask using a syringe pump at a rate of 1.7 mL per hour. After the addition was complete, the reaction was allowed to stir at room temperature for 2 hours prior to pouring it into 700 mL of ethyl acetate to precipitate the solid product. The solid was collected and dried in a vacuum desiccator to yield the final polymer product (4.20 g).

Synthesis of monomethoxy poly(ethylene glycol) hyperbranchedpolylysine-co-polycaprolactone star polymer (low molecular weight):

Monomethoxy poly(ethylene glycol) hyperbranched polylysine core (0.25 g) was placed in a reaction flask and purged with nitrogen gas. Via syringe, 10 mL of caprolactone and 10 mL of dimethylsulfoxide were added and the reaction was heated to 110^{o}C with stirring. Tin octanoate(0.0145mL) was then added and the reaction was allowed to stir at 110^{o}C for 24 hours. The solid polymer was then dissolved in dichloromethane and precipitated in cold methanol. The solution was filtered to collect the white solid which was dried in a vacuum desiccator to yield the final polymer.

Synthesis of monomethoxy poly(ethylene glycol) hyperbranchedpolylysine-co-polycaprolactone star polymer (high molecular weight):

Monomethoxy poly(ethylene glycol) hyperbranched polylysine core (0.2630 g) was placed in a reaction flask and purged with nitrogen gas. Via syringe, 7.5 mL of caprolactone was added and the reaction was heated to 130^{o}C with stirring. Tin octanoate(0.025mL) was then added and the reaction was allowed to stir at 130^{o}C for 24 hours. The solid polymer was then cooled to room temperature and dissolved in 30 mL dichloromethane. The solution was poured into 350 mL of cold methanol to precipitate the polymer before being filtered to collect the white solid. The polymer was dried in a vacuum desiccator to yield the final polymer.

Degradation studies:

The star polymer (7.21 g) was placed in an Erlenmeyer flask and dissolved in 75 mL of THF. To each 1.5 mL glass vial, a 1 mL portion of the star polymer solution was added. The solvent was allowed to evaporate over a 48 hour period, leaving a film on the bottom of each vial of approximately 0.100 (+/- 0.010) grams. To each vial the appropriate media was added; one third of the vials received phosphate buffer solution, one third received elastase in phosphate buffer solution (0.5 mg/mL) and the remaining third received trypsin in phosphate buffer solution (0.5 mg/mL). All samples were then incubated in a 37^{o}C water bath for one week. After a week, the solution was removed from each vial. Three vials from each solution type were allowed to dry and subsequently weighed. The remaining vials had 1 mL of fresh media containing the same initial concentration of enzyme added and were placed back in the 37^{o}C water bath. This process was continued each week for the duration of the eight week study.

RESULTS AND DISCUSSION

The general synthetic pathway can be seen in figure 1. Both the mPEG glycine core and the activated lysine monomer (**1**) were synthesized according to previously published procedures[1].In order to synthesize the

hyperbranched polylysine, the mPEG glycine core was stirred while slowly adding a dilute solution of activated lysine monomer.

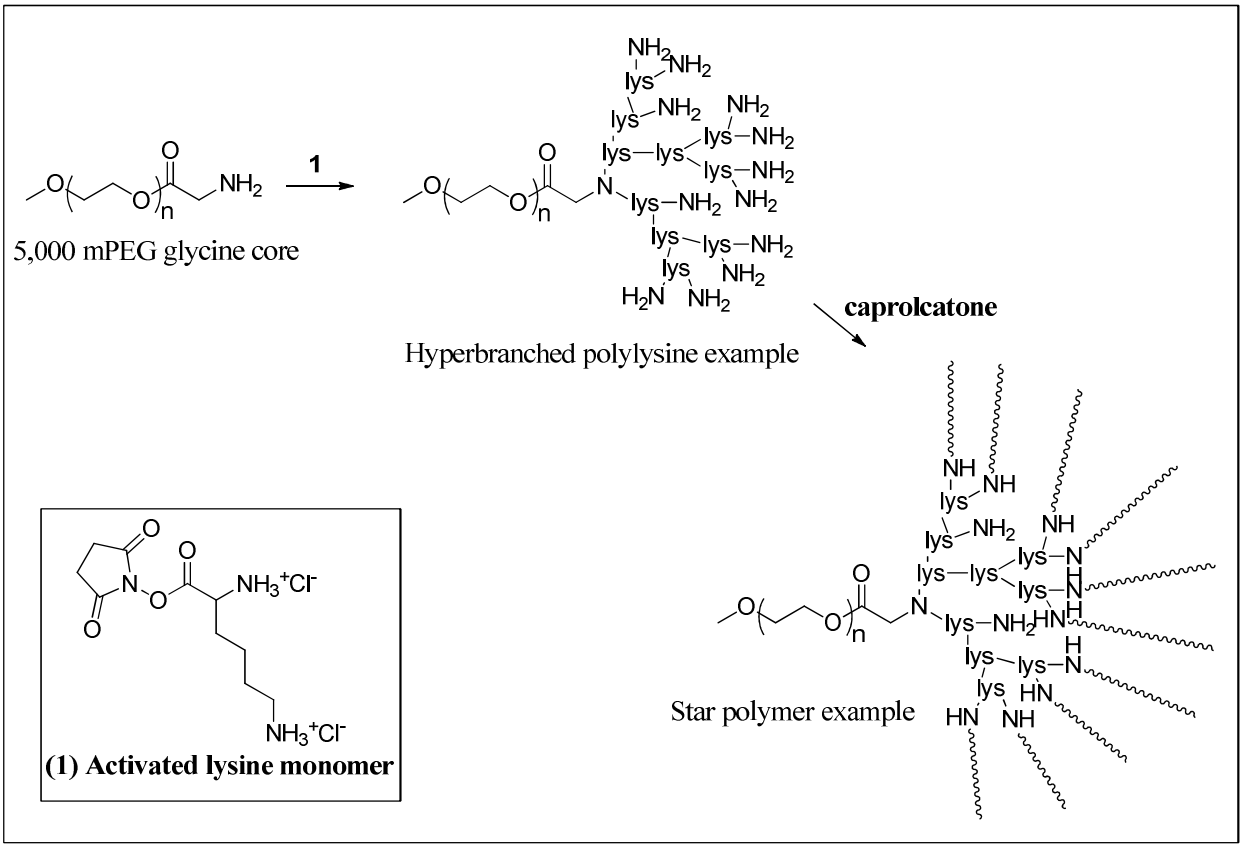

Figure 1.General Pathway for Star Polymer Synthesis. In the figure, 'lys' represents individual lysine residues and ' ∿∿ ' represent poly caprolactone

This process was based on a previously reported procedure utilized to prevent homopolymerization of the lysine monomer [4]. This one-pot slow addition to core hyperbranching method produced hyperbranched polymer analogous to the dendritic polylysine studied previously but in a fraction of the time (1 day vs. 4-14 days) and in a single step.

A standard ring opening polymerization of caprolactone was then initiated by the terminal amines using a tin octanoate catalyst in a second 12-24 hour reaction. By varying solvent and the amount of caprolactone monomer available for polymerization, varying lengths of PCL were prepared.

Two different star polymers were then tested for their ability to make films and show enzymatic degradation. Both polymers contained the same mPEG hyperbranched polylysine core and only varied in the amount of PCL content. The amount of PCL present in each sample was determined via ^{1}H NMR. The ratio of protons in the mPEG peak was compared to protons found in a PCL peak to determine the relative number of PCL repeats per mPEG chain. Figure 2 shows a typical ^{1}H NMR for the high molecular weight sample.

Since each molecule contains only one mPEG, this ratio provided the number of PCL repeat units per molecule. The lower molecular weight star polymer was found to contain and average of ~1300 PCL repeat units per mPEG. The higher molecular weight star polymer was found to contain ~5000 PCL units per mPEG.

Polymer films were made of each sample by dissolving the polymer in THF and subsequently allowing the solvent to evaporate leaving a solid polymer layer on the bottom of the vial. Films were made of the original dendritic star co-polymer in addition to the two new hyperbranched co-polymer stars. The degradation of each polymer was tested using two different enzymes and a phosphate buffer solution (PBS) control. Trypsin was chosen as it is a known lysine cleaving enzyme and elastase was also chosen due to its tendency to cleave hydrophobic peptide regions. The original dendrimer star co-polymer showed no change in mass over the eight week study duration. It

is hypothesized that with no free amines in the lysine units and a densely packed core region, the enzymes were unable to cleave the polymer structure.

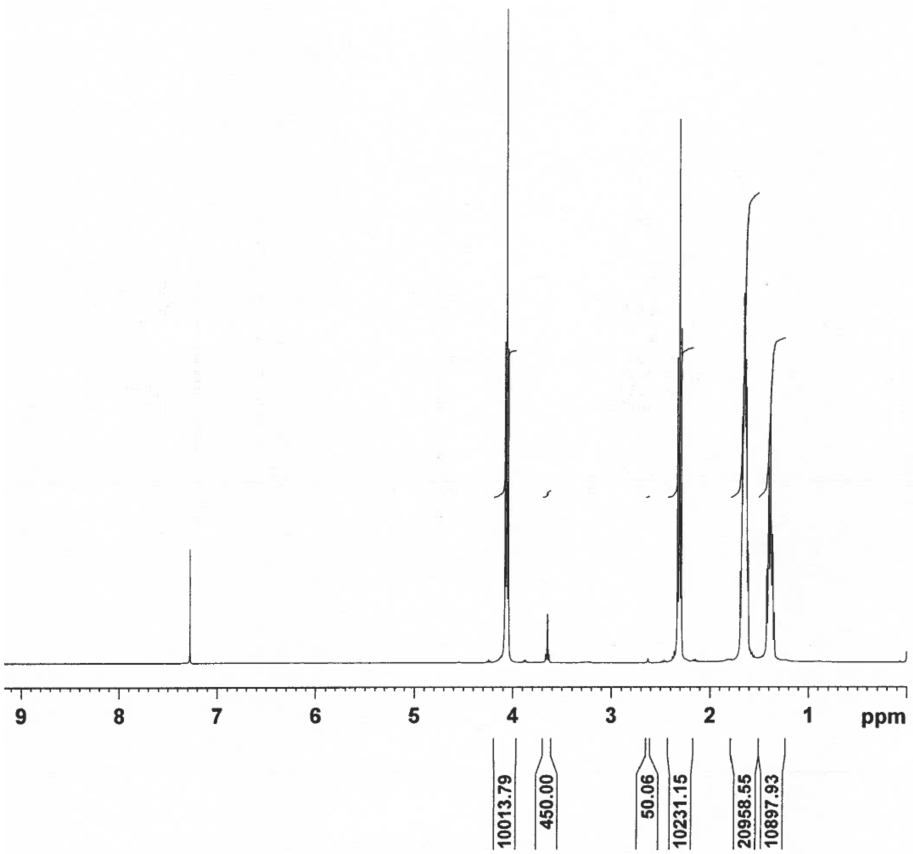

Figure 2. Typical ^{1}H NMR of high molecular weight star polymer. mPEG protons can be seen at ~3.6ppm

One of the two star polymer samples did show a significant increase in degradation over the duration of the eight week study. As seen in figure 3, the star polymer with the lower molecular weight PCL content saw a 30-45% mass loss over the 8 week period, indicating significant biodegradation. This shows significant promise for improved biodegradability utilizing the hyperbranched moiety. The star polymer with higher PCL content showed less than 1% mass loss over the eight week period, indicating the PCL content has a significant influence in the rate of degradation in the star polymers. This allows future work to optimize the PCL chain length to allow degradation while maintaining flexibility and strength of the scaffolds.

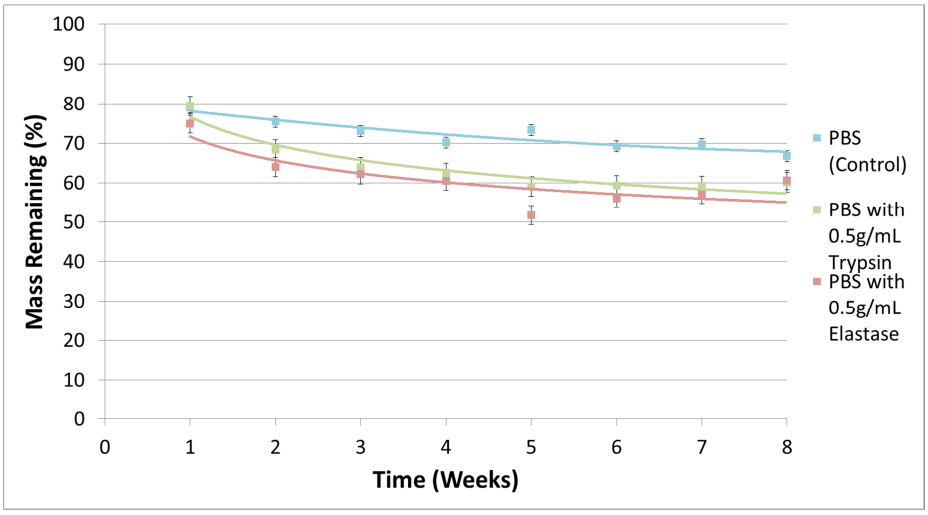

Figure 3. Graph of eight week degradation study for low molecular weight star polymer

CONCLUSION

The use of a hyperbranched polylysine facilitated the star polymer synthesis, allowing for a one-pot synthesis of the core molecule in 12 – 24 hours followed by the final star polymerization reaction. Thus, the use of hyperbranching allowed for a more facile synthesis of the star polymer in order to explore the polymer's ability to form flexible composites and show increased enzymatic degradation. While the degradation rate of the hyperbranched star polymers was increased versus the dendritic star polymer, the length of the PCL chains significantly influenced the hyperbranched star polymer's enzymatic degradation rate. While the degradation rate can be further optimized, this study demonstrated an increased degradation rate and a more facile method to explore star co-polymers for use in tissue engineering applications.

Acknowledgements
The authors would like to thank Western New England University for their generous support through a Faculty Development Grant.

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