Developing New Amphiphilic Diblock Copolymers for Delivery of Cytosolically Active Immunostimulants

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Introduction

Objectives

- To synthesize a library of polymers
- To characterize the polymers focusing on pH-responsive properties

Background

A current challenge in vaccine development is the ability to deliver immunostimulatory adjuvants to receptors in the cytosol. A method to overcome this problem is to utilize a pH-responsive nano-carrier, such a polymer consisting of both a hydrophilic and hydrophobic block.

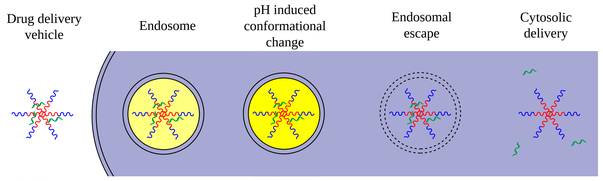


Figure 1. The blue represents the hydrophilic block of the polymer and the red represents the hydrophobic block. The green denotes the immunostimulant that is delivered to the cytosol.

Hypothesis

Changing the composition and length of the hydrophobic group of an amphiphilic diblock copolymer should alter its pH-responsive properties. Therefore, polymers with HMA should exhibit greater hemolytic activity and will form a more stable micelle than BMA containing polymers. These properties will affect its ability to deliver nucleic acid adjuvants.

Methods

Reverse addition fragmentation chain transfer (RAFT) polymerization was used to create BMA and HMA diblock copolymers, at three different compositions each.

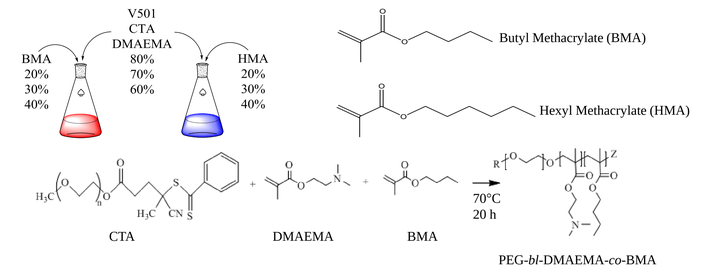
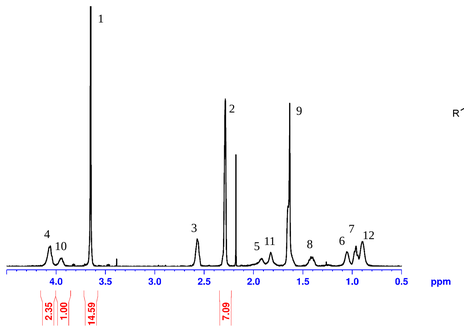


Figure 2. Copolymer synthesis was conducted in a one-pot fashion. In brief, initiator (cyanovaleric acid; V501), chain transfer agent (CTA; poly(ethylene glycol) 4-cyano-4-(phenylcarbonothioylthio)pentanoate), 2-dimethylaminoethyl methacrylate (DMAEMA) and hydrophobic monomer (butyl methacrylate (BMA) or hexyl methacrylate (HMA)) were combined in dioxane:DMF (60:40 v/v). The first block consists of PEG which provides hydrophilicity and biocompatibility while the second block carries pH-responsive properties.

Polymer Characterization



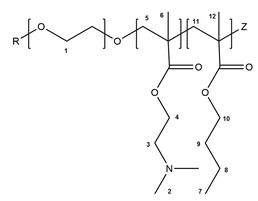


Figure 3. On the left is the Nuclear Magnetic Resonance (NMR) spectrum for the 30% BMA polymer. The number labels on the peaks correspond to the different hydrogen atoms of the polymer, shown on the right.

Polymer Characterization

Polymer	% BMA or HMA[a]	M_n[b] (g/mol)	PDI[c]	M_n[d] (g/mol)
20% BMA	19.3	26200	1.063	26001.33
20% HMA	21.0	28200	1.08	25736.49
30% BMA	30.5	26000	1.027	25520.72
30% HMA	30.0	26700	1.044	25457.78
40% BMA	38.5	30400	1.15	25964.63
40% HMA	37.1	33200	1.085	25457.78

Table 1. [a]^{1}H NMR. [b]^{1}H NMR. [c]Polydispersity Index from Gel Permeation Chromatography (GPC). [d]From GPC elution time.

Dynamic Light Scattering

Each polymer was dissolved in ethanol at a concentration of 50mg/mL then diluted to 10mg/mL with phosphate buffered saline (PBS). The results show that the 20% BMA, 20% HMA and 30% BMA polymers are unimeric for this pH range. The 30% HMA, 40% BMA and 40% HMA polymers appear to be micelles at pH 7.4

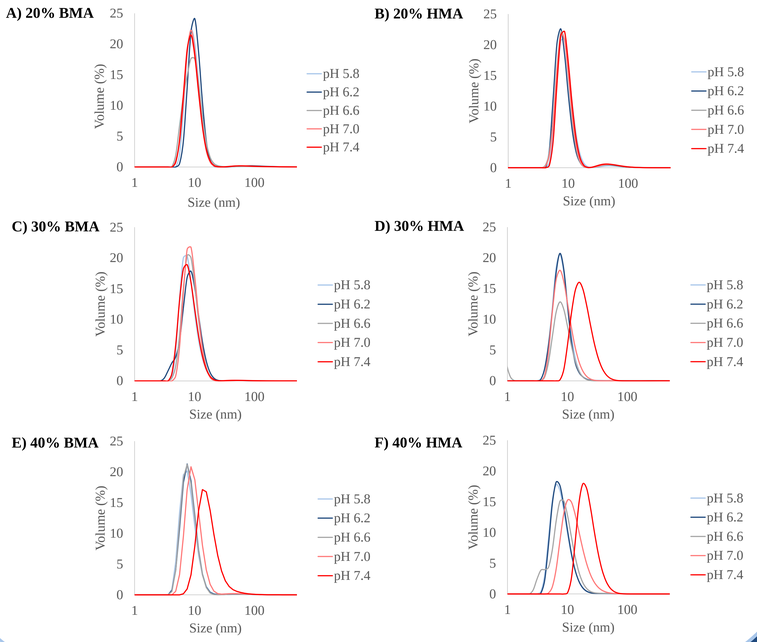


Figure 4. Results of DLS experiment, which were run in triplicate then averaged together.

Hemolysis

Polymer solution at different pH values were added to plate wells containing red blood cells (RBCs), which serve as a model membrane. The samples were incubated, centrifuged, and then the absorbance was measured at 541 nm. The results of hemolysis experiment show a sharp increase in membrane destabilizing activity at endosomal pH.

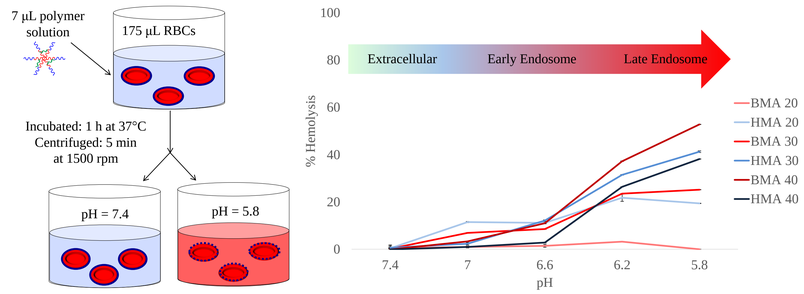


Figure 5. In the figure on the left, the well at pH 5.8 is red because it represents the release of hemoglobin, which occurs when the membrane breaks. In the graph, the bars represent standard error. The equation used was % Hemolysis = $100*(A_{541,sample}-A_{541,buffer})/(A_{541,triton}-A_{541,buffer})$.

Cell Viability

The cytotoxicity of PEG-*bl*-DMAEMA-*co*-BMA and PEG-*bl*-DMAEMA-*co*-HMA on B16, mouse melanoma cells, was evaluated using the CellTiter Blue Assay. Results suggest that these statistical copolymers are not cytotoxic (>80% cell viability) at a concentration of 10 µg/mL.

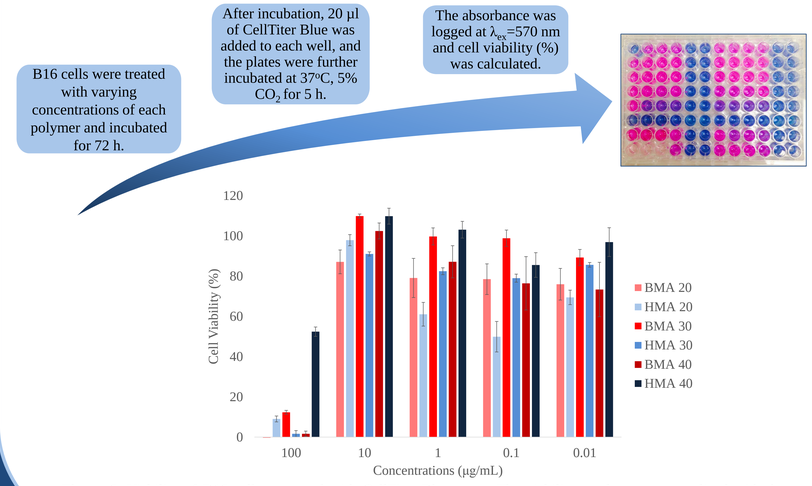


Figure 6. Viability of B16 cells measured with CellTiter Blue assay. The viability results were normalized with the positive control. Bars represent standard error.

Conclusion

The hypothesis was correct in that changing the length of the hydrophobic group effects the pH-responsive properties of polymer chains. For example, the DLS comparison between 30% BMA and 30% HMA shows that the 30% HMA polymer was able to form a micelle whereas its BMA counterpart did not. Also, the 20% BMA demonstrated minimal hemolysis whereas the 20% HMA did exhibit some pH-dependent membrane disruptive behavior. Similarly, incorporation of 30% HMA increased hemolysis relative to the same amount of BMA. However, the hypothesis that HMA would be more hemolytic at all compositions was not proven to be true, as the 40% BMA was significantly more membrane disruptive than any of the other polymers at late endosomal pH. The CellTiter Blue assay proved that all the polymers are viable at a concentration of 10 µg/mL. In conclusion, while it cannot be concluded that PEG-*bl*-DMAEMA-*co*-HMA is a better delivery vehicle than PEG-*bl*-DMAEMA-*co*-BMA, alkyl chain length can have a significant effect on the properties and offers a promising approach for controlling the delivery of immunostimulatory adjuvants.

Future Work

To continue this project, the first step is to complex the polymers with an immunostimulatory adjuvant then run an agarose gel to prove complexation has occurred. Next, an in vitro reporter cell assay should be completed to determine how successful the polymers are at drug delivery. The second step would be to increase the size of the polymer library. Additions to this library could include hydrophobic groups such as octyl, decyl, or phenyl. Also, side chains that include a double or a triple bond could be added to this library. Another possible continuation of this project would be to explore the effects of monomer composition on pH-responsive properties. The polymer library could be increased by including 50%, 60%, 70% or higher compositions of BMA and HMA.

Acknowledgements

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