Microgels for the Controlled Release of Proteins

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ABSTRACT SUMMARY

Protein therapeutics are playing an ever increasing role in disease treatment; however, their controlled release remains challenging. In this study a series of microgels with varying crosslinking densities was synthesized, characterized and evaluated for their potential as controlled release protein delivery devices.

INTRODUCTION

The advent of modern biotechnology has seen a remarkable increase in the number of therapeutic proteins and more potential candidates are in the pipeline.¹ Currently the FDA has approved more than 130 protein pharmaceutical products with an estimated annual growth rate of 7.08% projected until 2016.² While modern biotechnology has made the production of protein pharmaceuticals possible, there are substantial hurdles to be overcome. Protein function depends on its three dimensional structure, which can be compromised by both proteolytic/chemical degradation and physical/shear forces. To date, almost all therapeutic proteins are administered by the parenteral route and require frequent administration due to their short half-lives.² To effectively deliver protein therapeutics to their target, at proper dosage in a safe and minimally invasive manner, is a prominent challenge in the field. Therefore, there is an increasing need for well-defined controlled protein delivery systems.

Microgels have received recent attention for their possible use as controlled release protein delivery devices.³,⁴ This study presents a novel synthetic approach for the formation of microgels composed of poly(acrylic acid) (pAA) cross-linked with oligo ethylene glycol (OEG). The ability of the microgels to retain proteins and their ability to control the release of proteins was evaluated in in vitro.

EXPERIMENTAL METHODS

Microgels were formulated in two steps. First, pAA was synthesized by reversible addition-fragmentation chain-transfer (RAFT) polymerization. Second, pAA was crosslinked with OEG while in a microemulsion of DMSO droplets suspended in Pluronic® L35. A typical RAFT polymerization was performed as follows: CPA-DB (184 mg, 0.659 mmol) and A-CPA (46 mg, 0.164 mmol) were added into a Schlenk flask with a magnetic stir bar and dissolved in 36.6 mL of anhydrous methanol. Acrylic acid (9.5 mL, 0.138 mol) was then added into the Schlenk flask, a condenser column fitted to the flask and the entire system was purge with nitrogen for an additional 15 minutes. The reaction vessel was shielded from light and the polymerization initiated by lowering the reaction flask into a 60°C oil bath with continuous stirring for two days whereupon the Schlenk flask was placed in an ice bath and contents exposed to air. The product was dialyzed against deionized water for a week, then lyophilized.

Microgels were formed by crosslinking the pAA with the alcohol end-groups of OEG via esterification facilitated with 4-(4,6-dimethoxy-1,3,5-triazin-2-y1)-4-methylmorpholinium chloride (DMTMM) (Fig. 1).

In a typical reaction, 120 mg of pAA were dissolved in 1.5 mL of DMSO, then DMTMM and NMethylmorpholine were added and the solution stirred until complete dissolution. OEG was added to the solution and stirred until homogeneity was achieved. The reaction was then added to a beaker containing 40 g of Pluronic® L35 and stirred at 750 rpm using a Silverson L5M-A homogenizer for 4 hours whereupon microgels were separated by centrifugation at 9500 rpm and supernatant was decanted. The microgels were purified by at least 5 centrifugation/washing cycles with deionized water. Lastly, the microgels were suspended in a small volume of deionized water and lyophilized to dryness. The microgels were loaded with protein by incubation in a protein solution for 24 hours and protein loaded microgels were recovered by centrifugation. Protein release was measured by...
suspension of the microgels in 1 mL of PBS and incubating at 37 °C; the supernatant was collected and replaced at specific time points and released protein was quantified using the BCA method. The enzymatic activity of released lysozyme was evaluated by the *M. lysodeikticus* assay7.

RESULTS AND DISCUSSION

pAA of different sizes were synthesized using RAFT polymerization obtaining an Mn of 13,400 g/mol and 20,000 g/mol with polydispersity of 1.2 and 1.3 respectively. A series of microgel formulations with different crosslinking densities was synthesized (Table 1) and the size and shape of the microgels was evaluated by SEM. There was a general pattern showing that as the cross-linking density decreased the microgels became less spherical. The microgels effectively encapsulated and retained protein. Lysozyme percent loading was over 90% for all six microgel formulations that were tested (Fig 2a). Additionally, qualitative images of myoglobin and hemoglobin loading showed high protein uptake (Fig. 2b). Sustained release of lysozyme released from the microgels retained its activity at levels comparable to that of native lysozyme. This confirms that no significant damage to protein structure occurs while it is in the microgel microenvironment.

![Figure 3: Lysozyme cumulative fraction release from six microgel formulations. Bottom figures shows percent lysozyme activity as compared to native lysozyme.](image)

CONCLUSION

Microgels were synthesized and evaluated as possible devices for the controlled release of proteins. Microgels were able to encapsulate and retain, then release proteins in a controlled fashion. Furthermore, the proteins were not damaged by the microgel as shown by the retained lysozyme activity upon release. These results indicate that these microgels have potential as protein delivery devices.

REFERENCES


ACKNOWLEDGMENTS

Funding provided by a Colman Fellowship, NSF Graduate Research Fellowship and Cornell’s Office for Academic Diversity Initiatives. Cornell Center for Materials Research facilities were used supported by NSF MRSEC (DMR-1120296).