Enzymatically-Degradable Microgels for Pathophysiologically-Triggered Release of Therapeutic Agents

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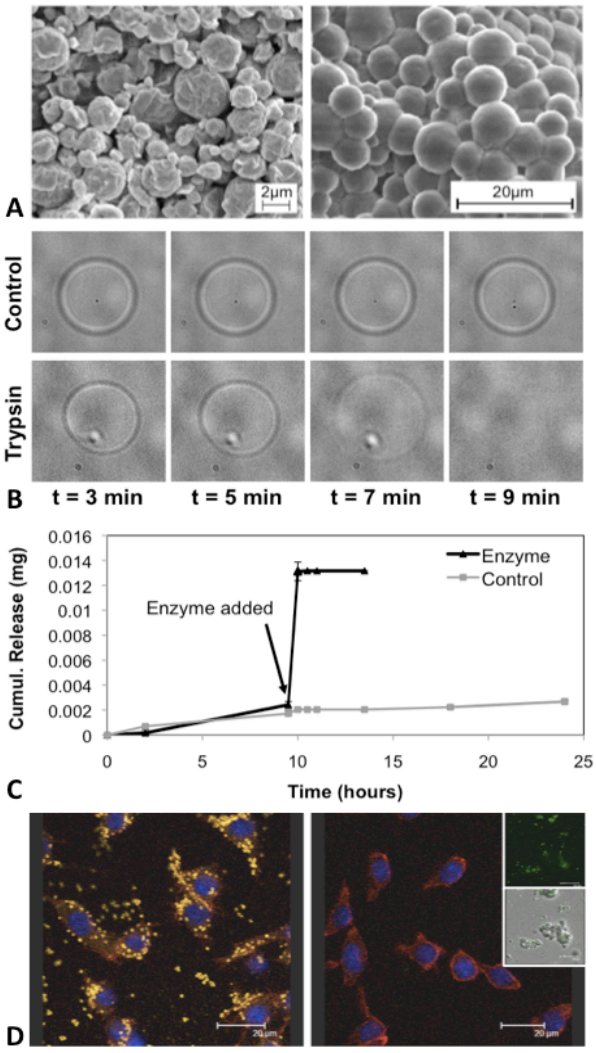
Statement of Purpose: Enzyme-degradable hydrogels have potential for applications in controlled drug delivery due to their highly specific degradation in response to pathophysiologically-up regulated enzymes in diseased tissues. In the present study, we demonstrate a microgel drug delivery system formed by Michael addition reaction during water-in-oil emulsion. These microgels are capable of localized, triggered release of encapsulated drug in response to pathophysiological stimulus (enzyme). Peptide sequences may be easily interchanged based on applicable enzyme/disease. Here, we discuss the potential for non-invasive delivery, with an emphasis on pulmonary delivery, as well as future directions in moving towards a multi-tiered nanoparticle-in-microgel system.

Methods: Microgels were synthesized using a four-arm-PEG-acrylate-10kDa (Laysan Bio, Inc., Arab, AL) and CGRGGC by a water-in-oil emulsion using various surfactants combinations, concentrations, and HLB values to vary particle size. Polymer/peptide solution was added to paraffin oil with surfactant, homogenized, and incubated at 37°C. Drug encapsulation was achieved by adding to the precursor solution. Particle size analysis post-swelling was performed on a Malvern Mastersizer; dry particle size observed by SEM. Time lapse studies to confirm the enzyme-triggered degradation of microgels were performed on a Zeiss Axiovert (60x) mounted with a Ti:S laser for optical trapping. A single microgel was optically trapped in the focal plane, exposed to trypsin, and observed for 10min in comparison to a control. To demonstrate the enzyme-triggered release of bioactives, Alexa Fluor® 594 labeled IgG (Invitrogen, Carlsbad, CA) was encapsulated, and the release profile was quantified using a fluorescence plate reader, along with time-lapse fluorescent imaging to visually confirm release. To demonstrate feasibility as a pulmonary delivery system, microgels were evaluated for "stealth" capabilities to avoid immediate clearance by macrophages using confocal microscopy and FACS to analyze uptake.

Results: Our results demonstrate well-formed microgels with a dry particle size distribution of 0.6-4.1µm (**A**). Optical trapping techniques provided visual confirmation of microgel degradation in response to enzyme (**B**). Microgels encapsulating IgG demonstrated rapid release in response to trypsin, whereas control samples efficiently retained the antibody (**C**). MTS Assay (Promega Corp, Madison, WI) confirmed there was no significant difference in A549 or HEK 293T cell viability at doses of 0.5 and 1.0mg/mL over 48 hours, as compared to the control samples (student t-test, $p<0.05$, data not shown). Raw 264.7 macrophages incubated with microgels and control fluorescent microparticles observed under confocal microscopy showed no uptake of microgels, but high uptake of control microparticles (**D**). These findings were also supported quantitatively by FACS analysis (data not shown).



A. SEM of lyophilized (left) and low vacuum dried (right) microgels
B. Time-lapse images showing enzymatic degradation of a microgel captured by optical tracking at 60x
C. Release study demonstrating enzyme-triggered release of fluorescently labeled IgG from microgels
D. Confocal microscopy showing uptake of control particles (left) versus no uptake of microgel (right, plain microgels at right inset)

Conclusions: We have successfully demonstrated the ability to create enzyme-degradable microgels with encapsulated drug by an *in emulsion* Michael addition cross-linking. The microgels exhibited rapid release in response to enzyme, the ability to retain drug without enzyme, and no adverse effects on multiple cell lines. Furthermore, microgels demonstrated avoidance of clearance by macrophages, as supported by both confocal microscopy and FACS analysis. They system shows much potential in applications for pulmonary drug delivery, and we are currently continuing work towards further characterization *in vitro* and *in vivo*.