Photodegradable microparticles for spatiotemporal control of growth factor delivery

Mark W. Tibbitt[1], April M. Kloxin[1,2], Bruce W. Han[1], & Kristi S. Anseth[1,2]
[1]Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO USA
[2]Howard Hughes Medical Institute, Boulder, CO USA

Statement of Purpose: Hydrogels are often used for controlled delivery of drugs, growth factors, and nucleic acid therapies both *in vivo* for regenerative medicine and *in vitro* for screening diagnostics and directing cell function.[1] Researchers have employed enzymatic hydrolytic degradation to acheive highly controlled and tailorable drug release profiles; however, there still exists a need for platforms to afford facile spatiotemporal control of growth factor delivery. Spatially specific release would be useful to direct anisotropic signalling and tissue morphogenesis. Toward this end, we have developed photoactive, protein-laden, hydrogel microparticles that afford user-defined release in the presence of cells at any point in space or time with light.

Here, photodegradable, PEG-based microparticles were synthesized and their size and release profiles were characterized. Cytocompatibility of the particles, irradiation, and cleavage products was demonstrated as well as bioactivity of released growth factors. Finally, spatial and temporal control of factor delivery was directed by selective irradiation.

Methods: A diacrylated, PEG-based, photodegradable crosslinker (PEGdiPDA) was synthesized as previously described.[2] PEGdiPDA and a PEG-tetrathiol (M_n~10,000 g/mol) were dissolved in PBS (pH 8.0) at 20 wt% and 30 wt%, respectively. Particles were synthesized via an inverse phase microemulsion.[3] Briefly, a macromer solution (10 wt% total macromer) at a 1:1 stoichiometric ratio of acrylates to thiols with 300 mM triethanolamine (TEOHA) and soluble protein were combined with an organic phase of hexanes and surfactants. The inverse emulsion was formed by agitation and the TEOHA catalyzed Michael-addition between the thiols and acrylates formed hydrogel microparticles within 1 hr (Figure 1). Particles were rinsed in hexanes to remove surfactants, then rinsed in 2-propanol, and finally in PBS.

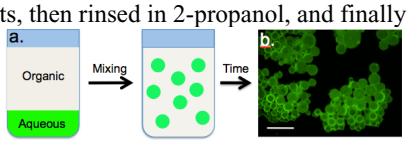

Figure 1: a. Photodegradable microparticles were formed with an inverse phase microemulsion. **b.** Image of particles with fluorescently-labeled BSA (scale bar, 60 μm).

Image analysis (ImageJ) was used to characterize the size of the microparticles. Degradation of particles was induced with flood (365nm at 10 mW/cm^2) or focused laser irradiation (Zeiss 710 LSM, 405nm, 25% intensity). Time-lapse confocal microscopy was used to image the release of a model protein, fluorescently-labeled bovine serum albumin (BSA), from degrading particles.

3T3 fibroblasts and PE25 cells were cultured in DMEM media with 10% FBS at 37 °C with 5% CO_2. For cytocompatibility and proliferation assays, 3T3s were seeded at 20K cells/cm^2. For bioactivity assays, PE25 cells were seeded at 30K cells/cm^2. Viability was measured with the Live/Dead Assay; metabolic activity was measured with AlamarBlue; and proliferation was measured with image analysis and EdU incorporation.

Results: BSA-laden, photodegradable microparticles were synthesized (Figure 1b) with an average diameter of 75 ± 2 1 μm (mean ± s.e.m.) as quantified with image analysis. Degradation of the particles was induced with flood or focused laser irradiation, leading to complete erosion within 8 minutes and 30 seconds of irradiation, respectively. Time-lapse confocal microscopy was used to quantify the release profiles from individual particles, which were described with a statistical-kinetic model of particle degradation and solute diffusion.

Cytocompatibility of the protein release was demonstrated by photodegrading BSA-laden particles in the presence of 3T3 fibroblasts. Viability and metabolic activity were the same for 3T3s in media alone and media with particles (10 mg/mL, wet weight, Figure 2a). Bioactivity of entrapped factors was demonstrated by releasing TGF-β1 (1 ng/mL) in the presence of PE25 cells, which respond to TGF-β1 by producing luciferase. Luciferase activity was significantly increased with TGF-β1 release as compared to control cells (Figure 2b).

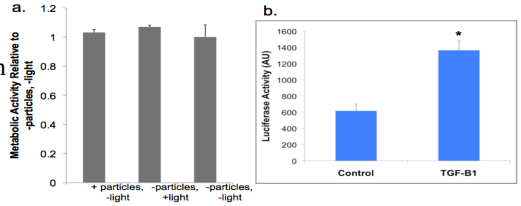

Figure 2: a. Metabolic activity of 3T3s is independent of degradaded particle or light presence. **b.** Luciferase activity is significantly upregulated ($p < 0.05$) in PE25 cells exposed to released TGF-β1 as compared to control cells with only media.

With a mixed population-laden particles, in focused light was used to induce degradation selectively to release BSA at t_1 and TGF-β1 at t_2. Further, photolithography was employed to spatially define FGF-2 release in the presence of 3T3s for directing cell proliferation in regions of interest and demonstrating spatially controlled growth factor release.

Conclusions: Photodegradable microparticles offer spatial and temporal control of bioactive growth factor release in the presence of cells. This platform offers new opportunities to study anisotropic signalling and direct graded tissue morphogenesis *in vitro*.

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References:

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