Engineering Cell-Triggered Release to Achieve Temporal Control

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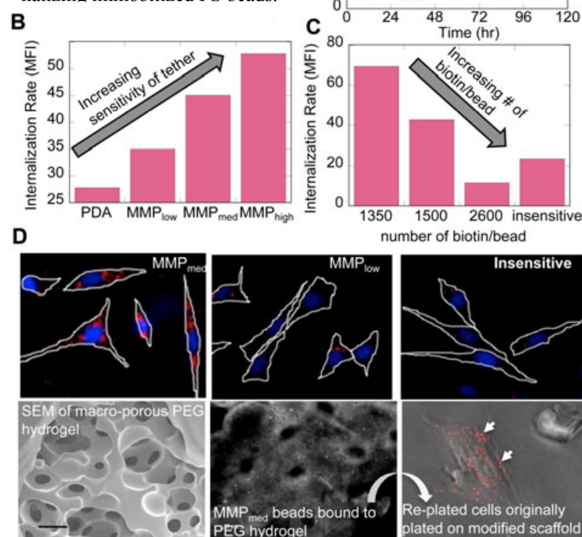
Figure 1. (**A**) Release of PS beads in condition media. PDA has no peptide tether. (**B, C**) Flowcytometry analyzed internalization rate as a function of peptide sequence and number of tethers. (**D**) Images of cells internalizing immobilized PS beads.



Statement of Purpose: Enzymatically degradable tethers have been utilized for the immobilization and release of growth factors and small drugs, which are only liberated by cleavage caused by cell secreted proteases, such as MMPs or plasmins, during local tissue remodeling. These proteases are known to be up-regulated during wound healing, microenvironment remodeling, and in diseased states and can, therefore, serve as triggers for bioactive signal delivery. Cell responsive systems, however, have yet to be developed for the delivery of multiple factors at distinct rates, which show efficacy in cellular environments. We have designed a strategy release nanoparticles through the action of cell-released MMPs. In our strategy the nanoparticles are immobilized to biomaterials through an MMP degradable peptide tether. The goal was to use peptide sequences that have been shown to degrade at different rates through the action of MMPs to achieve temporally controlled nanoparticle internalization by cells that overexpress MMPs.

Methods: *Particle modification:* MMP degradable peptides (MMP_{high}: Ac-KRGPQGIWGQDRCGR-NH_2, MMP_{med}: Ac-KRGPQGIAGQDRCGR-NH_2, MMP_{low}: Ac-KRGDQGIAGFDRCGR-NH_2) were reacted with NHS-PEG-biotin or NHS-LC-biotin or NHS-LC-acrylate to introduce biotin and acrylate functionalities, respectively. Carboxylate-modified, fluorescently labeled 40nm polystyrene beads (PS) were modified to possess biotinylated or acrylated peptide tethers using amine carboxylic acid chemistry with EDC as a catalyst (PS-MMP_x-Biotin or PS-MMP_x-AC). Control particles without the peptide crosslinker were synthesized in an analogous fashion using adipic acid hydrazide instead of the peptide. Dynamic light scattering and elemental analysis was used to determine the final size and degree of modification of the beads with biotin. *Release and internalization studies:* The MMP modified beads were immobilized to either tissue culture plates covalently modified with streptavidin or macro-porous PEG-diacrylate hydrogels. For release studies the surfaces were incubated in a variety of release mediums (cell conditioned media, collagenase I or PBS) and the surface was scanned for fluorescence with time. For internalization studies modified surfaces and hydrogels were seeded with either mMSCs or HEK293T or $HEK293T_{MMP-2}$ (which are cells transfected to overexpress MMP-2). At specified time points images were takes and cells were collected for flowcytometry analysis. *Macro-porous hydrogel synthesis*: Macro-porous PEG-diacrylate hydrogels were synthesized using 90-125µm diameter PMMA beads as the mold. Hydrogels were formed around tightly packed PMMA beads, after which the beads were degraded with acetone leaving behind a macro-porous hydrogel structure.

Results: We found that release rates of peptide-immobilized nanoparticles were a function of peptide sensitivity to proteases, the number of tethers between the nanoparticle, and the surface and the concentration of proteases used to induce release. No extensive particle internalization was observed when particles were modified solely with polymer chains, while peptide-modified particles were internalized to a significantly greater extent by cells, which express high levels of proteases. Flowcytometry data showed that protease-expressing cells internalized approximately 10-fold more particles when compared to non-protease expressing cells, suggesting that nanoparticle internalization could be controlled by the protease expression profile of the cells. Cellular internalization of the peptide-immobilized nanoparticles was also a function of the peptide sensitivity to proteases, the number of tethers between the nanoparticle and the surface and MMP expression profile of the cells. Similar trends were observed for peptide-immobilized nanoparticles inside micro-porous hydrogels, indicating protease sensitive tethers are effective in controlling release rate and internalization of nanoparticles (**Figure 1**).

Conclusions: By immobilizing nanoparticles through protease sensitive peptide tethers, release was tailored specifically for an intended cellular target, which over-expresses such proteases. Release was also shown to vary significantly in the presence or absence of cells, indicating the importance of such cellular release studies, which have generally not been conducted in previous sequential release studies. We observed that the protease (namely MMP) expression profile of the seeded cells was the main factor in determining the release and internalization kinetics of MMP-sensitive peptide-modified particles.

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