Controlled Release of Bioactive Transforming Growth Factor Beta 1 from Affinity Peptide Hydrogels

Joshua McCall,[1] Chien-Chi Lin[1,2] & Kristi Anseth[1,2]
[1]Dept. of Chemical & Biological Engineering and [2]Howard Hughes Medical Institute, University of Colorado, Boulder, CO USA

Statement of Purpose: Transforming growth factor beta 1 ($TGF\beta_1$) is a potent chemokine implicated in numerous cellular functions, including growth and differentiation of human mesenchymal stem cells (hMSCs). Biomaterial platforms capable of releasing this growth factor in a controllable manner present a powerful tool for dictating stem cell fate. In this work, we utilize a thiol-acrylate photopolymerization scheme to covalently incorporate affinity binding peptide sequences Trp-Ser-His-Trp (WSHW)[1] and Lys-Arg-Ile-Trp-Phe-Ile-Pro-Arg-Ser-Ser-Trp-Tyr (KRIWFIPRSSWY)[2], into poly(ethylene glycol) (PEG) diacrylate hydrogel networks. The objective of this work is to tune the release of bioactive $TGF\beta_1$ from these materials in order to control hMSC differentiation.

Methods: *Synthesis and Purification of Affinity Peptides:* Peptides were synthesized with a solid-phase peptide synthesizer and purified using reverse phase HPLC. Purified peptides were identified with MALDI-TOF mass spectrometry. *Preparation of Affinity Hydrogels:* Macromer solutions of PEG diacrylate (PEG_{DA}-10kDa) at a concentration of 20 mM and $TGF\beta_1$ (Peprotech) at a concentration of 25 nM were prepared with varying concentration of affinity peptides, including (CGGGGWSHW) and (CKRIWFIPRSSWY), along with 1 mM photoinitiator lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LAP)[3]. Solutions were exposed to 365 nm UV light at an intensity of ~10 mW/cm^2 for 3 min. *Release of $TGF\beta_1$:* Hydrogels were placed in 1.5 mL PBS release buffer at 4°C, and the buffer was collected at predetermined time points. Diffusion was assumed to be 1-D and with "sink" conditions. $TGF\beta_1$ concentration in the release supernatant was determined with an ELISA kit (BD Biosciences). *Bioactivity of Released $TGF\beta_1$:* Mink lung epithelial cells permanently transfected with a SMAD reported gene (PE-25 cells) were plated at a density of ~50,000 cells/cm^2 in 24-well plates. Affinity hydrogels were placed in transwell plates, and cells were incubated 20 hours in serum-free high-glucose DMEM (Gibco). The cell lysate was mixed with luciferase substrate and luminescence was measured with spectrophotometry.

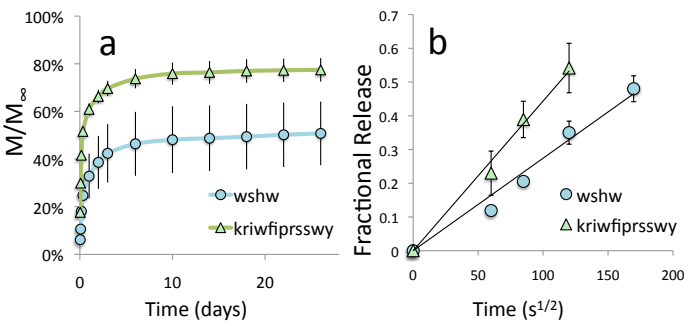

Figure 1. a) $TGF\beta_1$ released as a function of percent loaded for the two affinity peptide sequences (WSHW) and (KRIWFIPRSSWY) over a 26-day time period, b) materials exhibit different initial rate of $TGF\beta_1$ release.

Results: Affinity hydrogels containing binding peptides WSHW or KRIWFIPRSSWY, at a molar ratio of 1000 to $TGF\beta_1$ (R=1000), showed unique release profiles over a 26-day timescale (Figure 1a). The total amount of $TGF\beta_1$ released from the gels is due to different K_D values for the affinity interaction between respective peptide and $TGF\beta_1$. Comparison of initial release rate (Figure 1b) shows different effective diffusivities for the two peptide-functionalized hydrogels. This further confirms affinity-controlled diffusion from the polymer matrix. Furthermore, the bioactivity of released growth factor was confirmed with a PE-25 reporter cell assay. Hydrogels with affinity peptides (R=1000) showed luminescence approximately two-fold higher than that of gels without $TGF\beta_1$, similar to the response from gels with equivalent amounts of entrapped $TGF\beta_1$ (Figure 2).

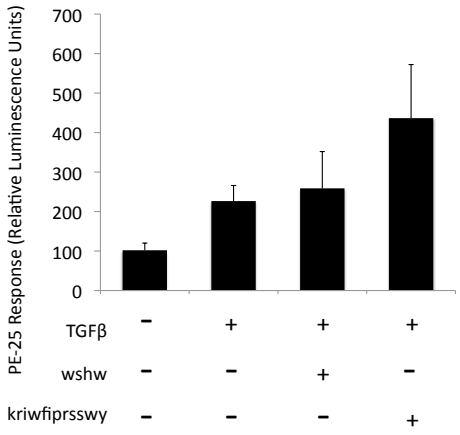

Figure 2. $TGF\beta_1$ released from affinity gels shows a similar fold-increase in relative luminescence from PE-25 cells, compared to gels made with entrapped $TGF\beta_1$.

Conclusions: A biomaterial platform utilizing a thiol-acrylate photopolymerization reaction scheme to incorporate WSHW or KRIWFIPRSSWY affinity peptide sequences into PEG hydrogels was developed to control the release of $TGF\beta_1$. PE-25 cell studies confirm that $TGF\beta_1$ bioactivity is maintained post-release. Tailorable release of $TGF\beta_1$ from affinity hydrogels is in development for controlling the differentiation of encapsulated mesenchymal stem cells.

References:[1]Young, G, et. al., Jour Biol Chem. 2004; 279.46:47633-47642, [2]Dotor, J, et. al., Cytokine. 2007;39:106-115, [3]Fairbanks, B, et. al., Biomaterials. 2009;30:6702-7, [6]The authors would like to thank our funding sources: NIH (1RO1DE12998), and Howard Hughes Medical Institute.