Sequestration of endogenous TGF-β for directed differentiation of mesenchyal stem cells

Sarah B. Anderson, Kristi S. Anseth
Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO USA
Howard Hughes Medical Institute, Boulder, CO USA

Statement of Purpose: It is well established that TGF-β promotes chondrogenic differentiation of mesenchymal stem cells (MSCs) *in vitro* (*1*). In the context of tissue engineering, TGF-β has been delivered to various cell types via microparticle release and hydrogel tethering, among others (*2, 3*). These approaches suffer a common weakness, in that recombinant TGF-β1 or TGF-β3 must be used since native TGF-β resides as an inactive complex in which the active site is masked by an inhibitory peptide called Latency Associated Peptide (LAP) (*4*). As a result, grafts containing recombinant TGF-β may contain unusually high concentrations of active TGF-β that could have promiscuous effects in the joint space, such as fibrosis or mineralization (*5*). Here, we develop a hydrogel system that sequesters native TGF-β to render it locally bioavailable, at endogenously relevant concentrations, by tethering the LAP-binding integrin complex $\alpha_V\beta_6$ to a PEG hydrogel.

Methods: *Cultured Cells:* PE25 cells are mink lung epithelial cells with a stably transfected luciferase reporter of TGF-β activity (*6*). Human MSCs were derived from bone marrow by ammonium chloride lysis. *Luciferase and Picogreen Assays:* Cells were treated with mature TGF-β1 (Peprotech) or latent TGF-β1 (R&D Systems). Cells were lysed and luciferase was measured using the Luciferase Assay System (Promega). DNA content was determined using the Quant-iT Picogreen Assay (Invitrogen). *Western Blots:* Proteins were separated by SDS-PAGE on 10% gels and transferred to PVDF membranes. Blots were probed for phosphorylated Smad2, Smad2/3 or β-actin (Cell Signaling Technologies). *Protein:* Integrin $\alpha_V\beta_6$ was thiolated with Traut's Reagent (Thermo Scientific). *Hydrogels:* Macromer solutions of 10wt% PEG diacrylate 10 kDa and thiolated integrin $\alpha_V\beta_6$ were photopolymerized with 0.05% I2959 photoinitiator. *ELISA:* Released latent TGF-β1 concentration was determined by ELISA using latent TGF-β1 capture and detection antibodies (R&D Systems).

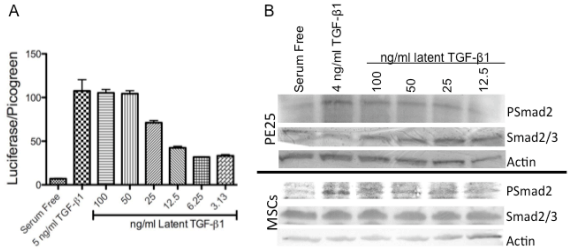

Figure 1. PE25 TGF-β reporter cells and MSCs respond to latent TGF-β1. Cells were treated with either mature TGF-β1 or latent TGF-β1, as indicated. (A) PE25 phospho-Smad2 luciferase reporter cells were analyzed for luciferase expression normalized to DNA content, as measured by Picogreen. (B) PE25 cells or MSCs were analyzed by Western Blot for the indicated proteins. PSmad2 indicates phosphorylated-Smad2.

Results:

To confirm that the phospho-Smad2-luciferase TGF-β reporter cell line PE25 and MSCs are capable of activating the endogenous, latent form of TGF-β1, cells were treated with either mature TGF-β1 or latent TGF-β1. The PE25 cells were evaluated for luciferase expression and phopho-Smad2 protein (Fig. 1A,B). MSCs were evaluated for phopho-Smad2 (Fig. 1B). TGF-β signals were detected in both PE25 cells and MSCs.

Latent TGF-β interacts with integrin $\alpha_V\beta_6$ through an RGD motif in LAP (*7*). We converted the primary amines of integrin $\alpha_V\beta_6$ to free sulfhydryls for facile incoporation into a PEG diacrylate network. Thiolated integrin $\alpha_V\beta_6$ was incorporated into a 10wt% 10 kDa PEG diacrylate monomer solution that was then photopolymerized to form a hydrogel network. Latent TGF-β1 was swollen into the hydrogel network and then release from the network was monitored (Fig 2). Incorporation of integrin $\alpha_V\beta_6$ to the hydrogel network increases the release of latent TGF-β1.

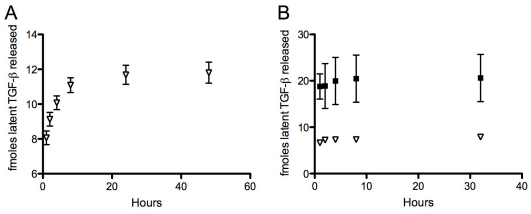

Figure 2. Integrin $\alpha_V\beta_6$ sequesters latent TGF-β1 in a 10wt% 10 kDa hydrogel network. Latent TGF-β1 concentration was measured by ELISA. (A) Hydrogels were swollen for 36 hours with latent TGF-β1 and then release from the network was monitored. (B) Hydrogels with tethered integrin $\alpha_V\beta_6$ (▽ 0 fmoles, ■ 50 fmoles) were swollen with latent TGF-β1 for 5 days and then release from the network was monitored.

Conclusions: A photopolymerizable hydrogel system was developed for sequestration of endogenous latent TGF-β. Bioactivity of latent TGF-β1 was confirmed in the reporter cell line PE-25 and in MSCs. Sequestration of latent TGF-β1 was observed in a hydrogel network to which integrin $\alpha_V\beta_6$ was tethered. Directed differentiation of MSCs with sequestered TGF-β is currently under investigation.

References: 1. M. F. Pittenger *et al.*, *Science* **284**, 143 (Apr 2, 1999). **2.** B. K. Mann, R. H. Schmedlen, J. L. West, *Biomaterials* **22**, 439 (Mar, 2001). **3.** J. S. Park, H. N. Yang, D. G. Woo, H. M. Chung, K. H. Park, *Tissue Eng Part A* **15**, 2163 (Aug, 2009). **4.** K. Miyazono, U. Hellman, C. Wernstedt, C. H. Heldin, *J Biol Chem* **263**, 6407 (May 5, 1988). **5.** E. N. Blaney Davidson, P. M. van der Kraan, W. B. van den Berg, *Osteoarthritis and Cartilage* **15**, 597 (2007). **6.** X. Hua, X. Liu, D. O. Ansari, H. F. Lodish, *Genes Dev* **12**, 3084 (Oct 1, 1998). **7.** J. S. Munger *et al.*, *Cell* **96**, 319 (Feb 5, 1999).