Impact of Reaction Conditions on PEGylated Fibrin Gelation and Cell Behavior

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Introduction: Microenvironmental cues are critical for regulating cell behavior and fate. The roles that mechanical signals play in regulating cell behavior have been reported recently [(1)], which suggests that an artificial matrix that can maintain appropriate characteristics for transplanted stem cells is needed to achieve the desired cell type. We have developed a PEGylated fibrin ECM mimic using difunctional BTC-PEG which can induce entrapped mesenchymal stem cell (MSC) differentiation towards cardiovascular cell types without adding soluble angiogenic growth factors. [(2)]In this study, we modified fibrinogen with various polyethylene glycol (PEG) derivatives and found that the critical factor affecting the matrix characteristics is the reaction half life of the PEG derivatives. We hypothesized that by adjusting the reaction conditions which affect hydrolysis, we could alter the properties of our PEGylated fibrin biomatrix and allow it to be a tunable system for tissue engineering.

Materials and Methods: PEG derivatives were used to PEGylated fibrinogen as previously described. [(2)] Human MSCs (hMSCs) were purchased from Cambrex and cultured in MSCBM medium (Cambrex) according to manufacturer's specifications. Passage 3-5 MSCs were washed with phosphate-buffered saline (PBS), trypsinized, and collected in MSCBM media without serum at 4X final cellular density. Equal volumes of the PEGylated fibrinogen solution and the cell suspension were mixed thoroughly and gels were formed immediately thereafter by the addition of thrombin. The final concentrations were: cells at $5x10^4$ cells/ml, fibrinogen at 10 mg/ml, thrombin at 12.5 U/ml, and PEG at a 10:1 molar ratio to fibrinogen. Media was added after incubating the gels for 10 min. Cell morphology was observed under a light microscope.
Homobifunctional NPC (3400 Da, SunBio, South Korea) were reacted with fibrinogen under different pH (pH 7.0, 7.5, 7.8, 8.2 and 8.5) as previously described [(2)]. BTC-PEG-BTC (3400 Da, Nektar, San Carlos, CA) and fibrinogen solution were included as controls. Crosslinked samples were either collected for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis on 4-20% Tris-HCl gels as previously described [(2)], or were used for cell seeding. For the latter, the PEGylated fibrin solutions were dialyzed overnight in Dispodialyzer membranes (Spectra/Por) in a 1x TBS solution at pH 7.8 as per the manufacturer's instructions prior to cell seeding.

Results and Discussion: The effect of pH on the degree of crosslinking between PEG and fibrinogen was observed using SDS-PAGE. Distinct banding pattern differences were evident by varying the reaction conditions. Compared to the fibrinogen control, the degree of crosslinking in NPC-PEG-NPC solutions increased as pH increased (Figure 1). These results confirm that by modifying the reaction conditions, such as by changing the pH, the concentration of PEGylated fibrin crosslinks can be changed proportionally.

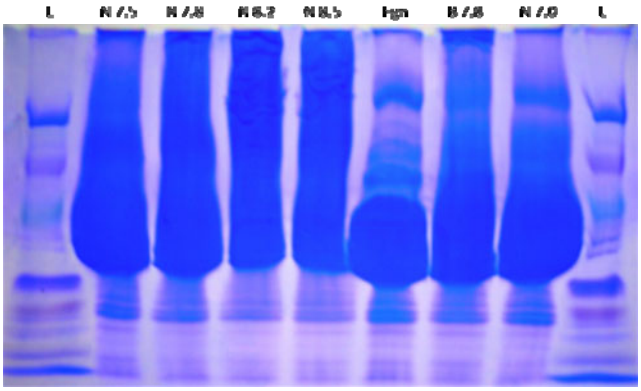

Figure 1: SDS-PAGE of NPC and BTC-PEGylated fibrin. Bands were stained with Coomassie Blue. Lane designations: L, ladder; N, NPC-PEG; Fgn, Fibrinogen; B, BTC-PEG. Numbers indicate the pH of the reaction.

Prior to cell seeding, the PEGylated fibrin solutions were dialysed to neutralize the pH. The final protein concentrations remained unchanged. The initial reaction pH had no effect on the ability of the PEGylated fibrin to gel upon addition of thrombin. MSC morphology varied in gels and was dependent on both the type of PEG as well as the reaction conditions.

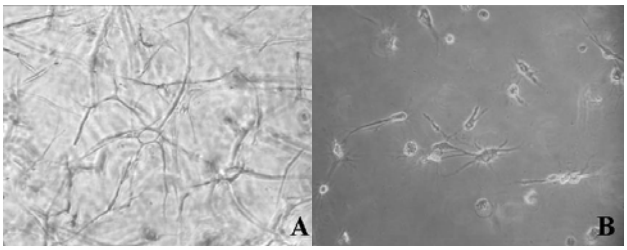

Figure 2: MSC morphology following embedding in PEGylated fibrin gels, (A) BTC-PEG, pH 7.8, (B) NPC-PEG, pH 7.8.

Changes in cell morphology were observed with BTC-PEG-BTC (Figure 2A) and NPC-PEG-NPC (Figure 2B). Morphological changes included the extent of cell spreading and proliferation *in vitro*. These differences demonstrate that MSC differentiation can be altered by the PEG derivative chosen as well as PEGylation reaction conditions.

(1) Engler, A. J., Sen, S., Sweeney, H. L. and Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell*, 126, 4 (Aug 25 2006), 677-689.
(2) Zhang, G., Wang, X., Wang, Z., Zhang, J. and Suggs, L. A PEGylated fibrin patch for mesenchymal stem cell delivery. *Tissue Eng*, 12, 1 (Jan 2006), 9-19.