Chemoattractant Degradation Products of Extracellular Matrix Bioscaffolds

Ellen P. Brennan, Janet Reing, Li Zhang, Julie M. Myers-Irvin, Stephen F. Badylak
McGowan Institute for Regenerative Medicine, University of Pittsburgh

Statement of Purpose: Xenogeneic and allogeneic extracellular matrix (ECM) has been successfully used as a resorbable scaffold for tissue engineering/regenerative medicine applications. Such scaffolds induce site-specific constructive remodeling of injured tissue and are completely degraded within 90 days of implantation [1]. The mechanisms by which remodeling occurs include cell recruitment, three-dimensional spatial reorganization, and vascularization. Degradation products of these scaffolds have been shown to possess antimicrobial activity against clinical strains of gram positive and gram negative bacteria in *in vitro* studies [2]. The objective of the present study was to investigate a separate biologic activity, chemoattractant activity, within the degradation products of ECM scaffolds.

Methods: Porcine urinary bladders were used to prepare urinary bladder matrix (UBM-ECM) as previously described [3]. UBM-ECM was then lyophilized, frozen, and comminuted into a particulate form. Particulate UBM-ECM was then degraded either by chemical and physical methods or by enzymatic treatment.

To digest UBM-ECM using acid and heat, a previously described procedure [4] was adapted. Lyophilized, particulate UBM-ECM was suspended in PBS with protease inhibitors, and dried by vacuum filtration on Whatman #42 filter paper. The hydrated retentate was collected and suspended in 0.5 N acetic acid. The suspension was transferred to a stirred autoclave reactor, brought to 120°C over 50 minutes, and held at 120°C for 30 minutes. The acid-digested suspension was then filtered in series, through cheesecloth, Fischer P8 filter paper, and Whatman #42 filter paper. The filtrate was snap-frozen and lyophilized, yielding a dry ECM digest.

Ammonium sulfate precipitation was performed to isolate fractions of the acid/heat UBM-ECM digest for evaluation of chemoattractant activity. The dry UBM-ECM digest was resuspended at a concentration of 10 mg protein per mL buffer in pH 6.8 buffer containing 0.1M sodium phosphate / 0.15M sodium chloride. Ammonium sulfate was added to the suspension to saturation percentages of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 100%. Samples of pellets and supernatants from each saturation percentage were retained and desalted, concentrated and pH neutralized using dialysis or centrifugal filter devices.

To digest UBM-ECM using pepsin, particulate UBM-ECM was added to 1 mg/mL pepsin in 0.01 N HCl for a final concentration of 10 mg UBM-ECM/mL suspension. The suspension was mixed at room temperature for 48 hours, at which time the UBM-ECM was fully digested.

UBM-ECM digests were assayed for chemoattractant activity for a multipotential cell population isolated from the healing blastema of an injured MRL mouse. The assays were performed in triplicate using the Boyden chamber cell migration assay (Neuro Probe).

Results/Discussion: Acid/heat degraded UBM-ECM fractions generated by ammonium sulfate precipitation induced increased migration of MRL blastemal cells (Figure 1). In particular, the fraction precipitated at 70% ammonium sulfate saturation increased the number of migrating MRL blastemal cells to roughly 400% the number of cells migrating to the control sample.

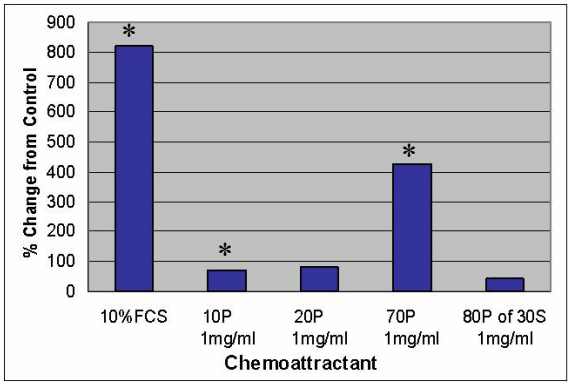

Figure 1. Migration of MRL blastemal cells toward ammonium sulfate precipitated fractions of acid/heat degraded UBM-ECM (* denotes $p < 0.05$)

Pepsin-digested UBM-ECM also induced a significant increase in MRL blastemal cell migration (Figure 2). The 200 µg/mL UBM pepsin digest showed a roughly 700% increase in MRL blastemal cell migration compared to the control sample.

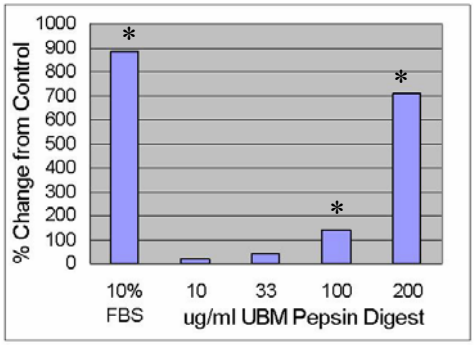

Figure 2. Migration of MRL blastemal cells toward pepsin digested UBM-ECM (* denotes $p < 0.05$)

Conclusions: Degradation products of UBM-ECM scaffolds produced in vitro induce migration of MRL blastemal cells. The recruitment of multipotential cells to the site of ECM scaffold remodeling may help explain the constructive remodeling outcomes that have been seen in both preclinical and clinical studies in which these scaffolds have been used.

References:

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4. Sarikaya A. Tissue Eng. 2002;8:63-71.