Controlled Generation of Chemokine Gradients in 3D Matrices for Directing T Cell Migration

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Statement of Purpose: Lymphocyte trafficking between lymphoid organs, the blood, and peripheral tissues is critical to proper function of the immune system, and chemokines are thought to play a key role in regulating lymphocyte migration.[1, 2] However, little is known about the quantitative requirements for chemokine concentration gradients to stimulate chemotaxis and chemokinesis in T cells. We are using a simplified *in vitro* model to quantitatively study human T cell motility in response to defined concentration gradients of the chemokine CCL21 produced by controlled release of chemokine from poly(ethylene glycol) (PEG)-based hydrogels, using videomicroscopy to track the migration behavior of individual lymphocytes in response to chemokine gradients. This work is a first step toward designing biomaterials and tissue engineering scaffolds that can direct the migration of cells to desired positions within a matrix.

Methods: Human T cells were isolated from peripheral blood leukocytes of anonymous healthy volunteers using Pan T cell or CD4 T cell magnetic bead-based negative selection (Miltenyi, Auburn, CA), and cultured in RPMI 1640 medium with 10% FCS overnight before use. Aqueous solutions containing PEG methacrylate (PEGMA) and PEG dimethacrylate (PEGDMA) were photopolymerized as previously described[3] within PDMS rubber molds to form cylindrical gels with total volumes of 5 µL. Recombinant CCL21 (R&D Systems, Minneapolis, MN) or 10 KDa FITC-dextran (Sigma) was loaded into PEG hydrogels by soaking gels in solutions for 18 hr, followed by washing to remove non-absorbed molecules. T cells were fluorescently labeled using carboxymethylfluorescein diacetate (Molecular Probes, Eugene, OR) following the manufacturers instructions. Labeled cells were then polymerized within a 1.8 mg/mL type I collagen gel (BD biosciences) surrounding the CCL21-loaded hydrogel (Fig. 1). Migration of the cells was tracked by time-lapse fluorescence videomicroscopy.

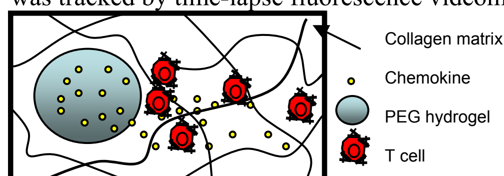

Figure 1. Schematic of experimental strategy.

Results/Discussion: The chemokine CCL21 or fluorescent dextran (as a labeled surrogate of the chemokine) were loaded into cylindrical PEG gels by soaking the gels in solutions of these molecules. Fluorescence imaging of dextran-loaded gels showed that overnight incubation was sufficient to load dextran throughout cylindrical 5 µL gels. We first characterized the diffusion-based release of fluorescent dextran from PEG gels embedded in collagen matrices. As shown in Fig. 2A, decreasing the crosslink density in PEG gels by decreasing the PEGDMA:PEGMA ratio allowed the rate of release and thus also the shape of the dextran concentration profile of released dextran to be varied. To determine whether concentration profiles of CCL21 released from PEG could stimulate T cell chemotaxis through collagen toward the PEG gel, 1:1 PEGDMA:PEGMA gels were loaded with 20 ng CCL21 and placed at the center of collagen gels containing fluorescently-tagged T cells. As shown in Fig. 2B, T cells within 500 µm of the edge of the PEG gel exhibited strongly directed migration toward the chemokine source, with many cells migrating directly up to the edge of the chemokine-releasing PEG matrix. Cells that reached the gel continued to migrate randomly in the vicinity of the gel but remained near the edge of the PEG gel. In the absence of chemokine, minimal motility of T cells was observed.

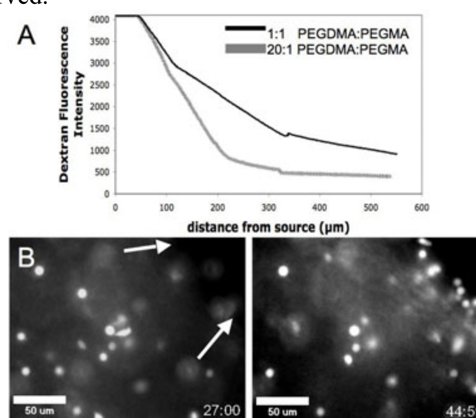

Figure 2. (A) Mean fluorescence intensity of labeled dextran diffusing out of PEG hydrogels into collagen. (B) Two videomicroscopy snapshots separated by 18 min showing the chemotaxis of T cells to a CCL21-releasing PEG gel. Arrows in the first panel denote the edge of the PEG gel.

Conclusions: We have shown that CCL21 gradients generated by controlled release of chemokine from PEG hydrogels can be used to direct T cell chemotaxis through collagen matrices. We are presently evaluating how changes in the concentration gradient of chemokine alter T cell attraction, and how the presence of polysaccharides such as heparan sulfate, which could anchor diffusing chemokines to the collagen matrix, influence T cell migration.

References

1. Campbell, DJ et al. Immunol Rev 2003; 195: 58-71.
2. Willimann, K et al. Eur J Immunol 1998; 28: 2025-34.
3. Stachowiak, AN, et al. Adv. Mater. 2005; 17: 399-403.