Development of a streptavidin-biotin hydrogel scaffold for modulating T cell activation

Niroshan Anandasivam[1], Derfogail Delcassian[1]
[1]Department of Bioengineering, University of California, Berkeley, Berkeley, CA

Statement of Purpose: T cell activation and proliferation are essential processes of the adaptive immune response. Emerging adoptive cell therapies rely on T cell activation and expansion strategies to produce clinically relevant numbers of T cells prior to patient reinfusion. However, the manufacturing timescale of these therapies is on the order of 1 month and is often rate-limited by slow expansion steps. Motivated by this issue, several others have shown how extracellular cues from the T cell microenvironment can be used to accelerate T cell activation. A biphasic response of T cells to substrate stiffness has been reported in polyacrylamide-based hydrogel systems [1-2]. Additionally, this response has been linked to changes in a wide array of critical cell functions, like migration, metabolism, and cell cycle progression [3]. Here, we develop a novel polyethylene glycol diacrylate (PEGDA) hydrogel scaffold with streptavidin acrylamide that serves as a screening platform for evaluating the effects of matrix stiffness in combination with ligand presentation on T cell activation.

Methods: PEGDA with streptavidin acrylamide (PEGDA-Strep) sandwich gels were fabricated on silane-treated glass slides. To functionalize hydrogels with ligands of interest, biotinylated anti-human CD3 and CD28 antibodies were incubated in solution with gel (Figure 1). Elastic moduli of hydrogels were measured and calculated using a dynamic shear rheometer. For activation studies, CD4+ T cells were seeded and grown on biotinylated ligand-coated hydrogels for 20 hours. Expression of activation markers (CD69 and CD25) was measured using flow cytometry, and IL-2 secretion in the cell supernatant was quantified.

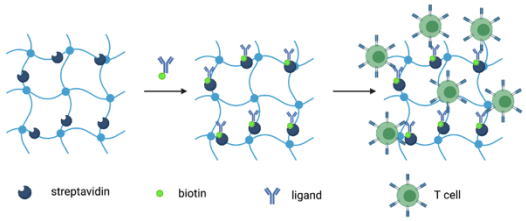

Figure 1: Schematic of immobilization of biotinylated ligand for presentation using a PEGDA-Strep scaffold.

Results: PEGDA-Strep gels were synthesized and mechanically characterized, with elastic moduli ranging from 1 kPa to 120 kPa (Fig. 2A). T cells grown on PEGDA-Strep hydrogels with immobilized anti-CD3 and anti-CD28 ligands exhibited a substrate stiffness-dependent activation response. Specifically, T cells seeded on PEGDA-Strep gels showed increasing expression of CD69 as elastic modulus was increased from 1 kPa to 120 kPa (Fig. 2B).

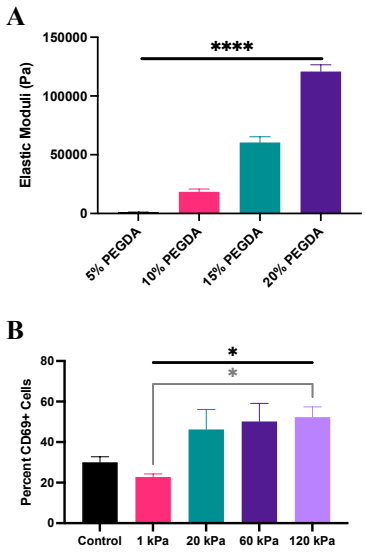

Figure 2. **(A)** Elastic moduli of PEGDA-Strep hydrogels of varying monomer concentrations. Data are mean ± SEM. n=3. Statistics show one-way ANOVA where ****P<0.0001 **(B)** CD69 expression of CD4+ cells grown on PEGDA-Strep hydrogel for 20 hours. Data are mean ± SEM. n=5. Statistics show one-way ANOVA (black) and pairwise comparisons (gray) where *P<0.05.

Conclusions: This work introduces a novel 2D hydrogel system for manipulating the T cell microenvironment that is bioorthogonal, nonadhesive, and highly amenable to 3D culture and scaleup via strategies like 3D printing. Here, we establish proof of concept for this system by detailing differences in the T cell activation response with respect to substrate rigidity. We show that increased stiffness leads to higher T cell activation on PEGDA-Strep gels that present stimulating antibodies in culture. Future work will continue investigating how mechanical and biochemical cues are integrated to alter T cell activation upon ligand binding through ligand screens. An improved understanding of the factors that impact T cell activation will provide much needed insight into manufacturing strategies for adoptive cell therapies.

References:

[1] Judokusumo, E. Biophys. J., 2012;**102**: L5-L7.
[2] Yuan, D.J. Biomaterials, 2021. **273**: 120797.
[3] Saitakis, M. eLife, 2017. **6**.