Surface-Initiated Photopolymerization to Fabricate Functionalized Coatings which Provide Local T cell Immunosuppression

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Statement of Purpose: Cell encapsulation has long been investigated as a means to achieve transplant immunoprotection, but encapsulation alone has generally proven to be insufficient to protect allograft tissue from rejection[1]. In the case of type I diabetes, β-cell transplants must be protected by systemic immunosuppression because encapsulation alone is inadequate to protect β-cells from small cytotoxic molecules produced by activated T cells, such as reactive oxygen species and cytokines[2]. The purpose of this work was to fabricate an immunologically-active polymeric coating for an encapsulation device which influences the local immune environment by mimicking a natural T cell regulation pathway. T cells are regulated *in vivo* via Fas/Fas ligand signaling, whereby cells located in immune-privileged regions express Fas ligand (FasL) and trigger T cell apoptosis upon binding the Fas receptor on a T cell surface[3]. Previous work has focused on conjugating anti-Fas antibody (DX2) directly to a polymer surface to trigger Fas-mediated apoptosis[4], but herein, we investigate a surface-initiated polymer coating, consisting of surface-anchored polymer chains with pendant DX2, as a means to increase DX2 surface density and mobility, and elicit T cell apoptosis. Further, we show that the incorporation of a T cell adhesion ligand, Intracellular Adhesion Molecule-1 (ICAM-1), into a DX2-functionalized coating increases T cell apoptosis.

Methods: *Acrylation of proteins:* Proteins were reacted with ACRYL-$PEG_{3,400}$-NHS in various molar ratios, to yield ACRYL-DX2, ACRYL-ICAM-1, and ACRYL-F-IgG (fluorescein-tagged IgG). *Incorporating proteins into surface-initiated polymer chains:* A polymeric base layer consisting of equal parts urethane diacrylate and tri(ethylene glycol) diacrylate, with 1.5 wt% 2,2-dimethoxy-2-phenylacetophenone (DMPA) initiator and 0.25 wt% tetraethylthiuram disulfide (TED) iniferter was used. 250 μg/ml ACRYL-F-IgG, 250 μg/ml ACRYL-DX2 and/or 25 μg/ml ACRYL-ICAM-1 were dissolved in 50% ACRYL-PEG_{400} in H_2O and photopolymerized on the TED-containing base layer for 0-900s under UV light (37 mW/cm^2, centered at 365 nm). Polymerized ACRYL-DX2 was quantified using a modified ELISA, whereby DX2-functionalized polymer coatings where incubated with soluble Fas receptor, anti-Fas ab, and a detection Ab. *Cell studies:* Jurkat T cells (Jurkats) & Fas-unresponsive Jurkats (I9.2) were incubated in media containing soluble ARYL-DX2 or on surface-initiated coatings containing ACRYL-DX2 and/or ACRYL-ICAM-1 for 24 hrs. The percentage of apoptotic T cells was quantified with an Annexin V apoptosis assay and metabolic activity was assessed with AlamarBlue reagent (both from Invitrogen).

Results: A 10:1, ACRYL-PEG-NHS:DX2 reaction stoichiometry yielded ACRYL-DX2 with no detectable loss in soluble biological activity. Surface-initiated photopolymerization times ranging from 120-180s produced the greatest detectable ACRYL-DX2 surface density, averaging 1.6±0.2 ng/cm^2. In Fig. 1a, the full 20 μm thickness of a representative polymer coating is visible via polymerized ACRYL-F-IgG. Fig. 1b and 1c demonstrate that a 150 kDa, rhodamine-tagged 2nd antibody (R-IgG) was free to diffuse throughout the full thickness, indicating high network accessibility.

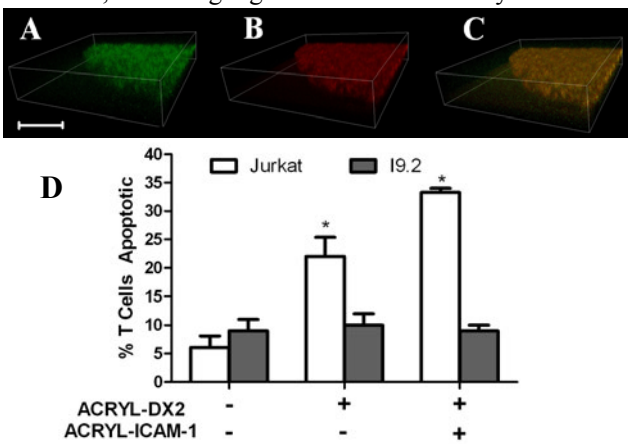

Figure 1. 20 μm thick coatings with (A) ACRYL-F-IgG (B) R-IgG staining (C) image merge. Scale bar represents 100 μm. (D) Coatings with DX2 and or ICAM-1 induced significant T cell apoptosis. * denotes $p<0.05$ significance from all other values.

ACRYL-DX2 was incorporated into the coating and detected at concentrations ranging from 1.4 – 1.7 ng/cm^2. As shown in Fig. 1d, surface-initiated polymer coatings containing ACRYL-DX2 induced significant, 22±3%, Jurkat apoptosis after 24 hr. Incorporating ACRYL-ICAM-1 and ACRYL-DX2 induced even greater Jurkat apoptosis, 33±1%, after 24 hr. In both cases, a significant increase in apoptosis was not observed in Fas-insensitive I9.2 cells, indicating the effect is Fas-mediated. Finally, a metabolic activity assay demonstrated a 55±8% reduction in Jurkat activity when cultured on ACRYL-ICAM-1 & ACRYL-DX2 coatings.

Conclusions: A polymeric coating was created via surface-initiated photopolymerization which induced significant Jurkat T cell apoptosis. Incorporating ACRYL-ICAM-1 along with ACRYL-DX2 further increased apoptosis and resulted in a significant reduction in T cell metabolic activity. This coating has significant potential to provide local T cell immunosuppression from the surface of an encapsulation device.

References: [1]Wilson JT, *Adv drug del rev* 2008; 60:124-45. [2]Jang JY, *Biomaterials* 2004;25:3663-9. [3]Palmer E, *Nat rev* 2003 May;3(5):383-391. [4]Cheung CY, *Bioconjugate Chem* 2006;17:1036-42. [5]The authors would like to thank our funding sources: NIH (1RO1DK076084) & GAANN fellowship to (PH).