Engineering Dendritic Cell Environments To Reduce Transplant Rejection By Induction Of Immune Tolerance.

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Statement of Purpose: Adaptive immune responses mediated by $CD8^+$ and $CD4^+$ T-cells are a major barrier to successful tissue transplantation. Systemically delivered immunosuppressants are given to transplant patients to reduce the risk of graft rejection, however, concurrently expose them to opportunistic infections by knocking down the entire immune system. This creates a need for engineering mechanisms to promote graft acceptance without compromising the patients' overall immune health. Professional antigen-presenting cells namely dendritic cells (DCs) have the ability to support immune tolerance in addition to their known function of promoting immunity[1]. It has been previously shown that DCs can be manipulated in culture to express a tolerance-inducing phenotype via treatment with anti-inflammatory agents such as interleukin -10 (IL-10)[2] or dexamethasone (dex)[3]. Consequently, generation of alternatively activated DCs (aaDCs) using maturation factors such as peptidoglycan (PGN)[3] or lipopolysaccharide (LPS)[4] along with donor cell lysates has been shown to induce donor-specific regulatory T-cells (T_{reg}s). We propose a multifunctional biomaterial approach to recapitulate this tolerogenic culture environment *in vivo* with a DC maturation-inert material namely, agarose (Ag), as the base scaffold[5]. Our technique involves developing a multifunctional Ag cryogel scaffold comprising of entrapped gelatin microparticles (gelMPs) to locally, sequentially deliver biomolecules, namely granulocyte monocyte colony-stimulating factor (GM-CSF – 14.3 kDa), followed by a tolerance-inducing agent, dex, maturation-inducing PGN (~46 kDa) and cell lysates from donor tissue. Upon implantation in a murine model, monocytes migrating to the scaffold, when exposed to GM-CSF (from Day 0-3), will develop into immature DCs. Subsequently (from Day 3-6), dex will stimulate tolerogenic DC phenotype and (on Day 6) PGN along with donor cell lysates will induce aaDCs to trigger donor-specific immune tolerance.

Methods: GelMPs were prepared from gelatin type B (Sigma-Aldrich) by emulsification-solvent extraction method and differentially crosslinked with 1.25-25mM glutaraldehyde (Sigma-Aldrich) solution at 4°C for 12 hrs and then with 25mM glycine solution for 1 hr at room temperature. Freeze-dried gelMPs were diffusionally loaded with model biomolecules, Alexa Fluor (AF) 647-Biotin (~1544 Da), AF 647-Ovalbumin (OVA) (~42.9 kDa) (Invitrogen) or cytokine, recombinant human GM-CSF (Peprotech Inc.) at 4°C for 12 hours. Ag cryogels were prepared with or without gelMPs by freezing 1.5% agarose (Affymetrix Inc.) solution, and controlled rate thawing in a cryostat (Leica) at 0.3°C/minute. Release from biomolecule from standalone MPs or MPs within the cryogel into PBS was determined by periodically sampling the supernatant and assessing concentration by reading fluorescence or by an ELISA.

Results: Differentially crosslinked gelMPs exhibit distinct release profiles for the respectively loaded small or large biomolecules such as AF 647-Biotin or AF 647-OVA. Similarly, differential release from Ag cyogels with loaded gelMPs is governed primarily by biomolecule size, MP number density and extent of gelMP crosslinking (Fig.1). MP number density negatively regulates driving force for biomolecule release to the external scaffold environment. These results provide the rationale for selecting a 10^{10} MP number density of 5mM crosslinked MPs in Ag scaffold as the most suitable formulation for delivering GM-CSF within days 0-3. We tested this hypothesis in a weeklong study to show that 69.28% of GM-CSF was released by Day 3 (Fig.2). Presently, development of multifunctional scaffolds with GM-CSF, dex and PGN loaded gelMPs is underway and release are being profiles analyzed. Subsequently, their ability to generate aaDCs from primary human iDCs will be assessed prior to application in a mouse transplantation model.

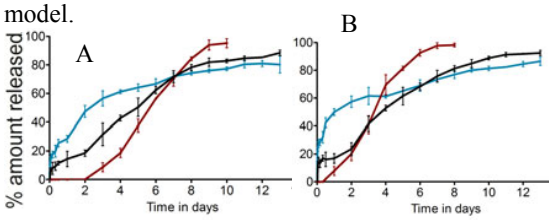

Figure 1: Release profiles of loaded-10mM gelMPs in Ag cryogel: % amount released normalized to amount loaded. **(A)** AF647-Biotin, **(B)** AF647-OVA. **MP number density per scaffold: (red)** 10^8, **(black)** 10^9 and **(blue)** 10^{10}.

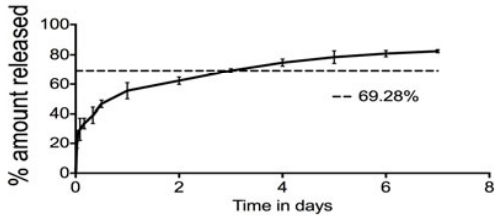

Figure 2: Release profiles of hGM-CSF loaded 5mM gelMPs in Ag cryogel: % amount released normalized to amount loaded.

Conclusion: Biomolecule release profiles can be fine-tuned by altering gelMP crosslinking density, MP number in the Ag cryogel, and loaded biomolecule molecular weight. Additionally, the Ag cryogel serves as a secondary diffusional barrier while acting as a DC maturation-inert matrix. It is anticipated that implantation of this multifunctional scaffold along with DC-phenotype modulators prior to allograft transplantation would educate the recipient's immune system for donor-specific acceptance of a subsequent allograft.

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References: 1. Banchereau J et al. Nature 392:245-252 (1998); 2. Muller G et al. J Invest Derm 119:836-841 (2002); 3. Rozkova D et al. Clin Immunol 120:260-271 (2006); 4. Emmer PM et al., Transplantation 81:1451-1459 (2006); 5. Park J. et al., Acta Biomateriala 8: 3606–3617 (2012); 6. Loznsky VI et al J App Poly Sci 108:3046–3062(2008)