Adhesive Peptide Gradient Regulation of Dendritic Cell Activation

Nathan D. Gallant[1], Abhinav P. Acharya[2], Benjamin G. Keselowsky[2].
[1]Department of Mechanical Engineering, University of South Florida
[2]J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida.

Statement of Purpose: Growing use of biomaterials as targeted therapeutics and tissue constructs has generated the need to understand biomaterial-induced immune responses and investigate the potential of utilizing biomaterials to modulate the immune system. Implantation of a biomaterial leads to the adsorption of several extracellular matrix proteins. Leukocytes such as dendritic cells (DCs) and macrophages can interact with adsorbed proteins and generate an immune response against the biomaterial. DCs are the key regulators of the adaptive and innate immune responses that can modulate lymphocyte function and in the process generate a pro-inflammatory or anti-inflammatory reaction. Hence, there is an interest to study adhesion based activation of DCs. This study employs adhesion peptide gradients[1] to activate DCs and modulate biomaterial-based immune responses.

Methods: Tunable gradients of terminal acid groups were generated on self assembled monolayers of alkyl silanes by controlled exposure to UV ozone.[2] A propargyl-derivatized linker was then attached to yield a surface possessing an increasing density of alkyne groups. Azide-functional GRGDS peptide was tethered to the surface via "click chemistry".[3,4] Gradients of carboxylic acid were used as controls. Gradient surfaces were coated with serum albumin and seeded with DCs isolated from the bone marrow of C57BL6/j mice[5] for 16 h. DCs were treated with monensin (BD Biosciences) for the last 5 h before staining for intra-cellular cytokines IL-12 and IL-10 and surface markers MHC-II and CD86.

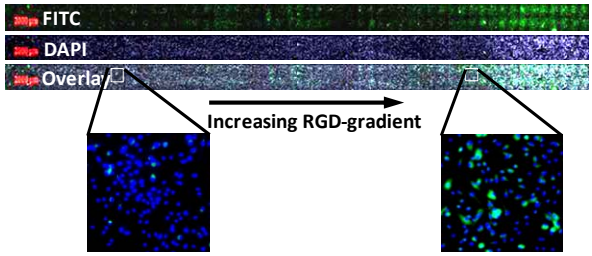

Figure 1: Dendritic cells cultured on RGD gradients and immunofluorescently stained were quantified for the expression of surface molecules MHC-II-stimulatory molecule and CD86-co-stimulatory molecule, intracellular anti-inflammatory cytokine IL-10, and pro-inflammatory cytokine IL-12p40.

Results: In this study, we cultured DCs on a gradient of RGD peptide that engages integrins such as, $\alpha_v\beta_3$ and $\alpha_V\beta_1$. The RGD ligand is present in several proteins, such as fibronectin that are known to induce maturation in DCs. Cultured DCs were stained for cell surface indicators of maturation including MHC-II, a stimulatory molecule (Figure 1), and CD86, a co-stimulatory molecule. It was observed that both MHC-II and CD86 had direct correlation with the surface density of RGD peptide. These results suggest that the increasing density of a specific adhesive ligand can modulate DC maturation state via outside-in signaling through integrin-ligand interaction. Furthermore, it was shown that in the absence of RGD-gradient or adhesive signaling, DCs had a constant basal level of MHC-II and CD86 expression. Furthermore, DCs cultured on the RGD-gradient demonstrate increasing production of IL-12p40 a pro-inflammatory cytokine. However, the production of IL-10, an anti-inflammatory cytokine, was not dependent on RGD surface density. These results suggest that increasing concentration of RGD peptide might induce a pro-inflammatory immune response via DCs and subsequently generate a T-cell response.

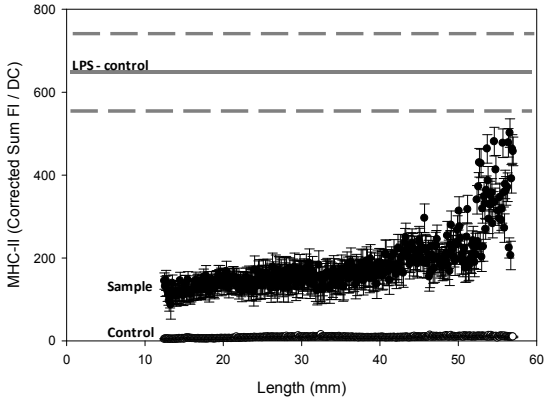

Figure 2: Surface expression of MHC-II stimulatory molecule is proportional to the RGD surface concentration

Conclusions: In this study, we have demonstrated that the DC maturation can be modulated by adhesion cues. Furthermore, we have developed a system in which the extent of DC maturation can be quantified spatially by image analysis on per cell basis. We have shown that the DC surface expression of MHC-II and CD86 is directly proportional to the surface density of RGD peptide. Interestingly, it was observed that the increasing surface density of RGD-peptide resulted in increasing pro-inflammatory cytokine IL-12p40 production whereas the anti-inflammatory cytokine IL-10 remained the same as negative control. This suggests that the extent of adhesion cues is an important factor in the maturation pathway of DCs and should be further studied. This work will provide tools to study implanted biomaterials from immunological perspective.

References: 1. Gallant ND. Adv Mater. 2007;19:965-969.
2. Roberson SV. Appl Surf Sci. 2002;200:150-164.
3. Huisgen R. Pure Appl Chem. 1989;61:613-628.
4. Kolb HC. Angew Chem Int Ed. 2001;40:2004-2021.
5. Acharya AP. Biomat. 2008;29:4736-4750.