Dendritic Cell Response to the Roughness and Chemistry of Titanium Surfaces

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Statement of Purpose: In order to either regenerate injured tissues or enhance vaccine efficacy, a variety of combination product, composed of biologics and biomaterials, have been developed. The success of these products lies in their ability to elicit a desired host response, which may be modulated by the biomaterial components. To achieve the desired host response, biomaterials can be used to control the immunological outcome by modulating the phenotype of dendritic cells (DCs), professional antigen-presenting cells (APCs) that bridge innate and adaptive immunity as well as play a central role in initiating immune tolerance.

The adjuvanticity of distinct biomaterials was shown to affect the maturation of DCs. For example, DC maturation was induced by poly(lactic-*co*-glycolic acid) (PLGA) or chitosan films, not induced by agarose or alginate films, and inhibited by hyaluronic acid films[1,2]. However, it was unclear which material properties contributed to such differential effects. In this study, DC response to surface roughness was analyzed on titanium (Ti) surfaces with defined roughness and chemistry. The results suggest that surface roughness enhances DC maturation; however, hydrophilicity may be an overriding material property that suppresses DC activation.

Methods: Ti disks were prepared from 1 mm thick sheets of grade 2 unalloyed Ti (Institut Straumann AG) and punched to fit into 24-well tissue culture plate. The methods used to produce pretreated (PT), grit-blasted and acid-etched (SLA), and hydrophilic SLA (modSLA) surfaces were previously reported[3]. The mean peak to valley roughness (Ra) for PT, SLA, and modSLA was 0.6 μm, 3.2 μm, and 3.2 μm, respectively. The water-air contact angle was 95.76°, 138.3°, and approximately 0°, respectively. The overall chemical composition of PT and SLA was similar, but modSLA had higher Ti and O, along with lower N and C compared to the other two surfaces.

Immature DCs (iDCs), derived from human peripheral blood mononuclear cells during a 5 day culture in the presence of inducing cytokines[2], were treated with Ti disks for 24 hours in 24-well plates. DC phenotype was compared to DC cultured on TCPS (TCPS, iDCs) and lipopolysaccharide-treated mature DC (TCPS + LPS, mDCs) controls. The loosely-adherent DC population was collected by gentle pipetting, while the adherent fraction was removed from the materials using cell dissociation solution (Sigma). The number of cells in both cell populations was quantified by Coulter Counter. The expression of surface markers, such as CD83, CD86, and HLA-DQ, was measured by flow cytometry. The cell culture supernatants were quantified for the production of cytokines and chemokines, including IL-1ra, IL-10, TNF-α, and MIP-1α, by multiplex cytokine analysis (Bio-Rad).

Results/Discussions: DCs expressed higher CD86 after treatment with PT or SLA, but the DCs treated with modSLA expressed CD86 at a level similar to iDCs (Figure 1). Because SLA and modSLA essentially have the same surface roughness but have very different water-air contact angles (138.3° vs 0°), this result suggests that hydrophilicity of a surface may be an overriding property that modulates DC phenotype.

The percent adherent DCs treated with all the Ti surfaces were significantly higher than on TCPS (iDCs), but not different among the different surfaces (39, 39, 38, and 20% on PT, SLA, modSLA, and TCPS, respectively). The percent cell recovery from the biomaterials was not significantly different from iDCs. It was unexpected that although modSLA was extremely hydrophilic, the % of adherent DCs on modSLA was not different from any other Ti surfaces, indicating that material properties other than hydrophilicity can affect DC adhesion.

The different Ti surfaces induced differential cytokine release in DCs: modSLA induced the least amount of anti-inflammatory cytokines IL-1ra and IL-10, the least amount of chemokine MIP-1α, and higher pro-inflammatory cytokine TNF-α.

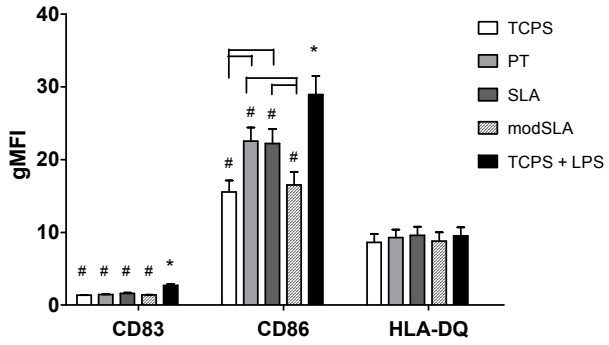

Figure 1: Surface marker expression on DCs upon treatment with Ti disks. Data are presented as mean ± SEM (n = 12 donors). *: $p<0.05$ different from TCPS (iDC); #: $p<0.05$ different from TCPS+LPS (mDC); brackets: $p<0.05$ between treatments.

Conclusions: DCs respond differentially to Ti surfaces with distinct roughness and chemistry. Because a hydrophilic surface with exactly the same roughness did not induce any enhanced CD86 expression but the same amount of cell adhesion, hydrophilicity may be an overriding material property that suppresses DC maturation, but other material properties may play a role in controlling DC adhesion to material surfaces.

Acknowledgements: Financial support from an NSF CAREER grant (BES-0239152) and NIH grants 1RO1 EB004633 and AR052102.

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