Monocyte phenotypic polarization on PEG hydrogels, polydimethylsiloxane, and TCPS is mediated by exogenous serum and surface-associated proteins including complement C3, fibrinogen, thrombin, and vitronectin

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Statement of Purpose: Monocytes are central in the host response to biomaterials and are phenotypically polarized by signals from the microenvironment. These polarized states have been broadly categorized: classically activated (M1) and alternatively activated (M2). M1 macrophages hold in common a predilection towards pro-inflammatory activities while subcategory M2c is considered to be a deactivation or anti-inflammatory state. Although these polarization states are broadly applied to maturing monocytes, there can be significant overlap between the polarization states and the full spectrum of macrophage phenotypes is far from being fully understood. Even though monocyte response to biomaterials has been actively studied, our current incomplete mechanistic understanding of this important interaction partly derives from the various in vitro experimental conditions employed and from which conclusions are drawn. Our current study addresses this disconnect by systematic analyzing how variations in the culture condition such as serum can affect biomaterial-mediated monocyte maturation. Furthermore, the role of surface-associated proteins such as complement factor C3 in regulating monocyte phenotypic polarization is elucidated.

Methods: Human peripheral monocytes cultured in medium supplemented with fetal bovine serum (FBS), autologous human serum (AHS), C3-depleted human serum with or without C3 replenishment were exposed to TCPS, polyethyleneglycol (PEG, synthesized from 3.4kDa PEG-diacrylate) hydrogels, and polydimethyl siloxane (PDMS) films and cultured up to 7 days. LPS was added to the culture medium as a positive control for the M1 activation state. At 2 hr, 1, 4, and 7 days, adherent cells were stained for CD86 and CD163 and evaluated under confocal microscope. Adherent cell density was quantified using the Live/Dead® assay. The concentration of six critical proteins of the M1 or M2 polarization state in the supernatant was quantified: interleukin-12 (IL-12(p40) and IL-12(p70)), growth-related oncogene-2 (GRO2), TNF-α, IFN- γ, IL-10, and IL-1β. Adsorbed proteins were surveyed using our established LC/MALDI-ToF/ToF-based proteomic analysis method (Ref) and selected proteins were quantified with ELISA at 2, 4, 12, 24, and 48 hr.

Results/Discussions: Adherent monocyte density and cytoplasmic spreading were comparable on TCPS and PEG hydrogels and were significantly higher in AHS-supplemented culture as compared to that with FBS. Adherent monocyte densities on TCPS supplemented with LPS and on PDMS films did not differ between the two serum types. These results have an important bearing on future studies involving primary monocytes. For example, monocyte adhesion to PEG-containing hydrogels may appear disproportionately low when cultured with FBS in vitro. From CD86, CD163, and protein release assays, we found that all cells were partially M1 activated through out the course of the culture. Moreover, the presence of LPS and biomaterials resulted in a heterogeneous polarization phenotype that was both M1- and M2-like. For example, TCPS with LPS induced a higher expression of pro-inflammatory cytokines and anti-inflammatory cytokine such as IL-10 than TCPS without LPS. Adherent monocytes on PEG supplemented with AHS produced more GRO, IL-1β, and TNF-α than cells cultured with FBS, indicating a more strongly activated M1 state. Adherent monocytes on PDMS with AHS exhibited a high concentration of GRO but not any other cytokines thus indicating that these cells may be in an M1 or M2 state. Under this condition, a low IL-10 concentration does not fit the M2a characteristic and the low concentration of TNF-α and IL-1β also conflicts with the M1 characteristic.

To gain insights into the molecular mechanism of monocyte phenotypic differentiation, various proteomic methods were employed. LC-MALDI results identified a dynamic adsorption/absorption isotherm of a complex composition of human serum proteins on TCPS and PEG hydrogels from 2 to 48 hr. This further verified the usefulness of this method in surveying the complex composition of serum proteins without a priori identification. ELISA was performed to complement the LC-MCALDI findings. Although the quantify of surface-associated proteins such as C3, vitronection, fibronectin, and thrombin was lower on PEG hydrogels than that on TCPS, the amount of these proteins on PEG hydrogels was still considerable up to 48 hr. The depletion of C3 from the culture serum resulted in a decrease in adherent monocyte density on all surfaces up to 7 days.

Conclusions: Monocytes exposed to different material and exogenous serum proteins (i.e., bovine vs autologous human source) have "material-specific" phenotypes that are not classically defined. These unique states straddle amongst multiple identified polarization states requiring the identification via multiple protein markers. A complex composition of serum proteins including C3, vitronectin, fibronectin, and thrombin adsorbed/absorbed onto PEG hydrogels, as well as TCPS, as potential ligands in mediating the observed high level of monocyte adhesion, cytoplasmic spreading, and phenotypic polarization. C3 plays an important role in mediating the observed surface-activated monocyte polarization.

References: Zuckerman ST et al. Biomaterials 30 (2009) 3825–3833