CD47 modified polymeric surfaces resist inflammatory cell attachment.

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Introduction: The host inflammatory response is a normal, protective reaction to biomaterial implants. However, the magnitude and duration of the response can have untoward effects upon the efficacy of the therapeutic device. Our group has targeted immune inhibitory receptors as a strategy to reduce the inflammatory response to biomaterials.

Signal regulatory protein alpha (SIRP-1α) is a transmembrane protein expressed in myeloid cells that negatively regulates the immune response. CD47 is a recently identified ligand of SIRP-1α. SIRP-1α binding to CD47 prevents phagocytosis of CD47 immobilized microbeads, in a species-specific manner, by monocyte derived macrophages (MDMs). Thus, CD47 appears to be a cellular marker of self that is capable of conferring immune evasion on expressing cells.

To test the hypothesis that surface immobilized CD47 can be used to generate a biomimetic surface capable of inhibiting inflammatory cell binding to synthetic surfaces, we employed a novel surface modification involving a polymeric photo cross-linker (PDT-BzPh) composed of 2-pyridyldithio groups (PDT) linked to the benzophenone (BzPh) photo-reactive groups to link the extracellular domain of CD47 to either polyurethane (PU) or polyvinyl chloride (PVC). The goals of this study are 1. Identify the role of SIRP-1α as it pertains to polymorphonuclear leukocytes (PMN) and MDM attachment to PVC and PU surfaces respectively. 2. Develop and characterize the surface modification chemistry to append molecular CD47 on PE surfaces. 3. Use in vivo and whole human blood models to assess the effect of CD47 surface immobilization upon inflammatory cell binding.

Materials and Methods: Biotinylated CD47 was immobilized on the surface of PU films ($1cm^2$) or PVC tubing by applying PDT-BzPh to PE films as a micelle suspension. Under UV-irradiation, BzPH groups form covalent bonds with the synthetic surface. The immobilized PDT groups were reduced to Thiol-groups by incubating the films with a solution of TCEP. Avidin (10mg/ml) was reacted with SPDP and purified by passing through a Sephadex column. The treated surfaces were reacted with the thiol reactive avidin and incubated overnight at room temperature. The avidin-immobilized films were then washed 5 times in dH_2O and biotinylated CD47 was immobilized on the surface.

The human neutrophil cell line, HL-60, were suspended to ~ 1.5 x 10^6 cells/ml in growth media supplemented with 10 µg/ml of IgG and/or anti- SIRP-1α antibody and the phorbol ester, phorbol 12-myristate 13-acetate (PMA). The resuspended cells were added to unmodified (control) PVC tubing or PVC tubing that was modified by surface immobilized CD47 (via avidin-biotin affinity) or avidin modified PVC tubing (avidin control). The tubes were capped at both ends and shaken for 3 hours at 37°C. The tubing was washed and attached cells were fixed with the addition of 4% paraformaldehyde. Cells attached to the PVC tubing were quantified by staining with the fluorescent dye DAPI (blue color on fluorescent micrographs) and visualized using a fluorescent microscope with the appropriate filter set.

We used the Chandler loop apparatus to investigate the effects of the CD47 modified PVC surfaces on whole blood under flow conditions. Briefly 10 mls of freshly drawn whole human blood (per an IRB approved protocol), supplemented with Sodium Citrate, was introduced to a closed loop (~ 33 cm in length) of CD47 modified or unmodified control PVC tubing. The tubing was rotated for three hours at 37ºC. At the end of the protocol, the tubing was washed with phosphate buffered saline and attached cells were quantified as above.

CD47, human (hCD47) or bovine (bCD47), was immobilized on PU films ($1cm^2$) and placed into subdermal pouches of male Sprague Dawley rats. After 10 weeks the animals were euthanized and PU films were removed and assessed for indicaitons of oxidative degradation using FTIR, SEM, and light microspcopy.

Results/Discussion: Transformed HL-60 attachment to PVC surfaces was significantly ($p < 0.001$) inhibited by the presence of 10 µg of SIRP-1α blocking antibody (29 ± 7.93 cells/200 x field) compared to 10 µg non-specific IgG antibody (77 ± 8.72 cells/200 x field).

The presence of hCD47 on PVC tubing also completely blocked HL-60 binding. In contrast, there was robust cell retention, as evident by ample DAPI (blue) staining, on unmodified PVC surfaces. Quantitative analyses showed that HL-60 attachment to PVC was modestly, but not significantly inhibited by the presence of surface immobilized avidin (103 ± 8.4 cells/200 x field v. 76.3 ± 10.5 cells/200 x field n=12).

Chandler Loop results showed robust cell attachment on the surface of the unmodified PVC tubing. In contrast there was scant evidence of cellular attachment in the CD47 modified PVC tubing. Quantitative analysis shows a 20-Fold greater cellular attachment in unmodified PVC tubing (control) versus CD-47 surface modified PVC tubing. These data confirm the anti-inflammatory effects of surface bound CD47 on PVC tubing.

All rats survived for the 10-week duration of the subdermal implant of CD47 modified and control PU films. FTIR analysis showed that CD47 inhibited aberrant ether cross-linking, a marker of MDM induced oxidative degradation of PU, three-fold.

Conclusions: We conclude that CD47 immobilized surfaces are resistant to inflammatory cell adhesion and that CD47 can confer resistance to synthetic surfaces to inflammatory cell mediated damage *in vivo*.