Blocking macrophage attachment to biomaterials with CD47

Stanley J Stachelek, Masako Ueda, Richard Tsai, Ivan Alferiev, Dennis Discher, Robert J. Levy

Introduction: Material degradation as a result of chronic inflammation of implantable polyurethane (PE) containing devices remains an important problem in biomaterials research. It was shown that reactive oxygen species (ROS) released from adherent monocyte derived macrophages (MDMs) were responsible for the structural damage observed in PE containing implants such as pace maker lead insulation. Modifying bulk preparations of PE with phenol based anti-oxidants effectively reduces the ROS mediated damage to PE films, but is limited by the fact that the anti-oxidant capacity is expended after reacting with the oxygen radical. Thus, additional approaches to addressing MDM mediated oxidative degradation of PE are required.

Signal regulatory protein alpha (SIRPα) is a transmembrane protein expressed in MDMs. SIRPα mediates diverse cellular functions through phospho-tyrosine signaling mechanisms. CD47 is a recently identified ligand of SIRPα. SIRPα binding to CD47 prevents phagocytosis of CD47 immobilized microbeads, in a species specific manner, by 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 MDM binding to the PE surface, we developed 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 a polyether polyurethane. The goals of this study are 1. Identify the role of SIRPα as it pertains to MDM attachment to PE surfaces. 2. Develop and characterize the surface modification chemistry to append molecular CD47 on PE surfaces. 3. Characterize, *in vitro*, the effect of CD47 surface immobilization on MDM attachment to PE.

Materials and Methods: The MDM cell line (THP-1) were transduced with the addition of 1.6 x 10^{-7} M of phorbol 12-myristate 13-acetate (PMA) to the media and were cultured on PE films in the presence of increasing concentrations (0, 2.5, 5, 10 μg) of anti-SIRPα antibody (SE7C2) directed against the protein's extracellular domain. Cells were allowed to attach to the PE films for 48 hours, at which time the films were washed and remaining cells were fixed with 4% paraformaldehyde and stained with the nuclei specific stain DAPI. Cell retention was determined by DAPI staining and counting 9 separate 200X fields. Additional studies examining the phosphorylated state of SIRPα were conducted on THP-1 cells grown on PE films for 2 days. At the end of the protocol, cells were lysed and incubated overnight with a polyclonal anti-SIRPα antibody in the presence of protein G conjugated agarose beads. The immunoprecipitated proteins were processed for the presence of phosphorylated tyrosine via Western Blotting.

Biotinylated CD47 was immobilized on the surface of PE films (1cm^2) by applying PDT-BzPh to PE films as a micelle suspension. Under UV-irradiation, BzPH groups form covalent bonds with PE macromolecules. PDT groups, on the PE films, 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. PE films 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 increasing concentrations (3.9ng, 7.8 ng, 15.6ng. 31.25ng, 62.5ng, 125ng, 250ng, or 500 ng) of biotinylated CD47 was added to each film.

To assess the immobilization of CD47 on the PE surface, a control film and CD47-immobilized films were incubated with FITC-conjugated mouse anti-CD47 antibody (B6H12) directed against the extracellular domain of human CD47. After washing the unbound antibody, films were analyzed for FITC fluorescence and the amount of bound antibody was determined from a standard curve generated from the measured fluorescence from known quantities of FITC conjugated antibodies. Cell attachment to PE-CD47 and control films was measured using activated THP-1 cells cultured, for 48 hours. After washing films in PBS, cell retention was assessed as above.

Results/Discussion: Transformed THP-1 attachment to PE surfaces was not affected by the presence of 10 μg of non-specific IgG antibody (96±7.9 cells/200 x field vs. 108±7.7 cells/200X field). However binding was significantly ($p < 0.001$) inhibited by the presence of 2.5 μg (64.4 ± 4.7 cells/200 x field) or 5 μg (60.5 ± 4.7 cells/200 x field) of SIRPα blocking antibody. There was a 2-fold further reduction in THP-1 binding to PE in the presence of 10 μg of anti-SIRPα blocking antibody. Western blot analysis of immunoprecipitated SIRPα from PE cultured THP-1 lysates showed the presence of phosphorylated tyrosine. These results indicate a SIRPα dependent mechanism in THP-1 binding to PE surfaces.

At the lowest CD47 surface concentration (~ 10 molecules/μm^2) there was significant ($p < 0.001$) reduction of THP-1 cell attachment (24.6 ± 8.2 cells/200 X field) compared to unmodified control surfaces (131.23 ± 21.6 cells/200 X field). There was a further four-fold reduction in THP-1 attachment to PE-CD47 surfaces at CD47 concentrations between 80-301 molecules/μm^2. Cell attachment was virtually abolished at CD47 concentrations greater than 600 molecules/μm^2

Conclusions: We conclude that the SIRPα signaling pathway is involved in MDM binding to PE surfaces. In addition this pathway can be targeted to prevent MDM attachment to PE surfaces by modifying the PE surface with the SIRPα ligand CD47 via photoactivation chemistry.