Anti-Inflammatory Capacity of Polymers Modified with a Synthetic Peptide Sequence of the CD47 Ig Domain

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Statement of Purpose: Implanted polymeric biomaterials trigger the foreign body reaction resulting in adsorption of blood proteins and platelets, monocyte/macrophage adhesion, and the release of pro-inflammatory cytokines, all of which contribute to clinical complications post-implantation and their ultimate failure[1,2]. Our lab has focused on appending recombinant CD47 to implanted polymers as a way of preventing the foreign body reaction and increasing the long-term success of these materials. CD47 is a ubiquitously expressed transmembrane protein with a known role in immune evasion and shows promise at conferring biocompatibility when appended to polymeric surfaces. Signal-regulatory protein alpha (SIRPα), the cognate receptor for CD47, is an immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing transmembrane protein expressed on cells of myeloid origin. Our lab has shown that CD47-SIRPα interactions down-regulate immune responses to polyurethane (PU) and polyvinylchloride (PVC) in *in vitro*, *ex vivo*, and *in vivo* models. Recently published work suggests that a peptide sequence encompassing the extracellular Ig domain of CD47 can confer the same level of inhibition of phagocytosis of opsonized microbeads as recombinant CD47[3]. We hypothesize that the CD47 peptide will confer similar biocompatibility as recombinant CD47 on PU. The use of peptides in place of recombinant proteins offers several advantages including cost reduction, more readily modifiable and increased biocompatibility. The goals of this study were to: 1. Append CD47 peptide to PU surfaces. 2. Quantify the amount of CD47 on the PU surfaces. 3. Assess if CD47 peptide binds SIRPα. 4. Evaluate CD47 peptide inhibition of cell attachment.

Methods: Polymer Modification: PU films were modified with recombinant human CD47 or modified with a peptide encompassing the extracellular Ig domain of human CD47 (CD47 peptide) using photoactivation chemistry as previously demonstrated[4]. Unmodified PU films served as controls. CD47 Quantification: CD47 recombinant and CD47 peptide levels on the films were quantified using an immunoassay with a fluorescently labeled antibody to the extracellular Ig domain of human CD47 and compared to a standard curve. SIRPα Binding Assay: SIRPα binding to unmodified, CD47 recombinant, or CD47 peptide modified PU films was quantified by an immunoassay using recombinant 6-His tagged SIRPα mixed with a fluorescently labeled anti-6-His tag antibody and compared to a standard curve. Cellular Adhesion Assays: THP-1 cells were differentiated using phorbol 12-myristate 13-acetate (PMA) and allowed to attach to unmodified and modified PU films (1 x 1 cm) for 3 days. Cells were fixed with 4% paraformaldehyde (PFA), stained with 4',6-diamidino-2-phenylindole (DAPI) for 30 mins, and counted using fluorescent microscopy. Chandler Loop Apparatus: Whole human blood was obtained under a protocol approved by the IRB from healthy donors not taking anti-inflammatory medications and perfused over unmodified, CD47 recombinant, and CD47 peptide modified films inserted into medical-grade PVC tubing. 10 ml of whole human blood was perfused over the films for 3 hr as described previously[4,5]. After perfusion, the films were removed and processed as described above to quantify cellular adhesion to PU films.

Results: The quantification of CD47 peptide demonstrated similar levels to CD47 recombinant appended to PU (CD47 recombinant: 203.2 ng/cm^2 vs. CD47 peptide: 174.6 ng/cm^2), demonstrating proof of concept that a peptide encompassing the extracellular Ig domain of CD47 can be appended to PU using photoactivation chemistry, as shown previously for recombinant CD47[4]. SIRPα binding assays showed that the CD47 recombinant and CD47 peptide both bind SIRPα (recombinant: 866.7 ng/cm^2 vs. peptide: 431 ng/cm^2), indicating that CD47 peptide is functional in binding its cognate receptor. CD47 recombinant and CD47 peptide significantly ($p < 0.0001$) inhibited THP-1 cell attachment to PU compared to unmodified control. Unmodified PU had an average of 64.9 attached cells (+/- 19.0) vs. 4.4 attached cells (+/- 2.5) for CD47 recombinant and 3.3 attached cells (+/- 1.7) for CD47 peptide. Both CD47 recombinant and CD47 peptide resulted in significantly less ($p = 0.0003$) cell attachment when exposed to whole human blood in the Chandler Loop Apparatus (unmodified: 48.7 attached cells +/- 31.3; CD47 recombinant: 13.4 attached cells +/- 3.3, and CD47 peptide: 11.7 attached cells +/- 4.6). However, for both the THP-1 adhesion assay and the Chandler Loop experiment, CD47 recombinant and CD47 peptide were not significantly different from one another, indicating that the appended CD47 peptide performs similarly to appended CD47 recombinant.

Conclusions: Our data indicate that a peptide encompassing the external Ig domain of human CD47 can be appended to PU using photoactivation chemistry, yielding similar concentrations as recombinant CD47. The CD47 peptide and recombinant CD47 both bind SIRPα, prevent THP-1 attachment, and prevent cell attachment in *ex vivo* human blood exposure assays. Functionalizing implantable materials with CD47 peptide can inhibit inflammatory cell interactions with polymeric surfaces, potentially promoting the long term success of implantable polymers and making them more cost effective and modifiable.

References: [1](Levy JH et al. Ann Thorac Surg 2003;75:S715-S720) [2](Anderson JM et al. Semin Immunol 2008;20:86-100) [3](Rodriquez PL et al. Science 2013;339:971-75) [4](Finley MJ et al. Biomaterials 2012;33:5803-11) [5](Stachelek SJ et al. Biomaterials 2011;32:P4317-26)