The use of CD47-modified biomaterials to mitigate the immune response

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Abstract

Addressing the aberrant interactions between immune cells and biomaterials represents an unmet need in biomaterial research. Although progress has been made in the development of bioinert coatings, identifying and targeting relevant cellular and molecular pathways can provide additional therapeutic strategies to address this major healthcare concern. To that end, we describe the immune inhibitory motif, receptor–ligand pairing of signal regulatory protein alpha and its cognate ligand CD47 as a potential signaling pathway to enhance biocompatibility. The goals of this article are to detail the known roles of CD47–signal regulatory protein alpha signal transduction pathway and to describe how immobilized CD47 can be used to mitigate the immune response to biomaterials. Current applications of CD47-modified biomaterials will also be discussed herein.

Keywords: CD47, signal regulatory protein alpha, biomaterials, inflammation

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Introduction

The cellular and molecular response at the interface between host tissue and the synthetic surfaces that comprise medical devices and therapeutic agents dictates the functional limits of biocompatibility. Clinical examples of aberrant biocompatibility are widely documented,^{1–6} and thus represent a significant financial and healthcare burden. For example, the efficacy of nanoparticle-based therapeutics is limited because they are commonly phagocytosized by resident macrophages and are sequestered in the liver, spleen, and lungs.⁷ Another example can be found with cardiopulmonary bypass circuits (CPBs), in which the perfusion of the patient's blood over the blood conduits and membrane oxygenator can elicit a systemic acute inflammatory response that correlates with adverse clinical outcomes following open heart surgery.^{8–14} The difference in the scale of the above mentioned examples, from nanoparticles to the extremely large surface area of a CPB circuit, reflects some of the underlying challenges related to cost and clinical application in developing therapeutic strategies to address the inflammatory response to biomaterials.

The host immune response to biomaterials is a carefully coordinated molecular and cellular reaction to multiple stimuli. Trauma is associated with the implementation of virtually all medical devices such as endovascular stents, CPBs, and hip replacements. The body's reaction to the damaging of blood vessels and surrounding tissue

represents the initial stages of the host response and is characterized by the almost immediate adsorption of blood proteins at the blood–biomaterial interface. The presence of adsorbed blood proteins, whose composition depends upon the material's physical properties, establishes a high-affinity matrix for the subsequent attachment and activation of platelets.^{15–18} Activated platelets respond by undergoing morphological changes and releasing pro-inflammatory signaling molecules that attract leukocytes to the site of injury and implantation.^{17–19} Polymorphonuclear leukocytes (PMN) are amongst the first type of white blood cells to respond to biomaterial insertion. PMN attach to the extracellular matrix composed of adsorbed blood proteins and fibrin whereupon they release pro-inflammatory cytokines to recruit additional contributors of the inflammatory response. In addition, PMNs release reactive oxygen species (ROS) such as hydrogen peroxide and super oxide with the overarching goal of removing the foreign material. For long-term implants, such as pacemaker leads, the host response changes from an acute inflammatory reaction to a chronic inflammatory response. This conversion occurs approximately three days after implantation and is characterized by the decreased presence of PMN and the increased presence of monocyte-derived macrophages (MDM). MDMs can further impede device function through their release of ROS and recruitment of fibroblasts that begin to isolate the implanted material by the formation of a fibrotic

Figure 1 Model ITIM receptor. The ITIM family receptors have a transmembrane region and an extracellular ligand binding domain. A cytoplasmic ITIM domain interacts with secondary cell signaling molecules such as SHP-1 and SHP-2. Through a series of transphosphorylation events, ITIM receptors inhibit immune cell activation events such as cytokine release and phagocytosis (A color version of this figure is available in the online journal.)

capsule around the site. Over time, MDMs can fuse to form foreign body giant cells that can persist for the lifetime of the implant.^{20–22} In spite of researchers having a thorough understanding of the cellular immune response to biomaterials, an effective strategy to address this issue remains elusive.

Medical device companies and academic laboratories have extensively pursued strategies to mitigate the host inflammatory response to biomaterials by focusing on one of the abovementioned processes, but the inflammatory response remains a significant obstacle. Altering the physical properties of biomaterial surfaces represents an anti-inflammatory strategy that aims to establish a bioinert surface with the overarching goal of preventing molecular and cellular interactions at the cell-biomaterial interface. For example, surface charge affects protein adsorption, and zwitterionic surfaces represent a recent innovation in inhibiting blood protein interactions with synthetic surfaces.^{23–25} At the nanometer and micrometer scale, leukocytes have a demonstrated affinity for specific surface topographies, and additional efforts are underway to modify the micro- and nanostructures at the material-tissue interface.^{26–28} Laboratories have reported successful bioinert coating strategies and research continues in these areas.

Immune cell tyrosine inhibitory motif and signal regulatory protein alpha

The efficacy of bioinert coatings is often hampered by the fact that no surfaces can completely block protein adsorption, and additional bioactive solutions may be required. One such approach is to focus on the biological processes that maintain immune system quiescence. Under normal

homeostatic conditions, the immune system is tightly regulated to ensure that the immune response is appropriate for the stimuli presented. This is achieved partly through a family of transmembrane proteins that express the immune cell tyrosine inhibitory motif (ITIM). As shown in Figure 1, the ITIM consists of a relatively short amino acid sequence located in the protein's cytoplasmic domain. Ligand binding of the ITIM-expressing protein elicits a series of phosphorylation-mediated cell signaling events initiated at the ITIM. Key downstream signaling molecules of ITIM proteins are Src homology proteins 1 and 2 (SHP-1, SHP-2) and inositol-phosphatase. This signal transduction cascade ultimately results in the cessation or reduction of the immune cell activation.

ITIM-expressing proteins represent a rather large family, and their potential contribution to biomaterials has been reviewed previously.¹⁷ The remainder of this review will focus on the ITIM protein signal regulatory protein alpha (SIRP α). SIRP α was first described in the 90s where expression was demonstrated to be in cells of myeloid origin and neurons.^{29,30} The structure of SIRP α (Figure 2) includes a single pass transmembrane domain, four extracellular immunoglobulin domains, and a cytoplasmic tail containing four ITIMs. The three known ligands of SIRP α are surfactant protein A (SP-A), surfactant protein D (SP-D), and CD47. Binding of SIRP α to its ligands initiates a phosphorylation cascade, initiated at the ITIM site, which involves downstream signaling by Src homology domain-containing proteins SHP-1 and SHP-2. Based upon the ligand, SIRP α signaling helps to maintain inflammatory cell quiescence.

SP-A and SP-D belong to the collectin family of molecules that participate in innate immune functions by

Figure 2 SIRP α and its ligands. SIRP α is expressed in cells of myeloid origin and platelets. It has three extracellular Ig domains that function as binding sites for the three known ligands (CD47, SP-A, and SP-B) of SIRP α . Upon ligand binding, SIRP α inhibits immune cell activation through its cytoplasmic ITIM domain (A color version of this figure is available in the online journal.)

recognizing microorganisms and facilitating their clearance by macrophages.^{31–34} Together, these proteins contribute to the innate immune response in the pulmonary organs. They have been demonstrated to opsonize apoptotic cells and facilitate their clearance by alveolar macrophages.^{31–34} Interestingly, reports have shown the proteins to possess both pro-inflammatory and anti-inflammatory properties, which have been shown to be regulated by SIRP α . Specifically, when SP-A and SP-D interact with SIRP α they appear to inhibit alveolar phagocytosis of apoptotic cells.³⁵ The precise mechanism, by which SIRP α regulates the dichotomous function of SP-A and SP-D, remains an ongoing subject of investigation.³⁵

Compared with SP-A and SP-D, the interactions between CD47 and SIRP α are arguably much better characterized, and the remainder of this review will focus on the immune inhibitory effects as a result of CD47 binding to SIRP α . CD47, also known as integrin-associated protein, is a ubiquitously expressed pentatransmembrane protein that has known physiological roles in cell attachment, spreading and immune evasion.^{36–39} In addition to functioning as a ligand for SIRP α , which will be discussed below, CD47 interacts with several other proteins. Within the same cell, CD47 can form a multiprotein complex with the following integrin heterodimers: $\alpha_{IIb}\beta_3$, $\alpha_v\beta_3$, and $\alpha_2\beta_1$.^{36–39} CD47 binding to integrin complexes has been shown to mediate attachment and spreading to a range of cell types such as platelets, endothelial precursor cells, and smooth muscle cells.^{36–39} Thrombospondin 1 (TSP-1) is secreted primarily

from activated platelets and has been identified as a CD47 ligand whereupon it inhibits the antithrombotic effects of nitric oxide (NO)-mediated signaling.^{40–42} CD47 interaction with the aforementioned integrin complexes and TSP-1 have potential significance in the fields of cancer research, regenerative medicine, and cardiovascular pathology and is the topic of ongoing investigations in a number of laboratories.

CD47–SIRP α signaling and phagocytosis

CD47 binding to SIRP α was first described in 1999.⁴³ At the immune synapse, the single Ig domain of CD47 binds to the NH₂-terminal domain of SIRP α and elicits the phosphorylation of the ITIM domains in the cytoplasmic region of SIRP α -expressing cells. As detailed below, the phosphate-mediated signaling cascade then elicits an increasing number of described cellular responses. CD47 and SIRP α interactions were originally defined by their capacity to inhibit macrophage engulfment of CD47-expressing cells and microparticles. Further analysis of this process indicated that the binding of CD47 to SIRP α initiated signal transduction events that dephosphorylated myosin 2a resulting in the depolymerization of actin fibers.⁴⁴ The molecular signaling cascade ultimately results in the inhibition of phagocytosis of CD47-expressing targets by SIRP α -expressing macrophages.

The inhibitory effect of CD47–SIRP α signaling on phagocytosis is largely species specific, and researchers have coined the terms “marker of self” or “don’t eat me”

signal when describing CD47.^{44–49} There are several examples of CD47-mediated biological inhibition of macrophage clearance. In mice, red blood cells (RBCs) lacking CD47 cells are rapidly cleared by splenic macrophages.⁴⁹ Hematopoietic stem cells upregulate their surface expression of CD47 to avoid clearance by macrophages.^{50,51} Similarly, CD47 has been shown to be overexpressed in ovarian carcinoma cells⁵² and leukemia cancer stem cells⁵¹ as a way to evade the immune system. These data strongly show the immunoprotective capacity of CD47 across a range of biological processes.

SIRP α and CD47 in the immune system beyond phagocytosis

Recent findings strongly suggest that CD47–SIRP α interactions influence leukocyte function beyond the canonical role as an inhibitor of phagocytosis. The dendritic cell (DC) is a poorly understood cell of the immune system, which initiates and maintains T-cell homeostasis.^{53–55} There are several subtypes of DCs, and the precise role of a particular DC depends upon their phenotypic expression of the CD4 or CD8 cell surface markers. For example, CD8[–] DCs are responsible for initiating and development of CD4⁺ helper T cells.^{56,57} Another DC subgroup, CD8⁺ DCs, plays a role in the induction of CD8⁺ cytotoxic T cells.^{56,57} Experimental evidence has shown that mice lacking a functional SIRP α have a reduced number of CD4⁺ and CD8[–] DCs.^{56,57} These data suggest that CD47–SIRP α contribute to maintaining an appropriate balance of DC subtypes.

In the spleen, several studies have shown that CD47–SIRP α signaling mechanisms play a key role in T-cell function. The process of T-cell activation partly occurs in concentrated regions, known as T-cell zones, of the spleen and lymph nodes.⁵⁸ These specialized regions provide the appropriate microenvironment for paracrine signaling events elicited by chemokines and cytokines. Matozaki and coauthors have reported that the T-cell zone in the spleen of SIRP α mutant mice was markedly reduced compared with wild-type mice.⁵⁶ In addition, the expression levels of chemokine (C-C motif) ligand (CCL) 19 and CCL 21, secreted from spleen stromal cells and play a role in attracting naïve T-cells,^{58,59} are reduced in the spleen of SIRP α mutant mice.⁶⁰ The release of CCL19 and CCL21 appears to be the result of an indirect effect upon the spleen stromal cells that are responsible for secretion of these chemokines.⁶⁰ Specifically, SIRP α on DCs or macrophages interacts with CD47 expressed on the surface of spleen stromal cell that then secretes CCL19 and CCL21.⁶⁰ Together, these data further demonstrate a role for SIRP α and CD47 in regulating the adaptive immune response.

The CD47–SIRP α -mediated regulation of the release of chemokines, cytokines, and extracellular matrix-related proteins appears to extend beyond dendritic cells. It was shown that the transcription of a number of cytokines and chemokines, such as CCL3 and CCL8, was significantly reduced when human whole blood was perfused over CD47-modified blood conduits. CCL3 and CCL8 are involved in the recruitment and activation of inflammatory cells. The same study also showed that the transcription of

matrix metalloproteinases (MMPs)-1, 7, 13, and 16 was increased beyond 100-fold when blood cells were perfused over the same CD47-modified polymeric surfaces. The effect of CD47–SIRP α signaling upon the secretion of these factors into extracellular milieu likely plays a critical role in maintaining homeostasis and can likely contribute to maintaining biocompatibility at the tissue–material interface. In addition, given the role of MMPs in matrix remodeling in late stage foreign body reaction, this finding can be important with respect to the issues associated with fibrotic barriers surrounding such long-term implants as metabolic sensors, hernia meshes, and breast implants.^{20,27,61,62}

Platelet interaction with synthetic surfaces represents one of the early and crucial steps that define the inflammatory response to biomaterials.^{17,20} Our laboratory was the first to demonstrate that SIRP α was expressed in platelets and that platelets are responsive to CD47-functionalized surfaces.⁶³ Using an *ex vivo* model system, which perfuses whole blood over CD47 immobilized or unmodified control surfaces, we showed that platelet activation was significantly inhibited as a function of CD47 exposure.⁶³ Specifically, we showed that the expression of the protein surface marker of platelet activation, P-selectin (CD62P), was significantly reduced after 3 h exposure to CD47-modified blood conduits.⁶³ Scanning electron microscopy demonstrated that the platelet attachment was markedly reduced on CD47-modified surfaces compared with unmodified control surfaces.⁶³ In addition, platelets that were adhered to CD47 surfaces had a noticeably rounder morphology and showed less evidence of spreading compared with the platelets from whole blood that was exposed to unmodified surfaces.⁶³ The fact that the platelets in these studies were not spreading, indicating activation, suggest that the actin cytoskeleton was not polymerized, which is consistent with previously described effects of SIRP α signaling upon myosin 2a and actin polymerization.

The exact role of SIRP α signaling in the platelet remains unclear. TSP-1, a known CD47 ligand, is primarily secreted from alpha granules of activated platelets.^{64–66} TSP-1 has been shown, via interactions with CD47, to exacerbate ischemia reperfusion injury by inhibiting signaling events mediated by NO.^{64–66} Thus, it is tempting to hypothesize that the SIRP α expression on platelets represents a feedback mechanism to limit the release of TSP-1 from platelet granules. Indeed, myosin 2a and actin have all been implicated in playing a role in a granule release. Given the negative role TSP-1 has on implanted medical devices as endovascular stents, further research is warranted.

Modifying biomaterial surfaces with CD47

The demonstrated and hypothesized regulatory effects of the CD47–SIRP α signaling pathway upon the immune system make it an attractive target to address the aberrant biocompatibility issues surrounding medical devices and therapeutics. Given the role of CD47 as the ligand for SIRP α , investigators have examined the efficacy of appending CD47, or selected domains thereof, to clinically relevant synthetic surfaces. Of course, it is imperative to ensure that the bioactivity of CD47 is not sacrificed as a result of the

immobilization procedure. As detailed below, several linking strategies are available to append bioactive CD47 to biomaterials' surfaces.

The high affinity of the avidin-biotin bond makes it a popular attachment strategy for appending therapeutic moieties to a wide range of substrates. Early analysis of CD47-SIRP α interactions involved the use of recombinant CD47-functionalized polystyrene microbeads. In these studies, the extracellular domain of CD47 was biotinylated and immobilized onto the avidin-coated beads. The avidin-biotin attachment strategy did not appear to effect CD47 physiology. Indeed, biotinylated CD47 appended to avidin-coated beads was one of the main experimental paradigms used to elucidate the inhibitory role of SIRP α in macrophage-mediated phagocytosis.^{44,46} Biotinylated CD47 was also appended to avidin-functionalized macro-scale surfaces, such as polyurethane films and polyvinyl chloride (PVC) blood conduits, and significantly reduced the interactions between inflammatory cells and the biomaterial.⁶⁷ These initial results established CD47 immobilization as a viable anti-inflammatory strategy.

Avidin has been reported to be immunogenic, and therefore may not be appropriate for all applications. In addition, the requirement of immobilizing avidin onto the surface introduces an additional procedure in the fabrication of inflammatory-resistant biomaterials. To alleviate the need for avidin, recombinant CD47 can be modified to include chemical reactive functional groups such as a C-terminus poly-lysine tag. This modification allowed for the addition of thiol-reactive groups by reacting the amines of the lysine with a heterobifunctional crosslinking agent such as succinimidyl 3-(2-pyridyldithio)propionate or succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate. To prime the polymeric surface with thiol-groups, these studies used a novel photoactivation chemistry in which a polymer (PDT-BzPh) composed of 2-pyridylthio groups (PDT) linked to the photo-reactive crosslinker, benzophenone (BzPh). The thiol-reactive CD47 was then covalently immobilized onto the activated polymeric surfaces.^{63,68} Of note is that BzPh is used commercially in sunscreens and represents an FDA approved photo-reactive crosslinker.^{69,70}

Recombinant CD47 represents the primary source of experimentally used CD47. In its present form, recCD47 is actually a chimeric protein composed of domains 3 and 4 of rat CD4 protein.⁴⁴ The purpose of this modification is to enhance recombinant CD47 protein secretion from the Chinese Hamster Ovary cell line. Although multiple laboratories have shown the expected biological response when cells or tissues are exposed to recCD47, the presence of rat peptide sequences makes the recCD47 a less than ideal choice for future clinical applications. More recently, a peptide sequence corresponding to the 22 amino acid Ig domain of the extracellular region of CD47 (pepCD47) has been shown to confer similar bioactive properties as the recombinant protein.⁷¹ Given the practical and economic advantages of peptide over recombinant proteins, research into the use of pepCD47 will likely benefit the research and development of CD47-based therapeutic strategies.

Examples of CD47-modified biomaterials

CD47-modified therapeutic delivery platforms

Given the fact that many cancer cells upregulate CD47 to evade macrophage clearance, interest has been focused on testing the efficacy of pharmacologic agents that function as CD47 or SIRP α antagonists. Monoclonal antibodies for CD47 have been explored as a treatment for cancers such as acute myeloid leukemia, non-Hodgkin's lymphoma, and bladder cancer.⁷² Chao *et al.* demonstrated that anti-CD47 antibody inhibits extranodal dissemination in a mouse non-Hodgkin's lymphoma model.⁷² In a follow-up study, Edris *et al.* revealed a decrease in tumor size and metastasis in a mouse leiomyosarcoma model.⁷³ Similar results were seen in inducing phagocytosis of human myeloma cells⁷⁴ and activating antitumor T cells.⁷⁵ Most impressively, a humanized blocking anti-CD47 antibody was shown to exert antileukemic effects both *in vitro* and *in vivo* leading to the eradication of acute myeloid leukemia in a xenotransplanted mouse model.^{72,74} At present, these *in vivo* and *in vitro* studies represent some of the leading examples of the efficacy of therapeutic strategies involving CD47-SIRP α signaling mechanisms.

The cancer-related studies detailed above help to demonstrate the therapeutic potential targeting the CD47-SIRP α pathway. However, systemic delivery of CD47 antibodies to tumor sites is limited by the need for high dosage levels and poor tumor targeting. Thus, a nanocarrier-based delivery system may be appropriate. Systemic drug delivery via nanoparticle injection has proven to be challenging due to nanoparticle clearance by macrophage phagocytosis. As such, CD47 offers a unique situation where the molecular target of interest, CD47 on cancer cells, can also be an attractive candidate to prolong the circulation time and availability of the therapeutic agent. The first example of recombinant CD47 attached to a surface for the purpose of evading host immune response is reported by Tsai *et al.*, where streptavidin-coated polystyrene beads are covalently labeled with biotinylated CD47 and cultured with peripheral blood monocytes and the human monocyte cell line (THP-1). In comparison with uncoated polystyrene beads, the recombinant CD47 coating on beads reduced phagocytosis by up to 50%, depending on the coating density.⁴⁴ The involvement of CD47 in the phagocytosis inhibition uptake was confirmed with a monoclonal antibody for CD47, where phagocytosis levels were comparable to uncoated polystyrene beads. In a follow-up study, a 21 amino acid CD47 peptide, consisting of the SIRP α -binding domain of CD47, was synthesized and attached to polystyrene beads.⁷¹ When injected into the bloodstream of NOD/SCID mice, circulation time was four times enhanced. Consequently, the delivery of paclitaxel to tumors, the resulting decrease in tumor size, and adverse side effects were improved in an *in vivo* mouse model.

Similar results were seen when artificial antigen presenting cells, fabricated from epoxy beads were co-cultured with macrophages.⁷⁶ This study demonstrated that phagocytosis inhibition was CD47 concentration dependent. Additionally, it was shown that CD47 did not interfere with the ability to expand antigen-specific T cells. *In vivo*

studies using these CD47-modified artificial antigen presenting cells showed increased stimulation of the production of CD8+ cells and a corresponding decrease in tumor area in mouse tumor model.

Hu *et al.*⁷⁷⁻⁷⁹ created (RBC) membrane-derived vesicles as a biomimetic coating for poly lactic co-glycolic nanoparticles. In these studies, CD47 is functionalized on nanoparticles in the exact conformation and density at which it is expressed on the surface of RBCs.⁷⁹ Vesicle-covered nanoparticles showed inhibition of macrophage phagocytosis and increased *in vivo* circulation time compared with poly-ethyleneglycol-covered nanoparticles showing significant particle retention in the blood at 72 h.^{77,79} Anti-CD47 antibody partially blocked the effects of the vesicle coating. RBC membrane vesicles were also used to coat gold nanoparticles, which are often used both as imaging agents and drug carriers.⁸⁰ Gao *et al.* was able to demonstrate decreased macrophage uptake of the coated nanoparticles compared with uncoated particles.⁸⁰ Ongoing research efforts continue to explore strategies to evade the immune system with the goal of developing drug delivery carriers with enhanced bioavailability.

CD47-modified polymers

Polyurethanes are block copolymers composed of a urethane hard segment and a polycarbonate or polyether soft segment. The widespread use of polyurethane components in medical devices is due in part to the polymer's superior durability and elasticity. They are commonly used as insulating material for pacemaker leads and metabolite sensors. However, polyurethane is subject to oxidative degradation caused by the release of ROS from adherent host MDM and neutrophils.^{81,82} This normal host response causes soft segment chain scission and aberrant crosslinking of the polyether or polycarbonate soft segments, which is then grossly observed as surface cracking of the poly(ether urethane) components of the cardiovascular device.⁸³⁻⁸⁵ Microscopic evidence of surface degradation has been observed by others and us to be present as early as 90 days post implantation.⁸³⁻⁸⁶ With respect to pacemaker leads, which commonly use polyurethane insulation, the duration from implantation to device failure has been shown to be as short as ~2.5 years.⁸⁷ Laboratories, including our own, have tried to address the issue of oxidative degradation of polyurethane by coblending⁸⁴ or covalently linking^{86,88} phenol-based antioxidants to polyurethane preparations. The limitations of this approach lie primarily in the fact that the antioxidants are expended once they react with hydroxy radicals. As such, the use of phenol-based antioxidants represents a temporary solution for addressing the oxidative degradation of polyurethane.

Our group has tested the ability of CD47 to inhibit the oxidative degradation of polyurethane by inflammatory cells. We appended recombinant biotinylated CD47 to the polyether polyurethane Tecothane TT1074A that had been surface modified, via photoactivation chemistry, with covalently linked avidin.⁶⁶ Salient *in vitro* findings from these studies included the observation that compared with unmodified control polyurethane films, attachment of the

human monocyte cell line, THP-1, to CD47-functionalized polyurethane was significantly reduced. In addition, we also noted that the inhibitory mechanism was species specific as bovine CD47 was not as efficient, compared with human-derived CD47, in blocking THP-1 binding. A seven-week subdermal implant model was used to assess the ability of surface immobilized CD47 to inhibit the oxidative degradation of polyurethane. Scanning electron microscopy demonstrated qualitative differences between CD47-modified and unmodified control explanted polyurethane. Specifically, unmodified control polyurethane samples showed extensive surface cracking that is consistent with oxidation-mediated damage.⁶⁶ Fourier Transformation Infrared Spectroscopy was used to provide quantitative assessment of oxidative degradation of the explanted samples. Results demonstrated that the CD47 surface modification significantly reduced oxidative degradation. Of particular importance for further long-term applications of CD47, we demonstrated that the CD47 modification was retained following seven-weeks *in vivo* in the oxidative environment of the rat subdermal implant. Together, these results strongly support the application of CD47-modified surfaces in such long-term polymeric implants as pacemaker lead insulation.

Another clinical application where immune response to blood-contacting materials can benefit from immune modulation is PVC blood conduits used in cardiopulmonary bypass and renal dialysis tubing. Post-procedural complications have been linked to inflammation and thrombotic reactions as a downstream response to protein adsorption and subsequent activation of platelets and leukocytes.^{16,89} Blood-contacting surfaces have been modified with heparin, thrombotic inhibitors, and self-assembled monolayers of alkylthiols⁹⁰⁻⁹²; however, there still remains an unmet need for biomaterials that contact large volumes of blood such as cardiopulmonary bypass tubing. Stachelek *et al.* first showed the attachment of CD47 to PVC and polyurethane surfaces and the reduction of human neutrophil and macrophage attachment.⁶⁶ Finley *et al.* further characterized these surfaces where it was demonstrated that CD47-coated PVC conduits significantly reduced attachment and activation of platelets and neutrophils.⁶⁸ When further examining the circulating platelets (platelets that did not attach to the tubing surface), it was found that the surface markers for activated platelets (CD62L and CD62P) were significantly reduced when blood was circulated through CD47-modified PVC tubing compared with unmodified tubing. Additional analysis showed that sterilizing the CD47-modified blood conduits via ethylene oxide exposure, did not negatively affect the anti-inflammatory properties of the CD47 modification. Given the effects CD47 has upon platelets and leukocytes, further exploration and development of CD47-modified blood conduits are warranted.

Conclusions

The inflammatory response to biomaterials contributes to a range of pathologies associated with medical devices. Bioactive strategies to mitigate the immune response to clinically used biomaterials can in theory enhance the

bioavailability of therapeutic agents and enhance the longevity and efficacy of medical devices. Targeting ITIM-expressing proteins represents a promising approach to address immune cell interactions at the cell–material interface. At present, the interaction between SIRP α and its cognate ligand CD47 represents a well-characterized ITIM signaling pathway with great promise for mitigating the inflammatory response to biomaterials.

Immunomodulation with CD47 has become a promising approach to develop long-circulating, immune evasive artificial surfaces. This approach is grounded in the “marker of self” feature of CD47, which is naturally presented by all cells in the body. As demonstrated herein, the capacity of CD47 to alter the immune cell response extends beyond CD47s canonical role in inhibiting macrophage phagocytosis. The role of SIRP α -CD47 signaling mechanisms within the context of T-cell physiology and the adaptive immune response represents an immense opportunity to develop a biomaterials approach in such fields as vaccine development. Similarly, the recent findings regarding the role of SIRP α in platelet activation supports future investigations into thrombus-resistant biomaterials. These data provide strong evidence in supporting the role of CD47 and SIRP α in regulating thrombosis as well as adaptive and innate immune processes. As such, the application of CD47-modified biomaterials has great potential across a range of medical devices and therapeutic drug delivery systems.

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