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Foreign body response to subcutaneous biomaterial implants in a mast cell-deficient *Kit$^{w\text{-}Sh}$* murine model

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ABSTRACT

Mast cells (MCs)_are recognized for their functional role in wound-healing and allergic and inflammatory responses – host responses that are frequently detrimental to implanted biomaterials if extended beyond acute reactivity. These tissue reactions impact especially on the performance of sensing implants such as continuous glucose monitoring (CGM) devices. Our hypothesis that effective blockade of MC activity around implants could alter the host foreign body response (FBR) and enhance the in vivo lifetime of these implantable devices motivated this study. Stem cell factor and its ligand c-KIT receptor are critically important for MC survival, differentiation and degranulation. Therefore, an MC-deficient sash mouse model was used to assess MC relationships to the in vivo performance of CGM implants. Additionally, local delivery of a tyrosine kinase inhibitor (TKI) that inhibits c-KIT activity was also used to evaluate the role of MCs in modulating the FBR. Model sensor implants comprising polyester fibers coated with a rapidly dissolving polymer coating containing drug-releasing degradable microspheres were implanted subcutaneously in sash mice for various time points, and the FBR was evaluated for chronic inflammation and fibrous capsule formation around the implants. No significant differences were observed in the foreign body capsule formation between control and drug-releasing implant groups in MC-deficient mice. However, fibrous encapsulation was significantly greater around the drug-releasing implants in sash mice compared to drug-releasing implants in wild-type (e.g. MC-competent) mice. These results provide insights into the role of MCs in the FBR, suggesting that MC deficiency provides alternative pathways for host inflammatory responses to implanted biomaterials.

1. Introduction

The host foreign body response (FBR) to implanted biomaterials impairs the performance of several implanted biomedical devices, particularly soft tissue sensor-actuator-type implants, such as pacemakers and continuous glucose sensors. The FBR is mediated by a complex series of tissue inflammatory events modulated by several cell types recruited as part of the normal wound-healing process. Dissolution, degradation and/or complete phagocytosis of certain foreign objects (e.g. pathogens, foreign proteins, nano- and microparticles) resolves the FBR and normal wound-healing is restored. Implants with prolonged tissue residence, generally lacking the ability to be cleared from a tissue site via natural mechanisms, alter the tissue wound-healing response in chronic inflammatory conditions, producing fibrous encapsulation of the foreign body and hallmark foreign body giant cells [1]. Fibrous encapsulation often isolates the implanted foreign body from normal host tissue sites. This capsule is dense and frequently poorly vascularized, altering sensor electrical responses, blocking transport of analytes to sensors, compromising functional performance and disposing these sites to infection. Several implant strategies including surface modifications, and active drug-releasing coatings containing anti-inflammatory agents and angiogenesis factors, have been used previously to mitigate FBR to subcutaneously implanted biomaterials, and specifically continuous glucose sensors, with varying results [2–8].

The intensity and impact of the FBR depend upon recruitment and reactivity of several key cell mediators, including polymorphonuclear neutrophils (PMNs), monocytes, macrophages, mast cells (MCs) and fibroblasts [9]. Each cell type has a specific role in modulating the local tissue response to wounding (i.e. implant placement) and subsequent healing, including chronic inflammation and the FBR [10]. The role of MCs in the FBR is associated with mediating the host response by secreting cytokines and cell mediators through cell activation and degranulation of their prominent intracellular granules [9]. MC precursors derive from hematopoietic

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stem cells in the bone marrow and are recruited through chemotactic inflammatory signaling to wound sites, where they mature and activate. MCs are found ubiquitously in all tissues, particularly those associated with vasculature and nerves, and also in proximity epithelial surfaces such as airways and skin. MC survival and differentiation depends on presentation of the ligand of their membrane-resident c-KIT receptor, also known as stem cell factor (SCF) [11–13]. The c-KIT receptor is primarily expressed on hematopoietic stem cells and MCs [14]. SCF/c-KIT interactions result in MC activation and degranulation, prompting secretion of vasodilators (histamine), chemokines, cytokines (e.g. IL-4, IL-13) and prostaglandins [15–18].

MCs act as a primary cell-based host defense mechanism, mediating allergic responses and inflammatory responses. They have also been shown to influence neovascularization and tissue remodeling [19]. MC degranulation in the presence of antigens or allergens produces cell secretion of granules containing IL-4 and IL-13 that provide chemotactic gradients to recruit macrophages, and histamine and serotonin to dilate the vasculature to facilitate greater access to the inflammatory cells arriving at the wound site [9].

The MC role in the implant-associated FBR was recently described using a wild-type C57BL/6 mouse subcutaneous model and a local pharmacological approach. Inhibition of tissue site MC activation and degranulation was attempted using local delivery of a tyrosine kinase inhibitor, masitinib [20]. Reduced thickness of the foreign body capsule formed around implants was demonstrated in vivo for up to 28 days post-implantation. A similar study using hernia mesh implants [21] demonstrated that blocking MC degranulation with cromolyn treatment reduced inflammation and fibrosis around the subcutaneous implant site. In the context of CGM sensor implants, Klueh et al. [22] recently probed the MC role in the performance of implanted CGM sensors in murine cutaneous implant models. When compared to MC-sufficient wild-type C57BL/6 cohorts, implants in knockout $Kit^{W\text{-}Sh}$ (sash) (MC-deficient) mutant mice exhibited stable CGM responses for up to 28 days, suggesting a critical MC role in modulating host tissue response to implanted sensors. Mechanisms for this observation require further study and elucidation.

Previous studies in MC-deficient sash mice also show normal wound-healing in cutaneous wounds in the absence of MCs [23]. Our prior work describes thinner fibrous capsule formation around masitinib-releasing implants than that around control implants in wild-type mice, suggesting that stabilizing MCs alone does not completely avoid FBR fibrosis [20]. These results have produced a further idea that the use of a MC-stabilizing drug in wild-type mice might alter the local tissue response to implants, but may not completely avoid it as fibrous encapsulation of foreign implants has also been observed in the absence of MCs [22,23]. Klueh et al. [22] report dramatically reduced inflammation and fibrosis around subcutaneous implants in MC-deficient mice, while Nauta et al. [23] observed no discernible differences in collagen concentrations in the scar tissue around cutaneous wound sites among MC-deficient and analogous wild-type mice. However, wound-healing models in these two studies were significantly different: CGM sensor insertion requires a percutaneous access of diameter ~600 μm [22], while cutaneous wounds [23] were 6 mm in diameter. Interestingly, glucose sensor CGM implants functioned normally, even with early capsules surrounding them, indicating that the early capsule is permeable to essential analytes (i.e. glucose and oxygen for this CGM) required for glucose sensing. Additionally, these studies have diametrically opposite conclusions regarding the role of MCs in modulating the FBR. Hence, this strategy needs further investigation to understand the complex MC mechanisms involved in FBR and if they can be exploited in the context of implant-associated healing and device integration.

Here, we evaluate the effect of the c-KIT-inhibiting drug, masitinib, in implant-associated MC functions in MC-deficient mutant sash mice. The intent is to assess the role of MCs in host tissue responses to implanted biomaterials. Masitinib is a relatively new tyrosine kinase inhibitor, shown to be an effective inhibitor of MC proliferation in vitro and in vivo [24], and therefore was chosen to release from the implanted CGM interface to inhibit MC degranulation in a device-based local delivery formulation. Apart from being a potent inhibitor of c-KIT, masitinib has also been shown to inhibit platelet-derived growth factor receptor (PDGFR), intracellular kinase Lyn and fibroblast growth factor receptor 3 (FGFR3), which play a role in tissue remodeling, inflammatory and allergic responses [24].

MC-deficient sash mice (B6.Cg-$Kit^{W\text{-}sh}$/HNihrJaeBsmGlliJ) have been used as the strain of choice for studying MC deficiency-related conditions [25]. In this context, polyester fiber model implants as sensor surrogates were coated with rapidly dissolving polymer film loaded with degradable polymer microsphere controlled release formulations of masitinib to alter the healing dynamics in subcutaneous tissues of MC-deficient sash mice implanted for 14, 21 and 28 days.

2. Materials and methods

2.1. Materials

Poly(lactic-co-glycolic acid) (PLGA) was purchased from Lakeshore Biomaterials (now Evonik Biomaterials, USA). Polyethylene glycol (PEG) (MW 20,000), polyethylene oxide (PEO, MW 100,000), polyvinyl alcohol (average MW 30–70 kDa) and solvents dichloromethane, chloroform, ethanol, methanol and acetonitrile were purchased from Sigma Aldrich, USA. Trifluoroacetic acid (TFA) was purchased from Fisher Scientific, USA. Masitinib drug was purchased from Selleck Chemicals, USA. Commercial polyester fiber (Trilene, 300 μm diameter, Berkley Fishing, USA) was cleaned using 70% ethanol before use. Ultrapure water (Millipore-filtered ASTM Grade II) was used for all experiments.

2.2. Implant fabrication

Implant fabrication was accomplished as described in a recent publication [20]. Briefly, PLGA polymer microspheres were fabricated using established solvent-evaporation techniques, either with masitinib at a concentration of 1 mg ml^{-1} or without masitinib (blank controls), and mixed with aqueous PEG/PEO solution at a concentration of 350 mg ml^{-1}. Polyester fiber sensor model implants were coated with the PEG–PLGA microsphere solution using a two-part aluminum mold and flash-freezing, and the resulting coating was lyophilized for 12 h to obtain the implants coated with a rapidly soluble polymer film containing drug-loaded microspheres. Both drug-releasing and control implants (i.e. PLGA microspheres, no drug) were fabricated using this procedure.

Intended masitinib load within each implant was calculated based on drug dosing values from Dubreuil et al. [24], with subcutaneous tissue density values obtained from Kyrzywicki and Chinn [26], and assumptions about the intended tissue delivery volume surrounding the implant (see Fig. 1A). A dose of 60 mg kg^{-1} day^{-1} was considered potent by assuming complete drug clearance each day from the tissue bed surrounding the implant. Subcutaneous tissue density of 1 g cm^{-3} and a targeted tissue drug exposure depth of 200 μm around each implant (illustrated in Fig. 1A) were considered, and the resulting dosing values were extrapolated for 30-day release for targeting final drug load on the implant. A margin of safety of 1.5× was used in calculating the drug loading/implant to accommodate this required drug load and assumptions.