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Modulation of mast cell adhesion, proliferation, and cytokine secretion on electrospun bioresorbable vascular grafts

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1. Introduction

An understanding of how mast cells participate in angiogenesis is important to further our knowledge about vascular development and remodeling¹. Mast cells are important to the pathogenesis of allergic, autoimmune and cardiovascular diseases, and cancer. However, in addition to playing a critical role in host defense, they are also involved in various physiological processes such as angiogenesis, tissue remodeling and collagen production²⁻⁵.

Biological responses to implanted materials involve neutrophil mediated detoxification, followed by macrophage activity to phagocytize debris and coordinate remodeling events. It was reported by Ashley *et al.* that these events occur in the presence of both eosinophils and mast cells⁶. They are known to mediate acute inflammatory responses to the implanted biomaterials⁷. Mast cell-derived Interleukin-4 (IL-4) and IL-13 were found to induce macrophage fusion while tumor necrosis factor alpha (TNF- α) was responsible for biomaterial adherent macrophage apoptosis. IL-4 and IL-13 lead to alternatively activated macrophages which play a role in allergic responses, elimination of parasites and matrix remodeling⁸. Using implantation of fibrinogen coated polyethylene terephthalate (PET) disks in mice, it was found that this biomaterial triggers the activation of mast cells shortly after implantation, releasing granular products including histamine. Mast cell-deficient mice exhibited reduced phagocyte accumulation on the implants. Administration of histamine receptor antagonists also greatly decreased recruitment and adhesion of neutrophils and monocytes/macrophages to implants⁹.

Mast cells can be activated by physical stimuli, immunogenic stimuli (Immunoglobulin E (IgE), complement, cytokines and growth factors) and neurogenic stimuli (neuropeptides)². Exposure of mast cells to an allergen leads to cross linking of the IgE-loaded surface receptor, Fc ϵ RI, with at least three biological effects. First, they can undergo degranulation; the process of regulated secretion in which preformed contents stored in their granules are rapidly released by exocytosis. Granule associated mediators include proteases, histamine, proteoglycans and cytokines such as TNF- α and IL-16. Following degranulation is the rapid enzymatic synthesis of lipid mediators derived from precursors stored in the cell membranes and in lipid bodies. These include, the arachidonic acid metabolites prostaglandin (PG) D₂, leukotrienes (LT) B₄⁺ and C₄, and platelet activating factor (PAF). Lastly, activated mast cells initiate transcription, translation and secretion of a variety of cytokines and chemokines depending on the mast cell type and stimulus. These include, TNF- α , granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), IL-3, IL-4, IL-5,

IL-6, IL-10, IL-13, IL-14 and IL-16, and chemokines, such as macrophage inflammatory protein (MIP)-1 α , MIP-1 β , T-cell activation gene 3, lymphotactin and monocyte chemoattractant protein -1 (MCP-1)¹⁰⁻¹³.

Under physiological conditions in several organs, mast cells are found in close vicinity to capillaries and lymphatic vessels and a direct correlation between the number of mast cells and blood vessel density has been demonstrated in the dermis¹⁴. Mast cells synthesize several potent angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), heparin, histamine, tumor necrosis factor (TNF- α) and IL-8. On the other hand, endothelial cells may also express the mast cell growth and chemotactic factor, SCF. This shows that endothelial cells may also elicit mast cell chemotaxis and survival, demonstrating a two way relationship between them^{15,16}. It has been shown that SCF is the primary cytokine involved in mast cell differentiation and activation¹⁷⁻²⁰. SCF (or c-kit ligand) is critical for mast cell survival, as mast cells cultured without SCF undergo apoptosis²¹.

In addition to acting on the vasculature, mast cells may augment wound healing by promoting extracellular matrix deposition. In fibroblasts, mast cell extracts elicit collagen synthesis and activate an enzyme involved in matrix remodeling; gelatinase A²². Similar to mast cell-endothelial cell interactions, mast cells can be attracted to fibroblasts via their SCF expression as both membrane-bound and secreted forms.

In this study, an investigation of mast cell interaction with electrospun scaffolds has been performed. Electrospinning holds the potential to create off-the-shelf bioresorbable vascular grafts as acellular implants that will be degraded gradually over a period of time, leaving behind no permanent synthetic materials to initiate an undesirable immune reaction. Wesolowski *et al.*²³ and Ruderman *et al.*²⁴ first described the concept of a slowly absorbable graft that allowed *in situ* regeneration to produce a “neoartery”. These grafts were composed of Dacron and polylactide yarns and were partially bioresorbable. Bowland *et al.* described the use of Vicryl (a copolymer of polyglycolide and polylactide) as a fully bioresorbable vascular graft²⁵. However, these grafts were susceptible to aneurysm formation. Greisler *et al.* utilized polydioxanone (PDO) absorbable vascular prosthetics in a rabbit aortic model. The results showed that the myofibroblast migration paralleled the macrophage-mediated degradation of the PDO structure. A confluent endothelial cell lining was present within two weeks. The compliance of the regenerated vascular tissue at one year resembled an artery²⁶.

The biomaterials chosen for this study were polycaprolactone (PCL), PDO, and silk fibroin (silk). PCL exhibits properties (i.e. compliance) that are conducive to arterial tissue engineering with an extended degradation time, and compatibility for endothelial cells and smooth muscle cells²⁷⁻³⁰. PDO has been extensively studied by our laboratory and has shown promise *in vitro*^{28,31-36}. Its flexibility, excellent strength retention, shape memory, low inflammatory response and slow degradation rate make it a suitable candidate for vascular tissue engineering. The use of silk as a vascular tissue engineering scaffold has escalated recently. Zhang *et al.* demonstrated the bioactivity of human aortic endothelial cells and human coronary artery smooth muscle cells on electrospun silk³⁷. It was

established by Soffer *et al.* that electrospun tubular silk constructs can withstand native arterial pressures while behaving in a similar manner to native vessels during creep testing³⁸.

The adhesion, proliferation and cytokine secretion of mast cells on electrospun bioresorbable grafts was investigated in the presence or absence of IL-3, SCF, IgE and IgE with a crosslinking antigen, dinitrophenol-conjugated albumin (DNP). We attempted to modulate the behavior of mast cells on electrospun scaffolds by adding these stimulating agents using tissue culture plastic (TCP) as the control. The stimulating agents were chosen and combined according to their proliferation and attachment-inducing potential. It is widely accepted that IL-3 alone is capable of maintaining mouse mast cell survival. However, SCF is required for mast cell attachment to substrates. The presence of IgE and antigen can further enhance the survival and attachment of mast cells induced by IL-3 and SCF. The results of various studies show conflicting reports on the effects of IgE in the absence of antigen. Its impact on mast cell survival and attachment has not been clearly defined yet³⁹. Overall, our data demonstrate mast cell adhesion, survival and IgE responsiveness when cultured with bioresorbable polymers, supporting the utility of these polymers in tissue grafts.

2. Materials & Methods

2.1 Electrospinning

Electrospinning was used to generate nanofibrous scaffolds of PCL, PDO, and silk. Silk fibroin was extracted from the cocoons of *Bombyx mori* silkworms (The Yarn Tree) through an established protocol⁴⁰. PCL (MW 60,000 kDa, Sigma Aldrich), PDO (Ethicon, Inc.) and silk polymer concentrations used in the study were 250, 100 and 100 mg/ml respectively in 1,1,1,3,3,3, Hexafluoro-2-propanol (HFP) to form flat sheets on a stainless steel mandrel (0.5 cm x 3cm x 15 cm). Disks 6 mm in diameter were punched out of these scaffolds and were placed in a 96 well plate. Fibronectin (100 µl) at a concentration of 50 µg/ml was added on top of the disk and the plate was allowed to sit in the incubator at 37°C for an hour. After an hour, the disks were moved to a new well. The scanning electron micrographs of the uncoated scaffolds are shown in Figure 1.

2.2 Cell Culture and Seeding

Bone marrow derived murine mast cells (BMMCs) were derived from C57BL/6 mice as described previously⁴¹. Briefly, BMMC cultures were derived from bone marrow harvested from C57BL/6 mouse femurs and tibias (Jackson Labs, Bar Harbor, ME). BMMC cultures were maintained in cRPMI supplemented with IL-3 containing supernatant from WEHI-3B and SCF containing supernatant BHK-MKL cells. The final concentration of IL-3 and SCF was adjusted to 1 ng/ml and 10 ng/ml respectively.⁴² After 3-4 weeks in culture, these populations were >99% mast cells, as judged by morphology and flow cytometry staining for expression of Fc_εRI, and Kit (data not shown). The resulting populations were generally used between weeks 4-12. The cells were rinsed with phosphate buffered saline (PBS) and cultured in complete RPMI (cRPMI) 1640 medium (Invitrogen Life Technologies) (10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mM sodium

pyruvate, and 1 mM HEPES; Biofluids), supplemented with 5 ng/ml of IL-3 (R&D systems) and 50 ng/ml of SCF (PeproTech). The cells were then divided into four groups and cultured for 18 hours in the presence of IL-3 (group 1), IL-3+SCF (group 2) and IL-3+SCF+IgE (1 µg/ml, clone C38-2, BD Biosciences) (group 3). After 18 hours, Group 3 was centrifuged and suspended in media containing dinitrophenyl-human serum albumin (DNP) antigen (100 ng/ml, Sigma-Aldrich) (group 4). This was done to cross link the IgE-loaded cell surface receptor Fc_εRI, triggering BMMC activation prior to scaffold seeding.

2.3 Cell Adhesion

Disks 6 mm in diameter were punched from the scaffolds, disinfected (by soaking in ethanol for 10 min followed by repeated rinses in PBS) and placed in a 96 well plate. Each disk was then coated with 100 µl of fibronectin (50 µg/ml) and placed in the incubator at 37°C for an hour. The disks were then moved to a clean well and BMMCs were seeded on fibronectin-coated electrospun PDO, PCL, and silk scaffolds and uncoated tissue culture plastic (TCP) under four different culture conditions. The culture conditions were; cRPMI media supplemented with IL-3 (group 1), IL-3+SCF (group 2), IL-3+SCF+IgE (group 3) and IL-3+SCF+IgE+DNP (group 4) as described in section 2.2. In all groups, mast cells were seeded at a density of 50,000 cells/well with 170 µL of media. The cells were allowed to attach for 7 hours in the incubator. At 7 hours, the cell seeded scaffold was moved to a clean well and the number of attached cells was quantified by MTS assay as described below.

2.4 Cell Proliferation

The numbers of cells on the scaffold were determined with a colorimetric cell titer assay (CellTiter 96[®] AQueous; Promega Corp., Madison, WI). The assay is composed of solutions of a tetrazolium compound, MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and an electron coupling reagent, PMS (phenazine methosulfate). Metabolically active cells convert MTS into the aqueous soluble formazan product. The quantity of formazan product can be measured by the amount of 490 nm absorbance. It is directly proportional to the number of living cells in culture. For this assay, disks 6 mm in diameter were punched from the scaffolds, disinfected (by soaking in ethanol for 10 min followed by repeated rinses in PBS) and placed in a 96 well plate. Each disk was then coated with 100 µl of fibronectin (50 µg/ml) and placed in the incubator at 37°C for an hour. The disks were then moved to a clean well and BMMCs were seeded on fibronectin-coated electrospun PDO, PCL, and silk scaffolds and uncoated tissue culture plastic (TCP) under four different culture conditions. The culture conditions were; cRPMI media supplemented with IL-3 (group 1), IL-3+SCF (group 2), IL-3+SCF+IgE (group 3) and IL-3 + SCF +IgE+DNP (group 4) as described in section 2.2. In all groups, mast cells were seeded at a density of 50,000 cells/well with 170 µL of media. In order to exclude any cells attached to TCP, the scaffold disks were moved to a new well plate and 100 µl of fresh media with 20% MTS solution (20 µl) was added and placed in the incubator for 2 hours. Simultaneously, standards were made with mast cells starting with a concentration 200,000 cells/well to zero (media alone). The number of cells was determined by interpolation from the standard curve by using a log-log fit. The assay was performed on day 1, 3 and 5. Each data point was calculated from triplicate wells.

2.5 Histology

For histological evaluation, disks of PDO, PCL and silk of all four groups were fixed in formalin on day 3. The paraffin embedded disks were cross-sectioned and stained with hematoxylin and eosin (H&E) to examine cell infiltration into the scaffolds.

2.6 Quantification of TNF- α , MIP-1 α and IL-13

10 mm disks (disinfected) of fibronectin-coated (50 μ g/ml) electrospun PDO and PCL scaffolds were placed in a 48 well plate and an 8 mm cloning ring was placed on top of the scaffolds to retain the cells during scaffold seeding within a defined circumference. Cells were seeded on fibronectin-coated electrospun PDO, PCL, and silk scaffolds and uncoated tissue culture plastic (TCP) at a concentration of $\sim 1 \times 10^6$ cells/ml. Media was added in the center of each scaffold (100 μ l of cells in media incubated for 45 min and followed by 200 μ l of media). Cell culture supernatants were collected after 24 hours and stored frozen at -20°C until analyzed by enzyme linked immunosorbent assay (ELISA). The amounts of TNF- α , MIP-1 α , IL-13 (PeproTech) secreted by the mast cells due to their interaction with the electrospun scaffold were quantified by ELISA as per the manufacturer's instructions.

2.7 Statistical Analysis

Data expressed in this paper is in the format of means $\pm$ standard error of mean (S.E.M). Data of one representative experiment carried out in triplicates are given. Each experiment was reproduced at least twice. All statistical analysis of the data was based on a Kruskal-Wallis one-way analysis of variance on ranks and a Tukey-Kramer pairwise multiple comparison procedure ($\alpha=0.05$) performed with JMP[®]IN 8 statistical software (SAS Institute). $P<0.05$ was considered significantly different.

3. Results

3.1 Mast Cell Adhesion and Proliferation

The percentage of adherent mast cells at the end of 7 hours on polymeric scaffolds and TCP are shown in Figure 2. On PCL, PDO and TCP, cells exposed to IgE alone (group 3) displayed greater attachment as compared to group 1 (IL-3) and group 4 (IL-3+SCF+IgE+DNP). Silk exhibited little to no attachment in all groups. SCF +IL-3 (group 2) promoted cell attachment at levels comparable to IgE on TCP and PDO.

Mast cell proliferation on the three scaffolds and TCP was studied at day 1, 3 and 5 with the results shown in Figure 3. Cells stimulated with IgE+DNP (group 4) showed significantly higher proliferation on day 5 as compared to groups 1 and 3 on the PCL and PDO scaffolds. By day 5, the group 4 cells proliferated 2-fold on PCL, 1.7-fold on PDO and 3.3-fold on TCP. Exposure of mast cells to IgE+DNP leads to cross linking of the IgE-loaded surface receptor Fc ϵ RI. Figure 3 demonstrates that although mast cells exposed to IgE+DNP initially adhered less than the cells exposed to IgE alone, antigen stimulation allowed for enhanced proliferation and a net gain in cell numbers over the IgE alone group. In fact, IgE in the absence of antigen did not support long-term proliferation and survival of mast cells in this assay. It has been reported that Fc ϵ RI aggregation enhances mast-cell proliferation, perhaps

owing to the autocrine effects of IL-3 and IL-4⁴³. Our data are consistent with these findings.

SCF is a powerful mast cell co-mitogen. Mast cells treated with IL-3+SCF (Group 2) showed a steady increase in proliferation from day 1 to day 5 for the PDO and PCL scaffolds as well as TCP. Two pieces of data from figure 3 were notable. First, IgE crosslinkage was a stronger mitogen than SCF when used in the presence of PDO or PCL. Second, silk not only offered poor adhesion (Figure 2) but also failed to support mast cell proliferation even in response to SCF or IgE crosslinkage. These data demonstrate the differential ability of the polymer substrates to support mast cell adhesion and expansion.

3.2 Histology

H&E staining revealed cell infiltration into the fibrous structures of PDO (Figure 4h) and PCL (Figure 4d) on day 3. Consistent with the poor cell binding in short-term assays, no cells were observed on the silk scaffold using H&E staining (Figure 4i, j, k, l). Mast cell adhesion molecules include various integrins, intercellular adhesion molecule-1 (ICAM1) and c-kit. It has been shown that mast cells adhere to plate bound laminin, fibronectin and vitronectin only when activated through FcεRI or by pharmacological stimuli such as phorbol 12-myristate-13-acetate (PMA) or calcium ionophore⁴⁴⁻⁴⁷. However, mast cells exposed to only IL-3+SCF attached to the PDO and PCL scaffolds. This proves that a combination of fibronectin, SCF and the scaffold nanostructure can elicit mast cell attachment. Consistent with Figure 2, mast cells exposed to IgE alone attached to the scaffold in greater numbers than IL-3+SCF (Figure 4b, c). It has been demonstrated that IgE primes mast cell adhesion to fibronectin using similar pathways as IgE +Ag⁴⁸.

As noted when studying proliferation in Figure 3, IgE+DNP stimulation led to the greatest number of adherent mast cells after 3 days of culture with PDO (Figure 4d) or PCL (Figure 4h). The increased number of cells is likely due both to strong adhesion and cell division. The histology analysis also revealed an important difference between the stimuli. In the presence of SCF, mast cells remained largely on the surface of the electrospun scaffold. However, in the presence of IgE+DNP, cellular infiltration dramatically improved. The cells migrated deeper into the fibrous structures of PDO and PCL scaffolds. These data suggest that FcεRI aggregation is a potent stimulus for mast cell attachment, proliferation and migration in the presence of electrospun bioresorbable graft structures.

3.3 Mast Cell Cytokine Secretion

To assess the functionality of mast cells adhered to electrospun scaffolds, we measured cytokine secretion. Mast cells secrete an array of cytokines and chemokines. We chose to measure TNF-α (Figure 5), MIP-1α (Figure 6) and IL-13 (Figure 7), which are well known for their roles in mast cell mediated inflammation⁴⁹⁻⁶⁰. IL-3 alone group and silk scaffold were omitted from this study due to their poor capacity for cell adhesion and proliferation. When compared to the background (media alone), only group 4 cells (IgE + DNP) produced any cytokines on the PDO and PCL substrates. While IL-3 and SCF are strong mast cell mitogens, neither is a potent inducer of cytokine secretion as a sole stimulus. In keeping with this, IgE+DNP was the only stimulus to consistently elicit cytokine production on

PDO, PCL and TCP. More importantly, mast cells cultured on PDO or PCL showed similar cytokine responses to the control group cultured on TCP. These data indicate that mast cell adhesion to bioresorbable polymers did not hinder their functionality.

4. Discussion

One of the main requirements of tissue engineered scaffolds is to provide cells with a suitable environment for cell attachment, which is the first step in the cellular growth process. The use of electrospinning for fabricating nanofibrous tissue engineering scaffolds has escalated at a dramatic rate in the recent years. Electrospinning is capable of producing fibers in micro/nanometer range, which mimic the structural dimension of the native extracellular matrix (ECM). The scaffolds produced by electrospinning possess a highly porous microstructure with interconnected pores and extremely high surface-area-to-volume ratio, which is conducive to cellular growth, attachment and organization⁶¹. Material interaction with the cells is guided by its physicochemical surface properties⁶². Surface modifications of the electrospun scaffolds can drastically improve the biological performance while retaining all the nanostructure features and properties⁶³. He *et al.* fabricated a collagen coated poly (L-lactic acid)-co-poly(ϵ -caprolactone) scaffold that exhibited enhanced spreading, viability and attachment of endothelial cells (EC)⁶⁴. It was observed in another study that fibronectin coating of polymeric substrates was required for Human Umbilical Vein Endothelial Cell (HUVEC) attachment and spreading⁶². In this study, fibronectin coating was found to be imperative for mast cell attachment and spreading on the electrospun scaffolds (data not shown). Plasma fibronectin modulates the foreign body response and fibrous encapsulation of implanted materials⁶⁵. Cellular transmembrane integrin receptors recognize the Arginine-Glycine-Aspartic acid (RGD) sequences within fibronectin. Once integrins bind to fibronectin, they activate a cascade of intercellular signaling pathways^{62,66}.

It has been documented that human mast cells attach to fibronectin and vitronectin only when stimulated by phorbol 12-myristate 13-acetate (PMA) and or/ calcium ionophore⁴⁵. In another study, it was found that following Fc ϵ RI-mediated activation mast cells exhibited flattening and spreading accompanied by cell translocation on laminin, fibronectin and matrigel⁴⁴. However, in this study mast cells adhered to fibronectin-coated electrospun scaffolds in the presence of SCF and IL-3 without any activation agents. But our data certainly did not refute the importance of mast cell activation in adhesion. In fact, we found that Fc ϵ RI aggregation augmented initial adhesion and supported proliferation on electrospun bioresorbable vascular graft materials. IgE binding to mast cells is referred to as a 'passive presensitization' step. In this study, IgE in the absence of antigen greatly augmented adhesion but did not support long-term survival and proliferation of mast cells in the absence of antigen. It is important to note that surface Fc ϵ RI on mast cells are nearly saturated in vivo. It has been reported previously that more than 80% of mast cell surface Fc ϵ RI are occupied by 7 weeks of age in mice⁶⁷. Therefore, passive presensitization is likely to occur, and may have important effects on mast cell adhesion to grafted polymers.

Xiang *et al.* showed that the survival effect of Fc ϵ RI aggregation occurs due to the expression of the anti-apoptotic Bcl-2-family protein A1. Mast-cell survival was abolished

in *AI1a*^{-/-} mast cells, and the increase in the number of mast cells that is induced by FcεRI aggregation *in vivo* was not observed in *AI1a*^{-/-} mice⁶⁸. In Figure 3, the decline in cell numbers by day 5 suggests that in the absence of antigen, continuous exposure to IgE is required for mast cell survival. In this study, IgE was not added to the culture medium that was used to feed the cells after day 1. It has been proposed that prolonged FcεRI aggregation is required for augmented proliferation of mast cells⁶⁹. It has been shown that the binding of monomeric IgE to FcεRI does not induce DNA synthesis, but it renders mast cells resistant to apoptosis induced by growth factor deprivation. However, continuous presence of IgE is required for this anti-apoptotic effect³⁹. It can therefore be concluded that the effects of IgE in the absence of antigen are short lived and are more prominent early on as compared to IgE+DNP, which has long-term effects on cells.

It should be noted that the MTS assay cannot efficiently quantify cells that have migrated into the three-dimensional structure of the scaffold and only provides a reliable estimation of the cell number on the surface. The polymeric materials pose unique challenges via various mechanisms including diffusion gradients and sequestering effects. Efforts are underway to validate the modified diphenylamine assay for cell quantification, recently published for use with three-dimensional polymeric scaffolds⁷⁰.

The physical properties of silk and its ability to promote endothelial cell growth and proliferation make it a promising candidate for vascular tissue engineering scaffolds. However, this study found that silk scaffold with or without fibronectin treatment was not conducive for mast cell adhesion. Our results are consistent with a study conducted by Panilaitis *et al.* showing that silk was largely inert and even inhibitory with respect to murine macrophage activation⁷¹.

Tissue regeneration requires multiple cells and cytokines acting together in concert in a proper sequence. Mast cells stimulated with IgE+DNP on PDO and PCL scaffolds as well as TCP produced high amounts of TNF-α, MIP-1α and IL-13. It has been demonstrated that mast cell derived TNF-α is a crucial component of host defense against bacterial infection and is involved in recruitment of leukocytes by establishing cytokine networks^{53,54}. In healing wounds, MIP-1α plays a critical role in macrophage recruitment. It has been reported that MIP-1α levels increase during the inflammatory phase, and decrease as inflammation resolves and repair proceeds. It is essential for T-cell chemotaxis to inflamed tissue and also plays a critical role in the regulation of trans-endothelial migration of monocytes, dendritic cells, and natural killer cells⁵⁸⁻⁶⁰.

IL-13 was also produced by mast cells adherent to the polymer scaffolds. IL-13 induces expression of endothelial adhesion molecules such as vascular endothelial adhesion molecule-1 (VCAM-1) and chemokines which are required for recruitment of granulocytes and monocytes into tissues⁷². IL-13 stimulates macrophages and fibroblasts to synthesize collagen and promotes fibrosis by stimulating macrophages to produce transforming growth factor (TGF-β)⁷³. These results demonstrate that mast cell cytokine synthesis can influence the biological response of various cell types that play an important part in tissue regeneration and angiogenesis. Improved knowledge of these mechanisms will allow us to control and modulate the reactions occurring at the host/implant interface.

Although many of the differences between human and mouse mast cells appear quite subtle, caution must be observed when interpreting animal model research for use in clinical applications. Animal studies are important in assessing and screening for risks associated with the use of biomaterials in humans. They also help in determining the mechanisms of immune modulation and the cell types affected following biomaterial exposure^{5,74}.

Evaluations that understand the behavior of mast cells on electrospun scaffolds at a molecular level may provide insight into the specific causes of effects observed in these studies. Such evaluations will help biomedical researchers in promoting biomaterial integration with the host tissue without any undesirable immune reactions.

5. Conclusion

The present study examined for the first time the cytokine expression and adhesion of mast cells on bioresorbable electrospun scaffold for potential use as a vascular graft. The study demonstrates that biomaterial exposure can affect mast cell adhesion and cytokine expression. This indicates that mast cells might play a role in the process of biomaterial integration into the host, tissue regeneration, and possibly angiogenesis. Mast cell attachment onto fibronectin coated electrospun scaffolds led to survival and proliferation, and these cells retained their IgE-induced cytokine production. Improved knowledge of mast cell responses to biomaterials will allow a better understanding of the regeneration process and modulation of both acute and chronic inflammation of implanted biomaterials.

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Figure 1.
SEM images of PCL (left) PDO (middle) and silk (right).

Figure 2.

Adherent cells expressed as percentage of seeded cells after 7 hours of culture. Symbols 'ψ' indicate a statistically significant difference from group 1 (IL3) and group 4 (SCF+IL-3+IgE +DNP) for a particular material. Symbol 'Φ' indicates statistical difference from all groups for that particular material.

Figure 3.

Mast cell proliferation on scaffolds and TCP. Symbols 'ψ' indicate a statistically significant difference from group 1 (IL-3) and group 3 (SCF+IL-3+IgE) for a particular material.

Symbol 'Φ' indicates statistical difference from all groups for that particular material.

Figure 4. H&E staining of mast cell seeded PCL, PDO and silk scaffolds on day 3. The blue arrows indicate the seeded surface.

Figure 5. Quantification of TNF- α release by ELISA on day 1. Symbol '*' indicates statistical difference from all groups for that particular material.

Figure 6. Quantification of MIP-1 α release on day 1. Symbol '*' indicates statistical difference from all groups for that particular material.

Figure 7. Quantification of IL-13 release on day 1. Symbol ‘*’ indicates statistical difference from all groups for that particular material.