The role of macrophage phenotypes M1 and M2 in the foreign body reaction

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ABSTRACT. Clinically successful integration of a biomaterial in a host tissue is a big challenge the tissue engineering field faces. The foreign body reaction consist of a series of inflammatory processes, which leads to fibrosis and encapsulation of the biomaterial. Macrophages play a key role in this reaction. They can adopt different phenotypes; a inflammatory macrophage phenotype (M1) and a regulatory or anti-inflammatory phenotype (M2). The severity of the foreign body reaction immediately after transplantation determines the success of transplantation and is greatly dependent of the biomaterials surface. Several immunomodulatory design strategies have been developed, trying to facilitate a switch in the host tissue macrophages from M1 to M2. In this thesis, the role of M1 and M2 macrophages will be discussed, as well how to activate macrophages to a desired phenotype. In addition, immunomodulatory strategies for better implant integration will be investigated.

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Introduction

The field of tissue engineering has emerged over the past decades, with biomaterials being the solution to improving or even restoring the function of diseased tissue. However, clinically successful integration of a biomaterial into host tissue is one of the biggest challenges. Upon implantation, the biomaterial finds itself in an inflammatory environment. Especially the foreign body reaction and the important role of macrophages in this reaction, poses a bump in the road for breakthrough developments. In brief, macrophages arrive at the site of tissue injury 24-48 hours post injury[1], where they participate in host defense, phagocytosis and secretion of inflammatory factors. Simultaneously, macrophages are also known to be essential for repair, regeneration and remodeling of tissues. The specific mechanisms remain unknown, but these contradictory roles of macrophages are related to the ability of macrophages to adopt different phenotypes. A transition from a inflammatory macrophage phenotype (M1) to a more regulatory or anti-inflammatory phenotype (M2) has been suggested[1–3].

The severity of the foreign body reaction and thus the success of implantation is greatly dependent of the biomaterials surface. Treatments or adjustment to biomaterials which facilitate a switch from the pro- to anti-inflammatory phenotype, will locally promote tissue remodeling instead of scar tissue formation. Therefore, immunomodulation has emerged as a potential solution. Several immunomodulatory biomaterial design strategies have been developed, such as adjusting the chemistry or physical properties of the biomaterials surface. Another approach is embedding pro-inflammatory agents in the scaffold.

This thesis will discuss the activation of the M1 and M2 macrophage phenotypes and their biofunctional roles in the foreign body reaction following implantation of a material. Furthermore, several immunomodulatory strategies, used to achieve the more favorable tissue-remodeling M2 phenotype will be investigated.

Biological responses after implantation

The foreign body reaction (FBR) is the end-stage of a series of inflammatory and wound-healing responses after implantation of a biomaterial, which leads to fibrosis. Other events happening prior to the foreign body reaction are blood protein adsorption, acute and chronic inflammation and the formation of granulation tissue[2,4,5] (**Figure 1)**. The main components in the FBR are macrophages and foreign body giant cells. Especially macrophages play an important role in the FBR and the rest of the inflammatory responses, as they secrete pro-inflammatory cytokines, chemokines and growth factors.

Blood protein adsorption and provisional matrix formation

Immediately after the surgical implantation of a foreign body into the host tissue, blood proteins adhere to the surface of the material and develop into a provisional matrix. The major component in this provisional matrix is fibrin. The fibrin is produced by activation of the coagulative and thrombosis systems and inflammatory products that are released by activated platelets, the complement system, inflammatory cells and endothelial cells [6,7]. Furthermore, the provisional matrix consists of adhesive molecules such as fibronectin, vitronectin and thrombospondin, as well as platelet granule components released during platelet aggregation[4]. The provisional matrix not only serves as an adhesion substrate for inflammatory cells, but it is also capable of mediating macrophage activity and proliferation and activation of other cells involved in the inflammatory and wound healing responses. This all is due to the presence of mitogens, chemoattractants, cytokines and growth factor within the provisional matrix.[5,8]

Acute inflammation

Depending on the extent of the injury, acute inflammation is of relatively short length. It can last from minutes to days. The main characteristic of acute inflammation is the presence of blood-derived polymorphonuclear leukocytes (PMNs), predominantly neutrophils. Following protein deposition, these PMNs migrate from the blood to the implant site and act as a first line of defense. The recruitment of PMNs to the implant site is triggered by host derived chemoattractants released from activated platelets and endothelial cells[9]. The PMNs encounter the provisional matrix at the implant site, where adhesion molecules present on the leukocytes interact with the blood-protein coated surface. An important group of adhesion molecules on PMNs include the CD11/CD18 family of integrins, a family of transmembrane glycoproteins that modulate cell-matrix and cell-cell interactions by acting as receptors or direct adhesion molecules[4]. This interaction triggers a phagocytic respons: activated PMNs secrete proteolytic enzymes and reactive oxygen species (ROS), which may or may not corrode the biomaterial surface, depending on the properties of the biomaterial[9]. Moreover, PMNs are capable of releasing chemokines, one of them being interleukin-8 (IL-8). PMNs are not only producers of IL-8, but also the main target, as IL-8 enhances PMN influx and priming[10]. Activated PMNs also secrete MCP-1 and MIP-1β, chemoattractants and activation factors for monocytes, macrophages and lymphocytes[9].

Chronic inflammation, granulation tissue and the FBR

When inflammatory stimuli persist at the implant site, chronic inflammation arises. This stage is characterized by the presence of monocytes, macrophages and lymphocytes, with the proliferation of blood vessels and connective tissue[4]. The chronic inflammatory response to biomaterials is always confined to the implant site and is mostly resolved within two weeks. Monocytes that were drawn to the implantation site undergo a phenotypic change and differentiate to macrophages. Subsequently, the macrophages secrete pro- inflammatory cytokines such as platelet-derived growth factor, TNF-α and granulocyte-stimulating factor to recruit more macrophages, and ROS and degradatieve enzymes in an attempt to phagocytize the biomaterial[11,12]. Macrophages also play an important role in wound healing and tissue regeneration. These different functions are performed by a different subset of macrophages, known as M1 and M2 macrophages. Their mechanisms and functions in the foreign body reaction will be discussed extensively later.

The activation of inflammatory macrophages initiate wound healing by attracting fibroblast and endothelial cells. They proliferate to form granulation tissue, the hallmark of healing inflammation[4,5,9]. The endothelial cells proliferate form pre-existing blood vessels and organize into capillary tubes to form new small blood vessels, while fibroblasts synthesize proteoglycans and later collagen [2,4]. Granulation tissue can be seen as the intermediate substrate tissue for subsequent fibrosis.

In an effort to further phagocytize the biomaterial, macrophages at the surface fuse to form foreign body giant cells (FBGCs). The foreign body reaction is composed of these FBGCs together with the components of granulation tissue. The form and topography of the material determines the cellular composition of the FBR. In general, flat or smooth surfaced biomaterials have a thin layer of macrophages with fibrosis compared to rough, high surface-to-volume and porous implants, which have a relatively greater number of macrophages and FBGCs[2]. The cytokines IL-4 and IL-13 have been identified as inducers of macrophage fusion *in vivo* and *in vitro*[13,14]. They stimulate the expression of several fusogenic molecules, including macrophage mannose receptor, CD44, CD47, E-cadherin and dendritic cell specific transmembrane protein (DC-STAMP)[5,9,12]. Over time, the granulation tissue becomes a dense collagen capsule, marking the final stage of the wound healing response to biomaterials. The capsule isolates the biomaterial and the FBGCs from the surrounding tissue, and in addition, the continuous release of ROS and degradatieve enzymes from the capsule may jeopardize the functioning of the biomaterial. Therefore, successful tissue repair requires resolution of the inflammation through the release of anti-inflammatory components or the downregulation of inflammatory mediators.

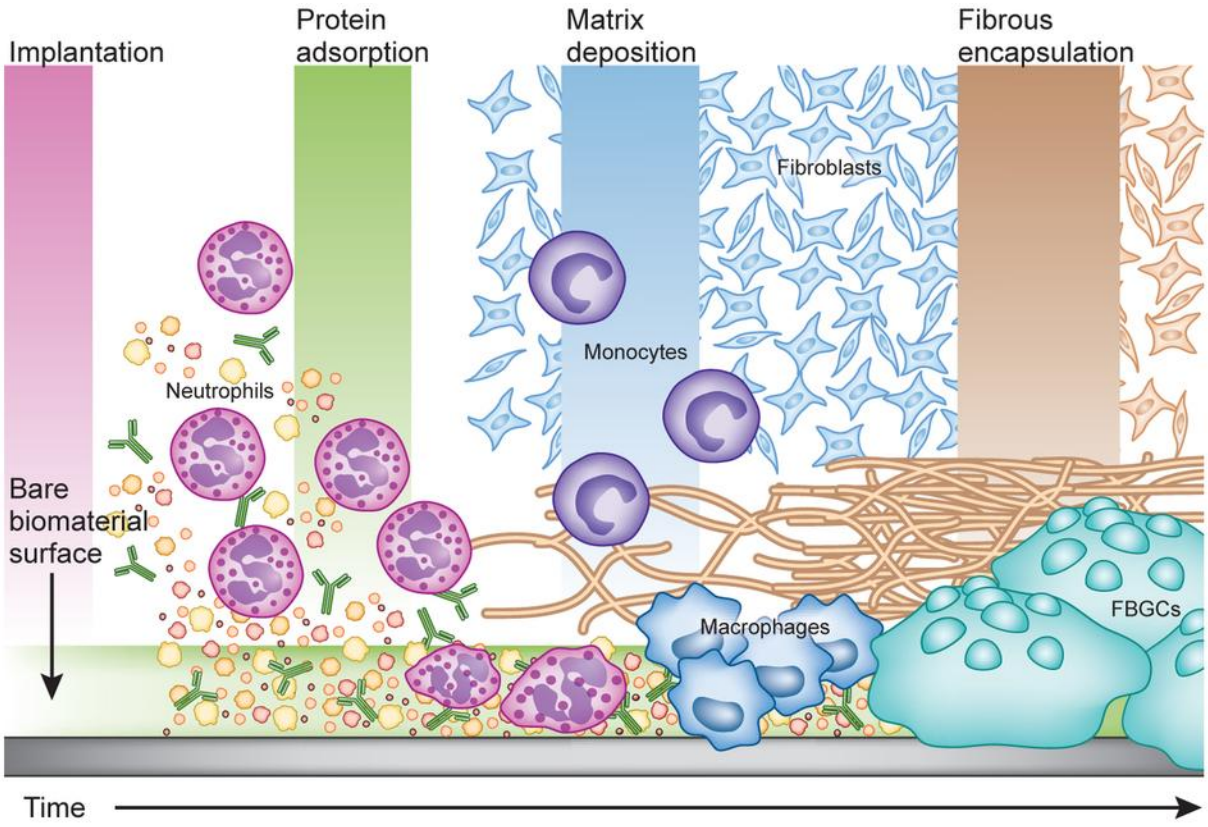

Figure 1. Immune response towards biomaterials. Immediately after transplantation spontaneous adsorption of proteins happens at the surface of the material. Inflammatory host cells encounter this adsorbed-protein layer and within hours polymorphonuclear leukocytes, mostly neutrophils, enter the implant site. These neutrophils dominate the acute inflammatory response by producing cytokines, chemokines and reactive oxygen species. In the next hours to days tissue-resident macrophages and monocytes are also recruited to the implant site. Because of persisting inflammatory stimuli, the inflammatory response switches to a chronic one, with macrophages producing signaling molecules that attract fibroblasts. The macrophages fuse into foreign body giant cells (FBGCs), while the fibroblasts produce collagen. This results in excessive fibrosis and the incapsulation of the biomaterial[15]. Image adapted from Grainger DW. All charged up about implanted biomaterials. Nat Biotechnol. 2013;31(6):507-509.

Macrophage phenotypes and their role in the FBR

Macrophages are part of the mononuclear phagocyte system, a family of professional phagocytes derived from hematopoietic progenitor cells[16]. The progenitor cells differentiate directly or via circulating monocytes into subpopulations of tissue macrophages. Depending on the tissue and thus the environment they are in, macrophages not only differ morphologically and phenotypically, but also functionally[3]. Macrophages are able to migrate to local sites of injury and infection, where they contribute to acute and chronic inflammation. In addition they can initiate tissue remodeling and resolve inflammation[17]. There are two different ways of differentiating and activating monocytes into macrophages, depending on specific growth factors, their receptors and cytokines. Mirroring the T helper 1-type (T_H1) and T_H2 nomenclature, the classical activation provides M1 cells and the alternative activation provides M2 cells.

Classical activation of macrophages

The classical activation of macrophages depends on interferon-γ (IFN-γ), a cytokine produced by specifically activated T_H1, hence the term M1 macrophages. Another important early source of IFN-γ are natural killer cells[18]. IFN-γ primes the macrophages for activation, but needs a second signal to actually activate them. This second signal is tumor necrosis factor α (TNF-α) produces by antigen-presenting cells or bacterial lipopolysaccharide (LPS)[19].

The most typical way to induce the transcription of TNF is when a Toll-like receptor (TLR) ligand acts in a MyD88-dependent manner. TNF will then cooperate with IFN-γ to activate the M1 macrophage (**Figure 2**). This signaling pathway leads to the activation of a cascade of kinases, which eventually results to the activation of nuclear factor kappa B (NF-κB). NF-κB is a key transcription factor and important in activating M1 macrophages, as it regulates the expression of a large number of inflammatory genes, such as TNFα, IL1b and IL6[20,21]. Upon activation, NF-κB is translocated to the nucleus, where it binds to the promoters of inflammatory genes.

Some TLR ligands also activate TIR-domain-containing adaptor protein inducing IFN-β (TRIF)-dependent pathways, which results in the production of IFN-β[22]. This endogenous IFN-β is able to replace IFN-γ. Therefore, the original two-signal requirement for the activation of this macrophage population can be overcome by certain TLR agonists that induce both TNF and IFN-β[18]. In this signaling pathway, interferon-responsive factor 3 (IRF3) is activated, leading to the expression and secretion of type I interferon. These secreted type 1 interferons bind to the type I interferon receptor (IFNAR), leading to activation of the transcription factor STAT1[20,21]. Genes activated through this pathway include chemokines CXCL9 and CXCL10[20].

The biological functions of classical activated macrophages are well known. They migrate to sites of inflammation where they encounter pathogens and phagocytize them. This killing of invaders is possible by an increase in the production of toxic oxygen species: the enzyme inducible nitric oxide synthase (iNOS) produces nitric oxide (NO) from arginine (**Figure 3**). Although M1 macrophages are very important effectors of the host defense, their activation must be tightly controlled. When not held in check, their produced cytokines and mediators can lead to damage to the host tissue.

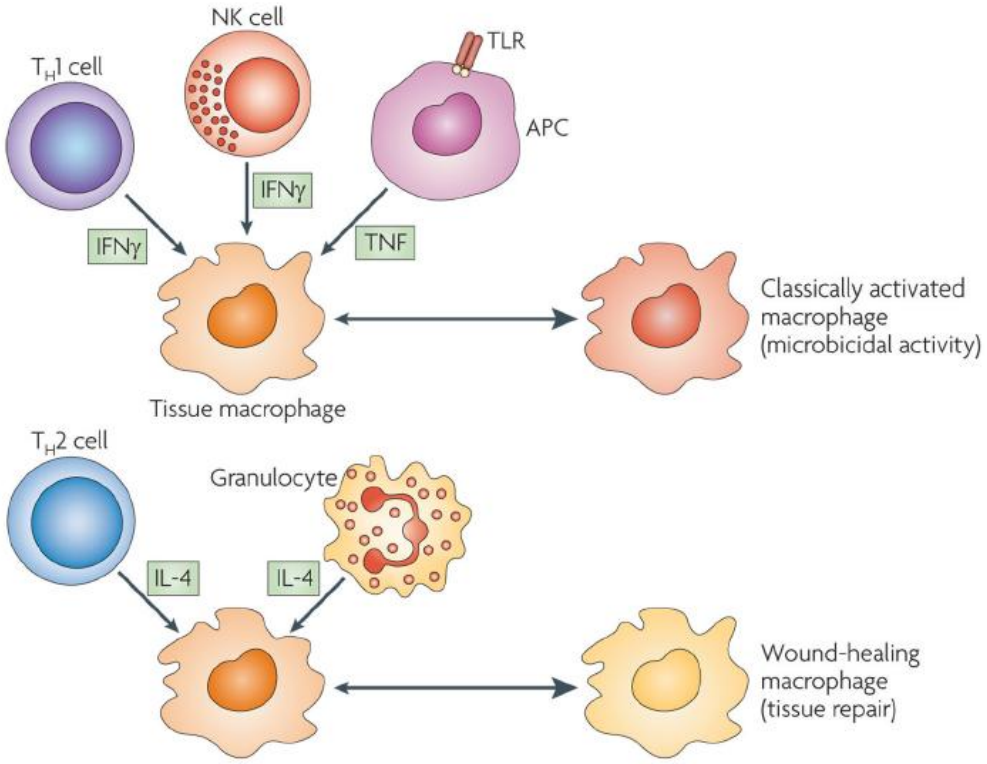

Figure 2. Cytokines produced by immune cells can give rise to macrophages with distinct physiologies. Classically activated macrophages arise in response to interferon-γ (IFNγ), which can be produced during an adaptive immune response by T helper 1 (TH1) cells or during an innate immune response by natural killer (NK) cells, and tumour-necrosis factor (TNF), which is produced by antigen-presenting cells (APCs). Wound-healing (alternatively activated) macrophages arise in response to interleukin-4 (IL-4), which can be produced during an adaptive immune response by TH2 cells or during an innate immune response by granulocytes. Adapted from Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008;8(12):958-969.

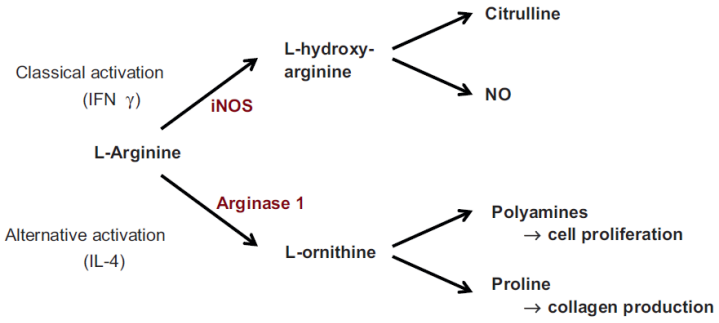

Figure 3. Arginine metabolism in macrophages is controlled by M1/M2 cytokines. Classically activated macrophages (M1) exhibit increased NOS activity, which promotes L-hydroxyarginine, L-citrulline, and NO production, contributing to their anti microbial activity. By contrast, alternatively activated macrophages (M2) show increased arginase and decreased NOS activity. Here, L-arginine is metabolized to urea and L-ornithine which is metabolized to produce polyamines, molecules that induce cell proliferation, or proline,the basic building block of collagen, promoting tissue repair. Adapted from Varin A, Gordon S. Alternative activation of macrophages: Immune function and cellular biology. *Immunobiology*. 2009;214(7):630-641.

Alternative activation of macrophages

Macrophages are alternatively activated by interleukin-4 (IL-4) and IL-13, which are cytokines that are produced in T_H2-type responses, hence the term M2 macrophages. There are more sources for these cytokines than T_H2 cells, including mast cells and basophils[23]. IL-4 and IL-13 have similar effects on macrophages and other target cells, but differ with regards to the range of cell types that respond to each cytokine. Receptors for IL-4 are mainly expressed on hematopoietic cells, whereas IL-13 receptors are also present on nonhematopoietic cells[17]. Macrophages express both receptor types.

Both the receptors share the IL-4Rα subunit, but there are specific IL-13Rα1 and IL-13Rα2 subunits. IL-4 binds IL-4Rα and then recruits either IL-2Rγ to form the type I receptor or the IL-13Rα1 chain to form the type II receptor (**Figure 4**). IL-13 however can only signal through type II receptor because it binds IL-13Rα1 and partners with IL-4Rα[24]. IL-13 can also bind IL-13Rα2, which is thought to be a decoy receptor[25]. After stimulation of the receptors, members of the Janus-activated kinase family (JAK), JAK-1 and JAK-3, become phosphorylated and activated. JAK-1 and JAK-3 in their turn induce phosphorylation of specific tyrosine residues of the receptor, which causes STAT-6 to bind to that phosphorylated domain[26]. STAT-6 itself becomes phosphorylated, leaves the receptor and forms a dimer with a second phosphorylated STAT-6 molecule. This dimercomplex translocates to the nucleus, where it regulates the transcription of a number of genes [17,26]. Another signal transduction pathway by IL-4R is mediated by IRS-2, inducing proliferative responses via the adaptor Grb-2 and PI-3 kinase[17,26].

These signaling pathways lead to expression of arginase 1 in macrophages, among others, while NO generated by iNOS is shut down. Arginase 1 is an enzyme that hydrolyzes arginine to urea and ornithine, a precursor for polyamines and proline[18,19,26,27] (**Figure 3)**. Polyamines promote cell growth and division, whereas proline is a key component of collagen[28]. Also, an increase in the expression of matrix protein such as fibronectin, βIG-H3 and fibrogenesis is induced, suggesting remodeling of the extracellular matrix[19]. Furthermore, the presence of alternatively activated macrophages is characterized by a high degree of vascularization and an increase of angiogenesis[26]. In conclusion, alternatively activated macrophages or M2 macrophages are associated with a more regulatory and recovery character, promoting tissue repair.

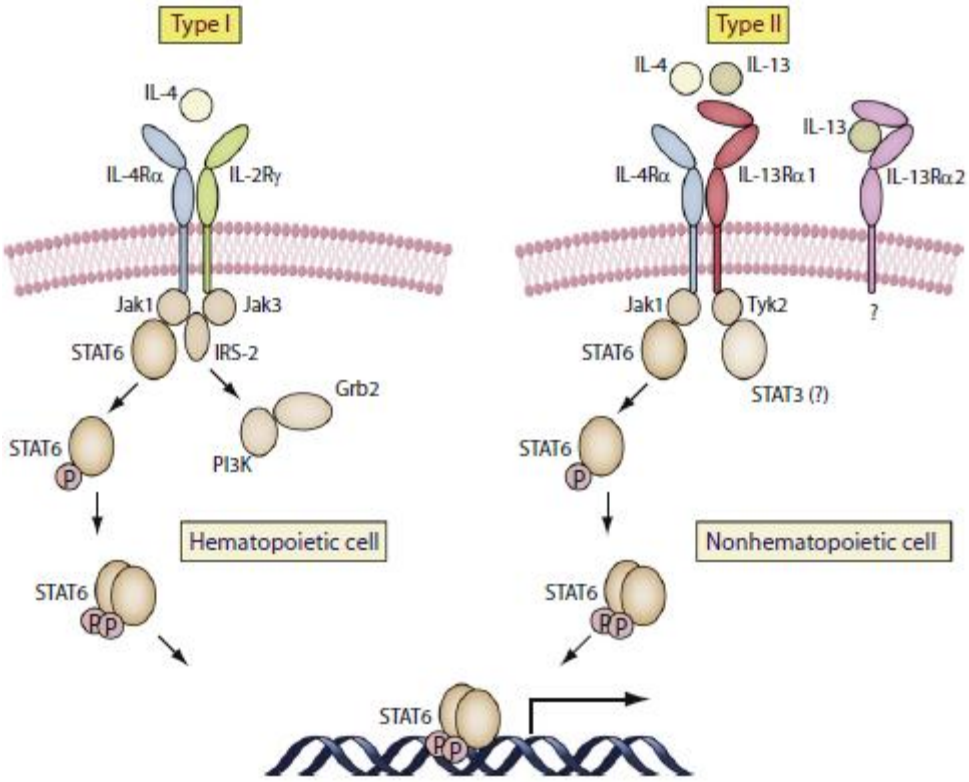

Figure 4. IL-4 and IL-13 signaling pathways. IL-4 binds IL-4Ra and then recruits either IL-2Rg chain (to form the type I receptor) or the IL-13Ra1 chain (to make the type II receptor), depending upon the cell type. However, IL-13 can only signal through type II receptor as it binds IL-13Ra1 and recruits IL-4Ra. The common signaling pathways shared by both the cytokines involve phosphorylation of STAT6, which leads to dimerization and translocation of STAT6 to the nucleus where it regulates the transcription of a number of genes. The type I receptor is predominantly found in cells of hemopoietic origin such as lymphocytes and granulocytes, resulting from restricted expression pattern of the IL-2Rγ chain. The type II IL-4 receptor is mainly expressed by cells of nonhemopoietic origin such as epithelial cells and fibroblasts. IL-13 also binds IL-13Ra2, with an extraordinarily high affinity which is thought to function as a decoy receptor, though evidence exists for its potential to signal in selected circumstances. Adapted from Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. Immunity. 2010;32(5):593-604.

Role of M1 and M2 in the FBR

In cutaneous wound healing, which has many resemblances with the FBR, a timeline can be sketched, with the different macrophage phenotypes appearing at different points of time. During the first days post-injury, the early inflammation phase dominates. In these 1 to 4 days, neutrophils and monocyte-derived macrophages respond to pro-inflammatory signals released from the microenvironment. These pro-inflammatory signals facilitate the polarization of infiltrating macrophages to the M1 phenotype[29]. At 5-7 days post-injury, the late inflammatory phase, marked by an accumulation of apoptotic cells, facilitates the polarization toward M2 macrophages. The M2 macrophages recruit fibroblasts into the site at 7-10 days post-injury by secreting effector molecules such as vascular endothelial growth factor (VEGF) and transforming growth factor β (TGF-β). After 10 days, the matrix deposition and tissue remodeling phase begins and the macrophage numbers begin to decrease[29].

In the FBR, a similar timeline is seen, but it is still not clear which macrophage phenotype is responsible for the formation of the fibrous capsule[11]. On the one hand M1 macrophages are

believed to increase the thickness of the fibrous capsule by upregulating the inflammatory response. On the other hand, the M2 cytokine IL-4 promotes formation of FBGC, ultimately causing the formation of the capsule[11]. One of the first studies that suggested macrophage phenotype as a predictor of the success or failure of biomaterials was performed by Badylak *et al*[30]. They used a rat model to determine the macrophage phenotype at the site of implantation of two biologic scaffolds, one derived from porcine small intestinal submycosa (SIS) and the other a carbodiimide crosslinked form of porcine-derived SIS (CDI-SIS) for 1, 2, 4 and 16 weeks. Constructive remodeling was shown in the SIS scaffolds at all time points; infiltration of M2 macrophage along with an organized layer of connective tissue at the 16-week time point was detected. The CDI-SIS showed the formation of FBGCs and fibrosis after 16 weeks post implantation. They concluded that biomaterials that promote the M2 phenotype are associated with constructive tissue remodeling compared to those that promote the M1 phenotype[30].

The opposite however, has also been shown. IL-4, which also causes M2 polarization, induced the formation of FBGCs through the secretion of PDGF and TGF-β in the study by Kao *et al*.[14]. In this study, a cage system was implanted subcutaneously in rats which provided a standard inflammatory environment. To test the effects of IL-4 on the FBR, anti-murine IL-4 (IL-4Ab), murine IL-4, normal goat nonspecific control IgG and PBS were directly injected into the cages and released into the implant site[14]. Via FBGC kinetic analysis, it was observed that FBGC density was significantly decreased by the injection of IL-4Ab compared to controls. On the other hand, the implants that released murine IL-4 showed a significant increase in the formation of FBGC compared to the IL-4Ab and controls. This data supported a role for IL-4 in mediating FBGC formation on biomaterials[14].

Engineering immunomodulatory biomaterials

The success and outcome of the implantation of a biomaterial can vary depending on the extent of the foreign body reaction. For a long time tissue-engineering methods have been used to achieve inert biomaterials. Nowadays, the idea of using immunomodulation to permit specific cell responses at the implant site that encourage wound healing has been adopted. The possibility of controlling macrophage behavior for a beneficial response, instead of inhibition of the inflammatory response, is becoming reality. Different strategies can be used, such as changing the chemical properties of the materials surface, controlling the release of anti- or pro-inflammatory cytokines from biomaterials, modifying the physical properties at the biomaterial host interface, and cell therapy methods via the direct inclusion of immune cells or induction of the arriving cells upon implantation[31] (**Figure 5**).

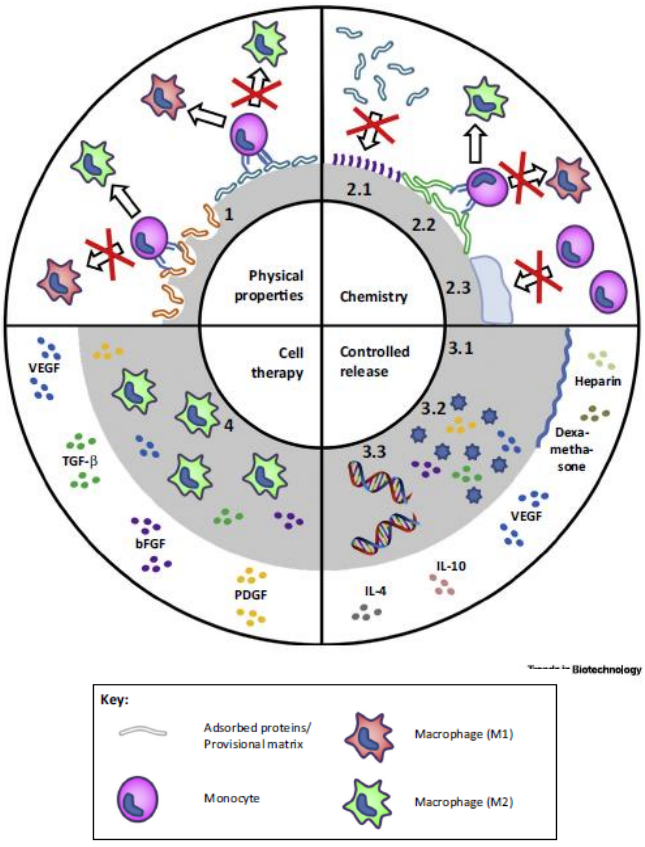

Figure 5. Schematic representation of different strategies that can be used to achieve immunomodulation. (**1**) The use of biomaterial physical properties such as stiffness or topography to control adsorption of specific proteins. (**2.1**) The use of non-biofouling coatings to prevent protein adhesion to the biomaterial surface; (**2.2**) use of biomimetic ECM components to control the integrin adhesion of monocytes and to disturb subsequent M1 activation and/or induce M2 activation of macrophages; (**2.3**) use of hydrogels to isolate implants from immune cells and thus to limit an inflammatory response. (**3.1**) The use of surface coatings for the delivery of soluble anti-inflammatory agents; (**3.2**) use of biomaterial embedded particles for the delivery of soluble anti-inflammatory agents; (**3.3**) use of gene delivery systems to induce residing cells to secrete anti-inflammatory agents. (**4**) The use of embedded immune cells secreting pro-angiogenic and pro-regenerative cytokines. Adapted from Vishwakarma A, Bhise NS, Evangelista MB, *et al*. Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response. *Trends in Biotechnology*. 2016.

Immunomodulation strategies using biomaterial chemistry

By modifying the surface chemistry of biomaterials protein adsorption can be modulated and, subsequently, cellular responses. There are several strategies for modifying the surface chemistry, such as the use of passive non-biofouling coatings, the use of biomimetic extracellular matrix (ECM) components or the use of hydrogels [31].

Firstly, the use of passive non-biofouling coatings will be discussed. In the first stages of the host response against the biomaterial, nonspecific protein adsorption and subsequently leukocyte adhesion takes place, events termed *biofouling*. With the non-biofouling strategy, implant surfaces are being coated with immune-isolating materials, making them non-interactive. Several chemical groups that resist protein adsorption have been identified, with polyethylene glycol (PEG) being the

standard for comparison[32]. The mechanism underlying the resistance to protein adsorption by PEG surfaces probably involves the ability of the polymer chain to keep hold of interfacial water combined with the resistance of the chain due to its tendency to remain an extended coil[33]. PEG and other hydrophilic polymers, such as poly(2-hydroxyethyl methacrylate), poly(N-isopropyl acrylamide), poly(acrylamide), and phosphoryl choline-based polymers, have been used as molecularly thin self-assembled monolayers (SAMs), polymer brushes and thin hydrogels, capable of resisting protein adsorption and leukocyte adhesion[32]. In another study, it is reported that a coating strategy based on thin films of poly(N-isopropyl acrylamide) (pNIPAm) microgels cross-linked with PEG, effectively prevented macrophage adhesion *in vitro*. The microgel coatings also reduced leukocyte adhesion and the expression of pro-inflammatory cytokines[34]. These passive strategies show promise *in vitro*, but is has been argued that it is more crucial to develop more bioactive strategies, instead of exclusion of all protein adsorption[31].

This brings us to the second strategy, which is using structures that mimic or directly use components of the ECM. By using adhesive domains of ECM proteins or ECM-derived proteins as a whole, a microenvironment can be created that is resembling the normal wound healing. For this purpose, artificial ECM coatings were developed by modifying collagen matrices with glycosaminoglycans (GAGs) and proteoglycans. Sulfated GAGs are known to bind with growth factors and cytokines, modifying their activity[35,36]. For example in a research done by Franz *et al*., it was demonstrated that collagen matrices containing high-sulfated hyaluronan are able to dampen pro-inflammatory M1 macrophages by controlling signaling pathways crucial for polarization to M1[37]. Moreover, M2-related cytokine IL-10 was secreted. Another GAG, chondroitin sulfate, was found to act immunosuppressive on different cells by reducing translocation of NF-κB, a key transcription factor of various pro-inflammatory mediators[38].

The third strategy uses hydrogels to shield the implant from immune cells and in this way limit an inflammatory response. Hydrogels offer many advantages over traditional surface modification strategies, including a viscoelastic network structure, incorporation of many chemical functionalities, nanoscale dimensions with complex architectures, tunable material characteristics, and the ability to deposit onto a variety of material substrates[32]. Peptide-modified PEG hydrogels have been used to prevent direct physical contact between encapsulated therapeutic cells, e.g. pancreatic islets and mesenchymal stem cells, and host tissue. The hydrogel made sure the cells were unaffected by infiltrating immune cells, while allowing diffusion of nutrients[39].

Immunomodulation using bioactive strategies

Coatings delivering anti-inflammatory agents show a more controlled and interactive approach to reduce leukocyte adhesion and activation. The agents can be either embedded into non-fouling coatings or delivered in soluble form from the coating (**Figure 6**). Pharmacological anti-inflammatory agents such as dexamethasone and heparin from reservoirs and coatings have shown reduced inflammation and fibrous encapsulation[32].

Dexamethasone is a synthetic glucocorticoid hormone that is used for the treatment of inflammatory responses in chronic inflammatory diseases, but also in biomedical research[40]. In a study by Morais *et al*., it was indeed shown that delivery of dexamethasone at the implant site resulted in a reduced

level of inflammation-mediating cells; decreased numbers of PMNs and the absence of macrophages, lymphocytes, and fibrous capsule formation were observed[41]. One unwanted side effect of dexamethasone however, is the inhibition of vascular endothelial growth factor (VEGF), preventing angiogenesis at the local tissue and thus delaying the healing process. Combined administration of VEGF and dexamethasone can overcome the anti-angiogenic effects of the corticosteroid[42,43].

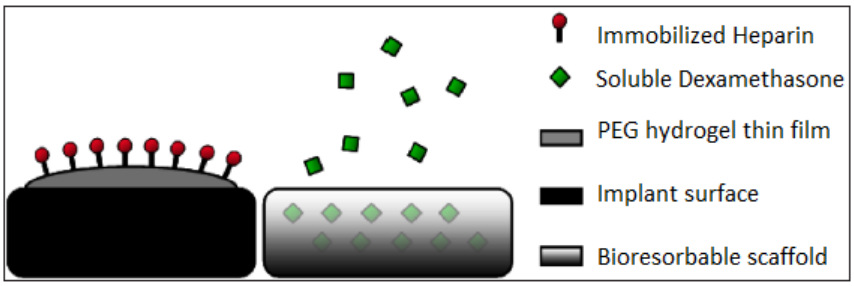

Figure 6. Bioactive implant coatings to deliver anti-inflammatory molecules. Representative schemes depict mechanisms for the active delivery of various immunomodulatory agents to reduce leukocyte adhesion and activation. Adapted from Bridges AW, García AJ. Anti-inflammatory polymeric coatings for implantable biomaterials and devices. *J Diabetes Sci Technol*. 2008;2(6):984-994.

Heparin is a highly sulfated glycosaminoglycan that is widely used as a blood thinner because of its strong anticoagulant activity. Just like other GAGs, it also exhibits anti-inflammatory properties. Heparin pretreatment significantly attenuates leukocyte transmigration through its actions on P- and L-selectin and the leukocyte-specific αMβ2 integrin, and it also binds cytokines and suppresses superoxide generation by neutrophils. Heparin-based coatings have reduced protein adsorption and leukocyte recruitment[32].

Besides these pharmacological agents, chemokines, cytokines and growth factors can also be promising molecules for release from bioactive coatings and downregulate specific inflammatory agents. They can be included directly into coatings or delivered by nucleic-acid based strategies. For direct inclusion, Hume *et al.*, used PEG hydrogels with immobilized TGF-β and IL-10 to reduce the maturation of dendritic cells, manipulating adaptive immunity[44]. The use of gene delivery systems is recently demonstrated in a study by Boehler *et al.*, in which lentiviral delivery of genes encoding for IL-10 represented a promising strategy for directing macrophage polarization toward a M2 phenotype[45].

Immunomodulation strategies using physical properties

Physical properties of the biomaterial, such as roughness, form and topography can determine the severity of the foreign body reaction. As mentioned before, implants with a high surface-to-volume ratio such as porous scaffolds show more macrophages and FBGCs than smooth surfaced implants do. Cells respond to ECM components in the nanometer scale in terms of adhesion, proliferation, migration and gene expression[9]. Imprinting topographic patterns at micron and nanometer scale on the surface may mimic the natural topography of the ECM. One study investigated the effects of polycaprolactone (PCL) nanofibrous scaffolds on the *in vivo* and *in vitro* foreign body reaction[46]. The results indicated that aligned PCL nanofibers minimized the host response, enhanced integration of the scaffold and induced a thinner fibrous capsule as compared to randomly oriented PCL fibers and

PCL film. McWorther *et al*. used a micropatterning approach to directly control macrophage cell shape, demonstrating that elongation of the cell itself, without exogenous cytokines, leads to expression of M2 macrophage markers. Thus alterations in cell shape associated with changes in ECM architecture may provide essential cues to modulate macrophage phenotype polarization[47].

Cell-based immunomodulation strategies

Immune cells can be beneficial because of induction of desired biological events, such as angiogenesis. Macrophages for example are capable of producing high concentrations of pro-angiogenic factors such as TNF-α and IL-1[31]. A possible way of addressing one major challenge in the field of tissue engineering, successful vascularization of complex engineered tissues, is using macrophages and their pro-inflammatory stimuli. Macrophages have indeed been found to induce formation of microvessel-like structures with an increase in the expression of E-selectin and ICAM-1, adhesion molecules which are strongly involved in the interaction between leukocytes and endothelial cells during the process of inflammation[48]. Other cells that have shown promise in their immunomodulatory properties are mesenchymal stem cells (MSCs). MSCs encapsulated within PEG hydrogels impaired the foreign body reaction compared to acellular hydrogels. Classically activated macrophages were attenuated and fibrous capsule thickness was reduced[49].

Discussion and concluding remarks

Since the discovery of alternatively activated M2 macrophages, a huge progress has been made in defining the underlying molecular processes of the polarized M1-M2 activation of macrophages and the different functions of these macrophages. The molecular determinants such as STAT, IRF and NF-κB are identified, but they may not be all the molecules involved in regulating M1-M2 polarization. As mentioned before, it is also still not completely clear which of the phenotypes causes the formation of the hallmark of the FBR, the fibrous capsule. An important step in fibrous encapsulation, is the development of new blood vessels. In a recent study by Spiller *et al.*[50], the direct role of macrophage phenotype in vascularization was assessed. They found proof contradicting the traditional paradigm, in which M2 macrophages are considered to promote angiogenesis and tissue regeneration, while the M1 macrophages are considered anti-angiogenic. In a murine subcutaneous implantation model, biomaterials were chosen to elicit different macrophage response. It was demonstrated that porous collages scaffolds were surrounded by a fibrous capsule, coincident with high expression of M2 macrophage markers. Scaffolds coated with LPS were degraded by inflammatory M1 macrophages, without evidence of fibrous encapsulation. Fascinatingly, glutaraldehylde-crosslinked scaffolds were infiltrated by high numbers of blood vessels and high levels of both M1 and M2 macrophages. This suggest that M1 and M2 macrophages are both required for scaffold vascularization[50]. However, clearly more research is needed to clear up the extent to which both phenotypes contribute to the FBR and fibrous encapsulation. With a better understanding of the immunological responses after transplantation, comes improvement of biomaterial development. In the future, the focus may be more on interacting with the host immune system instead of evading it.

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