Published in final edited form as:

Biotechnol Bioeng. 2012 August ; 109(8): 1898–1911. doi:10.1002/bit.24559.

Engineering Biomaterials to Integrate and Heal: The Biocompatibility Paradigm Shifts

James D. Bryers, Cecilia M. Giachelli, and Buddy D. Ratner

Department of Bioengineering, University of Washington, N310C Foege Hall, Box 355061, Seattle, WA 98195-5061; telephone: 206-221-5876; fax: 206-616-9763

Abstract

This article focuses on one of the major failure routes of implanted medical devices, the foreign body reaction (FBR)—that is, the phagocytic attack and encapsulation by the body of the so-called “biocompatible” biomaterials comprising the devices. We then review strategies currently under development that might lead to biomaterial constructs that will harmoniously heal and integrate into the body. We discuss in detail emerging strategies to inhibit the FBR by engineering biomaterials that elicit more biologically pertinent responses.

Keywords

tissue regeneration; foreign body response; macrophage phenotype

Introduction

The biomaterials device market has been projected to exceed \$250B in 2014 with millions of humans receiving implanted medical devices that save lives and improve the quality of life. Though, on the surface, this seems an admirable record, device failure and complication rates remain high (the FDA adverse events reporting system received 580 reports of device failure or complication in 2009) and many devices that could impact medical practice do not work at all or fail early. This article first focuses on one of the major failure routes of implant medical devices: the foreign body reaction (FBR)—that is, the phagocytic attack and encapsulation by the body of the so-called “biocompatible” biomaterials that comprise the devices. Then, we review strategies now under development that might lead to biomaterial constructs that will harmoniously heal and integrate into the body. This article will focus on inhibiting the FBR, and on triggering more biologically relevant responses as a path to better implant devices.

Be aware, this article challenges central ideas on how the biocompatibility of implant materials is understood today. The biocompatibility of a biomaterial is now evaluated based on the FBR to the material. After about 1 month, if a thin, avascular foreign body capsule (FBC) and a relatively quiescent implant site are observed, then the material is considered “biocompatible.” We will demonstrate how this description of biocompatibility is contradicted by recent findings, and thus, we recommend the paradigm of biocompatibility must shift with the times and new technologies.

The Foreign Body Reaction (FBR)

FBR Overview

The medical device industry is predicated on biomaterials designed over the past 50 years to be “biocompatible”—in essence biologically “inert,” which regrettably not only minimizes reconstructive healing but promotes the classic FBR and its most characteristic feature, the FBC. Only a brief overview of the processes contributing to the FBR will be provided here. The readers directed to a number of excellent review articles for greater detail (Anderson et al., 2008; Keselowsky et al., 2007; Wilson et al., 2005; Xia and Triffitt, 2006).

The host response to implanted biomaterials follows a cascade of events initially similar to, but ultimately diverging from, normal wound healing. Initial injury from the implantation procedure causes a perturbation of the homeostatic mechanisms, setting off the process of healing (Anderson, 2001). The classic view of the host response to biomaterials divides the response into several overlapping phases: blood–material interactions, acute inflammation, chronic inflammation, FBR, and fibrous encapsulation. A general timeline of the progression of the host response is shown in Figure 1.

When an implanted material comes in contact with blood, a layer of host proteins instantaneously adsorbs to the material surface; these include blood proteins such as fibrinogen (Fg), fibronectin, and vitronectin, as well as opsonins such as immunoglobulin G (IgG) and the complement-activated fragment C3b (Anderson, 2001; Wilson et al., 2005).

At the same time, inflammatory cells begin to exude out of the vasculature into the injured tissue, via specific interactions between up-regulated adhesion molecules on leukocytes and on endothelial cells (Pober and Cotran, 1990a,b). Acute inflammation lasts from minutes to days, and is dominated by neutrophils (Henson and Johnston, 1987; Lehrer et al., 1988; Malech and Gallin, 1987). Neutrophils normally phagocytose and degrade micro-organisms and foreign materials; but because implanted biomaterials are usually much larger than the leukocytes themselves, frustrated phagocytosis replaces the normal phagocytic response, where leukocyte products (e.g., lysosomal proteases and oxygen free radicals) are released in an attempt to degrade the foreign body (Henson, 1971, 1980). This initial process may be mediated by the proteins adsorbed onto the biomaterial surface. Neutrophils are usually cleared from the implant site within a day or two, and are replaced by monocytes, macrophages, and lymphocytes; together with the proliferation of blood vessels and connective tissue, the prolonged presence of these cells is generally taken as a hallmark of chronic inflammation (Cotran et al., 1999; Gallin and Synderman, 1999; Johnston, 1988; Williams and Williams, 1983). Although lymphocytes are found in the vicinity of implanted biomaterials, little is known of their role in the host response against biomaterials (Bergman and Zygourakis, 1999; Brodbeck et al., 2005; Yokoyama et al., 1986). As activated monocytes/macrophages initiate the healing response at the implant site, endothelial cells and fibroblasts begin to proliferate, giving rise to granulation tissue (Anderson, 2001). In normal wound healing (*sans* an implant), inflammation eventually resolves to yield regenerated tissue or, in cases where complete restitution of normal tissue structure is impossible, scar tissue (Ratner, 1996).

In the host response to implanted biomaterials, however, chronic inflammation is usually followed by the FBR. The FBR consists of varying proportions of granulation tissue components and the formation of foreign body giant cells (FBGC; multinucleated cells resulting from the fusion of macrophages). Such an accumulation of macrophages and FBGC at the tissue–material interface may persist throughout the lifetime of the implant (Anderson, 2001), though it is unclear whether these cells remain active or become quiescent over time. In most cases, the FBR eventually gives way to the fibrous

encapsulation of the implanted device, though the extent of encapsulation varies depending on the several factors, including the physical and chemical properties of the implanted material, the duration of implant, and the location of implant (Anderson, 2001).

Although there exists a general understanding of the sequential events in the host response to biomaterials, the molecular mechanisms driving these processes are still largely unknown. In particular, there is a large gap in our understanding of the mechanisms underlying the transition from inflammatory processes, which are also part of the normal wound healing cascade, to FBR and subsequent fibrous encapsulation, characteristic of the response against biomaterials. Approaches for designing materials with improved biocompatibility must be based on a thorough understanding of the molecular mechanisms that drive the host response culminating in FBR and fibrosis.

Inflammatory Cell Recruitment to the Implant Site

The mechanisms by which inert, nontoxic biomaterials cause the rapid and sustained accumulation of large numbers of phagocytic cells are not completely understood. Inflammatory cell recruitment requires enhanced leukocyte chemotaxis to the implant site, as well as leukocyte transmigration through the endothelium. Previous investigations have helped to elucidate several molecular mechanisms that underlie these processes.

A variety of molecular cues at the implant site may act as chemoattracts for phagocytic cells, mediating the initial recruitment of inflammatory cells. Initial injury from the implantation procedure may induce injured cells to release a variety of distress signals; some of the earliest such signals are small molecules such as ATP, uric acid, and bioactive lipids, leaking from damaged cells (Martin and Leibovich, 2005). Activated platelets release several growth factors, and activated mast cells likewise release various degranulation products, including histamine, proteoglycans, proteases, and cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-16 (Mekori and Metcalfe, 2000). Mast cells can also initiate the production of various other cytokines and chemokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF), IL-4, IL-6, IL-10, IL-13, macrophage inflammatory protein (MIP)- α , and monocyte chemotactic protein (MCP)-1. As leukocytes arrive at the site of injury, they too, begin to produce various growth factors and cytokines that attract more inflammatory cells. MIP-1 α and MCP-1 have been implicated in phagocyte chemotaxis toward the implant (Tang et al., 1998).

Phagocyte Adhesion and Activation

Following their chemotaxis toward the implant and transmigration through the endothelium, leukocytes adhere to the implant surface and become activated to carry out their functions. The molecular mechanisms that govern their adhesion and subsequent activation are widely considered to be important determinants of the outcome of the host–biomaterial interaction, and have thus been an active area of investigation.

For the most part, investigators have focused on the potential interactions between phagocytes—particularly macrophages—and biomaterial surface-adsorbed proteins. When materials are implanted, they instantaneously adsorb a layer of host plasma proteins. Therefore, it is commonly hypothesized that host cells interact with surface-adsorbed proteins, rather than with the biomaterial surface itself (Hu et al., 2001; Kao, 1999; Wilson et al., 2005). Albumin, Fg, and immunoglobulin (IgG) have been shown to predominate at the tissue–material interface of many implanted biomaterials, including poly(ethylene terephthalate) (PET), expanded poly(tetrafluoroethylene) (ePTFE), poly(urethanes) (PU), and poly(ethylene) (PE) (Andrade and Hlady, 1987; Anderson et al., 1990; Tang and Eaton, 1993). Albumin has primarily been shown to have a “passivating” effect on biomaterials,

since materials pre-incubated with albumin attract significantly less phagocytes than untreated controls (Tang and Eaton, 1993). Also, this albumin effect has been shown to be short-term. Hence, although albumin makes up approximately 70% of blood plasma proteins, it does not appear that surface-adsorbed albumin is responsible for phagocyte adhesion and activation.

Macrophages as the Primary Mediators of FBR

Growing Awareness of Macrophage Activation States

The mechanisms steering the cascade of responses of adherent phagocytes that lead to FBR and fibrosis remain unclear. Since the host fibrotic response to implanted biomaterials often leads to detrimental clinical consequences, the identification of such mechanisms is essential for attempting to engineer more biocompatible material surfaces.

Since neutrophils arriving at the implant site are, once “spent,” rapidly cleared by macrophages (Savill, 1996; Schwartz et al., 1999), the focus of most cell–biomaterial interaction studies has been the macrophage (MØ). MØ are widely considered the central cell type in directing and determining the outcome of the host response against implanted materials, because they are the predominant cell type found at the tissue–material interface in the later stages of the host response (Kao, 1999). While FBGC are also found in great numbers at the tissue–material interface, evidence suggests that MØ, and not FBGC, steer the outcome of the fibrotic response (Kyriakides et al., 2004).

One of the primary roles of MØ in the innate immune system is their recognition of pathogens by non-specific triggers such as microbial molecules or complement proteins, followed by phagocytosis and killing of pathogens, presentation of antigens for T-cell stimulation, and recruitment of additional immune system cells through cytokine secretion. When MØ receive additional stimulation from recruited immune cells, they become “activated,” changing both physical morphology and secretory profiles in response to the stimulation, thus amplifying the immune response.

In the last 10 years, a growing number of studies have revealed the heterogeneity of activated MØ (Gordon, 2003; Hume et al., 2002; Mosser, 2003). This heterogeneity is now believed to primarily arise during differentiation of MØ from circulating monocytes and monocyte precursor cells in response to environmental signals (Fig. 2) (Mantovani et al., 2004). “Classically” activated MØ cells, known as M1 macrophages (M1-MØ), result from stimulation byproducts of TH1 cells or natural killer cells, most commonly IFN- γ , either alone or in combination with microbial triggers such as lipopolysaccharides (LPS). M1 cells therefore are potent antibacterial effector cells and are characterized by IL-12 and IL-23 production, high production of reactive nitrogen and oxygen species (NO, superoxides, and hydrogen peroxide) and secretion of pro-inflammatory cytokines (TNF, IL-1, and IL-6) (Mosser, 2003; Mytar et al., 1999; Verreck et al., 2004). These cells are associated with promoting strong T helper cell-1 (TH1) immune responses as well as causing anti-proliferation and cytotoxic behavior. In the process of mediating the attack on invading pathogens, M1 cells can also destroy local tissue; without mitigation, this assault can lead to chronic inflammation (formation of FBGCs and encapsulation), autoimmune diseases, and other pathologies (Gordon, 2003).

“Alternatively” activated MØ (M2-MØ), were initially identified by Gordon and coworkers through IL-4 activation; a cytokine associated with TH2 cells (Stein et al., 1992). Later, IL-13 was also found to cause the same effect (Munder et al., 1998). More recently, subpopulations of M2 cells were defined as M2a, M2b, and M2c, based on stimulation by IL-4/IL13, immune complexes and toll-like receptor ligands, and IL-10, respectively. M2

cells have been termed “anti-inflammatory” MØ because they release high amounts of the deactivating cytokines IL-10 and transforming growth factor- β (TGF- β) and low amounts of inflammatory cytokines. IL-10 activates signal transducer and activator of transcription (STAT3), thereby leading to inhibition of pro-inflammatory cytokine secretion and nitric oxide release (Gordon, 2003; Murray, 2006). In addition, M2 cells induce arginase activity in mice, which acts against nitric oxide synthase-2 and also leads to tissue remodeling by secreting components of the extracellular matrix, including: fibronectin, OPN, and fibrin crosslinker transglutaminase (Gratchev et al., 2006; Munder et al., 1998). These cells therefore are generally associated with anti-inflammatory, immunosuppressive, and protective properties.

The M2 designation is rapidly expanding to include essentially all types of macrophage other than M1. This classification exists despite growing evidence indicating that the M2 designation encompasses cells with dramatic differences in biochemistry and physiology. Mosser and Edwards (2008) suggests that a more informative basis for MØ classification is the fundamental MØ functions that are involved in maintaining homeostasis, including: host defense, wound healing and immune regulation. This classification also helps to explain how MØ can exhibit characteristics that are shared by more than one MØ population. Such a revised notion thus brings characteristics of the *classically activated* (or host defense) MØ closer to the other two cell types, thus explaining observations of MØ that share characteristics of two populations. In fact, there may be many different shades of activation yet to be identified, resulting in a “spectrum” of MØ sub-populations based on function. For example, in a recent study, many cells that stained for specific cell surface markers of M1 also stained for M2, that is, in what appears to be an M1–M2 phenotype (Madden et al., 2010). This plasticity of MØ makes the task of assigning specific biochemical markers to each population difficult. However, the significance for understanding MØ heterogeneity is enormous because these cells can be biomarkers of diseases and have the potential to be used as surrogate markers of protection following drug treatment or even vaccination.

Approaches to Diminish or Control the FBR

Steroid Release

Steroid-releasing polymers for controlling the FBR were explored as early as 1966 (Dziuk and Cook, 1966) in an early effort to thwart the FBR on pacemaker leads. The FBR to electrical leads anchored in beating heart muscle led to a thick capsule that necessitated increasing the stimulation threshold (electrical field strength) of the pacing pulse needed to penetrate the capsule and stimulate muscle, thus reducing battery life. A dexamethasone (steroid)-releasing electrode was developed to reduce the inflammatory reaction (Stokes, 1988). The precise mechanism of action of dexamethasone is still unclear but the dexamethasone does down-regulate the production of factors associated with inflammation. While this innovation that targets inflammatory pathways has seen general success in reducing electrode encapsulation, the steroid action can also inhibit normal healing and reconstruction. For pacing applications, mechanical anchoring in heart muscle without full tissue site reconstruction is satisfactory and thus normal healing is not required. Steroid delivery will not be a general solution to the problem of the FBR because of the overall inhibition of reconstructive healing.

Non-Fouling Surfaces

Early stages of the FBR involve non-specific protein and biomolecule adsorption, a process termed “biofouling,” followed by subsequent leukocyte adhesion onto the surface. Once, it was believed that reducing biofouling would reduce subsequent adverse inflammatory responses, that is, leukocyte activation, tissue fibrosis, and foreign body response. Several

passive strategies were initially explored to achieve this goal. For example, pre-adsorption of material surfaces with less inflammatory proteins or cells is an attractive, relatively simple strategy (Amiji et al., 1992; Geelhood et al., 2007). However, such coatings suffer from a lack of stability as other proteins, such as Fg, can displace pre-adsorbed proteins such as albumin. In fact, covalently tethered non-adhesive proteins can be degraded by leukocytes, resulting in deposition of pro-inflammatory components. Similar approaches of pre-depositing cells onto surfaces prior to implantation may promote wound healing (Prichard et al., 2007), but issues related to cell sourcing, host responses to the donor cells, and long-term stability limit these strategies.

Non-fouling (i.e., protein adsorption-resistant) polymer coatings for biomaterials provides a more rigorous approach to reduce inflammatory responses. Such polymer surface coatings must satisfy the following constraints:

- use of nontoxic (biocompatible) materials,
- effectively inhibit in vivo biofouling,
- appropriate thickness and permeability to allow analyte transport,
- techniques to deposit coating onto a variety of materials and architectures, and
- must be mechanically, chemically, and electrically robust to withstand surface deposition, sterilization methods, implantation procedures, and in vivo environment.

Despite considerable research efforts, surface coatings that completely eliminate protein adsorption over the lifetime of a device have yet to be demonstrated. However, progress has been made in defining the mechanisms of protein adsorption, and several chemical groups that resist protein adsorption have been identified. Poly(ethylene glycol) (PEG, $[\text{CH}_2\text{CH}_2\text{O}]_n$) has proven to be the most protein-resistant functionality and remains the gold standard for comparison (Kingshott and Griesser, 1999). PEG chain density, length, and conformation strongly influence resistance to protein adsorption (Michel et al., 2005; Unsworth et al., 2005, 2008). Resistance to protein adsorption by PEG surfaces probably involves a combination of the ability of the polymer chain to retain interfacial water and the resistance of the polymer chain to compress due to its tendency to remain an extended coil conformation (Morra, 2000; Szleifer, 1997a,b). Other hydrophilic polymers, such as poly(2-hydroxyethyl methacrylate) (Wang et al., 2008), poly(*N*-isopropyl acrylamide) (Nolan et al., 2005; Singh et al., 2007), poly(acrylamide), and phosphoryl choline-based polymers (Goreish et al., 2004; Iwasaki et al., 1998; Kudo et al., 2006; Yang et al., 2000) also resist protein adsorption. Carboxybetaine-containing polymer brushes have been found to be particularly effective in inhibiting fouling at both low and high plasma protein concentrations (Zhang et al., 2008).

Such non-fouling coatings have been applied as molecularly thin self-assembled monolayers (SAMs), polymer brushes, and as thin or bulk hydrogels. SAMs are confined to inorganic planar surfaces and are only stable for a short-term in aqueous environments (Prime and Whitesides, 1993). Polymer brushes are more mechanically robust than SAMs and can be generated on non-planar surfaces. Surface-initiated polymerizations allow control over functionality, grafting density, and thickness of the polymer brushes (Edmondson et al., 2004; Zhao and Brittain, 2000). Hydrogels offer many advantages over traditional surface modification strategies, including presentation of a visco-elastic network structure, tunable material properties, incorporation of multiple chemical functionalities, nano-scale dimensions with complex architectures, and the ability to deposit onto a variety of material substrates (Hoffman, 2002; Kopecek, 2007; Mendelsohn et al., 2003; Nath and Chilkoti, 2002; Nayak and Lyon, 2004).

Although many of these coatings exhibit significant reduced protein adsorption and leukocyte adhesion in vitro, inconsistent results have been obtained regarding the ability of these polymeric coatings to reduce in vivo acute and chronic inflammatory responses (Park and Bae, 2003; Quinn et al., 1995; Ronneberger et al., 1996; Shen et al., 2002). Possible explanations for these mixed in vivo results include: insufficient non-fouling behavior, coating degradation or de-lamination, and inflammatory mechanism(s) independent from protein adsorption.

Matricellular Proteins

Matricellular proteins serve primarily as adaptors for cell–substrate interactions, rather than structural scaffold proteins within the extracellular matrix. Matricellular proteins include thrombospondins (TSPs) and osteopontin (OPN). Expression of these proteins is modulated during FBR and healing, and mounting evidence documents the role of these proteins in these processes.

OPN plays a role in both the classical foreign body response to biomaterials as well as the specialized host response to biomaterials that are prone to mineral accumulation, such as in the case of bioprosthetic valves. In the classical FBR, OPN regulates MØ migration as well as formation of FBGCs. Mice deficient in OPN show decreased accumulation of MØ surrounding subcutaneously implanted polyvinyl alcohol (PVA) sponges (Tsai et al., 2005). Indeed, OPN serves as a potent chemoattractant for MØ (Bruemmer et al., 2003; Giachelli et al., 1998; Panzer et al., 2001; Persy et al., 2003), and functional inhibition or genetic ablation of OPN in mice greatly reduces MØ recruitment in models of acute inflammation and wound healing (Bruemmer et al., 2003; Ophascharoensuk et al., 1999). Wound healing studies in mice also indicate that OPN is expressed during the acute inflammatory phase at very high levels in infiltrating leukocytes and other cell types where it appears to regulate leukocyte infiltration and activation as well as proper matrix organization (Liaw et al., 1998). Interestingly, down regulation of OPN at the wound site with antisense mRNA diminished macrophage infiltration and accelerated wound healing (Mori et al., 2008).

Despite its function to promote MØ accumulation, OPN actually inhibits FBGC formation in response to biomaterial implantation. In OPN-deficient mice, an increase in FBGC formation was observed surrounding implanted PVA sponges (Tsai et al., 2005). Furthermore, OPN inhibited MØ fusion to form FBGCs in vitro in a dose dependent manner (Tsai et al., 2005). Since FBGCs have been implicated as important mediators of biomaterial degradation and persistence of the FBR, OPN appears to play an important role in mediating biomaterial biocompatibility in vivo.

In calcification-prone materials, OPN serves as an endogenous inhibitor of biomaterial mineralization. OPN deficient mice display enhanced mineralization of implanted bioprosthetic valves (Steitz et al., 2002), and coating of valves with OPN greatly prevents mineral deposition (Ohri et al., 2005). The ability of OPN to inhibit bioprosthetic valve calcification depends on its ability to bind to nascent mineral deposits via phosphorylated serine and threonine residues, thus blocking crystal growth (Jono et al., 2000). OPN also promotes RGD-dependent cell interactions that lead to increased carbonic anhydrase expression and mineral regression (Rajachar et al., 2009; Steitz et al., 2002).

Kyriakides et al. (2001) quantified whether delivery of an antisense cDNA for the potent angiogenesis inhibitor thrombospondin 2 (TSP2) would enhance blood vessel formation and alter collagen fibrillogenesis in an implanted PVA sponge granuloma and surrounding capsule. Collagen solutions were mixed with plasmid to generate gene-activated matrices (GAMs) and applied to biomaterials that were then implanted subcutaneously. Sustained expression of plasmid-encoded proteins was observed at 2 and 4 weeks following

implantation. In vivo delivery of plasmids, encoding either sense or antisense TSP2 cDNA, altered blood vessel formation and collagen deposition in TSP2-null and wild-type mice, respectively. Untreated implants, implanted next to GAM-treated implants, did not show exogenous gene expression and did not elicit altered responses, suggesting that gene delivery was limited to implant sites.

Anti-Sense RNA and siRNA Approaches

Exposure of peripheral blood monocytes to IL-4 can recapitulate the fusion process in vitro. Jay et al. (2007) reportedly used IL-4 to induce multinucleation of murine bone marrow-derived MØ then observed changes in cell shape, including elongation and lamellipodia formation, before fusion. Because cytoskeletal rearrangements are regulated by small GTPases, they also examined the effects of inhibitors of Rho kinase (Y-32885) and Rac activation (NSC23766) on fusion. Rho kinase inhibitor Y-32885 did not prevent cytoskeletal changes or fusion but limited the extent of multinucleation. Rac inhibitor, NSC23766, on the other hand, inhibited lamellipodia formation and fusion in a dose-dependent manner. In addition, they found that in control cells, these changes were preceded by *Rac1* activation. However, NSC23766 did not block the uptake of polystyrene microspheres. Likewise, a short anti-sense RNA knockdown of *Rac1* limited fusion without limiting phagocytosis. Thus, phagocytosis and fusion can be partially decoupled based on their susceptibility to NSC23766. Furthermore, poly(ethylene-co-vinyl acetate) scaffolds containing NSC23766 attenuated foreign body giant cell formation in vivo. These observations suggest that targeting *Rac1* activation could protect biomaterials without compromising the ability of MØ to perform beneficial phagocytic functions at implantation sites.

Takahashi et al. (2010) tested the hypothesis that inhibition of the expression of the mammalian target of rapamycin (mTOR) in fibroblasts can mitigate the soft tissue implant FBR by suppressing fibrotic responses around implants. In their study, mTOR was knocked down using small interfering RNA (siRNA) conjugated with branched polyethylenimine (bPEI) in fibroblastic lineage cells in serum-based cell culture as shown by both gene and protein analysis. This mTOR siRNA knock-down led to an inhibition in fibroblast proliferation by 70% and simultaneous down-regulation in the expression of type I collagen in fibroblasts in vitro. These siRNA/bPEI complexes were released from PEG-based hydrogel coatings surrounding model polymer implants in a subcutaneous rodent model in vivo. No significant reduction in fibrous capsule thickness and mTOR expression in the FBCs was observed. The siRNA inefficacy in this in vivo implant model was attributed to siRNA dosing limitations in the gel delivery system, and the lack of targeting ability of the siRNA complex specifically to fibroblasts. While in vitro data supported mTOR knockdown in fibroblast cultures, in vivo siRNA delivery must be further improved to produce clinically relevant effects on fibrotic encapsulation around implants.

Synthetic Porous Polymer Scaffolds

Since the early days of medical implants fabricated from synthetic materials, the observation has been made that porous or rough materials will heal in a less fibrotic, more vascularized fashion than the same material fabricated into a smooth, solid form (Brauker et al., 1995; Karp et al., 1973; Picha and Drake, 1996; Sharkawy et al., 1997; von Recum et al., 1996). A wide variety of biomaterials, synthetic and natural, have been fabricated into porous 3D matrices and are described in many review articles (Badylak, 2007; Gunatillake and Adhikari, 2003; Malafaya et al., 2007). Scaffolds have been generated from natural polymers such as alginates (Bouhadir et al., 2001; Dar et al., 2002; Yang et al., 2002), chitosan (Cai et al., 2002; Chung et al., 2002; Lahiji et al., 2000; Madhally and Matthew, 1999; Mizuno et al., 2003; Tan et al., 2001; Zhu et al., 2002), collagen (Chvapil, 1977), glycosaminoglycans (GAGs) and elastin (Dantzer and Braye, 2001; Orgill et al., 1999;

Singla and Lee, 2002), gelatin (Huang et al., 2005; Mao et al., 2003; Santhosh and Krishnan, 2001), and fibrin (Bensaid et al., 2003; Jockenhoevel et al., 2001; Mooney et al., 1992). One system, a collagen/GAG based skin equivalent (Babu et al., 1989; Denuziere et al., 1998; Lindahl et al., 1998) is already in clinical use (Dantzer and Braye, 2001; Orgill et al., 1999) and has been under investigation for other applications such as heart valves, vascular grafts (Kessler and Byrne, 1999; Rothenburger et al., 2001; Spilker et al., 2001; Taylor et al., 2002; Yannas, 1997; Zaleskas et al., 2001) and vascular networks (Pieper et al., 2002). However, weak mechanical strength, limited fabrication options in altering mechanical and degradation properties limit their usage. Another system, decellularized submucosal ECM (Badylak, 2007), is widely used clinically; its good success in healing and tissue reconstruction is attributed to small, pro-healing peptides released as it rapidly degrades under macrophage attack (Vorotnikova et al., 2010).

Synthetic polyesters have been widely explored as tissue engineering materials, such as: poly(lactic acid) (PLA), poly(glycolic acid) (PGA), their copolymers (PLGA, PLLA, etc.) (Engelberg and Kohn, 1991; Gao et al., 1998; Lavik et al., 2002; Marra et al., 1999; Mikos et al., 1994; Mooney et al., 1996a; Nakamura et al., 1989; Oberpenning et al., 1999; Pattison et al., 2005; Ozawa et al., 2002), and poly(caprolactone) (PCL) (Hutmacher et al., 2001; Lowry et al., 1997). Various well-developed fabrication techniques exist for 3D scaffolds, including: free-form printing (Hutmacher et al., 2001), controlled rate freezing and lyophilization (Madhally and Matthew, 1999), porogen-leaching (Mikos et al., 1994), gas-foaming (Mooney et al., 1996b), and microfabrication (Wang and Ho, 2004).

Porous Biomaterials That Promote Healing Through MØ Recruitment and Controlled Differentiation

Unlike most porous scaffold materials described above, porous templated scaffolds (PTSs) are polymer constructs where every pore is exactly the same size, and pore interconnects are also uniform in size; *with both parameters being adjustable* (Beckstead et al., 2009; Linnes et al., 2007; Marshall et al., 2004; Osathanon et al., 2008, 2009; Saul et al., 2007) (Fig. 3). Uncrosslinked poly(methyl methacrylate) (PMMA) microspheres of a desired diameter are packed into a mold using sonic vibration to maximize packing. The entire mold is gently heated, which leads to sintering (fusion) of the spheres at their contact points. Next, the specific monomer selected for template construction is vacuum-infused in liquid form into the mold, surrounding the sintered beads. Monomer is polymerized in-place into a solidified crosslinked network. Finally, the PMMA micro-spheres are solubilized from within the crosslinked network, leaving a porous, interconnected structure. We have made PTSs from poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel, a biodegradable pHEMA-*co*-caprolactone (PCL) (Atzet et al., 2008), silicone elastomer, fibrin, alginate and polyurethane; all in pore sizes ranging from 10 to 160 μm (Beckstead et al., 2009; Bota et al., 2010; Linnes et al., 2007; Osathanon et al., 2008, 2009; Saul et al., 2007).

We have studied the healing of these materials in multiple in vivo environments (mouse subcutaneous, rat heart (Fig. 4), mouse percutaneous, rabbit vaginal wall, canine sclera, and in a rabbit drilled femur defect model). A sharp maximum in vascular density was noted with 35 μm pores (Fig. 5). Consistently, the 30–40 μm dia. pore size PTS shows excellent healing, regardless of its polymer composition or implant site (Beckstead et al., 2009; Linnes et al., 2007; Madden et al., 2010; Marshall et al., 2004; Osathanon et al., 2008, 2009; Saul et al., 2007).

The rich MØ infiltration in the 30–40 μm pore PTSs (Fig. 6) suggested the innovative hypothesis that perhaps MØ are being directed toward a healing phenotype. Similar successes with a polyHEMA PTS for regeneration of skin at a percutaneous wound site have been recently published (Fukano et al., 2006, 2010; Isenhath et al., 2007; Knowles et al.,

2005). Colleagues in Dermatology, UW Medical School, have noted that the PTSs with 35- μm pore size healed in a transcutaneous site (Fig. 7) with reconstruction of the epidermis and vascularized reconstruction of the dermis (Fukano et al., 2010).

Most recently, pHEMA hydrogel PTSs were implanted in a drilled bone defect in the femurs of old (18 month) rabbits. The control in these preliminary experiments was a drill hole simply allowed to fill with a clot. Within 4 weeks, with loading on the limb, good bone reconstruction was noted in the PTS group, and at 12 weeks continued bone growth was observed (Fig. 8). The contralateral control showed no apparent healing.

To determine the role M ϕ play in this improved healing response, non-porous slabs and 40 μm porous PTS materials were implanted into rat cardiac tissue for 4 weeks, then triple immunohistochemistry was performed to determine the phenotype of the M ϕ on the surface of the implants (Madden et al., 2010). Inducible nitric oxide synthase (NOS2, green) and M ϕ mannose receptor (MM2, blue) were used as proinflammatory M1 and pro-healing M2 markers, respectively, while CD68 (red) served as a total M ϕ cell marker. The number of M ϕ expressing only the M1 marker was about four fold higher on flat surfaces as shown in Figure 9, while the number of cells expressing either both markers or only the M2 marker was higher on sphere-templated materials. M ϕ on sphere-templated materials therefore showed a shift in activation polarization towards an M2 phenotype, thus providing a possible explanation for improved healing by these materials.

Why the 30–40 μm pore size PTSs promote healing and tissue integration, regardless of biomaterial or tissue, remains unknown. We *hypothesize* that the large numbers of M ϕ (Fig. 5) in the 30–40- μm pore PTSs are being ultimately directed to the M2 (regenerative) pathway rather than the tissue-destructive M1 and FBGC pathway, possibly by inhibit their ability to enter the spread (phagocytotic) phenotype. Figure 10 is a transmission electron micrograph of one pore in an explanted PTS scaffold after 14 days implantation, showing the fibroblast cells expected of local tissue regeneration (Fukano et al., 2010). However, the presence of M ϕ still within the pore structures leads us to a rather *controversial hypothesis* that perhaps M ϕ trans-differentiate into implant site-specific tissue cells. Recent literature has suggested that M ϕ may not be terminally differentiated, and that M ϕ may be capable of a high degree of plasticity (Jabs et al., 2005; Kuwana et al., 2006; Mooney et al., 2010; Sharifi et al., 2006). Unfortunately, current M ϕ cell targeting/identification technology relies primarily on differences in secreted cytokines and inflammatory molecules, thus preventing direct quantification of phenotype. Thus, more specific and mechanistically insightful markers for M1 and the various M2 sub-populations still remains a critical unmet need.

An imbalance in M1, M2(a–c) M ϕ , and FBGCs at an injury site can contribute to the persistence or even advancement of diseases associated with chronic inflammation. Non-porous biomaterials with surfaces decorated with simple adhesion molecules or porous biomaterials of random pore sizes may inadvertently promote M1 sub-populations and consequently lead to chronic inflammation and foreign body response (Wagner and Bryers, 2004; Wagner et al., 2004). Uniform 30–40 μm pore size PTSs appear to bias M ϕ polarization to M2(a–c) phenotype and subsequent healing.

Concluding Remarks

For centuries, medicine has had remarkably little ability to cure and regenerate, and has rather focused on palliative approaches to dealing with traumatic injury and other tissue damage. New strategies that directly tap into healing and regenerative biological pathways may lead the way to novel therapies that permit vascularized, non-fibrotic tissue

reconstruction. This article reviews some of the approaches that are under investigation to improve our ability to heal wounds and to circumvent the FBR.

At the University of Washington, we are currently developing new biomaterials for specifically controlling monocyte/MØ differentiation and polarization in order to control chronic inflammation and improve wound healing at biomedical device implant sites. Also, such materials may permit acellular (autologous) tissue engineering addressing major issues with cell sources. Such research needs to address four overarching questions: (1) Can we steer MØ down a healing/regenerative pathway? (2) Do MØ differentiate to other cell types and thus appear “stem-like?” (3) If new tissue is created, and the scaffold then biodegrades away, does the new tissue remain (i.e., have we created regenerated tissue)? And (4) if we implant in skin, we get skin; if we implant in bone, we get bone, etc. How general is this site-specific healing phenomenon and can we obtain insights to what is driving it?

The title of this article implies that the paradigm for biocompatibility is now in flux. Consider a slab of poly(HEMA) and a porous, templated structure with 40 µm pores made of the same poly(HEMA). The slab will heal with a classic FBR, an avascular, collagenous encapsulation. The exact same biomaterial in the PTS format will heal with little fibrosis, good vascularity and tissue reconstruction. We ask, “How can two so different biological reactions arising from the same material both be called biocompatible?” Thus, the paradigm of biocompatibility must shift to be consistent with new findings. With the shifting paradigm and the redefinition of biocompatibility will come improved outcomes and new therapies for patients suffering traumatic or disease-induced damage to tissues and organs.

References

- Amiji M, Park H, Park K. Study on the prevention of surface-induced platelet activation by albumin coating. *J Biomater Sci Polym Ed.* 1992; 3(5):375–388. [PubMed: 1419969]
- Anderson J. Biological responses to biomaterials. *Annu Rev Mater Res.* 2001; 31:81–110.
- Anderson J, Bonfield T, Ziats N. Protein adsorption and cellular adhesion and activation on biomedical polymers. *Int J Artif Organs.* 1990; 13:375–382. [PubMed: 2143174]
- Anderson JM, Rodriguez A, Chang DT. Foreign body reaction to biomaterials. *Semin Immunol.* 2008; 20(2):86–100. [PubMed: 18162407]
- Andrade J, Hlady V. Plasma protein adsorption: The big twelve. *Ann NY Acad Sci.* 1987; 516:158–172. [PubMed: 3439723]
- Atzet S, Curtin S, Trinh P, Bryant S, Ratner B. Degradable poly(2-hydroxyethyl methacrylate)-*co*-polycaprolactone hydrogels for tissue engineering scaffolds. *Biomacromolecules.* 2008; 9(12): 3370–3377. [PubMed: 19061434]
- Babu M, Diegelmann R, Oliver N. Fibronectin is overproduced by keloid fibroblasts during abnormal wound healing. *Mol Cell Biol.* 1989; 9:1642–1650. [PubMed: 2524650]
- Badylak SF. The extracellular matrix as a biologic scaffold material. *Biomaterials.* 2007; 28:3587–3593. [PubMed: 17524477]
- Beckstead BL, Tung JC, Liang KJ, Tavakkol Z, Usui ML, Olerud JE, Giachelli CM. Methods to promote notch signaling at the biomaterial interface and evaluation in a rafted organ culture model. *J Biomed Mater ResA.* 2009; 91A(2):436–446.
- Bensaid W, Triffitt JT, Blanchat C, Oudina K, Sedel L, Petite H. A biodegradable fibrin scaffold for mesenchymal stem cell transplantation. *Biomaterials.* 2003; 24:2497–2502. [PubMed: 12695076]
- Bergman A, Zygourakis K. Migration of lymphocytes on fibronectin-coated surfaces: Temporal evaluation of migratory parameters. *Biomaterials.* 1999; 20:2235–2244. [PubMed: 10614930]
- Bota PCS, Collie AMB, Puolakkainen P, Vernon RB, Sage EH, Ratner BD, Stayton PS. Biomaterial topography alters healing in vivo and monocyte/macrophage activation in vitro. *J Biomed Mater ResA.* 2010; 95(2):649–657.

- Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidized alginate and its potential application for tissue engineering. *Biotechnol Prog.* 2001; 17:945–950. [PubMed: 11587588]
- Brauker JH, Carr-Brendel VE, Martinson LA, Crudele J, Johnston WD, Johnson RC. Neovascularization of synthetic membranes directed by membrane microarchitecture. *J Biomed Mater Res.* 1995; 29:1517–1524. [PubMed: 8600142]
- Brodbeck W, MacEwan M, Colton E, Meyerson H, Anderson J. Lymphocytes and the foreign body response: Lymphocyte enhancement of macrophage adhesion and fusion. *J Biomed Mater Res.* 2005; 74A:222–229.
- Bruemmer D, Yin F, Liu J, Berger JP, Sakai T, Blaschke F, Fleck E, Van Herle AJ, Forman BM, Law RE. Regulation of the growth arrest and DNA damage-inducible gene 45 (GADD45) by peroxisome proliferator-activated receptor gamma in vascular smooth muscle cells. *Circ Res.* 2003; 93(4):e38–e47. [PubMed: 12881480]
- Cai K, Yao K, Cui Y, Lin S, Yang Z, Li X, Xie H, Qing T, Luo J. Surface modification of poly(D,L-lactic acid) with chitosan and its effects on the culture of osteoblasts in vitro. *J Biomed Mater Res.* 2002; 60:398–404. [PubMed: 11920663]
- Chung TW, Yang J, Akaike T, Cho KY, Nah JW, Kim SI, Cho CS. Preparation of alginate/galactosylated chitosan scaffold for hepatocyte attachment. *Biomaterials.* 2002; 23:2827–2834. [PubMed: 12069321]
- Chvapil M. Collagen sponge: Theory and practice of medical applications. *J Biomed Mater Res.* 1977; 11:721–741. [PubMed: 893491]
- Cotran, R.; Kumar, V.; Robbins, S., editors. *Pathologic basis of disease.* 6. Philadelphia, PA: Saunders Publishing; 1999.
- Dantzer E, Braye FM. Reconstructive surgery using an artificial dermis (integra): Results with 39 grafts. *Br J Plast Surg.* 2001; 54:659–664. [PubMed: 11728107]
- Dar A, Shachar M, Leor J, Cohen S. Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. *Biotechnol Bioeng.* 2002; 80:305–312. [PubMed: 12226863]
- Denuziere A, Ferrier D, Damour O, Domard A. Chitosan-chondroitin sulfate and chitosan-hyaluronate polyelectrolyte complexes: Biological properties. *Biomaterials.* 1998; 19:1275–1285. [PubMed: 9720891]
- Dziuk PJ, Cook B. Passage of steroids through silicone rubber. *Endocrinology.* 1966; 78(1):208–211. [PubMed: 5948426]
- Edmondson S, Osborne VL, Huck WT. Polymer brushes via surface-initiated polymerizations. *Chem Soc Rev.* 2004; 33(1):14–22. [PubMed: 14737505]
- Engelberg I, Kohn J. Physico-mechanical properties of degradable polymers used in medical applications: A comparative study. *Biomaterials.* 1991; 12:292–304. [PubMed: 1649646]
- Fukano Y, Knowles NG, Usui ML, Underwood RA, Hauch KD, Marshall AJ, Ratner BD, Giachelli CM, Carter WG, Fleckman P, Olerud JE. Characterization of an in vitro model for evaluating the interface between skin and percutaneous biomaterials. *Wound Repair Regen.* 2006; 14(4):484–491. [PubMed: 16939578]
- Fukano Y, Usui ML, Underwood RA, Isenhath S, Marshall AJ, Hauch KD, Ratner BD, Olerud JE, Fleckman P. Epidermal and dermal integration into sphere-templated porous poly(2-hydroxyethyl methacrylate) implants in mice. *J Biomed Mater Res A.* 2010; 94(4):1172–1186. [PubMed: 20694984]
- Gallin, J.; Synderman, R., editors. *Inflammation: Basic principles and clinical correlates.* 2. New York, NY: Raven Publishing; 1999.
- Gao J, Niklason L, Langer R. Surface hydrolysis of poly(glycolic acid) meshes increases the seeding density of vascular smooth muscle cells. *J Biomed Mater Res.* 1998; 42:417–424. [PubMed: 9788505]
- Geelhood SJ, Horbett TA, Ward WK, Wood MD, Quinn MJ. Passivating protein coatings for implantable glucose sensors: Evaluation of protein retention. *J Biomed Mater Res B.* 2007; 81(1):251–260.

- Giachelli CM, Lombardi D, Johnson RJ, Murry CE, Almeida M. Evidence for a role of osteopontin in macrophage infiltration in response to pathological stimuli in vivo. *Am J Pathol.* 1998; 152(2): 353–358. [PubMed: 9466560]
- Gordon S. Alternative activation of macrophages. *Nat Rev Immunol.* 2003; 3(1):23–35. [PubMed: 12511873]
- Goreish HH, Lewis AL, Rose S, Lloyd AW. The effect of phosphorylcholine-coated materials on the inflammatory response and fibrous capsule formation: In vitro and in vivo observations. *J Biomed Mater Res A.* 2004; 68(1):1–9. [PubMed: 14661243]
- Gratchev A, Kzhyshkowska J, Kothe K, Muller-Molinet I, Kannookadan S, Utikal J, Goerd S. M phi 1 and M phi 2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. *Immunobiology.* 2006; 211(6–8):473–486. [PubMed: 16920487]
- Gunatillake PA, Adhikari R. Biodegradable synthetic polymers for tissue engineering. *Eur Cell Mater.* 2003; 5:1–16. [PubMed: 14562275]
- Henson P. The immunologic release of constituents from neutrophil leukocytes. I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. *J Immunol.* 1971; 107:1535–1546. [PubMed: 5120396]
- Henson P. Mechanisms of exocytosis in phagocytic inflammatory cells. Parke–Davis award lecture. *Am J Pathol.* 1980; 101:494–511. [PubMed: 7004205]
- Henson P, Johnston RJ. Tissue injury in inflammation. Oxidants, proteinases, and cationic proteins. *J Clin Invest.* 1987; 79:669–674. [PubMed: 3546374]
- Hoffman AS. Hydrogels for biomedical applications. *Adv Drug Deliv Rev.* 2002; 54(1):3–12. [PubMed: 11755703]
- Hu W, Eaton J, Tang L. Molecular basis of biomaterial-mediated foreign body reactions. *Blood.* 2001; 98:1231–1238. [PubMed: 11493475]
- Huang Y, Onyeri S, Siewe M, Moshfeghian A, Madihally SV. In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. *Biomaterials.* 2005; 26:7616–7627. [PubMed: 16005510]
- Hume DA, Ross IL, Himes SR, Sasmono RT, Wells CA, Ravasi T. The mononuclear phagocyte system revisited. *J Leukoc Biol.* 2002; 72(4):621–627. [PubMed: 12377929]
- Hutmacher DW, Schantz T, Zein I, Ng KW, Teoh SH, Tan KC. Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling. *J Biomed Mater Res.* 2001; 55:203–216. [PubMed: 11255172]
- Isenhath SN, Fukano Y, Usui ML, Underwood RA, Irvin CA, Marshall AJ, Hauch KD, Ratner BD, Fleckman P, Olerud JE. A mouse model to evaluate the interface between skin and a percutaneous device. *J Biomed Mater Res A.* 2007; 83A(4):915–922. [PubMed: 17567856]
- Iwasaki Y, Ishihara K, Nakabayashi N, Khang G, Jeon JH, Lee JW, Lee HB. Platelet adhesion on the gradient surfaces grafted with phospholipid polymer. *J Biomater Sci Polym Ed.* 1998; 9(8):801–816. [PubMed: 9724895]
- Jabs A, Moncada GA, Nichols CE, Waller EK, Wilcox JN. Peripheral blood mononuclear cells acquire myofibroblast characteristics in granulation tissue. *J Vasc Res.* 2005; 42(2):174–180. [PubMed: 15767764]
- Jay SM, Skokos E, Laiwalla F, Krady MM, Kyriakides TR. Foreign body giant cell formation is preceded by lamellipodia formation and can be attenuated by inhibition of Rac1 activation. *Am J Pathol.* 2007; 171(2):632–640. [PubMed: 17556592]
- Jockenhoevel S, Chalabi K, Sachweh JS, Groesdonk HV, Demircan L, Grossmann M, Zund G, Messmer BJ. Tissue engineering: Complete autologous valve conduit—A new moulding technique. *Thorac Cardiovasc Surg.* 2001; 49:287–290. [PubMed: 11605139]
- Johnston RJ. Current concepts: Immunology. Monocytes and macrophages. *N Engl J Med.* 1988; 318:747–752. [PubMed: 3279314]
- Jono S, Peinado C, Giachelli CM. Phosphorylation of osteopontin is required for inhibition of vascular smooth muscle cell calcification. *J Biol Chem.* 2000; 275(26):20197–20203. [PubMed: 10766759]
- Kao W. Evaluation of protein-modulated macrophage behavior on biomaterials: Designing biomimetic materials for cellular engineering. *Biomaterials.* 1999; 20:2213–2221. [PubMed: 10614928]

- Karp R, Johnson K, Buoën L, Ghobrial H, Brand I, Brand K. Tumorigenesis by millipore filters in mice: Histology and ultrastructure of tissue reactions as related to pore size. *J Natl Cancer Inst.* 1973; 51(4):1275–1285. [PubMed: 4583375]
- Keselowsky BG, Bridges AW, Burns KL, Tate CC, Babensee JE, LaPlaca MC, García AJ. Role of plasma fibronectin in the foreign body response to biomaterials. *Biomaterials.* 2007; 28(25):3626–3631. [PubMed: 17521718]
- Kessler PD, Byrne BJ. Myoblast cell grafting into heart muscle: Cellular biology and potential applications. *Annu Rev Physiol.* 1999; 61:219–242. [PubMed: 10099688]
- Kingshott P, Griesser HJ. Surfaces that resist bioadhesion. *Curr Opin Solid State Mater Sci.* 1999; 4(4):403–412.
- Knowles NG, Miyashita Y, Usui ML, Marshall AJ, Pirrone A, Hauch KD, Ratner BD, Underwood RA, Fleckman P, Olerud JE. A model for studying epithelial attachment and morphology at the interface between skin and percutaneous devices. *J Biomed Mater Res A.* 2005; 74A(3):482–488. [PubMed: 15983994]
- Kopecek J. Hydrogel biomaterials: A smart future? *Biomaterials.* 2007; 28(34):5185–5192. [PubMed: 17697712]
- Kudo H, Sawada T, Kazawa E, Yoshida H, Iwasaki Y, Mitsubayashi K. A flexible and wearable glucose sensor based on functional polymers with soft-MEMS techniques. *Biosens Bioelectron.* 2006; 22(4):558–562. [PubMed: 16777401]
- Kuwana M, Okazaki Y, Kodama H, Satoh T, Kawakami Y, Ikeda Y. Endothelial differentiation potential of human monocyte-derived multipotential cells. *Stem Cells.* 2006; 24(12):2733–2743. [PubMed: 16888284]
- Kyriakides TR, Hartzel T, Huynh G, Bornstein P. Regulation of angiogenesis and matrix remodeling by localized, matrix-mediated antisense gene delivery. *Mol Ther.* 2001; 3(6):842–849. [PubMed: 11407897]
- Kyriakides TR, Foster MJ, Keeney GE, Tsai A, Giachelli CM, Clark-Lewis I, Rollins BJ, Bornstein P. The CC chemokine ligand, CCL2/ MCP-1, participates in macrophage fusion and foreign body giant cell formation. *Am J Pathol.* 2004; 165:2157–2166. [PubMed: 15579457]
- Lahiji A, Sohrabi A, Hungerford DS, Frondoza CG. Chitosan supports the expression of extracellular matrix proteins in human osteoblasts and chondrocytes. *J Biomed Mater Res.* 2000; 51:586–595. [PubMed: 10880106]
- Lavik E, Teng YD, Snyder E, Langer R. Seeding neural stem cells on scaffolds of PGA, PLA, and their copolymers. *Methods Mol Biol.* 2002; 198:89–97. [PubMed: 11951644]
- Lehrer R, Ganz T, Selsted M, Babior B, Curnutte J. Neutrophils and host defense. *Ann Intern Med.* 1988; 109:127–142. [PubMed: 3289431]
- Liaw L, Birk DE, Ballas CB, Whitsitt JS, Davidson JM, Hogan BL. Altered wound healing in mice lacking a functional osteopontin gene (spp1). *J Clin Invest.* 1998; 101(7):1468–1478. [PubMed: 9525990]
- Lindahl U, Kusche Gullberg M, Kjellen L. Regulated diversity of heparan sulfate. *J Biol Chem.* 1998; 273:24979–24982. [PubMed: 9737951]
- Linnes MP, Ratner BD, Giachelli CM. A fibrinogen-based precision microporous scaffold for tissue engineering. *Biomaterials.* 2007; 28(35):5298–5306. [PubMed: 17765302]
- Lowry KJ, Hamson KR, Bear L, Peng YB, Calaluce R, Evans ML, Anglen JO, Allen WC. Polycaprolactone/glass bioabsorbable implant in a rabbit humerus fracture model. *J Biomed Mater Res.* 1997; 36:536–541. [PubMed: 9294770]
- Madden LR, Mortisen DJ, Sussman EM, Dupras SK, Fugate JA, Cuy JL, Hauch KD, Laflamme MA, Murry CE, Ratner BD. Proangiogenic scaffolds as functional templates for cardiac tissue engineering. *Proc Natl Acad Sci USA.* 2010; 107(34):15211–15216. [PubMed: 20696917]
- Madihally SV, Matthew HW. Porous chitosan scaffolds for tissue engineering. *Biomaterials.* 1999; 20:1133–1142. [PubMed: 10382829]
- Malafaya PB, Silva GA, Reis RL. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliv Rev.* 2007; 59:207–233. [PubMed: 17482309]

- Malech H, Gallin J. Current concepts: Immunology. Neutrophils in human diseases. *N Engl J Med*. 1987; 317:687–694. [PubMed: 3041216]
- Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*. 2004; 25(12):677–686. [PubMed: 15530839]
- Mao JS, Liu HF, Yin YJ, Yao KD. The properties of chitosan-gelatin membranes and scaffolds modified with hyaluronic acid by different methods. *Biomaterials*. 2003; 24:1621–1629. [PubMed: 12559822]
- Marra KG, Szem JW, Kumta PN, DiMilla PA, Weiss LE. In vitro analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering. *J Biomed Mater Res*. 1999; 47:324–335. [PubMed: 10487883]
- Marshall AJ, Irvin CA, Barker T, Sage EH, Hauch KD, Ratner BD. Biomaterials with tightly controlled pore size that promote vascular ingrowth. *ACS Polymer Preprints*. 2004; 45(2):100–101.
- Martin P, Leibovich S. Inflammatory cells during wound repair: The good, the bad and the ugly. *Trends Cell Biol*. 2005; 15:599–607. [PubMed: 16202600]
- Mekori Y, Metcalfe D. Mast cells in innate immunity. *Immunol Rev*. 2000; 173:131–140. [PubMed: 10719674]
- Mendelsohn JD, Yang SY, Hiller J, Hochbaum AI, Rubner MF. Rational design of cytophilic and cytophobic polyelectrolyte multilayer thin films. *Biomacromolecules*. 2003; 4(1):96–106. [PubMed: 12523853]
- Michel R, Pasche S, Textor M, Castner DG. Influence of PEG architecture on protein adsorption and conformation. *Langmuir*. 2005; 21(26):12327–12332. [PubMed: 16343010]
- Mikos AG, Thorsen AJ, Czerwonka LA, Bao Y, Langer R, Winslow DN, Vacanti JP. Preparation and characterization of poly(L-lactic acid) foams. *Polymer*. 1994; 35:1068–1077.
- Mizuno K, Yamamura K, Yano K, Osada T, Saeki S, Takimoto N, Sakurai T, Nimura Y. Effect of chitosan film containing basic fibroblast growth factor on wound healing in genetically diabetic mice. *J Biomed Mater Res*. 2003; 64:177–181.
- Mooney D, Hansen L, Vacanti J, Langer R, Farmer S, Ingber D. Switching from differentiation to growth in hepatocytes: Control by extracellular matrix. *J Cell Physiol*. 1992; 151:497–505. [PubMed: 1295898]
- Mooney DJ, Baldwin DF, Suh NP, Vacanti JP, Langer R. Novel approach to fabricate porous sponges of poly(D,L-lactic-co-glycolic acid) without the use of organic solvents. *Biomaterials*. 1996a; 17:1417–1422. [PubMed: 8830969]
- Mooney DJ, Mazzoni CL, Breuer C, McNamara K, Hern D, Vacanti JP, Langer R. Stabilized polyglycolic acid fibrebased tubes for tissue engineering. *Biomaterials*. 1996b; 17:115–124. [PubMed: 8624388]
- Mooney JE, Rolfe BE, Osborne GW, Sester DP, van Rooijen N, Campbell GR, Hume DA, Campbell JH. Cellular plasticity of inflammatory myeloid cells in the peritoneal foreign body response. *Am J Pathol*. 2010; 176(1):369–380. [PubMed: 20008135]
- Mori R, Shaw TJ, Martin P. Molecular mechanisms linking wound inflammation and fibrosis: Knockdown of osteopontin leads to rapid repair and reduced scarring. *J Exp Med*. 2008; 205(1):43–51. [PubMed: 18180311]
- Morra M. On the molecular basis of fouling resistance. *J Biomater Sci Polym Ed*. 2000; 11(6):547–569. [PubMed: 10981673]
- Mosser DM. The many faces of macrophage activation. *J Leukoc Biol*. 2003; 73(2):209–212. [PubMed: 12554797]
- Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol*. 2008; 8(12):958–969. [PubMed: 19029990]
- Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase arginase balance: Competitive regulation by CD4(+) T cells correlates with Th1/Th2 phenotype. *J Immunol*. 1998; 160(11):5347–5354. [PubMed: 9605134]
- Murray PJ. Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr Opin Pharmacol*. 2006; 6(4):379–386. [PubMed: 16713356]

- Mytar B, Siedlar M, Woloszyn M, Ruggiero I, Pryjma J, Zembala M. Induction of reactive oxygen intermediates in human monocytes by tumour cells and their role in spontaneous monocyte cytotoxicity. *Br J Cancer*. 1999; 79(5–6):737–743. [PubMed: 10070862]
- Nakamura T, Hitomi S, Watanabe S, Shimizu Y, Jamshidi K, Hyon SH, Ikada Y. Bioabsorption of polylactides with different molecular properties. *J Biomed Mater Res*. 1989; 23:1115–1130. [PubMed: 2808460]
- Nath N, Chilkoti A. Creating “smart” surfaces using stimuli responsive polymers. *Adv Mater*. 2002; 14(17):1243–1247.
- Nayak S, Lyon LA. Ligand-functionalized core/shell microgels with permselective shells. *Angew Chem Int Ed Engl*. 2004; 43(48):6706–6709. [PubMed: 15593149]
- Nolan CM, Reyes CD, Debord JD, García AJ, Lyon LA. Phase transition behavior, protein adsorption, and cell adhesion resistance of poly(ethylene glycol) cross-linked microgel particles. *Biomacromolecules*. 2005; 6(4):2032–2039. [PubMed: 16004442]
- Oberpenning F, Meng J, Yoo JJ, Atala A. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat Biotechnol*. 1999; 17:149–155. [PubMed: 10052350]
- Ohri R, Tung E, Rajachar R, Giachelli CM. Mitigation of ectopic calcification in osteopontin-deficient mice by exogenous osteopontin. *Calcif Tissue Int*. 2005; 76(4):307–315. [PubMed: 15812576]
- Ophascharoensuk V, Giachelli CM, Gordon K, Hughes J, Pichler R, Brown P, Liaw L, Schmidt R, Shankland SJ, Alpers CE, Couser WG, Johnson RJ. Obstructive uropathy in the mouse: Role of osteopontin in interstitial fibrosis and apoptosis. *Kidney Int*. 1999; 56(2):571–580. [PubMed: 10432396]
- Orgill DP, Straus FH II, Lee RC. The use of collagen-GAG membranes in reconstructive surgery. *Ann NY Acad Sci*. 1999; 888:233–248. [PubMed: 10842636]
- Osathanon T, Linnes MP, Rajachar RM, Ratner BD, Somerman MJ, Giachelli CM. Microporous nanofibrous fibrin-based scaffolds for bone tissue engineering. *Biomaterials*. 2008; 29(30):4091–4099. [PubMed: 18640716]
- Osathanon T, Giachelli CM, Somerman MJ. Immobilization of alkaline phosphatase on microporous nanofibrous fibrin scaffolds for bone tissue engineering. *Biomaterials*. 2009; 30(27):4513–4521. [PubMed: 19501906]
- Ozawa T, Mickle DA, Weisel RD, Koyama N, Wong H, Ozawa S, Li RK. Histologic changes of nonbiodegradable and biodegradable biomaterials used to repair right ventricular heart defects in rats. *J Thorac Cardiovasc Surg*. 2002; 124:1157–1164. [PubMed: 12447182]
- Panzer U, Thaiss F, Zahner G, Barth P, Reszka M, Reinking RR, Wolf G, Helmchen U, Stahl RA. Monocyte chemoattractant protein-1 and osteopontin differentially regulate monocytes recruitment in experimental glomerulonephritis. *Kidney Int*. 2001; 59(5):1762–1769. [PubMed: 11318946]
- Park HJ, Bae YH. Hydrogels based on poly(ethylene oxide) and poly(tetramethylene oxide) or poly(dimethyl siloxane). III. In vivo biocompatibility and biostability. *J Biomed Mater Res A*. 2003; 64(2):309–319. [PubMed: 12522818]
- Pattison MA, Wurster S, Webster TJ, Haberstroh KM. Three-dimensional, nano-structured PLGA scaffolds for bladder tissue replacement applications. *Biomaterials*. 2005; 26:2491–2500. [PubMed: 15585251]
- Persy VP, Verhulst A, Ysebaert DK, De Greef KE, De Broe ME. Reduced postischemic macrophage infiltration and interstitial fibrosis in osteopontin knockout mice. *Kidney Int*. 2003; 63(2):543–553. [PubMed: 12631119]
- Picha GJ, Drake RF. Pillared-surface microstructure and soft-tissue implants: Effect of implant site and fixation. *J Biomed Mater Res B*. 1996; 30(3):305–312.
- Pieper JS, Hafmans T, van Wachem PB, van Luyn MJ, Brouwer LA, Veerkamp JH, van Kuppevelt TH. Loading of collagen-heparan sulfate matrices with β BFGF promotes angiogenesis and tissue generation in rats. *J Biomed Mater Res*. 2002; 62:185–194. [PubMed: 12209938]
- Pober J, Cotran R. The role of endothelial cells in inflammation. *Transplantation*. 1990a; 50:537–544. [PubMed: 2219269]
- Pober J, Cotran R. Cytokine-endothelial interactions in inflammation, immunity, and vascular injury. *J Am Soc Nephrol*. 1990b; 1:225–235. [PubMed: 2104268]

- Prichard HL, Reichert WM, Klitzman B. Adult adipose-derived stem cell attachment to biomaterials. *Biomaterials*. 2007; 28(6):936–946. [PubMed: 17074385]
- Prime KL, Whitesides GM. Adsorption of proteins onto surfaces containing end-attached oligo(ethylene oxide): A model system using self-assembled monolayers. *J Am Chem Soc*. 1993; 115:10714–10721.
- Quinn CP, Pathak CP, Heller A, Hubbell JA. Photo-crosslinked copolymers of 2-hydroxyethyl methacrylate, poly(ethylene glycol) tetra-acrylate and ethylene dimethacrylate for improving biocompatibility of biosensors. *Biomaterials*. 1995; 16(5):389–396. [PubMed: 7662824]
- Rajachar RM, Tung E, Truong AQ, Look A, Giachelli CM. Role of carbonic anhydrase II in ectopic calcification. *Cardiovasc Pathol*. 2009; 18(2):77–82. [PubMed: 18402839]
- Ratner BD. The engineering of biomaterials exhibiting recognition and specificity. *J Mol Recognit*. 1996; 9:617–625. [PubMed: 9174947]
- Ronneberger B, Kao WJ, Anderson JM, Kissel T. In vivo biocompatibility study of ABA triblock copolymers consisting of poly(L-lactic-co-glycolic acid) A blocks attached to central poly(oxyethylene) B blocks. *J Biomed Mater Res*. 1996; 30(1):31–40. [PubMed: 8788103]
- Rothenburger M, Vischer P, Volker W, Glasmacher B, Berendes E, Scheld HH, Deiwick M. In vitro modelling of tissue using isolated vascular cells on a synthetic collagen matrix as a substitute for heart valves. *Thorac Cardiovasc Surg*. 2001; 49:204–209. [PubMed: 11505315]
- Santhosh TR, Krishnan LK. Endothelial cell growth factor (ECGF) enmeshed with fibrin matrix enhances proliferation of EC in vitro. *Biomaterials*. 2001; 22:2769–2776. [PubMed: 11545311]
- Saul JM, Linnes MP, Ratner BD, Giachelli CM, Pun SH. Delivery of non-viral gene carriers from sphere-templated fibrin scaffolds for sustained transgene expression. *Biomaterials*. 2007; 28(31):4705–4716. [PubMed: 17675152]
- Savill J. Phagocyte recognition of apoptotic cells. *Biochem Soc Trans*. 1996; 24:1065–1069. [PubMed: 8968513]
- Schwartz B, Karsan A, Bombeli T, Harlan J. A novel beta 1 integrin-dependent mechanism of leukocyte adherence to apoptotic cells. *J Immunol*. 1999; 162:4842–4848. [PubMed: 10202028]
- Sharifi BG, Zeng ZH, Wang L, Song L, Chen HM, Qin MH, Sierra-Honigsmann MR, Wachsmann-Hogiu S, Shah PK. Pleiotrophin induces transdifferentiation of monocytes into functional endothelial cells. *Arterioscler Thromb Vasc Biol*. 2006; 26(6):1273–1280. [PubMed: 16614316]
- Sharkawy AA, Klitzman B, Truskey GA, Reichert WM. Engineering the tissue which encapsulates subcutaneous implants. I. Diffusion properties. *J Biomed Mater Res B*. 1997; 37(3):401–412.
- Shen M, Martinson L, Wagner MS, Castner DG, Ratner BD, Horbett TA. PEO-like plasma polymerized tetraglyme surface interactions with leukocytes and proteins: In vitro and in vivo studies. *J Biomater Sci Polym Ed*. 2002; 13(4):367–390. [PubMed: 12160299]
- Singh N, Bridges AW, García AJ, Lyon LA. Covalent tethering of functional microgel films onto poly(ethylene terephthalate) surfaces. *Biomacromolecules*. 2007; 8(10):3271–3275. [PubMed: 17877399]
- Singla A, Lee CH. Effect of elastin on the calcification rate of collagen-elastin matrix systems. *J Biomed Mater Res*. 2002; 60:368–374. [PubMed: 11920659]
- Spilker MH, Asano K, Yannas IV, Spector M. Contraction of collagen-glycosaminoglycan matrices by peripheral nerve cells in vitro. *Biomaterials*. 2001; 22:1085–1093. [PubMed: 11352089]
- Stein M, Keshav S, Harris N, Gordon S. Interleukin-4 potentially enhances murine macrophage mannose receptor activity—A marker of alternative immunological macrophage activation. *J Exp Med*. 1992; 176(1):287–292. [PubMed: 1613462]
- Steitz SA, Speer MY, McKee MD, Liaw L, Almeida M, Yang H, Giachelli CM. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol*. 2002; 161(6):2035–2046. [PubMed: 12466120]
- Stokes KB. Preliminary studies on a new steroid eluting epicardial electrode. *Pacing Clin Electrophysiol*. 1988; 11(11 Pt 2):1797–1803. [PubMed: 2463550]
- Szleifer I. Protein adsorption on tethered polymer layers: Effect of polymer chain architecture and composition. *Physica A*. 1997a; 244(1–4):370–388.
- Szleifer I. Polymers and proteins: Interactions at interfaces. *Curr Opin Solid State Mater Sci*. 1997b; 2(3):337–344.

- Takahashi H, Wang Y, Grainger DW. Device-based local delivery of siRNA against mammalian target of rapamycin (mTOR) in a murine subcutaneous implant model to inhibit fibrous encapsulation. *J Control Release*. 2010; 147:400–407. [PubMed: 20727922]
- Tan W, Krishnaraj R, Desai TA. Evaluation of nanostructured composite collagen–chitosan matrices for tissue engineering. *Tissue Eng*. 2001; 7:203–210. [PubMed: 11304455]
- Tang L, Eaton J. Fibrin(ogen) mediates acute inflammatory responses to biomaterials. *J Exp Med*. 1993; 178:2147–2156. [PubMed: 8245787]
- Tang L, Jennings T, Eaton J. Mast cells mediate acute inflammatory responses to implanted biomaterials. *Proc Natl Acad Sci USA*. 1998; 95:8841–8846. [PubMed: 9671766]
- Taylor PM, Allen SP, Dreger SA, Yacoub MH. Human cardiac valve interstitial cells in collagen sponge: A biological three-dimensional matrix for tissue engineering. *J Heart Valve Dis*. 2002; 11:298–306. [PubMed: 12056719]
- Tsai AT, Rice J, Scatena M, Liaw L, Ratner BD, Giachelli CM. The role of osteopontin in foreign body giant cell formation. *Biomaterials*. 2005; 26(29):5835–5843. [PubMed: 15949549]
- Unsworth LD, Sheardown H, Brash JL. Polyethylene oxide surfaces of variable chain density by chemisorption of PEO-thiol on gold: Adsorption of proteins from plasma studied by radiolabelling and immunoblotting. *Biomaterials*. 2005; 26(30):5927–5933. [PubMed: 15958239]
- Unsworth LD, Sheardown H, Brash JL. Protein-resistant poly(ethylene oxide)-grafted surfaces: Chain density-dependent multiple mechanisms of action. *Langmuir*. 2008; 24(5):1924–1929. [PubMed: 18217777]
- Verreck FAW, de Boer T, Langenberg DML, Hoeve MA, Kramer M, Vaisberg E, Kastelein R, Kolk A, de Waal-Malefyt R, Ottenhoff THM. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2, macrophages subvert, immunity to (myco)bacteria. *PNAS*. 2004; 101(13):4560–4565. [PubMed: 15070757]
- von Recum AF, Shannon CE, Cannon CE, Long KJ, Kooten TG, Meyle J. Surface roughness, porosity, and texture as modifiers of cellular adhesion. *Tissue Eng*. 1996; 2(4):241–253. [PubMed: 19877956]
- Vorotnikova E, Mcintosh D, Dewilde A, Zhang J, Reing JE, Zhang L, Cordero K, Bedelbaeva K, Gourevitch D, Heber-Katz E, Badyak SF, Brauhut SJ. Extracellular matrix-derived products modulate endothelial and progenitor cell migration and proliferation in vitro and stimulate regenerative healing in vivo. *Matrix Biol*. 2010; 29(8):690–700. [PubMed: 20797438]
- Wagner VE, Bryers JD. Poly(ethylene glycol)-polyacrylate copolymers modified to control adherent monocyte-macrophage physiology: Interactions with attaching *Staphylococcus epidermidis* or *Pseudomonas aeruginosa* bacteria. *J Biomed Mater Res A*. 2004; 69A(1):79–90. [PubMed: 14999754]
- Wagner VE, Koberstein JT, Bryers JD. Protein and bacterial fouling characteristics of peptide and antibody decorated surfaces of PEG-poly(acrylic acid) co-polymers. *Biomaterials*. 2004; 25(12):2247–2263. [PubMed: 14741590]
- Wang YC, Ho CC. Micropatterning of proteins and mammalian cells on biomaterials. *FASEB J*. 2004; 18(3):525–527. [PubMed: 14715699]
- Wang C, Yu B, Knudsen B, Harmon J, Moussy F, Moussy Y. Synthesis and performance of novel hydrogels coatings for implantable glucose sensors. *Biomacromolecules*. 2008; 9(2):561–567. [PubMed: 18166014]
- Williams G, Williams W. Granulomatous inflammation—A review. *J Clin Pathol*. 1983; 36:732–733.
- Wilson C, Clegg R, Leavesley D, Percy M. Mediation of biomaterial–cell interactions by adsorbed proteins: A review. *Tissue Eng*. 2005; 11:1–18. [PubMed: 15738657]
- Xia Z, Triffitt JT. A review on macrophage responses to biomaterials. *Biomed Mater*. 2006; 1(1):R1–R9. [PubMed: 18458376]
- Yang Y, Zhang SF, Kingston MA, Jones G, Wright G, Spencer SA. Glucose sensor with improved haemocompatibility. *Biosens Bioelectron*. 2000; 15(5–6):221–227. [PubMed: 11219733]
- Yang J, Goto M, Ise H, Cho CS, Akaike T. Galactosylated alginate as a scaffold for hepatocytes entrapment. *Biomaterials*. 2002; 23:471–479. [PubMed: 11761168]
- Yannas IV. Models of organ regeneration processes induced by templates. *Ann NY Acad Sci*. 1997; 831:280–293. [PubMed: 9616720]

- Yokoyama M, Nakahashi T, Nishimura T, Maeda M, Inoue S, Kataoka K, Sakurai Y. Adhesion behavior of rat lymphocytes to poly(ether)-poly(amino acid) block and graft copolymers. *J Biomed Mater Res.* 1986; 20:867–878. [PubMed: 3760003]
- Zaleskas JM, Kinner B, Freyman TM, Yannas IV, Gibson LJ, Spector M. Growth factor regulation of smooth muscle actin expression and contraction of human articular chondrocytes and meniscal cells in a collagen-GAG matrix. *Exp Cell Res.* 2001; 270:21–31. [PubMed: 11597124]
- Zhang Z, Zhang M, Chen S, Horbett T, Ratner B, Jiang S. Blood compatibility of surfaces with superlow protein adsorption. *Biomaterials.* 2008; 29(32):4285–4291. [PubMed: 18722010]
- Zhao B, Brittain WJ. Polymer brushes: Surface-immobilized macromolecules. *Prog Polym Sci.* 2000; 25:677–710.
- Zhu H, Ji J, Lin R, Gao C, Feng L, Shen J. Surface engineering of poly(D,L-lactic acid) by entrapment of chitosan-based derivatives for the promotion of chondrogenesis. *J Biomed Mater Res.* 2002; 62:532–539. [PubMed: 12221701]

Figure 1.
The general timeline of the cellular response against implanted biomaterials (Anderson, 2001).

Figure 2.

Inducers and responses of different polarized MØ populations. MØ exposure to IFN- γ and LPS drives M1 polarization, with potentiated cytotoxic and antitumoral properties, whereas M2-MØ are more prone to immuno-regulatory and protumoral activities. M2a- (induced by IL-4 and IL-13) and M2b-MØ (induced by immune complexes and TLR or IL-1R agonists) exert immuno-regulatory functions and drive type II responses, whereas M2c macrophages (induced by IL-10) are more related to suppression of immune responses and tissue remodeling. DTH, delayed-type hypersensitivity; IC, immune complexes; IFN- γ , interferon- γ ; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MR, mannose receptor; PTX3, the long pentraxin PTX3; RNi, reactive nitrogen intermediates; ROi, reactive oxygen intermediates; SLAM, signaling lymphocytic activation molecule; SRs, scavenger receptors; TLR, Toll-like receptor. After Mantovani et al. (2004).

Figure 3. Thirty five-micrometer pore size pHEMA sphere templated scaffold; scale bar =50 µm (**left**), scale bar =300 µm (**right**).

Figure 4. Survival and vascularization at 1 week of rat cardiomyocyte-seeded pHEMA 35 µm construct implanted subcutaneously. **A:** sarcomeric actin. **B:** rat endothelial cell antigen-1.

Figure 5.
Intra-pore vascular density after 4 weeks of subcutaneous implantation varies with pore size.

Macrophage infiltration *in vivo*

Figure 6. MØ infiltration in PTSs of different pore size; MØ stained with BM8 Mab marker (Fukano et al., 2010).

Figure 7. pHEMA 35-μm pore PTS implanted in dorsal murine model transcutaneously, regenerating dermis and epidermis layers (Fukano et al., 2010).

Figure 8.

A: Rabbit femur bone growing into the PTS (uninjured bone adjacent to the implant is at the top of the image). **B:** Control shows no bone tissue in-growth. **C:** Micro CT data on bone regrowth at 4 weeks.

Figure 9.

Macrophage phenotype in response to 4-week myocardial implants in Sprague–Dawley rats. Inset: Total MØ were identified with CD68+ staining (red). M1 and M2 phenotypes were determined by NOS2 (green) and MMR (blue), respectively. Overlaid images of CD68, NOS2, and MMR were analyzed to determine MØ phenotype. A majority of MØ in the porous implants expressed both NOS2 and MMR, although NOS2+/MMR– (darts) and NOS2–/MMR+ (arrows) MØ could be identified. MØ typically adhered to the material. The fraction of each activated state was determined for CD68+ MØ with significant increase in NOS2+/MMR+ MØ at all porous implant sites ($n=4$; $P<0.05$). There is a trend of increased NOS2–/MMR+ MØ in 40-μm porous constructs versus non-porous ($P=0.06$). Scale bar: 50 μm. (Madden et al., 2010).

Figure 10.

C57/Bl6 normal mouse implanted transcutaneously with poly(HEMA) porous construct; 35 µm pore size. Construct retrieved after 14 days; examined by TEM; cells colorized digitally (Fukano et al., 2010).