Immune responses to implants – A review of the implications for the design of immunomodulatory biomaterials

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A key for long-term survival and function of biomaterials is that they do not elicit a detrimental immune response. As biomaterials can have profound impacts on the host immune response the concept emerged to design biomaterials that are able to trigger desired immunological outcomes and thus support the healing process. However, engineering such biomaterials requires an in-depth understanding of the host inflammatory and wound healing response to implanted materials. One focus of this review is to outline the up-to-date knowledge on immune responses to biomaterials. Understanding the complex interactions of host response and material implants reveals the need for and also the potential of “immunomodulating” biomaterials. Based on this knowledge, we discuss strategies of triggering appropriate immune responses by functional biomaterials and highlight recent approaches of biomaterials that mimic the physiological extracellular matrix and modify cellular immune responses.

Abbreviations: aECM, artificial ECM; BMP, bone morphogenetic protein; CCL, CC chemokine ligand; COX-2, cyclooxygenase-2; CS, chondroitin sulfate; CXC, CXC chemokine ligand; DC, dendritic cells; DC-STAMP, dendritic cell-specific transmembrane protein; ECM, extracellular matrix; EGF, epidermal growth factor; ENA-78, epithelial cell-derived neutrophil attractant-78; ERK, extracellular signal-regulated kinases; FBG, foreign body giant cells; FBR, foreign body response; FGF, fibroblast growth factor; GAGs, glycosaminoglycans; GM-CSF, granulocyte macrophage colony-stimulating factor; HA, hyaluronic acid; HMMW-HA, high molecular weight HA; IL, interleukin; IL-1ra, IL-1 receptor antagonist; IFN, interferon gamma; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine; MI, mean fluorescence index; MHC-I/II, major histocompatibility complex class I/II molecules; MMPs, matrix metalloproteinas; NO, nitric oxide; NO2, nitric oxide synthase 2; PAMPs, pathogen-associated molecular patterns; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; PED, poly(ethylene oxide); PGs, proteoglycans; PMN, polymorphonuclear leukocytes; PRR, pattern recognition receptors; RGD, arginine–glycine–aspartic acid; ROS, reactive oxygen species; T, tissue factor; TGF-β, transforming growth factor beta; TH1, T helper 1 cells; TIMP, tissue inhibitor of metalloproteinas; TLR, toll-like receptors; TNF, tumor-nekrose-faktor alpha; TR, regulatory T lymphocytes; VEGF, vascular endothelial growth factor.

1. Introduction

The paradigm on the nature of biomaterials has been intensely refined within the last decades. Rather than being an inert material designed to minimize potentially deleterious host responses, Williams recently defined a biomaterial as “…a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure…” [1]. All materials when implanted into living tissue initiate a host response that reflects the first steps of tissue repair. Modern implant design is directed on making use of this immune response to improve implant integration while avoiding its perpetuation leading to chronic inflammation and foreign body reactions, and thus loss of the intended function [2]. Directing these processes requires an in-depth understanding of the immunological processes that take place at the interface between biomaterial and host tissue.

This review outlines the current knowledge on immune responses to biomaterials and debates on biomaterials designed to elicit appropriate host immune responses. Evidence will be presented that biomaterials mimicking the physiological extracellular matrix (ECM) may have the potential to modulate the immune system by enhancing or suppressing normal immune cell functions.
2. Host responses toward biomaterials

Biomaterial implantation is always accompanied by injury through the surgical procedure. Injury to the tissue or organ initiates an inflammatory response to the biomaterial starting with the formation of a provisional matrix. Implantation of engineered cell—material hybrids elicits an adaptive immune reaction toward the cellular component that influences the host response to the material component. When degradable devices are applied the immune response is additionally affected by degradation products and surface changes of the biomaterial that occur due to the degradation process.

2.1. Onset of the inflammatory response: blood proteins and alarmins induce granulocyte/monocyte activation

Nanoseconds after the first contact with tissue, proteins from blood and interstitial fluids adsorb to the biomaterial surface. This layer of proteins determines the activation of coagulation cascade, complement system, platelets and immune cells and guides their interplay which results in the formation of a transient provisional matrix and the onset of the inflammatory response [3,4] (Fig. 1A). The following section only delineates the complex interactions of coagulation, complement and platelets that occur on biomaterial surfaces. For a more detailed depiction we refer to recent reviews on biomaterial-associated protein adsorption, coagulation and complement activation [3,4].

2.1.1. Coagulation, complement and adhesion proteins — signals for integrins

Factor XII (FXII) and tissue factor (TF) are the initiators of the intrinsic and extrinsic system of the coagulation cascade, respectively. The intrinsic system is induced by contact activation of FXII on negatively charged substrates followed by a downstream cascade of protein reactions resulting in the release of thrombin [4,5]. Indeed, auto-activation of FXII has been shown to be catalyzed by surface contact with biomaterials [6]. It was suggested that contact activation of FXII is specifically promoted by biomaterials with anionic surfaces by imposing specific orientation on the surface adsorbed FXII facilitating its activation [7]. However, new findings demonstrate differences in the displacement of competing proteins on hydrophilic and hydrophobic surfaces to be the cause for enhanced FXII activation at negatively charged biomaterials [8,9]. Although auto-activated FXII on biomaterials initiates the generation of thrombin, the amount produced is not sufficient to induce clot formation [10]. Blood coagulation on biomaterials has been recently demonstrated to require the combination of both contact activation and platelet adhesion and activation [10,11]. Thrombin is one of the main activators of platelets. Low concentrations of thrombin released by FXII on biomaterials are suggested to activate platelets which in turn release mediators of the coagulation process and expose negatively charged phospholipids, thus providing the supposed catalytic surface for the coagulation cascade [10,12]. Platelet-derived prothrombinases (factor Va and factor Xa) of the extrinsic coagulation pathway assemble on the activated platelet surface and become activated. Subsequent thrombin formation occurs [12] that further activates platelets and coagulation factors of the intrinsic and extrinsic system amplifying the coagulation cascade on biomaterial surfaces [13]. Furthermore, thrombin initiates the cleavage of fibrinogen to fibrin forming the primary fibrous mesh around the biomaterial.

Fibrinogen is also known to spontaneously adsorb to biomaterial surfaces [14,15]. It was shown that an adhesion related conformational change of fibrinogen resulted in the exposure of two integrin binding domains capable to activate phagocytes [14,15]. It is suggested that phagocytes sense fibrinogen adherent to biomaterials as fibrin thus launching an inflammatory response occurring physiologically following clot formation [14]. Adsorbed fibrinogen also serves as adhesion substrate for platelets [16,17]. Besides thrombin and the adsorbed fibrinogen, TF expressed on damaged cells or on activated leukocytes at the implantation site as well as activated complement can induce activation of biomaterial attached platelets [18].

It is well established that complement is activated upon contact with biomaterial [19–21]. In inflammation complement activation occurs via three distinct pathways, the alternative, the classical and the lectin pathway which all converge on the level of C3 convertase activation that mediates formation and release of the anaphylatoxins C3a and C5a (reviewed in Ref. [22]). Activation of complement on biomaterial surfaces has been shown to be predominantly triggered by the classical and alternative pathway although there are also reports of lectin mediated complement activation [19–21]. However, complement activation is always associated with the biomaterial adsorbed protein layer. Attached IgG has been demonstrated to bind C1q resulting in the assembly of C1, the first enzyme of the classical pathway that promotes the initiation of the classical C3 convertase [23]. Additionally, C3 can spontaneously adsorb to the biomaterial surface while adopting a new conformation mimicking that of surface bound C3b of the alternative pathway [24]. The bound C3 then promotes the assembly of the initiating C3 convertase of the alternative pathway. C3b arising from the proteolytic cleavage of C3 by coagulation factors including FXIIa and thrombin can also directly attach to biomaterial surfaces and form an initiating C3 convertase [4,25]. Action of the C3 convertase results in the generation of C3b that binds to the biomaterial protein layer to form more C3 convertases thus launching the amplification loop of the alternative complement pathway [19,24]. Once the complement cascade is started high amounts of C3a and C5a are generated at the implantation site [26]. Both anaphylatoxins can contribute to the onset of inflammatory responses at implantation sites through their multitude of effector functions including triggering of mast cell degranulation, increasing vascular permeability, attracting and activating of granulocytes and monocytes and inducing granulocyte ROS (reactive oxygen species) release [22]. The induced complement proteins have also been shown to support platelet adhesion and activation on biomaterial surfaces [27] which in turn can propagate the coagulation cascade [10,12] but also promote TF expression by monocytes and granulocytes on biomaterial surfaces [28,29]. It becomes apparent that the coagulation cascade and complement system closely interact on the biomaterial surface and modulate each other’s activity [18]. The cross-talk between both systems operates synergistically in inflammatory cell activation.

Adhesion proteins of the ECM including fibronectin and vitronectin have also been described to attach to biomaterial surfaces [30]. Whereas fibrinogen and complement mainly contribute to the activation of inflammatory cells, fibronectin and vitronectin are critical in regulating the inflammatory response to biomaterials. Both proteins have been described to promote the fusion of macrophages to foreign body giant cells on biomaterial surfaces [31,32]. However, these proteins also support adhesion and spreading of osteoblastic cells to biomaterials which represents a crucial step for integration of orthopedic implants [33]. These seemingly opposed functions of fibronectin and vitronectin exemplarily reflect the high effector capacity of the adsorbed protein layer. Depending on the environment at the implant site, proteins adhering to biomaterials may either initiate and foster inflammation or assist healing.

Cell adhesion and activation on biomaterials primarily occurs via interaction of adhesion receptors with the adsorbed proteins. In this review we focus on the interaction of biomaterials with
Fig. 1. Immune response toward biomaterials. A) Adsorption of blood proteins and activation of the coagulation cascade, complement and platelets result in the priming and activation of PMNs, monocytes and resident macrophages. B) Danger signals (alarmins) released from damaged tissue additionally prime the immune cells for enhanced function via PRR engagement. C) The acute inflammatory response is dominated by the action of PMNs. PMNs secrete proteolytic enzymes and ROS, corroding the biomaterial surface. IL-8...
immunocompetent cells. Integrins represent the major adhesion receptors of leukocytes [34]. Protein ligands of integrins include fibrinogen, factor X, iC3b, fibronectin, vitronectin [35] – all of which have been shown to attach to biomaterial surfaces [36]. Indeed, cell adhesion to protein-coated biomaterials and subsequent cell activation have been described to be mediated by integrins [37–40]. Initial adhesion and spreading of phagocytes are primarily achieved through β2 integrins [14,37] which in turn leads to a change in the receptor profile including the up-regulation and enabling of further integrins [34]. Adherent monocytes differentiating to macrophages initiate β1 integrins [41] which in conjunction with β2 integrins mediate adhesion during biomaterial-associated macrophage fusion [32,40,42]. Clustering of integrins may also induce motility, phagocytosis, degranulation, as well as the release of ROS and cytokines, events which play an important role in the inflammatory response toward biomaterials.

2.1.2. Danger signals and pathogen recognition receptor

Besides recognition of biomaterials through adhesion receptors, there are other receptor–ligand interactions activating immunocompetent cells. Danger signals (also referred to as alarmins) have long been ignored as potential activators of leukocytes following biomaterial implantation (Fig. 1B). The role of alarmins in inflammation has been reviewed in detail elsewhere [43]. Alarmins are the endogenous equivalent of pathogen-associated molecular patterns (PAMPs) and include heat shock proteins, HMGB1 (high mobility group box 1), ATP and uric acid. They are rapidly released following injury by cells dying in a non-programmed way (necrosis) to signal associated tissue damage. Like PAMPs, alarmins are recognized by cells of the innate immune system such as macrophages and dendritic cells (DCs) via pattern recognition receptors (PRR) such as scavenger receptors, toll-like receptors (TLR) and C-type lectins which promote inflammation and immunity.

There is evidence that induced danger signals are capable of immune cell activation at biomaterial surfaces [44,45]. Upon biomaterial application alarmins may be released or induced by cells at the implant site that had been damaged due to the surgical procedure. Proteolytic enzymes leaking from the injured cells may additionally trigger the generation of extracellular danger signals [46]. These signals include fibrinogen or cleaved ECM components such as collagen peptides, hyaluronic acid (HA), fibronectin and laminin that adsorb to biomaterials. Thus, a coated biomaterial surface itself might act as a danger signal (Fig. 1A). Soluble or biomaterial-associated alarmins can interact with PRRs, preferentially TLRs on leukocytes and propagate the inflammatory response. Indeed, recent studies demonstrate a role of TLR4 in the host response to biomaterials in vitro and in vivo [47,48].

2.1.3. PMN activation

Immediately following injury and protein deposition, inflammatory cells — predominantly polymorphonuclear leukocytes (PMNs, granulocytes) — migrate from the blood toward the implant site. Circulating PMNs are rapidly recruited to infection sites by host- and pathogen-derived mediators, acting as a first line of defense against invading pathogens. The role of granulocytes in the innate immune response is extensively reviewed elsewhere [49]. PMN recruitment to implantation sites is triggered by host derived chemoattractants released from activated platelets and endothelial cells as well as injured cells (Fig. 1A,B). Mast cell degranulation and associated histamine release have been shown to play a role in directing PMNs and monocytes to implanted biomaterials in mice and humans [50,51]. Reaching the implantation site, PMNs encounter the protein-coated biomaterial surface and subsequent engagement of integrins and PRRs on the PMN surface triggers a phagocytic response and degranulation [52,53] (Fig. 1C). PMNs usually secrete proteolytic enzymes and ROS to promote and foster pathogen killing [49]. At the implant site, the destructive agents may corrode material surfaces as described for polyurethane [54]. Furthermore, the cytotoxic components damage surrounding tissue, prolonging the inflammatory response. Another adverse effect of biomaterial-induced PMN activation is the metabolic exhaustion and depletion of the granulocytes’ oxidative resources. Due to the continuous release of ROS the microbial killing capacity of PMNs is dramatically reduced, which has been related to severe biomaterial-centered infections [55].

PMNs also represent a significant source of immunoregulatory signals which they synthesize upon activation [56], interleukin-8 (IL-8) being among the most prominent chemokines. The primary targets of IL-8 are PMNs themselves. Various studies have reported granulocyte migration and prolonged presence of granulocytes within chitosan materials [57,58] due to persistent autocrine PMN attraction by IL-8 [59]. Activated PMNs also secrete MCP-1 and MIP-1β [49]. Both chemokines are known as potent chemoattractants and activation factors for monocytes, macrophages, immature DCs and lymphocytes [60]. Increased release of these chemokines by PMNs suppresses further PMN infiltration in favor of mononuclear cell influx [61]. Due to a lack of further activation signals PMN undergo apoptosis after having done their job as phagocytes and are engulfed by macrophages [61]. Within the first two days after biomaterial implantation PMN typically disappear from implantation sites [62].

2.2. Chronic inflammation: dual role of macrophages as inflammatory mediators and wound healing regulators in the foreign body reaction

Chronic inflammation develops as inflammatory stimuli persist at the implant site with macrophages representing the driving force in perpetuating immune responses [63]. Monocytes arriving at the implantation site undergo a phenotypic change differentiating to macrophages. Their activation leads to further dissemination of chemoattractants. Macrophages attached to the biomaterial can foster invasion of additional inflammatory cells by secreting chemokines like IL-8, MCP-1, MIP-1β [64] (Fig. 1D).

Macrophages also play a critical role in wound healing and tissue regeneration. Phagocytosis of wound debris, release of enzymes important for tissue reorganization and of cytokines and growth factors inducing migration and proliferation of fibroblasts are mediated by macrophages and constitute the initial steps toward effective tissue regeneration [65]. These different functions are typically promoted by different macrophage subsets, originally referred to as M1 (classically activated) and M2 (alternatively activated) macrophages [66]. Based on fundamental macrophage functions involved in maintaining homeostasis these subsets have been reclassified into classically activated, regulatory, and wound-healing macrophages [67] (Fig. 2). The latter two arise from released from PMNs enhances PMN influx and priming. In the transition from acute to chronic inflammation, PMNs stop secreting IL-8 in favor of cytokines promoting immigrating and activation of monocytes and macrophages. D) Macrophages are the driving force of chronic inflammation. Constant release of inflammatory mediators like TNFα, IL-6, and MCP-1 results in permanent activation of macrophages. Fusion-inducing stimuli like IL-4 and IL-13 promote the fusion of macrophages to FBGC, which form a highly degradative environment on the biomaterial surface. Furthermore, FBGC promote ECM remodeling and fibroblast activation resulting in excessive fibrosis and biomaterial encapsulation. E) Macrophage-derived cytokines and PRR engagement activate DCs on the biomaterial surface. Depending on the nature of the stimulus, DCs mature to either immunogenic or tolerogenic subtypes, amplifying or suppressing the inflammatory response.
a subdivision of the alternatively activated macrophage subset. Activation and function of these macrophage phenotypes is excellently reviewed by Mosser and Edwards [67]. The different macrophage populations are generated in response to either endogenous stimuli released by damaged cells or innate immune cells following injury or infection or to adaptive immune signals produced by antigen-specific immune cells [68,69]. Classically activated macrophages are typically triggered by interferon-γ (IFNγ) released by T helper 1 (TH1) cells during adaptive immunity or by natural killer (NK) cells during innate immunity and by TNFα produced by antigen presenting cells [67,68]. Classical stimulation prime macrophages to secrete inflammatory cytokines and to perform microbicidal activity mediated by increased synthesis of ROS and nitrogen radicals making them to a crucial part of host defense [67,68]. Wound-healing macrophages are generated in response to IL-4 produced by basophils, mast cells and granulocytes in early innate immune responses or by TH2 cells during adaptive immune responses [67,70]. Interleukin-4 programs macrophages to down-regulate pro-inflammatory mediators and to promote wound healing processes by contributing to the production of ECM and by activation of fibroblasts [67,68,70–72]. Although wound-healing macrophages exert anti-inflammatory activities they are not capable of down-regulating immune responses. Regulatory macrophages also arise during innate and adaptive immune responses. They are triggered in response to a variety of signals including apoptotic cells, prostanoids, IL-10, immune complexes, glucocorticoids [67,73]. However, to become fully activated the macrophages need a second signal such as a PRR-ligand [67,73,74]. The main task of regulatory macrophages is to limit inflammation and to dampen immune responses which they achieve by release of high levels of IL-10, a very potent immunosuppressive cytokine [67,73,74].

An immune response involves the action of all types of macrophages, classical activated macrophages in the early phase and regulatory and wound-healing macrophages in the resolution stage. It is still debated whether inflammatory macrophages emigrate from the site of inflammation to give rise for regulatory and wound-healing macrophages [75] or whether the macrophages alter their functional phenotype in response to progressive changes of signals during the course of inflammation [76]. There are reports showing that macrophages retain their functional adaptability and adjust their phenotype to changing environmental stimuli [76,77]. The remarkable plasticity of macrophages is making them to an interesting target in the context of immunomodulation.

### 2.2.1. Foreign body giant cell formation

Macrophages that attach and recognize a foreign material show typically a classically activated phenotype secreting inflammatory cytokines, ROS, and degradative enzymes and displaying high phagocytic capacity. Single macrophages are able to phagocytose particles up to a size of 5 μm [78]. If the particle size is larger, macrophages attempt to coalesce to FBGCs. The cytokines IL-4 and IL-13 have been identified to induce macrophage fusion on biomaterial surfaces in vivo and in vitro [79–81]. Activated T lymphocytes (CD4+ cells) at the implant site are assumed to be the source of both IL-4 and IL-13 and have been shown to enhance macrophage fusion on biomaterials [82]. However, a recent study investigating synthetic biomaterials in nude mice revealed CD4+ cells not to be essential for induction of a foreign body response (FBR) [83]. Although IL-4 was not present in the T cell-deficient setting, macrophage fusion was not impaired due to unaffected levels of IL-13 at the implant site. It was suggested that mast cells most likely serve as a source of IL-13 at the onset of inflammation and also sustain IL-13 production during the chronic inflammatory response to the biomaterial. Chemoattractant CCL2 was also reported to be involved in FBGC formation [84] though not by recruiting cells to the implant site but rather by guiding macrophage chemotaxis toward each other [85].

Moreover, the properties of the biomaterial surface are important for FBGC formation. Since biomaterials are immediately covered, it is the adsorbed protein layer that renders the surface fusogenic. A variety of proteins including collagen, fibronectin,
laminin, fibrinogen, and vitronectin have been tested on their capability to promote FBGC formation [32]. Although all proteins mediate initial monocyte adhesion, only vitronectin supports macrophage adhesion and fusion [32]. It has been shown that β1 and β2 integrins play a role in macrophage adhesion during biomaterial-associated fusion [42]. Nevertheless, both integrins seem not to mediate the crucial adhesion step required for macrophage fusion [86]. It is proposed that fusion-inducing stimuli and initial adhesion to the biomaterial induces the expression of multiple fusogenic molecules including mannose receptor (CD206) [87], CD44 [88], CD47 [89], DC-STAMP [90], and E-cadherin [91] rendering macrophages capable for fusion [85].

2.2.2. Release of ROS, degradative enzymes and MMPs
If the FBGCs do not succeed in phagocytosing the foreign material, they remain at the biomaterial–tissue interface and shape podosomal structures forming a closed compartment between their surface and the underlying substrate [92]. Various studies have shown that following fusion to FBGC, macrophages display a reduced phagocytic activity in coincidence with enhanced degradative capacity [93], a phagocyte-specific phenomenon referred to as frustrated phagocytosis. In an attempt to resorb the non-phagocytosable biomaterial, FBGCs secrete protons, enzymes, and ROS into the compartment between them [94,95]. This will lead to resorption of materials that are susceptible to degradation [96]. If the biomaterial is completely resorbed, associated inflammation may resolve as the causative agent is no longer present.

Matrix metalloproteinases (MMPs) are macrophage-derived proteolytic enzymes involved in the foreign body reaction to biomaterials [97–100]. The collagenases MMP-8 and MMP-13 and the gelatinases MMP-2 and MMP-9 could be detected in macrophages adhering to collagen disks post explantation [97]. Combined action of both gelatinases and collagenases has been suggested to promote degradation of collagen implants with MMP-9 as key enzyme. MMP-9 was also found to be induced in macrophages and fibroblasts during tissue remodeling in response to natural hydroxyapatite implanted in rats [100]. In this study MMP-9 alone was unable to breakdown the xenograft implant, but was involved in angiogenesis and ECM remodeling of the peri-implant connective tissue. Macrophages and FBGC cultured in vitro on various biomaterials have been shown to express MMP-9 [98]. The study further demonstrated reduced macrophage fusion by pharmacological inhibition of MMP-1, -8, -13, and -18 implicating a role of these MMPs in the fusion process. Although the MMP blocking assays of this study did not show an involvement of MMP-9 in the FBGC formation [98] comprehensive investigations analyzing the foreign body reaction in MMP-9 null mice clearly demonstrated an involvement of MMP-9 in macrophage fusion [99]. The study also revealed that MMP-9 may play a pivotal role in biomaterial encapsulation and angiogenesis [99].

The action of MMPs at the implant site is assumed to dramatically change the cellular environment around the biomaterial and to modify migration, differentiation and active state of macrophages and other immune cells [101]. Increased levels of MMP-9 have been suggested to be indicative of inflammation and associated with poor wound healing [102]. An environment rich in MMP-9 at the biomaterial site may thus perpetuate inflammation and act counter-regulatory on the wound healing process.

2.2.3. Fusion-induced macrophage phenotype switch
Fusion to FBGCs is typically associated with a phenotype switch of the macrophages from a classical to a more alternative activation state. The fusion-inducing cytokines IL-4 and IL-13 are known to promote wound-healing macrophages during inflammation [67,71,72]. This transition is reflected by alterations of the cytokine profile at the biomaterial site [64,103–105]. Early upon biomaterial recognition macrophages release IL-6, IL-1α, TNFα, IL-8, MCP-1, RANTES, ENA-78 similar to classically activated macrophages [64]. Over time the majority of these inflammatory cytokines is down-regulated in favor of IL-10, TGF-β, MDC, Eotaxin-2 as well as IL1 receptor antagonist (IL1ra) that resembles the anti-inflammatory cytokine/chemokine profile of alternatively activated macrophages. However, the activation state of fusing macrophages is not completely identical to the alternative phenotype since they still produce pro-inflammatory RANTES and MCP-1 [64]. ROS and degradative enzymes. Moreover, biomaterial-adherent macrophages sustain or even increase their IL-6 and TNFα production on materials that do not promote macrophage fusion without mandatorily undergoing a phenotype switch [64,105]. These findings support the dogma that implant surface properties dictate macrophage responses. More importantly, they show that macrophage behavior is predominantly governed by the fusion event rather than adhesion.

2.2.4. Impaired wound healing and excessive fibrosis
Little knowledge exists on the involvement of macrophages FBGCs in the healing response to biomaterials. Successful tissue repair requires resolution of the full inflammatory cascade through the release of anti-inflammatory mediators, clearance of chemokines, down-regulation of inflammatory mediators and receptors, and apoptosis of immune cells [106]. At implantation sites, FBGCs produce anti-inflammatory cytokines (IL-10, IL-1α) [64,103–105]. However, their immunosuppressive activity may be counter-regulated by the proteolytic and pro-oxidant microenvironment due to continuous release of ROS and degradative enzymes around the biomaterial [106,107]. FBGCs that have formed in response to IL-4 may release pro-fibrotic factors such as transforming growth factor beta (TGF-β) and platelet-derived growth factor (PDGF) that trigger the action of fibroblasts and endothelial cells as shown for IL-4 induced alternatively activated macrophages in vitro [71,72]. Activated fibroblasts start to synthesize and to deposit collagen that often results in material encapsulation [108,109]. However, the release of pro-fibrotic factors by FBGCs has never been clearly described. Although the mechanism is not well understood, it is assumed that continuous action of FBGCs result in prolonged fibroblast activation and excessive biomaterial-associated matrix deposition [110,111].

2.3. Dendritic cell responses to biomaterials
Synthetic biomaterials including ceramics, polymers or metallic materials are typically not immunogenic and are thought not to initiate an adaptive immune response, except for cases of metal hypersensitivity. However, lymphocytes have been found at sites of synthetic implants [44,112] suggesting their involvement in immune responses to biomaterials. DCs are key players of innate and adaptive immunity. They elicit adaptive immune responses by their ability of antigen presentation and T cell priming. Additionally, DCs possess immunoregulatory capacities as they play a role in the induction of antigen-specific T cell tolerance, T cell anergy and the activation and expansion of regulatory T cells (TReg) (reviewed in Refs. [113,114]). Whether DCs induce an immunogenic or a tolerogenic T cell response depend on many factors including the state of DC maturation and the cytokine environment [114]. According to the current concept of DC functions immature and semi-mature DCs are promoters of tolerance whereas fully mature DCs induce immunity [113]. Antigen presentation in the absence of co-stimulation by immature DCs typically promotes T cell anergy [115,116]. Semi-mature DCs expressing MHC and co-stimulatory molecules but unable to produce pro-inflammatory cytokines such as IL-12, TNFα,
IL-6 and IL-1β have been described to convert naïve T cells into CD4+/CD25+ Treg or IL-10 secreting CD4+ T cells (Tr1) [117–119]. The tolerogenic activity of semi-mature DCs can be additionally enhanced by their release of IL-10 [113,114]. Cytokines have been shown to play the major part in the induction of tolerogenicity. Besides IL-10 and TGF-β, two important immunosuppressive regulators, various cytokines and growth factors including IL-6 and TNFα at low concentrations as well as IL-16, granulocyte colony stimulating factor and hepatocyte growth factor have been reported to generate DCs with tolerogenic activity [114,120–125]. With respect to biomaterial application induction of tolerogenic DCs at the implant site would provide a powerful means to limit the immune response and to promote wound healing and biomaterial integration.

The role of DCs in biomaterial application has mostly been addressed in the presence of an immunogenic biological component. Biomaterials were found to exert adjuvant effects since they potentiated immune responses toward co-delivered antigens [126]. However, acting as an adjuvant requires the capability to prime DCs that has been shown to necessitate direct cell-material contact [127]. It is assumed that biomaterials activate DCs by triggering receptors and signaling cascades of the pathogen recognition system [127] (Fig. 1B and E). As described above, DCs may sense materials using PRRs including toll-like receptors and C-type lectins. The receptor-activating ligands are danger signals constituted by proteins and carbohydrate moieties in the adsorbed protein layer. A recent study suggests a substrate-dependent DC activation. Albumin or whole serum stimulated DCs to the release of IL-10 or to prime IL-4 producing T cells as typical for Th2 type responses [128]. In contrast, DCs cultured on collagen and vitronectin generated high levels of IL-12p40 that correlated with the release of IFNγ by T cells indicating a Th1 type response [128]. Because collagen and vitronectin function both as danger signals and adhesion substrates for integrins, integrin signaling cannot be ruled out as an alternative mechanism of DC activation to PRR engagement. Indeed, DC integrin binding to ECM proteins and subsequent DC maturation has been shown, and for fibronectin a dependency of DC maturation on β1 integrin binding was demonstrated [129,130].

Various biomaterial polymers (alginate, agarose, chitosan, HA, poly(lactic-co-glycolic acid)) have been shown to exert differential effects on DC maturation and activation [44,131,132]. DCs activated upon biomaterial contact develop an immunogenic phenotype similar to LPS-activated DCs which is characterized by increased expression of co-stimulatory molecules (CD80/CD86), major histocompatibility complex class II (MHC-II) molecules and the DC maturation marker CD83 [44,127]. Biomaterial-matured DCs are capable to promote T cell proliferation and secrete inflammatory cytokines (TNFα and IL-6) known to further amplify DC maturation by autocrine stimulation [44,127]. Nevertheless, some of the synthetic materials tested (e.g. alginate or HA) restrained DC maturation [131]. These cells develop a tolerogenic phenotype [125] that, upon encountering and presenting antigens, induce T cell tolerance. Depending on which PRR is engaged, DC maturation can be promoted or inhibited leading to immunity or tolerance, respectively [133]. Whereas immunogenic DCs may prolong the immune response to biomaterials and delay wound healing, tolerogenic DCs are capable to down-regulate the immune cells and resolve inflammation. Thus, induction of tolerogenic DC by designing the surface chemistry appears to be a promising strategy of modulating immune responses to biomaterials to improve biocompatibility.

2.4. T lymphocyte responses to biomaterials

T lymphocytes have been shown to adhere to synthetic biomaterials in vitro [62]. In co-culture with macrophages, they were found attached predominantly to macrophages and not to the biomaterial surface [82]. T lymphocytes have been demonstrated to promote macrophage adhesion and fusion via paracrine effects [82] (Fig. 1D). However, close association of lymphocytes and macrophages also suggests direct signaling which has been shown to dominate at later time points of their interaction [134]. During the initial response to biomaterials, lymphocytes and macrophages predominantly release inflammatory mediators [134–136]. These include the cytokines IL-1β, IL-6, TNFα and the chemokines IL-8, MCP, MIP-1β, ENA-78 all of which attract and activate inflammatory effector cells such as neutrophils, monocytes, T lymphocytes and natural killer cells. Interestingly, release of IL-1β and TNFα declined over time in favor of IL-10 and MMP-9, tissue inhibitor of MMPs (TIMP)-1 and TIMP-2—important mediators for ECM remodeling in wound healing [135]. These data nicely demonstrate the capability of T lymphocyte—macrophage interactions in guiding the inflammatory phases of the foreign body reaction. The absence of T cell activation in in vitro studies, however, questions the effect of lymphocytes in these processes. Neither IL-2 nor IFNγ were detected as a response to lymphocytes alone or in co-culture with macrophages to different synthetic biomaterials including silicone rubber, elastane 80A or polyethylene terephthalate [135–137]. On the other hand, activated T cells were identified during inflammatory biomaterial responses in vivo, suggesting the presence of the complete inflammatory environment as requirement for T cell activation in response to synthetic materials [137,138]. Nevertheless, the question remains how T cell activation is mediated during foreign body reaction. Synthetic biomaterials do not serve as an antigen. Given that the biomaterial is not degradable and that no bacteria transiently attach to its surface, T cell activation via antigen presentation does not occur. It has been suggested, that synthetic biomaterials may present functional groups on their surfaces acting as mitogens [137]. Mitogens are lectins that can trigger lymphocytes by cross-linking of glycoproteins on the lymphocyte surface. To date, mitogenic capabilities of biomaterials have not been demonstrated.

3. Extracellular matrix—a native modulator of cell activity in immune responses and tissue repair

The direct interactions of biomaterials and components of the host’s immune system described so far occur in vivo in the presence of the ECM. Several ECM proteins are potent regulators of monocyte/macrophage adhesion and activation and are involved in DC migration and maturation [139,140]. Bidirectional interactions of ECM, cells and growth factors/cytokines determine cellular behavior during all phases of biomaterial integration and wound healing including inflammation, cell proliferation and tissue remodeling. Moreover the ECM provides mechanical support and a three-dimensional scaffold for cellular organization. In addition it regulates cell behavior, storage and mobilization of signaling molecules as well as proteolytic degradation [141] (Fig. 3A). Collagen, elastin and fibrin form a fibrous structure that provides tensile strength or elasticity [142]. Non-fibrous proteins (predominantly fibronectin and laminin) linked to this scaffold supply domains for cell—matrix interaction [143]. The protein scaffold is embedded in a gelatinous, negatively charged matrix composed of glycosaminoglycans (GAGs). GAGs are long, unbranched carbohydrate chains consisting of repeating disaccharide units [144]. The sugar chains can be modified by sulfate groups as in chondroitin sulfate (CS), heparan sulfate, dermatan sulfate and keratin sulfate. HA is the only non-sulfated GAG. It is also not attached to a protein core whereas the sulfated GAGs are linked to serine rich proteins to form proteoglycans (PGs) [144]. The glycan matrix of the ECM serves as lubricant and provides a reservoir for signaling molecules. Additionally,
it participates in a variety of biological processes including cell-matrix interactions and activation of enzymes and mediators like growth factors and cytokines [145].

Cells including those involved in inflammation attach to the ECM via various surface receptors including integrins, selectins, syndecans and CD44 (Fig. 3B). They either recognize specific adhesive domains in the protein scaffold or bind to components of the glycan matrix e.g. HA, heparan sulfate and CS. This usually triggers intracellular signals that direct adhesion, migration, proliferation, differentiation, protein synthesis, and secretion [139,141,146]. These cellular functions are additionally regulated by signaling molecules (growth factors, cytokines and chemokines) that are trapped in the ECM. However, the ECM not only serves as a reservoir for signaling molecules, it rather regulates their distribution and mode of action. Components of the ECM (mostly PGs) retain the soluble mediators for example via electrostatic interactions between the negatively charged sulfate groups of the PGs and the positively charged surface of the signaling molecule [147]. This interaction has different biological consequences since it affects the local concentration, biological activity, and stabilization of growth factors [148,149] (Fig. 3C). Secreted growth factors usually have a very short half-life due to their high susceptibility to proteolytic degradation. Linkage to the ECM protects them from enzymatic cleavage. Moreover, binding to the ECM prevents growth factor diffusion within the compartment, providing a local store of functional molecules that persists long after their release has stopped [148]. This may result in a local concentration of growth factors needed for effective receptor signaling. Additionally, growth factor activity may be enhanced by localization within the ECM, allowing interaction with its specific ligands [149]. On the other hand, some growth factors may become inactive when bound to the ECM and can only act on their target when released by matrix proteolysis [141]. This requires action of ECM-degrading enzymes expressed by cells regulated by growth factors and ECM adhesion domains. Thus, growth factors and ECM proteins collaborate in creating a distinct cellular environment or “niche” that regulates tissue regeneration [150].
Proteolytic degradation of the ECM is also an essential feature of tissue repair and remodeling, and enables cell migration and invasion [148]. Numerous cell-secreted and cell-activated enzymes with ECM-degrading capacities have been identified including MMPs, elastase, and plasmin. MMPs play a predominant role in ECM degradation since they process and degrade virtually all structural ECM proteins. There are over 25 known MMPs grouped into collagenases, gelatinases, stromelysins, matrixins, membrane-type (MT)-MMPs and others performing multiple, sometimes overlapping, functions in ECM proteolysis (reviewed in Ref. [151]). However, MMPs are not only involved in ECM breakdown they also target non-ECM proteins, cell surface molecules and ECM-bound growth factors and cytokines and thus influencing cellular behavior [152]. For example, repressed T cell activity due to a down-regulation of the IL-2 receptor is mediated by MMP-9 thatcleaves the cytokine receptor from the T cell surface [152,153]. Membrane-type-MMP-1-mediated shedding of syndecan-1, a transmembrane protein involved in cell–cell and cell–matrix adhesion has been shown to stimulate cell migration [154]. Vascular endothelial growth factor (VEGF) restrained in the ECM has been shown to stimulate cell migration [154].

The action of MMPs is controlled at two levels, during transcription by cytokines and integrin clustering and after secretion by endogenous inhibitors including α2-macroglobulin and TIMPs [161–163]. Whereas cytokine and integrin signaling up- or down-regulate MMP expression, the protease inhibitors and here specifically TIMPs bind to the MMPs thereby determining their activity [163]. Precise control of MMP activity is crucial to ensure ECM remodeling under normal physiological conditions as dysregulation has been implicated in many diseases such as fibrosis, cancer, arthritis and vascular disease [162,164–166].

Taken together, the ECM not only provides support and a threedimensional scaffold for cells, it also plays a highly functionalized role in directing cell behavior by spatially and temporally concerted interaction with other cells, ECM components, degrading enzymes and signaling molecules all of which being relevant to tissue repair, host responses to biomaterial and ultimately biomaterial integration [167].

4. Modulating immune responses to biomaterials

For a long time biomaterial engineering focused on inert biomaterials. The concept of biologically inert materials is to minimize the host response by avoiding cell–material interactions. Inert biomaterials are recognized as foreign by the host but remain essentially unchanged and tolerated due to their encapsulation in fibrous tissue [168]. However, it has been realized that permitting specific cell responses may in fact be beneficial for biomaterial integration and improve implant performance [169]. For example osseointegration of titanium implants, classically inert biomaterials, could be improved by modification of the material surface allowing migration and adhesion of bone-forming cells from the surrounding tissues onto the implant [170].

Controlled tissue responses at the implant site are assumed to encourage wound healing. Increasing comprehension of the healing process point out that immune responses associated with inflammation and macrophage activation are crucial for tissue repair [171,172]. With growing knowledge on the processes of wound healing and host responses to biomaterials the field of biomaterial engineering has begun to address the development of materials with “immunomodulating” capacities. Ideally, the materials should affect normal immune cell function such that they promote healing and implant integration while sustaining specific implant function [2]. There are several publications reporting of modulated immune responses induced by silicon and blends of polydioxanone (PDO) and elastin or collagen [173–175]. The very comprehensive studies addressing modulation of functions of innate and adaptive immune cells revealed suppressed NK cell activity in response to all biomaterials whereas macrophage functions remained unaffected [173–175]. Blends of PDO and elastin or collagen were also shown to exert immunosuppressive effects on T and B cell-mediated immunity [174,175].

On the one hand the studies clearly point out the need for testing of biomaterials on their effects on both acquired and innate immune responses as a component of biocompatibility assessment [174,175]. On the other hand the studies nicely demonstrate the potential of biomaterials to modulate immune cell function. Encouraging the design of biomaterials capable of eliciting appropriate immune responses at implantation sites. Current strategies in the design of such biomaterials include alteration of material surface properties either passively via physicochemical features or actively with molecules or matrices designed to systematically target cell behavior.

4.1. Immunomodulation by surface modifications of biomaterials

Passive modulation of biomaterial surface properties aims at limiting macrophage adhesion, activation and fusion to FBGCs (Fig. 4A). Type, level and conformation of serum proteins that adsorb to biomaterial surfaces depend on the terminal chemistry of the biomaterial [36,38,39,176]. The adsorbed protein layer usually provides binding sites for protein-specific receptors (integrins, PRRs) on PMNs, monocytes and macrophages. Surface chemistry-dependent modulation of the protein layer may thus enable different receptor binding and signaling in the immune cells leading to altered cellular responses. Indeed, comprehensive proteomics studies revealed macrophages to change their protein expression profiles and cytokine/chemokine responses when cultured on surface-modified polymers displaying hydrophobic, hydrophilic, and/or ionic chemistries [64,177]. Macrophages attaching to hydrophilic and anionic biomaterial surfaces providing low integrin binding sites were shown to experience low integrin-mediated cell spreading, leading to macrophage apoptosis [178]. These findings provide a clue for surface modification of biomaterials to elicit desired PMN and macrophage activities.

Another strategy for guiding macrophage responses to biomaterials is the variation of roughness and surface topography [179,180]. In their natural environment cells respond to ECM components in the nanometer scale in terms of adhesion, proliferation, migration, and gene expression. Imprinting of patterns on micron and nanometer scales on material surfaces may mimic the natural topography of the ECM [179]. Topographic patterns are known to affect function of fibroblasts [181], epithelial cells [182], and endothelial cells [183]. A differing response of macrophages to micron-structured biomaterials has been demonstrated recently in the context of the foreign body reaction [184]. Parallel gratings imprinted on polymeric surfaces with line width ranging from 250 nm to 2 μm were shown to affect macrophage morphology and cytokine secretion in vitro, and macrophage adhesion in vivo independent of the biomaterial surface chemistry [184].
4.2. Immunomodulation by incorporation of bioactive molecules

Current methods of biomaterial functionalization include specific surface coatings and the incorporation of bioactive molecules like integrin adhesion sites as well as growth factors and anti-inflammatory mediators. Coating of biomaterials with artificial ECM — mimicking the biofunctions of the natural ECM as a tool for modulating immune cell behavior. Collagen provides natural binding sites for cell adhesion receptors (e.g. integrins). Proteoglycans are capable to interact with endogenous cytokines and growth factors allowing for their specific presentation to target cells.

4.2.1. Providing of integrin adhesion sites

Functionalization of biomaterial surfaces with specific integrin binding sites represents a powerful strategy in directing responses of inflammatory cells (Fig. 4A). Attachment of short oligopeptide sequences that make up receptor binding domains within adhesive proteins have been shown to promote cell-specific adhesion and function on biomaterials with arginine–glycine–aspartic acid (RGD) being the most prominent domain (reviewed in Refs. [186,187]). The RGD and PHSRN (proline-histidine-serine-arginine-asparagine) domains of fibronectin were identified to be crucial in regulating macrophage function via α5β1 and αvβ3 integrin signaling in vitro and in vivo [188,189]. Both domains, when imprinted at a specific orientation on the biomaterial, were found...
to initiate distinct intracellular “outside-in” signal pathways mediating macrophage adhesion and function upon ligation with integrins [190]. As one consequence, RGD and PHSRN domains mediated the formation of FBGC on biomaterial surfaces [189,190].

The incorporation of integrin ligands to biomaterials is often combined with polyethylene glycol (PEG) coatings (PEGylation), which renders biomaterial surfaces noninteractive (also referred to as “non-fouling”) [191,192]. Surfaces modified with PEG resist protein adsorption and are thus protected against passive cell attachment and subsequent cell activation [193]. The advantage of these combined approaches relies on the prevention of nonspecific cell—material interaction in favor of specific cell activation elicited by recognition of integrin adhesion sites on the biomaterial surface [194,195]. Further non-fouling coatings include dextran-based gels, poly(ethylene oxide) (PEO), and alginate [196–198].

4.2.2. Coupling of anti-inflammatory drugs to biomaterials

Another method of rendering biomaterials immunomodulatory is the incorporation of anti-inflammatory factors (Fig. 4A). Glucocorticoids are potent suppressors of immune responses (reviewed in Ref. [199]). They inhibit inflammatory cell activation by abrogating the synthesis of inflammatory mediators including several cytokines and chemokines, prostaglandins, leukotrienes, proteolytic enzymes, reactive oxygen radicals, and nitric oxide (NO). Simultaneously, they promote resolution of inflammation and of adaptive immune response by enhancing anti-inflammatory cytokine release and suppressing cellular [T helper (Th1-directed)] immunity in favor of humoral (Th2-directed) immunity and tolerance. Indeed, delivery at the implantation site of dexamethasone via coupling to biomaterials results in reduced implant-associated inflammation as shown by decreased numbers of PMNs in the initial inflammatory phase and the absence of macrophages, lymphocytes, and fibrous capsule formation in the later phase [200,201]. However, an unwanted side effect of dexamethasone treatment is the reduction of VEGF in the surrounding tissue impeding angiogenesis and delaying wound healing [200,202]. By combined administration of dexamethasone and VEGF this antiangiogenic effect could be overcome [202]. Though, dexamethasone needs to be supplied by the biomaterial throughout its lifetime. As soon as delivery is subsided, the anti-inflammatory effects fade and PMNs and macrophages start an inflammatory response [203].

Loading of biomaterial surfaces with NO-releasing coatings has evolved as an attractive strategy for durable control of immune responses [204]. Continuous and slowly liberation of NO results in reduced inflammatory cell recruitment and performance at the implant surface that sustains even after exhaustion of the NO reservoir [205]. Down-regulation of inflammatory cytokines such as IL-6 and MCP-1 as well as induction of nitrosated proteins are discussed to cause NO-mediated immunosuppression [206]. Furthermore, NO may induce macrophages to produce NO themselves explaining its long-lasting anti-inflammatory activity [207].

4.2.3. Delivery of growth factors

A complex signaling network of growth factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), granulocyte macrophage colony stimulating factor (GM-CSF), TGF-β, VEGF, and PDGF control adhesion, migration, proliferation, and differentiation of fibroblasts, keratinocytes, and endothelial cells in wound healing (reviewed in Ref. [208]). Although tissue cells are the primary targets of the growth factors, biomaterials decorated with these bioactive molecules can still be considered as immunomodulatory (Fig. 4A). Wound healing in adult tissue is always associated with an inflammatory response [209] and there is a tight cross-talk between immune cells and tissue cells regulating the healing process [210–215]. Fibroblasts, for example, have been shown to suppress MIP-1α release by activated macrophages [211] and to differently modulate IL-10 and IL-12 production by monocytes [212]. Monocytes develop fibroblast modulating activity as seen by enhanced MMP-2 expression of fibroblasts only in response to monocyte-derived GM-CSF. Vice versa, the activated fibroblasts amplify GM-CSF production in monocytes suggesting synergistic interactions during matrix remodeling [210]. Thus, modulating fibroblast, endothelial cell and keratinocyte function by loading biomaterials with growth factors also feeds back on the activity of monocytes and macrophages [202,216–219]. Moreover, growth factors also act directly on the immune cells, as shown for TGF-β1 and PDGF on macrophage chemotaxis and activation during wound repair [220].

4.3. Designing “immunomodulating” biomaterials based on artificial ECM – concepts and recent findings

The majority of functionalized biomaterials provide a single bioactive signal. Presentation of several bioactive factors is closer to the natural environment of the cells and may help tuning the desired responses. Interaction with the ECM regulates cellular behavior including migration, differentiation and proliferation making the ECM an attractive tool for endowing biomaterials with physiologic biofunctions (Fig. 4B).

4.3.1. Hydrogels

Considerable effort has been directed at mimicking the physiologic ECM microenvironment in the design of tissue engineering matrices. Natural and synthetic hydrogels are attractive matrices due to their high water content and three-dimensional structure resembling soft tissue [221]. Synthetic hydrogels are composed of polymers like PEG that are bio-inert. In different approaches the synthetic scaffolds have been rendered ECM-mimetic by grafting them with typical biofunctions including the presentation of receptor binding ligands for specific cell adhesion, the susceptibility to proteolytic degradation and remodeling by cell-derived proteases and the capability of binding and presentation of growth factors (reviewed in Ref. [222]).

Hydrogels that are made of materials from natural sources including ECM proteins (collagen, fibrin, gelatin) and polysaccharides (GAGs, dextran, alginate, chitosan) are already provided with ECM-derived biofunctions [223]. Although they usually have a low toxicity and rarely induce chronic inflammatory responses, the potential of pathogen transmission and immunogenicity of the materials restrain their use. A comparative study evaluating the morphologic host tissue response to five commercially available ECM-derived biologic scaffolds revealed that the scaffolds elicited distinct host responses depending on species of origin, tissue of origin, processing methods, and/or method of terminal sterilization [224]. However, natural ECM scaffolds are widely used for tissue engineering in regenerative medicine due to their simple design and economic fabrication in contrast to synthetic hydrogels.

4.3.2. Artificial ECM coatings for synthetic implants

The concept of modulating cellular responses through ECM-mimetic biomaterials has also been exploited to improve the biological acceptance and integration of synthetic implants. The first coatings that were developed made either use of adhesive domains of ECM proteins or ECM-derived proteins as a whole. Various studies reported of collagen coatings that supported cell attachment and activity on implants in vitro and significantly improved bone maturation and mineralization at implant—tissue interfaces in vivo [225,226]. Early appearance of mononuclear phagocytizing cells
and higher expression of bone-specific matrix proteins associated with increased early bone remodeling around the modified biomaterials were observed [227] indicating a modulatory capacity of the collagen matrices on both immune and tissue cells. Collagen coatings seem to promote specific cell implant interactions via integrin β1 as presented for rat calvarial osteoblasts [228]. Although, collagen was shown to mediate adhesion and spreading of osteoblasts to the implants, it had no effect on later processes such as proliferation, differentiation, and mineralization of the osteoblasts [229].

Two conclusions can be drawn from these findings. First, cellular functions are not controllable only by promoting adhesion and direct interaction with integrins and other cell surface receptors. It also requires indirect modulation through inducing specific growth factor responses. Second, to effectively modulate cell activity biomaterial coatings should comprise the action of signaling molecules. Biodegradable coatings locally releasing incorporated growth factors like bone morphogenetic protein (BMP), insulin-like growth factor (IGF), and TGF-β have been successfully tested to stimulate fracture healing and to improve biomaterial performance [230]. However, to yield proper biological effects, the growth factors have to be supplied in non physiologically high amounts at enormous costs. A more sophisticated approach therefore is to utilize the growth factor regulating property of the ECM. Artificial ECM (aECM) coatings were developed by modifying collagen matrices with GAGs and PGs [231] in order to create a biomaterial environment mimicking the situation in vivo (Fig. 5A).

Proteoglycans are suggested to be important mediators of osteoblast attachment and adhesion. Osteoblasts cultured on titanium were shown to synthesize sulfated GAGs that permeate the cell—biomaterial interface and promote adhesion [232]. Additionally, PGs should act as mediator between matrix and endogenous growth factors, thus improving cell activity around implants and influencing biomaterial integration [233]. Recent investigations focus on modifications of GAGs to influence their interaction with specific growth factors. Within the natural ECM the sulfate groups in GAGs represent one important growth factor binding site. Thus, incorporation of additional sulfate groups to GAGs should improve the biological properties of aECM coatings. For HA it was demonstrated that modification with sulfate groups increased the binding affinity for human BMP-4 [234]. Implant coatings containing sulfated GAGs may thus allow for enhanced osteoinductive activity by specifically interacting with bone cell stimulating factors.

In vitro experiments revealed aECM containing sulfated GAGs like CS promote cell adhesion as well as cell proliferation [235-236]. Several in vivo studies reported that addition of CS to collagen coatings improved the osteoconductive properties of both titanium implants [231,237] and hydroxyapatite bone cements [238,239]. When compared to uncoated implants and collagen matrices, bone remodeling and new bone formation around the implants were increased when CS was incorporated into the coatings (Fig. 5B–D). It was suggested that CS mediates attachment of growth factors to the ECM or cell surface thus stimulating both bone resorbing and bone-forming cells [231,237–239]. However, whether a specific growth factor response was modulated by the aECM coatings remains unclear. An interesting hint in this respect was provided by a study evaluating osseointegration of implants coated with aECM composed of collagen, GAG and BMP-4 [240]. Interestingly, collagen–GAG coatings alone proved to be as effective as those preloaded with BMP-4. The similar effects of both coatings might be due to the fact that only a very low amount of growth factor was used. Another explanation is the interaction of the collagen—GAG matrix with endogenous growth factors. The aECM coating might have stored growth factors at the implant site and presented them to cell receptors benefiting bone formation. Besides GAGs, other ECM components, either whole proteins (osteocalcin [241]), peptides (phosphoserine as an active part of osteopontin [242]), or functionalities (sodium citrate [242]) had positive effects on bone remodeling around hydroxypatite—collagen composite bone cements.

4.3.3. Immunomodulating effects of aECM coatings

ECM proteins are potent regulators of immune cell activity [139,140]. Thus, aECM coatings may have the capability to control
the inflammatory response which in turn has an important influence on bone regeneration and wound healing (Fig. 4B). Natural occurring GAGs have been identified to bind and modify inflammatory factors such as interleukins and chemokines [243–245]. An important immunoregulatory cytokine is IL-10, exhibiting both suppressive and stimulatory effects on the immune system. IL-10 acts anti-inflammatory on macrophages and DCs by reducing the production of pro-inflammatory cytokines and presenting antigens to T cells. Sulfated GAGs have been reported to bind human IL-10 and to modulate its activity [244]. Interestingly, GAG-promoted linkage of IL-10 to the surface of monocytes/macrophages resulted in enhanced activity of IL-10 on the immune cells [244]. The binding capability of the sulfated GAG was determined by grade of sulfation, position of the sulfate groups (C4 or CS) and linkage of the sulfate group to the sugar chain (N- or O-sulfation) indicating that subtle differences in the GAG structure and/or sequence might be sensed by signaling molecules and thus guides their interaction with the ECM. Differential binding to subsets of various sulfated GAGs was also observed for chemokines such as IL-8 [245]. Given that GAG structures vary among tissues and that chemokine interaction with GAG mediates their presentation to leukocytes, it is conceivable that GAGs participate in determining the specificity of leukocyte recruitment in vivo. This might be an important aspect for the design of aECM coatings to modulate the invasion of immune cells to the implantation site.

However, GAGs not only modulate the activity of inflammatory mediators they are also capable to directly interact with immune cells. In this respect, CS and HA are of special interest because they are known to act immunosuppressive on various cells. CS, for example, is used as a therapeutic agent in osteoarthritis since it exerts strong anti-inflammatory effects in the joint [246]. It was found that the immunosuppressive activity of CS is based on reduced nuclear translocation of NF-κB, a key transcription factor of various pro-inflammatory mediators, due to an inhibition of the ERK1/2 and p38MAPK pathway by CS [247]. Subsequent down-regulation of expression of MMPs, IL-1β, TNFα, COX-2 and NOS2 resulted in a reduced inflammatory reaction. Of special note is that the anti-inflammatory activity of CS was not only found at the level of chondrocytes and synovial cells but also on circulating peripheral blood mononuclear cells [248] suggesting an ubiquitous action of CS. Thus, CS may have the capacity to act as an immunosuppressive agent in a variety of inflammatory conditions associated with NF-κB activation including the foreign body reaction. Furthermore, both CS and HA were identified as antioxidants capable of reducing free radicals, protecting both cells and materials from ROS damage [249]. CS may therefore provide biomaterials with both immunosuppressive and antioxidant properties likely to modulate the inflammatory response toward resolution and healing.

The use of HA for aECM biomaterial coatings, however, is more complex. HA features different immunomodulatory activities depending on its molecular size [250]. Large HA polymers, as seen under physiologic conditions, have anti-inflammatory properties and promote tissue integrity and quiescence. However, during tissue injury and inflammation, HA becomes cleaved into smaller fragments that function as pro-inflammatory and immunostimulatory mediators potentially serving as endogenous “danger signal” triggering PRR of immunocompetent cells. Several studies have reported of low molecular weight HA activating inflammatory cytokine and chemokine production in PMNs and monocytes/macrophages as well as maturation of DCs via CD44 and/or TLR4 signaling, respectively [251–254]. Implant coating with HA might therefore seem counterproductive, since cleavage may cause...

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Fig. 6. Immunomodulating effects of aECM coatings on dendritic cells. A) Composition of the tested aECM coatings. B, C) Artificial ECM coatings attenuate DC maturation induced by collagen and LPS-stimulation: immature DCs (iDCs) were generated by culture of human CD14+ monocytes for 4 days in the presence of GM-CSF and IL-4. The iDCs were cultured for 24 h on either aECM or collagen alone with and without co-stimulation with LPS at 10 ng/ml. Expression of DC maturation markers and release of IL-12, the main cytokine for activation of T cell immunity were assessed by flow cytometry and ELISA, respectively. DC viability was assessed by staining with annexin V and propidium iodide. Viability of DC after 24 h culture on aECM was the same as in controls (DC cultured on plastic) and in all experiments over 85% (data not shown). At least three independent experiments were performed. Mann–Whitney U test was used for statistical analysis (*p < 0.05; **p < 0.005). Error bars represent standard deviation.
Further tissue injury. However, high molecular weight (HMW)-HA might continue acting anti-inflammatory even at sites of inflammation and promote and healing. In a model of non-infectious lung injury, up-regulation of endogenous HMW-HA production protected against acute inflammation due to reduced epithelial cell apoptosis and accelerated tissue repair [252]. HMW-HA has also been shown to promote the function of CD4+CD25+ Treg [255]. In uninjured or healing tissue, HMW-HA provides an important signal for Treg populations to persist, to resolve inflammatory processes and maintain homeostasis. There are reports of HA cross-linking to cables at sites of inflammation as part of a protective mechanism in inflammatory processes (reviewed in Ref. [256]). These HA cables are pro-adhesive to leukocytes, but suppress their activation by impeding the interaction with inflammation-promoting receptors on the underlying tissues. Moreover, the cross-linked HA structures are suggested to sequester pro-inflammatory mediators and to be more resistant to degradation in injured tissue, both being important events for limiting inflammation.

Although all these findings clearly emphasize the potential of HMW-HA and CS to gear inflammatory into regulatory processes suggesting their potential use for immunomodulating biomaterial coatings, data on controlling function of immunocompetent cells by aECM composed of collagen and GAGs are lacking. Our group recently addressed the immunomodulatory effects of aECM that was generated utilizing the natural self-assembly potential of type I collagen in combination with either HA or CS. HA was additionally modified by attaching of sulfate groups at low (S1) or high levels (S3) providing binding sites for endogenous growth factors and inflammatory mediators (Fig. 6A). Induction of tolerogenic DC function by aECM would provide a powerful tool to promote resolution of inflammation and to modulate T cell activity at the implantation site. In accordance with other studies [128] we found that collagen alone provokes DC maturation (Fig. 6B). Culture of immature DCs on collagen induces up-regulation of the co-stimulatory molecules CD80 and CD86 (Fig. 6B) as well as release of IL-12p40 (Fig. 6B), signals through which DCs direct differentiation of T cells toward an immune response. Of note, incorporation of the GAG derives into the collagen matrix attenuated the collagen driven DC maturation. Release of IL-12p40 and expression of maturation marker (MHC, CD86, CD80) were down-regulated following DC interaction with aECM (Fig. 6C). Moreover, DC maturation induced by LPS, a potent activator of DCs, is also diminished in the presence of aECM as seen by reduced expression level of MHC and CD86 as well as decreased secretion of IL-12p40 (Fig. 6C). Dendritic cells that have been prevented to mature are prone to develop a tolerogenic phenotype [113,114]. T cell stimulation in an environment lacking of co-stimulation (as typical for tolerogenic DC) can lead to T cell clonal anergy and adaptive tolerance [257]. As one consequence the inflammatory activity of T cells at the implantation site could become compromised either by T cell growth arrest or by down-regulating their effector functions. Additionally T cells with a regulatory phenotype could be induced capable to suppress naïve T cell responses but also the activity of innate immune cells like DC and macrophages through their release of immunosuppressive IL-10 and TGF-β. We are currently investigating T cell responses triggered by “aECM-activated” DC. Since our data suggest immunomodulatory capacities of aECM for DC we will further expand our investigations on innate immune cells including PMNs and macrophages.

5. Conclusions

For a long time, biomaterial science focused on inert materials which are recognized as foreign by the host but remain essentially unchanged and tolerated due to their encapsulation in fibrous tissue. However, it has been realized that permitting specific cell responses may be beneficial for biomaterial integration and improve implant performance. Cellular responses at the implantation site will also include immune responses to biomaterials resulting in their rejection or degradation. With expanding knowledge on these processes, biomaterial engineers have begun to develop materials capable of avoiding such detrimental immune responses. Current strategies for the design of these biomaterials focus on alteration of material surfaces either by modification of their physicomechanical features or by rendering them biologically active to systematically target cell behavior. Among the latter, functionalization of biomaterials with artificial ECM coatings appears to be particularly promising.

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