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Review

Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response

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Current state-of-the-art biomedical implants and tissue engineering methods promise technologies to improve or even restore the function of diseased organs. However, one of the biggest challenges to clinical success is the lack of functional integration. A series of cellular and molecular events following biomaterial implantation poses an important bottleneck for developing breakthrough solutions. With inflammation increasingly recognized as a crucial component influencing regeneration, immunomodulation or immuno-engineering has emerged as a potential solution to overcome this key challenge in regenerative medicine. We postulate possibilities to utilize biomaterial physicochemical modifications to modulate the host inflammatory response and develop strategies for effective biomaterial integration. Biomaterial-based immunomodulation strategies can significantly ameliorate the outcomes of medical implants and tissue engineering therapies.

Immune-Modulatory Biomaterials

The fields of biomaterials and tissue engineering are rapidly progressing towards developing mainstream solutions for a wide variety of healthcare problems. Biomaterials have played a central role in the implantable medical device industry and have improved the lives of millions of people worldwide. With synergistic progress in stem cell biology, biomaterials, and advanced fabrication techniques, there has been an exponential increase in the use of biomaterials in the tissue engineering field in previous decades. The result has been the generation of not only biohybrid artificial organs in combination with synthetic constructs but also fully functional bioengineered substitutes such as blood vessels, heart valves, kidneys, bladders, and airways [1,2].

Nevertheless, adverse immune reactions to biomaterials are crucial challenges resulting in a reduced quality of life for patients [3]. These adverse reactions are often seen to interfere with healing, leading to immediate acute outcomes such as immense pain, excessive inflammation, tissue destruction, or even isolation and rejection of medical devices. With tissue engineering implants containing living cells, inflammation can directly interfere with tissue mass-transfer requirements. The lack of detailed understanding of biomaterial–immune system interactions, resulting in significant pathological changes in the microenvironment, is a major barrier to developing effective biomaterial-based therapies. Developing strategies and solutions to avoid

Trends

Host response to biomaterial implantation: there is a continuous need to avoid or exclude undesired, adverse foreign-body host immune response to implanted biomaterials, representing one of the most important challenges in the biomedical sector.

Novel immundomodulatory biomaterials: discovering novel biomaterials that can mitigate the foreign-body response and enhance engraftment is a new trend in the clinical translation of biomaterial and tissue engineered products.

Harnessing inflammatory response: the trend is slowly shifting from developing biocompatible 'immune-evasive' biomaterials to 'immune-interactive' smart materials to harness the beneficial effects by modulating the inflammatory response towards healing and regeneration.

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or minimize undesired side effects in the use of biomedical devices/implants still represents an important challenge in the biomedical field. In addition, with the ever-increasing use of complex biomaterials in tissue engineering, it is important to define the broader biomaterial–host inflammatory interactions to not only predict the fate of these materials post-implantation but also modulate immune cell biology to overcome translational challenges in regenerative medicine. To address these complexities, innovative approaches for the control of immune response is of the highest priority. The objective is to modify the biomaterial design principles to create a microenvironment niche that controls the inflammatory response to implanted biomaterials and promotes tissue regeneration. Importantly, the term 'immunomodulatory biomaterials' in this paper is limited to the field of regenerative medicine and should not be confused with biomaterial-based immunotherapy utilized to treat immune-related diseases and cancers.

Factors Modulating the Interaction of Immune Cells with Biomaterials

The human immune system consists of innate and adaptive immune systems that play a distinct and significant role in reacting against any foreign material that is deemed to be 'dangerous'. In both types of responses there are numerous humoral and cellular factors that are essential for mounting effective immune responses. Figure 1 illustrates the cells and proteins involved in mediating these responses that occur in a coordinated and tightly controlled way. Host reactions to the biomaterial following implantation determine the success of integration and biological performance of implants such as biomedical devices, sensor electrodes, orthopedic implants, and tissue engineering scaffolds [4]. The outcome of biomaterial implantation varies depending on the extent of the **foreign-body response** (FBR, see Glossary) and subsequent homeostatic mechanism leading to the cellular processes of inflammation and wound healing. Figure 2 highlights the key players and events that influence multiple phases of immune response either towards fibrous tissue repair and encapsulation or rarely observed complete regeneration.

In the early stages of implant insertion, injury to blood vessels causes the extravasation of blood around the implant initiating the blood–material interaction cascade. Plasma components including proteins, lipids, sugars, and ions are adsorbed on the implant surface within minutes [5,6]. Factors such as the topography, roughness, chemistry, and energy of the surface affect

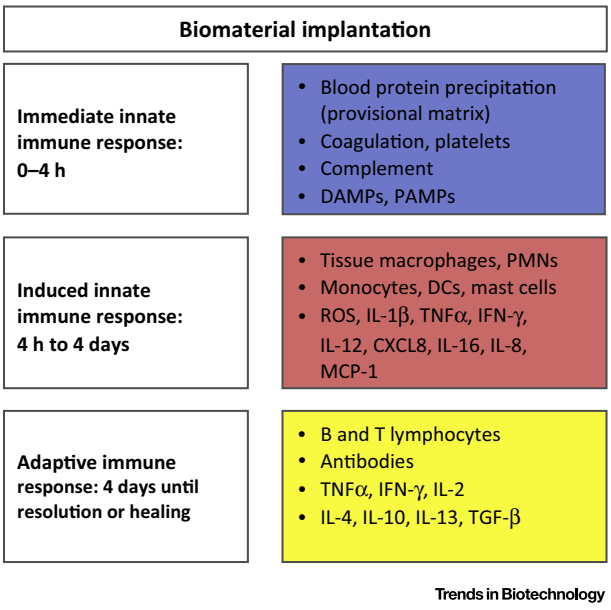

Figure 1. Overview of the Cells and Proteins Involved in Mediating Effective Immune Responses.

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the types and quantities of molecules that are adsorbed, and influence this composition and further recruitment and attachment of tissue-derived, inflammatory, vascular, and stromal cells over the course of several hours [7–12]. The blood exudate also consists of platelets forming a fibrin-rich clot by aggregation and coagulation. The clot serves as a depot for cytokines and growth factors which provide signals to initiate wound repair and serve as a transient provisional matrix for cell migration and attachment [13]. **Complement** proteins initiate the **innate immune response** in conjunction with specific structures such as **danger-associated molecular patterns** (DAMPs) and **pathogen-associated molecular patterns** (PAMPs). They are recognized by a limited number of pattern recognition receptors (PRRs) primarily expressed on macrophages and dendritic cells (DCs). This triggers release of several proinflammatory cytokines, chemokines that induce directed chemotaxis of other innate inflammatory cells [14], and maturation of DCs that leads to the activation of adaptive immunity via B and T lymphocytes.

While chemotaxis of polymorphonuclear neutrophils (PMNs) and recruitment of systemic monocytes differentiating into macrophages [15,16] is necessary for clearing debris and eradicating pathogens, the presence of biomaterial can intensify the inflammation by introducing antigens to the injury site. These migrating monocytes/macrophages attach to the transient provisional matrix on the biomaterial surface via integrins, which play a role in macrophage activation [17]. During the chronic inflammatory phase, the major source of cytokines is directly or indirectly activated T lymphocytes, particularly helper T cells expressing CD4 and their subsets Th1 and Th2. Their prolific cytokine production tends to largely influence the proinflammatory and anti-inflammatory responses [18]. B cells contribute to the immune response by generating antibodies. Lymphocytes are also capable of adhering to the material surface and are affected by pre-adsorbed proteins [19,20].

Both innate and adaptive immune cells associated with the above inflammatory process can be characteristic contributors to the resulting FBR. However, macrophages and T lymphocytes activated by mature antigen-presenting DCs appear to dominate the progression from chronic inflammation towards regeneration. For example, macrophages and resulting fusion into foreign-body giant cells (FBGCs) for phagocytosis of larger objects are a key innate component of material recognition and FBR [21]. The M1 subtype secretes numerous enzymes, including collagenases and different cytokines, such as TNF-α, IL-1, IL-6, IL-8, and IL-10, which further stimulate the inflammatory response. Alternatively, the activated M2 phenotype, which can be stimulated by several factors such as glucocorticoids, IL-4 or IL-13, releases anti-inflammatory cytokines to resolve inflammation [22–24]. If for any reason the host immune system fails to enhance M2 macrophage levels for switching to the healing stage, it could lead to the inability of macrophages to resolve the inflammation, resulting in a state named 'frustrated phagocytosis' [25,26]. While activated monocytes giving rise to specific tissue macrophages play a central role in the prevention of biomaterial integration, other host inflammatory cells at the site of inflammation such as dendritic cells and lymphocytes are also important in this process. The phenotype correlation of M1/M2 macrophages with the change in cytokine profile from Th1 to Th2 cell type suggests T lymphocytes as strong targets to promote resolution of inflammation and regeneration. The design approaches for biomaterials that are directed to **immunomodulation** must therefore account for the activation of immune cells, in particular when macrophages and lymphocytes can influence each other via direct and indirect mechanisms [27].

Immuno-Engineering Strategies for Biomedical Implants

Immunomodulation around implants and tissue-engineered structures can be achieved by using different strategies illustrated in Figure 3 (Key Figure) such as (i) changing the chemical properties of the base biomaterial, (ii) controlling the release of anti- or proinflammatory cytokines from biomaterials, (iii) modifying the physical properties at the biomaterial host interface, and (iv) cell

Glossary

Adaptive immune response: the response that develops later and involves-antigen specific lymphocytes.
Complement: a set of plasma proteins that act together as a defense against pathogens by the classical, alternative, or lectin pathways.
Danger-associated molecular patterns (DAMPs): molecules that can initiate a non-infectious inflammatory response.
Foreign-body response (FBR): a series of inflammatory events and wound-healing responses to biomaterials, leading to fibrosis.
Immunomodulation: the deliberate attempt to change the course of an immune response.
Innate immune response: a response to a foreign particle that is due to the presence of, and immediate activation of, the innate and relatively non-specific defense mechanisms of the body.
Pathogen-associated molecular patterns (PAMPs): molecules specifically associated with groups of pathogens and that are recognized by cells of the immune system.

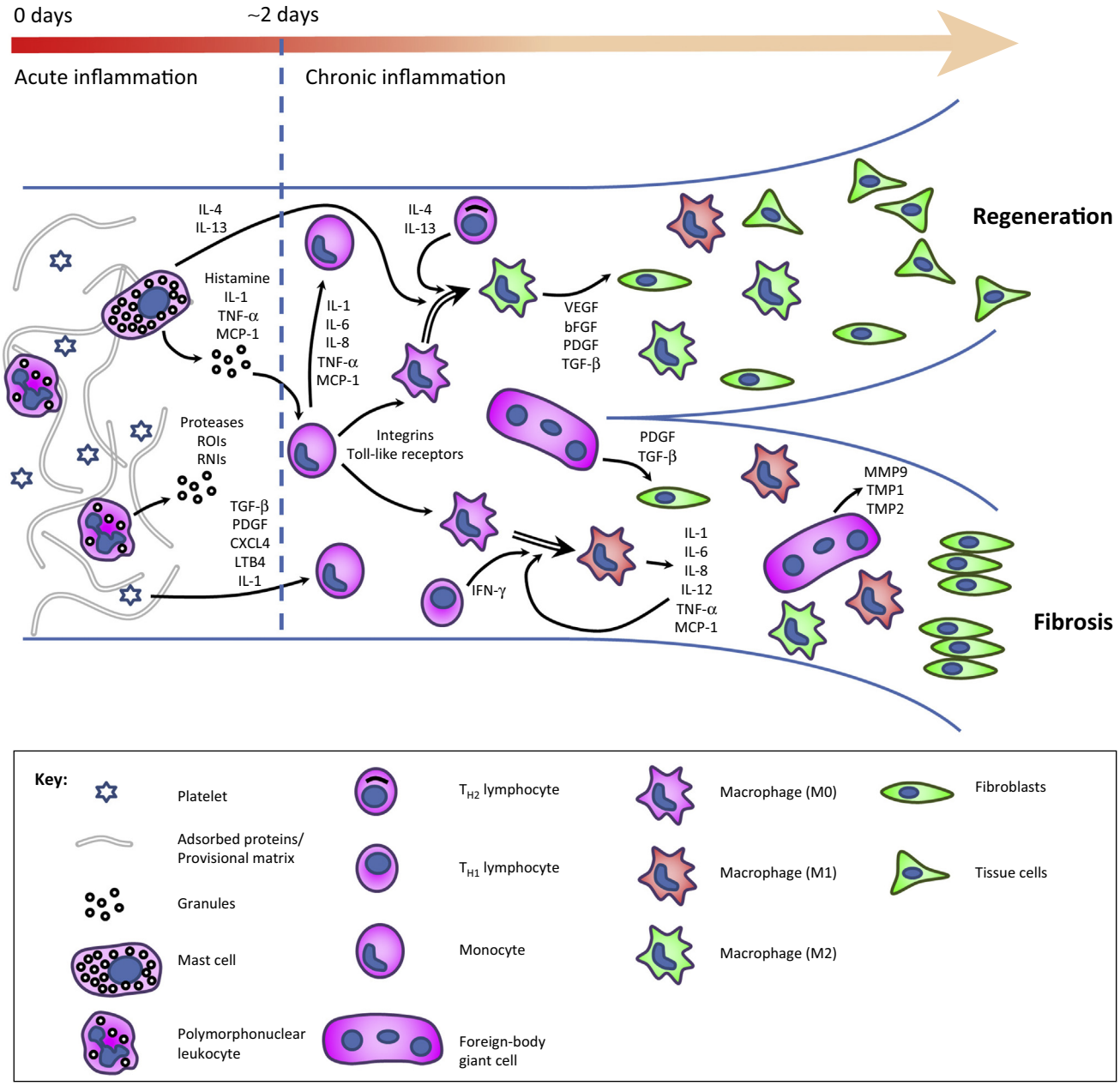


Figure 2. Overview of the Immune Response to a Biomaterial. Implantation of a biomaterial, and the accompanied damaging of blood vessels, triggers an acute inflammatory response. This phase starts with the absorption of proteins to the surface and the formation of a provisional matrix. The matrix contains a high amount of platelets that plug the injured blood vessels, but also serve as a reservoir for growth factors and cytokines. This environment results in the recruitment of polymorphonuclear leukocytes and mast cells, which characterize the acute inflammation phase. A range of growth factors are subsequently secreted by the platelets and through degranulation of the neutrophils and mast cells, which are key players to recruit macrophages to the site of implantation. The following stage is the chronic inflammatory phase defined by the presence of mononuclear cells. The secretion of factors by amongst others the monocytes propagates further recruitment of peripheral blood monocytes that can differentiate to become macrophages upon activation. During the initial phase of chronic inflammation, there is an increase in specific proinflammatory (M1) macrophages in the injured site. Based on, among others, stimulation by T_H lymphocytes, the polarization of the macrophage population can change to a pro-healing phenotype (M2). When this switch is made, tissue cells are stimulated towards regeneration of the implant site. However, if the environment fails to induce the switch to an abundance of M2 macrophages, the chronic inflammation phase will lead to fibrosis.

Key Figure

Schematic Representation of Different Strategies that Can Be Used To Achieve Immunomodulation Around Implants and Tissue-Engineered Structures

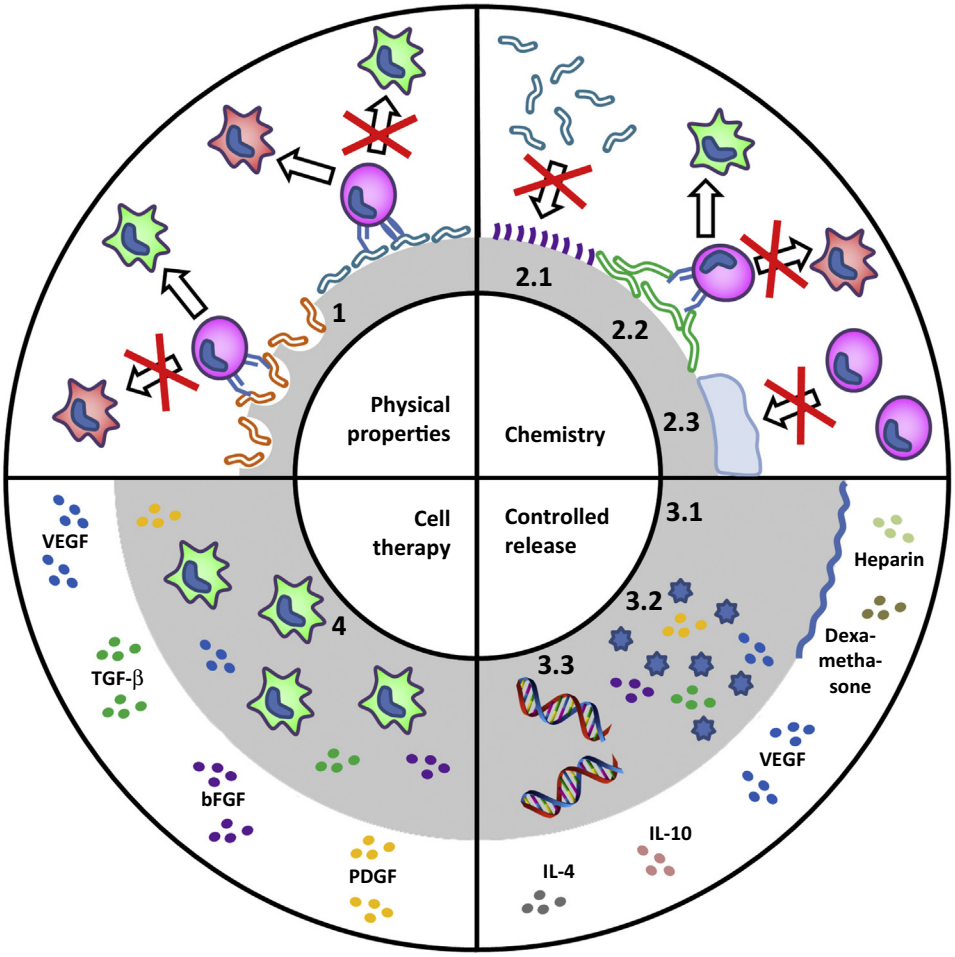


Figure 3. The activation of monocytes into mature tissue macrophages among other host inflammatory cells will play a central role in tissue remodeling and integration at the biomaterial-host interface. Various strategies include (1) The use of biomaterial physical properties such as stiffness or topography to control adsorption of specific proteins. This way, the integrin adhesion of monocytes and thus polarization of macrophages can be adapted. (2.1) The use of non-biofouling coatings to prevent protein adhesion to the biomaterial surface; (2.2) use of biomimetic ECM components to control the integrin adhesion of monocytes and to disturb subsequent M1 activation and/or induce M2 activation of macrophages; (2.3) use of hydrogels to isolate implants from immune cells and thus to limit an inflammatory response. (3.1) The use of surface coatings for the delivery of soluble anti-inflammatory agents; (3.2) use of biomaterial embedded particles for the delivery of soluble anti-inflammatory agents; (3.3) use of gene delivery systems to induce residing cells to secrete anti-inflammatory agents. (4) The use of embedded immune cells secreting pro-angiogenic and pro-regenerative cytokines.

therapy methods via the direct inclusion of immune cells or induction of the incoming cells upon implantation.

Immunomodulation Strategies Using Biomaterial Chemistry

Modifying the surface chemistry of biomaterials is a straightforward way to modulate protein adsorption and, subsequently, cell behavior. Tuning the surface properties and changes in the surface chemistry directly affect the biological behavior of immune factors.

Use of Passive Non-Biofouling Strategies

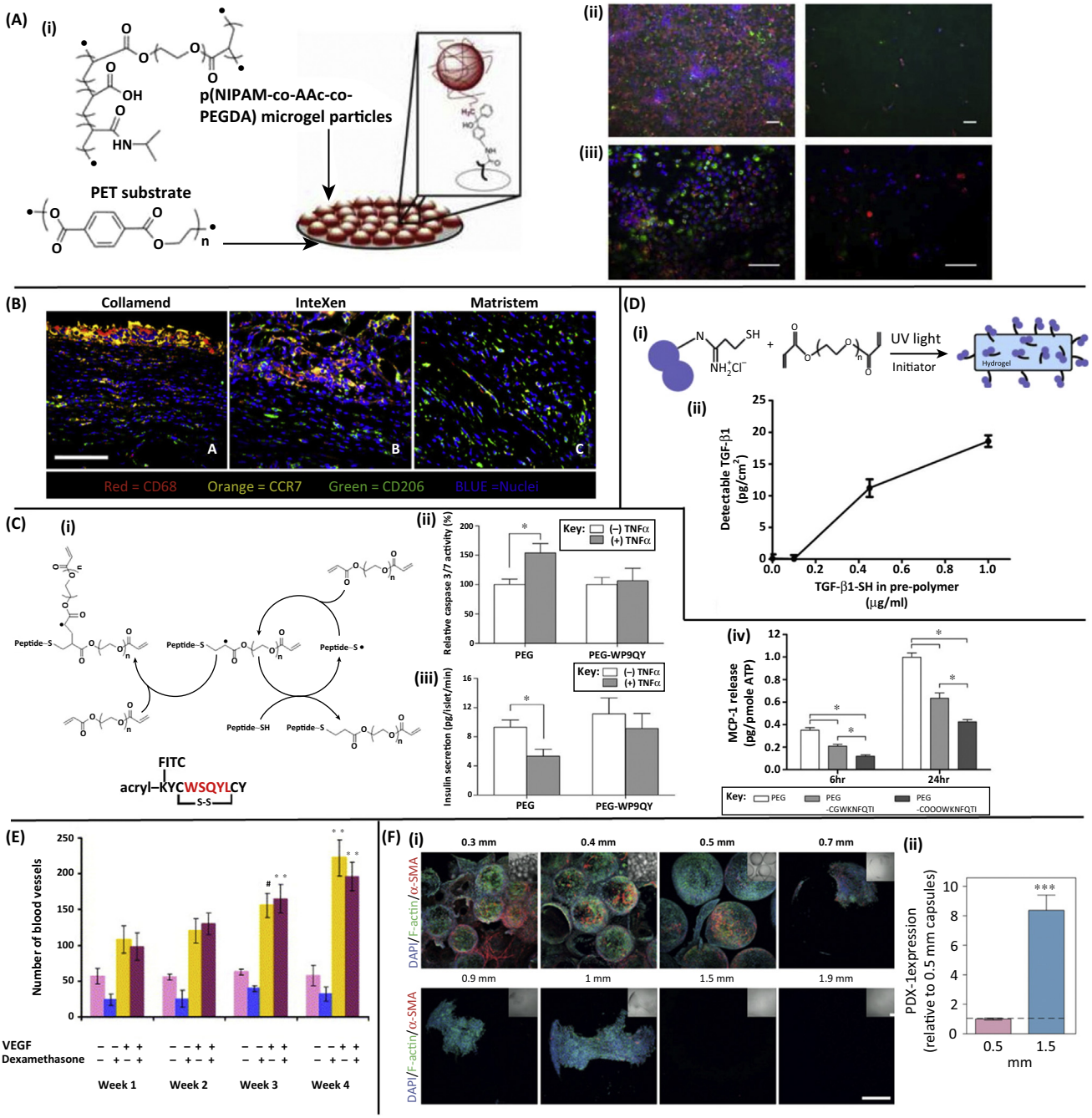
Efforts to attenuate the host inflammatory response to implants have led to the development of immune-isolating materials for coating implant surfaces [28]. Traditional surface-modification strategies, such as non-fouling dense hydrophilic polymeric films and brushes, work passively by reducing protein adsorption, leukocyte activation, and adhesion to decrease the FBR [29]. Semipermeable hydrogels have been widely researched as non-fouling implant coatings owing to their unique properties including high water content, ease of solute transport, and possibility to have different active groups for further chemical modifications [28,30]. Bridges *et al.* reported a coating strategy with poly(*N*-isopropyl acrylamide) (pNIPAm) films made of microgel particles that were crosslinked with short chains of poly(ethylene glycol) (PEG). The hydrogel effectively filled the non-uniformities of the surface of the model substrate and was able to prevent primary human macrophage adhesion *in vitro* and attenuate acute phase leukocyte adhesion and the levels of proinflammatory cytokines *in vivo* [28] (Figure 4A).

Although these passive strategies have shown promise *in vitro* and during the acute period of a few days following implantation, they lack long-term stability and have failed to show consistent favorable responses for *in vivo* chronic inflammation [31]. To address these challenges, Wong *et al.* reported a strategy to form a stable, defect-free, inert slippery interface by creating self-healing slippery liquid-infused porous surfaces (SLIPS) [32]. Moreover, recently it has been argued that, instead of a direct reduction of all protein adsorption, it is more crucial to control the unfolding of the adsorbed proteins and presentation of cryptic bioactive sites, which can trigger adverse reactions. In other words, the development of more bioactive strategies rather than the exclusion of the implanted material from all interaction with the surrounding tissue is needed [33].

Use of Biomimetic Extracellular Matrix (ECM) Components

Immunomodulation around biomaterials can be based on the properties of the biomaterials used [34]. Structures that mimic or directly use ECM components can create a microenvironment that can be conducive to the normal wound healing and repair mechanisms. For example Kajahn *et al.* demonstrated that the presence of highly sulfated hyaluronan (HA) can significantly disturb IL-6-, IFN-γ-, and MCP-1-mediated M1 activation and even induce M2-related cytokine IL-10 [35]. The effect can depend on the degree of sulfation of HA because sulfated glycosaminoglycans (GAGs) are known to interact with cytokines and growth factors, and have a direct effect on their bioactivity. Such artificial ECMs can be used as a coating to decrease the amount of inflammation with the aim of preventing chronic M1 activation of macrophages that are the driving factor behind impaired healing around implants. In a similar way, Brown *et al.* [36] demonstrated that the composition of implanted surgical meshes has a direct effect on the M2:M1 macrophage ratio in the implantation site, which can be an accurate predictor of the final healing outcome (Figure 4B).

Another common approach to directly use ECM components to create an immune-compatible microenvironment is de-cellularization of tissues to obtain cell-inductive scaffolds. De-cellularization enables the removal of most immunogenic components, including genetic material, membrane antigens (α-Gal), and MHC/HLA molecules. Indeed, it has been shown that xenogenic tissues implanted without de-cellularization induce a rejection response, whereas



Trends in Biotechnology

Figure 4. Immuno-Engineering Strategies for Biomaterial Implants. (A) (i) Chemical structures of unmodified poly(ethylene terephthalate) (PET) substrate and p(NIPAM-co-AAc-co-PEGDA) microgels used to functionalize the PET disks. (ii) Samples were implanted in mice peritoneal cavity for 48 h and immunostained for CD68 (macrophage marker, green), F-actin (red), and DNA (blue). As opposed to the PET (left panels), microgel-coated disks (right panels) had significantly less leukocyte adhesion and fewer macrophages around the implants. Reprinted with permission from Bridges *et al.* [32]. (B) Relative expression levels of M1 and M2 markers around surgical meshes of different composition *in vivo*. CD68 (macrophage marker), CCR7 (M1 marker), and CD206 (M2 marker); the ratio of M2:M1 markers was in good correlation with histological scores (as a measure of healing), where Matristem had the highest M2:M1 ratio and the best histological score. Reprinted with permission from Brown *et al.* [39]. (C) (i) Reaction scheme of thiol-acrylate photopolymerization used to engineer PEG hydrogels functionalized with FITC-tagged affinity peptide. (ii) PEG-WP9QY hydrogels have a cytoprotective effect to encapsulated mouse islets during TNFα (50 ng/ml) treatment, as assessed by relative caspase 3/7 expression (normalized to total islets in the gels and afterwards to the caspase 3/7 expression in non-treated islets) 2 days post-TNFα treatment and insulin secretion after 5 days of induction with TNFα. ($n = 3$, mean ± SEM, $P < 0.05$). (iii) MCP-1 release from MIN6 cells encapsulated in 10% PEG control hydrogels or functionalized PEG-peptide

(Figure legend continued on the bottom of the next page.)

de-cellularized structures are generally well tolerated even though they elicit IgG responses. Moreover, the de-cellularized ECM can contain immunomodulatory cytokines and growth factors. However, the processing parameters used to produce these ECM materials significantly affect their final fate upon implantation, owing to the presence of residual cells or cellular components, introduction of non-degradable crosslinks within the structure to improve its mechanical properties, and the remnants of de-cellularization or crosslinking reactions [37].

Use of Hydrogels for Immunoisolation of Encapsulated Therapeutic Cells

The implantation of donor therapeutic cells is a clear biomedical application for which immunomodulation is crucial. Host recognition and FBR are the key hurdles to the successful clinical implementation, such as donor pancreatic islets containing systems for type 1 diabetes treatment [38]. Semipermeable hydrogels can prevent direct physical contact between encapsulated islets and infiltrating immune cells around implants, but small molecules, including ROS, NO, and cytokines, can easily diffuse through the hydrogel network. Thus, in addition to delivering anti-inflammatory agents, polymeric hydrogels can also be designed to incorporate traps for capturing proinflammatory signals. Anseth and colleagues have explored the use of affinity hydrogels to sequester TNFα, a proinflammatory cytokine, and MCP-1, a monocyte chemoattractant protein, which are key mediators of proinflammatory response [39,40]. In this approach, the backbone of RGD-functionalized PEG hydrogels, commonly used for cell encapsulation, was modified with peptides that specifically and with a high affinity bind to these cytokines (Figure 4C). Their results indicate that the *in vitro* survival and function of encapsulated cells were significantly better in peptide-modified PEG hydrogels than in unmodified hydrogels; however, they have not investigated the *in vivo* performance.

Immunomodulation Using Bioactive Strategies

In recent years, significant advances have been made in developing bioactive strategies wherein anti-inflammatory and/or pro-wound-healing molecules are delivered locally from a reservoir or a coating. Some of these approaches are discussed in the following sections.

Delivery of Pharmacological Anti-Inflammatory Small Molecules

The delivery of soluble pharmacological anti-inflammatory agents such as dexamethasone, heparin, and superoxide dismutase from reservoirs and coatings has shown reduced inflammation and fibrous encapsulation [41–43]. The use of these drugs for long-term implant survival has long been limited by the complex pharmacokinetics of these drugs and reduced drug concentration and bioactivity over time, but novel sustained-release strategies using nanoparticle-embedded matrix coatings of dexamethasone have been successful for immunomodulation in neural prostheses over a period of 3 weeks [44,45].

Chemokines and their receptors are key regulators of the local inflammatory response by directly modulating the cellular infiltration around implants [46]. Cytokines and growth factors actively modulate the phenotype of the immune cells. Thus, these immune players are promising components for sustained release from bioactive coatings. Cytokines can be delivered either by their direct inclusion or by nucleic-acid delivery-based strategies for the sustained production

hydrogels incubated in IFN-γ (750 units/ml), IL-1β (10 units/ml), and TNFα (500 units/ml) (peptide = 100 mM, mean ± SEM, $n = 4$, $P < 0.05$). Reprinted with permission from Lin *et al.* [42,43]. (D) Direct immobilization of TGF-β1 in PEG hydrogels by the use of thiol-acrylate photopolymerization. Release of TGF-β1 significantly reduced the maturation of dendritic cells. Reprinted with permission from Hume *et al.* [51]. (E) Average vessels (per 25×10^{-3} mm^2 area of subcutaneous tissue) around biomaterial implants containing dexamethasone, VEGF, a combination of VEGF and dexamethasone, or no bioactive agents over 4 weeks in rats (#, $P < 0.01$; **, $P < 0.001$). Reprinted with permission from Patil *et al.* [60]. (F) (i) Degree of fibrosis of spherical alginate gels (0.5 ml in volume) of eight different sizes (0.3, 0.4, 0.5, 0.6, 0.7, 1, 1.5, and 1.9 mm) implanted into mice intraperitoneal cavity and retrieved after 14 days. Confocal images of retrieved spheres stained for cellular nuclei (DAPI), F-actin (phalloidin), and myofibroblast cells (α-SMA) show a significant decrease in cellular deposition and fibrosis formation with increasing size. Scale bar, 300 μm. (ii) 1.5 mm alginate capsules had significantly higher rat islet marker PDX-1 expression than 0.5 mm spheres as seen by qPCR analysis (mean ± SEM, $n = 5$ mice per treatment; ***, $P < 0.0001$). Reprinted with permission from Veiseh *et al.* [79].

and release of the cytokines by the cells present in the implantation site [47]. For direct inclusion, PEG hydrogels with immobilized cytokines such as TGF-β or IL-10 have been developed [48] (Figure 4D). These cytokines were still effective after their immobilization and suppressed the maturation of dendritic cells even under stimulation by lipopolysaccharide (LPS) or proinflammatory cytokines. For delivery via nucleic acid, some examples are adenoviral delivery of genes encoding IL-4 or IL-10 [49], or genes encoding the antibodies that would be able to neutralize the proinflammatory signals [50].

One of the commonly used delivery systems for immuno-engineering is polyelectrolyte multilayer coatings because the thickness of the coating can be easily controlled and the loading and controlled release of hydrophilic bioactive agents is easily achievable [51,52]. Other widely used methods are the tethering of cytokines or direct chemical conjugation. The main problem with the direct delivery of the cytokines is sustaining clinically relevant concentrations for long periods. This obstacle is the driving force behind the use of gene delivery-based systems where the induction of the desired cytokines or overexpression of the transcription factors can increase the expression of the related genes and keep the concentrations of the target cytokines at desired levels in the long term. Novel hydrogel-based gene delivery strategies have also been explored for delivery of antisense oligodeoxynucleotides to downregulate the levels of endogenous proinflammatory signals produced by local immune cells at the wound site [53]. The downside of the gene delivery methods is the intrinsic risks with viral delivery systems and the low efficacy of the non-viral methods at the moment. Moreover, the vectors utilized can be immunogenic, adding an additional level of complexity.

Strategies for Combined Delivery of Anti-Inflammatory Biologics

Recent studies have indicated that the combined delivery of glucocorticoids and anti-inflammatory cytokines (IL-6 and IL-10) can promote the repair phase of inflammation [54]. Although glucocorticoids attenuate inflammation around implants, their delivery can inhibit endogenous angiogenesis and can increase the risk of infection [55,56]. To address this issue, Burgess and colleagues studied the pharmacodynamic effects of the simultaneous delivery of dexamethasone and VEGF as an anti-inflammation and neo-angiogenesis combination therapy using PLGA microspheres/PVA hydrogel composites [57]. Using a rat model, they showed that implants coated with VEGF-containing hydrogels had significantly more mature blood vessels after 4 weeks following implantation, with or without dexamethasone (Figure 4E). Thus this combinatorial approach enabled overcoming the anti-angiogenic effects of glucocorticoids, and helped to promote healing around implants.

Immunomodulation Using Physical Biomaterial Properties

As shown in numerous works, biomaterial physical properties such as surface roughness, topography, and geometry are properties that play crucial roles in protein adsorption and the immune cell response. Topographical changes on the nanometer scale can modify the activity of adsorbed proteins via conformational changes during adsorption [58]. To influence cells directly, larger surface features, ranging between 10 and 100 μm, are generally used [59], but nano-sized topographies have also been explored to direct cell responses [60].

Using Surface Topography as a Cue

It has been shown that surface engineering, such as changing the surface hydrophilicity, nano/microtopography, and charge, can control events that are instrumental in the immune response to biomaterials. Leong and colleagues studied the effect of nano/microtopography in macrophage behavior in the FBR using gratings (500 nm to 2 μm parallel) imprinted on selected polymer surfaces [61]. Although they did not observe any distinctive trends from the differently sized gratings, they found that, compared to planar controls, different grating topographies induced changes in macrophage behavior on all polymer surfaces, independently of surface

chemistry. *In vitro* cytokine secretion levels and cellular morphology were affected, while *in vivo* larger size gratings altered the adhesion of cells in comparison to planar controls. McWhorter *et al.* reported that micro and nano-topographical features can effectively determine macrophage cell shape and orientation. Using micro-patterning, they demonstrated that elongation of the cells itself, without the addition of exogenous cytokines, results in the upregulation of M2 markers in mouse macrophages and a reduction in proinflammatory cytokine secretion. Moreover, they showed that cell elongation enhances the effect of M2-induction via IL-4 and IL-13 while it reduces their reaction to stimuli such as IFN-γ and LPS (M1-induction) [62].

More examples are available in orthopedic and dental applications where titanium is widely used [63]. Despite its good biocompatibility in comparison to other metals, the immune responses triggered by titanium are considerably stronger than for some other biomaterials, and inflammation is an important aspect of titanium implant failure. This has led to research focused on possible surface modifications to decrease the immune response to titanium structures. This can be achieved by several methods such as nano/microstructures [64] on titanium surfaces or chemical processes that change surface properties such as hydrophobicity and roughness [65]. For example, anodization of titanium surfaces, which causes the development of titanium dioxide nanotubes on the surface, has been shown to cause significantly less proinflammatory cytokine release [66]. Moreover, it has been shown that the titanium nanotube size can be optimized to reduce macrophage attachment (lowest attachment being at 60–70 nm nanotube diameter range) [67], and a significant decrease in macrophage migration was observed on nanostructured titanium surfaces [68]. The adhesion of macrophage has been seen to result in lymphocyte adhesion and activation [69].

With respect to chemical modifications, Alfarsi *et al.* recently demonstrated that hydrophilic modified sand-blasted, acid-etched (SLA) surfaces caused downregulation of 10 proinflammatory genes when compared to SLA surfaces only [70]. Li *et al.* have shown that the secretion of several proinflammatory cytokines (e.g., TNF-α, IL1-β, and MCP-1) was considerably higher on pristine titanium surfaces compared to heparin/fibronectin immobilized titanium surfaces owing to the lower hydrophilicity of the surface [71]. The impact of biomaterial topography and chemistry on function of antigen-presenting cells has recently been reviewed in detail by Rostam *et al.* [72].

Using Implant/Biomaterials Geometry as a Cue

Several studies have looked at the role of implant geometry, including size and shape, in controlling FBR [73–76]. For example, Salthouse *et al.* studied *in vivo* biocompatibility of rods extruded from various medical-grade materials with differently shaped cross-sections [75]. They noted that rods with circular cross-sections produced the least-extensive FBR, compared to pentagonal and triangular cross-sections. These important studies showed that the implant shape has a significant effect on macrophage behavior. Smooth, well-contoured surfaces cause less acute reactions than implants with sharp features, corners, and acute angles [77]. Recently, Veiseh *et al.* studied the role of implant geometry, particularly size of spherical biomaterials, on *in vivo* biocompatibility [77]. They examined spherical implants from 0.3 to 1.9 mm in diameter in rodents and primates, and observed that the larger implanted spheres, with diameters of 1.5 mm and above, significantly suppressed the FBR and fibrosis for a long period for a variety of materials including alginate hydrogels, metals, glass, and plastics (Figure 4F). The effect depended on a combination of large size and spherical geometry, but was independent of total implanted surface area.

Cell-Based Immunomodulation Strategies

Recent advances in the understanding of the immune responses to biomaterials and tissue-engineered constructs has led to the realization that immune cells can be beneficial in the indirect

induction of other desired biological events, such as angiogenesis. Many pro-angiogenic factors such as TNF-α and IL-1 are produced in high concentrations by immune cells such as macrophages. By treating osteoblast/endothelial cell co-cultures with macrophages derived from THP-1 cells, Dohle *et al.* induced the formation of microvessel-like structures with increases in expression of E-selectin and ICAM-1 [78]. Thus, using macrophages as a pro-angiogenic reservoir is a possible way of addressing one of the fundamental problems in tissue engineering – the proper vascularization of thick engineered tissues. Because methods for isolating specific blood-derived monocytes (such as $CD14^+$ monocytes) and their differentiation to desired cell types such as macrophages of a given phenotype are readily available, it is possible to develop such therapies with autologous cells.

The immunoregulatory properties of mesenchymal stem cells (MSCs) have been widely investigated. Swartzlander *et al.* reported that encapsulated MSCs attenuated the fibrotic response of the FBR compared to acellular hydrogels by downregulating the classically activated macrophages [79]. Based on the anti-inflammatory properties of MSCs, Kim *et al.* proposed an alternative method of producing M2 macrophages via co-cultures of monocytes with MSCs [80]. These macrophages, dubbed 'MSC educated macrophages', showed an anti-inflammatory phenotype with the exception of increased IL-6 production, and they can be used in conjunction with MSCs in regenerative medicine applications. However, such models need to be adjusted for each specific application because another study demonstrated that, even though co-culture of MSCs with macrophages improves osteogenic and adipogenic differentiation, it actually attenuates chondrogenic differentiation [81]. Another possibility is to induce macrophages by use of specific biomaterials and then use the secretions of the induced macrophages (conditioned medium) to differentiate other cells. Chen *et al.* used conditioned media from macrophages which were put in contact with β-tricalcium phosphate for inducing osteogenic differentiation of bone marrow-derived MSCs, with significant increases in osteogenic differentiation markers [82]. These studies demonstrated that the incorporation of macrophages to improve tissue formation is a viable method that can have a positive effect on tissue engineering and regenerative medicine systems.

Future Directions and Concluding Remarks

A better understanding of the significant role of innate immune cells in tissue remodeling and regeneration has made them an attractive target for improving the functionality of regenerative medicine efforts and implants [83]. A wide variety of strategies are available to orchestrate the initial immune response to implanted structures as detailed above. However, because the immune response is tightly regulated both spatially and temporally, more elaborate techniques will be necessary to attain optimal functional integration.

Apart from control of the innate immune responses, the next line of control can be achieved at the level of adaptive immunity via B cell and T cell responses [84]. Some of the most commonly used biomaterials, such as synthetic polymers and ceramics, can be spared of adaptive immunological responses owing to the lack of potential immunogenic components. However, new generations of biomaterials, where hybrids of organic and inorganic components, synthetic peptide structures, and cell-responsive polymeric components are increasingly used, necessitate the consideration of the **adaptive immune responses** to biomaterial structures. For a designed self-assembling polypeptide chain, for example, it is important to know whether the sequence and structure of the design resembles an antigen and can therefore be a functional epitope that triggers adaptive immunity. To optimize the adaptive immune response to biomaterials, a better understanding of the mechanism of events related to adaptive immunity, such as leukocyte attachment [85], is needed.

It is becoming increasingly important to ask fundamental immunological questions in the context of biomaterial development (see Outstanding Questions). In future, high-throughput systems

Outstanding Questions

Can we unravel the complexity of interactions between inflammation, fibrosis, and biomaterial rejection?

Can we examine the function of currently available biomaterials within the immune system?

How can we best design biomaterials that tune the immune response to promote integration and regeneration?

Is there evidence for the long-term efficacy and safety of immune-engineered biomaterials?

Can we develop a high-throughput system for the evaluation of patient-specific immune responses to different biomaterial formulations so as to design a personalized immunomodulatory regenerative approach?

which will be key to better mimic the FBR *in vitro* will allow the elucidation of biomaterial-specific responses in real-time [86,87], and will therefore substantially improve our ability to identify, predict, and control the immune response to implanted biomaterials. We believe, as the concepts surrounding the biocompatibility of biomaterials evolve, that the focus will shift from an evasion of the host immune system to an orchestrated interaction with it. This will also enable the discovery of new biomaterials.

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