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The role of biomaterials and scaffolds in immune responses in regenerative medicine: macrophage phenotype modulation by biomaterial properties and scaffold architectures

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Scaffolds are an integral part of the regenerative medicine field. The contact of biomaterials with tissue, as was clearly observed over the years, induces immune reactions in a material and patient specific manner, where both surface and bulk properties of scaffolds, together with their 3D architecture, have a significant influence on the outcome. This review presents an overview of the reactions to the biomaterials with a specific focus on clinical complications with the implants in the context of immune reactions and an overview of the studies involving biomaterial properties and interactions with innate immune system cells. We emphasize the impact of these studies on scaffold selection and upscaling of microenvironments created by biomaterials from 2D to 3D using immune cell encapsulation, seeding in a 3D scaffold and co-culture with relevant tissue cells. 3D microenvironments are covered with a specific focus on innate cells since a large proportion of these studies used innate immune cells. Finally, the recent studies on the incorporation of adaptive immune cells in immunomodulatory systems are covered in this review. Biomaterial–immune cell interactions are a critical part of regenerative medicine applications. Current efforts in establishing the ground rules for such interactions following implantation can control immune response during all phases of inflammation. Thus, in the near future for complete functional recovery, tissue engineering and control over biomaterials must be considered at the first step of immune modulation and this review covers these interactions, which have remained elusive up to now.

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1. Introduction

Scaffolds are an integral part of tissue engineering efforts where the control of their physicochemical properties creates the necessary microenvironmental conditions for complex organ generation. Starting with the inclusion of advanced three-dimensional (3D) printing technologies over the last 10 years, the forms and properties of scaffolds are constantly evolving towards the creation of smart, responsive scaffolds. The *in situ* printing using artificial intelligence, both for the design of scaffold architectures and their introduction to the dynamic environment of tissues and organs, improves their chances in potential clinical translation. The current developments in multi-ink 3D printing systems using multiple biomaterials also provide advanced environmental control over the micro-scale material properties of the scaffolds using multiple biomaterials. However, the level of sophistication achieved in scaffold architecture alone cannot overcome another important roadblock, the immune response to such structures both in the short and long term.

This is particularly due to the fact that immune responses by the host to foreign bodies such as biomaterials are not typical, because of the persistence of foreign body reactions, particularly if the biomaterial is not degradable or degrades slowly, modulated by two innate immune system cells, foreign body giant cells (FBGCs) and macrophages at the tissue/substrate interfaces.[1–3] The uniqueness of these interfaces and the specificity of the interactions between cells and implants depend on biomaterial chemistry and topography. Thus, in contrast to the traditional approach, which is based on the prevention of inflammation with the use of inert biomaterials, today biomaterial properties are used to modulate the immune response. In particular, *in vivo* application of these immunomodulatory implants yields very promising results in terms of recruiting

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and directing innate immune system cells along the direction of tissue regeneration. The level and type of response can be regulated through the design of biomaterials. For example, engineering the topography, changing the chemical and physical properties of biomaterial surfaces, releasing cytokines, and drugs from biomaterials can change the responses of the innate immune cells to an implanted biomaterial.[4–13]

For example, in a study, several different synthetic and natural polymeric or ceramic biomaterials with changing compositions were implanted in animal models (rat) subcutaneously for bone substitution and evaluated depending on the number of triggered multinucleated giant cells (MNGCs) by the nature of polymers. It was observed that biomaterials induced different cell types depending on their bulk or surface chemical and physical properties. For example, degradability of the biomaterials affects the immune cell recruitment differently, and nondegradable biomaterials induced only mononuclear cells, whereas degradable ones activated the formation of multinucleated cells.[14] In another study, two groups of surgical sutures made of nylon or polyglycolic acid polymers of the same size were implanted in a zebrafish model and Foreign Body Reactions (FBR) triggered by the sutures after the implantation were compared. It was reported that FBGCs were observed more around the polyglycolic acid sutures when compared with nylons.[15] Another study presented the use of electrospun polycaprolactone (PCL) nanofibers for tendon repair *in vitro* and *in vivo*. *In vitro*, co-cultures of macrophage and fibroblasts derived from human tendons were grown on nanofibers and it was reported that macrophage cells showed a distinct response to the alignment of the nanofibers. They elongated through the fibers and polarized to M2 phenotype which is a good inducer of implant integration to tissue. Similarly, *in vivo*, the same scaffolds were inserted into the Achilles tendons of the rats and non-aligned fiber scaffolds showed higher macrophage abundance with pro-inflammatory phenotypes after 7 days.[16] Another application of immune cell modulation by biomaterial properties was reported for the treatment of pelvic organ prolapse. In this study, electrospun poly L-lactic acid-*co*-poly ε-caprolactone nanofibrous meshes with and without endometrium derived mesenchymal stem cells were implanted in mice.[17] Earlier studies of the same group showed that nano- and microfiber meshes induced a favorable proliferation and differentiation of MSCs.[18,19] Results after 6 weeks of implantation showed that MSC seeded scaffolds induced M2 type macrophage polarization and increased tissue integration to the implant, whereas meshes without MSC induced M1 type macrophage phenotypes.[17] In another recent study, a novel design was created with 3D printing technology. With the use of camphene and polycaprolactone (PCL), microchannel structures (with 20–40 μm diameters) were obtained as a result of phase separation between these two polymers. Scaffolds with hierarchically deposited fibers with and without microchannels were implanted into mice subcutaneously. After 7 days, it was observed that M2 type macrophages were dominantly higher on micropatterned scaffolds than the controls without microchannels.[20]

These examples show that investigating the host response to different biomaterials is crucial for their suitable clinical use due to their specific characteristics such as fiber size and alignment.

Moreover, the individual aspect of the innate immune cells is much more pronounced than the other tissues; thus, establishing ground rules for the immune response to given properties has proven to be difficult. For example, a recently approved lifting solution for the treatment of submucosal resections (ORİSE) has been shown to induce granulomatous growths in some patients.[21] This synthetic polymer mixture, where the main component is a polyoxomer, can induce such reaction due to one or more components of its composition or the reaction can be based on the sensitivity of the individual patient, so the reaction cannot be generalized and the individuals that would be prone to such reactions need to be detected.[21] Other examples can be given of complications related to double encapsulation in breast implants. It was proposed that double capsules are observed when the surface of the implant is textured and rough. The first capsule formed on these textured surfaces may be separated due to an external force and result in seroma formation in between the primary and secondary capsules. Implants with smooth surfaces do not form secondary capsules since they allow tissue ingrowth through the primary capsule.[22] Textured breast implants may result in several complications linked to this chronic inflammation because of the development of a double capsule. This prolonged exposure to high amounts of pro-inflammatory cytokines, proteins, and other components related to inflammation may result in pain, swelling, seromas, DNA damage, and even cancer due to chronic inflammation.[23] For example, in a study, several double capsules were obtained from breast implants and analyzed in terms of bacterial growth and biofilm formation. SEM examination showed that on prosthesis interfaces (textured) there was increased bacteria growth and biofilm formation compared with intercapsular space interfaces (flat). This study suggested that the double capsule formation was originated mostly as a result of shear stress.[24] This shows that, even in passive implants, architecture plays a substantial role in the immune response over a long period. In the more dynamic environment of degrading/remodeling scaffolds, this role is even more significant.

Other examples of biomaterial-induced immune responses include aseptic loosening and osteolysis of implants used for hips and joints, which are caused by macrophage-mediated inflammatory reactions to implant-derived wear debris,[25] implant related oxidative stress due to the activity of monocytes and macrophages which generate degradative/reactive oxygen and nitrogen species (ROS and RNS),[26] allergic reaction, or hypersensitization (whose definition is giving strong immune reaction or response due to the existence of an antigen), possibly resulting from the use of the implants immunogenic to some patients,[27,28] and granuloma formation consisting of aggregates of macrophages, often including multinucleated giant cells, and lymphocytes observed mostly in breast[29] and dental[30] implants and tracheal/laryngeal stents.

Thus, these examples demonstrate that, in the efforts in complexifying the physical structure of scaffolds, the potential risks pertaining to the immune reaction to such complex architectures post-implantation and throughout their remodeling should also be taken into account.

Immunomodulation in tissue engineering could help better angiogenesis, tissue remodeling, and thus better tissue regeneration. Communication and cooperation between innate immune cells and stem/progenitor cells can achieve a balance between biomaterial degradation and tissue regeneration and guide the progression of tissue repair and inflammation. While phagocytic cells like macrophages aid in the process of regeneration of the tissue through debris clearance, at the later stages of inflammation, compensation between M1 and M2 macrophages is needed for tissue repair and the resolution of inflammation. Even though M1 macrophages are involved in the initial vascularization steps, if they persist in later stages, they may harm the tissue as a result of the secreted pro-inflammatory molecules. Besides, it is also known that clearance of necrotic tissue by M1 macrophages can help tissue regeneration. On the other hand, M2 macrophages with anti-inflammatory properties should be the dominant cell phenotype during tissue repair and wound healing, whereas their excessive presence may lead to fibrotic encapsulation rather than efficient healing.[31] Thus, there should be a balance between early M1 pro-inflammatory signals (which may result in tissue damage when not controlled) and late M2 anti-inflammatory signals (which may disrupt tissue healing if macrophages stay immature).[31] A recent study reported that the presence of M1 phenotypes more than M2 resulted in disruption of tissue repair. It was stated that excessive anti-inflammatory polarization with IL-4 cytokines might shift the balance of macrophages through a direction improving tissue regeneration.[32] On the other hand, during angiogenesis, innate immune system cells support the formation of new vascular structures by the release of soluble factors.[31] These key roles of innate immune system cells in tissue repair and regeneration should be taken into consideration in the construction of biomaterial-based implants and medical devices to modulate the immune response of the body. The main innovation in the use of innate immune cells in tissue engineering is the incorporation of a temporal control system for the regeneration process. Immunomodulation can act as a means of synchronization between the wound healing process initiated by the host and the remodelling process of the artificial organ. The second advantage is the improvement of the biomimicking capacity of the artificial organs by the incorporation of the immune cells, as this will provide the artificial organs with the resident immune cell component common in nearly all organs and tissues.

Although the effect of scaffold architecture on the innate immune response is known and was presented recently,[33,34] there are still not many studies on this perspective and the recent studies and literature reviews do not include the immunomodulatory effect of the biomaterial architecture at the *in vivo* level.[3,33–37]

Biomaterial risk assessment is the most critical step before the manufacturing and marketing of an implant; however, there is no regulation to check the immunomodulatory effect of 3D shape and architecture of the implants.[38] Thus, in this review, we aim to cover the recent studies that elucidate the behavior of macrophages in 3D biomaterial environments that define biomaterial property immune reaction relationships. Then, *in vitro* immune response models and on-chip innate immune system models are covered. The incorporation or recruitment of innate immune cells in regenerative medicine contexts with the use of scaffolds as a potential venue of immunomodulation is also presented.

2. Foreign body reaction (FBR)

The generation of adverse immune reactions in the host body is the most common result of inserting a biomaterial into the host tissue. The end-point of the host response given to implants in contact with their tissues is known as foreign body response (FBR), where the biomaterial is detected as a foreign substance/body by the host immune recognition system and FBR involves a complex cascade of reactions with the involvement of a variety of cells and immune components of the body.[39] There are two major results affecting the function of the biomaterial. First is the fibrous isolation/encapsulation of the biomaterial device which endangers the performance of the device and leads to device failure. The second is the deterioration or unwanted degradation of the scaffold due to the enzymes and reactive species released by innate immune system cells, which results in the activation of the innate immune system due to the products of degraded biomaterials entering the circulatory system.[39] Biomaterial insertion always comes with an injury at the host tissue. As a response to this injury, a series of events occurs as the inflammatory response which was described first by Anderson for implants.[40] Immediately after the implant insertion, plasma proteins adsorb on the implant surface. Then, various cells (mostly neutrophils and macrophages) reach and attach to the surface of the implant. Ultimately, if the inflammation is unresolved, fibrotic foreign body response occurs.[40,41] Immune reaction to a biomaterial based implant takes place with both innate and adaptive immunity.[42] Innate immunity recognizes pathogens new to the host, activates the preexisting mechanisms and prevents infection in a very fast manner (minutes/hours), whereas adaptive immunity targets highly specific antigens recognized previously, develops a long-term memory and occurs over a longer time (days/weeks).[3,42,43] This immune reaction can be acute or chronic and is decided by the specific properties of the inserted biomaterial and the host tissue. Both innate and adaptive immunities take a role in the processes of "acute" and "chronic" inflammations.

Foreign Body Reaction (FBR) is the last phase of immune response to a biomaterial and it involves two main cell types: macrophages and foreign body giant cells (FBGCs). FBR to implants is mostly directed by foreign body giant cells and

other cells of granulation tissue (such as fibroblasts and endothelial cells) depending upon the physical/chemical properties and surface roughness and patterns of the biomaterials. During FBR, at first, a granulation tissue forms, which is composed of fibroblasts and vascular endothelial cells proliferating around the implanted device and on the surface of the implant. With the proliferation of these vascular endothelial cells new blood vessels are formed from the preexisting vessels by angiogenesis (neovascularization).[44] Fibroblasts proliferate and take part in the synthesis of collagen and proteoglycans. Macrophages are critical in the formation of the innate immune response. These cells can respond to environmental cues and change their properties and phenotypes because of their extreme plasticity. They can modify their phenotype into M1 and M2 and this differentiation is known as polarization. M1 (pro-inflammatory) macrophages are classically activated and are cytotoxic and kill pathogens during acute inflammation.[45] They encourage inflammation and secrete pro-inflammatory cytokines and chemokines; IL-1, IL-6, IL-8, TNF-α, macrophage inflammatory protein-1 (MIP-1) and monocyte chemotactic protein-1 (MCP-1).[45] M2 (anti-inflammatory) macrophages are present in very large numbers and are very important in the progression of inflammation, since they play a role in the repair of damaged or injured tissues. They are the alternatively activated macrophages and promote tissue remodeling during chronic inflammation.[46] They are responsible for the secretion of immune modulating small molecules (cytokines and chemokines) such as IL-4, IL-10 and TGF-β, which play a role in tissue regeneration and wound healing.[47] Macrophage polarization is more of a spectrum than a distinct polarization of two phenotypes. M2 phenotype has four different subtypes: M2a, M2b, M2c and M2d (Tumor associated macrophages, TAMs) (Fig. 1).[47] These phenotypes are subdivided based on their functions.[48] Macrophages can phagocytose small particles with a size up to 5 μm in diameter. However, for the larger particles, they need to fuse and form foreign body giant cells (FBGC).[44] Macrophages and FBGC exist in the inflammation site together. These two cells form a sheet with varying thicknesses depending on the roughness, topography, and chemistry of the biomaterial surface. They regulate the degradation of the biomaterial by releasing phagocytic and degradative (oxidative) reactive species at the interaction zone of cells and biomaterial surfaces.[49]

FBGC formation with the fusion of macrophages has unique importance since it is the most definite indication of FBR that distinguishes FBR from a typical chronic inflammation. In this process, a large number of macrophages come together and fuse to form FBGC and the size of FBGC can reach up to hundreds of μm with dozens of nuclei.[2] They can stay at the implantation site during the whole lifetime of the implant device, remaining embedded in the host body.[2] FBGC existence at the implant surface is usually not desired since they give rise to degradative species such as ROS, NOS and enzymes. These reactive molecules can degrade the implanted material and cause

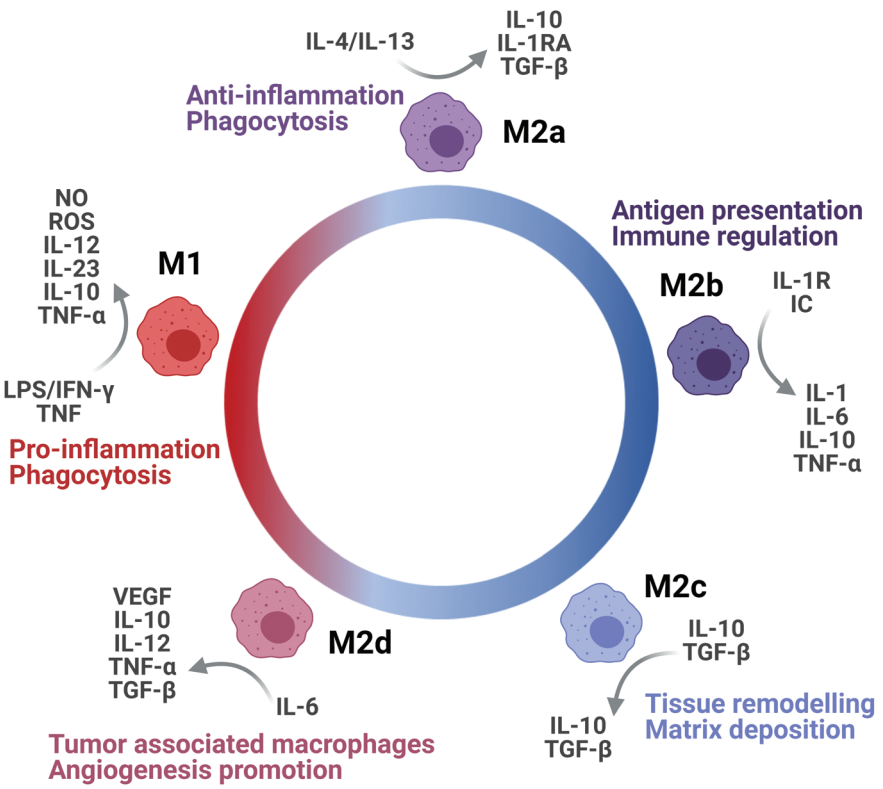

Fig. 1 Spectrum of macrophage phenotypes based on their functional activities in immune response and cytokines that activate and are triggered by these phenotypes (Created with BioRender.com).

device failure.[2] The extent of this degradation depends on the characteristics of the exterior sides of the biomaterial facing the tissue. As a result of the specific properties of implants, the amount and type of adsorbed proteins on their surfaces may vary and affect FBGC formation. For example, when monocyte and FBGC adhesions were evaluated on polyacrylamide and polyacrylic acid surfaces, it was shown that monocyte adhesion and FBGC formation were higher on cationic surfaces than on anionic surfaces.[50] Moreover, FBGC formation was evaluated after intramuscular insertion of poly(L-lactide-*co*-D/L-lactide) (PLA) implants into rats. Implants designed as membrane sheets and electrospun fiber meshes (uncoated and coated with a positively charged plasma polymer) were compared and it was reported that cell number was higher on fibers than on membranes due to the higher roughness with the fiber meshes. However, the cell number did not change significantly on coated and uncoated surfaces and surface roughness was a more effective parameter than the surface chemistry.[51]

Progression of inflammation with the formation of FBGC finally results in the development of fibrotic collagenous capsules covering the surface of the device. This fibrotic encapsulation isolates it from the host tissue. Both the local microenvironment of the implantation site and the biomaterial surface properties can affect macrophage adhesion, polarization, fusion and apoptosis.[52] Fibrous capsules mostly remain for the lifetime of the implanted material.[45]

There are two sequential but generally temporally overlapping events in the host determining the regeneration extent after the implantation; (i) migration of monocytes to inflammation site, their differentiation to macrophages and the degradation of the scaffold by macrophage activity, and (ii) induction of an anti-inflammatory healing process with the release of cytokines. This balance between the tissue regeneration and scaffold degradation must be sustained by modulating the properties of scaffolds without compromising their functionality. Development of a immune reaction against a medical device inserted into the host body is determined by the conditions and properties of the surrounding tissue, properties of the scaffold and the interactions at the interface between the implant and the host tissue. These three components of cell–biomaterial interactions dictate the performance of the implanted device.

2.1. Effect of biomaterial properties on macrophage phenotype

Macrophages are present in most tissues, but they start to proliferate after the insertion of an implant device and injury formation. They are differentiated from monocytes circulating in the blood and migrate to the implantation site in the first days of inflammation.[53]

The properties of the implanted material can affect the macrophage function. Due to their high plasticity, macrophages can be modulated by the scaffold and shift their phenotypes in response to environmental cues. This shift between two main macrophage phenotypes (M1/M2) is known as "macrophage polarization" and is induced by Th1/Th2 activation of T cells.[54]

Besides their role in the innate immune system, macrophages are also involved in the regulation of stem cell proliferation and differentiation. Thus, the design of biomaterials is important in both controlling the immune response and the repair and regeneration of tissue through the functions of macrophages. As was stated earlier, interactions of macrophage cells and implant devices are very important in the design and production of biomaterials and implants and in the enhancement of biomaterial/tissue interactions. In the literature, there are various studies showing the modulation of biomaterial properties for guiding the macrophage polarization towards desired phenotypes and these studies report that the function of macrophages in immune response is dependent both on the macrophage phenotype and the biomaterial type.[48] Their role in tissue repair and healing also depends on the type of tissue.[55] The influence of chemical cues on biomaterial surfaces on macrophage phenotypes is determined by the number of cell adhesion ligands,[55] surface chemistry[56] and the content of ECM.[57] The effect of physical cues, on the other hand, are determined by stiffness,[58] topography[59] and pore size[60–62] of the biomaterial. Also, mechanical forces, such as cyclic or magnetic loading, can also affect the phenotype, cytokine secretion and the regenerative function of macrophages.[63,64] It should also be noted that there is a material specific aspect on macrophage polarization and on biomaterials; macrophages almost never behave within the defined phenotypical profiles under cell culture conditions. Thus, the presence of biomaterial specific macrophage phenotypes should also be considered.

In this part of the review, we will center on the effect of biomaterial dimensionality on macrophage polarization. It is known that 2-dimensional (2D) and 3-dimensional (3D) microenvironments affect cell behavior in different ways.[65] In 2D, cells grow as monolayers which make their activities more homogenous, such as differentiation or polarization, interaction with neighboring cells and availability of nutrients and growth factors. However, since in natural tissue the cells are in a 3D microenvironment, only a group of cells survive and adapt to the conditions of 2D microenvironments. This results in the continuation of only a selected cell population on 2D. In 3D, complex structures, composition and properties of native tissue can be mimicked and the heterogeneity of the tissues and cells can be reflected.[66] Thus, it is expected that 2D and 3D biomaterial implants present the immune response in different ways and influence the morphology, polarization and activation of macrophages differently. The first property of cells affected by the dimension of the microenvironment is adhesion and morphology as a result of mechanosensitivity of the cells, which makes the cells sense the external signals from the environment and change the downstream signaling pathways.[67] In a study, the effect of dimensionality of the substrate on human macrophage polarization was studied. 2D collagen films and 3D collagen constructs were used to seed the macrophages. On 2D, flattened and spread cell shape was obtained, and in 3D collagens, round cell shapes with 20–30 μm diameter were obtained. Also, 2D and 3D scaffolds

affected the cytokine secretion in different ways. In 3D, macrophages secreted increased amounts of anti-inflammatory IL-10 and decreased pro-inflammatory IL-12 and TNFα, whereas on 2D, cells released elevated levels of these cytokines.[67] In another study, PLGA polymeric biomaterial was used in two different shapes and dimensions: 3D nanofiber meshes and 2D films. These substrates were seeded with human macrophages and it was shown that 2D films induced an increased number of M2 macrophages with a positive CD163 marker, whereas 3D nanofibers increased the number of M1 macrophages with positive 27E10 anti-inflammatory phenotype markers.[68]

2.1.1. Effect of 2D biomaterials on macrophages. On 2D biomaterials, the surface is the modulating unit of immune cell behavior and chemistry and topography of the surface are the two dominant properties affecting the macrophage functions. When the size of topographical cues on the biomaterial surface is in the nano/micro size, which is the sensible range of biological molecules by cells, these patterns can modulate the macrophage behavior without any bioactive agents.[69] For example, parallel patterns in a line shape with width sizes between 250 nm–2 μm are produced with three different polymers (PLA, PCL, and PDMS). On these patterned surfaces, macrophages were seeded and the effect of topography on the activity of macrophage (RAW 264.7) cells was studied. On the patterns with small widths, VEGF and TNF-α showed increased expression, whereas on the larger width patterns their expression was decreased. This trend was the same for all three different polymers.[59] The topography of the substrates also changed the morphology of macrophages. On all three patterned polymers with 2 μm width lines, cells were elongated in the direction of the patterns, whereas they were circular, not elongated on the unpatterned PDMS controls (Fig. 2A).[59] In another study, substrates of poly(L-lactic) acid (PLLA) were produced as smooth films and by electrospinning with micro and nano fibers and in organized/aligned and disorganized design. On surfaces with nanofibers, RAW 264.7 macrophages showed decreased inflammatory response with increased anti-inflammatory cytokines and chemokines when compared with control films and fibers in micron sizes. However, the proliferation of FBGCs was higher on smooth films than on the fibers with micro- and nano-sizes. Fiber organization did not affect the macrophage behavior as much as the dimensions (Fig. 2B).[70] In a similar study, polycaprolactone (PCL) was used in different forms, such as parallel or disorganized fibers, nanofibers produced with electrospinning, coverslips made of glass and surfaces modified with RGD and PCL films as controls. Fiber dimensions were in the nano-range and two different groups with varying diameters were used. On PCL surfaces with parallel line fibers, monocytes were not spread, and they were mostly in round shapes. Also, cell number was low. On the other three groups of PCL, the cell spreading was higher with elongated shapes and cell number was higher too (Fig. 2C).[71] Chemical modifications and crosslinking of the 2D surfaces can affect the macrophage phenotype significantly. For example, scaffolds of collagen were crosslinked using two different chemical crosslinkers (EDAC and Genipin). The mechanical properties of the two scaffolds were different due to the crosslinking. Macrophage cells (THP-1) attached, proliferated, and polarized the same when the stiffness of the surfaces was compared. However, on the surfaces treated with Genipin, both pro- and anti-inflammation were inhibited, whereas on the scaffolds treated with EDAC both inflammatory responses were activated.[72] In another study, it was shown that biomaterial degradation is a critical parameter changing macrophage behavior. Poly-ε-caprolactone-bisurea was used to produce electrospun constructs with changing diameters (2 and 6 μm). The organization of the fibers was aligned and random. Macrophages (THP-1) showed higher degradative activities (which are measured by the levels of ROS dependent peroxidation and NADPH oxidase) on aligned fibers with 6 μm diameters.[73] In a recent study, a processing method called melt electrowriting was used to produce substrates with fibers and pores in different shapes. Pores were designed with varying sizes (40 μm–100 μm) and their effect on macrophage activities was evaluated. It was shown that the macrophages aligned the most on the fibers with the smallest pore sizes and they also polarized into anti-inflammatory phenotypes. In another study, surfaces designed with nanodots (diameters ranging from 10 to 200 nm) were used to investigate the influence of roughness/topography on cytokine secretions by macrophages. It was reported that pro-inflammatory cytokines (IL6) increased on the most patterned surfaces rather than on smooth surfaces and also 200 nm nanodot surfaces showed higher cytokine release than on 50 nm.[74] Moreover, in another study, gelatin films produced with grooves having varying widths (2 to 40 μm) were used to study the influence of patterns on monocyte attachment and differentiation. Monocytes were seeded on micropatterned films with and without M1/M2 differentiation media. The morphology of these monocytes and their cytokine production levels were examined. It was reported that the patterns induced more cytokine secretion (such as IL-1β, IL-4, IL-12, TNF-α, CCL-18) in M1 media.[69] Another group cultured macrophage cells on titanium surfaces with topographies to investigate the impact of roughness on immune cell behavior and cytokine secretion. Several different topographies were created with different surface modification methods to obtain different surface roughness. It was reported that macrophages increased secretion of TNF-α which is an M1 inflammatory cytokine on surfaces with the highest roughness.[75] Moreover, the cells cultured on scaffolds with different shapes secreted different inflammatory markers. For example, markers of pro-inflammatory polarization were lower on all scaffolds with box, rounded, and triangle shapes whereas anti-inflammatory markers were lower on rounded scaffolds but higher on triangle ones (Fig. 2D).[76]

These examples show that 2D biomaterials can be designed to modulate macrophage polarization. Flexibility in designing 2D surfaces by modifying their physical, chemical and mechanical properties make them potential tools for *in vitro* studies. Although 3D biomaterials are better for mimicking the natural microenvironment for the cells, 2D biomaterials will always have the advantage of being simple to design and

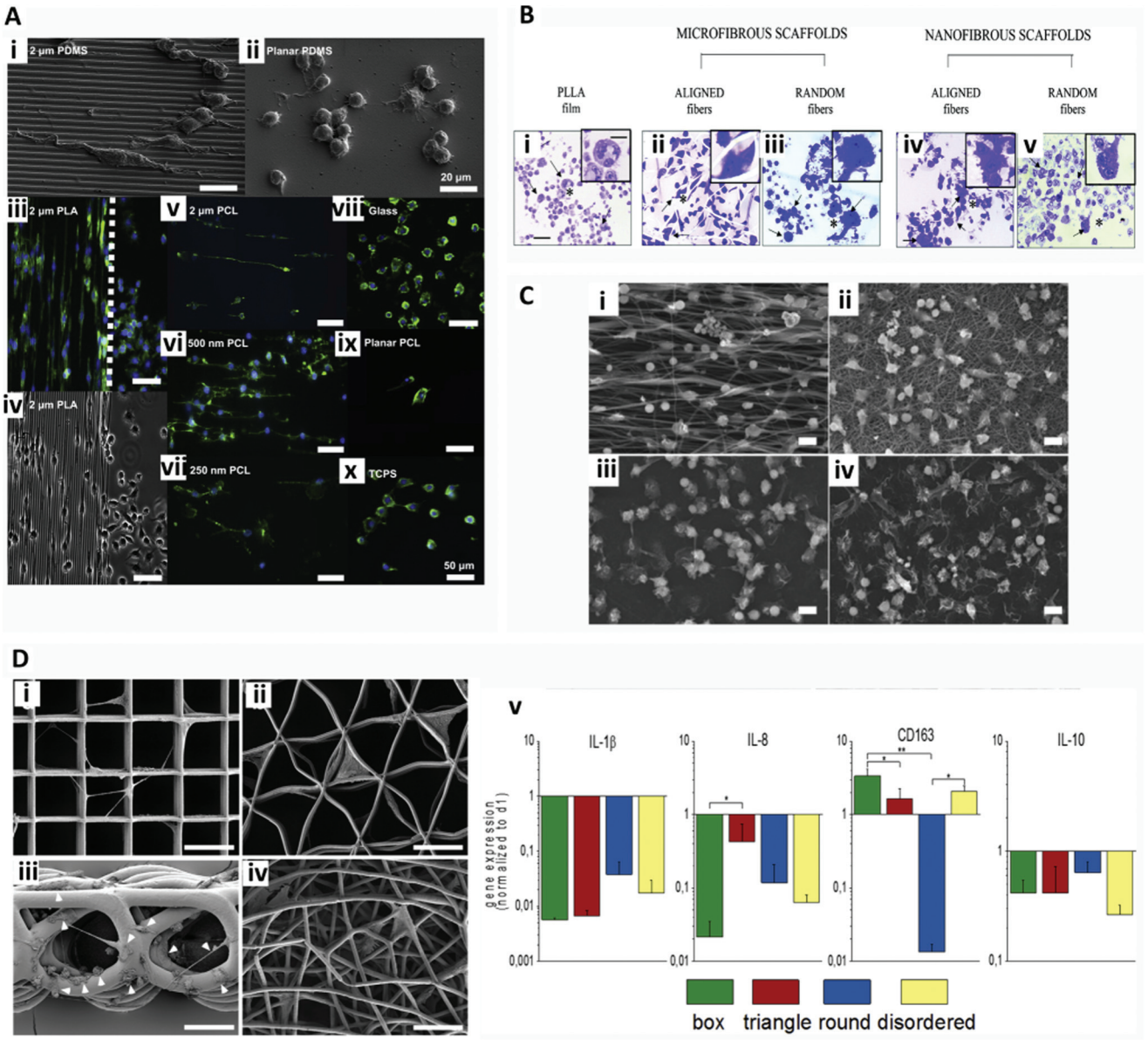

Fig. 2 Influence of 2D biomaterials on macrophage behavior. (A) Micrographs of macrophages grown on (i) 2 μm PDMS with line shaped topographies, (ii) smooth PDMS control, (iii) 2 μm PLA lines on glass, (iv) zone between the 2 μm PLA linear patterns and its smooth surface, PCL lines having widths (v) 2 μm, (vi) 500 nm, (vii) 250 nm, and control scaffolds made of (viii) glass, (ix) smooth PCL film and (x) tissue culture polystyrene (TCPS). Morphology of cells is different on substrates with changing patterns with different sizes (F-actin and cell nuclei: phalloidin Oregon-Green 488 (green) and DAPI (blue)).[59] (B) Histological staining of FBGCs on different PLLA scaffolds with micro- and nanofibers is shown and the arrows indicate active cells having spread shapes with increased filopodia, whereas asterisks show the magnified cells presented in the inserts (Stain: toluidine blue) (reprinted with permission. Copyright (2020) American Chemical Society).[70] (C) SEM images of monocytes on (i) PCL with parallel fibers, (ii) PCL with disorganized fibers, (iii) smooth PCL film, (iv) cover slip treated with RGD.[71] (D) Adhesion and alignment of macrophages are changing with the different porosity, structure and shapes of the PCL scaffolds (i, ii, iii, iv) and pro- and anti-inflammation markers are varying on different scaffolds (v).[76]

modify. Moreover, the use of 2D surfaces in immunomodulation *in vitro* can be advantageous when they are used as implant coatings. Implant surfaces designed with 2D topographies or chemical modifications can improve interactions at the tissue–implant interface and integration of the implant with the host body *via* immunomodulation.

2.1.2. Effect of 3D biomaterials on macrophages. In natural tissue, cells are enclosed with a complex three-dimensional structure named the extracellular matrix (ECM), which has functions in physical and chemical signaling cascades and provides cells with their mechanical properties. 3D scaffolds/substrates produced using biomaterials can possess properties to mimic physiological microenvironments of cells. They can be utilized to establish realistic compartments for housing, enrollment, and controlling host innate immune system cells. Moreover, the unique topography of ECM con-

sisting of components on micro- and nano-scales such as fibers and pores can modulate the immunomodulatory properties of host cells. Thus, 3D biomaterials are of choice for mimicking the natural tissues and controlling the immune response. Especially, hydrogels are commonly used for this purpose since they can provide a tunable 3D network. They consist of a huge quantity of water in their polymeric structure and particular viscoelastic properties. They include micro- and nanoporous structures like in natural tissues which make them suitable to simulate the physical features of ECM. Hydrogels and other 3D matrices are more advantageous than the 2D substrates due to their capacity to regulate properties, behavior and activities of cells, such as their shape, morphology, interactions and signaling with other cells and microenvironment. 3D substrates can achieve these control mechanisms by encapsulating the cells, and controlling the flow of bioactive molecules released by cells or provided from the environment with their porous structures.[77] The most recent studies on innate immune response to scaffolds show that the modulatory influences of 3D architectures on the behavior of macrophage cells are too crucial to overlook. For example, a novel 3D screening system for the determination of suitable biomaterial–cell combinations in the immune modulation was designed in a recent study.[78] In this study, alginate was used to form microcapsule hydrogels and to encapsulate stem cells and micro PCL spheres in these capsules. Then the outer surface of the microgels was coated with poly-L-lysine, alginate and chitosan with the layer-by-layer (LBL) method. Alginate hydrogel was then liquefied with the help of EDTA chelation but the outer coatings helped the microsphere maintain its shape. This platform was then tested on a 2D macrophage monolayer and it was reported that the order of the coating layer changed the polarization of the macrophages (Fig. 3A).[78] For example, when the outer layer was chitosan and the stem cells were encapsulated in the system, macrophages showed an anti-inflammatory and regenerative profile.[78] This kind of system can be very promising for the screening of various cell type-biomaterial pairings for the directed immune response. Another recent application of immunomodulatory 3D scaffolds is the healing of wounds formed as a result of diabetes through the personalized and controlled structure of the biomaterial based scaffold.[79] In this study, 3D PCL/F-127 scaffolds with nanofibers were produced with various shapes, coated with gelatin and BMSCs were seeded on them. Then, the cell seeded scaffolds were inserted into mice for the evaluation of wound healing. It was reported that the scaffolds with larger pore sizes caused M2 type macrophage polarization and better wound healing since they allowed cells to penetrate more through the scaffolds.[79]

Another important health issue related to macrophage presence in 3D environments is tumors and tumor-associated macrophages. This is particularly relevant in tissue engineering if the scaffold is to be placed after the tissue rejection due to tumor presence. For example, in a 3D tumor model study designed in a microfluidic device with an endothelial barrier, tumor migration and co-culture of tumor cells with macrophages were studied. Flow of lipopolysaccharide (LPS) and interleukin-4 (IL4) were provided through the capillary of the device and the polarization of macrophages (RAW264.7) was observed under the effect of these two different inducers. LPS resulted in M1 polarization whereas IL4 induced M2 phenotype. However, when the tumor cells were included in addition to macrophages, the polarization of macrophages did not change but tumor migration and invasion through the endothelial membrane were affected. It was observed that tumor cells migrated through the areas that macrophage cells located.[80] In another tumor model study, alginate hydrogels were used to design a 3D surrounding space for the encapsulation of cells. Three cells were cultured in the model: lung carcinoma cells in spheroid forms, fibroblasts associated with tumors, and macrophages (THP-1). It was reported that hydrogel with tri-cultured cells created an immunosuppressive microenvironment and directed the differentiation of macrophages into an M2 inflammation phenotype. Also, increased expressions of two tumor associated markers were detected in macrophage cells (CD163 and CD206) (Fig. 3B). In summary, this multi cell culture hydrogel system and the interactions between the cells and 3D microenvironment directed macrophage cells into more tumor cell like phenotypes which is called tumor associated macrophages (TAM).[81]

In a recent study, 3D collagen and HA scaffolds were produced and it was shown that the scaffolds having a higher amount of HA caused anti-inflammatory M2 phenotype polarization of macrophages (THP-1 cells).[82] In another study, both 2D and 3D substrates were used to co-culture two cells as stem cells and macrophages. In 3D matrices, markers related to both inflammation and chemotaxis were produced in lower amounts when compared with 2D substrates.[83] In a different study, two component hydrogel matrices with the combination of proteins (gelatin) and glycosaminoglycans (hyaluronic acid) were produced with different mechanical properties and loaded with macrophages (THP-1). In softer hydrogels with Young's modulus around 10 kPa, cells showed higher metabolic activity when compared with stiffer hydrogels having Young's modulus around 20 kPa.[84] In another study, two different hydrogels, gelatin methacryloyl (GelMA) and poly (ethylene glycol) diacrylate (PEGDA), were used to encapsulate macrophage cells and it was reported that THP-1 macrophages in hydrogels (GelMA) showed increased expression of M2 anti-inflammation marker (CD206), whereas they produced an increased level of M1 pro-inflammation marker (CD86) in PEGDA hydrogels (Fig. 3C).[85] These studies show that, as well as the architecture or dimension of the 3D microenvironment, the type of biomaterial is very effective on the immune cell behavior and response. In a recent study, 3D hydrogels of poly (ethylene glycol) (PEG) were used to load and culture macrophage cells (RAW264.7) and it was shown that matrix metalloproteinases and pro-inflammation markers (iNOS, COX2, TNF-α) increased in 3D, while anti-inflammation molecules (CD206, Arg1, TGF-β) decreased in the hydrogels. When the

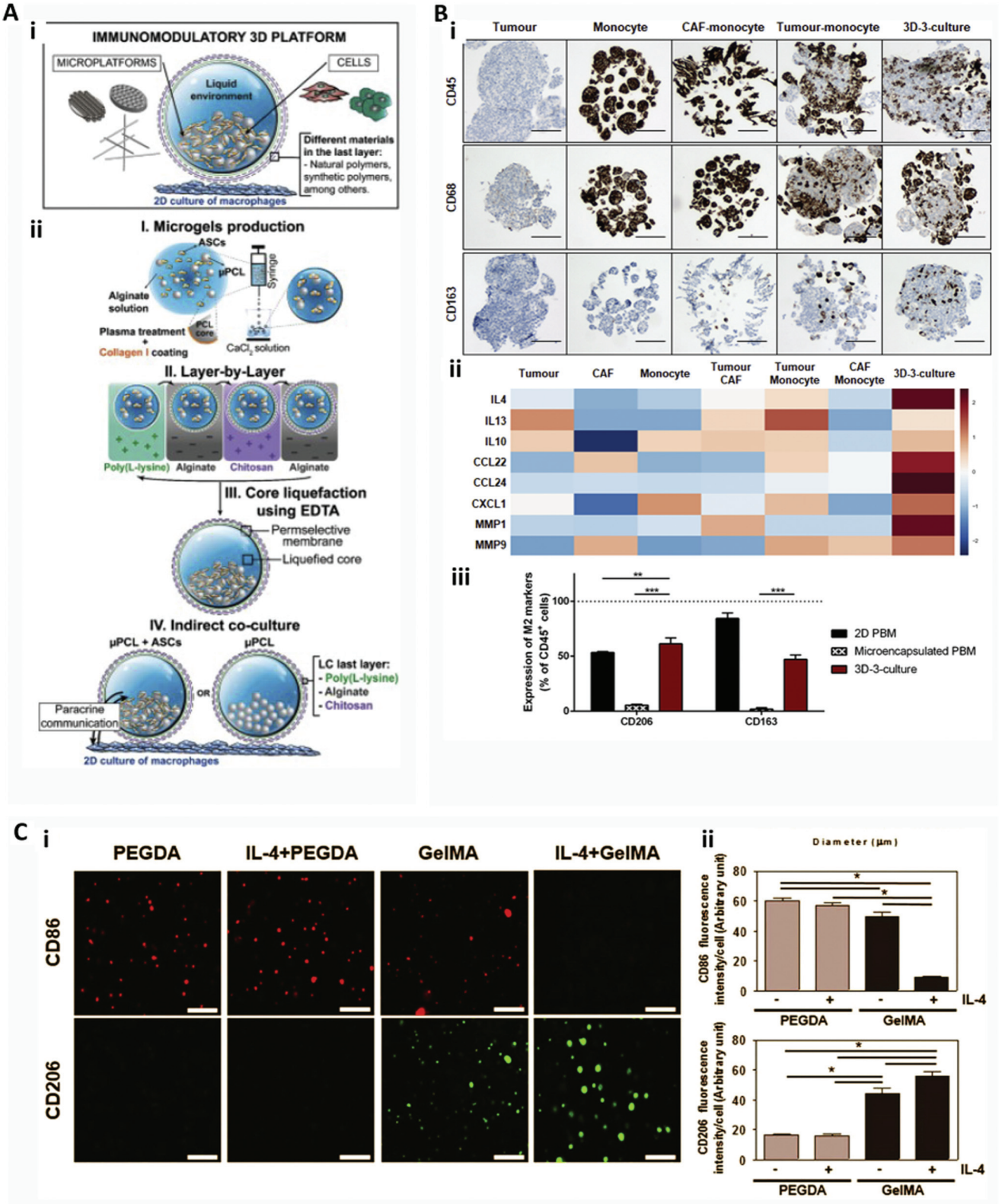

Fig. 3 Influence of 3D biomaterials on macrophage behavior. (A) A novel biomaterial–cell screening platform for the purpose of immunomodulation. (i) Application of stem cell encapsulating hydrogel system on 2D macrophage culture and (ii) the production steps of this platform using LBL method and liquefaction of hydrogel core.[78] (B) Tri-culture alginate hydrogel system was analyzed in terms of macrophage markers and cytokine secretions: (i) histology staining of cells for cell migration and macrophage polarization specific markers (ii) Heatmap for the expressions of macrophage related cytokines (iii) Quantification of antibody signal intensities for the detection of leukocyte (CD45) and M2 phenotype (CD163 and CD206) in both 2D and 3D microenvironments.[81] (C) Micrographs of macrophage polarization in 3D hydrogels were shown with immunostaining of the cells for pro- (CD86) and anti- (CD206) inflammatory markers (i) and the quantitative analysis of antibody signal intensities for the same markers were presented (ii).[85]

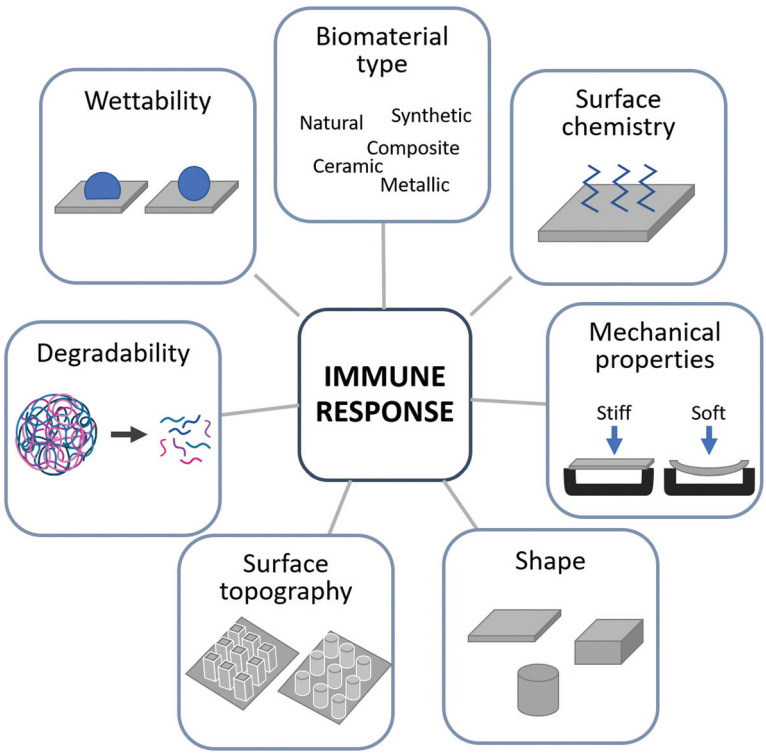

Fig. 4 Adjustable properties of biomaterials affecting immune response in contact with the host body.

activity of macrophage differentiation indicators was compared with changing stiffness of the hydrogels, it was observed that both pro- and anti-inflammation markers were induced more in stiff gels than in soft gels.[86] In another study, scaffolds were produced using a natural polymer chitosan and they were acetylated with changing degrees (5% and 15%). These porous scaffolds induced varying macrophage numbers, adhesion and polarization depending on their acetylation degrees. For example, 5% acetylation resulted in a decreased cell proliferation and a dominant M2 phenotype. However, 15% acetylated scaffolds induced M1 macrophages.[87] Results showed that 3D hydrogels could be used to control immune cell activities by modifying the enclosing 3D microenvironment and their properties, such as stiffness, chemistry, composition, crosslinking degree and design.

Studies with 3D biomaterials show that macrophage polarization, activation and function are modulated by both the nature of the biomaterial itself and the dimensionality of the microenvironment. In the use of biomaterials in immunomodulation or tissue renewal, dimensions of the microenvironment should be considered along with the chemical, physical and mechanical features of biomaterials.

3. Properties of scaffolds affecting immune response

Biomaterials have tunable properties which are useful in the immunomodulation of scaffolds and implants. Biomaterial type, surface chemistry, topography and wettability, shape, degradability and mechanical properties are the important factors deciding the components and severity of the foreign body reaction and immune response (Fig. 4). Thus, based on the application, an interplay and trade-off between various properties is required to achieve the optimum response. Having control over the ability of biomaterials to support cell adhesion, metabolic activity, differentiation, and to regulate the production of bioactive molecules and drugs can enable the control of innate host immunity. With the techniques commonly used for biomaterial processing, the derivation of highly tunable natural and synthetic biomaterial-based scaffolds and implants have become possible.

3.1. Biomaterial type

Natural polymers such as alginate, silk, chitosan, collagen, dextran, and hyaluronic acid release mostly nontoxic degradable compounds in the body. Their properties resemble the biological molecules already present in the host body and these bioactive natural biomaterials present adhesion regions for cells. As a result of the adhesion sites, natural biomaterials degrade easily with enzymatic degradation and they activate adverse FBR less than the synthetic biomaterials. However, their weak mechanical properties can limit their use for various applications.[88] *Synthetic materials* consist of molecules such as poly(lactic acid) (PLA), poly(ethylene glycol) (PEG), poly(lactic acid-*co*-glycolic acid) (PLGA), polymethyl methacrylate (PMMA), and poly(vinyl alcohol) (PVA). These polymers

can be easily designed to control the protein attachment to the implant surfaces which is the very early step of innate immune response.[53] Also, their controllable biomechanical and biodegradation properties make them very favorable for tissue engineering purposes. However, they may develop chronic inflammation which directs the fibrous isolation and encapsulation of implants.[88] Both natural and synthetic materials stimulate the proinflammatory responses by recruited innate immune system cells; however, the extent of this response depends on the surface properties rather than the bulk properties of the implants. For example, in a study, alginate, agarose, chitosan, hyaluronic acid, and poly(lactic acid-*co*-glycolic acid) (PLGA) were tested in terms of their initiation of the innate immune response. Dendritic cells were seeded on these polymeric biomaterials and their capacity to induce dendritic cell maturation was evaluated. On chitosan and PLGA films, cells showed an increased level of dendritic cell maturation marker and increased expression of B and T cell activation marker, whereas on alginate and hyaluronic acid films the expression levels of these two markers decreased. This higher number of the dendritic cells on chitosan and PLGA was explained with the higher hydrophobicity of these two polymers, which could create a more favorable surface for cell adhesion and spread.[89] In another study, immunity activation of ECM scaffolds (obtained from biological tissues) was compared with the synthetic scaffolds (polyethylene (PE) and polyethylene glycol (PEG)) and it was reported that ECM scaffolds made macrophages produce increased levels of CD206 (pro-regenerative marker), whereas PE and PEG resulted in decreased production of CD206. Also, more neutrophil cells were observed on synthetic scaffolds which was based on the higher stiffness of the synthetic scaffolds (Fig. 5A).[90]

Composites are designed by combining the properties of more than one material and show greater success in directing tissue regeneration after injury caused by implant insertion. With such control over the features of biomaterial surfaces, it can be possible to optimize the biological performances of the implants. Composite biomaterials reflect the properties of the constituent biomaterials and provide specific properties for cell guidance which normally do not exist in either biomaterial constituting the composite. With these combined properties, they can mimic the natural ECM which is good for cell adhesion. Also, designed adhesion regions and growth factors can be introduced into their structure for better cell attachment, proliferation and differentiation.[91] For example, in a study, composites of poly(ethylene glycol) (PEG) and collagen hydrogels with macroporous structures were synthesized. It was reported that only PEG hydrogels with high mechanical strength induced the secretion of cytokines/chemokines. However PEG-collagen composites showed increased mobility and migration of T and dendritic cells through the scaffold when compared with PEG scaffolds with the same pore sizes which showed no T cell attachment or migration.[92]

Metallic biomaterials are mostly used for the manufacturing of prosthetic and orthopedic implants and as composites for dentistry. Commonly used metal types are stainless steels, titanium, cobalt alloys, and alloys of more than one metal. Metals can undergo corrosion in contact with body fluids and release ions to the circulatory system. These released ions may form complexes with proteins and activate the innate immune cells even when they are biocompatible.[93] For example, the effect of several metal ions (cobalt, chromium, molybdenum, nickel) and particles of Co–Cr–Mo alloy on macrophages were evaluated and it was shown that these ions induced an increased macrophage activity and production and release of M1 type immune modulating cytokines (IL-1β and IL-18).[94,95] In a study, polydopamine coated titanium alloys were implanted in rats and it was reported that these metallic implants in soft tissues induced macrophage polarization into M2 phenotype.[96] In a recent study, microbeads of titanium were designed, and their surface properties were modulated with oxidation. After these metallic implants in microbead forms were oxidized, they caused decreased macrophage attachment and increased secretion of anti-inflammatory markers by these macrophages. They also increased the number of connective tissue cells attached to their surfaces.[97] As a result, these studies demonstrate that the use of metallic implants and their surface treatments can direct the behavior of host cells and can be used to control and personalize implant interfaces.

Ceramic biomaterials are mostly used to produce bone and dental implants. They are highly biocompatible due to their chemical and structural formulation. They are inert, hard, brittle, strong under compression and, due to these properties, they are very similar to native bone.[98] In various studies, it was shown that ceramics could change macrophage polarization. For example, in a study, biphasic calcium phosphate (BCP), β-TCP and hydroxyapatite ceramics were compared in terms of their macrophage polarization and it was reported that BCP showed both the highest osteoinduction activity and more M2 macrophage phenotype when compared with two other ceramics. β-TCP showed no osteoinduction and increased M1 macrophage phenotype and hydroxyapatite ceramics showed intermediate osteoinduction and induced both M1 and M2 macrophage phenotypes.[99]

3.2. Surface chemistry

The surface chemistry of biomaterials has an important function in the recruitment and activation of immunomodulatory cells. Functional groups at the outer face of implants are capable of controlling protein and cell adhesion, and subsequently, tissue reactions to implanted biomaterial surfaces. Implanted biomaterials are mostly hydrophobic, and proteins bind more strongly to hydrophobic surfaces than hydrophilic ones.[93,100] On the other hand, it was shown that wettability of the surface might not correlate with the effect of functional groups and surface chemical structure was the dominant factor determining the thickness of fibrous capsule formation *in vivo* (Fig. 5B).[101] Similarly, in several studies, it was reported that surfaces with $-NH_2$ and –OH groups induced more innate immune system cells and proteins migrate to the implant insertion site and form thicker fibrotic capsules around the implants when compared with surfaces with –CF and –COOH

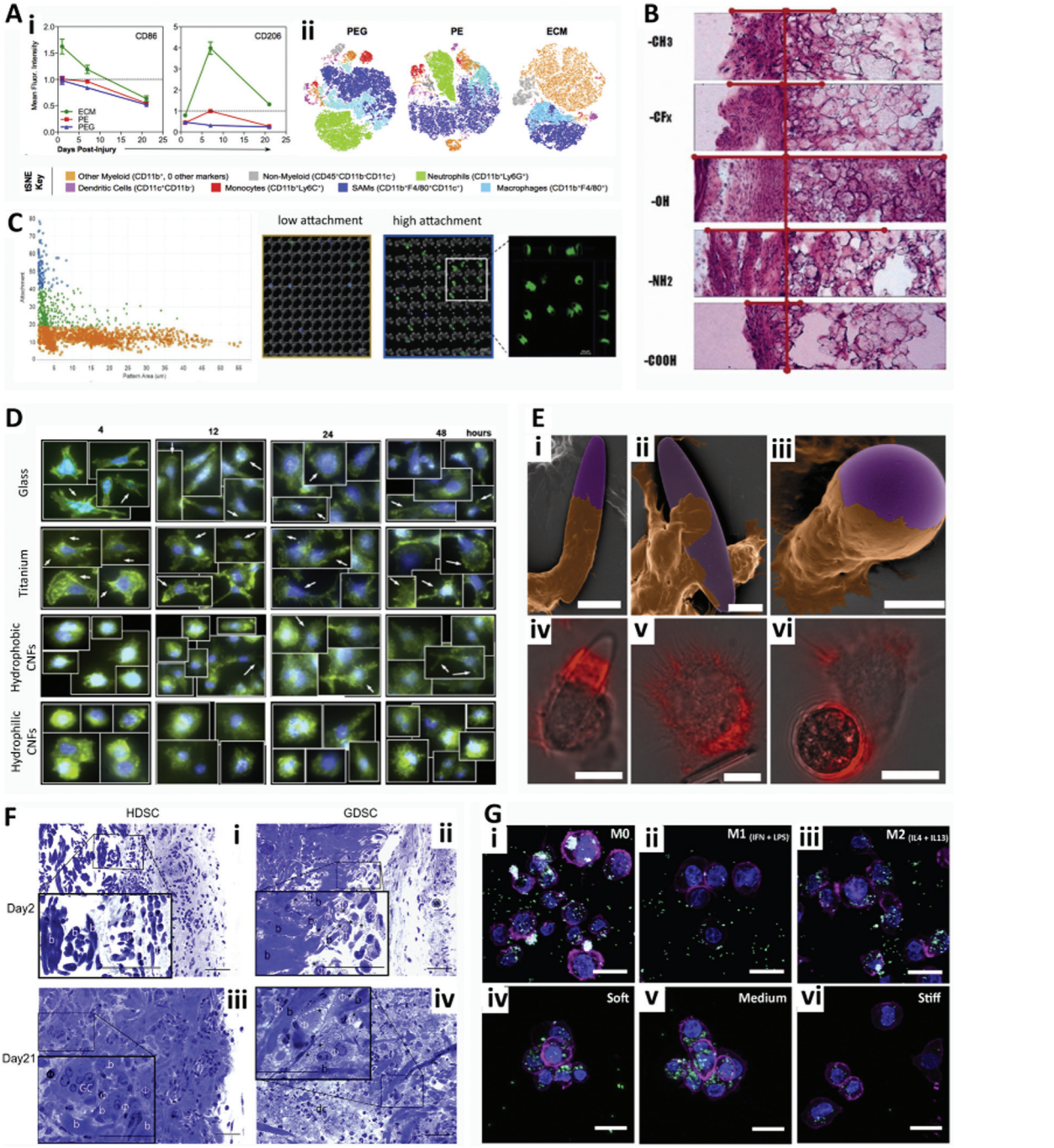

Fig. 5 Biomaterial properties can change the behavior of innate immune system cells. (A) Different innate immune responses are induced by synthetic and natural biomaterials: (i) CD86 and CD206 macrophage markers are evaluated with flow cytometry on ECM, PE and PEG on days 1, 7, and 21, (ii) immune cell recruitment to the microenvironment of scaffold implanted to a murine model is shown with multicolor flow cytometry analysis.[90] (B) Hematoxylin and eosin staining of tissues with different surface functional groups shows implant related innate immune responses is based on the density of fibrotic capsule and infiltration of cells (red lines).[101] (C) Macrophage cell attachment on pillars are presented with a scatter plot showing attachment *versus* total pattern area (μm^2) with high (blue), medium (green), or low (orange) attachment surfaces, and their confocal images (macrophage membrane: green and DNA: blue) (Creative Commons 4.0).[110] (D) On three different biomaterials (titanium, glass, carbon nanofiber), the filopodia and morphology of macrophages are presented on 4 time points (F-actin: green, DNA: blue) (filopodia are shown by white arrows).[112] (E) SEM micrographs (i–iii) and merged micrographs of brightfield and fluorescent images (iv–vi) are showing macrophage engulfment of the particles with different shapes and sizes (copyright (2020) National Academy of Sciences).[113] (F) Macrophage internalization of degraded scaffolds crosslinked with two different chemical crosslinkers are shown: no phagocytosis in hexamethylene diisocyanate (i, iii) whereas progressive phagocytosis in glutaraldehyde crosslinked scaffolds on day 2 (ii) and day 21 (iv).[117] (G) Micrographs obtained by confocal microscopy during the engulfment of latex beads with 1 μm diameter in M0 (i), M1 (ii) and M2 (iii) differentiation medium, and on soft (iv), medium (v), and stiff (vi) gels (actin cytoskeleton: purple, particles: green, nucleus: blue): cells in M1 differentiation media shows a decreased number of particles per cell and gels with average stiffness shows the increased particles internalized per cell.[118]

groups.[101–103] Control over surface properties of implants with surface modification methods (such as chemical grafting, self-assembly, plasma modification or polymerization) influences protein adsorption and successive immune cell reactions to implants.[100] The surface chemistry can also affect macrophage polarization, attachment and secretion of immune regulatory molecules. For example, implantation of poly(carboxybetaine methacrylate) (PCBMA) hydrogels with functional groups comprising zwitterionic properties to subcutaneous regions of mice induced more pro-inflammatory macrophages than poly (2-hydroxyethyl methacrylate) (PHEMA) scaffolds.[104] Also, in a study, macrophage polarization was investigated on meshes made of polypropylene. Meshes were coated with hydrogels of the decellularized porcine extracellular matrix obtained from the dermis of skin tissue and urinary bladder. It was reported that uncoated meshes induced M1 macrophage polarization, whereas ECM coated meshes resulted in an elevated number of M2-to-M1 polarized cells in mice.[105] Moreover, with the help of a proteomic analysis of polymeric surfaces, it was observed that fewer macrophages had migrated and attached and fewer foreign body giant cells were developed through the accumulation and fusion of macrophages on the hydrophilic and neutral surface with no charge than on hydrophobic and ionic surfaces.[106] In another study, nanorods with positive surface charge due to terminal amine groups directed anti-inflammatory M2 type polarization, while nanorods with negative surface charge due to carboxylic acid terminal groups induced proinflammatory M1 phenotype.[107] As can be seen, there is no clear-cut optimal immunomodulatory surface chemistry and surface chemistry should be modulated with respect to the needs of the host tissue with innate immune response also taken into account.

3.3. Surface topography

Surface topography of implanted biomaterials at the nano- and microscale affects cell morphology, adhesion, migration, proliferation and differentiation.[108] Modifying the surface topography is a useful way to change the immune cell reaction because these micro/nano surface features trigger changes in cell morphology and plasticity.[109] This regulation of surface properties helps control macrophage migration, proliferation, function, differentiation, polarization and fusion. For the design of the surfaces with topography, various methods such as photolithography, electron beam lithography, soft lithography, microcontact printing and hot embossing are used.[108] These techniques can create surface geometries at the nano- and microscale decorated with pillars, posts, gratings, ridges, pits and dots.[108] The reason for surface topography being highly effective on cell behavior stems from cell interactions with the integral elements of the ECM. The natural structure of ECM includes proteins and molecules on the nano- and microscale and components of ECM interact with cells at the nano/microscale which can be mimicked by designing the surface topography of an implant with the same dimensions.[100]

In a very recent study, the TopoChip platform was used to evaluate the influence of surface patterns in the micro/nano range on human macrophage adhesion and phenotype. The platform was designed with a large number of patterns in various shapes (circle, triangle, rectangle) with varying dimensions (3–23 μm diameter with 10 μm height). With the help of these different topographies and pattern sizes, the surfaces were produced with changing cell attachment properties. It was reported that the increased attachment of macrophages was on the micropillars with 5 μm diameter. Moreover, on TopoUnits with low cell adhesion surfaces (diameters higher than 10 μm), cells attached in between the micropatterns, whereas on surfaces with high cell adhesion properties, macrophages adsorbed micropillars through phagocytosis (Fig. 5C).[110]

3.4. Wettability

Wettability of the surfaces has a critical effect on immunogenicity since the innate immune cells recognize molecules with high hydrophobicity as foreign materials.[10] Thus, to prevent highly immunogenic reactions to surfaces with low wettability, hydrophilic polymers (polyethylene oxide (PEO) and polyethylene glycol (PEG)) are used in cell or drug carrier designs and in the construction of biomaterial devices considered for use in tissue engineering or as implants. With the help of these materials, scaffolds and implants gained nonimmune reactive properties like decreased adhesion of surface proteins and lower interactions with macrophage cells. For example, when macrophage cells were cultured on titanium with different wettability, they showed diverse effects on cytokine secretion and macrophage polarization: on hydrophobic titanium surfaces, the number of macrophages with pro-inflammatory M1 phenotype was increased with highly induced cytokine levels responsible for M1 type inflammation (such as IL-1β, IL-6, TNFα). However, on hydrophilic surfaces, M2 type anti-inflammatory macrophages were in a very high number and they also secreted anti-inflammation cytokines (such as IL-4, IL-10).[111] Macrophage morphology is affected by the surface wettability, too. It was shown that on hydrophobic carbon nanofiber surfaces, macrophages elongated more and the number of their filopodia was increased. However, on hydrophilic scaffolds, the cells remained rounded and not elongated with fewer filopodia even after 48 h (Fig. 5D).[112]

3.5. Shape

The shape of implanted devices has also been known to be effective on immune cell behavior and host response. Especially, the phagocytosis of the small particles (formed as a result of degradation of the scaffolds by macrophages) can be modulated by the particle shape and size.[93] It was shown that particle shape rather than their size was more effective in the internalization process of these small degradation products through phagocytosis. For example, particles of polystyrene (PS) were designed with varying geometric shapes (spheres and disks) in micrometer sizes. It was noted that macrophages phagocytosed disks with elliptical shapes through their major axis in a very short time, like 6 min, whereas did not engulf them even after 2 h through their minor axis. However, the

internalization of spheres was homogeneous (Fig. 5E).[113] In another study, the importance of implant geometry in the modulation of its biocompatibility was demonstrated *in vivo*. A mouse model with FBR resembling that of humans was used (C57BL/6 mouse) and hydrogels of alginate with spherical shapes with different diameters ranging between 0.3–1.9 mm were inserted in mice. When their immune reaction to spherical biomaterials was evaluated, it was observed that spheres with diameters more than 1.5 mm were more biocompatible with lower FBR and fibrosis than the spheres with smaller sizes.[114]

3.6. Degradability

The degradability of biomaterials can influence immune cell recruitment and responses; thus, crosslinking is important in immune modulation too. For example, hyaluronic acid scaffolds were enzymatically degraded into small fragments and these fragments had varying molecular weights. When they were incubated with dendritic cells and T cells, increased activation of dendritic cells and higher proliferation of T cells were reported with particles having a low molecular weight.[115] Moreover, it was presented that hyaluronic acid with low molecular weight induced proinflammatory macrophages, whereas particles with high molecular weight promoted anti-inflammatory phenotype.[116] On the other hand, the influence of a crosslinking agent was investigated and it was shown that collagen scaffolds crosslinked with glutaraldehyde induced a higher number of neutrophils than the polymers treated with hexamethylene diisocyanate since two crosslinkers caused degradation of the scaffolds in different durations. Samples crosslinked with glutaraldehyde were degraded after 28 days, whereas the samples crosslinked with hexamethylene diisocyanate crosslinker were not degraded in the same duration.[117] Also, phagocytosis of collagen scaffolds was determined by their degradation rate that samples with glutaraldehyde crosslinker were phagocytosed by macrophages whereas phagocytosis of samples crosslinked with hexamethylene diisocyanate was never observed. Thus it was concluded that only glutaraldehyde was able to develop a microenvironment suitable for the induction of neutrophils and macrophages (Fig. 5F).[117]

3.7. Mechanical properties

As well as the chemical and surface properties of implants, bulk features such as mechanical properties can also affect the behavior of innate immune system cells. Macrophages were shown to change their phenotypes and activation (such as differentiation or movement) as a response to the stiffness of the biomaterial, which proves the capability of stiffness in directing macrophage phenotype. Macrophages (THP-1 cells) were grown on collagen scaffolds which were coated with polyacrylamide gels and these gels were designed with different stiffnesses. Gel coated collagens with higher stiffness (323 kPa) induced pro-inflammatory M1 type macrophage cells and resulted in lower phagocytic activity. However, the two scaffolds with lower stiffness (11 kPa and 88 kPa) M2 cell profile and increased phagocytosis with a higher number of small particles were observed as engulfed in macrophages (Fig. 5G).[118] In a different research, the influence of strain force on the immune cell activity was investigated and cells derived from peripheral blood were seeded on scaffolds made of poly-ε-caprolactone polymers. After applying cyclic loads with varying stretchings (7%, 12% and controls with 0% load), innate immune response markers were evaluated in these cells after one week. It was shown that the cyclic load with the highest ratio resulted in the polarization of cells into M2 phenotype with induced anti-inflammatory markers.[63]

Further examples showing the effect of biomaterial properties (biomaterial type, surface chemistry, surface topography, wettability, shape, degradability, mechanical properties) on the behavior of innate immune system cells are summarized in Table 1:

4. Harnessing macrophages in regenerative medicine

Biomaterials that can manage immune cell response and tissue renewal activity of these cells are needed for the control of wound healing and tissue repair.[138] Harnessing the inflammatory response, in particular macrophages due to their unique plasticity, can be a useful approach to enhance tissue repair. There should be a balance in the abundance of two different phenotypes (M1 and M2) of macrophages since pro- and anti-inflammation activities of these cells decide the fate of tissue repair. This regulation is possible with the fine-tuning of biomaterial properties.[139] The presence of more cells with M1 phenotype than M2 macrophages can inhibit tissue repair.[140] Moreover, pro-inflammatory M1 macrophages and the cytokines produced by these cells play a role in angiogenesis, whereas M2 macrophages make angiogenesis steady but induce the proliferation of fibroblasts and construction of the extracellular matrix.[141] Additionally, macrophages take part in wound repair since they can encapsulate and phagocytose small sized molecules such as cellular debris, neutrophils, apoptotic cells and other foreign bodies.[142] These cells are also capable of producing chemokines, matrix metalloproteinases (MMPs), and various inflammatory molecules.[142] Both M1 and M2 macrophages and the molecules secreted by these cells activate blood vessel development, differentiation and activation of several cell types such as fibroblasts into myofibroblasts (for wound contraction and closure), parenchymal cells, stromal cells, stem cells and progenitor cell populations (for tissue healing and repair).[142] As a result of these critical roles in tissue repair and wound healing, various recent studies have focused on the modulation of macrophage–biomaterial interactions. For example, monocytes in the circulation system were directed by polymeric biomaterial films at the skin injury sites and after they accumulated on the polymer surfaces, they triggered vascularization and wound healing with the dominance of anti-inflammatory macrophages.[143] In another work, the activity of macrophages in the loss of muscle volume was evaluated and delivery of molecules with

Table 1 Effect of biomaterial properties on the behavior of innate immune system cells

Biomaterial property	Biomaterial	Cell type	Immunomodulatory function	Ref.
Biomaterial type (natural, synthetic, *etc.*)	Alginate, agarose, chitosan, hyaluronic acid, poly(lactic acid-*co*-glycolic acid) (PLGA)	Dendritic cells (DC)	PLGA, chitosan, and alginate activated DCs; HA and agarose prevented maturation of DCs	119
	Chitosan	Neutrophil	Increased IL-8 release and migration in neutrophils	120
	Polycaprolactone (PCL)	Macrophage	Induced M2 macrophage polarization, enhanced vascular remodeling and regeneration	121
	Polydimethylsiloxane (PDMS), polytetrafluoroethylene (PTFE), polystyrene (PS), and tissue culture polystyrene (TCPS)	Dendritic cells (DC)	PTFE and PDMS increased activation of dendritic cells	122
	Polypropylene meshes coated with decellularized ECM based hydrogel	Macrophage	Coated meshes directed macrophage polarization towards M2	105
	Self-assembled monolayers (SAMs) with functional groups (CH_3, OH, COOH, NH_2)	Dendritic cells (DC)	OH, COOH, and NH_2 caused more DC activation than CH_3	123
	Polyethyleneimine (PEI), polylysine, dextran, gelatin with cationic groups	T cells	Cationic groups triggered Th1 response with the toll like receptor-4 (TLR-4) induced IL-12	124
Surface topography	Micropatterned substrates designed with fibronectin patterns with 50 or 20 μm width gratings and 20 μm gaps coated with Pluronic F127	Macrophage	Macrophages elongated through the gratings and elongation induced M2 phenotype with decreased pro-inflammatory cytokines	125
	Titanium surfaces with micro- and nanogrooves (groove width: 0.15–50 μm, pitch: twofold of width, depth: 0.8–1.3 μm)	Macrophage	Grooves induced M2 macrophage phenotype	126
	Nanopatterned Silica with 30 nm groove depth	Macrophage	Nanogrooves induced more phagocytotic activity than smooth surfaces	127
	Poly(ε-caprolactone) (PCL), poly(dimethyl siloxane) (PDMS), poly(lactic acid) (PLA) surfaces with micro/nano patterns with 250 nm–2 μm width lines	Macrophage	Macrophages elongated more on 500 nm lines compared with smooth surfaces. Patterns with larger widths induced higher production of TNF-α and VEGF than smaller widths	59
Wettability	Microspheres of poly(D,L-lactic acid) (PLA), poly (D,L-lactic acid-*co*-glycolic acid) (PLGA), poly (monomethoxypolyethylene glycol-*co*-D,L-lactide)	Dendritic cells (DC)	PLA with high hydrophobicity directed higher antigen localization into DCs and increased CD86 marker	128
	Titanium	Macrophage	Titanium with hydrophilic surface decreased pro-inflammatory cytokines production	129
Shape	Polystyrene particles	Macrophage	Macrophages internalized particles with spherical shapes more than with high aspect ratios	113
	Silica particles	Macrophage	Particles with smaller diameter (submicron) activated inflammation more than the higher diameter particles (higher than 1 micron)	130
	Glass fibers with 0.1 μm–10 μm diameters	Macrophage	Particles with lower size induced production of immune reactive molecules *in vitro* and macrophage migration *in vivo*	131
	Poly(methyl methacrylate) (PMMA)	Macrophage	Increased M1 polarization was observed with macrophages inside the pores of scaffolds	61
Degradability	Hyaluronic acid	Dendritic cell	Low molecular weight (1500–5300 Da) showed induced dendrite function, cytokine production, and T cell number	115 and 132–134
	Hyaluronic acid	Macrophages	Low molecular weight induced M1 macrophage activation whereas high molecular wight induced M2 phenotype	116 and 135
Mechanical properties	Poly(ethylene glycol) (PEG) hydrogels	Macrophage	Higher stiffness (>100 kPa) directed pro-inflammatory M1 macrophage polarization	136
	Poly(lactic acid-*co*-glycolic acid) (PLGA)	Dendritic cells (DC)	Higher mechanical properties and porosity induced DC activation	62
	Agarose gels	Macrophage	Lower stiffness induced anti-inflammatory M2 macrophage polarization	137
	3D collagen construct	Macrophage	Higher stiffness directed M2 macrophage polarization, increased IL-10 and decreased IL-12 and TNF-α production	67
	Collagen gels	Macrophage	Lower stiffness (30 Pa) directed M1 macrophage activation more than higher stiffness (100 Pa)	67

biomaterials was used to trigger anti-inflammatory macrophages. With this targeted delivery, M2 type macrophages proliferated and were recruited to the damaged muscle tissue, and regeneration, healing, vascularization and deposition of collagen were achieved which resulted in an increase in muscle volume.[144] In a different study, scaffolds for bone tissue engineering obtained by the decellularization of natural tissues were used in the control, production, and release of cytokines (IFN-γ and IL-4) play a role in M1 and M2 macrophage polarization. Sequential release of cytokines was planned since the sequential differentiation of macrophages into M1 and M2 was shown to induce angiogenesis and tissue repair by the same group. In this study, delivery of M1 and M2 cytokines in an order resulted in enhanced vascular formation.[145]

In an *in vivo* study with rat models, tissue damage in their abdominal walls was healed with tissue regeneration modulated by macrophage activities. Autologous tissue insertions with acellular and cellular groups were used to trigger macrophage activation and acellular inserts resulted in M2 macrophage proliferation with improved tissue healing, whereas cellular groups induced mostly M1 phenotype which results in increased connective tissue formation.[146] Decellularized ECMs are commonly used in macrophage modulation studies since they have the advantage of avoiding inflammatory foreign body response after inserting into the host body. For example, decellularized ECMs were obtained from porcine colon tissues and they were used to produce sheets and hydrogels. These substrates induced M2 phenotype predominantly with anti-inflammatory, tissue regenerative capability *in vitro* and *in vivo.*[147] In another application, methacrylate based hydrogels with channel structures were synthesized for heart tissue engineering. These hydrogel channels in a parallel organization helped the alignment of micron sized cardiomyocyte cells for angiogenesis. Hydrogels with 30–40 μm pore sizes resulted in M2 type polarization, increased angiogenesis and decreased fibrotic response with tissue regenerative capacities.[148]

Furthermore, delivery of stem cells is widely applied as an approach in regenerative medicine due to their differentiating capacity and regenerative properties, but most importantly, they are known to provide strong immune controlling features and take a role in the secretion of molecules that directly modulate macrophage activation. For example, these stem cells were used to repair mouse skin damage and they were encapsulated in gelatin hydrogels together with macrophage cells. It was reported that encapsulation of both cell types and the interaction of the cells and their microenvironments help the regeneration of skin tissue and wound healing in mice.[149] Many recent studies show that there is a cooperation between stem cells and macrophages and this cooperation can be enhanced with the modulation of biomaterial properties. For example, it was shown that on collagen coated polyacrylamide gels with different stiffnesses (11 kPa, 88 kPa, 323 kPa) MSCs showed immune modulating properties when they were in M1 media regardless of the substrate stiffness. However, substrate stiffness was effective when the MSCs were seeded together with macrophage cells. On the substrates with 11 kPa and 88 kPa stiffness but not on the 323 kPa, macrophages secreted lower TNF-α and higher of IL-10.[150] Another study reported that hydroxyapatite nanoparticles induced IL-10 production in macrophages, which is an indication of anti-inflammatory phenotype, and also increased IL-10 resulting in differentiation of MSCs through an osteogenic phenotype with increased bone morphogenetic protein-2 (BMP-2) and alkaline phosphatase (ALP) gene expressions.[151] Similar results were also reported in a 3D environment in another recent study. In the study, 3D gelatin hydrogels were produced and MSCs and macrophages were encapsulated in the gels. When co-cultures of these two cells were compared with single cell cultures, it was shown that MSCs induced M2 polarization of macrophages, whereas M1 macrophages induced osteogenic differentiation of MSCs.[152] All these studies show that no reciprocal interaction of stem cells and macrophages can be modulated with the control of biomaterial properties.

5. Outlook: use of adaptive immune cells in regenerative medicine

Adaptive immune system cells consist of T and B cells with immunological memory. Their most critical role in the immune reaction is the disposal of foreign bodies after the reinfection of the host body by using their memories. The adaptive immune response includes mostly the chronic phase of inflammation and is regulated by T cells. The cells are activated into either Th1 or Th2 cells. After the activation of Th1 cells by IL-12 cytokine or antigen presenting cells (APCs) released from the spleen, they take a role in M1 macrophage polarization and disruption of tissue regeneration and transplanted cells. Th2 cells triggered by IL-10, on the other hand, alternatively induce M2 macrophage phenotype which takes a role in healing and repair of tissue.[153] When M1 macrophages and Th1 helper cells are induced at the right time, they can play a role in the removal of damaged tissue, stimulation of angiogenesis and promotion of stem cell proliferation. Stimulation of M2 macrophages and Th2 cells directs the remodeling of ECM, vascularization and differentiation of progenitor/stem cells but a prolonged Th2 stimulation results in fibrosis.[154] While T cells have a very crucial role in regeneration, there is not much known about the activity of B cells in the repair and renewal of the tissue.[53] However, due to the activities of T cells in tissue regeneration and repair, adaptive immune cells should be considered in immunomodulatory applications of the biomaterials. For example, it was reported that T cells with a positive CD8 surface marker and cytotoxic activity could interrupt the healing of fractured or damaged bone tissues with their negative role in osteogenesis and bone healing.[155] However, T cells with positive CD4 markers can enhance tissue regeneration depending on the activity of their subtypes (TH1, TH2, TH17, regulatory T (Treg) cells).[156,157] Especially, the regulatory Treg cells can enhance the activity of

stromal and progenitor cells for improved tissue regeneration.[156]

There are various studies using this unique regenerative property of T cells for the design and production of immunomodulatory biomaterials. For example, tissue-derived biomaterial scaffolds were shown to enhance the development of a pro-regenerative immune microenvironment and include adaptive immune cells, $CD4^+$ Th2 T cells.[158] In another study based on the substrate dependent use of dendritic cells, the cells cultured on extracellular matrix proteins assisted adaptive immune responses and induced increased T-cell numbers with increased T-helper cell activity.[159] At this stage, biomaterials with smart properties and produced with novel technologies gain importance to control the regeneration capacity of adaptive immune cells. There are several recent studies on the use of these biomaterials. For example, in a study, stem cell sheets were produced with ADSCs and then they were decellularized and implanted into rats. It was reported that these decellularized stem cell sheets induced anti-inflammatory cytokines in the hosts.[160] Another recent technology is the use of microneedles as drug delivery systems since they have an advantage over other injection methods in terms of delivery efficiency. In a study, polyvinyl alcohol (PVA), poly(*N*-vinylpyrrolidone) (PVP) and chitosan based microneedles were produced with an LBL method and drug loaded for targeting melanoma. It was reported that microneedle delivery systems showed both anti-tumor efficiency and increased T-lymphocytes at the tumor site.[161] Another novel method is the activation of chimeric antigen receptor (CAR-T) cells with the use of biomaterial systems for immune modulation. For example, poly(lactic acid-*co*-glycolic acid) (PLGA) microparticles were produced and their surface was functionalized with DNA fragments. These microparticles were used to activate CAR-T cells targeting HER2 positive tumor cells by the delivery at the tumor site and it was reported that this surface functionalized biomaterial based system achieved the activation of T cells.[162] Another example of smart scaffolds is the injectable hydrogels used for drug delivery and immunotherapies. In a recent study, a smart hydrogel system was designed using hyaluronic acid and polycaprolactone. After the injection of hydrogel, it turned into a microporous 3D microenvironment. By loading macrophage and dendritic cell stimulating factors in the hydrogel system, the increased number of dendritic cells were achieved at the injection site and T cells targeting melanoma tumor were activated.[163] A different study using 3D scaffolds reported that T cells were affected by the stiffness of the scaffold biomaterial and this property can be used to modulate T cell activity and immune response regulation. In this study, 3D alginate scaffolds with varying stiffness (4–40 kPa) were used to carry T cells and the scaffolds with higher stiffness induced T cell activation more.[164] Another smart biomaterial use in immune regulation is based on DNA origami or origami based designed smart biomaterials. With the origamic self-assembly of DNA or biomaterials, especially hydrogels, cell carrier scaffolds are designed with more controlled properties and cell responses to the microenvironment are modulated and directed.[165–167] These studies show the significance of adaptive immune cells and their interactions with innate immune cells in the creation of biomaterials for varied utilization, such as scaffolds for regenerative medicine applications, vaccines of which activity are based on synthetic particle based vaccines and for immunotherapies.

Conflicts of interest

NEV declares conflict of interest as the majority shareholder of Spartha Medical.

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