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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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1 **The Role of Scaffolds on Immune Response in Regenerative Medicine: Macrophage Phenotype**  
2 **Modulation by Scaffold Architectures**

3 **Revised title: The Role of Biomaterials and Scaffolds on Immune Response in Regenerative**  
4 **Medicine: Macrophage Phenotype Modulation by Biomaterial Properties and Scaffold**  
5 **Architectures**

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1 **Abstract:**

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

20

21 **Keywords:** macrophage, tissue engineering scaffold, phenotype plasticity, cell material-  
22 interaction, regenerative medicine

23

## 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 also their introduction to the dynamic environment of tissues and organs improve their chance in potential clinical translation. The current developments in multi-ink 3D printing systems using multiple biomaterials also provide an advanced environmental control over the microscale 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 response given by the host to foreign bodies such as biomaterials is not typical due to the persistency of foreign body reaction modulated by two innate immune system cells, foreign body giant cells (FBGCs) and macrophages, at the tissue/substrate interfaces <sup>1</sup>. The uniqueness of these interfaces and the specificity of the interactions between cells and implants depend on the 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, in contrast, biomaterial properties are used to modulate the immune response. Especially, *in vivo* application of these immunomodulatory implants yields very promising results in terms of recruiting 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 system cells to an implanted biomaterial <sup>2-11</sup>.

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 <sup>12</sup>. In another study, two groups of surgical sutures made of nylon or polyglycolic acid polymers with same sizes were implanted in a zebrafish model and Foreign Body Reaction (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 to nylons <sup>13</sup>. 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 tendon were grown on nanofibers and it was reported that macrophages 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 in to the Achilles tendons of the rats and non-aligned fiber scaffolds showed higher macrophage abundance with pro-inflammatory phenotypes after 7 days <sup>14</sup>.

1 Another application of immune cell modulation by biomaterial properties was reported for the  
2 treatment of pelvic organ prolapse. In this study, electrospun poly L-lactic acid-co-poly  $\epsilon$ -  
3 caprolactone nanofibrous meshes with and without endometrium derived mesenchymal stem  
4 cells were implanted in mice <sup>15</sup>. Earlier studies of the same group showed that nano- and  
5 microfiber meshes induced a favorable proliferation and differentiation of MSCs <sup>16,17</sup>. Results  
6 after 6 weeks of implantation showed that MSC seeded scaffolds induced M2 type macrophage  
7 polarization and increased tissue integration to the implant whereas meshes without MSC  
8 induced M1 type macrophage phenotype <sup>15</sup>. In another recent study, a novel design was created  
9 with 3D printing technology. With the use of camphene and polycaprolactone (PCL),  
10 microchannel structures (with 20-40  $\mu$ m diameters) were obtained as a result of phase separation  
11 between these two polymers. Scaffolds with hierarchically deposited fibers with and without  
12 microchannels were implanted into mice subcutaneously. After 7 days, it was observed that M2  
13 type macrophages were dominantly higher on micropatterned scaffolds than the controls without  
14 microchannels <sup>18</sup>.

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

17 Moreover, the individual aspect of the innate immune ~~system~~ cells is much more pronounced  
18 than the other tissues; thus, establishing ground rules on the immune response to given  
19 properties has proven to be difficult. For example, a recently approved lifting solution for the  
20 treatment of submucosal resections (ORiSE) has been shown to induce granulomatous growths  
21 in some patients <sup>19</sup>. This synthetic polymer mixture where the main component is a polyoxomer,  
22 can induce such reaction due to one or more components of its composition or the reaction can  
23 be based on the sensitivity of the individual patient, so the reaction cannot be generalized and  
24 the individuals that would be prone to such reactions need to be detected <sup>19</sup>. Other examples can  
25 be given on complications related to double encapsulation in breast implants. It was proposed  
26 that double capsule is observed when the surface of the implant is textured and rough. First  
27 capsule formed on these textured surfaces may be separated due to an external force and result  
28 in seroma formation in between the primary and secondary capsules. Implants with smooth  
29 surfaces do not form secondary capsules since they let tissue ingrowth through the primary  
30 capsule <sup>20</sup>. Textured breast implants may result in several complications linked to this chronic  
31 inflammation because of the development of a double capsule. This prolonged exposure to high  
32 amounts of pro-inflammatory cytokines, proteins, and other components related to inflammation  
33 may result in pain, swelling, seromas, DNA damage, and even cancer due to ~~the~~ chronic  
34 inflammation <sup>21</sup>. For example, in a study, several double capsules were obtained from breast  
35 implants and analyzed in terms of bacterial growth and biofilm formation. SEM examination  
36 showed that on prosthesis interface (textured) there was increased bacteria growth and biofilm  
37 formation than on intercapsular space interface (flat). This study suggested that the double  
38 capsule formation was originated mostly as a result of shear stress <sup>22</sup>. This shows that even in  
39 passive implants, architecture plays a substantial role ~~on~~ in the immune response in a long period.  
40 In the more dynamic environment of degrading/remodeling scaffolds, this role is even more  
41 significant.

42 Other examples of biomaterial induced immune responses include aseptic loosening and  
43 osteolysis of implants used for hip and joint which are caused by macrophage mediated  
44 inflammatory reactions to implant-derived wear debris <sup>23</sup>, implant related oxidative stress due to

1 the activity of monocytes and macrophages which generate degradative/reactive oxygen and  
2 nitrogen species (ROS and RNS) <sup>24</sup>, allergic reaction, or hypersensitization (whose definition is  
3 giving strong immune reaction or response due to the existence of an antigen) may be resulted  
4 from the use of the implants immunogenic to some patients <sup>25,26</sup>, and granuloma formation  
5 consisting of aggregates of macrophages, often including multinucleated giant cells, and  
6 lymphocytes observed mostly in breast <sup>27</sup> and dental <sup>28</sup> implants and tracheal/laryngeal stents.  
7 Thus, these examples demonstrate that in the efforts in complexifying the physical structure of  
8 scaffolds, the potential risks pertaining to the immune reaction to such complex architectures  
9 post-implantation and throughout their remodeling should also be taken into account.

10 Immunomodulation in tissue engineering can result in a better tissue regeneration, healing,  
11 angiogenesis, and tissue remodeling. Communication and cooperation between innate immune  
12 cells and stem/progenitor cells can make a balance between biomaterial degradation and tissue  
13 regeneration and decide the progression of tissue repair and inflammation. While phagocytic cells  
14 like macrophages result in the regeneration of the tissue through debris clearance, at the later  
15 stages of inflammation, compensation between M1 and M2 macrophages is needed for tissue  
16 repair. Even though M1 macrophages are involved in the initial vascularization steps, if they  
17 persist in later stages, they may cause harm to tissue as a result of the secretion of pro-  
18 inflammatory molecules. However, it is also known that clearance of necrotic tissue by M1  
19 macrophages can help tissue regeneration. M2 macrophages with anti-inflammatory properties  
20 should be the dominant cell phenotype in tissue repair and wound healing whereas excessive M2  
21 presence may lead to fibrotic encapsulation more than efficient healing <sup>29</sup>. Thus, there should be  
22 a balance between early M1 proinflammatory signals (may result in tissue damage when  
23 uncontrolled) and late M2 anti-inflammatory signals (may disrupt tissue healing if macrophages  
24 stay immature) <sup>29</sup>. A recent study reported that the presence of M1 phenotype more than M2  
25 resulted in disruption of tissue repair. It was stated that excessive anti-inflammatory polarization  
26 with IL-4 cytokines may shift the balance of macrophages through a direction improving tissue  
27 regeneration <sup>30</sup>. On the other hand, during angiogenesis, innate immune system cells support the  
28 formation of new vascular structures by the release of soluble factors <sup>29</sup>. These key roles of innate  
29 immune system cells in tissue repair and regeneration should be taken into consideration in the  
30 construction of biomaterial-based implants and medical devices to modulate the immune  
31 response of the body.

32 Although the effect of scaffold architecture on the innate immune response is known and  
33 presented recently <sup>31,32</sup>, there are still not many studies on this perspective and the recent studies  
34 and literature reviews do not include the immunomodulatory effect of the biomaterial  
35 architecture at the in vivo level <sup>31-36</sup>.

36 Biomaterial risk assessment is the most critical step before the manufacturing and marketing of  
37 an implant; however, there is no regulation to check the immunomodulatory effect of 3D shape  
38 and architecture of the implants <sup>37</sup>. Thus, in this review, we aim to cover the recent studies that  
39 elucidate the behavior of macrophages in 3D biomaterial environments and that define  
40 biomaterial property immune reaction relationships. Then, in vitro immune response models and  
41 on-chip innate immune system models are covered. The incorporation or recruitment of innate  
42 immune cells in regenerative medicine context with the use of scaffolds as a potential venue of  
43 immunomodulation is also presented.

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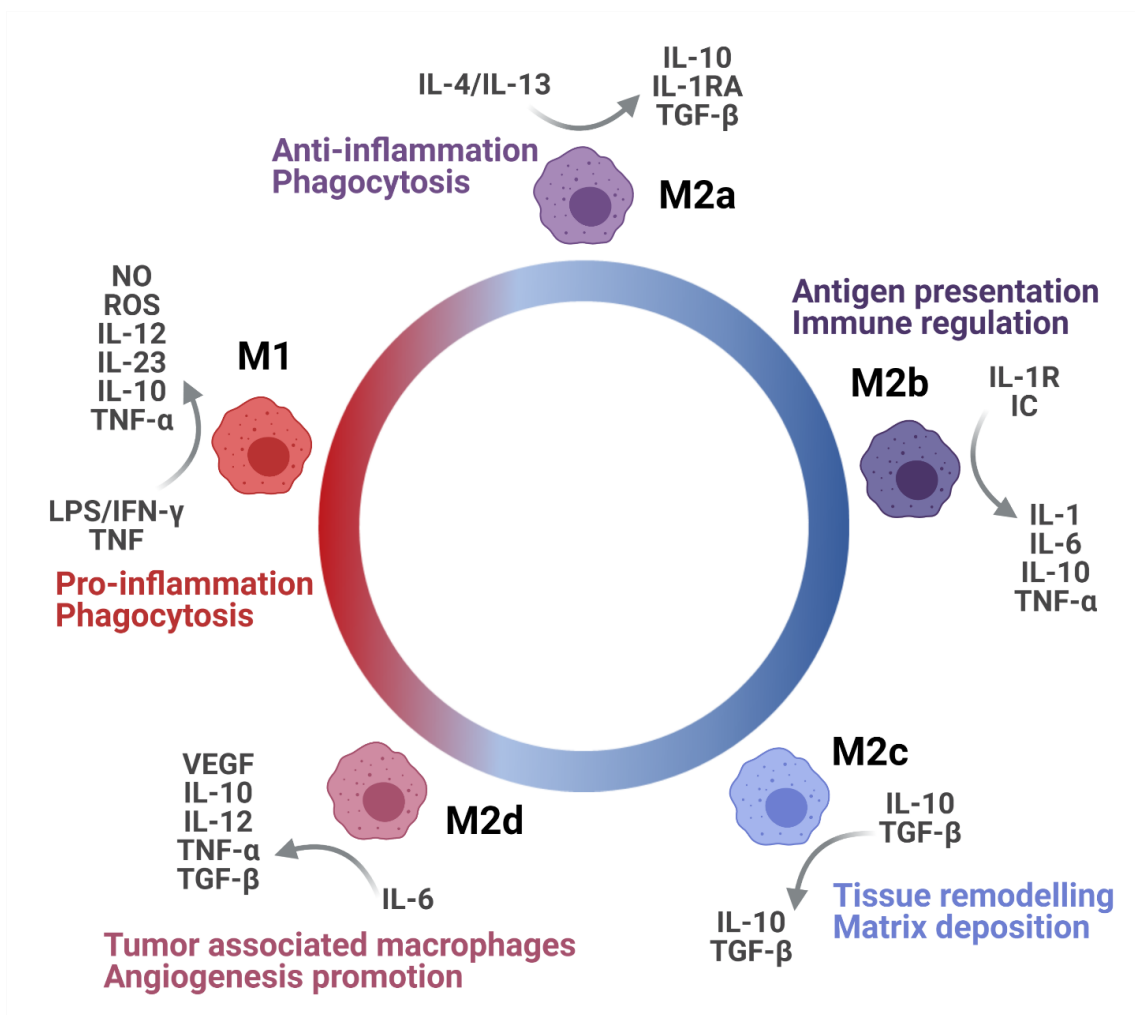
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## 2. Foreign Body Reaction (FBR)

The generation of adverse immune reactions in the host body is the most common result of the insertion of 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 inclusion of the various type of cells and immune components of the body<sup>38</sup>. There are two major problems affecting the function of the biomaterial: first is the fibrous isolation/encapsulation of the biomaterial device which endanger the performance of the device and leads to device failure and 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 degraded biomaterial products entering the circulatory system<sup>38</sup>. Biomaterial insertion always comes with an injury at the host tissue. As a response to injury, a series of events occurs as an inflammatory response which were described first by Anderson for implants<sup>39</sup>. 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<sup>39,40</sup>. Immune reaction to a biomaterial based implant takes place with both innate and adaptive immunity<sup>41</sup>. 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 in a long time (days/weeks)<sup>35,41,42</sup>. This immune reaction can be acute or chronic decided by the specific properties of the inserted biomaterial and the host tissue. Both innate and adaptive immunities take 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, and it is composed of fibroblasts and vascular endothelial cells proliferating around the implanted device and on the surface of implant. With the proliferation of these vascular endothelial cells new blood vessels are formed from the preexisting vessels by angiogenesis (neovascularization)<sup>43</sup>. Fibroblasts proliferate and take part in the synthesis of collagen and proteoglycans. Macrophages are the critical cells in the formation of the innate immune response. They 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<sup>44</sup>. They encourage inflammation and secrete pro-inflammatory cytokines and chemokines; IL-1, IL-6, IL-8, TNF- $\alpha$ , macrophage inflammatory protein-1 (MIP-1) and monocyte chemotactic protein-1 (MCP-1)<sup>44</sup>. M2 (anti-inflammatory) macrophages are present in very large numbers and are very important in the progression of inflammation since they play role in repair of damaged or injured tissues. They are the

1 alternatively activated macrophages and promote tissue remodeling during chronic inflammation  
 2 <sup>45</sup>. They are responsible for the secretion of immune modulating small molecules (cytokines and  
 3 chemokines) such as IL-4, IL-10 and TGF- $\beta$  which play a role in tissue regeneration and wound  
 4 healing <sup>46</sup>. Macrophage polarization is more of a spectrum than a distinct polarization of two  
 5 phenotypes. M2 phenotype has four different subtypes: M2a, M2b, M2c and M2d (Tumor  
 6 associated macrophages, TAMs) (**Figure 1**) <sup>46</sup>. These phenotypes are subdivided based on their  
 7 functions <sup>47</sup>. Macrophages can phagocytose small particles with a size up to 5  $\mu\text{m}$  in diameter.  
 8 However, for the bigger larger particles, they need to fuse and form *foreign body giant cells*  
 9 (FBGC) <sup>43</sup>. Macrophages and FBGC exist in the inflammation site together. These two cells form a  
 10 sheet with varying thicknesses depending on the roughness, topography, and chemistry of the  
 11 biomaterial surface. They regulate the degradation of the biomaterial by releasing phagocytic and  
 12 degradative (oxidative) reactive species at the interaction zone of cells and biomaterial surfaces  
 13 <sup>48</sup>.



14  
 15 **Figure 1:** Spectrum of macrophage phenotypes based on their functional activities in immune  
 16 response and cytokines activating and triggered by these phenotypes (Created with  
 17 BioRender.com).  
 18



1 FBGC formation with the fusion of macrophages has unique importance since it is the most  
2 definite indication of FBR that distinguishes FBR from a typical chronic inflammation. In this  
3 process, a large number of macrophages come together and fuse to form FBGC and the size of  
4 FBGC can reach up to hundreds of  $\mu\text{m}$  with dozens of nuclei<sup>49</sup>. They can stay at the implantation  
5 site during the whole life time of the implant device remain embedded in the host body<sup>49</sup>. FBGC  
6 existence at the implant surface is usually not desired since they give rise to degradative species  
7 such as ROS, NOS and enzymes. These reactive molecules can degrade the implanted material  
8 and cause device failure<sup>49</sup>. The extent of this degradation depends on the characteristics of the  
9 exterior sides of the biomaterial facing the tissue. As a result of the specific properties of implants,  
10 the amount and type of adsorbed proteins on their surfaces may vary and affect FBGC formation.  
11 For example, when monocyte and FBGC adhesions were evaluated on polyacrylamide and  
12 polyacrylic acid surfaces, it was shown that monocyte adhesion and FBGC formation were higher  
13 on cationic surfaces than on anionic surfaces<sup>50</sup>. Moreover, FBGC formation was evaluated after  
14 intramuscular insertion of poly(l-lactide-co-d/l-lactide) (PLA) implants into rats. Implants designed  
15 as membrane sheets and electrospun fiber meshes (uncoated and coated with a positively  
16 charged plasma polymer) were compared and it was reported that cell number was higher on  
17 fibers than on membranes due to the higher roughness with the fiber meshes. However, the cell  
18 number did not change significantly on coated and uncoated surfaces that surface roughness was  
19 a more effective parameter than the surface chemistry<sup>51</sup>.

20 Progression of inflammation with the formation of FBGC finally results in the development of  
21 fibrotic collagenous capsule covering the surface of the device. This fibrotic encapsulation isolates  
22 it from the host tissue. Both the local microenvironment of the implantation site and the  
23 biomaterial surface properties can affect macrophage adhesion, polarization, fusion and  
24 apoptosis<sup>52</sup>. Fibrous capsule mostly remains for the lifetime of the implanted material<sup>44</sup>.

25 Two simultaneous events in the host, migration of macrophages to inflammation  
26 site/degradation of the scaffold by macrophage activity and induction of an anti-inflammatory  
27 healing process with the release of cytokines determines the regeneration extent after the  
28 implantation. This harmony between the tissue regeneration and scaffold degradation must be  
29 sustained by modulating the properties of scaffolds without compromising their functionality.  
30 Development of immune reaction to an inserted medical device to the host body is determined  
31 by the conditions and properties of the surrounding tissue, properties of the scaffold and the  
32 interactions at the interface between implant and host tissue. These three components of cell-  
33 biomaterial interactions dictate the performance of the implanted device.

## 34 **2.1. Effect of biomaterial properties on macrophage phenotype**

35 Macrophages are present in most tissues, but they start to proliferate after the insertion of an  
36 implant device and injury formation. They are differentiated from monocytes circulating in the  
37 blood and migrating to the implantation site in the first days of inflammation<sup>53</sup>.

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

1 Besides their role in the innate immune system, macrophages are also involved in the regulation  
2 of stem cell proliferation and differentiation. Thus, the design of biomaterials is important in both  
3 controlling the immune response and the repair and regeneration of tissue through the functions  
4 of macrophages. As was stated earlier, interactions of macrophage cells and implant devices are  
5 very important in the design and production of biomaterials and implants and in the  
6 enhancement of biomaterial/tissue interactions. In the literature, there are various studies  
7 showing the modulation of biomaterial properties for guiding the macrophage polarization  
8 towards desired phenotypes and these studies report that the function of macrophages in  
9 immune response is dependent both on the macrophage phenotype and the biomaterial type<sup>47</sup>.  
10 Their role in tissue repair and healing also depends on the type of tissue<sup>55</sup>. The influence of  
11 chemical cues on biomaterial surfaces on macrophage phenotypes is determined by the number  
12 of cell adhesion ligands<sup>55</sup>, surface chemistry<sup>56</sup> and the content of ECM<sup>57</sup>. The effect of physical  
13 cues, on the other hand, are determined by stiffness<sup>58</sup>, topography<sup>59</sup> and pore size<sup>60–62</sup> of the  
14 biomaterial. Also, mechanical forces, such as cyclic or magnetic loading, can also affect the  
15 phenotype, cytokine secretion and the regenerative function of macrophages<sup>63,64</sup>. It should also  
16 be noted that, there is a material specific aspect on macrophage polarization and on biomaterials,  
17 macrophages nearly never behave within the defined phenotypical profiles under cell culture  
18 conditions. Thus, the presence of biomaterial specific macrophage phenotypes should also be  
19 considered.

20 In this part of the review, we will center on the effect of biomaterial dimensionality on  
21 macrophage polarization. It is known that 2-dimensional (2D) and 3-dimensional (3D)  
22 microenvironments affect cell behavior in different ways<sup>65</sup>. In 2D, cells grow as monolayers which  
23 make their activities more homogenous such as differentiation or polarization, interaction with  
24 neighboring cells and availability of nutrients and growth factors. However, since in natural tissue,  
25 the cells are in a 3D microenvironment, only a group of cells survive and adapt to the conditions  
26 of 2D microenvironment. This results in the continuation of only a selected cell population on 2D.  
27 In 3D, complex structure, composition and properties of native tissue can be mimicked and the  
28 heterogeneity of the tissues and cells can be reflected<sup>66</sup>. Thus, it is expected that 2D and 3D  
29 biomaterial implants present the immune response in different ways and influence the  
30 morphology, polarization and activation of macrophages differently. The first property of cells  
31 affected by the dimension of the microenvironment is adhesion and morphology as a result of  
32 mechanosensitivity of the cells which makes the cells sense the external signals from the  
33 environment and change the downstream signaling pathways<sup>67</sup>. In a study, the effect of  
34 dimensionality of the substrate on human macrophage polarization was studied. 2D collagen films  
35 and 3D collagen constructs were used to seed the macrophages. On 2D; flattened and spread cell  
36 shape, and in 3D collagens; round cell shapes with 20–30  $\mu\text{m}$  diameter were obtained. Also, 2D  
37 and 3D scaffolds affected the cytokine secretion in different ways that in 3D, macrophages  
38 secreted increased amounts of anti-inflammatory IL-10 and decreased pro-inflammatory IL-12  
39 and TNF $\alpha$ , whereas on 2D, cells released elevated levels of these cytokines<sup>67</sup>. In another study,  
40 PLGA polymeric biomaterial was used in two different shapes and dimensions: 3D nanofiber  
41 meshes and 2D films. These substrates were seeded with human macrophages and it was shown  
42 that 2D films induced an increased number of M2 macrophages with a positive CD163 marker

1 whereas 3D nanofibers increased the number of M1 macrophages with positive 27E10 anti-  
2 inflammatory phenotype marker <sup>68</sup>.

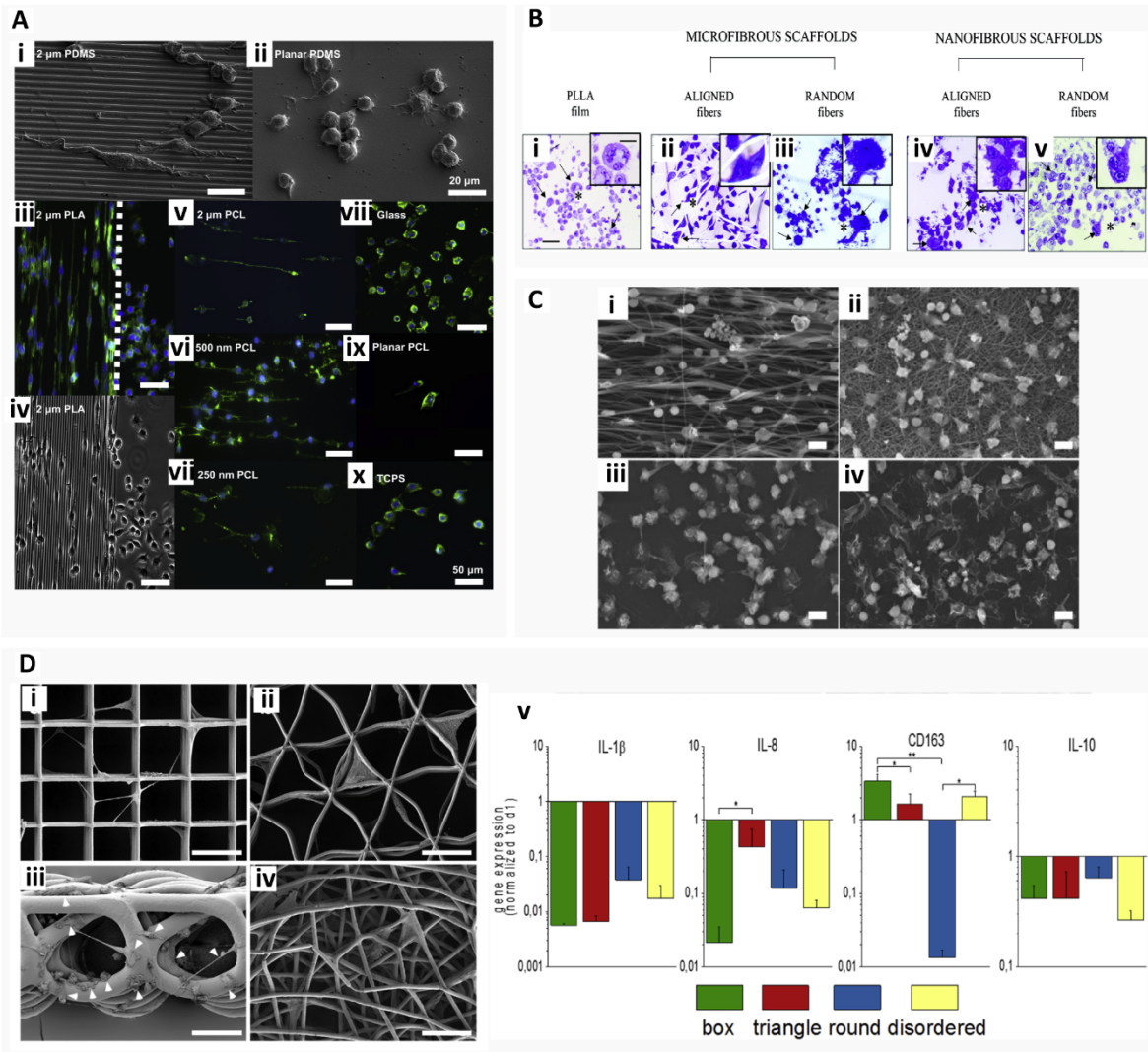
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#### 4 **2.1.1. Effect of 2D biomaterials on macrophages**

5 On 2D biomaterials, the surface is the modulating unit of immune cell behavior and chemistry  
6 and topography of the surface are the two dominant properties affecting the macrophage  
7 functions. When the size of topographical cues on the biomaterial surface is in the nano/micro  
8 size which is the sensible range of biological molecules by cells, these patterns can modulate the  
9 macrophage behavior without any bioactive agents <sup>69</sup>. For example, parallel patterns in a line  
10 shape with width sizes between 250 nm–2 μm are produced with three different polymers (PLA,  
11 PCL, and PDMS). On these patterned surfaces, macrophages were seed and the effect of  
12 topography on the activity of macrophage (RAW 264.7) cells were studied. On the patterns with  
13 small width, VEGF and TNF-α showed increased expression whereas on the larger width patterns  
14 their expression was decreased. This trend was the same for all three different polymers <sup>59</sup>. The  
15 topography of the substrates also changed the morphology of macrophages. On all three  
16 patterned polymers with 2 μm width lines, cells were elongated in the direction of the patterns  
17 whereas they were circular, not elongated on the unpatterned PDMS controls (**Figure 2A**) <sup>59</sup>. In  
18 another study, substrates of poly(l-lactic) (PLLA) were produced as smooth films and by  
19 electrospinning with micro and nano fibers and in organized/aligned and disorganized design. On  
20 surfaces with nanofibers, RAW 264.7 macrophages showed decreased inflammatory response  
21 with increased anti-inflammatory cytokines and chemokines when compared with control films  
22 and fibers in micron sizes. However, the proliferation of FBGCs were higher on smooth films  
23 than on the fibers with micro- and nano- sizes. Fiber organization did not affect the macrophage  
24 behavior as much as the dimensions (**Figure 2B**) <sup>70</sup>. In a similar study, polycaprolactone (PCL) was  
25 used in different forms such as parallel or disorganized fibers nanofibers produced with  
26 electrospinning, coverslip made of glass and surface modified with RGD and PCL films as controls.  
27 Fiber dimensions were in nano- range and two different groups with varying diameters were used.  
28 On PCL surfaces with parallel line fibers, monocytes were not spread, and they were mostly in  
29 round shapes. Also, cell number was low. On the other three groups of PCL, the cell spreading  
30 was higher with elongated shapes and cell number was higher too (**Figure 2C**) <sup>71</sup>. Chemical  
31 modifications and crosslinking of the 2D surfaces can affect the macrophage phenotype  
32 significantly. For example, scaffolds of collagen were crosslinked using two different chemical  
33 crosslinkers (EDAC and Genipin). The mechanical properties of the two scaffolds were different  
34 due to the crosslinking. Macrophage cells (THP-1) attached, proliferated, and polarized the same  
35 when the stiffnesses of the surfaces were compared. However, on the surfaces treated with  
36 Genipin, both pro- and anti-inflammation was inhibited whereas on the scaffolds treated with  
37 EDAC both inflammatory responses were activated <sup>72</sup>. In another study, it was shown that  
38 biomaterial degradation is a critical parameter changing macrophage behavior. Poly-ε-  
39 caprolactone-bisurea were used to produce electrospun constructs with changing diameters (2  
40 and 6 μm). The organization of the fibers were aligned and random. Macrophages (THP-1)  
41 showed higher degradative activities (which are measured by the levels of ROS dependent

1 peroxidation and NADPH oxidase) on aligned fibers with 6  $\mu\text{m}$  diameters <sup>73</sup>. In a recent study, a  
2 processing method called as melt electrowriting was used to produce substrates with fibers and  
3 pores in different shapes. Pores were designed with varying sizes (40  $\mu\text{m}$ -100  $\mu\text{m}$ ) and their effect  
4 on macrophage activities were was evaluated. It was shown that the macrophages aligned the  
5 most on the fibers with the smallest pore sizes and they also polarized into anti-inflammatory  
6 phenotype. In another study, surfaces designed with nanodots (diameters ranging from 10 to 200  
7 nm) were used to investigate the influence of roughness/topography on cytokine secretions by  
8 macrophages. It was reported that pro-inflammatory cytokines (IL6) increased on the most  
9 patterned surfaces than on smooth surfaces and also 200 nm nanodot surfaces showed higher  
10 cytokine release than on 50 nm <sup>74</sup>. Moreover, in another study, gelatin films produced with  
11 grooves having varying widths (2 to 40  $\mu\text{m}$ ) were used to study the influence of patterns on  
12 monocyte attachment and differentiation. Monocytes were seeded on micropatterned films with  
13 and without M1/M2 differentiation media. Morphology of these monocytes and their cytokine  
14 production levels were examined. It was reported that the patterns induced more cytokine  
15 secretion (such as IL-1 $\beta$ , IL-4, IL-12, TNF- $\alpha$ , CCL-18) in M1 media <sup>69</sup>. Another group cultured  
16 macrophage cells on titanium surface with topographies to investigate the impact of roughness  
17 on immune cell behavior and cytokine secretion. Several different topographies were created  
18 with different surface modification methods to obtain different surface roughness. It was  
19 reported that macrophages increased secretion of TNF- $\alpha$  which is a M1 inflammatory cytokine on  
20 surfaces with the highest roughness <sup>75</sup>. Moreover, the cells cultured on scaffolds with different  
21 shapes secreted different inflammatory markers. For example, markers of pro-inflammatory  
22 polarization were lower on all scaffolds with a box, rounded, and triangle shapes whereas anti-  
23 inflammatory markers were lower on rounded scaffolds but higher on triangle ones (**Figure 2D**)  
24 <sup>76</sup>.

25 These examples show that 2D biomaterials can be easily designed to modulate macrophage  
26 polarization. Flexibility in designing 2D surfaces such as their physical, chemical and mechanical  
27 properties make them potential tools for *in vitro* studies. Although 3D biomaterials are better to  
28 mimic the natural microenvironment for the cells, 2D biomaterials will always have the  
29 advantageous of being simple to design and modify. Moreover, the use of 2D surfaces in  
30 immunomodulation *in vitro* can be advantageous when they are used as biomaterial based  
31 implant coatings. Implant surfaces designed with 2D topographies or chemical modifications can  
32 improve tissue-implant interface interaction and implant integration to the host body.



1  
2 **Figure 2:** Influence of 2D biomaterials on macrophage behavior. **A)** Micrographs of macrophages  
3 grown on (i) 2  $\mu$ m PDMS with line shaped topographies, (ii) smooth PDMS control, (iii) 2  $\mu$ m PLA  
4 lines on glass, (iv) zone between the 2  $\mu$ m PLA linear patterns and its smooth surface, PCL lines  
5 having widths (v) 2  $\mu$ m, (vi) 500 nm, (vii) 250 nm, and control scaffolds made of (viii) glass, (ix)  
6 smooth PCL film and (x) tissue culture polystyrene (TCPS). Morphology of cells are different on  
7 substrates with changing patterns with different sizes (F-actin and cell nuclei: Phalloidin Oregon-  
8 Green 488 (green) and DAPI (blue))<sup>59</sup>. **B)** Histological staining of FBGCs on different PLLA scaffolds  
9 with micro- and nanofibers are shown and the arrows indicate active cells having spread shapes  
10 with increased filopodia whereas asterisks show the magnified cells presented in the inserts  
11 (*Stain:* Toluidine blue) (Reprinted with permission. Copyright (2020) American Chemical Society)  
12 <sup>70</sup>. **C)** SEM images of monocytes on (i) PCL with parallel fibers, (ii) PCL with disorganized fibers, (iii)  
13 smooth PCL film, (iv) cover slip treated with RGD<sup>71</sup>. **D)** Adhesion and alignment of macrophages  
14 are changing with the different porosity, structure and shapes of the PCL scaffolds (I, ii, iii, iv) and  
15 pro- and anti-inflammation markers are varying on different scaffolds (v)<sup>76</sup>.

16

### 1 2.1.2. Effect of 3D biomaterials on macrophages

2 In natural tissue, cells are enclosed with complex three-dimensional structures named  
3 extracellular matrix (ECM) which have functions in physical and chemical signaling cascades and  
4 provides cells their mechanical properties. 3D scaffolds/substrates produced using biomaterials  
5 can possess properties to mimic physiological microenvironments of cells. They can be utilized to  
6 establish realistic compartments for housing, enrollment, and controlling host innate immune  
7 system cells. Moreover, the unique topography of ECM consisting of components in micro- and  
8 nano- scales such as fibers and pores can modulate the immunomodulatory properties of host  
9 cells. Thus, 3D biomaterials are of choice for mimicking the natural tissues and controlling the  
10 immune response. Especially, hydrogels are commonly used for this purpose since they can  
11 provide a tunable 3D network. They consist of a huge quantity of water in their polymeric  
12 structure and particular viscoelastic properties. They include micro- and nanoporous structures  
13 like in natural tissues which make them suitable to simulate the physical features of ECM.  
14 Hydrogels and other 3D matrices are advantageous than the 2D substrates due to their capacity  
15 to regulate properties, behavior and activities of cells such as their shape, morphology,  
16 interactions and signaling with other cells and microenvironment, organization, spacing, density  
17 of micro/nano components, and their availability to the soluble molecules. 3D substrates can  
18 achieve these control mechanisms by encapsulating the cells, and controlling the flow of bioactive  
19 molecules released by cells or provided from the environment with their porous structures<sup>77</sup>. The  
20 most recent studies on innate immune response to scaffolds show that the modulatory influence  
21 of 3D architectures on the behavior of macrophage immune cells are too crucial to overlook. For  
22 example, a novel 3D screening system for the determination of suitable biomaterial-cell  
23 combination in the immune modulation was designed in a recent study<sup>78</sup>. In this study, alginate  
24 was used to form microcapsule hydrogels and to encapsulate stem cells and micro PCL spheres in  
25 these capsules. Then the outer surface of the microgels were coated with poly-L-lysine,  
26 alginate and chitosan with layer-by-layer (LBL) method. Alginate hydrogel was then liquefied with  
27 the help of EDTA chelation but the outer coatings helped the microsphere maintain its shape. This  
28 platform was then tested on a 2D macrophage monolayer and it was reported that the order of  
29 the coating layer changed the polarization of the macrophages (**Figure 3A**)<sup>78</sup>. For example, when  
30 the outer layer was chitosan and the stem cells were encapsulated in the system, macrophages  
31 showed an anti-inflammatory and regenerative profile<sup>78</sup>. This kind of system can be very  
32 promising for the screening of various cell type-biomaterial pairing for the directed immune  
33 response. Another recent application of immunomodulatory 3D scaffold is the healing of wounds  
34 formed as a result of diabetes through the personalized and controlled structure of the  
35 biomaterial based scaffold<sup>79</sup>. In this study, 3D PCL/F-127 scaffolds with nanofibers were produced  
36 with various shapes, coated with gelatin and BMSCs were seeded on them. Then, the cell seeded  
37 scaffolds were inserted into mice for the evaluation of wound healing. It was reported that the  
38 scaffolds with larger pore sizes caused M2 type macrophage polarization and better wound  
39 healing since they allowed cells to penetrate more through the scaffolds<sup>79</sup>.

40 Another important health issue related with to macrophage presence in 3D environments is  
41 tumors and tumor associated macrophages. This is particularly relevant in tissue engineering if  
42 the scaffold is to be placed after the tissue rejection due to tumor presence. For example, in a 3D

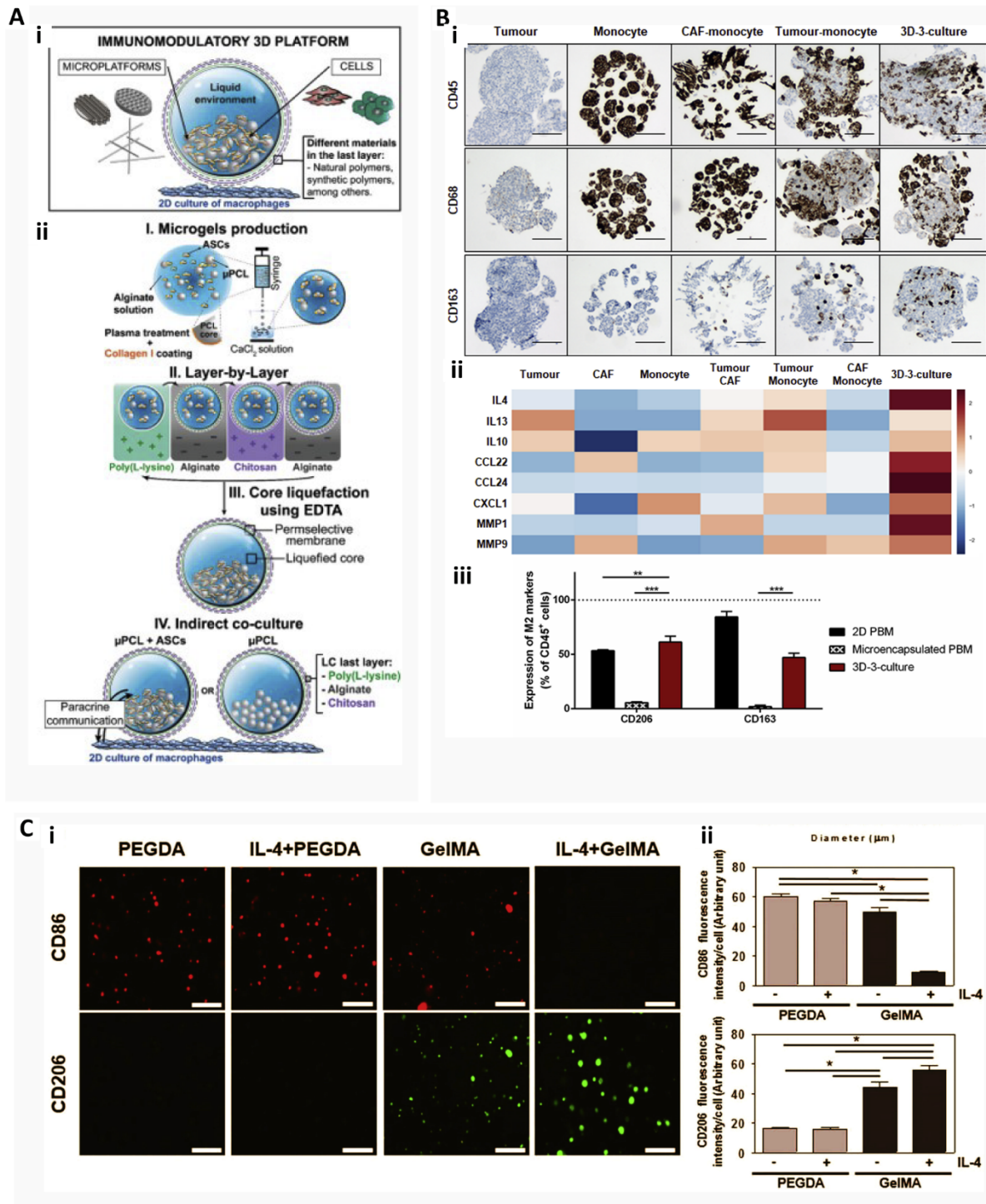
1 tumor model study designed in a microfluidic device with an endothelial barrier, tumor migration  
2 and co-culture of tumor cells with macrophages were studied. Flow of lipopolysaccharide (LPS)  
3 and interleukin-4 (IL4) were provided through the capillary of the device and the polarization of  
4 macrophages (RAW264.7) were was observed under the effect of these two different inducers.  
5 LPS resulted in M1 polarization whereas IL4 induced M2 phenotype. However, when the tumor  
6 cells were included in addition to macrophages, the polarization of macrophages did not change  
7 but tumor migration and invasion through the endothelial membrane were affected. It was  
8 observed that tumor cells migrated through the areas macrophage cells located <sup>80</sup>. In another  
9 tumor model study, alginate hydrogels were used to design a 3D surrounding space for the  
10 encapsulation of cells. Three cells were cultured in the model: lung carcinoma cells in spheroid  
11 forms, fibroblasts associated with tumor and macrophages (THP-1). It was reported that hydrogel  
12 with tri-cultured cells created an immunosuppressive microenvironment and directed the  
13 differentiation of macrophages into an M2 inflammation phenotype. Also, increased expressions  
14 of two tumor associated markers were detected in macrophage cells (CD163 and CD206) (**Figure**  
15 **3B**). In summary, this multi cell culture hydrogel system and the interactions between the cells  
16 and 3D microenvironment directed macrophage cells into more tumor cell like phenotypes which  
17 is called as-tumor associated macrophages (TAM) <sup>81</sup>.

18 In a recent study, 3D collagen and HA scaffolds were produced and it was shown that the scaffolds  
19 having a higher amount of HA caused anti-inflammatory M2 phenotype polarization of  
20 macrophages (THP-1 cells) <sup>82</sup>. In another study, both 2D and 3D substrates were used to coculture  
21 two cells as stem cells and macrophages. In 3D matrices, markers related to both inflammation  
22 and chemotaxis were produced in lower amounts when compared with 2D substrates <sup>83</sup>. In a  
23 different study, two component hydrogel matrices with the combination of proteins (gelatin) and  
24 glycosaminoglycans (hyaluronic acid) were produced with different mechanical properties and  
25 loaded with macrophages (THP-1). In softer hydrogels with a-Young's modulus around 10 kPa,  
26 cells showed higher metabolic activity when compared to stiffer hydrogels having Young's  
27 modulus around 20 kPa <sup>84</sup>. In another study, two different hydrogels, Gelatin methacryloyl  
28 (GelMA) and poly(ethylene glycol) diacrylate (PEGDA), were used to encapsulate macrophage  
29 cells and it was reported that THP-1 macrophages in hydrogels (GelMA) showed increased  
30 expression of M2 anti-inflammation marker (CD206) whereas they produced an increased level  
31 of M1 pro-inflammation marker (CD86) in PEGDA hydrogels (**Figure 3C**) <sup>85</sup>. These studies show  
32 that as well as the architecture or dimension of the 3D microenvironment, the type of biomaterial  
33 is very effective on the immune cell behavior and response. In a recent study, 3D hydrogels of  
34 poly(ethylene glycol) (PEG) were used to load and culture macrophage cells (RAW264.7) and it  
35 was shown that matrix metalloproteinases and pro-inflammation markers (iNOS, COX2, TNF- $\alpha$ )  
36 increased in 3D while anti-inflammation molecules (CD206, Arg1, TGF- $\beta$ ) decreased in the  
37 hydrogels. When the activity of macrophage differentiation indicators were was compared with  
38 changing stiffness of the hydrogels, it was observed that both pro- and anti-inflammation markers  
39 were induced more in stiff gels than in soft gels <sup>86</sup>. In another study, scaffolds were produced  
40 using a natural polymer chitosan and they were acetylated with changing degrees (5% and 15%).  
41 These porous scaffolds induced varying macrophage numbers, adhesion and polarization  
42 depending on their acetylation degrees. For example, 5% acetylation resulted in a decreased cell  
43 proliferation and a dominant M2 phenotype. However, 15% acetylated scaffolds induced M1

1 macrophages <sup>87</sup>. Results showed that 3D hydrogels ~~can~~ could be used to control immune cell  
2 activities by modifying the enclosing 3D microenvironment, and their properties such as stiffness,  
3 chemistry, composition, crosslinking degree and design.

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



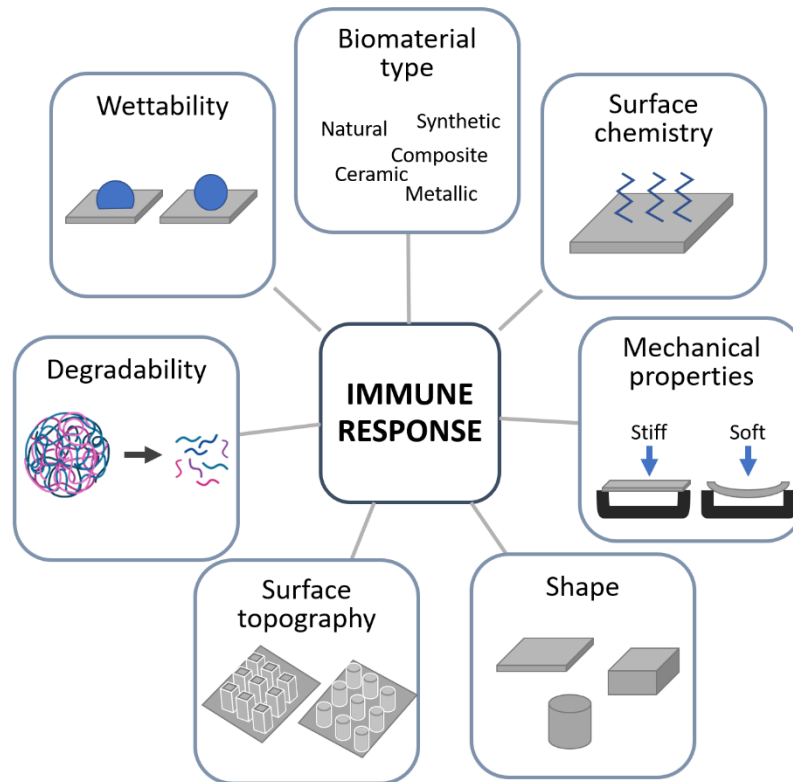


1  
 2 **Figure 3:** Influence of 3D biomaterials on macrophage behavior. **A)** A novel biomaterial-cell  
 3 screening platform for the purpose of immunomodulation. (i) Application of stem cell  
 4 encapsulating hydrogel system on 2D macrophage culture and (ii) the production steps of this  
 5 platform using LBL method and liquefaction of hydrogel core <sup>78</sup>. **B)** Tri-culture alginate hydrogel  
 6 system was analyzed in terms of macrophage markers and cytokine secretions: (i) Histology

1 staining of cells for cell migration and macrophage polarization specific markers (ii) Heatmap for  
 2 the expressions of macrophage related cytokines (iii) Quantification of antibody signal intensities  
 3 for the detection of leukocyte (CD45) and M2 phenotype (CD163 and CD206) in both 2D and 3D  
 4 microenvironments <sup>81</sup>. **C)** Micrographs of macrophage polarization in 3D hydrogels were shown  
 5 with immunostaining of the cells for pro- (CD86) and anti- (CD206) inflammatory markers (i) and  
 6 the quantitative analysis of antibody signal intensities for the same markers were presented (ii)  
 7 <sup>85</sup>.

8  
 9 **3. Properties of scaffolds affecting immune response:**

10  
 11 Biomaterials have tunable properties which is are useful in the immunomodulation of scaffolds  
 12 and implants. Biomaterial type, surface chemistry, topography and wettability, shape,  
 13 degradability and mechanical properties are the important factors deciding the components and  
 14 severity of the foreign body reaction and immune response (**Figure 4**). Thus, based on the  
 15 application, an interplay and trade-off between various properties is required to achieve the  
 16 optimum response. Having control over the ability of biomaterials to support cell adhesion,  
 17 metabolic activity, differentiation, and to regulate the production of bioactive molecules and  
 18 drugs can enable the control of innate host immunity. With the techniques commonly used  
 19 for biomaterial processing, the derivation of highly tunable natural and synthetic biomaterial-  
 20 based scaffolds and implants have become possible.  
 21



1 **Figure 4:** Adjustable properties of biomaterials affecting immune response in contact with the  
2 host body.

3

4 ***Biomaterial type:***

5 *Natural polymers* such as alginate, silk, chitosan, collagen, dextran, and hyaluronic acid release  
6 mostly nontoxic degradable compounds in the body. Their properties resemble the biological  
7 molecules already present in the host body and these bioactive natural biomaterials present  
8 adhesion regions for cells. As a result of the adhesion sites, natural biomaterials degrade easily  
9 with enzymatic degradation and they activate adverse FBR less than the synthetic biomaterials.  
10 However, their weak mechanical properties can limit their use for various applications <sup>88</sup>.

11 *Synthetic materials* consist of molecules such as poly(lactic acid) (PLA), poly(ethylene glycol)  
12 (PEG), poly(lactic acid-co-glycolic acid) (PLGA), polymethyl methacrylate (PMMA), and poly(vinyl  
13 alcohol) (PVA). These polymers can be easily designed to control the protein attachment to the  
14 implant surfaces which is the very early step of innate immune response <sup>53</sup>. Also, their controllable  
15 biomechanical and biodegradation properties make them very favorable for tissue engineering  
16 purposes. However, they may develop chronic inflammation which directs the fibrous isolation  
17 and encapsulation of implants <sup>88</sup>. Both natural and synthetic materials stimulate the  
18 proinflammatory responses by recruited innate immune system cells, however the extent of this  
19 response depends on the surface properties rather than the bulk properties of the implants. For  
20 example, in a study, alginate, agarose, chitosan, hyaluronic acid, and poly(lactic acid-co-glycolic  
21 acid) (PLGA) were tested in terms of their initiation of the innate immune response. Dendritic  
22 cells were seeded on these polymeric biomaterials and their capacity to induce dendritic cell  
23 maturation were evaluated. On chitosan and PLGA films, cells showed an increased level of  
24 dendritic cell maturation marker and increased expression of B and T cell activation marker  
25 whereas on alginate and hyaluronic acid films the expression levels of these two markers  
26 decreased. This higher number of the dendritic cell on chitosan and PLGA was explained with the  
27 higher hydrophobicity of these two polymers which could create a more favorable surface for cell  
28 adhesion and spread <sup>89</sup>. In another study, immunity activation of ECM scaffolds (obtained from  
29 biological tissues) was compared with the synthetic scaffolds (polyethylene (PE) and polyethylene  
30 glycol (PEG)) and it was reported that ECM scaffolds made macrophages produce increased levels  
31 of CD206 (pro-regenerative marker) whereas PE and PEG resulted in decreased production of  
32 CD206. Also more neutrophil cells were observed on synthetic scaffolds which was based on the  
33 higher stiffness of the synthetic scaffolds (**Figure 5A**) <sup>90</sup>.

34 Composites are designed by combining the properties of more than one material and show  
35 greater success in directing tissue regeneration after injury caused by implant insertion. With such  
36 control over the features of biomaterial surfaces, it can be possible to optimize the biological  
37 performances of the implants. Composite biomaterials reflect the properties of the constituent  
38 biomaterials and provide specific properties for cell guidance which normally do not exist in either  
39 biomaterial constituting the composite. With these combined properties, they can mimic the  
40 natural ECM which is good for cell adhesion. Also, designed adhesion regions and growth factors  
41 can be introduced into their structure for better cell attachment, proliferation and differentiation  
42 <sup>91</sup>. For example, in a study, composites of poly(ethylene glycol) (PEG) and collagen hydrogels with  
43 macroporous structures were synthesized. It was reported that only PEG hydrogels with high

1 mechanical strength induced the secretion of cytokines/chemokines. However PEG-collagen  
2 composites showed increased mobility and migration of T and dendritic cells through the scaffold  
3 when compared with PEG scaffolds with the same pore sizes which showed no T cell attachment  
4 or migration <sup>92</sup>.

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

23 *Ceramic biomaterials* are mostly used to produce bone and dental implants. They are highly  
24 biocompatible due to their chemical and structural formulation. They are inert, hard, brittle,  
25 strong under compression and due to these properties, they are very similar to the native bone  
26 <sup>98</sup>. In various studies, it was shown that ceramics ~~can~~ could change macrophage polarization. For  
27 example, in a study, biphasic calcium phosphate (BCP),  $\beta$ -TCP and hydroxyapatite ceramics were  
28 compared in terms of their macrophage polarization and it was reported that BCP showed both  
29 the highest osteoinduction activity and more M2 macrophage phenotype when compared to  
30 other two ceramics.  $\beta$ -TCP showed no osteoinduction and increased M1 macrophage phenotype  
31 and hydroxyapatite ceramics showed intermediate osteoinduction and induced both M1 and M2  
32 macrophage phenotypes <sup>99</sup>.

33  
34 **Surface chemistry:** Surface chemistry of biomaterials has an important function for the  
35 recruitment and activation of immunomodulatory cells. Functional groups at the outer face of  
36 implants are capable of controlling protein and cell adhesion, and subsequently tissue reactions  
37 to implanted biomaterial surface. Implanted biomaterials are mostly hydrophobic, and proteins  
38 bind more strongly to hydrophobic surfaces than hydrophilic ones <sup>93,100</sup>. On the other hand, it was  
39 shown that wettability of the surface may not correlate with the effect of functional groups and  
40 surface chemical structure was the dominant factor determining the thickness of fibrous capsule  
41 formation *in vivo* (**Figure 5B**) <sup>101</sup>. Similarly, in several studies, it was reported that surfaces with -  
42 NH<sub>2</sub> and -OH groups induced more innate immune system cells and proteins migrate to the  
43 implant insertion site and form thicker fibrotic capsules around the implants when compared to  
44 surfaces with -CF and -COOH groups <sup>101-103</sup>. Control over surface properties of implants with

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

21  
22 **Surface topography:** Surface topography of implanted biomaterials at the nano- and microscale  
23 affects cell morphology, adhesion, migration, proliferation and differentiation <sup>108</sup>. Modifying the  
24 surface topography is a useful way to change the immune cell reaction because these micro/nano  
25 surface features trigger changes in cell morphology and plasticity <sup>109</sup>. This regulation of surface  
26 properties helps control macrophage migration, proliferation, function, differentiation,  
27 polarization and fusion. For the design of the surfaces with topography, various methods such as  
28 photolithography, electron beam lithography, soft lithography, microcontact printing and hot  
29 embossing are used <sup>108</sup>. These techniques can create surface geometries at the nano- and  
30 microscale decorated with pillars, posts, gratings, ridges, pits and dots <sup>108</sup>. The reason of  
31 surface topography being highly effective on cell behavior stems from cell interactions with the  
32 integral elements of the ECM. The natural structure of ECM includes proteins and molecules in  
33 the nano- and microscale and components of ECM interact with cells at nano/microscale which  
34 can be mimicked by designing the surface topography of implant at the same dimensions <sup>100</sup>.  
35 In a very recent study, TopoChip platform was used to evaluate the influence of surface patterns  
36 at micro/nano range on human macrophage adhesion and phenotype. The platform was designed  
37 with a large number of patterns in various shapes (circle, triangle, rectangle) with varying  
38 dimensions (3–23  $\mu\text{m}$  diameter with 10  $\mu\text{m}$  height). With the help of these different topographies  
39 and pattern sizes, the surfaces were produced with changing cell attachment properties. It was  
40 reported that the increased attachment of macrophages was on the micropillars with 5  $\mu\text{m}$  in  
41 diameter. Moreover, on TopoUnits with low cell adhesion surfaces (diameters higher than 10  
42  $\mu\text{m}$ ), cells attached in between the micropatterns whereas on surfaces with high cell adhesion  
43 properties, macrophages adsorbed micropillars through phagocytosis (**Figure 5C**) <sup>110</sup>.

44

1 **Wettability:** Wettability of the surfaces have a critical effect on immunogenicity since the innate  
2 immune system cells recognize molecules with high hydrophobicity as foreign materials <sup>8</sup>. Thus,  
3 to prevent highly immunogenic reaction to surfaces with low wettability, hydrophilic polymers  
4 (polyethylene oxide (PEO) and polyethylene glycol (PEG)) are used in cell or drug carrier designs  
5 and in the construction of biomaterial devices considered for use in tissue engineering or as  
6 implants. By With the help of these materials, scaffolds and implants gained nonimmune reactive  
7 properties like decreased adhesion of surface proteins and lower interactions with immune  
8 macrophage cells. For example, when macrophage cells were cultured on titanium with different  
9 wettability, they showed diverse effects on cytokine secretion and macrophage polarization: On  
10 hydrophobic titanium surfaces, the number of macrophages with pro-inflammatory M1  
11 phenotype was increased with highly induced cytokine levels responsible for M1 type  
12 inflammation (such as IL-1 $\beta$ , IL-6, TNF $\alpha$ ). However, on hydrophilic surfaces, M2 type anti-  
13 inflammatory macrophages were in a very high number and they also secreted anti-inflammation  
14 cytokines (such as IL-4, IL-10) <sup>111</sup>. Macrophage morphology is affected by the surface wettability,  
15 too. It was shown that on hydrophobic carbon nanofiber surfaces, macrophages elongated more  
16 and the number of their filopodia were was increased. However, on hydrophilic scaffolds, the  
17 cells remained rounded, and not elongated with less fewer filopodia even after 48 h (**Figure 5D**)  
18 <sup>112</sup>.

19  
20 **Shape:** Shape of implanted devices has also been known effective on immune cell behavior and  
21 host response. Especially the phagocytosis of the small particles (formed as a result of  
22 degradation of the scaffolds by macrophages) can be modulated by the particle shape and size <sup>93</sup>.  
23 It was shown that particle shape rather than their size was more effective in the internalization  
24 process of these small degradation products through phagocytosis. For example, particles of  
25 polystyrene (PS) were designed with varying geometric shapes (spheres and disks) in micrometer  
26 sizes. It was noted that macrophages phagocytosed disks with elliptical shapes through their  
27 major axis in a very short time like 6 min whereas did not engulf them even after 2 h through their  
28 minor axis. However, internalization of spheres was homogeneous (**Figure 5E**) <sup>113</sup>. In another  
29 study, the importance of implant geometry in the modulation of its biocompatibility was  
30 demonstrated in vivo. A mouse model with FBR resembling to human was used (C57BL/6 mouse)  
31 and hydrogels of alginate with spherical shapes with different diameters ranging between 0.3-1.9  
32 mm were inserted in mice. When their immune reaction to spherical biomaterials was evaluated,  
33 it was observed that spheres with diameters more than 1.5 mm were more biocompatible with  
34 lower FBR and fibrosis than the spheres with smaller sizes <sup>114</sup>.

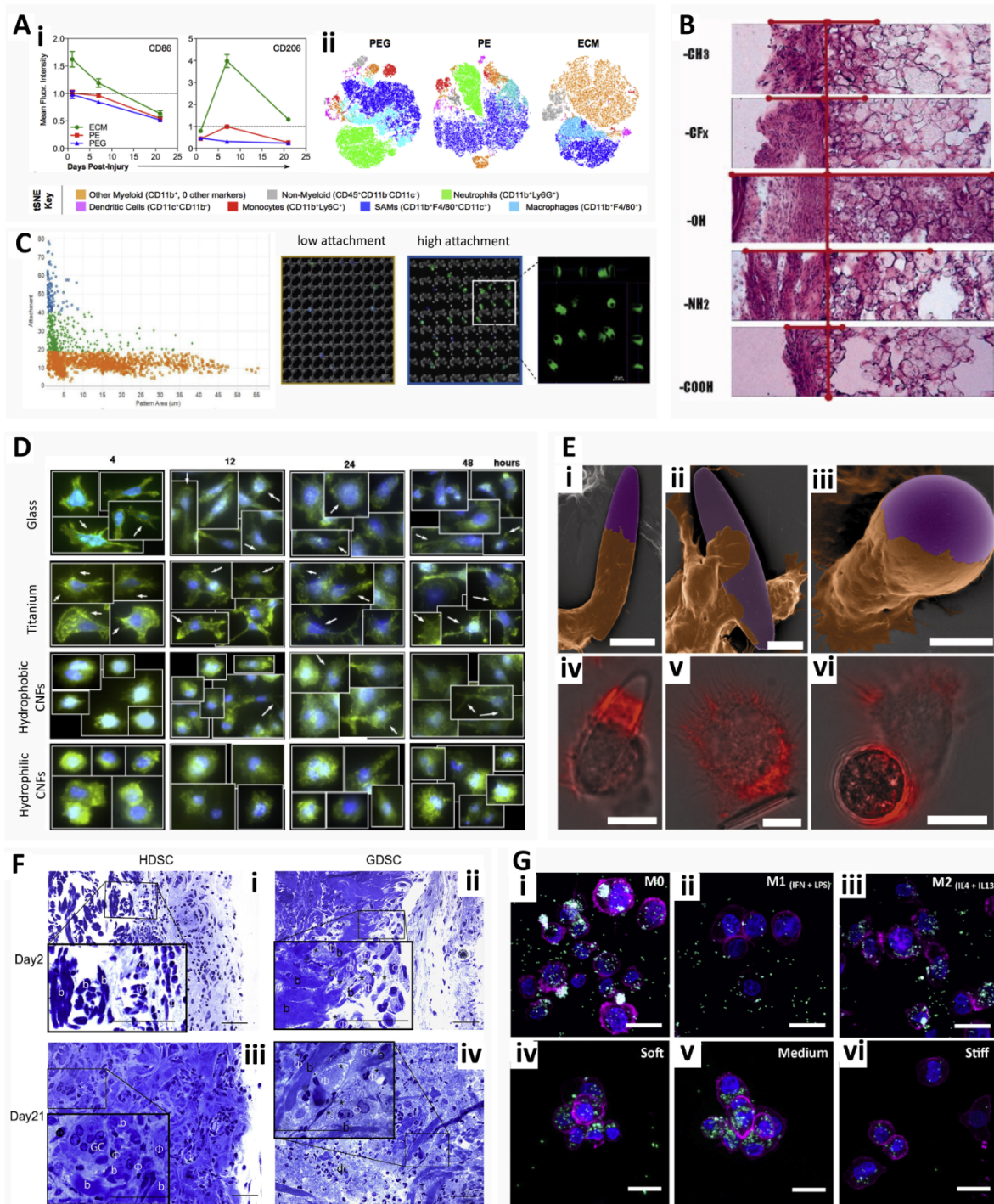
35  
36 **Degradability:** Degradability of biomaterials can influence immune cell recruitment and  
37 responses; thus, crosslinking is important in immune modulation too. For example, hyaluronic  
38 acid scaffolds were enzymatically degraded into small fragments and these fragments had varying  
39 molecular weights. When they were incubated with dendritic cells and T cells, increased  
40 activation of dendritic cells and higher proliferation of T cells were reported with particles having  
41 a low molecular weight <sup>115</sup>. Moreover, it was presented that hyaluronic acid with low molecular  
42 weight induced proinflammatory macrophages whereas particles with high molecular weight  
43 promoted anti-inflammatory phenotype <sup>116</sup>. On the other hand, the influence of crosslinking  
44 agent was investigated and it was shown that collagen scaffolds crosslinked with glutaraldehyde

1 induced a higher number of neutrophils than the polymers treated with hexamethylene  
2 diisocyanate since two crosslinkers caused degradation of the scaffolds in different durations.  
3 Samples crosslinked with glutaraldehyde were degraded after 28 days, whereas the samples  
4 crosslinked with hexamethylene diisocyanate crosslinker were not degraded in the same duration  
5 <sup>117</sup>. Also, phagocytosis of collagen scaffolds was determined by their degradation rate that  
6 samples with glutaraldehyde crosslinker was phagocytosed by macrophages whereas  
7 phagocytosis of samples crosslinked with hexamethylene diisocyanate was never observed. Thus  
8 it was concluded that only glutaraldehyde was able to develop a microenvironment proper for  
9 the induction of neutrophils and macrophages (**Figure 5F**) <sup>117</sup>.

10  
11 **Mechanical properties:** As well as the chemical and surface properties of implants, bulk features  
12 such as mechanical properties can also affect the behavior of innate immune system cells.  
13 Macrophages were shown to change their phenotypes and activation (such as differentiation or  
14 movement) as a response to the stiffness of the biomaterial which proves the capability of  
15 stiffness in directing macrophage phenotype. Macrophages (THP-1 cells) were grown on collagen  
16 scaffolds which are coated with polyacrylamide gels and these gels were designed with different  
17 stiffnesses. Gel coated collagens with higher stiffness (323 kPa) induced pro-inflammatory M1  
18 type macrophage immune cells and resulted in lower phagocytic activity. However, the two  
19 scaffolds with lower stiffness (11 kPa and 88 kPa) M2 cell profile and increased phagocytosis with  
20 a higher number of small particles were observed as engulfed in macrophages (**Figure 5G**) <sup>118</sup>. In  
21 a different research, the influence of strain force on the immune cell activity was investigated and  
22 cells derived from peripheral blood were seeded on scaffolds made of poly-ε-caprolactone  
23 polymers. After applying cyclic load with varying stretchings (7%, 12% and controls with 0% load),  
24 innate immune response markers were evaluated in these cells after one week. It was shown that  
25 the cyclic load with the highest ratio resulted in the polarization of cells into M2 phenotype with  
26 induced anti-inflammatory markers <sup>63</sup>.

27





1  
 2 **Figure 5:** Biomaterial properties can change the behavior of innate immune system cells. **A)**  
 3 Different innate immune responses are induced by synthetic and natural biomaterials: i) CD86  
 4 and CD206 macrophage markers are evaluated with flow cytometry on ECM, PE and PEG on days  
 5 1, 7, and 21, ii) Immune cell recruitment to the microenvironment of scaffold implanted to a  
 6 murine model is shown with multicolor flow cytometry analysis<sup>90</sup>. **B)** Hematoxylin and Eosin  
 7 staining of tissues with different surface functional groups shows implant related innate immune



1 responses is based on the density of fibrotic capsule and infiltration of cells (red lines) <sup>101</sup>. **C)**  
2 Macrophage cell attachment on pillars are presented with a scatter plot showing attachment  
3 versus total pattern area ( $\mu\text{m}^2$ ) with high (blue), medium (green), or low (orange) attachment  
4 surfaces, and their confocal images (macrophage membrane: green and DNA: blue)(Creative  
5 Commons 4.0) <sup>110</sup>. **D)** On three different biomaterials (titanium, glass, carbon nanofiber), the  
6 filopodia and morphology of macrophages are presented on 4 time points (F-actin: green, DNA:  
7 Blue) (Filopodia are shown by white arrows) <sup>112</sup>. **E)** SEM micrographs (i–iii) and merged  
8 micrographs of brightfield and fluorescent images (iv–vi) are showing macrophage engulfment of  
9 the particles with different shapes and sizes (Copyright (2020) National Academy of Sciences) <sup>113</sup>.  
10 **F)** Macrophage internalization of degraded scaffolds crosslinked with two different chemical  
11 crosslinkers are shown: No phagocytosis in hexamethylene diisocyanate (i, iii) whereas  
12 progressive phagocytosis in glutaraldehyde crosslinked scaffolds on day 2 (ii) and day 21 (iv) <sup>117</sup>.  
13 **G)** Micrographs obtained by confocal microscopy during the engulfment of latex beads with 1  $\mu\text{m}$   
14 diameter in M0 (i), M1 (ii) and M2 (iii) differentiation medium, and on soft (iv), medium (v), and  
15 stiff (vi) gels (Actin cytoskeleton: purple, particles: green, nucleus: blue): Cells in M1  
16 differentiation media shows a decreased number of particles per cell and gels with average  
17 stiffness shows the increased particles internalized per cell <sup>118</sup>.

18  
19 Further examples showing the effect of biomaterial properties (biomaterial type, surface  
20 chemistry, surface topography, wettability, shape, degradability, mechanical properties) on the  
21 behavior of innate immune system cells are summarized in **Table 1**:

22  
23  
24  
25

Biomaterial property	Biomaterial	Cell type	Immunomodulatory function	References
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 <sub>3</sub> , OH, COOH, NH <sub>2</sub> )	Dendritic cells (DC)	OH, COOH, and NH <sub>2</sub> caused more DC activation than CH <sub>3</sub>	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

<b>Surface topography</b>	Micropatterned substrates designed with fibronectin patterns with 50 or 20 $\mu\text{m}$ width gratings and 20 $\mu\text{m}$ 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 $\mu\text{m}$ , pitch: twofold of width, depth: 0.8-1.3 $\mu\text{m}$ )	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( $\epsilon$ -caprolactone) (PCL), Poly(dimethyl siloxane) (PDMS), Poly(lactic acid) (PLA) surfaces with micro/nano patterns with 250 nm–2 $\mu\text{m}$ width lines	Macrophage	Macrophages elongated more on 500 nm lines compared to smooth surfaces. Patterns with larger widths induced higher production of TNF- $\alpha$ and VEGF than smaller widths	59
<b>Wettability</b>	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

<b>Shape</b>	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 $\mu\text{m}$ -10 $\mu\text{m}$ diameters	Macrophage	Particles with lower size induced production of immune reactive molecules <i>in vitro</i> and macrophage migration <i>in vivo</i>	131
	Poly(methyl methacrylate) (PMMA)	Macrophage	Increased M1 polarization was observed with macrophages inside the pores of scaffolds	61
<b>Degradability</b>	Hyaluronic acid	Dendritic cell	Low molecular weight (1500–5300 Da) showed induced dendrite function, cytokine production, and T cell number	115,132–134
	Hyaluronic acid	Macrophages	Low molecular weight induced M1 macrophage activation whereas high molecular weight induced M2 phenotype	116,135

<b>Mechanical properties</b>	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- $\alpha$ production	67
	Collagen gels	Macrophage	Lower stiffness (30 Pa) directed M1 macrophage activation more than higher stiffness (100 Pa)	67

1

## 2 **4. Harnessing macrophages in regenerative medicine**

3 Biomaterials which can manage immune cell response and tissue renewal activity of these cells  
4 are needed for the control of wound healing and tissue repair <sup>138</sup>. Harnessing the inflammatory  
5 response, in particular macrophages due to their unique plasticity, can be a useful approach to  
6 enhance tissue repair. There should be a balance in the abundance of two different phenotypes  
7 (M1 and M2) of macrophages since pro- and anti-inflammation activities of these cells decide the  
8 fate of tissue repair. This regulation is possible with the fine-tuning of biomaterial properties <sup>139</sup>.  
9 The presence of more cells with M1 phenotype than M2 macrophages can inhibit tissue repair  
10 <sup>140</sup>. Moreover, pro-inflammatory M1 macrophages and the cytokines produced by these cells play  
11 a role in angiogenesis whereas M2 macrophages make angiogenesis steady but induce the  
12 proliferation of fibroblasts and construction of extracellular matrix <sup>141</sup>. Additionally, macrophages  
13 take part in wound repair since they can encapsulate and phagocytose small sized molecules such

1 as cellular debris, neutrophils, apoptotic cells and other foreign bodies <sup>142</sup>. These cells are also  
2 capable of producing chemokines, matrix metalloproteinases (MMPs), and various inflammatory  
3 molecules <sup>142</sup>. Both M1 and M2 macrophages and the molecules secreted by these cells activate  
4 blood vessel development, differentiation and activation of several cell types such as fibroblasts  
5 into myofibroblasts (for wound contraction and closure), parenchymal cells, stromal cells, stem  
6 cells and progenitor cell populations (for tissue healing and repair) <sup>142</sup>. As a result of these critical  
7 roles in tissue repair and wound healing, various recent studies are focusing on the modulation  
8 of macrophage-biomaterial interactions. For example, monocytes in the circulation system were  
9 directed by polymeric biomaterial films at the skin injury sites and after they accumulated on the  
10 polymer surfaces, they triggered vascularization and wound healing with the dominance of anti-  
11 inflammatory macrophages <sup>143</sup>. In another work, the activity of macrophages in the loss of muscle  
12 volume was evaluated and delivery of molecules with biomaterials ~~were~~ was used to trigger anti-  
13 inflammatory macrophages. With this targeted delivery, M2 type macrophages proliferated and  
14 were recruited to the damaged muscle tissue, and regeneration, healing, vascularization and  
15 deposition of collagen were achieved which resulted in an increase of muscle volume <sup>144</sup>. In a  
16 different study, scaffolds for bone tissue engineering obtained by the decellularization of natural  
17 tissues were used in the control, production, and release of cytokines (IFN- $\gamma$  and IL-4) play a role  
18 in M1 and M2 macrophage polarization. Sequential release of cytokines was planned since the  
19 sequential differentiation of macrophages into M1 and M2 was shown to induce angiogenesis  
20 and tissue repair by the same group. In this study, delivery of M1 and M2 cytokines in an order  
21 resulted in enhanced vascular formation <sup>145</sup>.

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

37 Furthermore, delivery of stem cells is widely applied as an approach in regenerative medicine due  
38 to their differentiating capacity and regenerative properties, but most importantly, they are  
39 known to provide strong immune controlling features and take a role in the secretion of  
40 molecules that directly modulate macrophage activation. For example, these stem cells were  
41 used to repair the skin damage of mouse and they were encapsulated in gelatin hydrogels  
42 together with macrophage cells. It is reported that encapsulation of both cell types and the

1 interaction of the cells and their microenvironments help the regeneration of skin tissue and  
2 wound healing in mice <sup>149</sup>. Many recent studies show that there is a cooperation between stem  
3 cells and macrophages and this cooperation can be enhanced with the modulation of biomaterial  
4 properties. For example, it was shown that on collagen coated polyacrylamide gels with different  
5 stiffnesses (11 kPa, 88 kPa, 323 kPa) MSCs showed immune modulating properties when they  
6 were in M1 media regardless of the substrate stiffness. However, substrate stiffness was effective  
7 when the MSCs were seeded together with macrophage cells. On the substrates with 11 kPa and  
8 88 kPa stiffness but not on the 323 kPa, macrophages secreted lower TNF- $\alpha$  and higher of IL-10  
9 <sup>150</sup>. Another study reported that hydroxyapatite nanoparticles induced IL-10 production in  
10 macrophages which is an indication of anti-inflammatory phenotype and also increased IL-10  
11 resulted in differentiation of MSCs through an osteogenic phenotype with increased bone  
12 morphogenetic protein-2 (BMP-2) and alkaline phosphatase (ALP) gene expressions <sup>151</sup>. Similar  
13 results were also reported in a 3D environment in another recent study. In the study, 3D gelatin  
14 hydrogels were produced and MSCs and macrophages were encapsulated in the gels. When co-  
15 cultures of these two cells were compared with single cell cultures, it was shown that MSCs  
16 induced M2 polarization of macrophages whereas M1 macrophages induced osteogenic  
17 differentiation of MSCs <sup>152</sup>. All these studies show that not reciprocal interaction of stem cells and  
18 macrophages can be modulated with the control of biomaterial properties.

19

## 20 **5. Outlook: Use of adaptive immune cells in regenerative medicine**

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

1 regulatory Treg cells can enhance the activity of stromal and progenitor cells for improved tissue  
2 regeneration <sup>156</sup>.

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

42



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7 **Conflict of interest**

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

9

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