Acta Biomaterialia 10 (2014) 613–622

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Impact of 3-D printed PLA- and chitosan-based scaffolds on human monocyte/macrophage responses: Unraveling the effect of 3-D structures on inflammation

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ARTICLE INFO

Article history:
Received 4 June 2013
Received in revised form 24 October 2013
Accepted 29 October 2013
Available online 5 November 2013

Keywords:
Tissue engineering
3-D scaffolds
Rapid prototyping
Human macrophages
Inflammation

ABSTRACT

Recent studies have pointed towards a decisive role of inflammation in triggering tissue repair and regeneration, while at the same time it is accepted that an exacerbated inflammatory response may lead to rejection of an implant. Within this context, understanding and having the capacity to regulate the inflammatory response elicited by 3-D scaffolds aimed for tissue regeneration is crucial. This work reports on the analysis of the cytokine profile of human monocytes/macrophages in contact with biodegradable 3-D scaffolds with different surface properties, architecture and controlled pore geometry, fabricated by 3-D printing technology. Fabrication processes were optimized to create four different 3-D platforms based on polylactic acid (PLA), PLA/calcium phosphate glass or chitosan. Cytokine secretion and cell morphology of human peripheral blood monocytes allowed to differentiate on the different matrices were analyzed. While all scaffolds supported monocyte/macrophage adhesion and stimulated cytokine production, striking differences between PLA-based and chitosan scaffolds were found, with chitosan eliciting increased secretion of tumor necrosis factor (TNF)-α, while PLA-based scaffolds induced higher production of interleukin (IL)-6, IL-12/23 and IL-10. Even though the material itself induced the biggest differences, the scaffold geometry also impacted on TNF-α and IL-12/23 production, with chitosan scaffolds having larger pores and wider angles leading to a higher secretion of these pro-inflammatory cytokines. These findings strengthen the appropriateness of these 3-D platforms to study modulation of macrophage responses by specific parameters (chemistry, topography, scaffold architecture).

1. Introduction

Implantation of a biomaterial elicits an inflammatory reaction, which influences the behavior of different cell populations involved in the regenerative process determining implant success [1]. The emerging view is that activation of the inflammatory process can lead to implant rejection, but is also key for efficient tissue regeneration and repair [2,3]. Having the capacity to modulate the inflammatory response will lead to more effective tissue engineering (TE) strategies. Indeed, it has been recently shown that materials modified with inflammatory molecules can impact on human natural killer cell behavior, and lead to increased mesenchymal stem/stromal cell (MSC) recruitment [4].

Inflammation is an extremely complex, multistage process involving numerous cell types and mediator signals. Macrophages are dominant infiltrating cells that respond rapidly to biomaterial implantation and play a crucial role in regulating the inflammatory response and tissue remodeling, by secreting large amounts of bioactive mediators that can initiate inflammation, cell migration and differentiation, tissue remodeling and blood vessel formation [1,2]. The mediators secreted will depend on the material properties, and will direct subsequent inflammation and/or tissue repair. If macrophages fail to phagocytose a certain material or foreign body, cells may fuse and form multinucleated giant cells (MGC), which have

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http://dx.doi.org/10.1016/j.actbio.2013.10.035

thus been related with the foreign body response. It is generally believed that macrophages can polarize towards either a pro-inflammatory or anti-inflammatory phenotype, exhibiting regulatory, pro-regenerative and angiogenic functions [5].

Monocyte/macrophage activation and cytokine secretion are affected by surface chemistry and topography [6–8]. Additionally, porous structures promote faster healing and form a thinner fibrous capsule than dense solid implants [9,10], suggesting that the implant architecture can play a role in foreign body reaction. Thus, not only the intrinsic material properties but also the geometry/architecture and surface cues of the 3-D scaffolds may be of importance in the design of new surfaces and scaffolds that can tailor macrophage activation towards a regenerative pathway.

Polylactic acid (PLA) and chitosan (Ch) are two well-known biodegradable polymers that are widely used in scaffolds for TE purposes. Both materials possess distinct chemical, physical and biological properties that may affect the inflammatory response. The inflammatory impact of the US Food and Drug Administration (FDA) approved PLA has been mainly reported in terms of the effect of its acidic degradation by-products upon implantation [11]. Though there is some literature on the PLA inflammatory response in vitro, only a few works have tackled the behavior of cells in 3-D structures [12,13]. As for Ch, some contradictory results have been reported on the inflammatory response elicited when the polymer was presented in different forms [14,15]. Recently, it was shown that 2-D Ch surfaces drive polarization of human macrophages towards an anti-inflammatory phenotype and dendritic cells towards a pro-inflammatory profile [16]. However, the effect of PLA and Ch 3-D scaffolds on primary human monocyte/macrophage responses has not been fully established.

Rapid prototyping (RP) is an emergent tool in the biomaterials field that enables fabrication of 3-D structures with well-defined and reproducible geometries and architectures. By combining polymers together with RP, it is possible to build 3-D scaffolds with different characteristics and well-distinguished geometric and physico-chemical features, allowing the study of the effect of these factors on inflammatory cell responses.

The hypothesis behind this work was that both the chemical composition and the architecture of 3-D scaffolds affect macrophage responses. We hypothesized that the geometry of pores could affect macrophage behavior and, in particular, cytokine secretion, as it has already been shown for other cells, particularly for MSCs, that differentiation depends on the geometry of the substrate (in two dimensions), by affecting the curvature of the cytoskeleton actin fibers [17]. Thus, here we set out to determine which impacted on macrophage behavior the most: the chemical composition or the geometry of the scaffold. Therefore, this work aims at: (i) the controlled fabrication of novel 3-D platforms of PLA and Ch using RP, and (ii) the study of the macrophage morphology and cytokine profile in these matrices. To this end, Ch scaffolds with two different geometries (orthogonal and diagonal) and PLA-based orthogonal scaffolds made of PLA and a combination of PLA and a biodegradable calcium phosphate glass (G5) were fabricated. Fabrication of Ch scaffolds with two defined geometries allowed us to evaluate the effect of scaffolds architecture while keeping the surface properties and material chemistry constant. In contrast, by adding G5 glass to PLA scaffolds, the effect of the surface topography/chemistry was investigated, while enhancing both the biological and mechanical performance of the scaffolds [18,19], due to G5's bioactive properties [20]. The impact of these 3-D scaffolds on the secretion of the cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, IL-12/23(p40) and tumor growth factor (TGF)-β1 was analyzed. TNF-α and IL-6 are pro-inflammatory cytokines commonly analyzed when studying the inflammatory response induced by different biomaterials. Timely production of the appropriate amounts of TNF-α and IL-6 is critical for the efficient repair of, for example, bone [3], and macrophages usually associated with an inflammatory M1 or a regenerative M2b phenotype produce increased amounts of these two cytokines [21]. We also quantified the amounts of p40, thus detecting the monomer p40 and the heterodimeric cytokines IL-12 or IL-23, which consist of p40 bound to either p35 or p19 chains, respectively. These two pro-inflammatory cytokines of the same family can be produced at high levels by M1 macrophages and stimulate the differentiation of proinflammatory T_H1 and T_H17 cells [22]. Regarding cytokines with anti-inflammatory properties, we analyzed IL-10 and TGF-β. IL-10 is one of the most studied anti-inflammatory cytokines, as it regulates many steps of an immune response, being crucial in restraining inflammation [23]. TGF-β has a role in controlling immune homeostasis [23] and is also an important player in regulating bone repair [3].

2. Materials and methods

2.1. Materials

Poly(95L/5DL)lactic acid (PURAC) and polyethylene glycol (PEG; MW = 400 Da; Sigma Aldrich) were dissolved in chloroform (5% w/v) and combined to obtain a polymer blend solution (5% w/w PEG) [18]. A completely biodegradable calcium phosphate glass (G5, molar composition: $44.5P_2O_5$–$44.5Ca_2O$–$6Na_2O$–$5TiO_2$) was used in the form of particles (<40 μm) and added to the solution (50% w/w) [24]. Endotoxin-free Ch powder (Chitosan 123, France-Chitine), with Mw = 324 ± 27 × 10^3 and 11–12% of N-acetylation [4], was purified as previously described [25]. Ch solution (3% w/v) was prepared by hydrating Ch overnight in MilliQ water, dissolving in acetic acid (2%, Sigma) overnight at 4 °C under constant stirring and centrifuging for 5 min at 800*g*.

2.2. Scaffolds fabrication

A nozzle-deposition system also known as a direct-print tool (Tissue Engineering 3-Dn-300, Sciperio/nScrypt Inc. Orlando, Florida; available in the Rapid Prototyping service of the Biomedical Networking Center, CIBER-BBN and IBEC www.ibecbarcelona.eu/biomaterials) was used to fabricate the 3-D scaffolds. It utilizes a computer-aided design/computer-aided manufacturing approach. The dispensing process allows flexible alteration of parameters such as speed of deposition, air pressure in the pneumatically actuated pump and dispensing height. Two different architectures were designed and fabricated (Fig. 1): (i) an orthogonal displaced doubled layer (orthogonal) configuration, with distance between struts D = 1 mm and 500 μm for Ch and PLA-based scaffolds, respectively; and (ii) an orthogonal–diagonal double layer design (diagonal) with distance $D1$ = 1 mm between the struts axes of the orthogonal layers and $D2$ = 700 μm between the struts axes of the diagonal layers. In the case of the PLA-based scaffolds, the orthogonal design was used. In the case of Ch, scaffolds were produced in two different geometries – ChO, with orthogonal struts, and ChD, with diagonal and orthogonal struts. Three-dimensional structures were built by layer-by-layer deposition. In the case of PLA-based scaffolds, a printing pressure between 40–80 psi, a motor speed of 3 mm s^{-1} and a G27 (200 μm) nozzle were used. The syringe temperature was 40 ± 5 °C, and room temperature was 25 ± 2 °C. In the case of Ch scaffolds, a printing pressure around 40 psi, a motor speed of 7 mm s^{-1}, and a Teflon tip with diameter of 150 μm were used. Additionally, NaOH (8% w/v) in ethanol (70%) was dispensed drop by drop on top of the printed struts to cross-link the polymer structure during the fabrication process [26]. Ch scaffolds were kept protected from light overnight at room temperature in NaOH (8% w/v) and washed three times for 5 min each with MilliQ water.