Seminars in Immunology 29 (2017) 62–71

Contents lists available at ScienceDirect

Seminars in Immunology

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

Influence of scaffold design on host immune and stem cell responses

Ashwin Nair[a,*], Liping Tang[a,b,*]

[a] Joint Biomedical Engineering Program, University of Texas at Arlington, Arlington, TX 76019 and University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390

[b] Department of Biomedical Science and Environmental Biology, Kaohsiung Medical University, Kaohsiung 807, Taiwan

ARTICLE INFO

Keywords:
Tissue engineered scaffolds
Inflammatory responses
Protein
Cytokines
Stem cells
Macrophages
Fibrocytes
Tissue regeneration
Foreign body reactions

ABSTRACT

The combined culture of isolated stem cells in tissue engineering scaffolds represents a popular strategy for the regeneration of specialized tissues. Despite of improved outcomes in some tissues, this stem cell-seeded tissue engineering strategy has not led to significant tissue regeneration as expected. The lower-than-expected outcome may be caused by overwhelming immune responses to scaffold materials and poor survival of seeded stem cells following implantation. This review is aimed at summarizing the success and failure of this strategy and also shedding some light on new directions to design scaffolds for promoting regenerative responses via autologous stem cells. The first half of this review summarizes the influence of scaffold physical and chemical properties on immune cell responses to scaffold implants. The second half focuses on the influence of scaffold design to alter immune and stem cell responses for achieving desirable tissue regeneration.

1. Introduction

Tissue engineering represents an exciting paradigm shift by providing a new treatment to replace or to replenish a large number of tissues. Scaffolds, that provide the structural template for tissue regeneration, are one of the major components of tissue engineering strategies. The implantation of man-made medical devices sets into motion a series of native immune responses – starting with hemostasis, inflammatory responses, and tissue regeneration. Following implantation, medical implants such as tissue engineering scaffolds are bathed with blood and tissue fluids. This environment and subsequent cellular responses launch the so called foreign body response – a cascade of events that attempt to stop blood loss, prevent infection and start wound healing responses. Since most scaffolds are made of synthetic degradable polymers, it is critical to understand how the interactions between inflammatory cells and synthetic implants would impact subsequent host responses.

1.1. Acute biomaterial-mediated inflammatory responses

The adsorption of plasma proteins on to biomaterial surfaces occur almost instantly (within seconds) following implantation of scaffold or device. As a result of this fast response, biomaterial surfaces are covered with layer(s) of host proteins prior to interaction with immigrated immune cells [1]. A wide variety of plasma proteins are known to bind onto biomaterial implants, mediated mainly by hydrophobic interactions [2]. The amounts of the adsorbed plasma proteins have direct relationship with their concentrations in blood. However, based on the extent of conformational changes, plasma proteins may have varying affinity to biomaterial surfaces [3]. Plasma proteins with high conformational change, such as fibrinogen, may be difficult to be replaced by other plasma proteins even if they are abundant. However, those proteins with minimal potential for conformational change, such as albumin, may be replaced by other plasma proteins [1,4]. This highly dynamic process in which proteins predominant in blood serum with low surface affinity are replaced by proteins with higher surface affinity and concentrations is called Vroman Effect [5]. Typically, initial adsorption of albumin is replaced by globulins that are then followed by fibrinogen, fibronectin, factor XII and high molecular weight kininogens [5]. It should be noted that adsorbed proteins also denature to a greater extent upon increasing contact with hydrophobic surfaces [4,6]. Key among these adsorbed proteins is fibrinogen, in which conformational changes lead to the exposure of hitherto hidden epitopes, proving causal to inflammatory cell accumulation and activation [4,7,8]. An interesting study has shown that fibrinogen domains have different affinity to hydrophobic surfaces and such differential responses lead to the exposure of inflammatory epitope at the D domain of fibrinogen molecules [9].

During an acute inflammatory phase, inflammatory cells like monocytes/macrophages (MØ) and polymorphonuclear leukocytes (PMN) are recruited and accumulate at the interface between biomaterial implants and surrounding tissue. Studies have shown that the

* Corresponding authors at: Bioengineering Department, Box 19138, University of Texas at Arlington, Arlington, TX 76019-0138, United States.
E-mail addresses: anair@uta.edu (A. Nair), ltang@uta.edu (L. Tang).

http://dx.doi.org/10.1016/j.smim.2017.03.001
Received 12 August 2016; Received in revised form 17 March 2017; Accepted 24 March 2017
Available online 26 April 2017

activation and subsequent degranulation of mast cells and histamine release are known to play a critical role in this process [10,11]. In fact, this histamine mediated inflammatory cell recruitment that is enabled by adsorbed fibrinogen can be minimized by administration of H1 and H2 histamine receptor antagonists [10,11]. A biomaterial-mediated acute inflammatory phase that lasts for a few days to a week depends on the nature and site of the implant, and the extent of implantation-associated tissue injury.

1.2. Chronic foreign body reactions

Continued presence of the implant can lead to chronic inflammation that is characterized by the presence of MØ, lymphocytes and foreign body giant cells at the implantation site. [12] A hallmark of chronic inflammation is the conversion of the fibrin clot formed during the acute inflammatory phase into a granulation tissue with abundant MØ and fibroblasts. These cells release biochemical cues that trigger angiogenesis and influx of fibroblasts to produce collagen and fibrotic tissue. In the case of "normal" wound healing responses, the granulation tissue is replaced by collagen, elastin, glycoproteins and proteoglycans at the end of healing processes. However, in the case of permanent (or non-degradable) implants that may present persistent inflammatory stimuli, chronic inflammation leads to continuous activation of fibroblasts to form a thick collagenous fibrous capsule that "walls off" the implant from the rest of the body [13,14]. The formation of fibrotic tissues have been associated with the impaired function of many medical devices, including breast implants, insulin catheters, neural implants, etc [13,14].

1.3. Key biomolecules in triggering biomaterial-mediated tissue response

The recruitment of activated immune cells and thrombocytes produce a wide variety of chemokines, cytokines and growth factors, which participate in biomaterial/scaffold implant-associated inflammatory reactions. For example, activated mast cells release cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-Iβ) which aids the inflammatory response [15,16]. Recruited platelets and/or MØ have been shown to produce cytokines like platelet factor-4, platelet derived growth factor (PDGF), and transforming growth factor-beta (TGF-β) that can trigger recruitment of fibroblasts as well as mesenchymal and other progenitor cells. In fact, the administration of PDGF was found to stimulate influx and proliferation of inflammatory cells and fibroblasts which in turn increased collagen formation improving healing outcome [17]. On the other hand, previous studies have shown that biomaterial-mediated inflammatory responses increase the production of IL-4 [18], IL-13 [19], fibroblast growth factor-2, TGF-β and TNF-α. [20] Specifically, tissue MØs release a number of cytokines like IL-1, TGF-β1 and PDGF. Elevation of IL-1 and TGF-β1 heralds the fibrotic capsule formation and production of collagen I and III by myofibroblasts. IL-13 has been shown to promote fibroblast proliferation and collagen production [21].

Recent studies have provided increasing evidence indicating that biomaterial and scaffold properties may affect MØ responses and subsequent tissue reactions. Many recent studies have uncovered that the balanced interactions between M1 MØ-mediated pro-inflammatory responses and M2 MØ-associated regenerative reactions are crucial in determining the outcome of the remodeling process. M1 MØ may be activated by cytokines like interferon-γ (IFN-γ) which leads to production of reactive oxygen species and a number of pro-inflammatory cytokines like IL-1β, IL-6, 12, 23 and TNF-α. M2 MØ are activated by cytokines like IL-4, 13 and 10 [22]. It must be noted that, in the case of natural scaffold materials, ECM proteins and their degradation products may promote the differentiation of M2 MØ [23]. Overall, it is well established that biomaterial and scaffold implants would trigger immune responses to different extent. However, it remains mostly unclear whether and how scaffold properties would affect immune reactions.

2. Factors affecting scaffold-mediated inflammatory responses

Almost all scaffold implants prompt different extent of foreign body reactions and/or immune cell responses. It is also well established that the physical and chemical properties play a pivotal role in dictating the degree of tissue reactions to scaffold implants. To enhance cell adhesion, infiltration and tissue integration, tissue engineering scaffolds are designed to be highly porous and in various shapes, sizes and with various topographical as well as surface characteristics for different applications. While a lot of emphasis has been placed on changing scaffold properties to improve the responses of "desired" cells, little attention has been placed on how "unwanted" immune cells would respond to specific scaffold designs. In fact, it is well established that the foreign body response mounted by the host may significantly hamper the survival of transplanted cells embedded inside the tissue engineering scaffolds [24–26]. The influence of different physical, chemical and biological properties of tissue engineering scaffolds on immune cell responses will be discussed in the following sections.

2.1. Effect of scaffolds' physical property on inflammatory responses

Scaffolds are often embedded with drug/biomolecules-loaded micro- and nano-particles for improving cell migration, proliferation and/or differentiation [27,28]. When released from scaffolds, micro-/nano-particles (< 10 μm) are effectively phagocytosed by MØs. Implants larger than these and measuring up to 100 μm are not phagocytosed by a single MØ. Instead, multiple MØ fuse together to form foreign body giant cells that then engulf the implant. However, in the case of larger and thicker implants (> 100 μm), these fused MØs exhibit a phenomenon called "frustrated phagocytosis" that may result in the release of a number of cytotoxic products, such as reactive oxygen species, and proteolytic enzymes that degrade the implant and surrounding healthy tissue [19,29].

Although the size of the implant is important, the shape or geometry is also a very important scaffold design parameter. Almost three decades ago, most studies involving understanding inflammatory response to implantable materials typically used spherical materials [30,31]. A study on various medical grade polymers in circular, triangular and pentagonal shapes implanted in rodent gluteal muscles for 14 days showed highest enzymatic activity in triangular shaped followed by pentagonal and circular implants [32]. Interestingly, a separate study reported that both the shape and sizes of the implant play a major role in phagocytosis. By investigating MØ uptake of variously sized polystyrene particles (1–12.5 μm) made in different shapes (spheres, oblate and prolate ellipsoids, elliptical and rectangular) *in vitro*, a study has found that MØ uptake depended on attachment sites [33]. Briefly, attachment to major axis of elliptical disks led to rapid internalization as opposed to minor axis attachment. Amongst all the particle shapes, irrespective of the size, phagocytosis occurred readily when MØs attached to the dome or ring regions as compared to concave regions [33]. This phenomenon was attributed to the formation of actin cups in the MØ which occurred immediately after MØ attachment to the major axes. While there is no agreement on the ideal shape of the implant, it is believed that circular shaped implants with smooth contours are better tolerated by the immune system [32,34]. A recent study sought to evaluate the biocompatibility of spherical implant geometry on *in vivo* biocompatibility. Alginate hydrogel particles (0.3 mm to 1.9 mm diameter), used extensively in a number of medical applications including in the immune-isolation of donor pancreatic islets for Type-1 diabetes, were implanted in the intraperitoneal space of C57/BL6 mice. It was found that spherical microspheres that were greater than 1.5 mm in diameter were able to keep the MØ response elicited to a minimum for at least six months [34]. However, it must be noted that not all implants are circular in shape