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Review

In vivo cellular reactions to different biomaterials—Physiological and pathological aspects and their consequences

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ABSTRACT

Biomaterials are widely used in guided bone regeneration (GBR) and guided tissue regeneration (GTR). After application, there is an interaction between the host immune system and the implanted biomaterial, leading to a biomaterial-specific cellular reaction. The present review focuses on cellular reactions to numerous biomaterials *in vivo* with consideration of different implantation models and microenvironments in different species, such as subcutaneous implantation in mice and rats, a muscle model in goats and a femur model in rabbits. Additionally, cellular reactions to different biomaterials in various clinical indications within the oro-maxillofacial surgical field were considered. Two types of cellular reactions were observed. There was a physiological reaction with the induction of only mononuclear cells and a pathological reaction with the induction of multinucleated giant cells (MNGCs). Attention was directed to the frequently observed MNGCs and consequences of their appearance within the implantation region. MNGCs have different subtypes. Therefore, the present review addresses the different morphological phenotypes observed within the biomaterial implantation bed and discusses the critical role of MNGCs, their subtypes and their precursors as well as comparing the characteristics and differences between biomaterial-related MNGCs and osteoclasts. Polymeric biomaterials that only induced mononuclear cells underwent integration and maintained their integrity, while polymeric biomaterials that induced MNGCs underwent disintegration with material breakdown and loss of integrity. Hence, there is a question regarding whether our attention should be directed to alternative biological concepts, in combination with biomaterials that induce a physiological mononuclear cellular reaction to optimize biomaterial-based tissue regeneration.

1. Introduction

Currently, a wide range of different biomaterials is available to support hard and soft tissue regeneration following the principles of guided bone and guided tissue regeneration (GBR/GTR). In this context, biomaterials are used as scaffolds to hold a place for delayed tissue regeneration in bone defects as well as to prevent premature soft tissue ingrowth into the defect area [1]. After biomaterial implantation, an interaction between the host immune system and implanted biomaterial occurs, resulting in a biomaterial-specific tissue response during a complex biological process [2]. Two types of cellular reactions towards biomaterials have been observed. They are a cellular reaction based on physiologically existing mononuclear cells, such as macrophages, lymphocytes and fibroblasts, and a foreign body reaction based on the additional presence of multinucleated giant cells [3]. The inflammatory pattern induced by biomaterials includes an early innate immune response from macrophages, whereas lymphocytes, as a part of the adaptive immune system, play a crucial role in the foreign body reaction and formation of foreign body multinucleated giant cells (MNGCs) [4]. In the last decade, our group has conducted numerous systematic studies in standardized *in vivo* implantation models to assess the cellular reaction towards different biomaterials. Additionally, several clinical studies have included a histological evaluation of the cellular reaction to a variety of biomaterials to determine the induced inflammatory pattern. The presence of multinucleated giant cells (MNGCs) as a part of the foreign body reaction within the implantation bed of biomaterials was frequently observed in almost all investigated biomaterials [5–9]. However, the role of these cells *in vivo* is still unexplored. These observations highlight the crucial need to understand the critical role of MNGCs within the biomaterial-based regeneration process as well as their origin. Previously, the formation of biomaterial-related MNGCs was described as a process of frustrated phagocytosis [10]. During this

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process, macrophages that are incapable of degrading the implanted biomaterial fuse to form MNGCs [2]. Macrophages and probably MNGCs are heterogeneous and have different subtypes [11]. Some macrophage and MNGC types have the potential to express tartrate-resistant acid phosphatase (TRAP), a degradation enzyme that was originally detected in osteoclasts [12,13], raising the discussion of whether biomaterial-related MNGCs are indeed osteoclast-like cells. Furthermore, *in vitro* studies have shown that the formation of MNGCs is induced by very specific cytokines, particularly interleukin-4 (IL-4) and interleukin-13 (IL-13) [14], which are predominantly released by persistent lymphocytes [15,16]. Based on these findings, *in vitro* models were established using IL-4 to form human monocyte-derived MNGCs and study the process of MNGC formation as well as the characteristics of MNGCs [17,18]. In addition, the formation of MNGCs depends on the biomaterial surface properties and capacity to absorb specific molecules, which probably contributes to the induction of specific macrophage phenotypes that have the potential to fuse to MNGCs [19,20]. *In vitro* models are important for studying specific cells types and their molecular interactions as an isolated cell system to better understand a particular mechanism [10,14]. However, these models are intentionally created systems that cannot mimic the complex *in vivo* native environment in which MNGCs are induced and formed. By contrast, *in vivo* models allow for assessment of the cellular response towards biomaterials within the native environment of the cells. Moreover, developments in immunohistochemistry permit the detection of different signaling molecules to further identify the phenotypes, the role of MNGCs within the original implantation bed and MNGC interaction with the peri-implantation region. Using this platform, our group has performed several studies in different implantation models of both large and small animals as well as clinical studies focusing on the tissue response towards various biomaterials. The present review systematically outlines the *in vivo* cellular inflammatory response to different biomaterials, with special attention paid to the formation of MNGCs, their phenotypes and their consequences in relation to different biomaterials and implantation environments.

2. Immune cells involved in the formation of multinucleated giant cells

After biomaterial implantation, an interaction between the implanted biomaterial and host immune system involving the innate and adaptive immune responses occurs [21]. First, a provisional blood clot is formed on the surface of the biomaterial, which is followed by sterile acute inflammation progressing to chronic inflammation and a foreign body reaction in most cases [2]. The key cells involved in the formation of multinucleated giant cells in response to biomaterials are macrophages and lymphocytes [10].

2.1. Macrophages

It is generally accepted that macrophages are precursor cells of multinucleated giant cells [4]. Macrophages, as a part of innate immunity, are among the first lines of defense for the body [22]. In addition to their phagocytic activity, these cells are involved in wound healing and repair [23] as well as in maintaining tissue integrity through their capacity to release various growth factors and cytokines [24,25]. Moreover, macrophages exist as different subtypes, reflecting their activation as pro-inflammatory cells (M1) that are mainly involved in phagocytosis and inhibiting anti-inflammatory cytokines. By contrast, anti-inflammatory or regulatory (M2) macrophages adopt a different phenotype to support wound healing [25]. The reversible polarization of macrophages plays a crucial role in the biomaterial tissue reaction and MNGC formation [26]. The classification of differentiated macrophages generally depends on the induced parameter and expression pattern. During the innate immune response, M1 macrophages are activated by TNF and IFN, which are released by natural killer cells [27]. This macrophage phenotype expresses inducible NO synthase (iNOS) [22], whereas M2 macrophages are preliminarily induced by IL-4 from basophilic cells during the innate immune response and express arginase [28]. To identify the inflammatory pattern of macrophages, several markers have been established. M1 macrophages are positive for iNOS, TNF alpha 1, CCR7, CD-80, CD-86, SOCS3 and CD-64, while M2 are positive for Argin.1, CD-206, CD-163, and SOCS1/2 [2,22,25,26,29] (Table 1). In this context, the local microenvironment appears to determine the macrophage phenotype. Moreover, macrophages exhibit high plasticity, allowing for their transition between the M1 and M2 phenotypes according to the dominant conditions [30]. It is generally accepted that macrophages are precursor cells of MNGCs, especially foreign body MNGCs [2]. However, the *in vivo* influence of M1 and M2 macrophages on MNGC formation and differentiation into possible subtypes needs to be further elucidated.

Table 1
Markers of (M1) pro- and (M2) anti-inflammatory activated macrophages [2,22,25,26,29].

M1	M2
iNOS	Argin 1
TNF alpha 1	CD 206
CCR 7	CD163
CD86	TGM2
SOCS 3	SOCS 1/2
CD 80	CD 23

2.2. Lymphocytes

When innate immunity limits are reached, T lymphocytes, which are part of adaptive immunity, take over [22]. T lymphocytes are important for wound healing, biomaterial responses and foreign body reactions [23]. Two different types of T lymphocytes are activated by antigen recognition [31,32]. Th1 lymphocytes release IL-2, TNFß 1 and IFNγ. These signaling molecules induce macrophage polarization and activation to the M1 pro-inflammatory phenotype [22] and support the differentiation of CD-8 cells to cytotoxic cells [33]. Furthermore, this cascade has been shown to be involved in the rejection reaction to cardiac xenotransplantation in a murine model [34].

Th2 lymphocytes release IL-4, IL-5, IL-6 and IL-10. These cytokines stimulate the differentiation of macrophages in M2 phenotypes [20]. This pathway has been described to support transplant tolerance in animals [35,36]. In addition, IL-4 plays a crucial role in the fusion of macrophages and formation of foreign body MNGCs [37]. Its interaction with different adhesion molecules, such as integrin ß 1/2 [38], and upregulation of the expression of mannose receptor (CD-206) were found to be critical in understanding the process of MNGC formation [39].

3. Multinucleated giant cells and their subtypes

Different types of multinucleated giant cells (MNGCs) have been found in different microenvironments, the subtypes depending on their precursor cells and formation process [40–42].

3.1. Osteoclasts

Bone-related giant cells, i.e., osteoclasts, are important for bone regeneration and remodeling [40]. These cells are derived from bone marrow early monocytes circulating in the blood [40]. In contrast to other multinucleated giant cells, a recent study has shown that the precursors of osteoclasts do not express CD-68 [43]. To form MNGCs, adhesion molecules play an important role. Integrin αvβ3 is the dominant integrin in osteoclastogenesis and is considered to be an osteoclast marker [38,44,45], whereas macrophages and macrophage-