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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-

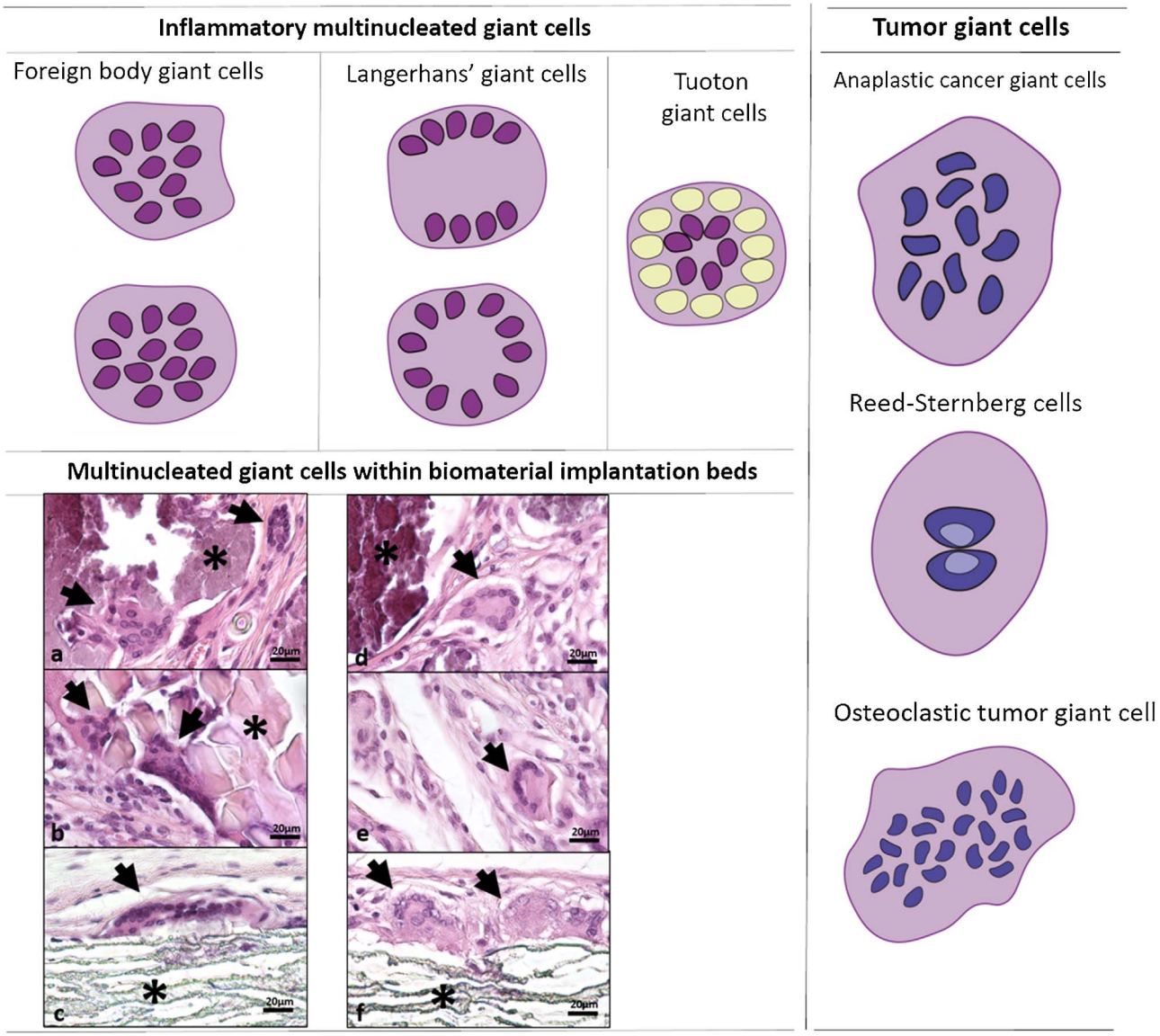

Fig. 1. Illustrative artwork of the histopathological characteristics of different multinucleated giant cells subtypes. Exemplary histological micrographs of the morphologically different subtypes of multinucleated giant cells observed within the subcutaneous implantation beds of biomaterials. a) Foreign body MNGCs with heterogeneously distributed nuclei (black arrows) within the implantation bed of a nanostructured bone substitute material (*) on day 15. b) Foreign body MNGCs with heterogeneously distributed nuclei (black arrows) within the implantation bed of silk fibroin (*) on day 60. c) Foreign body MNGCs with heterogeneously distributed nuclei (black arrows) within the implantation bed of expanded polytetrafluoroethylene (*) on day 60. d) Langerhans'-like MNGCs with peripherally oriented nuclei in a circle (black arrow) within the implantation bed of a nanostructured bone substitute material (*) on day 10. e) Langerhans'-like MNGCs with peripherally oriented nuclei in a horseshoe arrangement (black arrow) within the implantation bed of silk fibroin (*) on day 15. f) Langerhans'-like MNGCs with peripherally oriented nuclei in a circle (black arrow) within the implantation bed of expanded polytetrafluoroethylene (*) on day 30. All histological stains are H and E and in ×400 magnification.

derived MNGCs do not express this specific integrin [45]. Another molecule involved in the differentiation of osteoclasts is RANKL (receptor activator of NF-kappa B ligand), one of the regulating cytokines of osteoclastogenesis that is accepted as an osteoclast marker [40,46–48]. Moreover, *in vitro* studies have shown that the calcitonin receptor is expressed on osteoclasts and not on foreign body MNGCs [37,49]. In this context, the calcitonin receptor is a further marker of osteoclast identification.

3.2. Disease-related MNGCs (Langerhans' MNGCs)

In addition to osteoclasts, additional MNGC subtypes were found in relation to pathological processes, such as in tumors and foreign body granulomas [41,42]. These disease-related MNGCs are present in tuberculosis, sarcoidosis, histiocytosis and xanthoma. Generally, different types of MNGCs exhibit specific morphological characteristics. Pathological MNGCs, such as Langerhans' giant cells that appear in tuberculosis and sarcoidosis, generally have peripherally oriented nuclei, forming a ring or horseshoe or showing peripheral polarization into two poles [41] (Fig. 1). Langerhans' MNGCs are formed by the fusion of macrophages activated by pathogens, such as the tuberculosis bacterium [50]. An *in vitro* study showed that after stimulation of macrophages and their fusion into Langerhans' MNGCs, these cells expressed less mannose receptor (CD-206) than their precursor macrophages, reflecting the loss of the phagocytic function [50]. However, in this and in a further *in vitro* study, Langerhan's MNGCs were positive for MHC-II (HLA-DR) [50,51], demonstrating that after macrophage fusion, the formed MNGCs preserve some characteristics of their precursors, such as the antigen-presenting function, while losing others, such as phagocytosis. The preserved properties might depend on the local microenvironment and involved signaling molecules in the process of fusion. Langerhans' MNGC fusion depends on the CD-40-CD-40-Ligand

interaction and IFNγ, and adhesion molecules, such as integrin ß1 and ß2, play an important role in the fusion process [52–54]. Additionally, inducible nitric oxide synthase (iNOS) has been shown to be expressed in MNGCs in bovine tuberculosis [55] and wild boar tuberculosis [56]. This molecule is also upregulated in pro-inflammatory macrophages [29]. These findings show that this MNGC type can exhibit pro-inflammatory characteristics [29].

Another MNGC subtype was found in xanthoma disease. This type of MNGC was termed Touton giant cells, and these cells have lipid vacuoles in their cytoplasm as well as centrally located nuclei (Fig. 1) [41].

3.3. Foreign body MNGCs

When macrophages fail to eliminate foreign particles by phagocytosis, they fuse to form MNGCs during chronic inflammation, comprising a foreign body reaction [2]. Foreign body MNGCs are present in foreign body granuloma. The general morphological characteristic of their nuclei is that they are heterogeneously distribution throughout the cytoplasm (Fig. 1). The number of nuclei varies up to 100 uniformly shaped nuclei, and the nuclei are similar to those of macrophages [42]. Foreign body MNGC formation via activated macrophage fusion was shown to depend on IL-4 and IL-13 [4,37]. In contrast to osteoclasts, MNGC formation is related to a different type of integrin than that involved in osteoclast formation. Integrins ß1 and ß2 have been described in the process of foreign body MNGC fusion, while Integrin ß 3 has not [38]. Additionally, the expression of several cytokines and receptors characterized foreign body MNGCs *in vitro*. Similar to their precursors, foreign body MNGCs express CD-68, a glycoprotein expressed in macrophages [4,37,57]. Furthermore, the mannose receptor (CD-206) was found in foreign body MNGCs during and after fusion *in vitro* [4]. MHC-II (HLA-DR), a molecule involved in antigen presentation, was also expressed in foreign body MNGCs [4]. CD-86 is related to T-cell activation and was found in pro-inflammatory macrophages and foreign body MNGCs [4,37], while expression of this molecule was absent in osteoclasts and during formation [58]. Therefore, CD-86 can be considered to be a marker for foreign body MNGCs.

3.4. Tumor-related MNGCs

Anaplastic cancer giant cells are large and have more hyperchromatic nuclei that vary in shape and size. Reed-Sternberg cells contain two nuclei and are found in malignant tumors, such as Hodgkin's lymphoma [42]. In addition, giant cell bone tumors have evenly distributed nuclei within the cytoplasm (Fig. 1) [40,42].

Table 2 summarizes the most common molecules expressed in osteoclasts, Langerhans' MNGCs and foreign body MNGCs. In this context, it is noteworthy that most of these markers were established using *in vitro* models. To date, MNGCs detected *in vivo* within biomaterial implantation beds are generally considered to be foreign body giant cells. However, their characteristics and classification in possible subtypes have not been fully explored.

Table 2
Most expressed molecules in osteoclasts, foreign body giant cells and Langerhans' giant cells (+ = expressed; − = not expressed;? = no data found).

Molecule	Osteoclasts	Foreign body giant cells	Langerhans' giant cells
CD 206	−	+	±
Calcitonin	+	−	?
RANKL	+	−	?
HLA-DR	−	+	+
CD 68	−	+	+
Integrin ß 3	+	−	−
Integrinn ß 1/2	−	+	+
CD 86	−	+	?

4. Implantation environments and study designs

The present review focuses on the cellular reaction to numerous biomaterials *in vivo*, including the subcutaneous implantation model in mice and rats, critical size femur defects in rabbits and the muscle implantation model in goats. Additionally, translational studies, including clinical and histological evaluations at different localizations within the oro-maxillofacial surgical field, are considered in this review (Table 3). Interest is directed to the frequently described MNGCs and related biological processes (Fig. 2). The findings summarized below should provide an overview of the *status praesens* of the cellular reaction to biomaterials that are clinically applicable and the ubiquitous presence of MNGCs while focusing on their persistence, morphology and significance.

4.1. Subcutaneous implantation model in mice

Several biomaterials have been studied using an established subcutaneous implantation model in mice. A wide variety of synthetic, xenogeneic and phycogenic biomaterials of different origins showed different inflammatory patterns, including MNGC induction.

4.1.1. Polymeric biomaterials

When examining collagen-based biomaterials, two cellular reaction types were observed: a physiological reaction by mononuclear cells and pathological reaction characterized by multinucleated giant cells. Two non-cross-linked porcine-derived collagen-based biomaterials showed an exceptional tissue response after implantation [3,59]. These biomaterials only induced mononuclear cells (MNCs) over a period of 60 days; they maintained their native structure and were well integrated into the host tissue without any signs of breakdown or foreign body reaction [3,59]. In addition to the induction of only mononuclear cells, a mild vascularization pattern was observed without transmembranous vascularization, reflecting the process of integration and preserved capacity as a functional barrier to prevent total influx of peri-implantation connective tissue [3,59] (Fig. 3A).

By contrast, other non-cross-linked, collagen-based, porcine-derived biomaterials evoked a foreign body reaction characterized by multinucleated giant cells (MNGCs) after 10–15 days and persisted up to 30 days [5,6]. The occurrence of MNGCs within the implantation bed of these biomaterials was associated with enhanced vascularization, loss of the native structure, material breakdown and premature ingrowth of peri-implantation connective tissue into the biomaterial central region [5,6]. These factors are characteristic of the disintegration process in which the biomaterial undergoes breakdown by biodegradation [5]. After induction of an increased number of MNGCs, which started to invade the biomaterial and destroy its native structure, highly vascularized granulation and connective tissues characterized the ingrowth into a biomaterial body, leading to the premature loss of its function and integrity (Fig. 3B).

Interestingly, despite similar xenogeneic origins, different collagen-based biomaterials showed two different cellular reaction types within the same microenvironment, i.e., a physiological mononuclear cell-based immune response [3,59] and a foreign body reaction dominated by multinucleated giant cells [3,5,6]. These observations underline the observation that the formation of MNGCs depends on the surface properties of the biomaterials and their capacity to promote cell adhesion, absorb specific proteins and influence macrophages to adapt a phenotype with the potential to fuse and form MNGCs [4,19,60].

In a similar manner, MNGCs were observed in the implantation bed of silk fibroin, a silk-based biomaterial derived from *Bombyx mori* cocoons [3]. The MNGCs induced over 60 days showed various morphological appearances, numbers of nuclei and sizes (Fig. 1b,e). This biomaterial underwent breakdown after MNGC induction, leading to disintegration as a consequence of the induced MNGCs and influx of peri-implantation tissue into the biomaterial body [3]. Another study

Table 3
Systematic overview of the included implantation models, biomaterials and observed biological process in relation to the cellular reaction.

Implantation model	Biomaterial	Immune response	Biological process
Subcutaneous implantation in mice	- Collagen-based biomaterials [3,59]	- MNCs	- Integration
	- Collagen-based biomaterials [5,6]	- MNGCs	- Disintegration
	- Silk fibroin [3,13,61]	- MNGCs	- Disintegration
	- Expanded Polytetrafluorethylene (ePTFE) [3]	- MNGCs	- Encapsulation
	- High-temperature sintered bovine bone substitute materials [8]	- MNGCs	- Foreign body reaction
	- Deproteinized bovine bone substitute material [62]	- MNGCs	- Initial foreign body reaction
	- Bioglass [63]	- MNGCs	- Foreign body reaction
	- Hybrid-Bioglass [63]	- MNGCs	- Foreign body reaction
Subcutaneous implantation of pre-cultured biomaterials in mice	- Silk fibroin + human Osteoblasts and endothelial cells [65]	- MNGCs	- Foreign body reaction
	- Monocytes pre-seeded biphasic hydroxyapatite-beta-tricalcium phosphate bone substitute material [66]	- MNGCs	- Foreign body reaction
	- Phycogenic hydroxyapatite-based material [64]	- MNGCs	- Foreign body reaction
Subcutaneous implantation rats	- Beta-tricalcium phosphate [67]	- MNGCs	- Foreign body reaction
	- Biphasic hydroxyapatite-beta-tricalcium phosphate bone substitute material [68]	- MNGCs	- Foreign body reaction
	- Collagen-embedded hydroxylapatite-beta-tricalcium phosphatesilicon dioxide [69]	- MNGCs	- Foreign body reaction
	- Silica matrix embedded nanocrystalline hydroxyapatite [70]	- MNGCs	- Foreign body reaction
	- Hydroxyapatite [71]	- MNGCs	- Foreign body reaction
	- Beta-tricalcium phosphate [71]	- MNGCs	- Foreign body reaction
	- Biphasic calcium phosphate ceramics [71]	- MNGCs	- Foreign body reaction
	- Beta-tricalcium phosphate, methylcellulose and hyaluronic acid composition [72]	- MNGCs	- Foreign body reaction
Muscle implantation in goats	- Porous beta-tricalcium phosphate [73]	- MNGCs	- Foreign body reaction
	- Hydroxyapatite/silicon dioxide-based nanocrystalline bone [73]	- MNGCs	- Foreign body reaction
Femur model in rabbits	- Beta-tricalcium phosphate, methylcellulose and hyaluronic acid composition [74]	- MNGCs	- Foreign body reaction
Sinus lift in humans	- Nano-structured hydroxyapatite [7,9,78]	- MNGCs	- Foreign body reaction
	- Bovine deproteinized bone substitute material [7,9,78]	- MNGCs	- Initial Foreign body reaction
	- Nano-crystalline hydroxyapatite based bone substitute [79]	- MNGCs	- Encapsulation
	- High-temperature sintered bovine bone substitute materials [8]	- MNGCs	- Foreign body reaction
Socket preservation in humans	- Beta-tricalcium phosphate, methylcellulose and hyaluronic acid composition [80]	- MNGCs	- Foreign body reaction
Periodontal regeneration in human	- Non-cross linked collagen matrix [59,81]	- MNCs	- Integration
Head skin defect in humans	- Non-cross linked collagen matrix [82]	- MNCs	- Integration

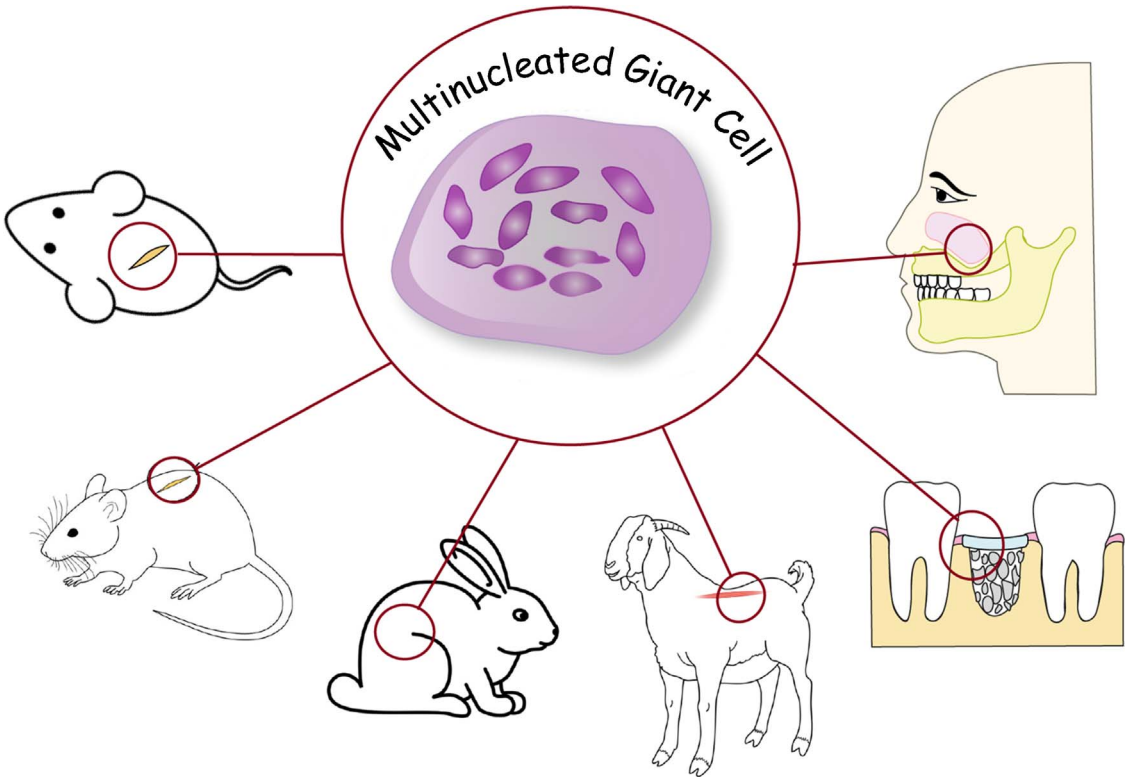

Fig. 2. Illustrative overview of the considered evaluation models and resulting cellular reaction to specific biomaterials.

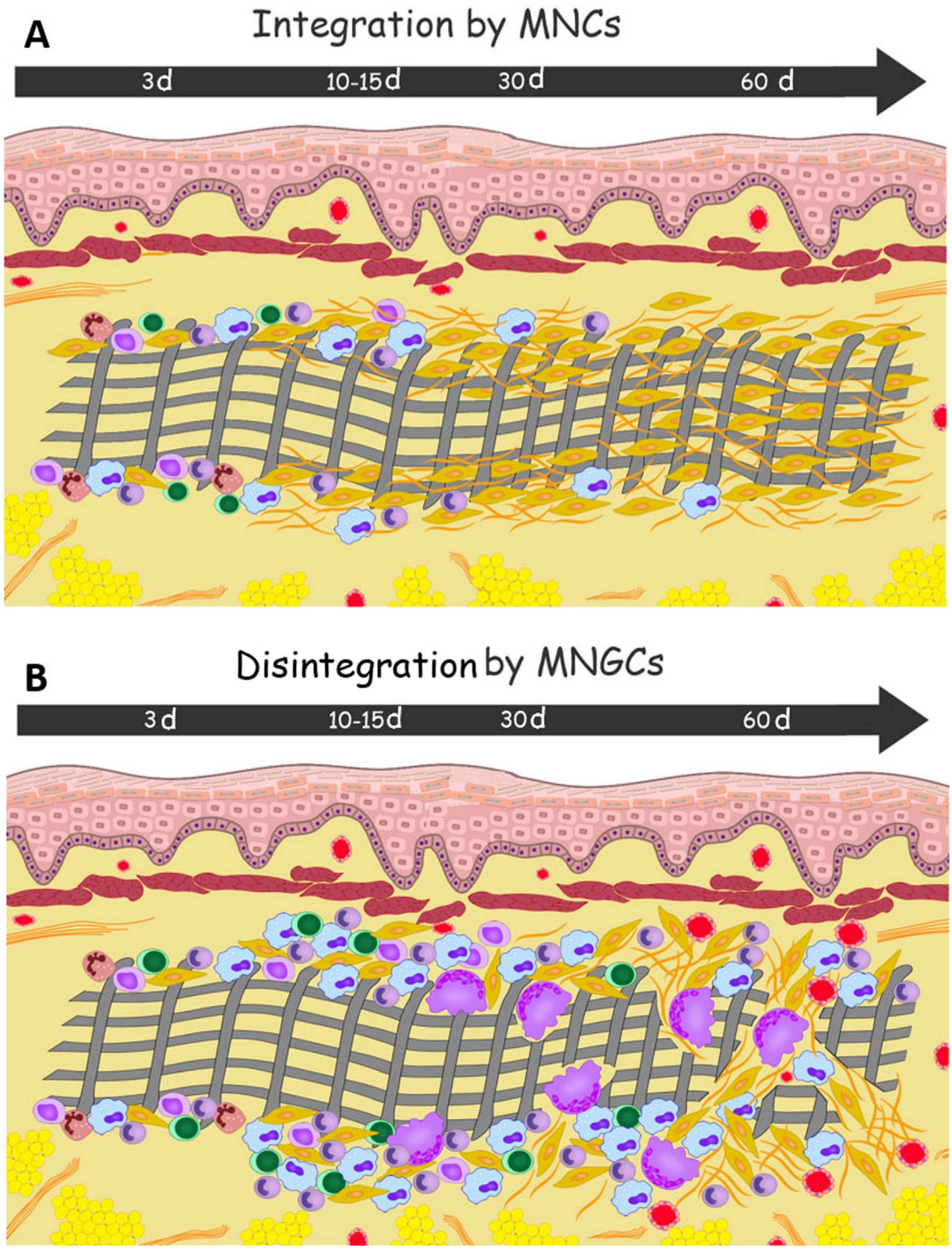

Fig. 3. Illustrative artwork of the cellular reaction towards collagen-based biomaterials in a subcutaneous implantation model. A) A mononuclear-based cellular reaction with a time course (time in days), reflecting the resulting biomaterial integration. B) The presence of MNGCs and subsequent disintegration with the time course.

evaluated the potential of MNGCs induced by silk fibroin to express pro- (COX-2, CCR7 and NF- kappa B) and anti-inflammatory (HO-1 and CD-206) molecules. The results underline the heterogeneity of these cells and their ability to exist in two different functional states, *i.e.,* the pro- and anti-inflammatory states, at the same time [13]. Moreover, the influence of the fine tuning of a formic acid treatment on a silk fibroin membrane was investigated *in vivo*. The data showed that the increased formic acid treatment time of 60 min led to a higher number of MNGCs and increased degradation compared to silk fibroin treated with formic acid for 30 min [61]. These findings indicated that alteration of the chemical characteristics influences the cellular reaction *in vivo* [61]. However, to date, there are no *in vivo* data about the role of different surface chemistry and physicochemical characteristics of biomaterials in the induction of different MNGC subtypes.

Furthermore, within the same environment, induction of MNGCs led to the encapsulation of a non-resorbable synthetic membrane consisting of expanded polytetrafluorethylene (ePTFE) after 60 days as a result of a foreign body reaction that is represented by the sustained presence of MNGCs from the tenth day after implantation [3]. The presence of MNGCs was continuous over 60 days. However, morphological

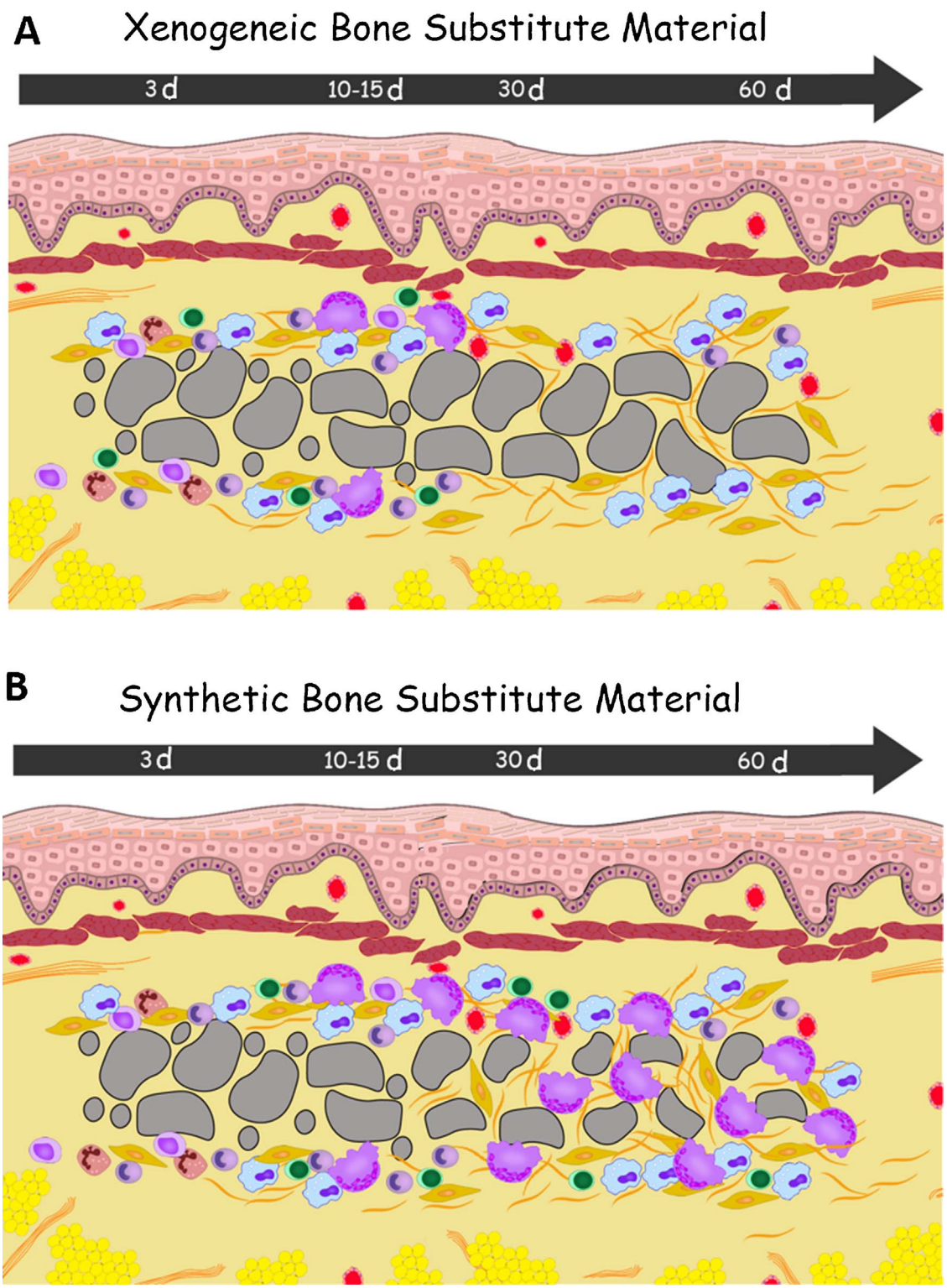

Fig. 4. Illustrative artwork of the cellular reaction towards bone substitute materials in subcutaneous implantation model with the time course. A) The cellular reaction towards a xenogeneic bone substitute material showing an initial MNGC presence, which decreases with the time course (time in days), resulting in maintained structure and biomaterial integration. B) The cellular reaction towards synthetic bone substitute materials with a high number of MNGCs and their persistence, resulting in premature connective tissue ingrowth.

differences in terms of the shape, size and nuclear orientation of the induced MNGCs were observed at the different time points (Fig. 1c,f).

4.1.2. *Bone substitute materials*

Different bone substitute materials were evaluated using the subcutaneous implantation model as a standardized evaluation method to focus on the cellular inflammatory response evoked by the specific material. In this context, a high-temperature sintered, bovine-derived bone substitute material induced a large number of MNGCs at 10 days after implantation. Most of the observed MNGCs showed TRAP expression. This foreign body reaction was consistent over 60 days without a significant decrease in the MNGC number [8]. The presence of these cells was accompanied by significantly higher vascularization compared to the control group with sham operations [8]. However,

within the limits of this study, no clear evidence of encapsulation or significant degradation could be identified [8]. Notably, another bovine-derived deproteinized bone substitute material, which was sintered at a lower temperature, showed a different inflammation pattern. In this case, the induction of MNGCs was related to the small-sized substitute granules. Therefore, the maximum number of MNGCs was observed at the early study time point of 10 days and decreased significantly with the time course because the small substitute granules were gradually degraded (Fig. 4A). Additionally, in contrast to the high temperature sintered bone substitute material, in this case, the MNGCs were mostly TRAP-negative and were associated with enhanced vascularization [62]. These findings reflect a mild tissue response and initial MNGC formation in terms of a "temporary" foreign body reaction, but also highlight that the level of inflammation and persistence period of the tissue reaction in terms of MNGCs might influence the regeneration process [62] (Fig. 3A).

Furthermore, synthetic biomaterials, such as pure or hybrid bioglasses, demonstrated the induction of MNGCs. However, a mild level of inflammation was evoked by hybrid bioglass compared to pure bioglass, maintaining the integrity of the former [63].

Cell-based tissue engineering has shown that the addition of human blood to a phycogenic hydroxyapatite-based substitute material results in a higher number of MNGCs and vascularization compared to pure biomaterial implantation [64]. In this context, it might be that processing the biomaterial surface with blood prior to its implantation induced changes in the surface properties in favor of MNGC formation. Similarly, pre-cultivation of biomaterials in a combined *in vitro-in vivo* study illustrated the influence of additional human cells on the host tissue response and presence of MNGCs. Thus, pre-cultured silk fibroin using human osteoblasts and endothelial cells showed enhanced vascularization, which was attributed to the presence of osteoblasts and endothelial cells, although MNGC formation was evident [65]. Moreover, human monocytes that were pre-seeded on synthetic bone substitute materials enhanced the vascularization rate in the implantation bed without influencing the number of induced MNGCs [66]. These observations suggest that the presence of MNGCs does not affect the function of other important cell populations, such as monocytes.

In conclusion, in the subcutaneous implantation model in mice, two types of cellular reactions were evident: a physiological reaction involving the induction of only mononuclear cells and an apparent pathological reaction including the additional induction of MNGCs. However, based on the biomaterial properties and level of inflammation, different biological processes were observed, including disintegration, encapsulation and degradation. Moreover, morphological differences in MNGCs were found over time. These findings underline the possible functional heterogeneity of MNGCs within this implantation model.

4.2. Subcutaneous implantation model in rats

Synthetic bone substitute materials based on hydroxyapatite or beta-tricalcium phosphate and their combinations with further components were evaluated using a subcutaneous implantation model in rats. The influence of the bone substitute granule size on the cellular response was demonstrated *in vivo* [67]. This study included five synthetic beta-tricalcium phosphate-based granules with different sizes and shapes. All granules evoked the formation of MNGCs as a foreign body reaction. However, both the number of MNGCs and related inflammatory level were different according to the evaluated granule size and shape. Thus, it was established that small round granules and polygonal morsels with a size of 50–250 μm and an intergranular porosity of 25–35% evoked a maximum number of MNGCs among all of the other investigated granules [67]. Additionally, this study implemented immunohistochemical staining and revealed that MNGCs express vascular endothelial growth factor (VEGF). This observation was correlated with the enhanced vascularization observed within the implantation bed after the formation of MNGCs [67]. Moreover, both TRAP-negative and TRAP-positive MNGCs were present in comparable numbers in the implantation bed of the biomaterials.

Another study investigated biphasic bone substitute materials based on hydroxyapatite and beta-tricalcium phosphate that had different granule sizes. The data gathered indicated that small sized granules induced a significantly higher number of MNGCs, especially TRAP-positive MNGCs. These observations correlated with the enhanced vascularization rate [68]. These findings again highlight the fact that the specific biomaterial properties are of considerable significance for the type of cellular reaction induced. Additionally, this study underlined the phagocytotic capacity of macrophages and MNGCs as well as allowed for recognition of induced MNGCs as a foreign body giant cell type as a result of the lack of morphological characteristics of osteoclasts, such as a ruffled border [68]. Moreover, the cellular reaction towards collagen-embedded hydroxyapatite–beta-tricalcium phosphate–silicon dioxide bone substitute granules showed the formation of MNGCs, particularly a high number of TRAP-positive MNGCs [69]. However, this study suggested that the material degradation was predominantly due to mononuclear cells, such as macrophages, whereas MNGCs adopted the form of a foreign body reaction [69]. Further investigations of synthetic bone substitute materials have focused on silica matrix-embedded, nano-crystalline hydroxyapatite bone substitute material as a composite of beta-tricalcium phosphate-based synthetic biomaterials. The cellular reaction was represented by MNGCs, which led to biomaterial degradation within 90 days [70]. However, the morphological properties of the observed MNGCs differed over the investigation time points (Fig. 1 a, d). In this context, both TRAP-positive and TRAP-negative MNGCs contributed to degradation and enhanced the vascularization rate at the early time point of 10 days. The number of MNGCs decreased with the progression of degradation, suggesting that MNGCs were the main phagocytotic cells associated with this biomaterial [70].

Additionally, the chemical composition of synthetic biomaterials, such as the combination of beta tricalcium phosphate and hydroxyapatite, impacted the cellular reaction and number of MNGCs compared to beta tricalcium phosphate or hydroxyapatite alone. Therefore, pure hydroxyapatite attracted significantly higher numbers of MNGCs compared to pure beta tricalcium phosphate, while the inflammatory pattern of beta tricalcium phosphate and hydroxyapatite composition showed MNGC formation with decreased numbers over the study period of 30 days [71] (Fig. 4 B). In this study, TRAP-positive and TRAP-negative MNGCs were involved in the cellular reaction in a similar manner. An injectable paste-like composition of beta tricalcium phosphate, methylcellulose and hyaluronic acid was also thoroughly studied. This composition served as a placeholder that prevented connective tissue ingrowth up to 30 days, while undergoing slow degradation from the periphery towards the central region. The cellular reaction towards this biomaterial included MNGCs that were mostly TRAP-positive [72].

In summary, the data from the various studies indicated that all synthetic bone substitute materials of different compositions attracted the formation of MNGCs. These cells were present with varying morphological phenotypes, i.e., differently oriented nuclei and various sizes, while TRAP expression varied in the form of TRAP-positive and TRAP-negative MNGCs. The number of induced MNGCs and level of inflammation depended on the physico-chemical properties of the evaluated biomaterials.

4.3. Muscle model in goats

Ectopic implantation of bone substitute materials in goats was performed to assess the osteoinductive capacity of the synthetic bone substitute materials. The cellular reaction for both porous beta tricalcium phosphate and hydroxyapatite combined with silicon within the microenvironment of goat muscle and showed the presence of

MNGCs, leading to degradation after 181 days [73]. The presence of these cells was referred to as a foreign body reaction, while the detection of TRAP-positive MNGCs was associated with the degradation of both biomaterials [73]. Moreover, this biomaterial showed a lack of osteoinduction within this microenvironment.

4.4. Femur model in rabbits

In this implantation model, the regenerative capacity of a synthetic injectable bone substitute material, consisting of beta tricalcium phosphate, methylcellulose and hyaluronic acid, was investigated. Within the study period of 6 months, the biomaterial underwent rapid degradation, resulting in enhanced new bone formation. The cellular reaction showed MNGCs as a foreign body reaction towards the biomaterial within this microenvironment [74].

4.5. Sinus lift in humans

Sinus lift is a surgical intervention for bone regeneration prior to dental implantation. In this context, bone substitute materials are used to support regeneration in atrophic bone [75]. In past years, MNGCs were frequently observed in relation to biomaterials in bone augmentation environments. Because of their TRAP expression activity, morphological multinucleation and assumed degradation potential, these cells were described as “osteoclast-like cells” [76,77]. However, comparable studies have shown that a beta-tricalcium phosphate-based synthetic bone substitute material induced a significantly higher number of MNGCs than a xenogeneic substitute material, reflecting the inflammatory potential of the different biomaterials [9]. Thus, the synthetic biomaterial underwent faster degradation and was replaced by a higher level of connective tissue compared to the xenogeneic biomaterial. By contrast, the percentage of newly formed bone was higher in the augmentation region of the xenogeneic biomaterial, which induced a lower number of MNGCs. In this context, the high number of MNGCs associated with the synthetic biomaterial did not contribute to a higher level of new bone formation, and the enhanced vascularization accompanied by their presence appears to have no significant positive effect on the regeneration process [7]. Focusing on the role of MNGCs in the implantation bed of synthetic and xenogeneic bone substitute materials within the clinical implantation environment, it could be concluded that MNGCs, especially TRAP-positive MNGCs, are foreign body giant cells rather than osteoclast-like cells due to their lack of osteoclastic function and influence on new bone formation [7].

Furthermore, several studies reported on the role of adhesion molecules, such as integrins in osteoclasts, and MNGC formation [38,45]. While integrin ß 3 has been described as being involved in the function and adhesion of osteoclasts [44], integrin ß 2 was required for the formation of IL-4-induced MNGCs [38,40]. On this basis, immunohistochemical evaluation of MNGCs within the sinus augmentation region involved an evaluation of biomaterial-adherent MNGCs in the augmented region and MNGCs in the residual bone area. The results showed that biomaterial-adherent MNGCs are only integrin-ß2 positive and are therefore marked as foreign body giant cells, whereas MNGCs within the residual bone were positive for integrin ß 3, reflecting their osteoclastic origin and function [78]. In addition, the persistence of biomaterial-adherent MNGCs within the implantation bed of a synthetic bone substitute material was asssociated with its encapsulation, as shown in a case report that included a biopsy from a sinus augmentation region after three years [79]. These findings support the hypothesis that biomaterial-induced MNGCs are foreign body giant cells and lead to the encapsulation of the biomaterial rather than its degradation. However, the level of inflammation and their initial or persistent occurrence do appear to influence the regeneration pattern and resultant biological process.

In summary, synthetic bone substitute materials induced a higher number of MNGCs and underwent faster degradation compared to xenogeneic bovine-derived bone substitute metals, whereas the amount of new bone regeneration showed no correlation with the number of induced MNGCs.

4.6. Socket preservation in humans

Socket preservation is a clinical intervention to avoid bone loss after tooth extraction and support the regeneration process within the resulting bone defect. A clinical study addressed the application of a synthetic bone substitute material of beta-tricalcium phosphate basis, which was reinforced with methylcellulose and hyaluronic acid. The cellular reaction towards the biomaterial showed the presence of MNGCs within the augmented region, reflecting a foreign body reaction [80]. Moreover, the biomaterial was nearly degraded after four months and new bone formation was observed [80].

4.7. Periodontal regeneration in humans

Guided tissue regeneration is a central topic in periodontology. A non-cross-linked collagen matrix was used to regenerate tooth recession as well as a peri-implant gingiva in two independent studies. In addition to satisfactory clinical results, histological analysis of the harvested biopsies from the treated region revealed a mononuclear reaction towards the biomaterial. In this case, no MNGCs were found within the implantation bed of this biomaterial. The biomaterial was integrated within the implantation region without any signs of foreign body reaction [59,81].

4.8. Extraoral skin regeneration in human head and neck regions

Application of a collagen-based tissue substitute material was demonstrated for the first time in the extraoral head and face region. Using a non-cross-linked collagen matrix, skin defects within the head and face were successfully regenerated in a case series. Focusing on the cellular reaction towards the biomaterial, histological evaluation of a biopsy from the treated area showed the induction of mononuclear cells alone. Therefore, the collagen-based matrix was fully integrated within the application region without any signs of foreign body reaction or MNGC formation [82].

5. Discussion and future insights

The present review reports on the cellular inflammatory response to various biomaterials that are used every day in the clinic, while considering different local environments and implantation models to evaluate their regenerative capacity. Basically, two different types of cellular reactions were observed: a physiological reaction with only mononuclear cells and a pathological reaction characterized by multinucleated giant cells (MNGCs) [3,5,6,8,59,62]. Regardless of the biomaterial origin or implantation environment, MNGCs were frequently observed in the biomaterial implantation beds. Different species, including rats, mice, goats, and rabbits as well as humans, showed a similar biomaterial-specific tissue response, especially the ubiquitous presence of MNGCs.

The presence of MNGCs in relation to biomaterials and their role in the regeneration process is still not fully understood. To the best of our knowledge, no prior studies investigated the morphological differences of MNGCs within the *in vivo* biomaterial implantation bed over time. This review draws attention to the morphologically different phenotypes of biomaterial-related MNGCs (Fig. 1a–f). The various studies suggest that there are different biomaterial-related MNGC phenotypes. Based on the histopathological characteristics, the correlations between Langerhans’ MNGCs and MNGCs found within the biomaterial implantation bed were assessed. At early time points, such as days 15 and 30, MNGCs showed peripherally oriented nuclei, similar to those of Langerhan’s cells. These cells were also sporadically found at late time

points, such as 60 days or later. Moreover, common features of Langerhans' MNGCs and foreign body MNGCs in terms of the expressed receptors and proteins are obvious (Table 1). Langerhans' MNGCs are inflammatory MNGCs that are induced during a pathological process [50] and exhibit a pro-inflammatory character [55], while foreign body giant cells are induced by anti-inflammatory cytokines, such as IL-4, which inhibit the pro-inflammatory reaction [16]. Nevertheless, their morphological similarities raise questions about whether there is any correlation between the pathological form of Langerhans' MNGCs and MNGCs observed in relation to biomaterials. However, there would appear to be evidence to reactivate the assumption that Langerhans' MNGCs might be precursor cells of foreign body giant cells [83,84].

A recent study showed that cellular debris present within biologic scaffold biomaterials induces pro-inflammatory activation of macrophages *in vivo* and *in vitro* [85]. Only single studies have investigated the impact of the physico-chemical properties of biomaterials on the activation of macrophages *in vivo*. To determine the influence of cross-linking, a biological porcine-derived extracellular matrix (ECM) from the small intestinal submucosa was studied in a native, cross-linked form *in vivo*. Macrophage polarization was observed as anti-inflammatory M2 profiles in the native ECM, whereas cross-linking led to the activation of mostly M1 macrophages, reflecting their pro-inflammatory pattern in the ECM [20]. However, The M2 activation profile is also involved in pathological processes, such as sarcoidosis, leading to enhanced myofibrosis [86]. Therefore, the influence of the biomaterial components and processing seems to influence the polarization of macrophages and formation of MNGCs. In this context, there might be a harmonic balance between M1 and M2 polarization and kinetics in their transition over time according to the dominant microenvironment and interaction between the host tissue and local environment near the biomaterial, thus playing a role in the biomaterial-based regeneration process. The data presented showed that non-cross linked, highly purified collagen biomaterials induced only mononuclear cells over 60 days, leading to their integration within the host tissue [3,59]. Several clinical studies showed the benefit of these specific biomaterials for soft tissue regeneration with a different localization [59,81,82]. The histological findings demonstrated a reproducible physiological cellular reaction towards these biomaterials, based on mononuclear cells alone. In this case, the successful regeneration pattern in the absence of MNGCs raises the question of whether the induction of MNGCs by other biomaterials contributes to the regeneration process or if we should focus on biomaterials that induce mononuclear cells alone.

Following these outcomes, and based on the fact that macrophages are precursors cells for MNGCs, investigations on MNGC polarization showed that MNGCs within the implantation bed of natural silk fibroin express both M1 and M2 markers, underlining their heterogeneity [13]. Immunohistochemical investigation of the macrophage and MNGC phenotypes is a main topic of ongoing research in our laboratory to determine the biomaterial-related inflammatory pattern and thus better understand the role and function of these cells. Moreover, further methods, such as laser capture microdissection and gene expression determination, could be beneficial for performing detailed investigations of the macrophages and MNGCs in their native microenvironment and relation to specific biomaterials.

Surface characteristics are not only crucial for the polarization of macrophages but they also play a critical role in the formation of foreign body MNGCs [19]. An *in vivo* study has shown that processing beta tricalcium phosphate with plasma prior to it subcutaneous implantation leads to the formation of cathepsin-K positive MNGCs, while processing with purified fibrin leads to the formation of cathepsin-K negative MNGCs [87]. These data again highlight that the surface characteristics of the biomaterial might be a stimulus for the formation of MNGCs with different phenotypes. Basically, adhesion of macrophages to the biomaterial is significant for their survival. It was previously shown that a hydrophilic surface prevents macrophage adhesion, leading to increased macrophage apoptosis. This phenomenon was suggested to reduce the fusion of macrophages and formation of foreign body MNGCs [88]. However, macrophages that fail to maintain adhesion might undergo fusion to form MNGCs to avoid apoptosis [89]. The mechanism of MNGC apoptosis has been morphologically investigated [90]. However, no studies describe the time point and mechanisms for MNGC apoptosis. It is possible that foreign body MNGCs undergo apoptosis after full degradation of the biomaterial. These findings are related to the presented observation on the size of biomaterial granules and induced MNGCs, especially small sized particles. Thus, a biphasic biomaterial showed temporary induction of MNGCs, which was related to the small granules. After their degradation, the number of MNGCs significantly decreased [62]. The different morphological characteristics of MNGCs observed within the biomaterial implantation bed permit the hypothesis that there are different types of biomaterial-related MNGCs. It might be that some MNGCs are "persistent", leading to a classical foreign body reaction towards encapsulation and further phenotypes that are more of an "inflammatory" nature, lasting for a short time period. These data suggest that some MNGCs might be induced to rapidly eliminate the biomaterial and then undergo apoptosis. However, the basics of guided tissue engineering require biomaterials that are intended to remain in place and serve as a scaffold for a defined time period to support tissue regeneration [91]. Therefore, rapid elimination is counterproductive and does not contribute to improved regeneration in the long term. Therefore, in the clinical setting it must be thoroughly considered where to apply biomaterials that induce MNGCs and find suitable indication fields for the specific biomaterials regarding their cellular reaction.

Looking at the data presented here and the biological processes accompanied by the induction of MNGCs, such as disintegration with premature breakdown by biodegradation in polymeric biomaterials that leads to premature loss of integrity and function [5,6] as well as rapid degradation or encapsulation of bone substitute materials [79], it has to be critically questioned whether these cells contribute to the regeneration process and whether we should accept their presence within the implantation bed. MNGCs have been shown to exhibit a phagocytotic potential [17,68], which could involve them in the cellular-mediated degradation of biomaterials [68,70,74].

The capacity of MNGCs to perform phagocytosis is a topic of discussion in the literature. *In vitro* studies have shown that the mannose receptor (CD-206) is upregulated in foreign body giant cells derived from human monocytes [46], which shows that foreign body giant cells exhibit phagocytosis features. Moreover, studies in basic cell research have shown that foreign body MNGCs exhibit phagocytic features due to the existence and involvement of the endoplasmic reticulum [17]. Further *in vitro* studies have suggested that the phagocytic capacity of foreign body MNGCs is specialized for large particles [92]. Another aspect might be that the phagocytic potential of biomaterial-related MNGCs depends on the characteristics of the specific biomaterial and its degradation capacity. In an animal study using mandible defects, two types of MNGCs were recognized in relation to two different bone substitute materials, including xenogeneic and synthetic biomaterial. The synthetic biomaterial induced a MNGC phenotype with phagocytic capacity, leading to its degradation, whereas the induced MNGCs within the microenvironment of the xenogeneic bone substitute material showed different morphological characteristics and a reduced phagocytosis potential [93]. These findings are in accordance with the presented histomorphometrical outcomes evaluating clinical samples after application of xenogeneic and synthetic bone substitute materials, which showed more degradation in the case of the synthetic biomaterial [7]. Furthermore a phycogenic bone substitute material showed clear signs of degradation with the induction of MNGCs [64]. These bone substitute materials are used for clinical application and contribute to bone regeneration. However, the differences in the cellular reaction, number of the induced MNGCs and their persistence showed different patterns. Especially within the bone environment, discussion about the

similarities between biomaterial-related MNGCs and osteoclasts question whether these cells might contribute to bone regeneration and remodeling in relation to bone substitute materials. Accordingly, every bone substitute material implantation might be automatically accompanied by the induction of MNGCs, which have to be accepted as such. However, consideration that these cells represent in this context a non-physiological reaction and the lack of data about their role in the regeneration process demand further examination.

Nevertheless, the potential for degradation and phagocytosis is physiologically inherent in macrophages [25,68,94]. In this regard, the question arises to what extent the unphysiological degradation by MNGCs could be beneficial for the regeneration process because premature degradation will lead to loss of function of biomaterials as placeholders and functional barriers to support tissue regeneration, especially in maxillofacial surgery. Moreover, biomaterials that induce a physiological mononuclear-based cellular reaction alone underwent full integration while maintaining their structure and function. These biomaterials showed slow thickness reduction over a study period of 60 days, which might be a result of the physiological degradation by macrophages [59]. Indeed, MNGCs have the potential to release VEGF and enhance the vascularization pattern [67]. However, cell-based tissue engineering has shown that the addition of human peripheral blood or monocytes isolated from human peripheral blood contributes to enhance vascularization *in vivo* [64,66]. Moreover, the development of an autologous blood concentrate system in advanced PRF (platelet-rich-fibrin)-based matrices, which are derived from the patient's own peripheral blood, provides a strong alternative to support tissue regeneration [95,96]. This blood system is based on concentrating the peripheral blood by centrifugation following a low-speed centrifugation concept (LSCC) to generate PRF-based matrices, which include a high number of leukocytes and platelets, providing a reservoir for growth factor release [97]. The combination of PRF-based matrices manufactured following LSCC, with biomaterials that induce mononuclear-based cellular reactions, is a promising alternative to maintain the physiological reaction and biologically modify biomaterials by influencing cell–cell communication to enhance their regeneration potential in terms of essential functions, including vascularization.

6. Conclusion

The investigation of different biomaterials in numerous microenvironments showed two principal types of cellular reactions. These were a physiological reaction based on the induction of mononuclear cells alone and a pathological reaction characterized by multinucleated giant cells (MNGCs). The biomaterial physico-chemical properties influence the rate and nature of the inflammatory pattern, number of the MNGCs and their period of persistence. The occurrence and duration period of these cells were associated with enhanced vascularization, disintegration in polymeric biomaterials and encapsulation of non-resorbable biomaterials. Synthetic bone substitute materials induced many MNGCs and underwent rapid degradation compared to xenogeneic bone substitute materials. However, to date, little is known about the function and role of MNGCs in the biomaterial-based regeneration process. Therefore, the question arises regarding whether we should accept the presence of MNGCs in the biomaterial implantation beds or aim to optimize further biological concepts and focus on biomaterials that induce a physiological mononuclear-based cellular reaction for optimized tissue regeneration.

Conflicts of interest

The authors declare that they have no conflicts of interest. This work was funded by funds of the FORM-lab.

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