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REVIEW ARTICLE

Osteoinduction and osteoimmunology: Emerging concepts

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1 | INTRODUCTION

Monocytes and macrophages are pivotal cell types located in the bone marrow that have important roles in the human body. They represent some of the first cell types that interact with foreign pathogens and implanted medical devices. Classic studies have demonstrated that macrophages are rapidly recruited to infectious and injury sites, where they play critical roles in innate immunity. Here, these cells were shown to have broad roles and be responsible for regulating tissue homeostasis, including innate and adaptive immunity, wound healing, hematopoiesis, and malignancy.[1]

Biomaterials, once implanted into the human body, result in a foreign body reaction (FBR) that causes a period of inflammation largely involving monocytes and macrophages. This can also lead to excessive inflammation, tissue destruction, fibrous encapsulation, and incomplete osseointegration.[2] Thus, the study of these materials, including the interactions between the immune system and skeletal system, becomes extremely vital, especially given that millions of people suffer from bone defects and immune conditions such as osteoporosis, osteoarthritis, and diabetes.[3] Traditional studies have often predominantly focused on the integrations between biomaterials and their "osteogenic capacity," and much less attention has been given to the interaction between immune cells and biomaterials despite being the first cell type in contact with biomaterials. For instance, a systematic review of dental and orthopedic implants found that the majority of research (over 90%) focused primarily on the in vitro behavior of osteoblasts on implant surfaces, while only a small percentage (roughly 10%) was dedicated to immune cells, including monocytes, macrophages, osteoclasts, leukocytes, and multinucleated giant cells (MNGCs).[4] Thus, the field of bone biomaterial research has largely omitted their importance over the years, but our research group has specifically focused on the interplay of osteal macrophages (OsteoMacs) and their key role around bone biomaterials.[5,6]

Today, an entire field investigating the interplay and connection between the skeletal system and immune cells has been developed to address these exciting research topics. Osteoimmunology therefore focuses on strategies aimed at enabling biomaterials to modulate local immune environments from proinflammatory toward tissue resolution favoring healing and regeneration.[5–7] Over the years, the recognition and importance of osteoimmunology has led to a shift in the traditional evaluation of biomaterials toward one that focuses more on the effects of immune cells around bone biomaterials. Thus, while there remains a lack of knowledge regarding the complex interactions between the immune and skeletal systems upon biomaterial implantation, researchers have indicated the importance of this pairing in recent years to improve the osteoinductivity of bone biomaterials.[8]

This review article outlines the advancements made in the field of osteoimmunology, emphasizing the role of the osteoimmunomodulatory properties of biomaterials and their impact on osteoinduction. First, the various immune cell types involved in bone biomaterial integration are discussed, including the prominent role of osteal macrophages (OsteoMacs) during bone regeneration. Thereafter, key biomaterial properties, including topography, wettability, surface charge, and adsorption of cytokines, growth factors, ions, and other bioactive molecules, are discussed in terms of their impact on immune responses. These findings highlight and recognize the importance of the immune system and osteoimmunology, leading to a shift in the traditional models used to understand and evaluate biomaterials for bone regeneration.

2 | IMMUNE CELLS

Bone is constantly renewed in healthy individuals with the participation of the immune system to a large extent. While it was once

thought that bone turnover involved three primary cell types, osteoblasts (bone-forming), osteocytes (bone-maintenance), and osteoclasts (bone-resorbing), more recently, the immune system and cells have been largely implicated in their cross talk. These effects have been further demonstrated in many knockout experiments in which immune cells are inactivated in bone tissues, and a dramatic reduction in bone development and formation has been observed. These cross talk mechanisms between the bone and immune system have essentially formed the field of osteoimmunology, and these extend into biomaterial osteoinduction discussed later in this article. An excellent review article by Yang and Liu[9] addressed this topic during bone regeneration (Figure 1). This article summarizes their role in bone regeneration from the aspects of immune cells and immune cytokines.

Interestingly, while immune cells are known to participate in the regulation of bone homeostasis, bone cells also impact the activity of immune cells, dictating their two-way communication between bone and immune systems.[10] The immune microenvironment dictates the healing, repair, and regeneration of bone tissue, with OsteoMacs being key players around bone biomaterials that determine the ability of bone tissue to regenerate.[11] Bone turnover has been shown to be affected by several immune-related diseases, such as osteoporosis, hyperparathyroidism, osteoarthritis, and several other immune diseases.[12–16]

2.1 | Neutrophils

Neutrophils are the most abundant leukocytes in mammalian blood and are heavily implicated in innate immunity. Upon tissue injury, they are usually the first to be recruited to the injury to clear invasive pathogens and microorganisms. These cells also initiate the acute inflammatory response.[17] Histological animal studies have further confirmed that their aggregation is visualized during bone injury, playing a key role in the early stages of bone repair. Furthermore, their depletion in animal models leads to an impairment in bone healing following fracture.[9]

A second important role of neutrophils is the recruitment of monocytes and macrophages via secretion of inflammatory cytokines.[18] Studies have reported that bacteria producing endotoxin-lipopolysaccharide (LPS) stimulate neutrophils to express receptor activator of nuclear factor kappa B ligand (RANKL).[19,20] Interestingly, in contrast to $CD3^+$ T lymphocytes, neutrophils only express RANKL as a membrane protein and do not secrete soluble RANKL. This finding suggests that neutrophils activate osteoclast precursors only through direct intercellular contact.[20,21] Neutrophils have also been implicated in bone regeneration. For instance, in long-term glucocorticoid use leading to osteoporosis as well as chronic gout, neutrophils directly inhibit osteoblast function, leading to a reduction in bone formation.[22,23] These cells have also been shown to play important roles in osteoarthritis and periodontitis.[24,25] In summary, diverse research has demonstrated that while neutrophils are active in and initiate the immune system during fracture healing and/or biomaterial implantation, functioning as immune cells, they also regulate bone homeostasis in both anabolic and catabolic pathways.

2.2 | Dendritic cells

Dendritic cells (DCs) are most widely known as functional antigen-presenting cells. The surface of DC membranes highly expresses major histocompatibility complex II (MHC II), which effectively activates the immune function of T cells and initiates an immune response.[26] Their role is largely dictated during fracture healing and

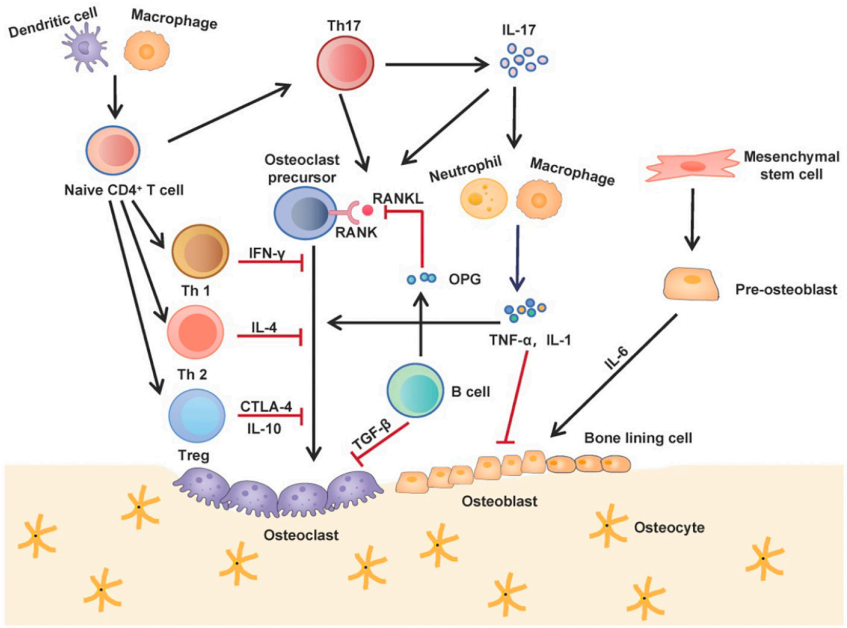

FIGURE 1 Interactions between the immune and skeletal systems. T-cell subsets (Th1, Th2, Th17, and Treg) play an important role in bone repair and regeneration. Th17 cells, known as osteoclastogenesis-supporting T cells, secrete IL-17 to upregulate RANKL expression, and induce inflammatory cytokines such as TNF-α and IL-1 from innate immune cells. These cytokines further activate osteoclast precursor cells and inhibit osteoblast function. In contrast, Th1, Th2, and Treg cells inhibit osteoclastogenesis by secreting the cytokines INF-γ, IL-4, CTLA-4, and IL-10. B cells antagonistically block the effect of RANKL by secreting OPG and induce osteoclast apoptosis by secreting TGF-β. Reprinted with permission from Yang and Liu.[9]

biomaterial implantation. Under normal physiological conditions, DCs are not found in or adjacent to bone tissues with no obvious abnormality of the skeletal system in DC-deficient mice.[27] However, under tissue injury or systematic conditions such as rheumatoid arthritis, a growing population of both immature and mature DCs accumulate in active lesion sites and bone tissue.[28] Similarly, in chronic inflammatory diseases such as periodontitis, DCs aggregate into periodontal pockets and form aggregates with T cells to activate osteoclasts to destroy bone.[29] Therefore, it is believed that DCs may indirectly affect inflammation-related bone loss by activating and regulating T-cell function.

2.3 | Innate lymphoid cells

Innate lymphoid cells (ILCs) are a more recent described subset of innate lymphocytes classified into three principal groups according to their transcription factor expression and cytokine secretion.[30] Group 1 ILCs (ILC1s) consist of NK cells and ILC1s, which produce IFN-γ. ILC2s secrete IL-13 and IL-5, while ILC3s consist of lymphoid tissue inducer cells and ILC3s, which secrete IL-22 and IL-17.[31] Notably, various animal models have shown that ILCs are present in high numbers in periodontal tissues, with a ligature-induced periodontal model displaying significant increases in each subset.[32] Human periodontal tissues also show remarkable similarities to those of the murine model.[32,33] While ILCs remain one of the "newer" researched cell types in bone tissues as well as periodontally diseased tissues, their implications in bone homeostasis are clearly observable, and future research is needed to better understand their role.

2.4 | T cells

T cells are lymphocytes involved in the adaptive immune system that are derived from hematopoietic stem cells. They produce various cytokines and growth factors that play a key role in the process of bone remodeling and regeneration. According to their surface receptors, T cells are divided into either αβ T cells or γδ T cells. αβ T cells are further subcategorized into $CD4^+$ and $CD8^+$ T cells.[9]

2.5 | T helper: (Th1)/Th2 cells

Naive T cells can differentiate into either Th1 or Th2 cells. The role of Th1 cells is primarily to eradicate intracellular pathogens through the secretion of interferon γ (IFN-γ), IL-2, and tumor necrosis factor α (TNF-α). Th2 cells, in contrast, play a role in B-cell activation and elimination of extracellular pathogens through the expression of IL-4, IL-5, IL-6, and IL-10.[34] Previous studies have shown that RANKL is primarily expressed by Th1 cells.[35] In addition, during postmenopausal estrogen deficiencies, the osteoclastogenic effect of Th1 cells is the major source and cell type implicated in lower bone mass caused by menopause.[36]

2.6 | Th17 cells

Th17 cells are a subtype of $CD4^+$ T cells implicated in bone. Under activation by transforming growth factor β (TGF-β) and IL-6, T cells differentiate into Th17 cells, which are responsible for promoting bone resorption through the secretion of IL-17, IL-22, and IL-26.[37] IL-17 also indirectly promotes osteoclast function by recruiting and activating other immune cells to secrete TNF-α and IL-1.[38] Thus, Th17 cells, known as osteoclastogenesis-supporting T cells, play a negative role in bone regeneration through their expression of Il-17.

2.7 | T regulatory cells (Tregs)

T regulatory cells (Tregs) are a subset of T cells with immunosuppressive functions and thus play a positive role in bone regeneration. These cells secrete pro-tissue resolution cytokines, such as TGF-β and IL-4, and maintain autoantigen immune tolerance and homeostasis of the immune system. Basic research studies have shown that transgenic mice with elevated levels of Tregs display higher bone mineral density and lower bone resorption than wild-type mice.[39] Furthermore, basic research studies have demonstrated that systematic injection of Tregs effectively reduces the levels of inflammatory factors in the local area of trauma, thus leading to an improvement in osteogenic ability.[35] In vitro studies have also shown that Tregs directly enhance the function of osteoblasts and improve their osteogenic differentiation.[40] Other studies have shown that Tregs also interact with $CD8^+$ T cells to upregulate WNT10b, which acts on mesenchymal stem cells and osteoblasts to induce bone formation.[41] Moreover, Tregs decrease the differentiation and function of osteoclasts.[42,43] Therefore, Tregs play a direct and important role in both improving bone formation and attenuating bone resorption by osteoclasts. These cells are also recruited to sites of tissue inflammation, attenuate the inflammatory process, and regulate immunopathologic reactions after injury.[44] Therefore, Tregs indirectly promote tissue regeneration by controlling neutrophil behavior and macrophage polarization.[45,46]

2.8 | B cells

B cells are also derived from the hematopoietic stem and are an important source of antibody synthesis and secretion important to adaptive immunity. These cells work very closely with the immune system, whereby the differentiation and maturation of B cells occurs in the bone marrow cavity, where bone marrow-derived stem cells provide a stable microenvironment for B-cell differentiation.[47] This phenomenon is most pronounced during bone-related diseases such as multiple myeloma,[48,49] rheumatoid arthritis,[50] and osteoporosis.[51] Notably, in bone tissues, B cells and plasma cells are the main sources of osteoprotegerin (OPG). OPG blocks the effect of RANKL and reduces osteoclast activity, therefore leading to increases in bone mass.[52,53] In summary, the role of B cells in bone regeneration is most pronounced during pathological conditions.

2.9 | Macrophages

Macrophages have been the cell type most studied with respect to their role in the immune system as well as their role around bone biomaterials leading to osteoinduction. Notably, Allison Petit's research group is given credit for terming a subset of osteal macrophages found in bone tissues "OsteoMacs." Original observations described in the mid-1980s sought to characterize the role of osteal macrophages in bone biology.[54] Hume et al. were one of the first to clearly demonstrate that periosteal and endosteal tissues contained a discrete population of resident tissue macrophages in line with traditional bone cell nomenclature.[55,56] OsteoMacs constitute approximately one sixth of all cells residing in bone marrow and display a stellate morphology allowing them to achieve extension coverage of bone surfaces, suggesting that they may form a comprehensive communication network (Figure 2).[56] This subset of $CD68^+$ cells has been shown to be derived from a resident population of macrophages, such as macrophages found in other tissues.[57–59] More recent research has clearly confirmed that macrophages may subdivide and proliferate from resident tissues, in contrast to the original theories positing that these cells are derived from monocyte precursors from the bloodstream.[60–63]

3 | M1 AND M2 MACROPHAGES

The general role of OsteoMacs has been described as immune surveillance cells in the bone microenvironment. Several previous studies have demonstrated that this subset of macrophages can function as phagocytes,[64,65] can detect bacterial products,[66,67] and can respond to antigens.[65,68] In vitro cell culture systems have further provided evidence by demonstrating how primary murine osteoblast cultures can respond to pathophysiological levels of lipopolysaccharide (LPS), characteristics of the M1 macrophages.[56]

Initial macrophage experiments classified macrophages into two specific cell types, classic M1 proinflammatory macrophages and M2 tissue resolution/wound healing macrophages (Figure 3). Classic proinflammatory stimuli in response to LPS include TNF-α,[69,70] IL-6,[71,72] and IL-1β[69,73] all contributing to tissue inflammation and osteoclastogenesis. M2 macrophages typically produce TGF-β and arginase, both of which are implicated in tissue repair processes.[74–77] Table 1 presents a general overview of the differences observed between M1 and M2 macrophages.

Phenotyping macrophages has typically been carried out with cell surface markers, including CD11b, CD68, macrophage antigen-2, and F4/80. Phenotyping macrophages is a complex process since subsets from animals such as rodents are different than those in humans, making it difficult to rapidly advance the field using animal models. Some researchers have even suggested that human macrophages are "fundamentally" different from their mouse counterparts and thus should be studied as entirely separate entities.[78,79]

Figure 3 presents an overview of general cell types derived from the monocyte lineage. In vitro differentiation of macrophages toward the M1 phenotype is optimally induced with IFN-γ and LPS and TNF-α, whereas M2 macrophages are typically produced with either IL-4 or IL-13.[80] In vitro culture with IL-4 causes upregulation of two key M2 markers, TGF-β and arginase, which are largely assumed to participate in tissue regeneration.[74–77,80] Moreover, IL-4 increases expression of the mannose receptor CD206. Since M2 macrophages have a wide variety of characteristics as originally defined, more recent research has subdivided their classification into M2a/b/c to further express the differences found between certain M2 macrophages[81] (Figure 3). Briefly, the M2a phenotype is produced by exposure to IL-4 + IL-13 acting through IL-4Rα to increase the expression

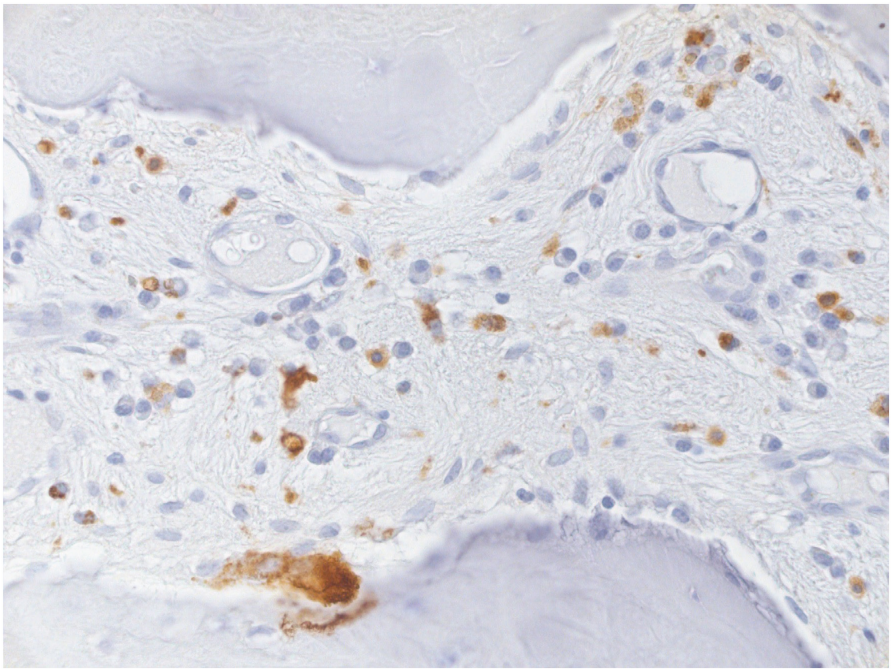

FIGURE 2 Paraffin section from human bone immunolabeled for CD68. Human bone with bone matrix and immature bone marrow after augmentation. The large positive cells are osteoclasts on bone matrix, whereas the other positive cells are $CD68^+$ macrophages in roughly a 1–6 ratio.

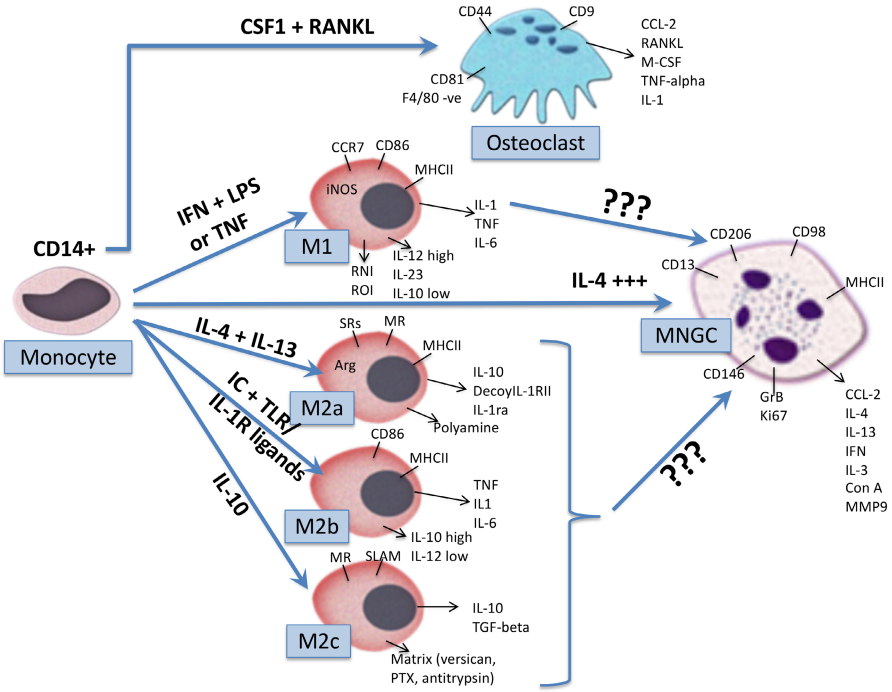

FIGURE 3 Monocyte differentiation includes the expression of markers of osteoclasts, M1, M2a, M2b, and M2c macrophages and MNGCs. Reprinted with permission from Miron and Bosshardt.[5]

of CD206, arginase, and TGF-β.[75,82–85] The M2b phenotype has been described following exposure to a combination of IgG-immune complexes and IL-1R ligands, in turn increasing IL-10 production and decreasing IL-12, largely contributing to anti-inflammatory properties.[86,87] Cell culture with IL-10 or glucocorticoids produces the M2c phenotype characterized by high IL-10 and low IL-12 production,[87] as well as increased surface receptor CD163.[88,89] While the aim of this review article is not to give a background on the specificity of the various M2 macrophage subgroups, it remains important to note that various cell culture models have distinct M2 macrophage characteristics. For an excellent overview on this topic, the reader is kindly directed to a recent review article on the M1 and M2 paradigms of macrophage activation.[1]

These observations as well as others indicate the potential cross-lineage plasticity and cross talk between osteoblasts and hematopoietic cells in vitro,[90–92] which makes it difficult to clearly define the cells responsible for specific functions reported in primary "osteoblast" cultures. This issue is best exemplified in a study by Chang et al.,[56] who showed that by removing macrophages from primary osteoblast cultures, a 23-fold reduction in mineral deposition was observed. The researchers concluded that it was the OsteoMacs, and not the osteoblasts as originally hypothesized, within these in vitro culture systems that responded to pathophysiological concentrations of cytokines, and their removal from calvarial cultures significantly decreased in vitro mineralization by osteoblasts.[56]

3.1 | OsteoMac function in osteoblast mineralization

Preliminary findings from primary osteal tissues clearly demonstrated that OsteoMacs play a pronounced role in osteoblast function and differentiation.[56] Interestingly, depletion of OsteoMacs in vivo by various knockout systems has also been shown to markedly reduce bone formation.[56,93] Macrophages may produce a number of potent bioactive growth factors for osteoblasts including transforming growth factor β (TGF-β),[94] osteopontin,[95] 1,25-dihydroxy-vitamin D3,[96] and BMP-2.[97] These factors are known inducers of extracellular matrix deposition and new bone formation and are classic characteristics of M2 macrophages. The plasticity of macrophages suggests that their trophic role in bone tissues is highly regulated by changes to the microenvironment. OsteoMacs are capable of promoting anabolic function in certain conditions, whereas in others, they are responsible for creating and directing an inflammatory environment.

3.2 | OsteoMacs and bone modeling

Bone modeling is an anabolic process involving new bone deposition and is unlike bone remodeling, which involves the careful and coordinated balance between osteoclasts and osteoblasts.[98,99] A previous report showed that macrophages are localized at the bone modeling site on cortical diaphyseal endosteal bone surfaces without the presence of osteoclasts in the vicinity.[56] This process has been described as "forming a canopy-like cell structure" where OsteoMacs were found to encapsulate the functionally mature osteoblasts, suggesting that they are heavily involved in the bone modeling process.[56,100] Once again, the functional importance of OsteoMacs was demonstrated by knockout systems in which macrophages were depleted using a Fas-induced apoptosis (Mafia) transgenic mouse model, which can induce macrophage depletion via synthetic ligand treatment.[101] In this system, the OsteoMac canopy architecture was disrupted, leading to a complete loss of mature osteoblasts and bone modeling at the bone interface.[101]

TABLE 1 Summary of in vitro culture conditions of M1 and M2 macrophages.

	M1 macrophage	M2 macrophage
Activator	IFN-γ, TNF-α, LPS	IL-4, IL-13
Proinflammatory cytokines	IL-1B, TNF-α, IL6, IL12	Low
iNOS	High (in rodents only)	Low
Anti-inflammatory cytokines	Low	TGF-B high, IL-10 Low
CD206	Low	High
Dectin-1	Low	High
Ym1	Low	High
Phagocytosis/Endocytosis	High	Decreased phagocytosis of implanted particles
Matrix proteins	MMP9	FN, TGFB1, MMP1, MMP12, TG, F13A1
Markers		
Human	CD64, IDO, SOCS1, CXCL10	MRC1, TGM2, CD23, CCL22
Mouse	CXCL9, CXCL10, CXCL11, NOS2	Mrc1, tgm2, Fizzl, Ym1/2, Arg1
Transcription factors		
Human	pSTAT1, IRF5	IRF4, SOCS1*, GAT3* SOCS3
Mouse	pSTAT1, pSTAT6-ve, Socs1	pSTAT6, pSTAT1-, Soc2
Cytokines		
Human	TNF, IL6, IL1b, IL12A, IL12b, IL23A	IL-10
Mouse	TNF, IL-6, IL-27, Tnf23a	IL-10, IL-6
Chemokines		
Human	CXCL10, IL8, CCL5, CXCL9	CCL4, CCL13, CCL17, CCL18
Mouse	CXCL11, CCL18-ve	CCL17 CCL24, CXCL13, CCL1, CCL22, CCL20

It was originally proposed that during bone remodeling, osteoclasts provide a "coupling signal" to promote and coordinate osteoblast activity.[98] Interestingly, with the numerous advancements made in the field of osteocyte biology, it has recently been proposed that osteocytes are also implicated in the bone remodeling process by dictating both osteoblast and osteoclast activity.[102] Given that bone modeling animal models lack osteocytes and osteoclasts during the developmental stages of bone modeling, it was proposed that OsteoMacs may be the cells responsible for coupling-like signals dictating osteoblast function. While evidence from the literature has previously suggested that TGF-β and ephrin B2 are possible coupling factors between osteoclasts and osteoblasts,[98,103–105] macrophages have also been shown to produce TGF-β[94] and ephrin B2,[106,107] suggesting that OsteoMacs are also capable of fulfilling such roles. Nevertheless, more research is still necessary to further understand the role of OsteoMacs during bone modeling in the absence of osteoclasts and osteocytes.

Interestingly, depletion of OsteoMacs in vivo using the macrophage Fas-induced apoptosis (Mafia) mouse caused a complete loss of osteoblast bone formation at the bone surface, demonstrating that OsteoMacs are an integral component of bone tissues and play a pivotal role in bone homeostasis.[56] These proposed models show that OsteoMacs function to both detect alterations in the local environment and guide bone formation in vivo. In response to anabolic stimuli, these cells function to recruit mesenchymal progenitor cells and induce their proliferation and differentiation toward bone-forming osteoblasts. OsteoMacs subsequently provide ongoing anabolic signals to the underlying osteoblasts.[56]

3.3 | OsteoMacs and bone remodeling

As described earlier, bone remodeling involves the fine balance between bone-resorbing osteoclasts and bone-forming osteoblasts.[98,99] Resorption signals, including RANKL and CSF-1, and local calcium concentrations,[108] are expressed by bone lining cells and osteocytes and are necessary for directing osteoclastogenesis and bone resorption. It was previously proposed that osteoclasts subsequently provide the coupling signal coordinating osteoblast activity to facilitate bone deposition and mineralization.[98] A previous study

showed that during this process, osteoclasts are only located at the leading edge of the formation phase and have moved or undergone apoptosis before new bone formation is completed.[109] Therefore, certain investigators have posed the question: "what cellular/molecular mechanism drives osteoblasts to initiate mineralization and complete the remodeling cycle following osteoclast apoptosis?"[110]

As previously mentioned, OsteoMacs have been shown to form a cellular canopy structure around osteoblasts during bone modeling. This process was postulated to create an enclosed compartment for local communication and coordination during the complex remodeling process.[100] Although osteoclasts have been proposed to have a dominant role in orchestrating the recruitment, proliferation and initial differentiation of preosteoblasts during bone remodeling based on the release of cytokines from resorbed bone, the various roles of OsteoMacs in combination with their anatomical location and canopy architecture have recently suggested that they may also be necessary for optimal mineralization by osteoblasts.[56] Furthermore, due to their close proximity to bone surfaces and well-known ability to detect dying cells,[111] OsteoMacs are an obvious candidate to detect and respond to bone damage, a critical event for osteoclast recruitment, thus initiating the bone remodeling phase.[112]

Alexander et al.[113] demonstrated that osteal macrophages promote intramembranous bone healing in vivo in a mouse tibial model. The authors used a very similar approach to the previous study by knocking out macrophage populations using Mafia mice and clodronate liposome delivery. Following tibial injury, these researchers demonstrated that the depletion of OsteoMacs led to significantly reduced intramembranous ossification bone healing, whereas administration of CSF-1 in animal models led to an increase in OsteoMac number at the injury site, which concurrently increased new matrix deposition and mineralization.[113] A study with a similar animal model also demonstrated that fracture healing via periosteal callus formation also requires OsteoMacs for both the initiation and progression of early endochondral ossification.[93] Furthermore, a separate group found that depletion of macrophages using Mafia mice led to early skeletal growth retardation and progressive osteoporosis (25% reduction in bone mineral density, 60% reduction in number of mesenchymal progenitor cells) by 3 months.[114] Of particular interest, animals that were treated with anabolic factors such as PTH showed a significantly higher level of OsteoMacs, further suggesting their important role in bone remodeling.[115]

It is difficult to technically assess whether osteoclasts or macrophages are more important for bone remodeling and regulating osteoblast activity. The main reason is that most of the mutations to date that affect macrophages also have a large impact on osteoclasts since they are derived from the same precursor cells.[116] It is therefore extremely difficult to knock down only macrophages without compromising osteoclast activity.[116] In contrast, it is possible to abolish osteoclasts by specifically targeting OPG by blocking the actions of RANKL.[117] Since there is a close lineage relationship between macrophages and osteoclasts,[118] the current in vivo models could benefit from future refinement, as considerable cellular plasticity is found between these two cell types.[119] Further specific investigation on the role of OsteoMacs versus osteoclasts and their contribution to bone remodeling is needed to clearly delineate all the cellular participants and molecular factors in osteoblast coupling.

3.4 | OsteoMacs: Key players around bone biomaterials

As major advancements have been made in the field of osteoimmunology over the past decade, further elucidation of the response of these cell types to various bone biomaterials is critical. Immune cells play a pivotal role in determining the in vivo fate of bone biomaterials by either facilitating new bone formation around bone-implanted devices or creating an inflammatory fibrous tissue encapsulation. Macrophages are known to be the major effector cells in immune reactions to biomaterials, where they are indispensable for osteogenesis. Knockout models have demonstrated that a loss of macrophages around bone grafting materials may entirely abolish their osteoinductive potential, thus confirming their primary role in the immune system modulation later responsible for guiding osteogenesis.[120]

Over the years, complex studies from basic research have revealed the dynamic interactions between the skeletal system and immune system.[10,55,56] Furthermore, the main factors responsible for directing their phenotypes toward more specialized cell types in response to biomaterials also remains poorly characterized.

Several leukocytes (T lymphocytes, B lymphocytes, dendritic cells [DCs], natural killer [NK] cells, macrophages, monocytes, and neutrophils) hosted in bone[121] are involved in the inflammatory process as well as in the subsequent bone repair and remodeling stages after biomaterial implantation (Figure 4). However, OsteoMacs and their plastic phenotype have been the most studied, leading to M1 and M2 phenotypes dictating the integration of various bone biomaterials. Macrophages are key modulators of both inflammation and bone remodeling with an ability to adapt to exogenous stimuli that influences the healing cascade.[122–126]

These cells also have the ability to engulf particles below 5 μm[127] or fuse together into multinucleated giant cells (MNGCs) to engulf particles up to 100 μm.[128] The effects of these differences as well as various subsets of M1-MNGCs and M2-MNGCs were recently proposed.[129,130] Thus, both macrophages and MNGCs and potentially other subsets of immune-related cells can shift between various phenotypes depending on external stimuli. This phenomenon largely impacts their secretion of proinflammatory versus pro-tissue resolution cytokines.[129,130]

More recently, the acquired phenotypes that can rapidly shift from M1 to M2 have been studied for timing during successful bone healing.[131] Thus, the key is the timing of the switch between them, which depends on the composition and kinetics of the total biochemical milieu to which they are exposed. M1 macrophages predominantly exist during the early stages of acute inflammation, where they perform cleaning duties and release oxidative metabolites,[126] proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and ROS.[132] At later stages, their plasticity allows a rapid shift toward

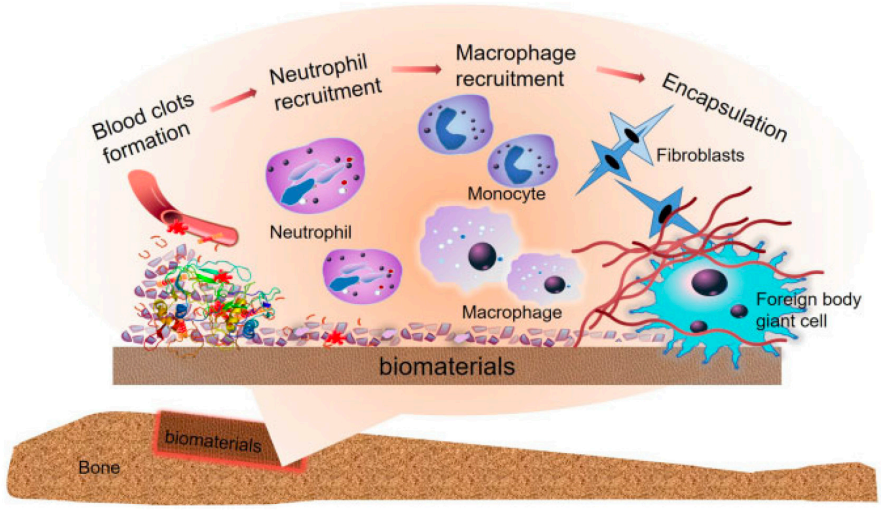

FIGURE 4 Biomaterials elicit immune reactions. As the implantation proceeds, the blood clots consist of protein, growth factors, cytokines, and MMPs adsorbed on the biomaterial surface and the injured area, which trigger a series of reactions in the immune system. Neutrophils are recruited, and then, monocytes accumulate and differentiate into activated macrophages, which lead to the secretion of various cytokines and take up biomaterials as foreign bodies by forming a fibrin matrix around the biomaterials. Reprinted with permission from Xie et al.[138]

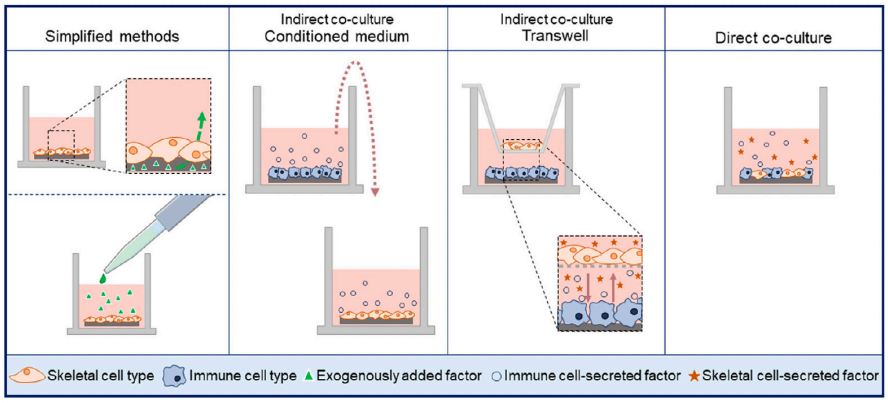

FIGURE 5 Schematic of the main experimental configurations to study the osteoimmunomodulatory properties of biomaterials: Simplified methods, indirect coculture using conditioned medium or transwells, and direct coculture. Reprinted with permission from Mestres et al.[8]

M2 macrophages, which are predominantly responsible for healing and express important anti-inflammatory cytokines such as IL-4 or IL-10 or enzymes such as arginase-1. M2 macrophages are divided into four subsets, as highlighted in Figure 2, depending on their role during repair. Generally, they are classified as M2a, stimulating fibroblast and extracellular matrix formation (ECM)[80,82]; M2b, responsible for balancing the inflammatory process[133]; M2c, responsible for matrix renovation and vascularization[81,134]; and M2d, important pro-angiogenic modulators.[135]

3.5 | In vitro testing of OsteoMacs on bone cells

Multiple cell types within the family of immune cells, both primary and cell lines, have been used to study the osteoimmunomodulatory properties of biomaterials. Among the immune cells, the commercially available monocyte/macrophage cell line RAW 264.7 is most common. The main reasons to investigate macrophages are that they are one of the first cell types involved during biomaterial integration and play a major role in osteoimmunology.[136]

Unfortunately, the typical experimental setups used to assess the biological properties of biomaterials are still strongly centered on conventional approaches that involve standard tissue culture plastic. Regardless of the experimental setup, more specific evaluations should focus first on determining the immune cell response upon biomaterial contact and subsequently examine how this response affects the differentiation of osteoblasts/MSCs.[8] The immune response is assessed by identifying the inflammatory profile of macrophages as either M1 (proinflammatory) or M2 (anti-inflammatory) through the study of expressed genes, released cytokines, or surface markers.[8]

More importantly, the immune system and skeletal system interact with one another constantly, and previous reports on OsteoMacs have shown up to a 23-fold decrease in osteoblast differentiation and mineralization when OsteoMacs were removed from culture. Therefore, there is great interest in further evaluating biomaterials using coculture systems. Typically, three main methods are utilized to investigate coculture systems (Figure 5).

1. Immune cells are cultured directly on the biomaterial. Following a period of time, the culture medium is collected (termed conditioned medium) and then applied to osteoblasts seeded on biomaterial surfaces. This process allows immune cells to indirectly impact osteoblasts.
2. The second method involves a transwell assay where immune cells are seeded in the upper compartment and the osteoblasts

are seeded on the biomaterial surface in the lower compartment. This technique allows a more back-and-forth communication style between both cell types and evaluates their relationship in a second indirect model.
3. The third way is in direct coculture systems where osteoblasts and immune cells are cultured simultaneously on biomaterials. Typically, cells are seeded in a 6:1 ratio of osteoblasts/immune cells since these are the typical ratios found in human bone tissues.

4 | OSTEOINDUCTIVE BIOMATERIALS

The field of osteoinduction has seen major progress over the past decade. In 2012, a review article by our group titled "Osteoinduction: a Review of Old Concepts with New Standards" highlighted many features related to both bone biomaterials and growth factors and their ability to induce ectopic bone formation.[137] The osteoinductive potential of bone biomaterials was summarized as requiring three main criteria (Figure 6).[137]

1. The biomaterial and/or its combination with growth factors should be able to rapidly recruit mesenchymal stem cells to its surface.
2. These stem cells should then differentiate toward the osteoblast lineage.
3. These cells show ectopic bone formation.

While the article summarized the research to date on the topic, 10 years ago, this represented a quite simplistic understanding of the events required during osteoinduction as well as the pronounced role of immune cells during these interactions.

Today, it is known that immune cells are the first to come into contact with biomaterials, with increasing research specifically on the interaction of immune cells and biomaterials. Properties of biomaterials such as topography, wettability, surface charge, and the release of cytokines, mediators, ions, and other bioactive molecules can affect the immune responses to interfere with the skeletal system (Figure 7). An excellent review article recently summarized all the events that take place and modifications to biomaterials to improve their osteoimmunomodulatory properties.[138]

4.1 | Osteoimmunomodulation by surface topography and architecture

One of the goals of designing biomaterials is to trigger appropriate immune responses with the goal of facilitating and lowering inflammation and increasing integration. The design and modifications to biomaterial surfaces have drawn the most attention since the osteoimmunomodulatory properties can be largely manipulated to stimulate immune cell function with the aim of improving bone formation.[139] The ability of macrophages to polarize on biomaterial surfaces has therefore been largely studied and is sensitive to their physicochemical properties. Therefore, the regulation of immunomodulatory properties on biomaterial surfaces has been an excellent strategy to meditate the local environment for bone regeneration.[140] Surface roughness is an important modification method to regulate osteoblast and osteoclast behavior, which has previously been highly researched, including sand blasting and acid etching of various biomaterial surfaces.[141] Additionally, surface roughness not only influences the secretion of cytokines but also has an effect on angiogenesis and BMSC function, enhancing the function of CD206, Arg1 (M2 marker), and the anti-inflammatory factors IL-4, IL-10, and IL-1ra.[142] Interestingly, much research has now demonstrated that regulating surface properties such as surface roughness has a major impact on protein layer adsorption, which affects downstream cellular events.[140] In this regard, modification of the physical surface properties of biomaterials can regulate the activation of immune cells, especially macrophages.

4.2 | Osteoimmunomodulation by wettability

Surface wettability has been another prominent area of research to improve biomaterial integration. Hamlet et al.[143] demonstrated that titanium implants that were made more hydrophilic (modSLA) were better able to integrate into the body. The improved hydrophilic implant surfaces promoted the expression of CD163 and Arg1, favoring M2 macrophage polarization.[143] In another similar study, it was found that macrophages cultured on hydrophilic implant surfaces decreased proinflammatory mediators, including TNF-α, IL-1, and CCL2.[144] Additionally, it was reported that these surfaces allowed less contamination, which improved osseointegration.[141] Similar to surface roughness, wettability impacts surface protein adsorption, clot formation, and immune system responses.

4.3 | Osteoimmunomodulation by surface charge

The surface charge of biomaterials has also been reported as an important factor dictating protein adsorption and immune cell responses.[145] Interestingly, Brodbeck and colleagues compared biomaterials with an anionic functional group of poly(acrylic acid) and a cationic functional group of poly(dimethylamino propyl acrylamide) and determined that the anionic group increased IL-10 and decreased IL-8 expression. Thereafter, this group also impacted the positive outcomes in osteoblast differentiation.[146] Therefore, the surface charge can affect many immune responses via various modifications to chemical groups or roughness. More future work is required to better understand how various factors affect surface charge characteristics.

4.4 | Osteoimmunomodulation by decellularized ECM

Surface protein adsorption is highly implicated in biomaterial integration. Therefore, a strategy involving directly coating biomaterials with various extracellular matrix (ECM) components has been developed.[147]

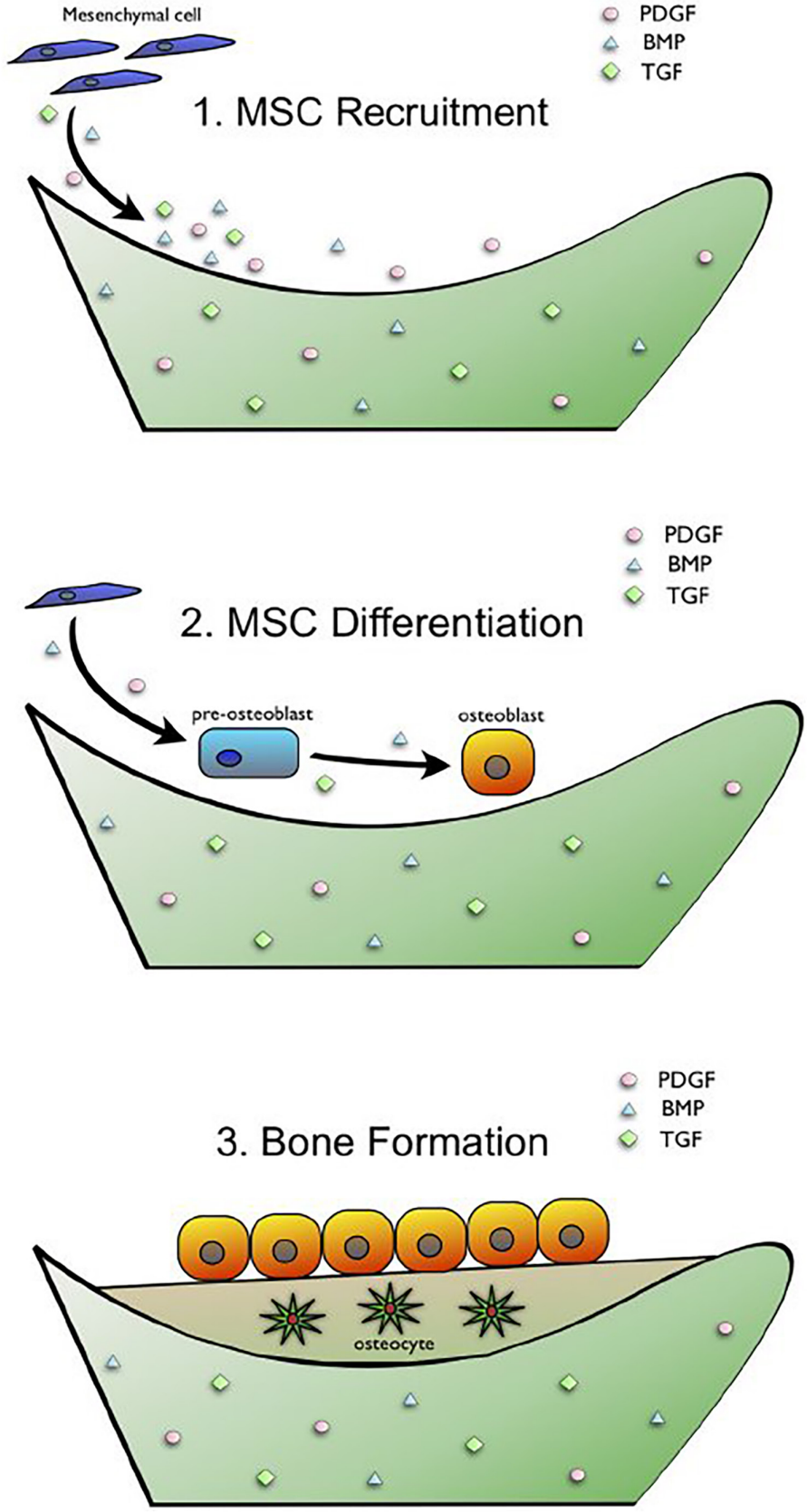

FIGURE 6 Principles of osteoinductive materials. Principle 1: Osteoinductive materials should be capable of recruiting MSCs to bone graft surfaces through growth factor release. Principle 2: The material should promote MSC differentiation into osteoblasts. Principle 3: Osteoblasts must be capable of forming ectopic bone in vivo. Reprinted with permission from Miron and Zhang.[137]

The ECM matrix has the ability to modify signaling molecules, activate various enzymes, and regulate cytokine release and behavior.[148] Therefore, and in summary, ECM decellularization and protein adsorption is a promising strategy to decrease immune responses and control protein adsorption to the surface of biomaterials prior to their implantation.[149,150]

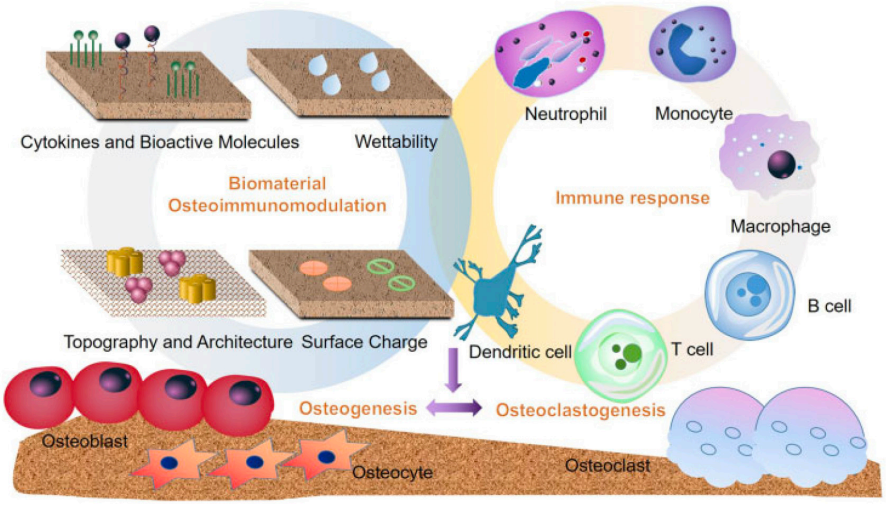

FIGURE 7 Modification strategies such as topography, wettability, surface charge, cytokines, and bioactive molecule release of bone biomaterials can modulate the osteoimmune environment. Reprinted with permission from Xie et al.[138]

4.5 | Osteoimmunomodulation by delivering cytokines and bioactive molecules

The final strategy that has been well investigated has been the delivery of biomolecules onto implantable biomaterials. Therefore, various cytokines have shown antiosteoclastogenic properties and have been used in various research projects.[151] Risser et al.[152] designed biomaterials with the release of IL-4 to control immune cell responses. While this remains a novel strategy that requires further research, various approaches have been investigated to modulate immune cell behavior using bioactive molecules (such as drugs, mediators, or ions).

4.6 | Osteoinduction of synthetic bone grafts

Perhaps, the most studied biomaterials to date with respect to their osteoinductive potential has been the development of synthetic bone grafts fabricated from various calcium phosphates. These materials, when transplanted into intramuscular and subcutaneous areas, lead to ectopic and orthotopic bone formation in preclinical studies and effective fracture healing in clinical trials.

Years ago, it was initially thought that the dissolution and precipitation of an apatite layer on CaP materials was the trigger inducing bone formation.[153] It was further proposed that either direct application of BMPs or bone growth factors from body fluids could adsorb on the surface of CaPs, thereby attracting stem cells to their surface to induce bone.[154]

In an article titled "A proposed mechanism for material-induced heterotopic ossification," the authors propose and demonstrate that intrinsic osteoinduction is the result of calcium and/or phosphate depletion, thus explaining why not only the material (surface) composition but also the material volume and architecture (e.g., porosity, pore size) play a decisive role in this process.[6]

Thus, the popular belief used to explain intrinsic osteoinduction is based on the assumption that there is at some point during implantation the release of calcium and phosphate ions, thus leading to supraphysiological calcium and phosphate concentrations. These supraphysiological concentrations are assumed to drive stem cells into the osteogenic lineage. The opposite effect seems to be much more likely.[6,108] This statement is based not only on in vitro and in vivo data but also on thermodynamic considerations. Indeed, calcium and phosphate concentrations decrease in cell culture media in contact with osteoinductive materials due to apatite precipitation.[155–160] In vivo, the formation of a biomimetic layer (observation "a" herein), which by definition consumes calcium and phosphate ions, is a prerequisite for intrinsic osteoinduction.[161–165] Thermodynamically, physiological fluids are supersaturated toward hydroxyapatite at pH 7.4.[166–168] According to Bohner and Lemaître,[166] the supersaturation of serum is in the range of $10^{1.4}$ (≈ 25), which means a very crude approximation that 96% of all calcium and phosphate ions could precipitate to reach the chemical equilibrium between hydroxyapatite and serum. Assuming that this concept is correct, designing a material that triggers a strong mineralization reaction should lead to a strongly osteoinductive material. In support of this statement, the osteoinductive potential of eight calcium phosphate bone graft substitutes was recently predicted by an in vitro mineralization test.[169]

4.7 | Intrinsic osteoinduction relies on physical, chemical and biological factors (reprinted with permission from Bohner and Miron[6])

The observations made over the past 50 years in the field of intrinsic osteoinduction underline the importance of physical, chemical, and biological factors for this currently unexplained phenomenon:

1. The formation of a biomimetic apatite layer on the material is a prerequisite[161–165,170] but not a determinant for intrinsic osteoinduction.[171,172]

2. Intrinsic osteoinduction occurs first on the surface of pores present in the core of a material and then spreads toward the periphery.[154,164,170,173–175] This contrasts with osteoconduction, which starts first at the periphery and then spreads into the material (Figure 1).[176]
3. Intrinsic osteoinduction is more often observed in large animals than in small animals.[176–180]
4. The scaffold architecture plays a very important role in intrinsic osteoinduction. Bone is generally found in concavities rather than convexities, and an increase in microporosity positively affects osteoinduction.[171,181–186]
5. Intrinsic osteoinduction does not depend on the chemical composition because it has been observed in polymers,[165] metals,[163,187,188] calcium phosphate-polymer composites,[161] and calcium phosphates. However, calcium phosphates are particularly prone to induce bone formation.[162,189]
6. Ingrowth of blood vessels into the material is a necessary[184,186] but not sufficient condition for intrinsic osteoinduction.
7. Intrinsic osteoinduction is a very slow process: Bone formation may take a few weeks up to 1 year to occur.[162,174,190] This process is in contrast with the very rapid (1–3 days) woven bone formation in bone defect healing.[191]
8. Although both calcium and phosphate ions are considered to play a key role in intrinsic osteoinduction,[154,164,171,180,189,192] many studies have noted the importance of Ca ions and the Ca sensing receptor.[157,182,185,186,193–197]
9. Cartilage formation has been observed and suggested to occur during intrinsic osteoinduction,[181,198] but it is generally accepted that intrinsic osteoinduction provokes intramembranous ossification[180,189,199,200] with the formation of woven bone[165,171,173,175,178,193,200,201] and then lamellar bone.
10. Macrophages and osteoclasts[120,175,202–208] are considered to play an essential role in intrinsic osteoinduction.

Therefore, the geometry of the biomaterial is certainly a critical parameter for bone induction. Studies have demonstrated that for CaPs to exhibit osteoinductive properties, both a macroporous structure and surface microporosity are prerequisites.[175] Macropores are needed primarily to produce concavities,[209] whereas microporosity is controlled by the sintering temperature, with lower sintering temperatures resulting in higher surface microporosity. It has further been speculated that low oxygen tension in the central region of the implants might provoke osteoinduction and depletion of calcium and/or phosphate ions in the center of an implanted material could induce bone formation via the calcium-sensing of immune and bone cells.[6,108]

4.8 | Bone regeneration enhanced by M2 macrophages

Today, it has been well established that the immune response to bone biomaterials is critical for optimal implantation. While an increasing number of studies have indicated that bone biomaterials cause foreign responses and create inflammation, the host-to-scaffold immune response has been highly studied to improve clinical outcomes. Therefore, modifications to surfaces have become common study points to improve biomaterials through macrophage polarization.[210,211] Advancements in nanoporous structures have facilitated a shift toward the M2 phenotype by releasing higher levels of osteogenic factors, including BMP2, BMP6, TGF-B1, VEGF, and WNT10b.[212] Similar strategies have been adapted on titanium implant surfaces, whereby nanotubes (NTs) with small diameters (NT-30) favored better osteoblast differentiation than NT-100 via macrophage polarization.[213] Ma and colleagues fabricated hydrophilic NT TiO2 surfaces with tube sizes of 30 and 80 nm via anodization at 5 and 20 V. NT20 favored an M1 macrophage phenotype, whereas polarization toward the M2 phenotype was found in the NT5 group.[214]

Another strategy used to favor M2 macrophage polarization has been the use of small biomaterials attached to biomaterial surfaces. Wendler and colleagues utilized iloprost, a prostacyclin (PG12) analog with anti-inflammatory properties, leading to an improvement in bone regeneration in an in vivo model.[215] Further groups have utilized proregenerative mediators such as saffron, an antioxidant and anti-inflammatory molecule, as well as lithium chloride to favor bone regeneration via M2 macrophage polarization.[216,217] Additional biomaterials added to various bone grafts include hierarchical intrafibrillar mineralized collagen embedded with strontium-incorporated calcium silicate (Sr-CS), which was shown to have a profound bone regenerative ability via polarization of M2 macrophages via IL-4 secretion.[218,219] These combined studies highlight the fact that macrophage polarization toward an M2 phenotype has routinely been found to favor greater osteogenic differentiation.

5 | DISCUSSION

The interplay between the skeletal and immune systems has been increasingly researched, identifying very early key players in the regulation of osteoclasts.[220–223] Later, Arron and Choi defined the field of osteoimmunology when demonstrating that T lymphocytes regulate osteoclast activation.[10] Since then, osteoimmunology has been one of the fastest growing fields of active research that focuses on the cross talk between the immune system and skeletal cells.

The field of osteoimmunology aims to modulate the local immune response to quickly shift from a proinflammatory state in favor of proresolution and regeneration.[7] This review article focused on the intimate connection between these two systems and further discussed strategies to improve biomaterials. Thus, in summary, novel strategies should focus on providing more effective bone biomaterials by focusing on immune responses and not only osteoblast behavior.

While current research has attempted to regulate immune responses to biomaterials, the field remains in its infancy with a lack of clear understanding of precise ways to optimize immune cell

behavior on bone biomaterials. Thus, future studies focusing on the roles of various immune cells discussed in this article and their behavior specific to bone regeneration will provide more effective strategies for the future treatment of bone loss.

Furthermore, small biomolecules can substantially impact immune systems. For instance, monoclonal antibodies and resolvins have been utilized for the treatment of bone damage caused by immune-related diseases such as rheumatoid arthritis. Drugs such as tocilizumab (target: IL-6R) and adalimumab (target: TNF-α) have been approved and shown to be successful for the management of rheumatoid arthritis.[224,225] Potentially, the adsorption of specific immune-related small biomaterials may further enhance bone regeneration by targeting immune system responses. Exosomes, for instance (and as discussed in later articles in this issue), are further therapeutic targets that may contribute to meaningful new therapeutic strategies that may aid in bone regeneration via the modulation of immune cells.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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