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Review Article

Crosstalk between Macrophages and Mesenchymal Stem Cells Regulated by Biomaterials and Its Role in Bone Regeneration

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Impaired bone healing caused by severe trauma, infection, and tumor resection is an extremely common phenomenon. Therefore, the treatment of bone defects represents a major clinical challenge worldwide. In such situations, the application of biomaterials is necessary for filling defects and promoting bone regeneration. Bone repair biomaterials having osteoconductive and osteoinductive properties can act as an appropriate template for the formation of new bone and induce the osteogenic differentiation of preosteocytes and stem cells. Thus, the influence of biomaterials on the regulation of osteogenesis of mesenchymal stem cells (MSCs) has been widely studied. However, immune response is also critical in bone healing; macrophages play pivotal and dynamic roles in bone regeneration. The interfacial properties of biomaterials that affect the adsorption of proteins and the adhesion function of cells require great attention in the field of bone tissue engineering because they are related to the crosstalk with the immune and bone or stem cells. Thus, selection of biomaterials or specific surface coatings may reduce local undesirable inflammatory responses and promote bone regeneration. This review provides a detailed overview of bone regeneration mechanisms and the interaction between immune cells and MSCs. Moreover, the influence of biomaterials on the regulation of functions of MSCs and macrophages and the macrophage-related inflammatory response triggered by biomaterials and its specific role in osteogenesis are discussed.

1. Introduction

Traumata and degeneration of skeletal tissues, such as bone, cartilage, ligament, and tendon, are among the most frequent clinical problems and often require the use of implants or grafts to restore their function [1]. Although bone exhibits good recovery and regeneration potential, impaired bone healing after trauma or tumor resections is extremely common [2, 3]. Thus, surgical interventions are required for healing large bone defects, which are challenging in orthopedic medicine [4]. Because of their osteogenic, osteoinductive (capable of recruiting immature cells and stimulating them to develop into preosteoblasts), and osteoconductive (the ability to support bone growth on its surface) abilities, autologous bone grafts have been considered the gold standard in clinical approaches for treating bone defects [5]. However, grafts have several flaws and disadvantages, such as limited tissue availability, pain, and donor-site morbidity [6]. Although allogenic bone grafting can be an alternative, it has several limitations, including suboptimal osteoactivity, donor incompatibility, and an increased risk of disease

transmission, compared with autografting [4]. Therefore, new approaches, such as the use of biomaterials and tissue engineering, have emerged to augment the natural healing capacity of bone. Actually, the primary healing mechanism in the regeneration of large bone defects is bone ossification, which is controlled by various inductive factors and the recruitment of inflammatory cells involved in the regulation of osteoprogenitor cell activities and bone remodeling [7]. Therefore, new strategies capable of creating a natural bone healing environment emerge as a promising approach for the regeneration of biologically functional bone tissues.

It was once believed that only two types of cells, namely, osteoblasts and osteoclasts, are involved in the process of bone healing, which play essential roles at different time stages and locations [8]. Osteoblasts originating from mesenchymal stem cells (MSCs) are the predominant bone-forming cells [9], whereas osteoclasts are a type of multinucleated cells derived from monocytes, which are responsible for bone resorption [10]. Currently, it is well accepted that the complex interplay of cells, including MSC-osteoblasts and monocyte-macrophage-osteoclast lineage, is pivotal in the formation, remodeling, and repair of tissue [11]. Therefore, it is essential to create a bone healing microenvironment considering the importance of the strong crosstalk between the skeletal and immune systems.

Over the last few decades, the advancement of tissue engineering, particularly the improvement in biomaterials, has been largely propelling the development of bone tissue regeneration [12]. Nevertheless, growing evidence suggests that the physical and chemical properties of biological materials influence local tissue inflammatory response, which positively or negatively affects osteogenesis [13]. For example, Reifenrath and coworkers found that the implantation of screws composed of the magnesium alloy ZEK 100 caused a higher inflammatory response accompanied by a larger bone volume loss than MgCa0.8 screws [14]. However, Wang et al. found that calcium phosphate ceramics promoted the secretion of inflammatory cytokines and growth factors from macrophages, which enhanced osteogenesis [15]. Several other studies proved that biomaterials could affect the osteogenesis of stem cells by regulating macrophage activation [16]. Hence, in the field of bone tissue engineering, great attention should be paid to the interfacial properties of biomaterials to adjust their crosstalk with the immune cells in order to effectively reduce local undesirable inflammatory responses and promote bone regeneration.

In this review, we first provide a general overview of the bone regeneration mechanisms and the interaction between immune cells and MSCs. Further, the influence of biomaterials on the regulation of the biological behavior as well as the function of macrophages and MSCs is emphasized. In addition, the macrophage-related inflammatory response triggered by biomaterials and its specific role in osteogenesis is discussed.

2. The Composition and Healing Mechanism of the Bone

Bone is the main component of the musculoskeletal system, which plays a pivotal role in the support, protection, and motion of the body. Mature bone is composed of minerals (~65% by weight), organic matrix (~10%), and water (~25%) [17]. The inorganic bone matrix accounts for 99% of the calcium, 85% of the phosphorous, and 40%–60% of the magnesium and sodium of the body's storage [18]. Inorganic matrix mainly exists in the form of hydroxyapatite and provides the majority of bone strength, including stiffness, and resistance to compressive forces [18]. Its removal renders bone soft, malleable, and spongy, for example, osteomalacia or Rickets secondary to vitamin D deficiency [19]. Although the organic matrix secreted by osteoblasts predominantly comprises type I collagen, it also contains proteoglycans, glycoproteins, and growth factors [18]. These growth factors include the bone morphogenic proteins (BMPs) and other transforming growth factors, family factors, interleukin-1, interleukin-6, osteocalcin, osteonectin, and bone sialoprotein [20, 21]. These factors play important roles in osteogenesis as well as mineralization and remodeling of bones [21]. Organic matrix gives bone its form and provides resistance to tensile forces. One of the important components of the bone matrix essential for the remodeling and regeneration of bone is BMPs, which were discovered in 1965 by Urist [22]. BMPs are members of the transforming growth factor superfamily, which include 20 known members. Embryologically, they are important mediators of cardiac, nerve, and neural crest development and possess critical osteoinductive properties applicable in postnatal bone formation and healing.

Osteogenesis is a key process during bone regeneration and remodeling [23]. In this process, osteoblasts emerge from the differentiation of osteogenic progenitor cells in the periosteum and are responsible for the synthesis and mineralization of bone during initial bone formation and later bone remodeling [24, 25]. Osteoblastogenesis, that is, the differentiation of osteogenic cells such as MSCs into osteoblasts, requires a certain microenvironment and is regulated by an interplay of cytokines, components, and mechanical characteristics of the matrix [26]. Osteoblasts are eventually surrounded by the growing bone matrix; as the matrix calcifies, the cells get trapped in it and become osteocytes, that is, bone cells [27, 28]. The extracellular matrix (ECM) proteins can interact with cells via integrin adhesion receptors [29] that are involved in cell signaling transduction, thus influencing the attachment, proliferation, and differentiation of cells [30]. Moreover, ECM proteins bind to soluble growth factors, including BMPs, and regulate their distribution, activation, and presentation to cells, which integrate complex, multivalent signals to cells in a spatially organized and regulated manner [31, 32]. More recently, it has been demonstrated that mechanical characteristics of the matrix, including stiffness and deformability, also provide inputs into cell behavior [33, 34].

The regeneration of bone tissue after injuries is also inseparable from the interplay with the immune system. Bleeding after bone injury results in the delivery of different type of blood cells such as platelets and granulocytes as well as blood monocytes to the injury site. Monocytes then differentiate into macrophages under the effect of cytokines (M-CSF, IL-4, etc.) that are present in the wound area. In the early phases of bone regeneration, M1 macrophages mediate acute inflammation, regulate MSCs recruitment, and initiate bone tissue regeneration [35]. The initial inflammation can induce the recruitment of MSCs and immune cells, including monocytes [36]. Subsequently, the recruited macrophages may positively regulate the differentiation of MSCs into osteoblasts by secretion of cytokines, including IL-6 and TNF-α [36]. However, for prolonged inflammation, the remaining proinflammatory cytokines negatively affect bone regeneration [37].

3. Role of Macrophages in Bone Regeneration

Inflammation is a complex defensive process of the body reflecting its response to various noxious stimuli. Immune cells, such as neutrophils, monocytes, macrophages, T cells, and dendritic cells, play an important role in the regulation of the immune response against infections and injuries [38]. Various types of immune cells are involved in the inflammatory response; however, macrophages are indispensable and key players because they secrete various cytokines that control inflammation and participate in tissue regeneration [39]. Macrophages are grossly classified as the following phenotypes: inflammatory macrophages (M1) and anti-inflammatory macrophages (M2) [35]. M1 phenotype is the "classically activated" subset of macrophage, whereas M2 phenotype is known as "alternatively activated" macrophages owing to differing activation signals compared with the M1 subset [40]. M1 macrophages secrete proinflammatory cytokines such as IFNγ, TNFα, and IL-6, which affect osteoblasts by inhibiting their differentiation and promoting apoptosis, thus hindering the production of collagen by osteoblasts, which is necessary for mineralization [37]. In contrast, M2 macrophages produce TGF-β, VEGF, and IL-10, which inhibits osteoclast formation and supports bone deposition [37, 41]. A fine balance in M1/M2 macrophage function appears mandatory to fracture healing and successful regeneration. Indeed, macrophages play essential roles not only in early inflammation but also in the later phase of bone healing, which relies on the M1/M2 macrophage phenotype switch [42]. M1 macrophages are necessary for the initiation of the regeneration process; however, prolonged infiltration of proinflammatory macrophages causes chronic inflammation and negatively influences bone healing [43, 44]. A key element in bone healing is the shift from the proinflammatory M1 macrophage, important during the initial healing step, to the anti-inflammatory M2 phenotype, during stages in which endochondral ossification is expected [42]. In addition, macrophages phagocytose necrotic and apoptotic cells and recruit MSCs to the injured site to promote osteogenesis [45]. In this context, increasing attention has been paid to the role of macrophages in tissue regeneration.

Osteoclasts are usually regarded as the "resident macrophages" of bone. However, a recently identified resident population of nonosteoclast macrophages in the skeleton has been found to play diverse roles in bone biology. It was found that they participated in bone formation and regulated bone homeostasis [46]. Nicolaidou et al. demonstrated that monocytes and macrophages facilitate mineralization. More specifically, monocytes induced osteogenesis in MSCs through the STAT3 activation [47]. Additionally, oncostatin M (OSM) secreted by macrophages has been considered a specific macrophage factor that supports the osteoblastic potential of MSCs [48]. Further evidence of the ability of macrophages to support mineralization has been verified by the increased mineral deposition when vascular smooth muscle cells were cocultured with macrophages [49]. Moreover, the importance of macrophages in bone healing has been demonstrated in different animal models [34]. For example, Wang et al. have found that magnesium-calcium phosphate cement upregulated the bone-repair-related release of the cytokine TGF-β1, downregulated proinflammatory cytokines, including TNF-α and IL-6, and facilitated bone healing [50]. Mice treated with a broad-spectrum promyeloid factor, namely, macrophage colony-stimulating factor (CSF-1), showed an increase in bone formation and bone mass [51–53]. Zhu et al. found that crocin induced polarization of anti-inflammatory (M2) macrophage and promotion of cocultured BMSCs' osteogenic differentiation [54]. In addition, a recent study has indicated that macrophage deficiency hindered the regeneration of the tail fin and patterning of bony rays in zebrafish [55].

4. Role of MSCs in Bone Regeneration

MSCs can both self-renew and self-differentiate. A wide variety of MSCs, including BMSCs, adipose-derived stromal cells (ADSCs), and umbilical cord mesenchymal stromal cells (UC-MSCs), exist. BMSCs are considered the progenitor cells for bone formation; they can differentiate into multiple cell types, such as osteoblasts, chondrocytes, adipocytes, and smooth muscle cells [56–58]. Previous studies have demonstrated that BMSCs play an essential role in the maintenance of bone homeostasis and the treatment of various bone disorders [59, 60].

The proficient recruitment of MSCs to the defect site is a critical process during bone regeneration [61]. Chemotactic factors released by macrophages at the bone defect site play a critical role in MSC homing and recruitment. It is known that MSCs express at least 19 chemokine receptors, such as CXCR1 and CCR1 [62]. Local hypoxia in the initial stage of fracture is caused by vascular disruption and induces the production of chemotactic factors, including stromal cell-derived factor-1 (SDF-1 and CXCL12), which in turn promotes the chemotactic recruitment of numerous cell types, such as MSCs and other progenitor cells [63, 64], thus facilitating bone regeneration [65]. MSCs can also recruit other MSCs or progenitor cells to the injury site via paracrine action such as stromal cell-derived factor 1 [66]. Therefore, several attempts have been made to stimulate the

recruitment/mobilization of stem cells for accelerating bone regeneration. For example, stromal-derived factor 1 (SDF-1) induced stem cell mobilization and homing [67]. The local delivery of SDF-1 to bone injury sites during bone defect healing can recruit MSCs/precursor cells, which results in tissue-specific differentiation and significant bone regeneration [68]. A similar study demonstrated that cobalt contributes to the recruitment of MSCs because it activates HIF-α, which then accelerates the bone healing process [69]. Moreover, the recruitment of MSCs for efficient tissue regeneration is largely affected by the local microenvironment. The chemical, topographic, and mechanical properties of the ECM have been widely investigated to study their influence on attachment, migration, and differentiation of MSCs [70].

The osteogenic differentiation potential of MSCs is also of great importance for bone regeneration. Multiple intermediate processes involved in the differentiation of MSCs into cells of osteogenic lineage are important targets for cytokines, hormones, and ECM, thereby regulating bone regeneration. For instance (Figure 1), a large number of signaling pathways have been found to induce conversion between osteogenesis and adipogenesis of MSCs, which is primarily related to the following two key transcription factors: Runx2 (the key modulator of osteogenic differentiation) and PPAR (the master regulator of adipogenic differentiation) [71]. The presentation of growth factors, such as BMP-2, TGF-β1, and FGF2, to MSCs, has been found to target Runx2 and to activate and regulate osteogenesis [72–74]. In addition to proosteogenic effects, the supply of Nel-like protein type I (NELL-1) significantly reduced adipose differentiation of ADSCs while promoting osteogenic differentiation [75, 76]. Moreover, the recently discovered transcriptional activator with PDZ-binding motif (TAZ) is another critical transcriptional modulator capable of stimulating osteogenesis while simultaneously blocking the differentiation of MSCs into adipocytes [77]. It is noteworthy that the mechanical signals transduced from the cellular microenvironment (substrate topographical, stiffness, etc.) can affect the cell fate determination of MSCs via YAP/TAZ activity regulation [78]. Experiments have demonstrated that YAP/TAZ localizes outside the nucleus and are inhibited when cultured on a soft matrix, whereas it localizes in the nucleus and is transcriptionally active when cells are cultured on a stiff matrix [79]. Moreover, YAP/TAZ is inhibited when cells are restricted to small adhesive areas [79, 80].

5. Crosstalk between MSCs and Macrophages and Its Role in Bone Regeneration

Recent studies have revealed a clear understanding that successful bone healing is based on a carefully coordinated crosstalk between macrophages and MSCs in the body (Figure 2(a)) [81]. Particularly, responses of the innate immune system can affect the formation, remodeling, and healing of bones, and this process is quite complex [82]. Among the variety of immune cells, macrophages play a significant role in the regulation of bone repair [83]. Previous studies have demonstrated that inflammatory macrophages, which secrete TNF-α and IL-1β, suppressed osteoblastogenesis [84]. In contrast, other studies suggested that several foreign materials result in an M1-dominant macrophage phenotype, which enhances bone formation and healing [85, 86]. Consistent with *in vivo* studies, the *in vitro* studies also showed the positive effect of M1-dominant macrophages on strengthening osteogenic differentiation of MSCs [87]. Omar et al. found that MSCs treated with conditioned media (CM) containing lipopolysaccharide-(LPS-) stimulated monocytes exhibited an enhanced osteogenic differentiation (see Figure 2(b)) [88]. In addition, it was demonstrated that M1 macrophages were capable of secretion of OSM, which promoted the osteoblastogenesis of MSCs *in vitro* [48]. Conversely, other studies reported that M2, but not M1, macrophages enhanced osteogenic differentiation of MSCs [89]. Indeed, M1 versus M2 alternatively activated macrophages, which played key roles in successful osteogenic differentiation and bone repair. M2 phenotypes can be attributed to a properly regulated inflammatory response being an important physiological process that promotes tissue repair after injury [81]. However, the exact role of the M1/M2 paradigm in response to osteogenic differentiation of MSCs is still not completely understood. More recently, several studies have implicated that M1 and M2 dominant macrophages cooperatively regulate the osteogenic differentiation of MSCs. More specifically, M1 macrophages enhanced osteogenic differentiation at an early stage through the recruitment of stem cells to injured sites, whereas M2 macrophages may subsequently facilitate matrix mineralization (Figure 2(c)) [90].

Macrophage-derived osteoclasts are also important for the regulation of MSC-based bone regeneration. Quint and Pederson et al. found that cytokines (BMPs, WNTs, Sphingosine 1-phosphate, etc.) secreted by osteoclasts induced the migration and osteogenic differentiation of MSCs [91, 92]. Another notable research showed that MSCs transplanted with biphasic calcium phosphate (BCP) attracted circulating monocytes and induced their differentiation into osteoclasts and promoted osteogenesis. Interestingly, local injection of clodronate or anti-RANKL antibodies (a key factor for osteoclast differentiation and activation) abolished osteoclasts formation and suppressed osteogenic differentiation. This fact has reemphasized the considerable role of osteoclasts in MSC-mediated osteogenic differentiation and bone formation [85]. Overall, these studies demonstrated that macrophage polarization is of great importance for distinct roles in the bone regeneration mediated by MSCs, which is similar to the repair process of the normal tissue (from a proinflammatory to a preoperative stage).

MSCs can modulate immune cells through cell contact by binding to receptors on the surface of immune cells [93, 94] and secretion of cytokines, including TGF-β, hepatocyte growth factor, and prostaglandin E2 (PGE2), as well as other anti-inflammatory factors [95, 96], and have a pivotal role in maintaining immune homeostasis [97]. Previous investigations on the immunomodulatory function of MSCs have been concentrated on the interaction between MSCs and B lymphocytes, dendritic cells, and natural killer

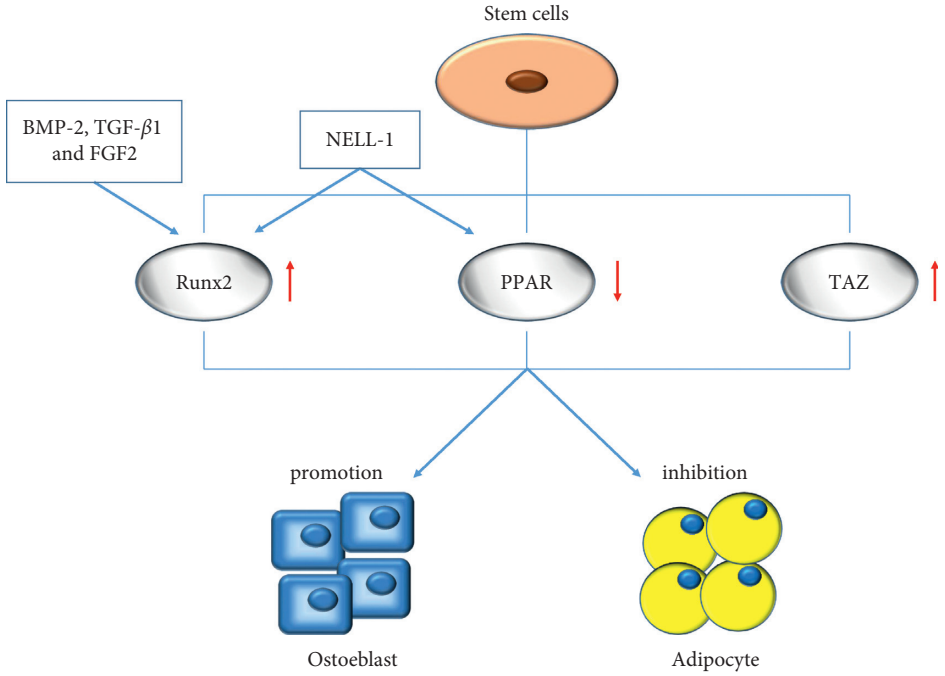
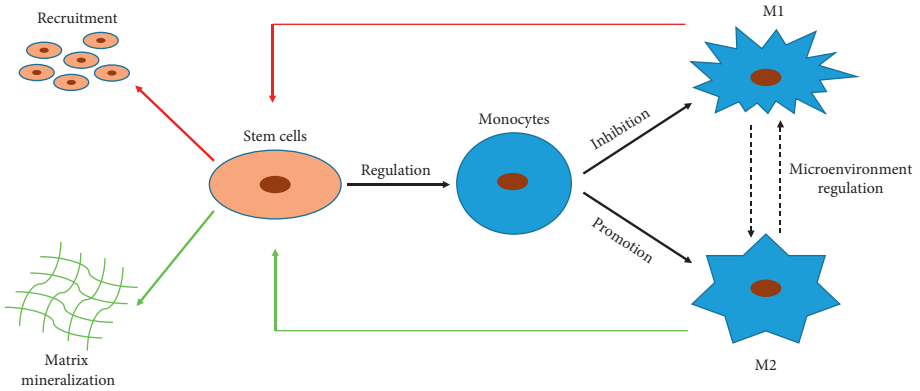


Figure 1: Schematic representation of signaling pathways which induce the switch between osteogenesis and adipogenesis in MSCs.

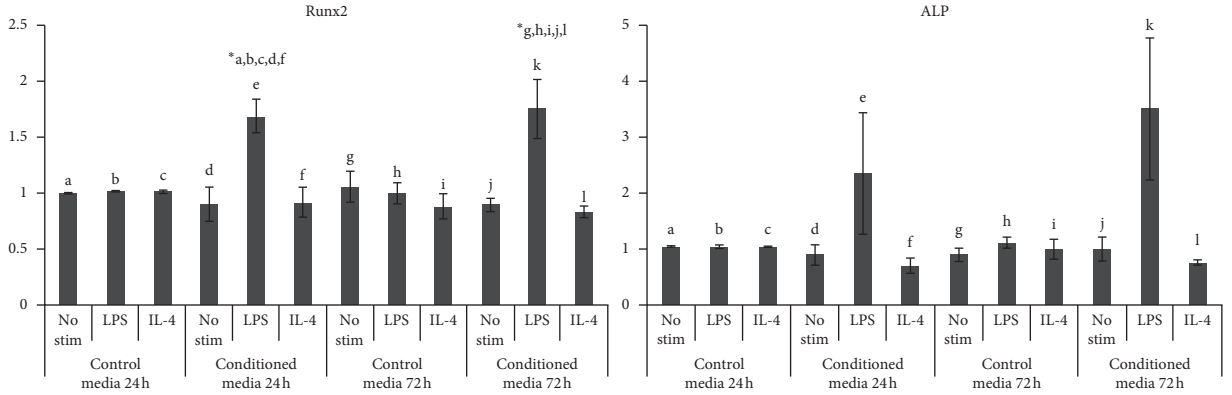
cells [98]. In recent years, the use of MSCs in the repair of damaged tissues and modulation of the inflammatory response has gained increasing attention with respect to macrophage regulation. MSCs have the ability to differentiate into osteoblasts and their immunomodulatory action affects osteogenesis [99]. The mixing of MSCs with BCP particles enhanced the recruitment of macrophages to the site of BCP implantation and accelerated bone regeneration [85, 86]. Moreover, it was observed that the production of inflammatory cytokines, including TNF-α, IL-6, IFN-γ, and IL-12p70, by bone marrow-derived macrophages cultured with MSCs or in MSC-conditioned medium was significantly suppressed after stimulation with TLR7/8 ligand (the most potent activator of monocytes) [100]. Simultaneously, the production of anti-inflammatory cytokines, including IL-12p40 and IL-10, increased. Interestingly, recent studies found that exosomes secreted by MSCs not only promoted tissue repair but were also involved in the regulation of inflammatory responses. Although the MSC-derived exosomes could enhance the enrichment of CD163+ M2 macrophages, they reduce the infiltration of CD81+ M1 macrophages and thus decrease the release of related inflammatory factors (Figure 2(d)) [101]. Several studies demonstrated that allogeneic MSCs could induce the differentiation of monocytes toward an anti-inflammatory M2 phenotype, which may occur through the STAT-3 and NF-κB pathways [102, 103]. Furthermore, MSCs can be polarized into either a proinflammatory or immunosuppressive phenotype based on Toll-like receptors. They are polarized into a proinflammatory phenotype by TLR4 stimulation and into an immunosuppressive phenotype by TLR3 stimulation, which is determined by MSC1 and MSC2, respectively [104].

6. Role of Biomaterials in the Regulation of Macrophage Behavior

Biomaterials have a long record of replacing lost or damaged tissues and supporting the healing process [105]. To date, various polymers [106], ceramics [107], and metals [108] have been applied as implant materials to replace and regenerate damaged tissues. When a biomaterial is implanted in the body, it leads to an extensive and complex inflammatory response related to the surgical intervention and the properties of material, which recruits various types of immune cells to the region of the implanted biomaterial by triggering a series of biochemical signals [109]. Thereafter, owing to stimulation by various proinflammatory cytokines produced by other inflammatory cells, including mast and dendritic cells, the recruited monocytes can differentiate into macrophages, which adhere to the implanted biomaterials and subsequently affect the bone healing process [109]. In this process, the biophysical and biochemical properties of the biomaterials play a crucial role in directing the phenotypic transformation of macrophages [110]. The inflammatory response is related to the proteins adsorbed on the implanted materials which formed a provision matrix at the biomaterial's surface [111]. For example, the amount of adsorbed vitronectin and fibronectin on the implant surface is pivotal for monocyte adhesion and giant cell formation via their integrin-mediated interaction with these proteins [112, 113]. Additionally, the provision matrix contains and releases several other cytokines and GFs of diverse nature that are capable of modulating the attraction and activity of macrophages and other immune cells [111]. The properties of the biomaterials can strongly influence the adsorption of proteins in terms of types, concentration, mode, and



(a)



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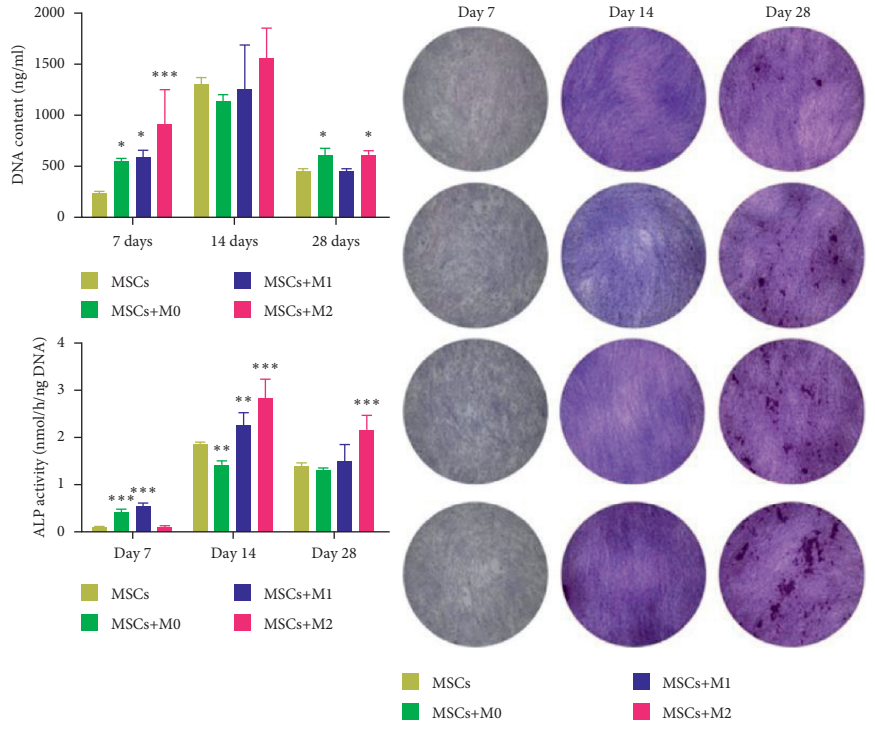

(c)

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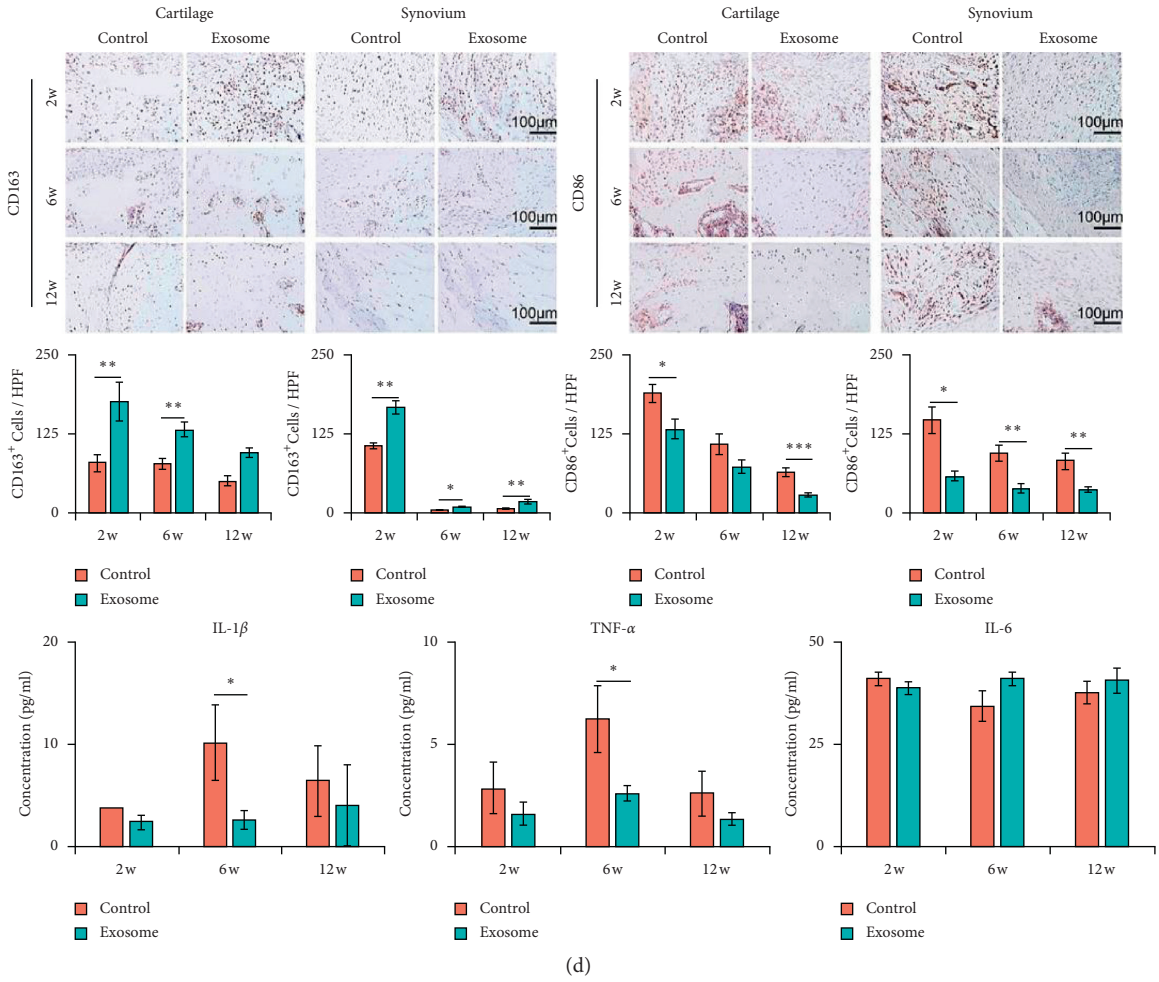


Figure 2: (a) The schematic diagram indicating the crosstalk between MSCs and macrophages. (b) The relative gene expression of Runx2 and ALP in MSCs. Gene expression of Runx2 and ALP in hMSCs after culturing in unconditioned control media (Ctrl) or monocyte conditioned media (CM) for 72 h [88] (Copyright 2011, Elsevier). (c) Proliferation and ALP activity of MSCs indirectly cocultured with M0, M1, and M2 macrophages [90] (Copyright 2017, Springer). (d) Effects of MSC exosomes on macrophage infiltration and proinflammatory cytokine production during cartilage repair [101] (Copyright 2018, Elsevier).

conformation [114]. Moreover, it was observed that the physical properties of biomaterials, such as surface topography, stiffness, and porosity, are key factors for regulating macrophage polarization [115]. Hamlet et al. proved that the secretion of the proinflammatory factor IL-6 increased in M1-dominant macrophages cultured on Ti, which exhibited microroughness [116]. Thus, it became evident that the microroughness of the biomaterial promoted the secretion of inflammatory factors, triggering both the M1 and M2 phenotypes. Moreover, the pattern of the surface, such as the diameter and alignment of fibers, influences the activation of macrophages. The aligned poly (L-lactic acid) (PLLA) fibers with micro- or nanoscale diameter enhanced macrophage adhesion compared with randomly aligned or flat PLLA. In the presence of LPS stimulation, the secretion of inflammatory cytokines was significantly less in macrophages cultured on fibrous PLLA than those cultured on flat PLLA films, indicating that fibrous materials reduce classical activation in macrophages [117]. Substrate stiffness and applied forces on substrates also affect macrophage activation. In the presence of LPS stimulation, murine macrophages showed increased expression of TNF-α, IL-10, IL-1β, and IL-6on poly (ethylene glycol) hydrogels with higher stiffness [118]. Pores in scaffolds also play a key role in tissue engineering applications because they allow permeation of the scaffold with oxygen, nutrients, and even proteins. However, the size of the pores and porosity of scaffolds are considerable factors that affect immunomodulation and osteogenesis because cells interact differently with environments in nano-, micro-, and macroscale materials [119]. Sussman et al. investigated the influence of microsized pores on macrophage polarization phenotypes using poly (methyl methacrylate) (PMMA) and poly (2-hydroxyethyl methacrylate) (pHEMA) scaffolds. Implants with pore sizes of 34 μm increased the levels of IL-1R1 and inducible nitric oxide synthase (iNOS) (M1 phenotype marker) upon

implantation into mice [120]. Conversely, it was found that the morphology of macrophages was strongly regulated by micropatterned substrates. More specifically, the macrophages that were forced into an elongated morphology because of the surface structures showed greater M2-dominant polarization (Figure 3(a)) [121]. Additionally, the chemical composition of biomaterials affects protein adsorption as well as differentiation and activity of macrophages, which regulates the inflammatory response [115]. Accordingly, it was found that the characteristics of biomaterials related to the presence of specific functional groups, such as the surface charge and wettability, played a key role in the inflammatory reaction [109]. Self-assembled monolayers with different wettability and charge properties were used as model biomaterials for macrophage/fibroblast coculturing. It was demonstrated that the hydrophobic CH3 surface caused the strongest inflammatory reactions, whereas the hydrophilic/anionic COOH surface caused the least inflammatory response (Figure 3(b)) [122]. They also found that the surface coating of glycosaminoglycans (GAGs) and chitosan (Chi) composed polyelectrolyte multilayers, particularly the heparin-Chi multilayers, and strongly reduced the inflammatory responses [123]. Conversely, polyethylene terephthalate films with hydrophilic/neutral surfaces promoted macrophage activation, which generated markedly high amounts of different cytokines and chemokines [124]. In addition, a carbon nanotube-covered surface that was modified by plasma polymerization with oxygen-containing functional groups and the chemical properties of oxygen groups enhanced the adhesion and activation of macrophages, resulting in polarization to M1 phenotype [125]. Therefore, the dynamic interactions between biomaterials and immune cells have attracted increasing attention in the field of tissue engineering.

7. Role of Biomaterials in the Regulation of MSC Behavior

Indeed, the modulation of cellular adhesion is important for the control of recruitment in the early stage osteogenic differentiation of stem cells [126]. However, most man-made biomaterials lack favorable surfaces for cell attachment owing to missing specific cell-recognizable signals, such as adhesive ligands for integrins [127–129]. Additionally, chemical composition and surface properties greatly affect the regulation of protein adsorption and subsequent cell attachment and differentiation [126, 130, 131]. Therefore, surface functionalization of implant materials has emerged as a promising method to obtain an interface with enhanced bioactivity between implants and tissues [127, 132].

ECM includes proteins such as fibrillar collagens and elastin as well as adhesive proteins such as fibronectin (FN) and proteoglycans (e.g., aggrecan). ECM components have been widely used to create a bioactive interface owing to the excellent biological properties of ECM, which can offer a suitable microenvironment and provide significant cues for the adhesion, migration, and differentiation of MSCs [133]. Lee et al. engineered FN type III 9 and 10 domains fused to elastin-like polypeptides (FN-ELPs). The recombinant MSCs cultured on plates coated with FN-ELP had significantly greater adhesion activity, proliferation, and osteogenesis than the cells cultured on noncoated plates [134]. Vitronectin and FN are the frequently studied proteins that enhance cell attachment because they are selectively recognized by the cell transmembrane receptors integrins owing to the presence of the "RGD" (Arg-Gly-Asp) amino acid sequence [135]. Hence, the RGD sequence has been frequently used to modify biomaterial surface. For example, investigations have revealed that immobilized RGD peptides can significantly promote the adhesion of hBMSCs. In addition, it was shown that peptide density and the spacing between clusters of RGD peptides could determine integrin receptor binding and subsequent signal transduction, which was visualized by the expression of focal adhesion of stem cells [136]. We have previously shown that the formation of polyelectrolyte multilayers using bone matrix components, namely, collagen I (Col I) and chondroitin sulfate (CS), has a superior effect on promoting the adhesion and osteogenic differentiation of hADSCs compared with a combination of collagen I with hyaluronan that is not found in bone (Figures 4(a) and 4(b)) [137]. In addition to ECM proteins, growth factors have been frequently applied for making biomaterials bioactive toward cells, including the support of the differentiation of MSCs. It was reported that self-assembled, microsphere-incorporated hMSC sheets could form cartilage in the presence of exogenous transforming growth factor β1 (TGF-β1) or with TGF-β1 released from incorporated microspheres. Moreover, improved cartilage formation was found in the microsphere-incorporated cell sheets [138]. Zhang et al. prepared poly (lac-tic-co-glycolic acid) (PLGA) microspheres coated with multilayer polyelectrolytes ((HA-CS)2-Hep-BMP-2-Hep-(CS-HA)2) as the multibarrier microcarriers for osteogenic growth peptide and BMP-2. The immobilization of the microspheres at the surface of a highly interconnected porous hydroxyapatite (HAP) scaffold largely promoted *in vitro* and *in vivo* osteogenic differentiation (Figure 4(c)) [139]. In a study by Wang et al., hydroxyapatite (HAP)/TiO_2 composite coatings were first prepared on titanium (Ti) surface by one-step microarc oxidation; next, pure Chi and bone morphogenic protein-2- (BMP-2-) encapsulated CS coatings were loaded on the HA/TiO_2 surfaces, which accelerated the adhesion, migration, and proliferation of osteoblastic cells [140].

Topographical cues are other features of biomaterial surface that can modulate the morphology and subsequently cellular functions, such as cytoskeletal organization, gene, and protein expressions of MSCs [141]. Seunghan et al. altered the dimensions of titanium oxide surface nanotubes and observed that small (30 nm in diameter) nanotubes promoted adhesion without noticeable differentiation, whereas larger (70–100 nm in diameter) nanotubes elicited a significant hMSC elongation (10-fold increased). The latter induced cytoskeletal stress and selective differentiation into osteoblast-like cells [142]. Kim and coworkers found that hDPSCs presented a linear arrangement on a nanopatterned surface and irregular arrangement on a conventional surface, which was accompanied by a more significant adipogenic differentiation. However, gene expression analysis

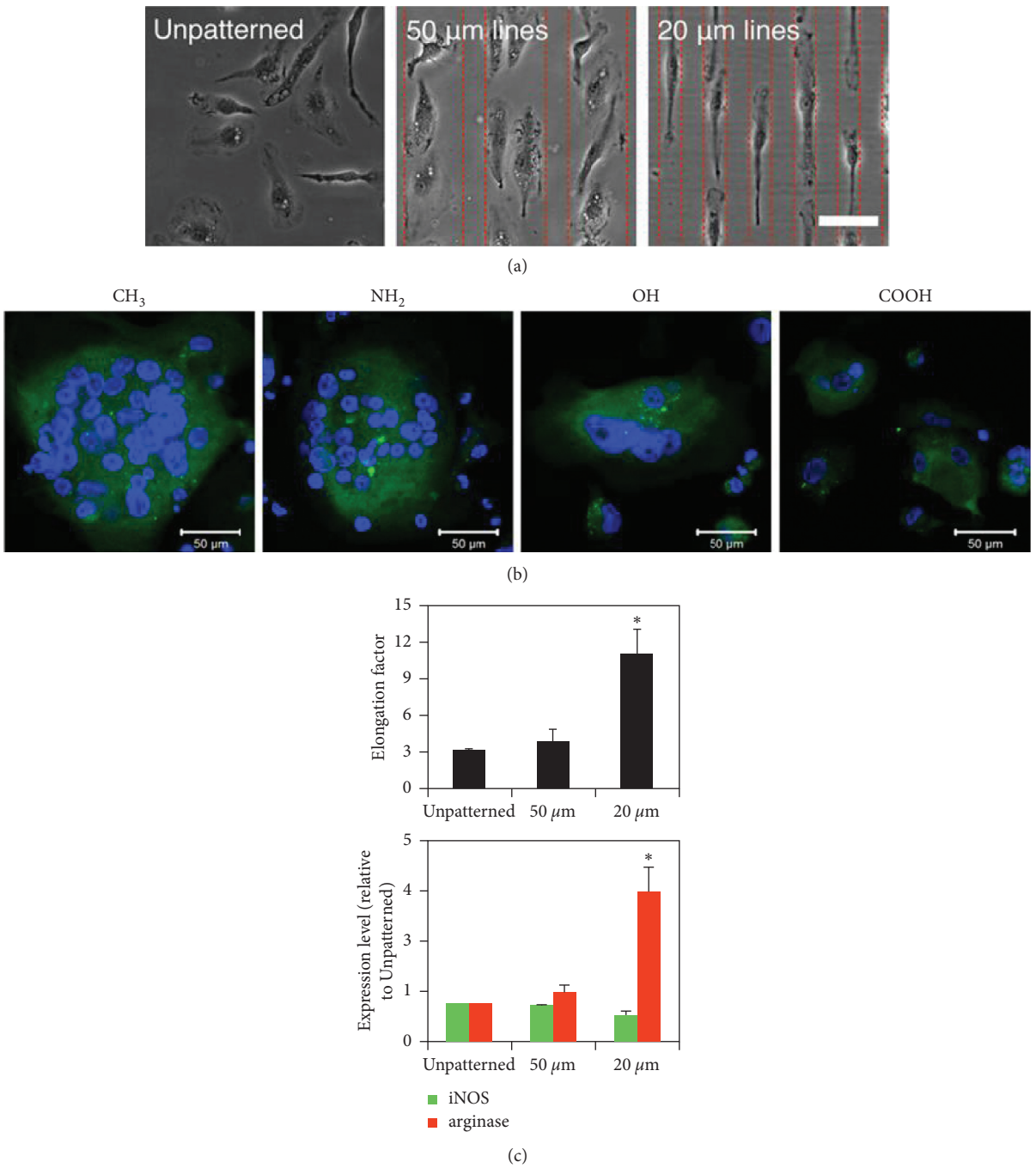
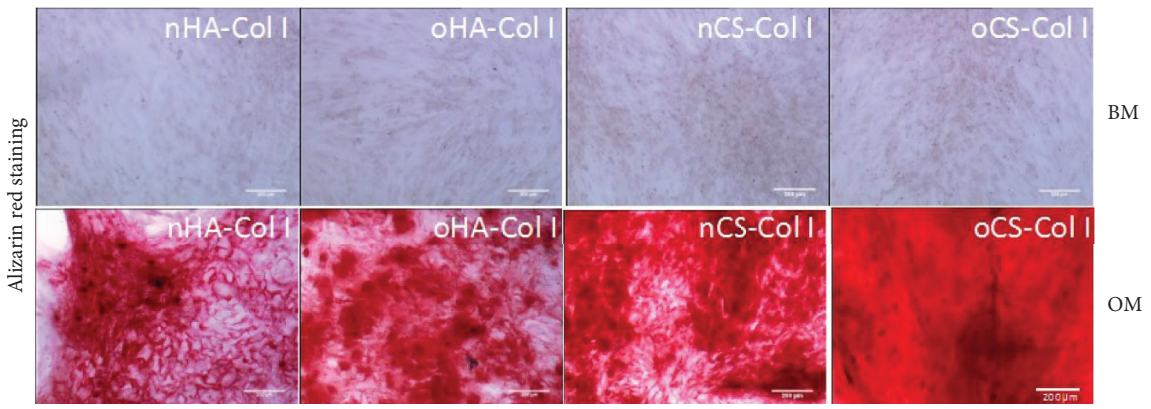


Figure 3: (a) The elongation of cells by micropatterning drives macrophage polarization [121] (Copyright 2013, National Academy of Sciences). (b) Expression of $\beta 1$ integrin was determined by fusing macrophages/FBGCs in macrophage monocultures at CH_3, NH_2, OH, and COOH surfaces [122] (Copyright 2015, Elsevier).

revealed significantly higher expression of LPL in the nanopatterned group than in the conventional group after induction of osteogenic differentiation [143]. Dalby et al. presented the topographies, originally produced by colloidal lithography and polymer demixing on silicon and then embossed (through an intermediate nickel shim) into polymethyl methacrylate, which stimulated the osteoprogenitor cell differentiation toward an osteoblastic phenotype (Figure 4(d)) [144].

In addition to topographical cues, it has been evidenced that stiffness and elasticity of the surrounding matrix have profound effects on the regulation of MSCs fate [145]. For example, Rowlands and George's groups have demonstrated the importance of stiffness and ECM protein microenvironments by culturing MSCs on polyacrylamide gel substrates (with varying stiffness) coated with tissue-specific ECM proteins. The gel-protein substrates supported the proliferation of MSCs in a stiffness-dependent manner.



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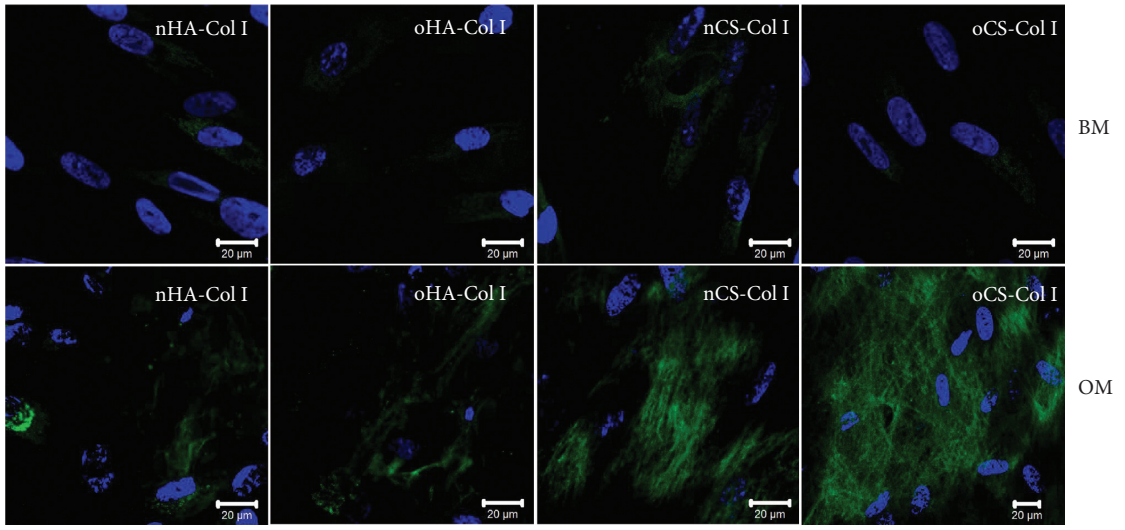

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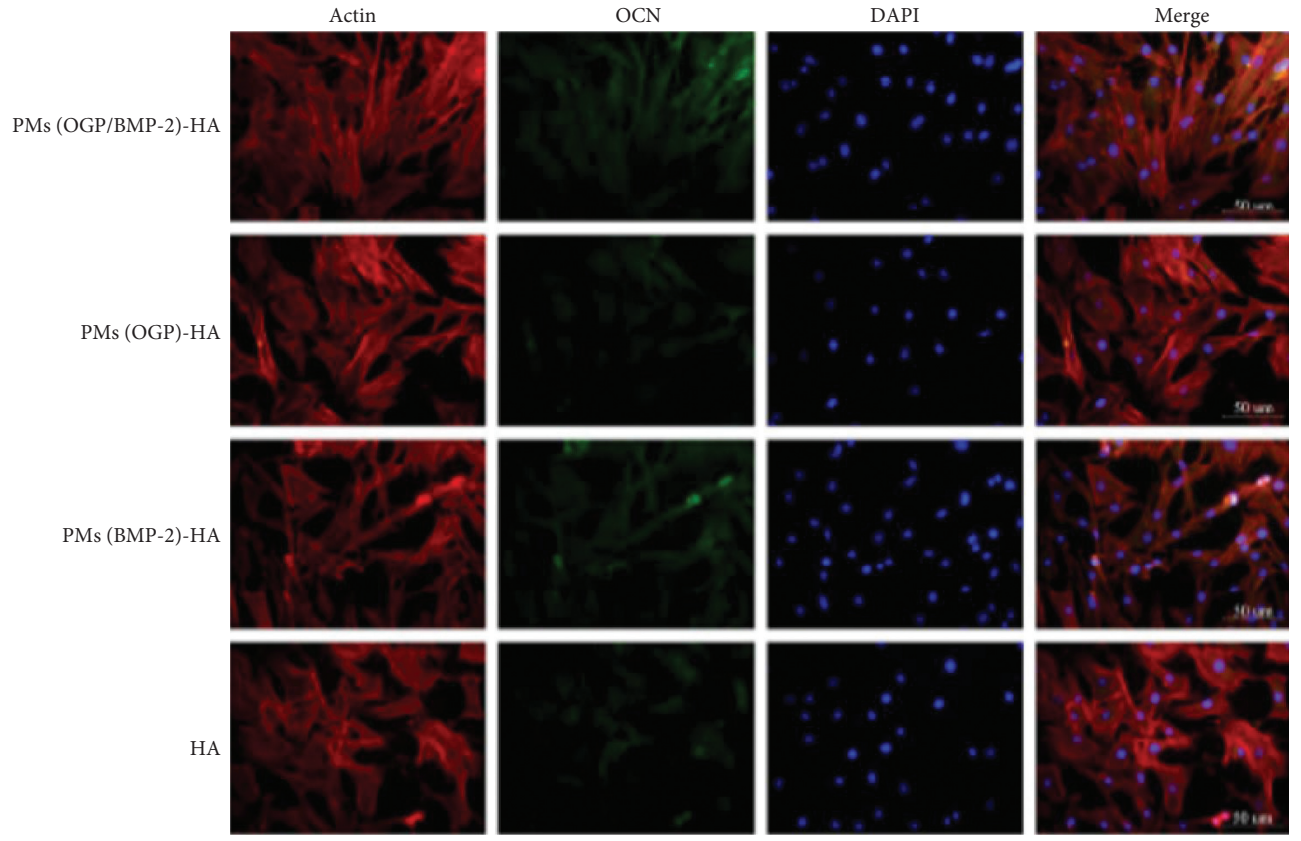

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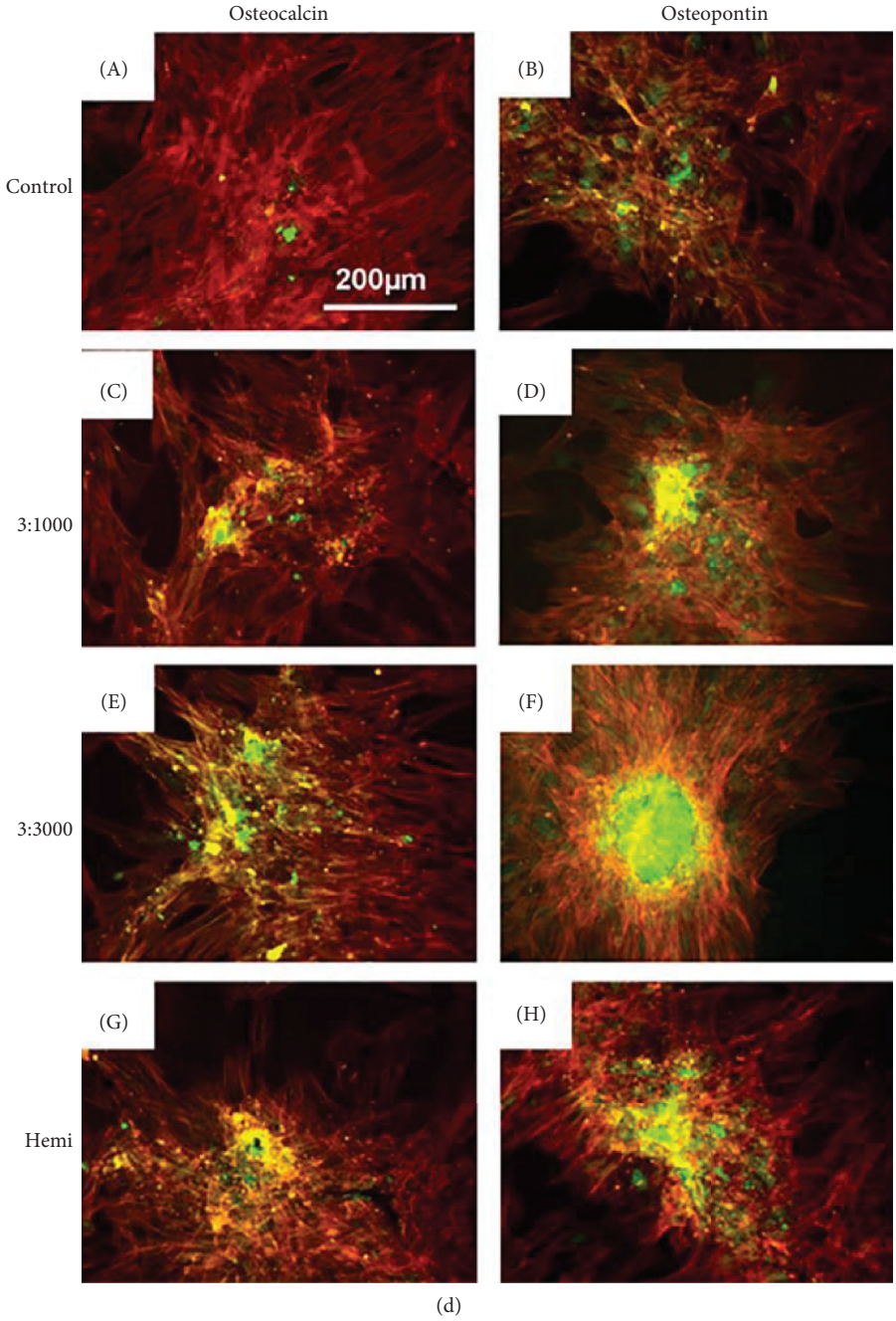


Figure 4: (a) Histochemical staining of calcium phosphate with Alizarin Red S at day 21 after osteogenic differentiation. (b) Immunofluorescence staining of type I collagen (Col (I)) in hADSCs at day 21 after osteogenic differentiation in the presence of BM (basal medium; upper panel) and OM (osteogenic differentiation medium; lower panel) [137] (Copyright 2016, Elsevier). (c) Immunofluorescence and western blotting analysis of osteogenic marker protein in BMSCs cocultured with different scaffolds [139] (Copyright 2017, Royal Society of Chemistry). (d) Osteocalcin (OC) and osteopontin (OPN) fluorescent images for hMSCs cultured on the control and test materials [144] (Copyright 2006, Elsevier).

Moreover, a much higher number of cells were detected on high-stiffness substrates than on low-stiffness gels [146]. Saha et al. revealed that substrate modulus could guide neurogenesis in neural stem cells, in which soft matrices promoted the extension of dendritic processes [147]. Moreover, a pioneering study by Engler and coworkers demonstrated the importance of matrix stiffness in guiding cell differentiation. The authors studied the adhesion of MSCs to collagen-coated polyacrylamide hydrogels of various stiffness [148]. The commitment of MSCs to a specific lineage was based on similarity with the native matrix of committed cells. MSCs cultured on soft polyacrylamide gels (<1 kPa) mimicking the elasticity of the brain developed neuronal-like characteristics. MSCs cultured on

intermediate stiffness gels (~10 kPa) mimicking the characteristics of muscle committed to myoblast phenotype, whereas those cultured on stiff gels (>30 kPa) mimicking permineralized bone developed osteogenic properties [148].

8. The Role of the Inflammatory Response in Biomaterial-Induced Bone Regeneration

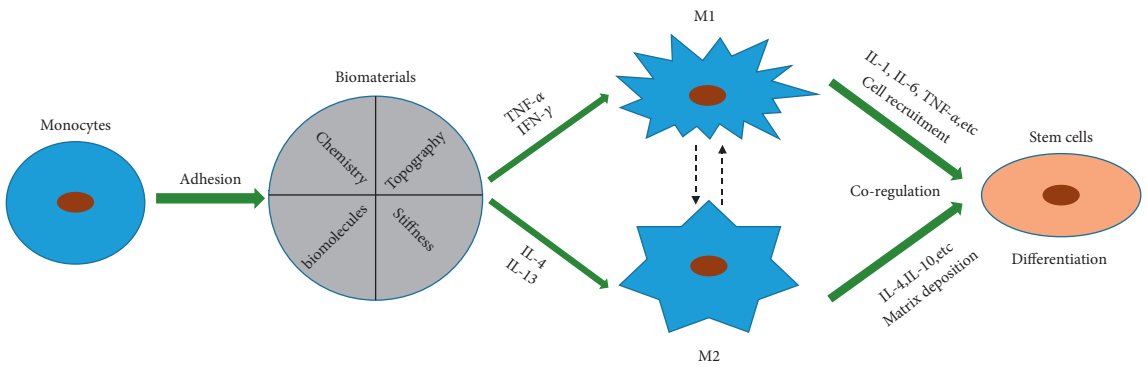
Recently, the crosstalk between inflammatory response and osteogenesis has attracted the attention of researchers. In the past, most studies focused on designing biomaterials that directly regulate the differentiation of MSCs. However, recent studies aimed to use bioactive materials to promote osteogenesis via immunomodulation [50, 149–151]. Several recent observations demonstrated that the implant-mediated cytokine production triggers the recruitment and differentiation of stem cells (Figure 5(a)) [152]. For example, a positive linear relationship between the accumulation of inflammatory cells and recruitment of stem cells was found for biomaterials with varying proinflammatory properties [153]. Therefore, for the tissue engineering community, it is important to understand the effects of biomaterials on macrophages to control osteogenic differentiation.

Surface properties of biomaterials, such as chemical composition, surface physical properties, and bioactive molecules, have demonstrated the decisive roles in the interactions between implants-mediated inflammatory response and stem cells [16]. Inflammatory reactions of biomaterials affect the affinity of the stem cells toward an implant and the function of osteoblastic cells. Biomineralization has been widely performed for the fabrication of artificial bone substitutes. Mineralized surfaces can promote the osteogenic differentiation of stem cells. Thus, osteoimmunology of minerals has also been considered a hot and promising field in bone tissue engineering. Wang et al. observed the crosstalk between immune and stem cells cultured on magnesium-calcium phosphate cement by sintering. Although the macrophages seeded on the cement increased the secretion of TGF-β1, the secretion of IL-6 and TNF-α decreased [50]. These changes are beneficial for osteogenic differentiation. Chen et al. found that β-tricalcium phosphate (β-TCP), used to coat magnesium scaffolds, promoted macrophages to secrete IL-1RA and BMP-2, thereby inducing a bone healing process and enhancing osseointegration capability (Figure 5(b)) [150]. Other bioactive materials were also reported to affect the crosstalk of macrophages and stem cells. Zhu et al. demonstrated that bioactive glass (BG)/sodium alginate hydrogel induced the polarization of macrophages *in vitro* and *in vivo* toward the M2 phenotype, upregulated the expression of anti-inflammatory genes, and enhanced the synthesis of ECM and differentiation of stem cells [154]. Qiu et al. fabricated an injectable periosteal ECM hydrogel, which not only induced the recruitment and M2-polarization of macrophages but also promoted the differentiation of MSCs into osteoblasts [155].

Wan and coworkers demonstrated that the matrix stiffness and inflammatory factor (interleukin-1 (IL-1b)) played opposite roles in the regulation of osteogenic differentiation, with the inflammatory cytokine IL-1 inhibiting matrix stiffness-induced osteogenic differentiation (Figure 5(c)) [156]. Similarly, He et al. found that macrophages encapsulated in low-stiffness transglutaminase cross-linked gelatins (TG-gels) exerted a positive effect on the osteogenesis of cocultured BMSCs. In contrast, macrophages encapsulated in high-stiffness TG-gels negatively affected cell osteogenic differentiation when either CM-based incubation or Trans-well-based coculture was used [157].

Bioactive molecules can be loaded on the implant surface by physical or chemical modification. Studies on osteoimmunomodulation by delivering biological molecules have gained comprehensive attention. For instance, Loi et al. investigated the crosstalk between preosteoblasts and macrophages in a direct coculture. IL-4 was used to induce macrophages into the M2 phenotype and subsequently observed increased OSM secretion and osteogenic differentiation [158]. Moreover, Guihard et al. found that OSM, an IL-6 family cytokine produced by activated circulatingCD14$^+$ or bone marrow CD11b$^+$ monocytes/macrophages, can promote osteoblast differentiation and matrix mineralization of MSCs while inhibiting adipogenesis via signal transducer and activator of transcription (STAT) signaling [48]. It is noteworthy that Spiller et al. used a bone substitute with a short release of IFN-γ (M1) and sustained release of IL-4 (M2). To achieve this sequential release profile, IFNg was physically adsorbed onto the scaffolds, whereas IL-4 was attached via biotin-streptavidin binding. The authors showed that the combined M1 and M2 phenotype induction could better promote osteogenic properties [159]. Delivery of growth factor also regulated both immunomodulation and bone formation. Wei et al. prepared a gelatin sponge loaded with BMP-2 and implanted it in mouse subcutaneous tissue for immunoregulation. The results proved that BMP-2 delivery acted as a direct inducible factor for osteogenesis and an indirect regulator of osteogenesis via immune suppression (Figure 5(d)) [160]. Furthermore, the nucleic acid is one of the important factors affecting osteoimmunomodulation. In the study by Li et al., long noncoding ribonucleic acid (lncRNA) MALAT1, an important endogenous regulator of the proliferative, angiogenic, and immunosuppressive properties of MSCs, was used to culture stem cells. Subsequently, the level of indoleamine2,3-dioxygenase in MSC increased and induced M2 dominant macrophage polarization and finally promoted osteogenic differentiation of stem cells [161].

In recent years, remarkable progress has been made in various interdisciplinary fields related to biomaterials research, such as material chemistry, nanotechnology, and material fabrication techniques. However, the biomaterial-immune system interactions and the effects of inflammatory changes (owing to the biomaterials) on the change in tissue healing process remain largely unknown. Thus, research and development of novel biomaterials should be focused on exploring various surface modification strategies for fine-tuning the control of immune systems. This will allow either avoiding unnecessary inflammation or reprogramming the inflammatory cells to promote the wound healing process



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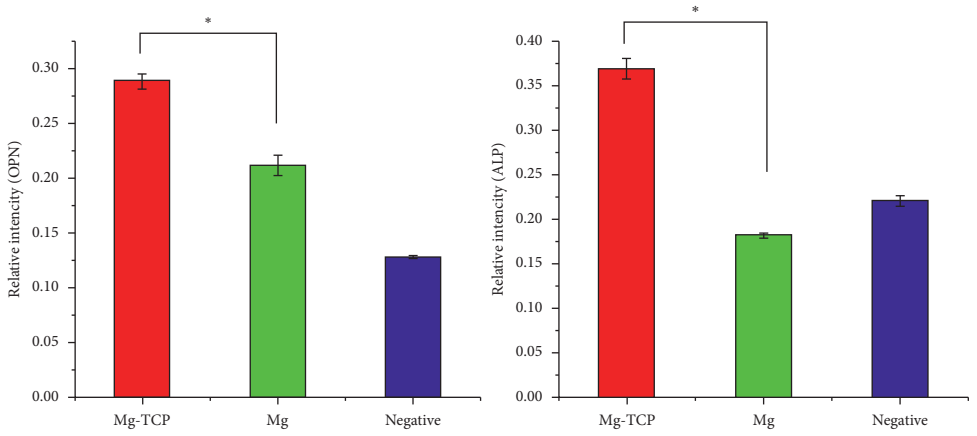

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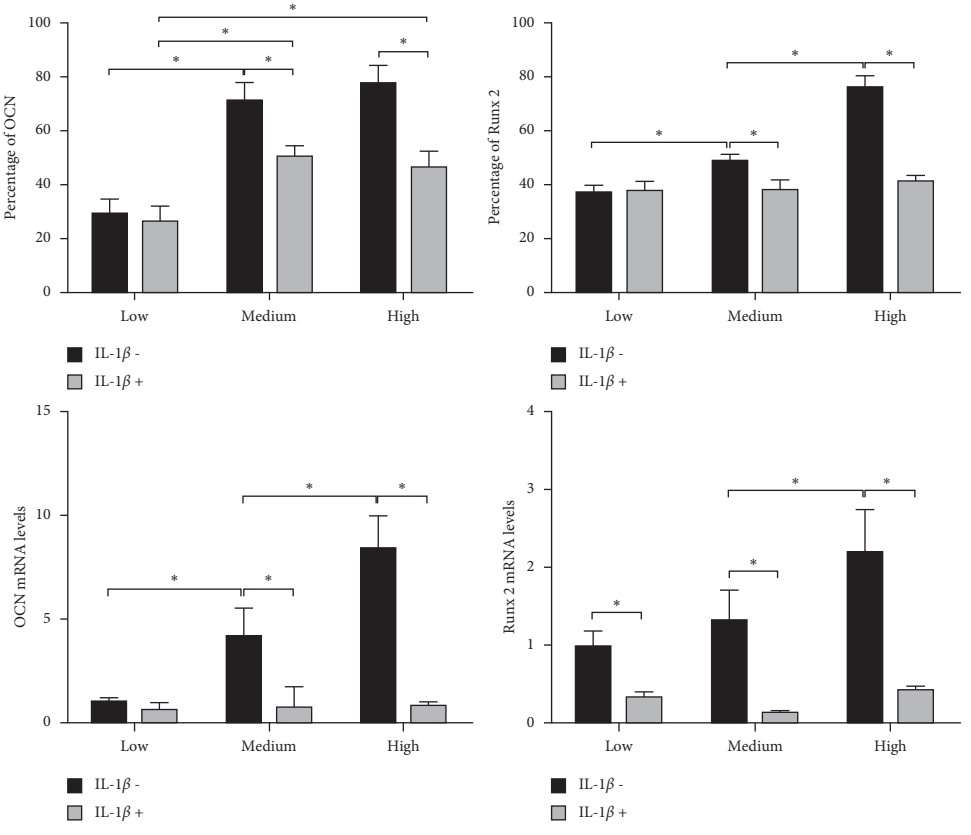

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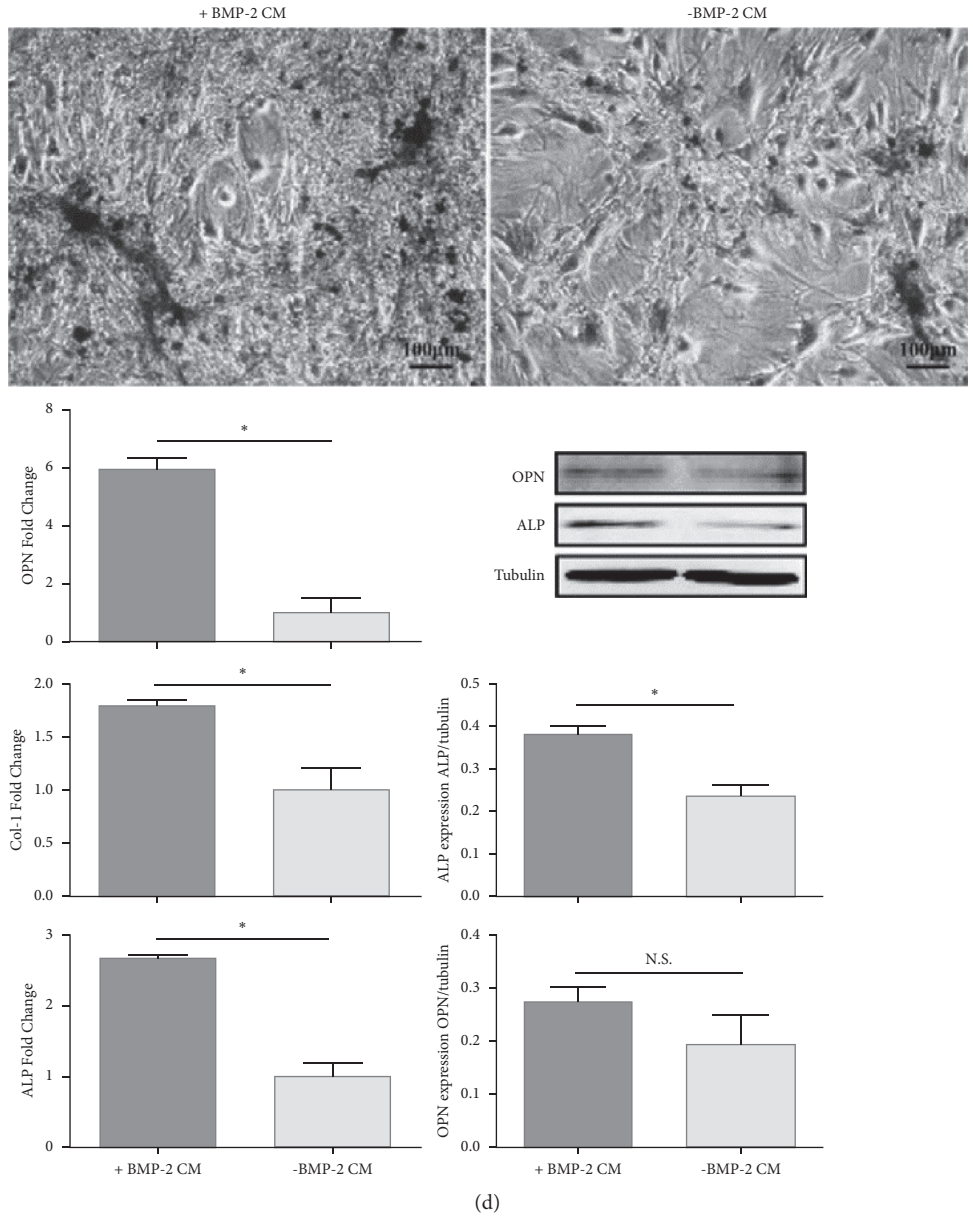

Figure 5: (a) Biomaterials regulate the inflammatory response of macrophages and its role in osteogenesis. (b) The western blotting analysis of OPN and ALP expression by BMSCs cultured in the culture medium (negative) and macrophage/scaffold conditioned medium [150] (Copyright 2104, Elsevier). (c) The quantification of the staining of OCN and Runx2 using ImageJ software and the expression of OCN analyzed by real-time PCR [156] (Copyright 2019, Biophysical Society). (d) Alizarin Red S staining of BMSCs treated with macrophage-derived conditioned medium (CM) (+BMP2 CM or −BMP2 CM) in the osteogenic differentiation condition for 21 days; gene expressions of ALP, OPN, and Col I detected by real-time PCR and representative western blots of ALP and OPN after BMSCs were cultured with macrophage-derived CM for 3 days [160] (Copyright 2018, Mary Ann Liebert Inc.).

[16]. Thus, understanding biomaterial-immune cell interactions will significantly contribute to bone regeneration and will open promising venues for the development of bone tissue engineering.

9. Conclusion and Perspectives

The application of biomaterials in bone healing greatly relies on the understanding of the fine balance between the inflammatory response of immune cells and repair activity mounted by stem cells. The success of biomaterials for bone regeneration depends not only on how well the biomaterials integrate with in vivo local bone microenvironments but also on the regulation of the key bone healing events. Although surface functionalization of biomaterials can induce enhanced adhesion, proliferation, and differentiation of stem cells, which has been extensively investigated, the role of inflammation as a mediator has not yet been completely understood. Inflammatory reactions accompany the entire osteogenesis process and play pivotal regulatory roles. Thus,

the response of the macrophages to biomaterials and its role in driving the osteogenic differentiation of MSCs is worth further investigation. Recent studies have revealed that the surface and mechanical properties of biomaterials can be efficiently used to adjust the functions and interplay of the stem and immune cells. Consequently, macrophages have been considered a key cell type in the inflammatory response, and the transformation of macrophage polarization in time and space is a key event that affects osteogenesis. Paracrine action can be the primary mode that affects the osteogenesis of stem cells. Additionally, the influence of biomaterials on the regulation of inflammatory response and its role in osteogenic differentiation of progenitor and stem cells is mainly restricted to *in vitro* studies and must be further assessed *in vivo*. Moreover, the study of inflammation should be extended to other critical cells of the immune system, including dendritic and T cells.

Conflicts of Interest

The authors declare no conflicts of interest with respect to the authorship and/or publication of this review.

Acknowledgments

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