REVIEW ARTICLE
Iran J Allergy Asthma Immunol
October 2018; 17(5):398-408.

Modulation of Macrophage Polarization for Bone Tissue Engineering Applications

Zahra Jamalpoor[1], Alireza Asgari[2], Mohammad Hossein Lashkari[3], Abbas Mirshafiey[4], and Monireh Mohsenzadegan[5]

[1] Trauma Research Center, AJA University of Medical Sciences, Tehran, Iran
[2] Aerospace Medicine Research Center, AJA University of Medical Sciences, Tehran, Iran
[3] Department of Surgery, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
[4] Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[5] Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

Received: 21 August 2017; Received in revised form: 3 December 2017; Accepted: 13 December 2017

ABSTRACT

Innate immune cells play a crucial role in bone development and repair. Macrophages are the main effector cells in immune responses to implants and are indispensable for bone healing success. The heterogeneity and plasticity of macrophages make them a prime target for immune system modulation to enhance bone repair and regeneration. It is believed that the polarization of macrophage phenotype towards the anti-inflammatory M2, rather than the inflammatory M1 phenotype, promotes osteogenesis. Tissue-engineered bioimplants are potentially capable of producing signals to modulate macrophage polarization. Therefore, development of smart immunomodulatory bioimplants via manipulation of their properties seem a promising strategy for tuning immune responses to optimize bone repair without any unwanted inflammatory reactions. The purpose of the present review is to summarize the currently available studies performed on the effects of macrophage polarization, especially towards M2 phenotype, both in bone repair and in bioimplant-stimulated osteogenesis. Moreover, this literature highlights the need to focus future studies on the development of smart immunomodulatory implants capable of switching macrophage polarization-enhancing bone implant-host tissue integration.

Keywords: Bone repair; Bone tissue engineering; Macrophage polarization; Osteogenesis

INTRODUCTION

Immediately after the implantation of the tissue-engineered bone substitute, macrophages as the primary effector cells are recruited into the implant site and elicit unavoidable inflammatory response, namely foreign body reaction, which may or may not favor the repair. Since macrophage-induced inflammation is almost the sole determinant of the long-term fate of bioimplants, their properties are to be

Corresponding Author: Monireh Mohsenzadegan, PhD; Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Iran University of Medical Sciences (IUMS) Hemmat Highway, Tehran, Iran. Tel: (+98 21) 8670 4681, Fax: (+98 21) 8862 2533, Email:monirehmohsenzadegan@gmail.com, mohsenzadegan.m@iums.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

carefully designed in order to escape unwanted immune response.[1]

Biocompatibility is one of the properties of tissue-engineered constructs. Traditionally, those constructs that were inert by the view of the elements of the host immune system were considered favorable. However, in the contemporary view, this approach does not suffice. The constructs need be immunomodulatory, to force the immune cells, in particular, macrophages, adapt an active positive role in bone repair.[2,3]

Macrophages are heterogeneous and plastic in nature in a sense that their phenotypes/polarizations span a wide spectrum from inflammatory M1 to anti-inflammatory or pro-regenerative M2 cells. Microenvironmental signals within the injury or implant site modulate the polarization of macrophages, potentially letting regenerative processes to settle down.[4-7] Despite the fact that macrophages are the first to rush into the injury site, and also remain in situ for long, they have not attracted enough attention of researchers to unveil their non-immune functions. Miron and colleagues reported in a systemic review that only 10% of investigations in the area of orthopedic and dental implants have included immune cells behavior on implant surfaces.[8] It seems crucial, however, to revisit both the distinct and the integrative roles of different macrophage populations in bone repair.

In the present review, we attempt to summarized macrophage characterizations, modulation, and their interaction with implanted bone substitutes to shed light to the path of developing smart immunomodulatory bone implants, which may be the key advancement to benefit from the regenerative capacity of this powerful endogenous cell source.

Macrophages

Macrophages Origin and Function

Macrophages are differentiated immune cells. They are activated in response to diverse environmental signals and participate in different functions including phagocytosis of microbial and parasites pathogens, efferocytosis of apoptotic cells, presenting antigen cells to T lymphocytes, inflammation, vascularization, wound healing and immunomodulation.[9-11] Macrophages can be categorized into two groups according to their origin: tissue-residing and tissue-circulating**.**

Tissue-residing macrophages are derived from the yolk sac in the utero during early stages of the embryogenesis.[12] Liver Kupffer cells, sinusoidal macrophages in the spleen, alveolar macrophages in the lung, and microglial cells in the brain are examples of tissue-reside macrophages.[8,13] Due to high proliferation rate, tissue-reside macrophages can maintain local population during tissue injury.[14] OsteoMacs are bone residing macrophages. They are one of the first cell types coming in contact with bone bioimplants and potentially determining the success or failure of the implants. Due to anatomical location, it is assumed that OsteoMacs act as precursors for osteoclast and/or multinucleated giant cells (MNGCs) participating in bone tissue.[8] OsteoMacs are involved in bone tissue development, homeostasis, modeling, and remodeling.[15-17] It has been shown that OsteoMacs regulate osteoblast function and are needed for mineralization in vitro and in vivo[18] and depletion of OsteoMacs not only reduces the number of osteoblasts but also raises cytokines secretion such as angiopoietin-1, KIT ligand, and CXCL12.[19]

Tissue-circulating macrophages are derived from circulating monocytes ($CD115^+$ $CD11b^+$ $CD14^+$ $CD16^-$ cells), which in turn are derived from bone marrow hematopoietic stem cells.[2,20] For further reading Das et al and Ogle et al are recommended.[2,10]

Macrophage Heterogeneity and Plasticity

Macrophages are a heterogeneous cell population and show a wide spectrum of polarization/phenotype from classically pro-inflammatory M1 until alternative anti-inflammatory M2 phenotype related to their functional diversity.[6,21] Macrophage polarization into M1 pro-inflammatory or M2 anti-inflammatory/wound healing macrophages is very importance in bone regeneration and hence the significance of bone/biomaterial interfaces lies within their abilities to polarize macrophages either into M1 or M2 macrophages.

Pro-Inflammatory M1 Macrophages

The pro-inflammatory M1 phenotype is classically activated by injury, infection, bacterial products [lipopolysaccharide (LPS)] and cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ.[22] They produce high levels of reactive oxygen species (ROS), nitric oxide (NO) and pro-inflammatory cytokines such as interleukin (IL)-1, IL-2, IL-6, IL-12, TNF-α and IFN-γ.[22,23] The M1 produced inflammatory

cytokines participate in boosting host defense against pathogens, clear necrotic tissues and activate some immune system components such as natural killer cells and T helper (h)1. However, over-stimulation of M1 macrophages is associated with the production of IL-1, IL-6, and IL-23 cytokines resulting in the differentiation of T cells into the pathogenic TH17 subset leading to tissue damage and giving rise to autoimmune disease pathogenesis.[24]

Anti-Inflammatory M2 Macrophages

The anti-inflammatory M2 phenotype is alternatively activated by different signals such as IL-4 and/or IL-13 coming from basophils and mast cells, IL-1 receptor ligands or IL-10, immune complexes and toll-like receptors (TLRs).[25,26] M2 macrophages are visualized by the expression of several surface receptors such as the mannose receptor CD206, CD163 (a scavenging receptor), dectin-1 and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN).[27,28] These cells are characterized by the production of anti-inflammatory cytokines such as IL-10, Chemokine (C-C motif) ligand (CCL)-18, and CCL-22 and low expression of IL-12.[24] M2 macrophages may produce a number of osteogenic growth factors such as BMP-2,[29] TGF-β,[30] osteopontin,[31] 1,25-dihydroxy-vitamin D3.[32] The growth factors code for extracellular matrix deposition and new bone formation and are classical features of the M2 macrophage.[8]

M2 macrophages, a non-uniform population, are further subdivided into M2a, M2b, M2c, and M2d categories based on signals activated, cell surface markers, and a wide spectrum of functions. Naïve macrophages can be switched into M2a phenotype by IL-4 and/or IL-13, and are involved in immune responses against parasites mediated by Th2 cells. M2a macrophages, also called wound-healing macrophages, are pro-fibrotic identified by high surface expression of IL-4R and FcεR, Dectin-1, CD163, CD206 and CD209.[33] Moreover, they express high levels of arginase-1 in response to IL-4 that depletes L-arginine, thereby suppressing T cell responses and depriving iNOS of its substrate. Arginase-1 provides precursors for collagen and fibroblast stimulating factor, thus supporting their role in extracellular matrix sediment and wound closure.[24] Given the role of M2a macrophages in the remodeling of extracellular matrix and wound stabilization, however, they must be carefully investigated to prevent unfavorable fibrotic changes in an injured tissue or surrounding biomaterial implants.[34]

M2b macrophages are considered as an immune-regulating member of the M2 macrophage family and are instigated by IL-1R ligands, LPS, and immune complexes. In addition to IL-10, they produce IL-1, IL-6, and TNF-α.[2] M2c macrophages increase in number in presence of IL-10, TGF-β and glucocorticoids. This subgroup was defined as deactivated or anti-inflammatory macrophages and is known to be involved in tissue repair, remodeling, and angiogenesis.[35] They produce anti-inflammatory cytokines such as IL-10 and TGF-β and express multiple receptors, such as CD163, CD206 and RAGE receptors.[36] The M2d macrophages, when activated by IL-6 and adenosines produce various cytokines including IL-10, TGF-β, and IL-12 as well as vascular endothelial growth factor (VEGF)-A.[37,38] For further details, the following well-written review are suggested.[39]

Macrophage Modulation

Non-Aided M2 Macrophage Polarization in Bone Healing

Bone healing is a complex four-stage process comprising of inflammation, soft callus formation, hard callus formation and bone remodeling stages.[40] Immediately after bone injury and following the orchestrated microenvironmental signals such as secretion of pro-inflammatory cytokines, macrophages infiltrate into injury site and initiate bone repair via triggering inflammatory cascade and secretion of both pro-inflammatory and regenerative cytokines.[41] Macrophage recruitment into the injury site is mediated by CC chemokine CCL-2 and its receptor, chemokine (C-C motif) receptor (CCR)-2.[2,42] Early inflammatory phase created by macrophages are critical for successful bone repair, for any macrophage depletion result in impaired vascularization, reduced formation of callus, and delayed maturation of cartilage.[42,43] Figure 1 schematically shows macrophage polarization during early and late phases of bone repair. Monocytes and uncommitted M0 macrophages polarize towards M1 macrophages in the presence of IFN-γ, LPS and TNF-α found in abundance at inflammatory injury microenvironment.[44,45] M2 macrophages produce lesser OSM in comparison with M1 macrophages.

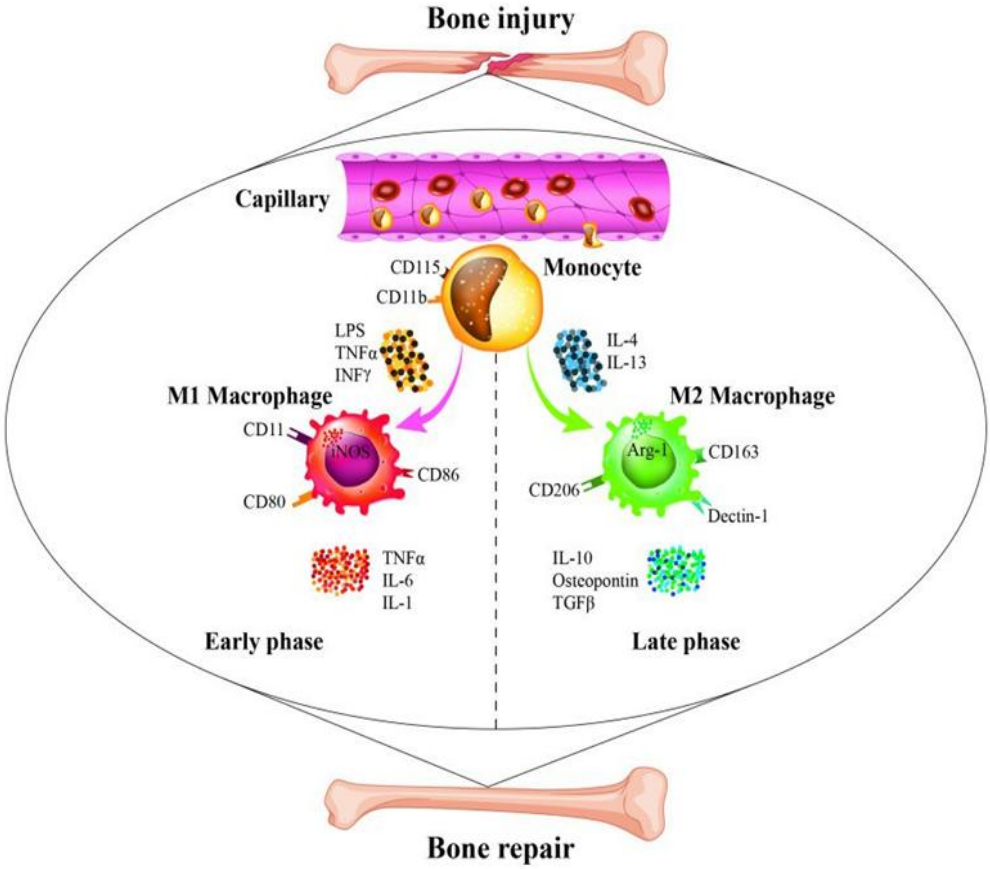

Figure 1. Bone repair process in early and late phases. Following an injury, the monocytes penetrate into the site of injury out of blood circulation via diapedesis. During the early phase of repair, the inflammatory microenvironment (microbial products; lipopolysaccharide (LPS), interferon (IFN-γ) and tumor necrosis factor (TNF-α) pushes macrophages towards M1 polarization. It has been shown that early secretion of pro-inflammatory cytokines including TNF-α, interleukin (IL)-6, IL-1, macrophage colony-stimulating factor (M-CSF), and inducible nitric oxide synthase (iNOS) are necessary for early bone repair. During the late phase of repair, changes in microenvironment due to IL-4 and IL-13 secretion originating for T helper (h)2 and the process of efferocytosis (clearance of apoptotic cells) stimulate the macrophages toward M2 polarization. These macrophages produce IL-10, transforming growth factor beta (TGF)-β and osteopontin that enhance bone repair.

TNF-α, secreted by M1 macrophages, plays an essential role in elevating postnatal bone repair through an increase in recruitment of osteoprogenitor cell or osteogenic cell activation in the context of intramembranous bone formation. Gerstenfeld and colleagues demonstrated that the intramembranous ossification, as well as type I collagen and osteocalcin mRNAs expression, were immensely reduced in double TNF-α gene knockout mice ($p55^{-/-}p75^{-/-}$). They concluded that pro-inflammatory cytokines are necessary for successful bone healing.[46] In addition to TNF-α, secretion of other inflammatory cytokines (e.g., IL-6, IL-1, IL-12, IL-23, macrophage colony-stimulating factor -M-CSF-, iNOS, and oncostatin M -OSM-) by M1 macrophages have been proven in various in vivo studies.[27,47-49] OSM as a major M1 macrophage cytokine regulates osteogenic differentiation of MSCs and matrix mineralization.

In the late inflammatory phase, when inflammatory cytokines subside and the levels of T-lymphocyte cytokines (e. g., IL-4, and IL-13) rise, M0 and M1 macrophages will gradually polarize towards M2 anti-inflammatory/regenerative macrophages. M2 cytokine secretion profile IL-4, IL-13, IL-10, IL-1ra, Arginase 1 and chitotriosidase lead to a downregulation of the inflammatory responses and upregulation of

regenerative pathways such as angiogenesis, extracellular matrix formation, and remodeling.[50-52] Potent bioactive growth factors for osteoblasts including BMP-2,[53] BMP-4[54] and Wnt family members[16] produced mainly by M2 macrophages are inducers of extracellular matrix deposition and new bone formation.[8]

Guihard et al claimed[25] that bone formation was stimulated by M1-derived OSM in conditions of inflammation, infection and/or injury, not attributable to M2 macrophages, despite their known role in tissue repair.[55,56]

Wu et al created a bone injury model, osteonecrosis, by injection of methylprednisolone (corticosteroid medication) in mice. During the early stage of osteonecrosis, M1 inflammatory cells infiltrate into the necrotic zone and secrete high levels of TNF-α. However, in the late stage of osteonecrosis, the expression of TNF-α gradually decrease, followed by the appearance of a large M2 cell population in the necrotic area. The authors speculated that a high number of M2 macrophages could account for reduced inflammation, hence promoting tissue repair. Their claim was further supported and confirmed by the histologic findings of the new bone formation around the necrotic bone.[57]

Considering the significance of M2 macrophages in bone regeneration in the late phase of injury, in the proceeding sections, we will discuss drugs, cytokines and biomaterial agents potentially capable of polarizing macrophages towards M2 macrophage phenotype.

Tissue Engineering-Aided M2 Macrophage Polarization in Bone Healing

Modification of an implant surrounding milieu may potentially bring about macrophage polarization, where their functions will follow suite. To adopt such a strategy, one can look into signals stemming from three main components of any tissue engineered construct. Not only that, their respectful components may display a crucial role in macrophage modulation. What needs to keep our gaze into is the M1/M2 ratio, a critical dynamic index that oscillates temporally according to the necessities of any particular phase of the healing. Accordingly, any core constituents of the construct can chemically be targeted with the aim of making them immunomodulatively smart, bringing about the just-needed temporal variations in macrophage phenotypes and different landscapes of signaling molecules, all in favor of rapid and more natural healing of the wound. The following subsections are an attempt to cover the state of the art in this strategy.

Scaffold-Aided M2 Macrophage Polarization

Bone scaffolds are synthetic ECM made from different biomaterials such as biodegradable natural and synthetic polymers and ceramic using diverse fabrication techniques such as electrospinning and Freeze drying.[58-60] These 3D matrices should support osteogenic cell attachment, recruitment, growth, proliferation, and differentiation. In addition, they should induce or support vascularization.[61] Thus physical, chemical and mechanical scaffold's properties should be regulated carefully,[62] since Scaffold properties have a significant influence on host immune responses, they may raise detrimental or otherwise beneficial reactions that remarkably modify the healing process. In smart immunomodulative scaffolds, these properties should be manipulated in such an optimized manner that can switch macrophage polarization towards M2 phenotype. However, keeping the balance seems critical, for an excessive number of M2 macrophages may have the opposite effect in stimulating the immune system.[63]

Physicochemical properties of scaffolds including geometry, topography, pore size, porosity, surface charge and hydrophobicity can influence implant-host interaction and hence alter the cell surface adherence and function.[2] Lee et al showed that chemical surface modification using divalent cations (Ca^{2+} and Sr^{2+}) combined with the nanostructured Ti surface dramatically polarize J774 macrophages cell line into regenerative M2 macrophage phenotype. They concluded that surface bioactive ion modification may prove beneficial by reducing inflammatory condition.[64] Similarly, Chen et al demonstrated that macrophages in response to β-Tricalcium phosphate β-TCP extracts (i.e., one of the main combustion products of bone) were switched to M2 phenotype. The polarization led to the release of osteoinductive molecules and anti-inflammatory cytokines, such as BMP2 that promoted the differentiation of bone marrow-derived mesenchymal stem cells (BMSCs) into the osteoblast.[3]

Magnesium ion (Mg^{2+}) is another divalent used in the fabrication of scaffolds, however, it evokes an excessive inflammatory response, restricting its efficacy in bone tissue engineering.[65] To control the detrimental osteoimmunomodulatory feature of Mg^{2+}

scaffolds, Chen and colleagues coated Mg^{2+} scaffolds with β-TCP. They demonstrated that such scaffolds can switch the macrophage into the M2 phenotype, giving rise to BMP2 and vascular endothelial growth factor (VEGF), enhancing osteogenic differentiation of BMSCs.[3] In another report, Chen et al suggested that the potential underlying mechanisms may rest in inhibition of the TLR receptor pathway and reciprocal activation of the BMP2 signaling pathway.[66]

In dental implantology in vitro conditions, Barth et al, showed that high RAW 264.7 cell density (mouse macrophages cell line) on rough SLA (sandblasted and acid etched) implants were associated with the early bone formation. It was found that SLA promoted polarization of macrophages towards M2-like phenotype through upregulation of the macrophage attractant chemokines such as MCP-1and MIP-1a and concomitant downregulation of the secretion of the M1-typical chemokines such as IP-10. These findings indicate that early bone formation occurs more rapidly on the macrophage-rich SLA implants with M2 polarization promoting implant-induced healing and osseointegration.[67] For further details in regards to the role of scaffold's different biomaterials in macrophage modulation, the reader is recommended to read the review by Sridharan et al.[39]

Cell-Aided M2 Macrophage Polarization

In bone tissue engineering, a variety of primary and/or stem cells, either in 2D/3D forms, mono/cocultured, are introduced to the site of injury, where they themselves may demonstrate osteogenic activities or may recruit native cells to show osteogenic/angiogenic activities by the production of selective growth factors. Hoovering upon more recent investigations in the type of cells utilized, our understanding of the reciprocal role of macrophages in implants is superficial. Amongst few studied carried out, one encounters a possibility of either direct or indirect interaction between the implanted cells with host macrophages. Horwood and colleagues[68] and Sridharan et al[39] have covered the relevant details in their reviews.

MSCs play a central role in bone repair due to their osteogenic potential and capacity. Gong et al showed that coculture of MSC with macrophages polarizes macrophages towards M2 phenotype along with an increase in alkaline phosphatase (ALP), osteogenic markers and bone mineralization paralleled by a decrease in M-derived cytokines.[69] Another study showed that there is direct cell-to-cell interaction between macrophages and osteogenic cells.[70] Guihard et al reported that M1 inflammatory macrophages, and not M2 cells, produce OSM via prostaglandin (PG)- E2 and cyclooxygenase (COX)-2 pathway,[71] differentiating MSCs into osteoblasts.[25] Since there is not a general consensus among researchers in this area, further detailed studies are required to investigate the interaction between MSC-macrophage modulation during developmental osteogenesis and bone repair.

Signaling Molecules-Aided M2 Macrophage Polarization

Signaling molecules are an ensemble of cytokines, growth factors, peptides, nucleotides, and drugs that activate or inactivate specific signaling pathways, capable of osteogenic induction. Routes to up the level of the signaling molecules are diverse: injected directly into the site, released from implanted scaffolds, or even secreted from already seeded cells. Alvarez and colleagues[72] have reviewed the means of immunomodulative signaling molecules release from scaffolds in their more recent stunning review. Regardless of the mode of administration, the quantity of signaling molecules at the site of implant is to match the exact phase of the bone healing. Activation of M2 macrophages favors the osteogenesis by further releases of anti-inflammatory cytokines. On the contrary, over-activation of M1 macrophages would entail a rise in inflammatory cytokines, postponing the healing process. Therefore, a temporally adjusted balance in the ratio of M1/M2 is what is expected of smart immunomodulative constructs.

Schlundt et al induced the M2/Th2 phenotype through local administration of IL-4 and IL-13 in the fractured area in C57BL/6 mice leading to a higher bone formation in comparison to the untreated control group during the 3-week treatment period.[73] The results address the bone repair potentials of M2 macrophage polarization. In another study, Loi et al showed that pre-osteoblastic MC3T3 cells co-cultured with M1 macrophages increase ALP activity, osteocalcin concentration, and matrix mineralization. The osteogenesis by M1-MC3T3 co-cultures was further enhanced by macrophage phenotype modulation to M2 via IL-4 treatment 72 hours after seeding. The findings indicate that modulating M1 macrophages into the M2-like phenotype through IL-4 treatment can mimic the physiological shift from inflammation to tissue

regeneration.[74] Furthermore, Silfversward et al showed that male mice depleted of Th2-associated anti-inflammatory cytokines, including IL-4 and IL-13, had reduced cortical bone mass and strength, indicating that these cytokines are somehow involved in the modulation of bone regeneration.[75] However, the same researchers have recently shown that IL-4 and IL-13 depleted mice demonstrated no alterations in fracture healing or heterotopic bone formation, despite an earlier reduction in cortical bone mass in mice. In addition, they reported an altered expression of autonomous nerve… and markers of neovascularization in the same depleted mice. The authors suggested that the relatively weak effects of IL-4 and IL-13 in bone formation can be supported by other factors including insulin-like growth factor (IGF)-1, growth hormone (GH), transforming growth factor-β1 (TGF)-β1 and bone morphogenetic proteins (BMPs).[76]

In patients suffering from chronic arthritic disorders and other end-stage degenerative conditions, total joint replacement (TJR) is strongly indicated. However, wear particles from artificial joints appear in the same space as time goes by. Rao et al aimed to control the inflammatory reactions that occur in response to wear particles production, specifically polarization of M1 macrophages towards the M2 phenotype by IL-4. They reported that wear particles induce a pro-inflammatory process for osteolysis, may potentially be modulated into M2 macrophages, promoting tissue healing and angiogenesis rather than periprosthetic osteolysis and implant loosening.[45]

Clodronate (brand name: Bonefos) is an anti-osteoporotic drug approved for the prevention and treatment of osteoporosis in men and post-menopausal women to decrease complications of hyperparathyroidism, vertebral fractures, hypercalcemia due to underlying medical condition, multiple myeloma and fracture-related pain because of its potent anti-inflammatory and analgesic effects via a decrease in inflammatory cytokines such as IL-1β, IL-6, and TNF-α.[77] Cho et al showed that in clodronate liposome model, any depletion of mature phagocytic macrophages or M1 macrophage was associated with increased trabecular bone mass and increased parathyroid hormone-induced anabolism. Moreover, clodronate liposomes increased efferocytosis and gene expression associated with M2 macrophages as well as expression of genes associated with bone formation including Wnt3a, Wnt10b, and TGF-β1.[16] The results indicate that clodronate induces macrophage polarization towards M2 and osteogenesis through up-regulation of Wnts and TGF-β1 production in bone. Another study reported that clodronate-induced macrophage depletion does not disrupt early bone healing phase, but severely delayed hard callus formation. They observed that M2 macrophages were obviously predominant during the ossification phase and concluded that in order to meet a successful bone repair M1/M2 macrophage ratio should be well-balanced.[73]

CONCLUSION

This review is meant to serve as a pillar for tissue engineering to advance basic and clinical studies with aims of modulating the microenvironment of injury site for optimal bone regeneration. Macrophages are heterogeneous and plastic in nature; the two main phenotypes are M1 and M2, the absolute number and the ratio of which vary in different stages of repair. Signals present in the environment trigger phenotype switching. Inflammatory M1 macrophages are those involved in the secretion of inflammatory factors prolonging the repair process. M2 macrophages, on the other hand, are those cells that hasten the healing process by releasing anti-inflammatory factors.[78] It is noteworthy that neither a reduction in the number of M1 macrophages nor an increase in M2 macrophages *per se* would favor the success of repair. Immunocompatibility refers to the overall attributes of the implant where the key players not only deactivate the foreign body reaction but also allow the implant to enjoy the integration with the host tissue via the immune system enhancement. Components of the scaffolds and their physicochemical properties, as well as types of the biomaterials utilized, cytokines and type of cells seeded, are the main factors encoding the immunomodulatory features of the smart implant. Therefore, in-depth acquisition of further evidence of key elements and their interplay, as well as an adequate and effective spatiotemporal macrophage phenotype switching, are what determine the outcome of the implant.

Future Prospective

There seems to be an intrinsic algorithm that runs the switching for and against different stages of the battle at the site of the implant. Any repair strategy that

mimics best the subtending spatiotemporal interactions and hijacks the capabilities of the immune system in their own favor seem promising in rendering implants smart.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support extended by AJA University of Medical Sciences, Tehran, Iran and Iran University of Medical Sciences, Tehran, Iran.

REFERENCES

1. Bartneck M, Heffels K-H, Pan Y, Bovi M, Zwadlo-Klarwasser G, Groll J. Inducing healing-like human primary macrophage phenotypes by 3D hydrogel coated nanofibres. Biomaterials 2012; 33(16):4136-46.
2. Ogle ME, Segar CE, Sridhar S, Botchwey EA. Monocytes and macrophages in tissue repair: Implications for immunoregenerative biomaterial design. Exp Biol Med 2016; 241(10):1084-97.
3. Das TP, Suman S, Damodaran C. Induction of reactive oxygen species generation inhibits epithelial–mesenchymal transition and promotes growth arrest in prostate cancer cells. Mol Carcinog 2014; 53(7):537-47.
4. Brown BN, Ratner BD, Goodman SB, Amar S, Badylak SF. Macrophage polarization: an opportunity for improved outcomes in biomaterials and regenerative medicine. Biomaterials 2012; 33(15):3792-802.
5. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci 2008; 13(15):453-61.
6. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; 23(11):549-55.
7. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 2000; 164(12):6166-73.
8. Mohsenzadegan M, Fayazi MR, Abdolmaleki M, Bakhshayesh M, Seif F, Mousavizadeh K. Direct immunomodulatory influence of IFN-β on human astrocytoma cells.Immunopharmacol Immunotoxicol 2015; 37(2):214-9.
9. Das A, Ganesh K, Khanna S, Sen CK, Roy S. Engulfment of apoptotic cells by macrophages: a role of microRNA-21 in the resolution of wound inflammation. J Immunol 2014; 192(3):1120-9.
10. Das A, Sinha M, Datta S, Abas M, Chaffee S, Sen CK, et al. Monocyte and macrophage plasticity in tissue repair and regeneration. Am J Pathol 2015; 185(10):2596-606.
11. Jafarnezhad-Ansariha F, Yekaninejad MS, Jamshidi A-r, Mansouri R, Vojdanian M, Mahmoudi M, et al. The effects of β-d-mannuronic acid (M2000), as a novel NSAID, on COX1 and COX2 activities and gene expression in ankylosing spondylitis patients and the murine monocyte/macrophage, J774 cell line. Inflammopharmacology 2017:1-10.
12. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5(12):953-64.
13. Ovchinnikov DA. Macrophages in the embryo and beyond: much more than just giant phagocytes. Genesis 2008; 46(9):447-62.
14. Yona S, Kim K-W, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 2013; 38(1):79-91.
15. Raggatt LJ, Wullschleger ME, Alexander KA, Wu AC, Millard SM, Kaur S, et al. Fracture healing via periosteal callus formation requires macrophages for both initiation and progression of early endochondral ossification. Am J Pathol 2014; 184(12):3192-204.
16. Cho SW, Soki FN, Koh AJ, Eber MR, Entezami P, Park SI, et al. Osteal macrophages support physiologic skeletal remodeling and anabolic actions of parathyroid hormone in bone. Proc Natl Acad Sci U S A 2014; 111(4):1545-50.
17. Miron RJ, Zohdi H, Fujioka-Kobayashi M, Bosshardt DD. Giant cells around bone biomaterials: Osteoclasts or multi-nucleated giant cells? Acta Biomater 2016; 46:15-28.
18. Chang MK, Raggatt L-J, Alexander KA, Kuliwaba JS, Fazzalari NL, Schroder K, et al. Osteal tissue macrophages are intercalated throughout human and mouse bone lining tissues and regulate osteoblast function in vitro and in vivo. J Immunol 2008; 181(2):1232-44.
19. Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood 2010; 116(23):4815-28.
20. Kaur S, Raggatt LJ, Batoon L, Hume DA, Levesque J-P, Pettit AR, editors. Role of bone marrow macrophages in controlling homeostasis and repair in bone and bone marrow niches. Semin Cell Dev Biol 2017; 61:12-21.
21. Murray PJ. Macrophage polarization. Annu Rev Physiol 2017; 79:541-66.
22. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003; 73(2):209-12.

23. Genin M, Clement F, Fattaccioli A, Raes M, Michiels C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. BMC cancer 2015; 15(1):577.
24. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8(12):958-69.
25. Guihard P, Danger Y, Brounais B, David E, Brion R, Delecrin J, et al. Induction of osteogenesis in mesenchymal stem cells by activated monocytes/macrophages depends on oncostatin M signaling. Stem cells 2012; 30(4):762-72.
26. Woo SR, Corrales L, Gajewski TF. Innate immune recognition of cancer. Annu Rev Immunol 2015; 33:445-74
27. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 2009; 27:451-83.
28. Fard NA, Tabrizian N, Mirzaei R, Hadjati J, Zavareh FT, Nodeh ARS, et al. Efficacy and Safety of G2013 as a Novel Immunosuppressive Agent on Differentiation, Maturation and Function of Human Dendritic Cells. Iran J Public Health 2017; 46(2):216-221.
29. Champagne C, Takebe J, Offenbacher S, Cooper L. Macrophage cell lines produce osteoinductive signals that include bone morphogenetic protein-2. Bone 2002; 30(1):26-31.
30. Assoian RK, Fleurdelys BE, Stevenson HC, Miller PJ, Madtes DK, Raines EW, et al. Expression and secretion of type beta transforming growth factor by activated human macrophages. Proc Natl Acad Sci U S A 1987; 84(17):6020-4.
31. Takahashi F, Takahashi K, Shimizu K, Cui R, Tada N, Takahashi H, et al. Osteopontin is strongly expressed by alveolar macrophages in the lungs of acute respiratory distress syndrome. Lung. 2004; 182(3):173-85.
32. Kreutz M, Andreesen R, Krause SW, Szabo A, Ritz E, Reichel H. 1, 25-dihydroxyvitamin D3 production and vitamin D3 receptor expression are developmentally regulated during differentiation of human monocytes into macrophages. Blood 1993; 82(4):1300-7.
33. Rőszer T. Understanding the mysterious M2 macrophage through activation markers and effector mechanisms. Mediators Inflamm 2015; 2015:816460.
34. Knipper JA, Willenborg S, Brinckmann J, Bloch W, Maaß T, Wagener R, et al. Interleukin-4 receptor α signaling in myeloid cells controls collagen fibril assembly in skin repair. Immunity 2015; 43(4):803-16.
35. Jetten N, Verbruggen S, Gijbels MJ, Post MJ, De Winther MP, Donners MM. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo. Angiogenesis 2014; 17(1):109-18.
36. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014; 41(1):14-20.
37. Wang Q, Ni H, Lan L, Wei X, Xiang R, Wang Y. Fra-1 protooncogene regulates IL-6 expression in macrophages and promotes the generation of M2d macrophages. Cell Res 2010; 20(6):701-12.
38. Ferrante CJ, Pinhal-Enfield G, Elson G, Cronstein BN, Hasko G, Outram S, et al. The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4Rα) signaling. Inflammation 2013; 36(4):921-31.
39. Sridharan R, Cameron AR, Kelly DJ, Kearney CJ, O'Brien FJ. Biomaterial based modulation of macrophage polarization: a review and suggested design principles. Materials Today. 2015;18(6):313-25.
40. Schindeler A, McDonald MM, Bokko P, Little DG, editors. Bone remodeling during fracture repair: The cellular picture. Semin Cell Dev Biol 2008; 19(5):459-66.
41. Ren PG, Huang Z, Ma T, Biswal S, Smith RL, Goodman SB. Surveillance of systemic trafficking of macrophages induced by UHMWPE particles in nude mice by noninvasive imaging. J Biomed Mater Res A 2010; 94(3):706-11.
42. Sinder BP, Pettit AR, McCauley LK. Macrophages: their emerging roles in bone. J Bone Miner Res 2015; 30(12):2140-9.
43. Xing Z, Lu C, Hu D, Yu Y-y, Wang X, Colnot C, et al. Multiple roles for CCR2 during fracture healing. Dis Model Mech 2010; 3(7-8):451-8.
44. Valladares RD, Nich C, Zwingenberger S, Li C, Swank KR, Gibon E, et al. Toll-like receptors-2 and 4 are overexpressed in an experimental model of particle-induced osteolysis. J Biomedical Materials Research Part A 2014; 102(9):3004-11.
45. Rao AJ, Gibon E, Ma T, Yao Z, Smith RL, Goodman SB. Revision joint replacement, wear particles, and macrophage polarization. Acta Acta Biomater 2012; 8(7):2815-23.
46. Gerstenfeld L, Cho T-J, Kon T, Aizawa T, Cruceta J, Graves B, et al. Impaired intramembranous bone formation during bone repair in the absence of tumor necrosis factor-alpha signaling. Cells Tissues Organs 2001; 169(3):285-94.

47. Gibon E, Lu L, Goodman SB. Aging, inflammation, stem cells, and bone healing. Stem Cell Res Ther 2016; 7(1):44.
48. Einhorn TA, Majeska RJ, Rush EB, Levine PM, Horowitz MC. The expression of cytokine activity by fracture callus. J Bone Miner Res 1995; 10(8):1272-81.
49. Guihard P, Boutet M-A, Brounais-Le Royer B, Gamblin A-L, Amiaud J, Renaud A, et al. Oncostatin m, an inflammatory cytokine produced by macrophages, supports intramembranous bone healing in a mouse model of tibia injury. Am J Pathol 2015; 185(3):765-75.
50. Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003; 3(1):23-35.
51. Song E, Ouyang N, Hörbelt M, Antus B, Wang M, Exton MS. Influence of alternatively and classically activated macrophages on fibrogenic activities of human fibroblasts. Cell immunol 2000; 204(1):19-28.
52. Maresz K, Ponomarev ED, Barteneva N, Tan Y, Mann MK, Dittel BN. IL-13 induces the expression of the alternative activation marker Ym1 in a subset of testicular macrophages. J Reprod Immunol 2008; 78(2):140-8.
53. Muller PA, Koscsó B, Rajani GM, Stevanovic K, Berres M-L, Hashimoto D, et al. Crosstalk between muscularis macrophages and enteric neurons regulates gastrointestinal motility. Cell 2014; 158(2):300-13.
54. Kan L, Liu Y, McGuire TL, Berger DMP, Awatramani RB, Dymecki SM, et al. Dysregulation of local stem/progenitor cells as a common cellular mechanism for heterotopic ossification. Stem cells 2009; 27(1):150-6.
55. Benoit M, Desnues B, Mege J-L. Macrophage polarization in bacterial infections. J Immunol 2008; 181(6):3733-9.
56. Hamilton JA, Tak PP. The dynamics of macrophage lineage populations in inflammatory and autoimmune diseases. Arthritis Rheum 2009; 60(5):1210-21.
57. Wu X, Xu W, Feng X, He Y, Liu X, Gao Y, et al. TNF-a mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. Int J Immunopathol Pharmacol 2015; 28(3):351-61.
58. Jamalpoor Z, Mirzadeh H, Joghataei MT, Zeini D, Bagheri-Khoulenjani S, Nourani MR. Fabrication of cancellous biomimetic chitosan-based nanocomposite scaffolds applying a combinational method for bone tissue engineering. J Biomed Mater Res A 2015; 103(5):1882-92.
59. Jamalpoor Z, Ebrahimi M, Amirizadeh N, Mansoori K, Asgari A, Nourani M. Fibrin sealant as scaffold can be a suitable substitute to autograft in short peripheral nerve defect in rats. J Dev Biol Tissue Eng 2011; 3(6):75-9.
60. Pati F, Song TH, Rijal G, Jang J, Kim SW, Cho DW. Ornamenting 3D printed scaffolds with cell-laid extracellular matrix for bone tissue regeneration. Biomaterials 2015; 37:230-41.
61. Karimpour Malekshah A, Talebpour Amiri F, Ghaffari E, Alizadeh A, Jamalpoor Z, Mirhosseini M, et al. Growth and chondrogenic differentiation of mesenchymal stem cells derived from human adipose tissue on chitosan scaffolds. J Babol Uni Med Sci 2016; 18(9):32-8.
62. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. Trends Biotechnol 2012; 30(10):546-54.
63. Anderson JM, Rodriguez A, Chang DT, editors. Foreign body reaction to biomaterials. Semin Immunol 2008; 20(2):86-100.
64. Lee C-H, Kim Y-J, Jang J-H, Park J-W. Modulating macrophage polarization with divalent cations in nanostructured titanium implant surfaces. Nanotechnology. 2016; 27(8):085101.
65. Staiger MP, Pietak AM, Huadmai J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials 2006; 27(9):1728-34.
66. Chen Z, Mao X, Tan L, Friis T, Wu C, Crawford R, et al. Osteoimmunomodulatory properties of magnesium scaffolds coated with β-tricalcium phosphate. Biomaterials 2014; 35(30):8553-65.
67. Barth KA, Waterfield JD, Brunette DM. The effect of surface roughness on RAW 264.7 macrophage phenotype. J Biomed Mater Res A 2013; 101(9):2679-88.
68. Horwood NJ. Macrophage polarization and bone formation: a review. Clin Rev Allergy Immunol 2016; 51(1):79-86.
69. Gong L, Zhao Y, Zhang Y, Ruan Z. The macrophage polarization regulates MSC osteoblast differentiation in vitro. Ann Clin Lab Sci 2016; 46(1):65-71.
70. Nicolaidou V, Wong MM, Redpath AN, Ersek A, Baban DF, Williams LM, et al. Monocytes induce STAT3 activation in human mesenchymal stem cells to promote osteoblast formation. PloS one 2012; 7(7):e39871.
71. Mirshafiey A, Taeb M, Mortazavi-Jahromi S, Ansariha FJ, Rehm BH, Esposito E, et al. Introduction of β-D-mannuronic acid (M2000) as a novel NSAID with immunosuppressive property based on COX-1/COX-2 activity and gene expression. Pharmacol Rep 2017; 69(5):1067-72.
72. Alvarez MM, Liu JC, Trujillo-de Santiago G, Cha B-H, Vishwakarma A, Ghaemmaghami AM, et al. Delivery strategies to control inflammatory response: Modulating

M1–M2 polarization in tissue engineering applications. J Control Release 2016; 240:349-63.
73. Schlundt C, El Khassawna T, Serra A, Dienelt A, Wendler S, Schell H, et al. Macrophages in bone fracture healing: their essential role in endochondral ossification. Bone 2018; 106:78-89.
74. Loi F, Córdova LA, Zhang R, Pajarinen J, Lin T-h, Goodman SB, et al. The effects of immunomodulation by macrophage subsets on osteogenesis in vitro. Stem Cell Res Ther 2016; 7(1):15.
75. Silfverswärd CJ, Larsson S, Ohlsson C, Frost A, Nilsson O. Reduced cortical bone mass in mice with inactivation of interleukin-4 and interleukin-13. J Orthop Res 2007; 25(6):725-31.
76. Silfverswärd CJ, Sisask G, Larsson S, Ohlsson C, Frost A, Ljunggren Ö, et al. Bone formation in interleukin-4 and interleukin-13 depleted mice. Acta Orthop 2008; 79(3):410-20.
77. Österman T, Virtamo T, Lauren L, Kippo K, Pasanen I, Hannuniemi R, et al. Slow-release clodronate in prevention of inflammation and bone loss associated with adjuvant arthritis. J Pharmacol Exp Ther 1997; 280(2):1001-7.
78. Mirshafiey A, Mohsenzadegan M. TGF-beta as a promising option in the treatment of multiple sclerosis. Neuropharmacology 2009; 56(6-7):929-36.