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Review

New insight of immuno-engineering in osteoimmunomodulation for bone regeneration

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ABSTRACT

With the continuous development of bone tissue engineering, the importance of immune response in bone tissue regeneration is gradually recognized. The new bone tissue engineering products should possess immunoregulatory functions, harmonizing the interactions between the bone's immune defense and regeneration systems, and promoting tissue regeneration. This article will interpret the relationship between the bone immune system, bone tissue regeneration, as well as the immunoregulatory function of bone biomaterials and seed stem cells in bone tissue engineering. This review locates arears for foucusing efforts at providing novel designs ideas about the development of immune-mediation targeted bone tissue engineering products and the evaluation strategy for the osteoimmunomodulation property of bone biomaterials.

Contents

1. Introduction

Although bone tissue engineering continues to explore strategies to promote bone tissue healing, bone defects remain one of the most common clinical problems [1]. Biomaterial scaffolds and seed cells are two major elements in bone tissue engineering. Traditional studies of bone tissue engineering products used to repair injury

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are deficient in systemic research on the immune system and the remodeling of the regenerated microenvironment. In recent years, significant progresses have been made in the modification of biological materials and the application of seed cells. One of the consensuses is that new bone tissue engineering productions should possess immunomodulatory functions, with the ability to remodel immune environment and reconstruct tissue regeneration process [2,3].

Osteoimmunology reveals the close relationship between the immune system and the skeletal system. Both systems have many common cytokines and signaling molecules, suggesting that immune cells play a crucial role in bone formation [4]. Optimizing the bone immunomodulatory roles can cause a moderate inflammatory response that balances the formation of osteogenesis and osteoclasts, thereby facilitating bone regeneration. Therefore, increasing researches have focused on the interaction between bone tissue engineering and immune system [5,6]. There is a close relationship between the immune system and the skeletal system, which makes the claim that stimulation of immune cells may contribute to the success and failure of implantation.

This review aims to discuss the relationship between immune cells and bone cells, the effect of tissue engineered bone implantation on the immune microenvironment in the trauma site and the design of tissue engineering bone with the feature of immunoregulation. It's urgent to improving the pre-clinical evaluation system to estimate the immuneregulation function of tissue-engineering bone.

2. Relationship between bone immune system and bone tissue regeneration system

So far, for the sake of promoting tissue repair, the application of tissue engineering to has achieved certain achievements, which are attributable to the treatment strategies in the later stages of wound healing, such as promoting differentiation of stem cells. The immune system is a highly flexible network system which guards the integrity of the organization and regulates the characteristics of the local immune micro-environment [7]. The immune system clears tissue debris and dead cells during tissue repair, recruiting and promoting the proliferation and vascularization of tissue precursor cells [8–10]. Immune homeostasis is indispensable to tissue development, regeneration and repair [11]. Trauma provokes a local and systemic cascade of immune responses, which induces the migration of tissue cells to the site of injury, thereby triggering tissue defense and repair responses. In the past, the immune system was merely considered to reject biological materials [12]. However, recent studies have found that innate immune components are significant determination of the biomaterials remodeling [13].

An overactive immune response in the bone tissue after trauma can direct foreign body reaction. The implant is covered and wrapped by hyperplastic fibrous tissue and cannot be well integrated with the bone tissue. A proper bone immune response will promote a successful integration between the host bone tissue and the implantation surface. Therefore, the immune response is a key factor in determining the fate of the implantation in the body [3].

In addition to causing chronic inflammation, the immune response plays an important role in the integration and osteogenic process of grafts [2]. Inflammatory cytokines IL-1, TNF-αand IL-6 can promote osteoclast differentiation, enhance bone resorption activity and inhibit osteoblast activity and bone formation; anti-inflammatory cytokines like IL-4, IL-10, IL-13 present the opposite effect [14]. Apart from osteoblasts, B cells has also been shown to be the major source of bone marrow-derived osteoprotegerin, accounting for 64% of the total [15], indicating that B cells are the major suppressors of osteoclastogenesis under physiological conditions.

Therefore, making use of bone tissue engineering to promote bone healing, we must take full advantage of the role of immunomodulation to promote bone formation, regulate the immune microenvironment of bone tissue regeneration, and mobilize the body regeneration system. The term “osteoimmunomodulation” is used to describe this process [4].

3. Biomaterial-mediated bone regeneration immune response

In innate immunity, macrophages have been paid much attention due to their plasticity and polymorphism in the interaction between a biological material and a host [16–18]. Macrophages are highly malleable and can adapt to environmental signal changes by altering their phenotype and physiological function [19]. Therefore, macrophages become the primary targets of research in the innate immune system during biological material-mediated immune regulation.

When the innate immune defense line is broken, the macrophage presents the antigenic information about the biological material to the T cells in the adaptive immunity, causing the $CD4^+$ T cells to differentiate into Th1 or Th17 cells and resulting in a strong T cell rejection response [20]. On the other hand, the physiochemical properties of biomaterials also advance the polarization of $CD4^+$T to Th2 phenotype. Th2 cells boast not only anti-inflammatory effects, but also can promote M2 polarization by the secretion of IL-4. Owing to the polymorphism of its subsets, $CD4^+$T as a component of adaptive immunity is becoming the chief target of investigation in injury repair promotion.

3.1. Macrophage responses in biomaterial-mediated bone regeneration

Macrophage is the most important effector cell in innate immune cells. The long-term immune response and inflammatory response caused by biomaterials are mainly determined by macrophages [21]. M1 has typical surface markers, CD11c and CCR7. M1 causes local inflammation and tissue fibrosis by secrete IL-1, IL-6, IL-8, IL-12, TNF-α and MCP-1, and meanwhile, promotes Th1 cells to secrete Inflammatory cytokines, such as IL-2, IFN-γ, TNF-α, TNF-β, etc., finally resulting in Th1-type inflammatory response [22]. M2 has the typical surface markers CD163 and CD206. M2 secretes cytokines, such as VEGF, bFGF, PDGF, TGF-β, etc. to promote tissue repair, and contribute to the secretion of cytokines, such as IL-4, IL-5, IL-6, IL -10, IL-13 by Th2 cell, leading to Th2-type inflammatory which possesses anti-inflammatory effects and benefit the tissue healing [13].

Apart from the effects on the inflammatory process, macrophages also affect the physiology and pathology of bone [23]. It is well-known that macrophage is progenitor of osteoclasts, participating in bone tissue remodeling and material degradation. Macrophages can also express and secrete a large number of regulatory molecules, such as BMP2, TGF-β, etc., that support osteogenesis [24]. Macrophage is a essential condition for the effective osteogenic mineralization, and the lack of them will shrink the osteogenic potential of osteoblasts [25].

The immune response determines the fate of the bone substitute material in the body, with the formation of new bone or inflammatory fibrous tissue cysts. Macrophages are the major effector cells in the immune response of implants and are indispensable for osteogenic function. However, there are few reports on the effect of macrophages in the process of biomaterial-mediated osteogenesis.

β-tricalcium phosphate (β-TCP) is a widely recognized osteogenic inducing biomaterial which has been broadly employed in clinical bone regeneration. β-tricalcium phosphate is commonly used as a biomaterial model to study the effect of macrophages on material-induced bone formation. β-tricalcium phosphate convert macrophages phenotype into M2 type, associated with the activation of the calcium-sensing receptor (CaSR) pathway. At the same time, it was also found that β-tricalcium phosphate is able to significantly increase the expression of bone morphogenetic protein 2 (BMP2) in M2. This suggests that macrophages may be involved in the process of osteogenesis stimulated by β-tricalcium phosphate [26]. When conditioned media of co-cultured macrophages and β-tricalcium phosphate extract is applied to bone marrow mesenchymal stem cells (BMSCs), osteogenic differentiation of bone marrow mesenchymal stem cells is significantly enhanced, indicating that macrophages plays a vital role in the process of bone formation induced by biomaterial [27].

3.2. T-cell response in biomaterial-mediated bone regeneration

The functional polarization of macrophages to pro-inflammatory M1 or anti-inflammatory M2 relies on T cell involvement [28]. The transition of cytokines profiles from Th1 cells to Th2 cells is related to the M1/M2 phenotype, which suggests that T cells are target cells in the process of inflammation and regeneration. Therefore, not only are macrophage considered as the target cells for immune regulation and tissue regeneration promotion, but also CD4+ T cells become a controlled object for promoting tissue repair, by virtue of their multiple phenotype and versatility. When biological materials are designed, it is necessary to modulate immunity and reveal the mechanism of activation of immune cells, especially the mechanism of the interaction between macrophages and T lymphocytes [29].

Some biomaterials are capable of inducing the body to produce COX-2 after implantation, which in turn causes prostaglandin (PGE2) production. It is generally accepted that PGE2 is a regulator of inflammatory activation, promoting local vasodilation and reinforcing the recruitment and activation of neutrophil, macrophage and mast cell in the early inflammatory phase [30].

PGE2 has been reported to inhibit Th17 cell development and activity through inhibiting IL-12p70 production. PGE2 can also promote Th17 cell development and IL-17 production by enhancing IL-23 expression, eventually leading to inflammation and tissue damage [31]. Therefore reducing the number of Th1 and Th17 cells in the local microenvironment will help to improve the survival and osteogenic differentiation of the implanted MSCs.

Muscle is a potential source of osteoprogenitor cells. Muscle are known to facilitate bone repair, which is often attributed to their high vascular density. In the study of muscle trauma, it was found that biological scaffolds promote tissue regeneration by remodeling the tissue immune microenvironment. Biostimulation-induced tissue regeneration relied on Th2 cells activated by mTOR/Rictor signaling pathway. IL-4 secreted by Th2 cells can promote macrophage polarization [32] and IL-4 polarized macrophages serve as an imperative factor in tissue repair. By the manipulation of adaptive immunity to promote systemic and local regenerative immune responses through biological tissue engineering, the goal of promoting tissue repair is promising to be achieved [32]. A Th2 cell response is considered an essential driving force during the repair of the quadriceps injury. It was found that under physiological conditions, myeloid cells reach a peak one week after injury, and simultaneously a small amount of CD4+ and CD8+ T cells infiltrate at the site of muscle injury, which peak after three weeks. After the acellular matrix material is implanted, the proportion of CD4+: CD8+ T cells will be increased, with the raise of CD+4 T proportion (about 40% of CD4+T in physiological condition and about 70% after treatment of biological scaffold).

CD4+FoxP3+ regulatory T cells will also be present in small amounts and gradually increase over time. Biomaterials induce gene expression of Th2 cells, characterized by increased IL4 expression and decreased Ifng and bx21 (key genes for Th1 cells) [32]. In addition, the expression of Jag2 encoding the Notch ligand Jagged2 is elevated. Jagged2 helps T help cells differentiate into Th2 cells [33]. IL4 encodes Th2 cytokines that play a crucial role in the healing process of muscles.

CD4+ T cell subsets (Th1, Th2, Treg and Th17) can be transformed into each other, so that the body's immune effects and immune suppression be refined in a delicate and complex equilibrium. Thus, the application of novel immunomodulatory biomaterials in regulating the balance of Th1/Th2 and Th17/Treg plays a crucial role in the process of inflammation and bone regeneration [34].

4. Modulatory effects of mesenchymal stem cells (MSCs) in bone regeneration

MSCs, multi-functional stromal cells, are widely sourced and able to differentiate into different phenotypic lineages in vivo and vitro, such as cartilage, bone, fat and nerve [35–38]; providing a great hope for tissue engineering and cell therapy for many diseases, especially the bone disease and bone formation.

Cell therapy is a promising bone defect reconstruction strategy. Mesenchymal stem cells are favored for their ability to regulate immunity—inflammatory reaction and differentiate into bone [39]. The process of the bone tissue regeneration is controlled by a variety of cell proliferation, differentiation, migration, and apoptosis, including osteoblasts, osteoclasts, immune cells, and their Progenitor cells [40]. Previous studies have focused on the ability of transplanted cells to differentiate at the site of bone defects. Now, more and more studies have found other functions of MSCs involving immunomodulatory effects during bone repair [41].

4.1. The immunomodulatory effect of MSCs on macrophages

MSCs-mediated macrophage response is crucial to inflammation and tissue repair [42]. The interaction between MSCs and macrophages helps macrophages to function normally in the immune response [43,44]. The factors released by MSCs exert effects on the polarization, and function of macrophages [45,46], thereby affecting the intensity, duration, and regulation of tissue regeneration. MSCs can secrete various cytokines and chemokines, which recruit macrophages and endothelial cells to repair injuries and promote wound healing [47]. Among them, VEGF-α is not only a significant mediator of physiological angiogenesis, but also an important factor in bone augmentation [48].

Previous studies of MSCs and macrophages interactions indicated that PGE-2 produced by MSCs could convert M1 into M2, or enhance the selective activation of M2 [28]. PGE-2 is able to enhance the expression of IL-10 and TGF-β in macrophages [49]. Anti-inflammatory M2 suppresses immune activity by interacting with NK cells, CD8+ cells and tregs [50].

In addition to PGE-2, paracrine interleukin-1 receptor antagonists (IL-1Ra) can also mediate macrophage M2 polarization [51], and micro-RNAs secreted by MSC are also involved in the regulation of macrophages [52]. MSCs can also modulate the function of macrophages by means of exosome; for instance, CCR2-positive exosomes have an effect on CCL2 recruiting and activating macrophages [53].

4.2. The immunomodulatory effect of MSCs on T cells

Much of the research in immunomodulation effect of MSCs and their secreted factors has examined that they contribute to regulating T cell activation and functional differentiation. Transplanted MSCs can inhibit the function of T cells and impede the infiltration of immune cells at the site of inflammation [54], indicating that MSCs can affect the migration of T cells. When stimulated by inflammatory factors (such as IFN-γ and TNF-α), MSCs overexpress CXCL9 and CXCL10, which recruit T cells round themselves [55] and regulate T cell activation and differentiation. Activated MSCs can produce immunomodulatory factors, such as INOS, IDO, TGF-β, PGE-2, to inhibit T cell proliferation [49]. IDOs have the ability to modulate the response of T cells in inflammation [56]. In addition to IDO, heme oxygenase-1 (HO-1) secreted by MSCs can also suppress T cells [57]. On the other hand, MSCs inhibit T cell activation through different pathways. MSCs can produce FasL and TRAIL which induce apoptosis of T cells and inhibit the activity of T cells [58,59]. Interestingly, when the intensity of inflammation is insufficient to induce iNOS or IDO expression, MSCs can oppositely initiate the T cell infiltration [60].

It has been reported that MSCs can regulate inflammatory response by secreting TGF-β, PGE-2, Notch1, and IL-10 that are able to enhance Treg cell differentiation and inhibit Th17 [61]. In addition to the direct effect on T cells, MSCs can also influence innate T cells through its paracrine-regulated innate immune cells which contanin dendritic cells and macrophages [46,62]. The activation of T cells requires the interaction between T cell receptors and the costimulatory ligand of antigen-presenting cells [62]. MSCs factors have an impact on the level of costimulatory ligands in APCs [63].

In addition, the proteome secreted by MSCs regulates the expression of IL-12, TGF-β, IL-1 and IL-10, in antigen-presenting cells which regulate the differentiation of T cell subsets [49,64]. Through the regulation of T cells and their secreted factors, MSCs plays a beneficial role in the process of bone repair.

4.3. Effect of host immune system on survival of transplanted MSCs

MSCs are expected to change the face of regenerative medicine. Although biomaterials are used to control and manipulate the fate of transplanted MSCs in order to obtain high quality tissue regeneration [65,66], immune rejection caused by host immune cells and pro-inflammatory cytokines remains a major challenge in controlling the fate of transplant stem cells. MSCs-mediated bone regeneration is partially controlled by the local microenvironment, including growth factors, host immune cells, and cytokines [67]. In particular, pro-inflammatory T cells produce IFN-γ to down-regulate the expression of osteogenesis-regulating factors and enhance TNF-α signaling in apoptosis to inhibit MSCs-mediated bone regeneration [67]. Current studies demonstrate that pro-inflammatory T cells, rather than macrophages, affect MSCs-mediated bone tissue regeneration [68].

In bone tissue engineering, an important role of biomaterials is to provide a physiological environment for MSCs, ensure the viability of the encapsulated MSCs, and regulate their function and fate. It has been found that encapsulated hydrogel can physiologically protect implanted MSCs from host immune cells and cytokines, and regulate signal exchange between immune cells and MSCs, especially during the early stages of implantation [68]. Nanotechnology has also been introduced and used to promote cell growth and differentiation. Nanomaterials provide well-structured and tunable surfaces in nanopores for cells adherence and promote cell growth and differentiation [69].

5. Evaluation of immunomodulatory function for bone tissue engineering production

With the continuous development of the interdiscipline of materials and medicine, the design concept of biomaterials has gradually shifted from the evasion of the host immune system to the design of biological materials with the advantage of immunomodulation function. Therefore, it is increasingly important to accurately evaluate the immunoregulatory properties of bone tissue engineering productions. In the process of interaction among biological material, seed cells and host cells, since macrophages and CD4+ T cells have a strong plasticity and functional diversity, they are paid much more attention among all immune cells, and are becoming the key cells to evaluate the immunomodulatory function of biological materials.

Lipopolysaccharide (LPS), also known as lipopolysaccharide or endotoxin, is present on the outer membrane of gram-negative bacteria, with the ability to induce a strong immune response. LPS can also convert macrophages into the pro-inflammatory M1 phenotype, leading to the release of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6 [70,71]. Due to its strong pro-inflammatory effects, LPS is commonly used to activate macrophages for use in inflammation related studies [72]. Biomaterials or tissue engineering products are selected to intervene in the inflammation, and then the anti-inflammatory properties of biomaterials could be determined [73]. Biological material-induced regeneration of the immune microenvironment requires the involvement of Th2 cells because IL-4 secreted by Th2 cells can promote M2 polarization. Therefore, bone tissue engineering products with the ability to promote the differentiation of CD4+ T cells toward Th2 can also proof its function of regulating the immune microenvironment and promoting tissue regeneration and repair.

However, few consensuses has been achieved on how to apply macrophages and CD4+ T cells to detecting the immunomodulatory properties of biological tissue engineering products. Due to the insufficient scientific research on this issue, the uniform standards is absent in the evaluation of immune properties of tissue engineering products [73]. Therefore, with the continuous development of immuno-immune repair-type bone tissue engineering, it is necessary to develop a set of effective, unified and recognized evaluation systems to evaluate the immunomodulatory properties of bone tissue engineering products.

6. Conclusion

The coordinated regulation of bone tissue regeneration and the immune system clearly reflects the extreme complexity of the interaction between them. In the interaction of bone tissue regeneration and the immune system, understanding and application of the osteoimmunomology are becoming more and more important. M2 macrophages secrete growth cytokines to promote tissue repair, and Th2-type inflammatory responses with anti-inflammatory effects also improve tissue healing. Thus M1/M2, Th1/Th2 and Th17/Treg transformation balance should be designed. Establishing a immunomodulation strategy for bone regeneration will become a promising direction.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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