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Stimulation and Acceleration of Bone Regeneration and Fracture Repair by Biomaterials with Immunomodulatory Properties
(A review paper)

Abstract
Background: One of the issues related to stimulating and accelerating bone regeneration and bone repair is the role of the immune system in fracture healing, known as osteo-immunology.
Method: In these review 57 articles of science direct and pumped database is investigated for potential role and mechanism of the immune system's response to bone fracture.
Results: biological materials such as tumor necrosis factor alpha (TNF-α), lipopolysaccharide (LPS), interleukin-17 (IL-17) protein, lipotic Acid (LTA) could regulate the immune system, which have the ability to improve the ossification process and faster healing.
Conclusion: The direction of future research was predicted regarding the emergence of new therapeutic compounds derived from bone-building materials such as bone growth factors and substances that regulate the behaviour of the immune system in order to regenerate bone.
Keywords: Biocompatible materials, Bone regeneration, Immunomodulation, Osteogenesis

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Vahid Zarghami, PhD[1]; Fereshteh Moharramzadeh Jeghanab[2]; Mohammad Ali Shokrgozar, PhD[1]

[1]National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran.

[2]Institute for Nanoscience & Nanotechnology, Sharif University of Technology Tehran, Iran.

Corresponding author:
V Zarghami, PhD
Email Address:
zarghami.mse@gmail.com

Introduction

The skeleton supports the movement and mechanical stability of the body and has vital role in various biological processes such as mineral metabolism and blood cell production [1-2]. To perform these vital functions, bone is transformed into tissue that has a significant innate ability to regenerate after injury [3]. Unlike most other tissues, bone tissue is able to heal completely without the formation of scar tissue, so that newly formed bone is usually indistinguishable from existing healthy bone [3].

Bone fracture repair is used for studying the biology of bone regeneration widely and shows that this process similar the same biological events that are observed during fetal skeletal development. Fractures often heal indirectly, so a callus of tissue is deposited [4-5]. Using local and systemic factors expressed after injury, progenitor cells from tissues. Differentiations of cells are absorbed at the site of injury and they differentiate into the tissue cells that produce chondrocytes and osteoblasts cells. Chondrocytes replace the primary granulation tissue with a soft cartilaginous callus, then calcify and rapidly perform the defect-fixing function [6-7]. After hypertrophy and mineralization of the cartilaginous pattern, intrusive osteoblasts of an organic matrix consisting of Type I collagen precipitates proteoglycans and bone-specific proteins that form phosphate crystals that make up the bone mineral matrix [7-8].

In addition to the indirect path of bone formation, populations of resident cells that have been shed for bone formation after fracture can directly contribute to bone formation without causing cartilage precursor [1]. The entangled bone in the fracture callus, which is mechanically weak, provides the initial stability of the defect and is replaced by a very fine layer of bone, so that the bone density and structure adapt to the mechanical loads applied to it [1].

During adult life, this bone regeneration is an ongoing process and there is a precise coordination between ossifying osteoblasts and bone resorbing osteoclasts. The immune system, from which osteoclasts originate, has been shown to be an important regulator of the interaction between osteoblasts and osteoclasts during growth, repair, and disease [9]. Interactions between the immune system and bone growth are discussed in a scientific field called "osteoimmunology" [10].

Despite the inherent capacity of bone repair, failure to repair bone may require surgical intervention to correct the defect and re-establish the mechanical and biological conditions required for bone repair [11]. Incomplete repair of bone fractures is observed in 5-15% of trauma patients and is associated with risk factors such as comorbidities, old age and adverse characteristics of injury [12].

In cases where the required bone tissue formation is greater than the bone's ability to repair itself, increased bone repair is also necessary. In animal model research, such bone deficiency is mimicked by critical size defects, which is defined as the smallest defect size that will not heal naturally regardless of the time elapsed [13]. For example, it is assumed that long bone defects do not heal when they are larger than approximately twice the diameter of the bone. In addition to repairing large bone defects, surgery to induce bone formation outside the main margin of the bone may also be necessary, as is common in spinal fusion surgery [13].

Treatments aimed at repairing incurable bone defects or creating new bone usually use a type of bone graft. Bone grafts can be bone grafts from the patient or other alternatives, such as freshly frozen or processed allogeneic bone, natural or artificial bone alternatives, or any combination of these with or without specific bone growth stimulants. They can have different biological properties that are involved in bone formation [14].

Autologous bone grafting is considered optimal because it acts primarily as a structural matrix for the migration, adhesion, and matrix formation of bone cells, a process called osteoconduction [15]. Bone grafts from the patient's own body contain native growth factors that cause the use of endogenous bone-producing cells at the surgical site and induce differentiation into osteoblasts [15]. Precursors in fresh bone grafts may have paracrine effects such as the production of growth factors, cytokines, or hypoxia-related factors that support osteogenesis or angiogenesis [16].

In this paper, first the conventional methods of stimulating bone formation and reconstruction of bone defects and fractures in three general sections of synthetic biomaterials, cells and bone growth factors are reviewed, and then the role of the immune system in stimulating and regenerating bone growth is discussed. Also, new approaches and methods of multifunctional and integrated confrontation between new and conventional methods for bone regeneration are introduced.

Methods

In this review 57 article of Science Direct and Pub Med database is reviewed to explain the role and mechanism of the immune system's response to bone fractures.

Results

Bone growth improvers

The use of osteoconductive biological materials, osteoporogenic cells, and osteoconductive stimuli or the creation of a favourable (mechanically) spatial environment can all create a more favourable condition for bone formation [17]. Conventional methods of stimulating bone formation and reconstruction of bone defects and fractures are reviewed in four general categories according to Figure 1.

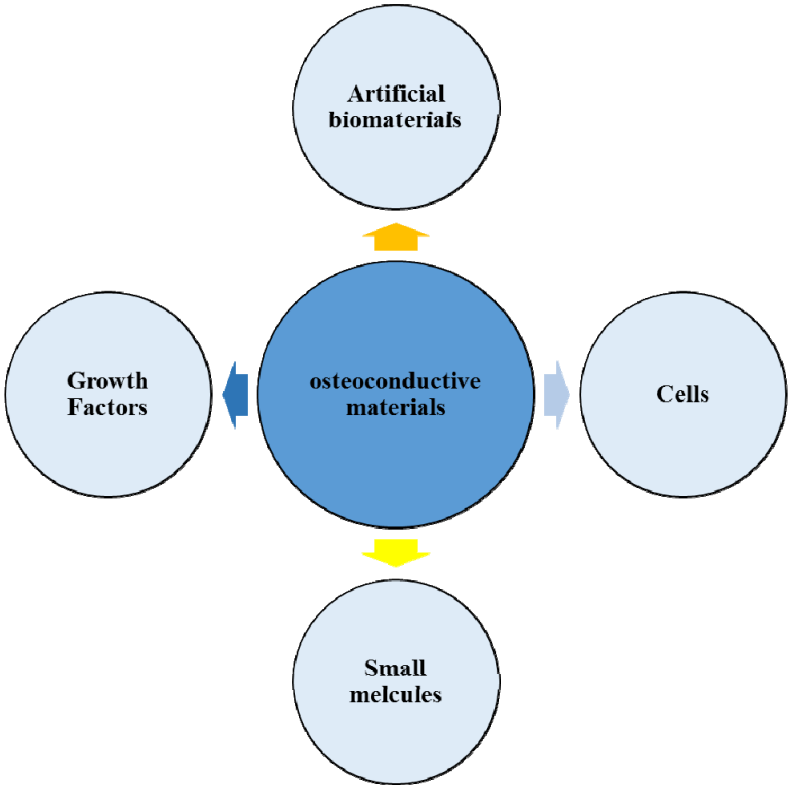

Figure 1. General classification of osteoconductive materials

3-1-1- Use of synthetic biomaterials

Numerous synthetic biomaterials have been produced that can be used as the basis for bone grafting, materials that mimic the inorganic and structural phase of bone, such as calcium phosphates, or materials such as bioactive glass or hyaluronic acid that can induce an appropriate cellular response that has been shown to enhance bone formation [18-20]. This occurs mainly through the osteoconductive mechanism, based on which their behaviour can be targeted by changing the parameters of materials such as chemical composition, macro structure and surface microstructure. Despite of these products in clinical practice, there has been only a slight shift in the use of autologous bone to the use of such bone replacements, the fact that the benefits of artificial bone alternatives are controversial [21-22]. Osteoinduction is often seen in calcium phosphate ceramics, and in preclinical models, more bone formation is usually seen in bone defects treated with osteoinduction ceramics compared to non-osteoinductive ceramics. However, the clinical efficacy of osteoinductive ceramics has not yet been determined [23]. Current biomaterials require the use of osteoinactive compounds such as bone growth factors in large bone defects [24].

3-1-2- Using of cells

Various cellular sources that contribute to bone formation and chondrogenesis during fracture healing include resident osteoblasts and precursor cells extracted from bone marrow, bone connective tissue, and surrounding muscles in vivo studies [25]. Studies show that bone precursor cells utilized from the bloodstream also enter the fracture callus [26]. Among the various bone-producing cells, mesenchymal stromal cells (MSCs) are the most widely used in bone reconstruction medicine [27]. Through the differentiation process, native mesenchymal stem cells can play a role in healing bone fractures because evidence suggests that MSCs mostly in the periphery of arteries and enter the damaged area to facilitate regeneration [28].

3-1-3- Using of growth factors

Normally, the process of bone regeneration, including the process of cartilage formation, ossification and angiogenesis, various growth factors for cell growth and differentiation are activated to complete the ossification process [29]. Initially, transformant growth factor (TGF)

-β and platelet-derived growth factor (PDGF) are released by platelets as soon as blood clots form, providing migration and mitogenic conditions for white blood cells and MSCs. Cartilage differentiation processes, ossification are regulated by a group of (TGF) -βs, of which bone morphogenetic proteins (BMPs) are very important [30]. Use of morphogenetic bone proteins (BMPs) in bone fractures development has developed. This protein is a non-collagenous glycoprotein and 15 different types of it have been identified so far [31]. Studies also show that angiogenic factors such as PDGF, VEGF or FGF only have stimulatory effects on the healing of defects and fractures if combined with osteoinductive growth factors such as BMP-2 [31].

3-1-4- Using of small molecules

Recently, small molecules have been proposed as one of the bone-building stimulation options for recombinant protein therapy due to their unique benefits. To date, therapies for orthopaedic applications rely heavily on bone growth factors. This trend is likely to be reversed as new drug discovery strategies lead to the discovery of many small molecule compounds and the development of their applications. Some of these small molecules have already been identified with the potential for bone formation in humans. Some of these substances include ascorbic acid (vitamin C), vitamin D3, dexamethasone, tetracycline, statins, retinoic acid, alendronate, etc [32].

3-2- Immune system and its role in bone regeneration

3-2-1- The role of acute inflammation in the reconstruction and healing of bone fractures

Repairing damaged bone largely replicates the fetal skeletal process. The main processes include cartilage formation, ossification, angiogenesis and apparent bone regeneration [29]. Unlike systemic ossification seen during skeletal formation, fracture healing is a process that requires topical application and differentiation of suitable cells for repair. Bone healing therefore relies on an acute inflammatory response to provide basic local symptoms for the onset of bone formation [5]. Numerous observations point to the fact that acute inflammation developed after bone injury has strong regenerative effects. Primary fracture hematoma is a source of inflammatory cells and cytokines that subsequently react with resident cells and bone-forming cells that have penetrated the site [33]. The finding that hematoma resection leads to delayed or hypertrophic disconnection is evidence that the primary fracture hematoma contains essential signals of bone regeneration [34].

Balanced inflammatory response after bone injury generally does not last more than 7 days and is strongly involved in bone formation [35]. Certain inflammatory signals are required, and suppression of the immune response with anti-inflammatory drugs interferes with bone healing. Systemic inflammatory diseases such as rheumatoid arthritis or temporary acute inflammation after poly-trauma can lead to reduced bone repair. This fact shows the importance of a balanced local inflammatory response for the formation of new bone [4].

3-2-2- Immune cells as regulators of bone formation

The immune system generally uses two mechanisms: 1) the innate response, which is mostly used to provide non-specific immediate actions, and 2) the acquired response, which performs a specific action and usually starts later [36]. In most responses, there is a dependent relationship between innate and acquired immune system cells. For example, following soft tissue damage, innate and acquired immunity are involved and complement each other to mediate tissue regeneration. Similarly, in fracture healing, subsets of innate and acquired immune cells can be observed at the fracture site during acute inflammation [37]. Studies showed that genetically modified mice have confirmed their functional role in fracture healing [38].

3-2-2-1- Intrinsic immunity in bone formation

Neutrophils enter the defected area within minutes. Inhibition of neutrophils in fracture studies leads to bone loss and impairment of callus mechanical properties. Their regenerative functions are likely to be mediated by the effects of monocyte uptake and extracellular matrix synthesis [39]. In addition to bone-covering macrophages,

there are also inflammatory macrophages that penetrate the defect site and have more flexibility [40]. In a combined model of spinal fusion injury and localized inflammation, bone formation in muscle is eliminated after selective reduction of macrophages [41]. Macrophages contribute to bone formation through a variety of actions, including the production of cytokines or growth factors that lead to bone formation, and by the secretion of factors that promote angiogenesis and vascular regeneration [42]. Proinflammatory cytokines of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and macrophage-derived Oncostatin M are readjusted during acute inflammation. Each of these cytokines has an operational role in bone repair, as shown by studies of genetically modified mice, and may have different actions in chemical conduction, osteoblast differentiation, angiogenesis, or cartilage production, especially TNF-α in these processes has an important role [43]. While proinflammatory cytokine-secreting macrophages (M1 macrophages) predominate during the acute phase of inflammation, the phenotype changes to a precursor cell type (M2 macrophages) with several prognostic growth factors such as VEGF, PDGF, FGF, and growth factor such as insulin (IGF) -1 expresses [44]. It has been suggested that disruption of this process is due to poor ossification and angiogenesis in delayed bone healing models [45].

3-2-2-2- Acquired immunity in bone formation

Lymphocytes have been shown to play an important role in bone regeneration, which determines the local activity of osteoclasts. Lymphocytes can also be an important effect in the elimination of bone inflammation in uncontrolled inflammatory conditions [46]. Recently, the regenerative effects of acquired immune cells on bone repair have been considered. Mice lacking functional lymphocytes show abundant callus formation, but their fractured calluses have bone quality and lower levels of bone markers. Selective proinflammatory T lymphocytes help destroy callus [47-48].

3-3- Biomaterials have the property of regulating the immune system and their effect on bone formation

Four biomaterials have the property of regulating the immune system, that the effect of them on bone formation has been studied and proven (Figure 2).

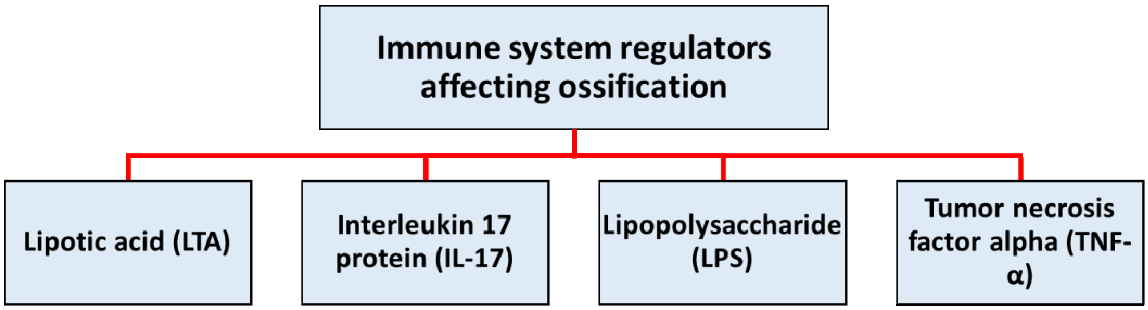

Figure 2. Four biomaterials that regulate the immune system for bone regeneration

3-3-1- Tumor necrosis factor alpha (TNF-α)

Tumor necrosis factor-alpha (TNF-α) is an inflammatory cytokine belonging to the family of proteins produced by macrophages / monocytes during acute inflammation and is responsible for a wide range of signalling events in cells, leading to necrosis or apoptosis. TNF-alpha exerts many of its effects by binding to a 55-kDa cell membrane receptor called TNFR-1 or a 75-kDa cell membrane receptor called TNFR-2. Both of these receptors belong to the TNF family [49]. Numerous studies have shown that TNF-α, in combination with the host reservoir of mesenchymal stem cells, is the main determinant of the success of bone repair in defects and fractures. It has been shown that patients with poor bone repair usually have inappropriate TNF-α responses, which inadequate levels have led to non-transplantation in this patient population. The use of TNF-α in topical fracture healing has

led to favourable recovery results in in vitro and in vivo tests [50].

3-3-2- Lipopolysaccharide (LPS)

One of the most studied bacterial surface molecules is the glycolipid known as lipopolysaccharide (LPS), which is the main skin material of gram-negative bacteria. LPS, first noticed in the early 1900s, was known for its ability to stimulate the immune system, known as endotoxin. It was later found that LPS inhibits the permeability of bacteria on the cell surface and is a major factor in antibiotic resistance, with gram-negative bacteria exhibiting many antimicrobials. Not surprisingly, these important properties of LPS have been widely used in the literature for over a hundred years [51]. Creates with a hypertrophic and immature callus to accelerate the ossification process. In vitro results on mesenchymal stem cells also indicate that LPS increases the rate of cell differentiation and ossification [52].

3-3-3- Interleukin 17 protein (IL-17)

Interleukin 17 (IL-17, also known as IL-17A) is a key cytokine that links T cell activation to neutrophil activation. Similarly, IL-17 can promote innate immunity to pathogens or be involved in the pathogenesis of inflammatory diseases such as psoriasis and rheumatoid arthritis. IL-17, in addition to promoting inflammation, neutrophils have strong osteoclastogenic effects that may be involved in the pathogenesis of periodontitis, rheumatoid arthritis and other diseases associated with bone immunopathology [53]. Recent studies on the use of this substance to treat bone defects together with T cells, it indicates the acceleration of the ossification process and the differentiation of stem cells into ossification of cells [54].

3-3-4- Lipotic acid (LTA)

Lipotaic acid (LTA) is an adhesive amphiphile on the surface of gram-positive bacteria and regulates autolytic wall enzymes. It is released from bacterial cells mainly after lysozyme bacterialization, cationic peptides from leukocytes or beta-lactam antibiotics. It binds either to non-specific target cells, to membrane phospholipids, or, more specifically, to CD14. It stimulates the secretion of neutrophils and macrophages, acidic hydrolase, highly cationic proteinases, antibacterial cationic peptides, growth factors and cytotoxic cytokines, which synergistically strengthen skeletal bone [55]. Limited information indicates that LTA for bone regeneration is useful in vitro. In a study conducted by LTA for the treatment of mouse femur fractures, the results showed that LTA treatment resulted in timely bone formation, ossification and rapid healing of fractures in mice with femoral defects. In vitro, LTA directly increased dermal factors induced by MC3T3-E1 cell differentiation indices, including alkaline phosphatase activity, calcium deposition, and osteopontin expression. As a result, the findings show that LTA has promising properties for bone regeneration [56].

4- The use of a combination of bone growth improvers and immune-regulating substances, the direction of future research

Researchers are currently laying the groundwork for a future research approach that leverages the properties of bone growth and differentiation enhancers as well as immunosuppressive agents to build bone and repair fractures and bone defects quickly. For example, recent studies on the simultaneous use of lipopolysaccharide (LPS) and growth factor BMP-2 have shown that the synergistic effect of these two substances on cell differentiation and ossification [56]. It is predicted that in the future, with the identification of other immune system regulators, more research will be done to take advantage of the simultaneous properties of these two types of osteogenic and immune system regulators, defining different properties and unique applications in bone repair and fracture treatment [57] (Figure 3).

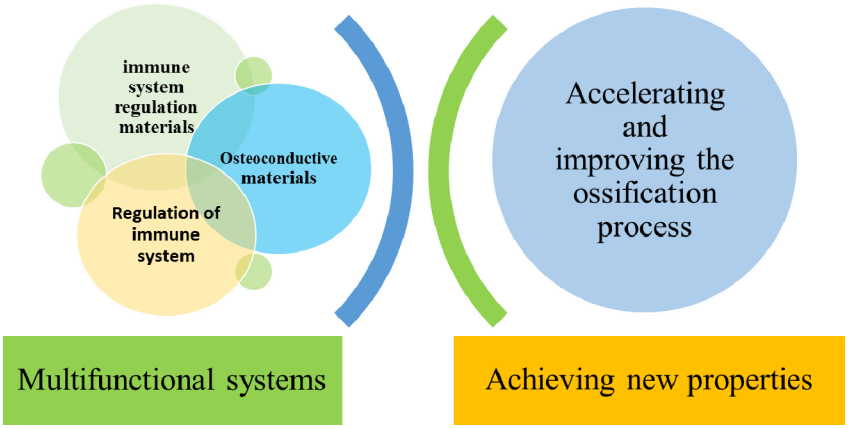

Figure 3. Achieving new properties in accelerating and improving the ossification process through the simultaneous use of osteoconductive biomaterials and immune system regulators

Conclusion

In this paper, first the conventional methods of stimulating bone formation and reconstruction of bone defects and fractures in three general parts of the use of artificial biomaterials, cells and bone growth factors were studied. Then the role of the immune system in stimulating and regenerating bone and immune stimulants participation in ossification was studied. Studies show that the immune system, as the most complete system facing bone defects and fractures, performs its inherent function in such a way that fractures are properly treated. However, in cases where the fracture and defects are larger and more than critical, external treatment usually requires surgery. In this way, the use of biological substances such as tumor necrosis factor alpha (TNF-α), lipopolysaccharide (LPS), protein interleukin 17 (IL-17), lipotic acid (LTA) as substances that regulate the immune system, stimulants Osteogenesis and regulating the function of the immune system to better deal with the problem and damage. It was predicted that the combined use of these materials with conventional osteogenic materials such as synthetic materials, bone growth factors could be promising new therapeutic compounds in the future that the duration of treatment for bone fractures will be decreased.

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