The Bone Regeneration

Subjects: Surgery
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Bone regeneration is a complex process that is influenced by tissue interactions, inflammatory responses, and progenitor cells.

Keywords: Bone regeneration

1. Introduction

Bone regeneration is a sophisticated process influenced by a variety of factors. In young healthy patients, bone tissue shows high self-repair abilities. However, systematic factors, such as an increased age, disease or obesity can negatively affect bone regeneration [1][2][3]. Large defects due to heavy trauma, multiple fracture, infection, or tumor resection, are also disruptive for proper tissue healing [4][5]. Notably, 5–10% of all fracture healing is disturbed, might take months longer or is even impossible [2][6].

The current gold standard for bone defect reconstruction is bone grafting where autologous bone tissue is transplanted to bridge the gap in the bone defect zone. The graft structure is similar to the original bone, which enables growth and regeneration. However, there are limitations to this therapy, such as donor site morbidity and availability of suitable autologous material [5].

A promising approach is bone tissue engineering, which has been successfully applied in a few clinical trials [7][8][9][10]. One approach is to transplant osteogenically induced stem cells into the bone defect zone, which then support the healing process. Within the fracture zone, the cells undergo further osteogenic differentiation, secretion of osteogenic factors, and recruitment of osteoblast progenitor cells. Stem cells can also be combined with allogeneic, alloplastic or xenogeneic scaffolds. These structures are seeded with the cells and support healing by their osteoinductive and/or osteoconductive properties [11].

Stem cells can be isolated from embryonic, fetal and adult tissue. Alternatively, cells are induced into the pluripotent stem-cell state: induced pluripotent stem (iPS) cells. Embryonic, fetal, or reprogrammed cells are associated with major safety, regulatory and ethical problems [12]. In contrast, adult stem cells can easily be isolated from a variety of tissues including adipose-derived stem or stromal cells (ASCs) from adipose tissue, with high osteoinductive and osteogenic potential [11]. These adult stem cells are called mesenchymal stem cells (MSCs). Beside ASCs, bone marrow mesenchymal stem cells (bmMSCs) are another type of MSCs, which also show the typical characteristics of all MSCs. Bone marrow biopsy allows isolation of bmMSCs, which is a procedure with risk of additional morbidity that provides only a low yield of cells, when compared to the surgical procedure for harvesting ASCs: ASCs can be easily harvested through noninvasive procedure and have a significantly higher yield of cells than that obtained for bmMSCs [13]. Moreover, ASCs have a higher proliferation capacity and more colony-forming units compared to bmMSCs [14][15]. Cell therapy requires high numbers of cells for successful application. This could require artificial cell expansion to reach sufficient numbers [16]. However, cell culture increases senescence with every passage, with the consequence of reduced proliferation, changes in morphology, which both could influence the cell function. bmMSCs are more susceptible to senescence and have a shorter life span then ASCs [14][15].

Bone tissue engineering is a sophisticated process, in which there is an interplay between stem cell properties, osteogenic pathways and secretome. Here we review these concepts.

2. Bone Regeneration

Bone tissue is able to remodel and self-renew, which happens throughout a human’s lifespan. Their extra-cellular matrix, which consists of water, minerals (e.g., hydroxyapatite, calcium fluoride, and calcium carbonate), and proteins (mostly

type I collagen) [17], undergoes constant remodeling. Equilibrium is obtained by synthesizing osteoblasts and degradation from osteoclasts [3]. bmMSCs influence this through their secretome and their ability to develop into osteoblasts [3][18].

2.1. Fracture Healing

Fracture healing can take place in two ways: primary healing requires that the fragments are in close contact and immobilized. This happens when a fracture is immediately treated after trauma. A small amount of granular tissue and callus forms between both fracture ends. The cutting cone, which consists of osteoclasts, creates zones between both ends, while osteoblasts interconnect these zones. The new bone is then formed and the fracture is closed [2][19].

However, most fractures close through secondary healing, also called endochondral ossification. This process is divided into four stages: hematoma formation (days 1–5), soft callus formation (days 5–11), hard callus formation (days 11–28), and bone remodeling (day 28–months later) [2].

Trauma causes the fracture itself and additionally leads to rupture of blood vessels inside the bone, which creates a hematoma. The hematoma creates a clot inside the fracture, which induces the recruitment of immune cells, including macrophages, monocytes, and lymphocytes. These cells influence the subsequent process of osteogenesis [20][21]. They initiate and modulate the fracture healing process. In particular, macrophages are crucially involved in bone healing through their secretome [20][22] and by forming a layer above the osteoblast, which is called an osteomac [23]. The macrophages have different phenotypes, such as M1 (the so-called activated or proinflammatory phenotype) and M2 (the alternatively activated or anti-inflammatory phenotype), which are induced during different phases and, in turn, influence different processes of bone healing. The exact mechanisms and participations during bone tissue regeneration are not completely understood [24][25]. The M1 phenotype appears to support the inflammatory response and reduces regenerative osteogenic potential. Moreover, M2 phenotypes have pro-osteogenic effects though their secretome. Kang et al. [25] studied healing of rat calvaria defects and show that M2 secretomes support fracture healing. Furthermore, macrophage deficient mice have fewer MSCs in their bones, decrease bone mineralization and longer fracture healing time [22]. On the other hand, a high and prolonged inflammatory intensity impairs, or even completely inhibits, the tissue healing process [3].

In the second step, granulation tissue, which is rich in fibroin, develops inside the fracture. The growth factors secreted by immune cells include vascular endothelial growth factor (VEGF), which induces vascularization and the outgrowth of blood vessels. Moreover, MSCs are recruited to the fracture site, and they start to proliferate and differentiate into chondroblasts, osteoblasts, and fibroblasts. Chondroblasts help with the creation of a soft cartilage callus inside the fracture [2][19].

The soft cartilage callus is incrementally replaced by a hard bone callus during the subsequent days. This is accomplished by the collaboration of osteoblasts, osteoclasts, chondroclasts and chondroblasts. Vascularization also occurs deeper into the callus, thus facilitating MSCs to invaginate. This process, in turn, fosters the creation of a hard callus. Osteo-progenitor cells start the creation of woven bone from periosteal. At the end of this stage, the callus is completely replaced by bone tissue [2][19].

To complete the healing, the bone must be remodeled, which is achieved through the equilibrium of osteoclast resorption and osteoblast rebuilding. The remodeling aims to create compact bone at the center and lamellar bone at the edge. This process can take months to complete [2][3][20].

2.2. Impaired Bone Healing

Fracture healing in 5–10% of patients can fail or be delayed for months [2][6], and this can either be caused by systemic risk factors, such as obesity, malnutrition, smoking, anemia, endocrine conditions, disease and aging [1][2][3] and/or local risk factors extensive fractures from massive trauma, multiple and open fractures, radiotherapy or infection [5].

Older people suffer from reduced bone mass and thus experience more frequent and severe bone fractures [3][26]. Moreover, their MSCs have a reduced commitment to osteogenic lineage and are primed for adipogenesis [27]. Studies have shown that several osteogenic transcription factors are reduced in older MSCs, including MAF bZIP transcription factor [28], Forkhead box P1 [29], and Core-binding factor β [30]. Accordingly, fracture healing is impaired among the elderly, and recovery takes longer. In animal studies, older rats show less bone regeneration, reduced vascularization of their callus, less cartilage, and decreased ossification [31].

Diseases, such as diabetes type I and type II, osteoporosis, and osteogenesis imperfecta, are also a major factor in interrupted fracture healing [3]. Osteoporosis leads to less Ca^{2+} deposition in the bones, making the structure weaker. As such, trauma can result in larger fractures, which often require additional treatment [32]. The impact of osteoporosis on fracture healing has been disputed in conflicting studies [33][34].

Most cases of osteogenesis imperfecta are due to genetic mutation in collagen type I. The disease is associated with more brittle bones and more fractures. The healing of these fractures in many cases results in non-union, which often requires a longer healing time [3][35][36].

Patients with diabetes generally show reduced bone regeneration. In 87% of cases, fractures need a longer healing period. These patients have a 3.4 times higher risk for complications [37]. Thereby, myostatin regulates bone formation, and blocking myostatin improves fracture healing in patients with type 2 diabetes [38]. Furthermore, diabetes is associated with advanced glycation end-products, which are proteins linked to aldose. These proteins can bind to the receptor of advanced glycation end products (RAGE) and lead to a proinflammatory response. They are also linked to an increased number of osteoclasts [37][39], reduce osteoblast ability for bone repair, and decreasing bone mineralization [40].

Moreover, fracture healing can be impaired through infections. *Staphylococcus aureus* is a common pathogen in healthcare settings and is often associated with soft tissue complications [41][42]. The pathogen is also responsible for 30–42% of all infections during bone healing, which can appear because of an open fracture, or bone fixation. Thereby, bone regeneration is disturbed, and antimicrobial therapy is necessary [43].

In an aging society where there is an increase in lifestyle diseases, such as diabetes, it is crucial to have efficient treatment for delayed or failed fracture healing. The method currently used is bone grafting.

2.3. Interaction of Inflammatory and Bone Cells during the Fracture Healing and Bone Re-Generation—The Nrf2–Keap1 System

In the mechanism of intractable fractures, oxidative stress is considered one of the main factors that interfere with fracture healing. Oxidative stress is generally caused by an imbalance between oxidation and reduction. During the early phase of fracture healing, reactive oxygen species (ROS) are generated under inflammatory and ischemic conditions [44]. However, the influence of ROS can be normally restricted by protective antioxidant enzymes, capable of stabilizing or deactivating free radicals before cellular components are attacked [45][46][47]. On the other hand, excessive oxidative stress potentially can occur after the fracture in patients with underlying diseases that inherently expose to oxidative stress, as well as disruptive or compound fractures [45]. Excessive ROS can lead to chronic inflammation [48], decrease in osteoblast function and differentiation [49][50], whereas they can activate bone resorption through elevating osteoclast differentiation and function [51]. Thus, these modifications of bone metabolism by oxidative stress affect bone remodeling and regeneration [52].

The Nuclear factor erythroid 2–related factor 2 (Nrf2)-Kelch-like erythroid cell-derived protein with cap ‘n’ collar homology-associated protein 1 (Keap1) system plays an important role in the regulation of the biological response to oxidative stress. In the basal condition, Nrf2 is regulated by the stress sensor Keap1. Under conditions of oxidative stress, stabilized Nrf2 is translocated into the nucleus where it binds to antioxidant-response elements (ARE) in the promoter regions of target genes, resulting in the activation of a variety of antioxidant genes [53][54]. Recently, attention has focused on the role of Nrf2 in fracture healing process. A previous report showed that Nrf2 deficiency decreased fracture callus by using *Nrf2*-knockout (KO) mice [55]. Moreover, Nrf2 can be involved in the control of excessive inflammatory responses [56], the promotion of osteogenesis in MSCs, and angiogenesis through VEGF expression [57], in early phase of fracture healing. In the remodeling phase, Nrf2 also regulates the balance of bone metabolism by suppressing oxidative stress-induced osteoclastogenesis [58]. In light of bone tissue engineering, it is expected that Nrf2 would be a future therapeutic strategy for fracture healing or bone regeneration in patients with intrinsic oxidative stress such as diabetes type II or osteoporosis [45].

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