Chapter 9

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Currently, there is a significant clinical need to develop innovative approaches to treat large bone defects and bone fractures. With the development of bone substitutes, many patients with missing teeth or bone defects have gained satisfactory outcomes. However, for patients with adverse bone conditions and well-recognized risk factors, such as alcoholism, osteoporosis, or with locally infected bone defects, there is a significant need to improve the properties of bone substitutes to achieve more satisfactory bone regeneration.

New methods for bone regeneration include gene, cell, and cytokine therapies. Traditional Chinese Medicines have been reported to substitute for autografts and allografts whose clinical applications are hampered by various limitations. Among these therapies, we chose two new methods. First, cytokine therapy is advantageous in terms of its safety, feasibility, and potential for clinical application compared with gene and cell therapies. Second, Traditional Chinese Medicines have attracted increased research attention because of their lower price and fewer side effects. Traditional Chinese Medicines have been used to promote bone formation in fractures in China for over a thousand years, involving the use of agents that possess high bioactivity with minimal side effects. Therefore, we examined the function of different cytokines (BMPs) and Traditional Chinese Medicines (NGR1) as osteoinducers in adverse bone conditions.

1. To investigate different dimerization types and doses of BMPs to antagonize the inhibitory effect of ATRA on osteoblastogenesis and to explore the molecular mechanism of the inhibitory effect of ATRA on osteoblastogenesis.

BMPs have been introduced to accelerate bone regeneration. BMP2 and BMP7 have been approved by the food and drug administration (FDA) as a medical device. However, because of the high cost associated with their use, they have not been widely used clinically. BMP2/7 heterodimers have a significantly higher potency in inducing bone regeneration, and thus could be an ideal alternative to BMP homodimers. ATRA, a metabolite of alcohol, causes accelerated bone resorption, bone fragility, and spontaneous fractures. BMP2/7 could have a promising application potential to significantly promote bone regeneration and implant osteointegration for patients with alcoholism. Alcoholism can result in a low bone mineral density and compromised regenerative capacity, thus delaying implant osteointegration. One of the underlying mechanisms is the inhibitory effect of ATRA on osteoblastogenesis. BMP2/7 could antagonize the inhibitive effect of ATRA and enhance osteoblastogenesis. In chapter 2, we compared the efficacy of heterodimeric BMP2/7 and the respective homodimeric proteins in restoring ATRA-inhibited osteoblastogenesis. We also investigated the molecular mechanism of the inhibitory effect of ATRA on osteoblastogenesis. We found that osteoblastogenesis could be significantly inhibited by ATRA alone, while BMP2/7 enhanced it, with a higher dose-efficiency than BMP2 or BMP7. In the presence of ATRA, BMP2/7 was advantageous only in enhancing cell viability and ALP activity. Compared with BMP2 or BMP7, it was not better, or was worse, in restoring osteocalcin expression, mineralization, or the expression of two key osteogenic genes, DLX5 and RUNX2. Irrespective of their dimeric type or potency, the selected BMPs could antagonize the inhibitory effect of ATRA on osteoblastogenesis. However, heterodimeric BMP2/7 was not superior to BMP2 or BMP7 in restoring ATRA-inhibited osteoblastogenesis. Bone remodeling and regeneration are controlled carefully by osteoblasts and osteoclasts. BMP2/7 has been proven to be more rapid and efficient for bone regeneration, by enhancing osteoblastogenesis, as well as osteoclastogenesis. However, in clinical practice, cytokines induce a series of side effects, such as overstimulation of osteoclastic activity and ectopic bone formation in unintended area. We showed that ATRA could regulate osteoblastogenesis, which was over-
enhanced by BMP2/7. Thus, in chapter 3, we hypothesized that ATRA could modulate osteoclastogenesis in the presence of BMP2/7, with the aim of enhancing bone regeneration. ATRA significantly inhibited cell viability, TRACP activity, and the expression of all tested osteoclastogenic genes in the presence of BMP-2/7. In the presence of ATRA-receptor inhibitors (RARα-antagonist, RARβ-antagonist, and RARγ-antagonist), osteoclast cell numbers were significantly decreased. ATRA could modulate osteoclastogenesis in the presence of BMP2/7, and inhibit osteoclast differentiation. In osteoclastogenesis, ATRA did not function via activating the RAR receptor. Thus, ATRA has a promising application potential to significantly regulate bone regeneration for patients with bone tumors or hyperactive osteoclast-induced bone loss.

2. To develop a new, single component to treat bone defects: NGR1. To investigate the effects of NGR1 on osteoblastogenesis in an in vitro time-course and dose-dependency study.

We found that BMP2/7 could promote not only osteoblastogenesis, but also osteoclastogenesis, which might be unsuitable to repair bone defects with overstimulated osteoclastic activities, such as in osteoporosis. The Traditional Chinese Medicine PNS has been used to cure osteoporosis for thousands of years in China. NGR1, the main active saponin of PNS, has attracted increased research attention. Unlike other pharmacologically active saponins that occur in both PNS and other ginsengs, NGR1 exists only in PNS. Thus, in chapter 4, we assessed the effects of NGR1 on the osteoblastogenesis of a pre-osteoblast cell line (MC3T3-E1) in an in vitro time-course and dose-dependency study. NGR1 exhibited a bell-shape dose pattern in promoting the proliferation and ALP activity of pre-osteoblasts, with the peak occurring at 50 μg/ml. NGR1 markedly increased the expression of osteocalcin and mineralization at 1000 μg/ml in a dose-dependent manner. Thus, NGR1 could significantly promote the osteoblastogenesis of pre-osteoblasts, which suggested a promising application potential for bone regeneration in patients with osteoporosis.

Over recent decades, in the fields of orthopedics, oral implantology, and maxillofacial surgery, the repair of infected bone defects has remained a challenge because the residual bacteria are nearly impossible to completely eliminate by debridement and is beyond the self-healing of bone defects. Infected bone defects are conventionally treated using systemic or local administration of antibiotics to control infection and by the subsequent implantation of bone grafts, such as autografts and allografts. However, these treatment options are time-consuming and usually have less than optimal efficacy. To solve these problems, novel biomaterials with both antibacterial and osteoinductive properties have been developed.

3. To develop a novel bone-defect-filling material with a sequential release system comprising an initial burst release of a powerful antibacterial agent (Hydroxypropyltrimethylammonium chloride chitosan (HACC), followed by the controlled release of BMP2. To summarize the current knowledge concerning novel biomaterials with dual functions in treating infected bone defects, and to review biomaterials with antibacterial and osteoinductive properties.

To provide a viable treatment option for infected bone defects, we developed a novel antibacterial and osteoinductive BMP2-BioCaP/HACC complex with the sequential release of an antibiotic and osteoinductive agent. The system comprised the short-term delivery of HACC, followed by the slow and sustained release of BMP2. The BMP2-BioCaP/HACC complex could rapidly eliminate antibiotic-resistant bacteria and efficiently promoted new bone formation, both in vitro and in vivo, making this novel material a promising technique to repair infected critical-sized bone defects. In chapter 6, we summarized the current knowledge concerning novel biomaterials with both antibacterial and osteoinductive properties, and then proposed an ideal local biomaterial
system to overcome the difficulties and consequently repair infected critical-sized bone defects. The biomaterial system functions as both an antibiotic carrier and a bone substitute, to clear the infection and contribute to the subsequent bone regeneration process. Compared with the current clinically used antibiotics, many novel antibacterial biomaterials have shown very promising application potential because of their broader bactericidal spectrum, lack of resistance, and good biocompatibility. BMPs, particularly BMP2, are the most potent osteoinductive factors for inducing in vitro osteoblastogenesis and in vivo osteogenesis. Antibacterial and osteoinductive drugs can be incorporated into a co-delivery system using the following modes: (A) superficial adsorption/binding with a chemical bond; (B) an internal encapsulation; (C) a mixed carrying mode with superficial adsorption and internal encapsulation; and (D) surface coating. By manipulating the carrying modes, the antibacterial and osteoinductive drugs can be released in a variety of ways with different kinetics (burst or slow) and temporal characteristics (simultaneous or sequential). The antibiotics used for local delivery should have a broad spectrum of activity and a low rate of bacterial resistance. Osteoinductive bone grafts are more favorable to improve bone regeneration. BMPs are used to functionalize the biomaterials with potent osteoinductive properties. By manipulating the carrying modes and release kinetics, these biomaterials could be optimized to maximize their antibacterial and osteoinductive functions with minimal cytotoxicity. Research over the last decade has demonstrated the promising application potential of these novel biomaterials with the dual functions to treat infected bone defects.

4. To design a low pH-triggered silver-releasing TNT-AL-AgNPs implant to control peri-implant infection and test the antibacterial efficiency of the released AgNPs. To evaluate the biocompatibility of TNT-AL-AgNPs, as well as their effect on osteoblast morphology and differentiation in vitro.

In chapter 7, we developed a novel pH-dependent silver nanoparticle releasing titanium implant to control peri-implant infection. Broad-spectrum antimicrobial AgNPs were successfully loaded into TNT via pH sensitive AL, without affecting the physicochemical characteristics of TNT. A pH of 5.5, mimicking the pH level in peri-implant surface during bacterial infection, could trigger the release of the AgNPs release from TNT. The released AgNPs efficiently controlled bacterial growth in vitro. This novel implant was biocompatible and osteoinductive. Our findings suggested that low pH-triggered AgNPs release from a TNT-AL-AgNPs implant could be a potent therapeutic approach to control peri-implant infection.