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General review

Bone regeneration strategies with bone marrow stromal cells in orthopaedic surgery

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ABSTRACT

Bone is the most transplanted tissue human with 1 million procedures every year in Europe. Surgical interventions for bone repair are required for varied reasons such as trauma resulting non-union fractures, or diseases including osteoporosis or osteonecrosis. Autologous bone grafting is the gold standard in bone regeneration but it requires a second surgery with associated pain and complications, and is also limited by harvested bone quantity. Synthetic bone substitutes lack the osteoinductive properties to heal large bone defects. Cell therapies based on bone marrow or ex vivo expanded mesenchymal stromal stem cells (MSCs) in association with synthetic calcium phosphate (CaP) bone substitutes may be alternatives to autologous bone grafting. This manuscript reviews the different conventional biological and synthetic bone grafting procedures as well as the more recently introduced cell therapy approaches used in orthopaedic surgery for bone regeneration. Some clinical studies have demonstrated safety and efficacy of these approaches but regeneration of large bone defects remain challenging due to the absence of rapid and adequate vascularisation. Future directions in the field of bone regeneration are presented, such as testing alternative cell sources or in situ fabrication of vascularized bone grafts in patients.

1. Introduction

Bone maintenance comprises a tightly coupled balance between bone formation and bone resorption. When this is interrupted by trauma in the form of a bone fracture, bone tissue possesses an innate capacity to repair itself. Indeed apart from the liver, bone is the only tissue in the human body with the capacity of scarless self-renewal [1]. Bone healing is a complex, multi-stage process involving different cell types, extracellular matrices and a myriad of signalling molecules [2]. When a perturbation of the bone-healing cascade occurs, it may result in a delayed bone healing or non-union fracture. A surgical intervention and bone regeneration strategies are required to help fracture repair. In addition to non-union fractures, instances requiring bone-healing approaches include bone diseases such as osteoporosis, osteonecrosis and bone cancer. The current gold standard for regenerating bone defects remains biological bone grafting, with the bone graft retrieved either from the patient themselves (autograft) or from a donor (allograft). Synthetic bone graft substitutes made form biomaterials can also be employed to fill bone voids. More recently, recent progress in both material science and biology has resulted in the possibility of bone tissue engineering, which combines cells and biomaterials. Mesenchymal stromal stem cells (MSCs), which can be retrieved from the patient's bone marrow, can be combined with synthetic three-dimensional scaffolds and this approach has been proposed as a potential alternative to overcome the critical shortcomings associated with biological or synthetic bone grafts.

At present, in the bone tissue engineering field, three strategies make use of the patient's own bone marrow cells to engineer autologous osteogenic grafts. The first approach consists of aspirating bone marrow, followed by centrifugation [3] to concentrate mononuclear cells, and then immediate implantation into the bone defect with or without a synthetic bone substitute. However, this strategy has not led to reproducible bone which may be due to the low number of MSCs in the bone marrow (e.g. 0.01%

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of mononucleated cells) [4]. In the second approach, the harvested MSCs are cultured for 2–3 weeks in a cell therapy unit in order to isolate and amplify the MSC fraction. Several tens of millions of these cells are then injected alone into a bone defect or seeded onto a suitable scaffold shortly before implantation. These hybrid MSC + biomaterial grafting materials have shown bone-inducing ability in animal models [5]. The third strategy comprises harvesting bone marrow, isolating and expanding the osteoprogenitor cells for several weeks, then seeding them on to a scaffold, where they are cultured for a further few weeks to promote the formation of a bone-like tissue layer on the implant [6]. This hybrid construct is finally transplanted into orthotopic site to regenerate a bone defect. As such, the latter two strategies require several weeks of culturing under strict aseptic conditions making the clinical applications for bone tissue engineering extremely difficult in terms of regulations. In spite of this, the therapeutic value of MSCs are evidenced by the growing number of on-going clinical trials to treat bone defects [7]. However, even though it has been over 30 years since the first efforts in this area, only few bone tissue-engineering techniques have been translated into clinical trials and none of them has become the standard of care. This manuscript reviews the different approaches currently used in orthopaedic surgery for bone regeneration. After considering the challenges of two major indications, non-union fractures and osteonecrosis of the femoral head, strategies for bone induction, augmentation and regeneration will be considered. A particular emphasis will be placed on cell therapy applications in bone healing.

2. Non-union fractures

In the regular follow-up of patients after bone fracture, the course of fracture consolidation is reviewed by conventional, two orthogonal projection plain radiographs. Therefore, the development of a bone healing can be monitored clinically and through imaging. In general, physiological bone repair results in the production of a mechanically strong bone after a few weeks of immobilization and weight bearing suppression for lower limb fractures. In spite of bone's extraordinary healing capability, many cases of long bone fractures require therapeutic intervention to facilitate bone healing and regeneration. An estimated 5% to 10% of fractures result in delayed union or non-union [8] causing patients to endure impaired function as well as repeated hospitalizations and surgeries. Radiographs in Fig. 1 show examples of long bones non-union fractures. In these radiological images, a large persistent radiolucent zone in the fracture gap can be observed indicating a non-union. Risk factors for long bone non-union fractures include smoking, infection, postoperative fracture gap, polytrauma, and a high degree of initial fracture displacement [9]. Non-union may also result from lack of post-surgery mechanical stability or, conversely, an excessive rigid fixation of osteosynthesis material. Furthermore, important local conditions in the fracture environment such as inadequate blood supply and soft tissue injury can impact bone healing. The reduced numbers and efficacy of host bone marrow progenitor cells is also thought to play a role.

The treatment of non-union fractures is a major challenge in orthopaedic surgery. The total estimated cost of these complications is between 10,000 € and 100,000 € per patient in Europe [10,11]. Surgical interventions aim to enhance mechanical stability through modification of osteosynthesis and reduction of the fracture gap, to increase of compressive motion between fracture fragments, to improve the biological environment by the introduction of new cells, vascular supply and eventually grafting of autologous bone tissue into the fracture gap. The current gold standard of treatment for atrophic non-union includes surgical stabilization and autologous bone grafting with success rates as high as 70%–95%. However, autologous bone grafting is a limited source of material and can be associated with complications, including persistent donor site pain in 16–26% of patients at 3 to 12 months follow-up [12,13]. Increasingly, alternative methods have been tested with variable success in clinical trials. For instance collagen sponges loaded with recombinant human bone morphogenetic proteins-2 and 7 (rhBMP-2 and -7). However, the supraphysiological dosages of these growth factors have resulted in several clinical complications, in particular, the increase of cancer risk [14]. Other alternative treatments of non-unions consist of the percutaneous injections of concentrated autologous bone marrow (CABM) or ex vivo culture expanded MSCs as well as their combinations with synthetic bone fillers in open surgery. These new cell therapy treatments are currently being investigated to facilitate bone healing in clinical trials and will be discussed in further detail in the following sections.

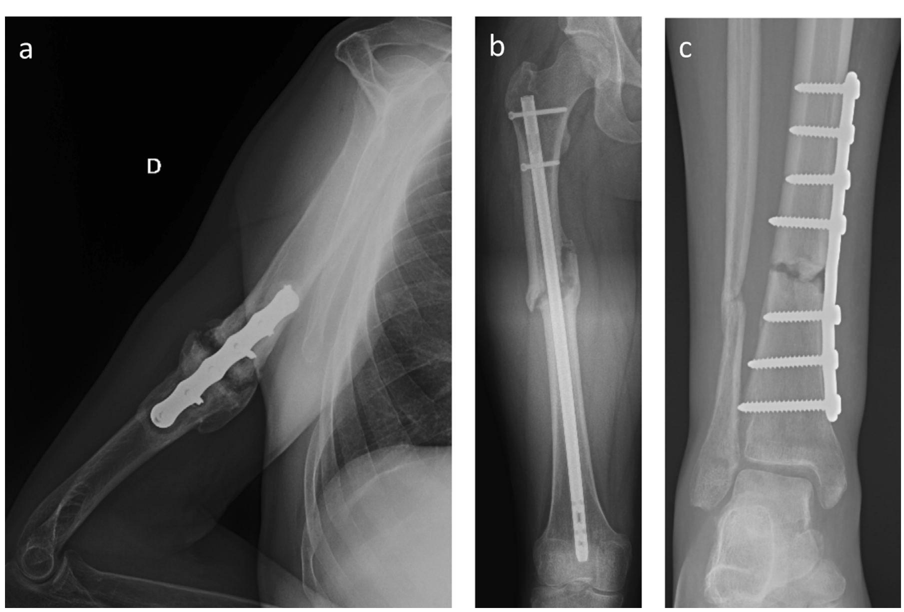

Fig. 1. Plain radiographs of long bone non-union fractures (a: humerus; b: femur; c: tibia).