Recent biological trends in management of fracture non-union

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Abstract
Bone regeneration is a complex, well-orchestrated physiological process of bone formation, which can be seen during normal fracture healing, and is involved in continuous remodelling throughout adult life. Currently, there is a plethora of different strategies to augment the impaired or “insufficient” bone-regeneration process, including the “gold standard” autologous bone graft, free fibula vascularised graft, allograft implantation, and use of growth factors, osteoconductive scaffolds, osteoprogenitor cells and distraction osteogenesis. Improved “local” strategies in terms of tissue engineering and gene therapy, or even “systemic” enhancement of bone repair, are under intense investigation, in an effort to overcome the limitations of the current methods, to produce bone-graft substitutes with biomechanical properties that are as identical to normal bone as possible, to accelerate the overall regeneration process, or even to address systemic conditions, such as skeletal disorders and osteoporosis. An improved understanding of the molecular and cellular events that occur during bone repair and remodeling has led to the development of biologic agents that can augment the biological microenvironment and enhance bone repair. Orthobiologics, including stem cells, osteoinductive growth factors, osteoconductive matrices, and anabolic agents, are available clinically for accelerating fracture repair and treatment of compromised bone repair situations like delayed unions and nonunions. A lack of standardized outcome measures for comparison of biologic agents in clinical fracture repair trials, frequent off-label use, and a limited understanding of the biological activity of these agents at the bone repair site have limited their efficacy in clinical applications.

Key words: Biological; Fracture repair; Nonunion; Cell therapy; Bone substitutes

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Core tip: Successful fracture healing requires mechanical stability and a viable biologic microenvironment. Fractures with compromised biology will benefit from treatment options that can augment the biologic potential at the site of bone repair. An ideal bone graft...
should be osteoinductive, osteoconductive, osteogenic, angiogenic and should provide mechanical support and promote physiologic healing without any significant adverse effects. Regenerative strategies like the use of bone morphogenetic proteins, platelet rich plasma, stem cells and anabolic agents are promising in the treatment of fractures either acute or fracture non-union.

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INTRODUCTION

Healing of the fracture is a multifactorial metabolic process. If these factors impaired, healing process is interrupted resulting in fracture nonunion[1]. The majority of fractures heal without any complications, but literature reported non-union of all fractures ranged between 5% to 10%[2].

Biological stimuli for regeneration of bone involve the interplay of four critical elements, namely: (1) osteoinductive growth factors (induce differentiation of stem cells to osteoblasts); (2) stem cells that respond to osteoinductive signals (osteogenic); (3) an intact vascular supply, and, finally; and (4) a scaffold that supports cellular attachment, proliferation, and ingrowth (osteocductive matrix)[3].

This article provides a review of the biologic agents that can enhance bone healing either clinically available or are still under trials.

BIOLOGIC ENHANCERS OF BONE REPAIR

Bone grafting, scaffolds and bone substitutes

Autologous bone graft is a commonly performed surgical maneuver to enhance bone healing and being considered as the “gold standard” as it contains all properties required in a bone graft material: osteoinductive [bone morphogenetic proteins (BMPs) and other growth factors], osteoconductive (scaffold) and osteogenesis (osteoprogenitor cells) and has a success rate of 50%-80%[3].

The iliac crest is the commonly used donor sites. But harvesting its complications and needs an additional surgical procedure[4].

Allogeneic bone graft bypasses the harvesting problems and graft quantity. It is available in many forms, such as demineralised bone matrix, cancellous and cortical, corticocancellous, osteochondral and whole-bone segments[5]. But They have decreased osteoinductive properties and with no cellular component, their main drawbacks are the issues of rejection, immunogenicity, transmission of infection, and cost[6].

Bone-graft substitutes are alternatives to autologous or allogeneic bone grafts. They are composed of scaffolds, such as collagen, hydroxyapatite, b-tricalcium phosphate[7] that enhance the proliferation of bone cells for bone regeneration[8].

Aspiration concentrate of the bone marrow

It contains stem cells that could differentiate into osteoblasts in response to osteoinductive signals[7]. Classically, the iliac crest is the main donor for bone marrow aspiration, but alternative sites, including the vertebral body, proximal humerus, proximal tibia, have also been described[9].

Connolly et al[10] were among the first to demonstrate the efficacy of percutaneous bone marrow injection in the treatment of nonunited fracture tibia. In a cohort of 20 tibial nonunions, 90% healed in averaged 6 mo after injection. In a retrospective study involving 60 atrophic tibial nonunions Hemigou et al[11] demonstrated complete healing in 88.3% that were treated with a single injection of bone marrow aspirate.

Percutaneous bone marrow grafting is a minimally invasive treatment. It avoids the complications associated with the open graft harvest procedure. However, this technique, if used alone, may not be sufficient to induce healing of complex fractures with large bone gaps[12].

Platelet rich plasma

Platelet concentration counts in a healthy individual between 1.5-4.5 × 10^7 μL. To be labeled as platelet rich plasma (PRP), a platelet count of 4-5 times of the baseline should be present in the platelet concentrate[13].

Platelets contain granules which contain multiple growth factors and cytokines that play an important role in the early responses of bone repair and also help the regeneration of tissues with low healing potential[14].

PRP preparation includes drawing of blood into a tube containing an anticoagulant followed by centrifugation then treated with calcium chloride and bovine thrombin which forms a gel-like substance for direct application[15].

Hakimi et al[15] compared combined PRP with autologous cancellous graft and isolated autologous cancellous graft in long bones of minipigs. There was a significantly better bone regeneration in case of combined PRP and graft. Yamada et al[16] combined mesenchymal stem cells with PRP in a canine model that resulted in a higher maturation of bone.

PRP is autologous and nontoxic, with no risks of immunogenic reactions. However, the use of bovine thrombin leads to the development of auto-antibodies against factors V and XI, and thus the risk of life-threatening coagulopathies[17].

BMPs

They are involved in early limb development and enhance maturation and function of differentiated cells (chondrocyte and osteoblast)[18]. They bind to their
receptors (serine/threonine kinase receptors) which are responsible for modulating gene transcription[19].

BMP-2 and BMP-7 are the most intensively studied BMPs in the recombinant technology. Their role in the treatment of fractures nonunion has been evaluated in multiple trials and small case series[20].

Adult patients with a diaphyseal fracture tibia with a residual bone defect were randomly received either an autogenous bone graft or a combination of an allograft and rhBMP-2 on a collagen sponge. Healing rates in the autograft group was 66.6% and in the rhBMP-2 group was 86.6%[21].

In a prospective randomized trial, tibial nonunions that required internal fixation and supplemental bone grafting were randomly received either rhBMP-7 or fresh autograft bone, rhBMP-7 (81% healing rate) demonstrated clinical equivalence with respect to fracture union compared with the autograft group (85% healing rate) at 9 mo (P = 0.0524) and 2 years (P = 0.93)[22].

However, in a prospective study, Ekrol et al[23] reported conflicting results with the use of rhBMP-7. Thirty patients with a distal radius malunion were stabilized with a fixator or a plate and were randomly received either an autogenous bone graft or a combination of an allograft and rhBMP-2. The autogenous bone graft group had higher healing rates and shorter time to union (P = 0.02). However, the study sample size was small and there was no power analysis presented in the study for sample size calculation. The rhBMP-7 treatment group had higher rates of inflammatory swelling and osteolysis at the site of malunion site.

RhBMPs are among the most common biologic agents used for enhancing bone repair. However, there are certain hurdles limiting their efficacious use in humans. First, rhBMPs have a short half-life and complete healing large bone defects need more than single dose[24]. Second, the ideal carrier matrix for rhBMPs is yet to be identified[25]. Third, supraphysiologic doses (in milligrams) of rhBMPs are being used in humans, and its long-term effects are not clearly known. Consequently, rhBMPs are not FDA-approved in the pediatric age group, in pregnant patients, or in the presence of tumors. Finally, there are complications associated with rhBMPs that are either related to the initial inflammatory response induced by the proteins (neck swelling, seroma, neuritis) or an extension of their osteoinductive function (heterotopic ossification, paraplegia, transient osteopenia)[26].

**Fibroblast growth factor**

Fibroblast growth factor (FGF) receptor promotes expression of multiple genes that are involved in all stages of osteogenesis. FGF signaling also controls expression of multiple genes that are involved in all stages of osteogenesis. FGF signaling also controls expression of multiple genes that are involved in all stages of osteogenesis. FGF signaling also controls expression of multiple genes that are involved in all stages of osteogenesis. FGF signaling also controls expression of multiple genes that are involved in all stages of osteogenesis. FGF signaling also controls expression of multiple genes that are involved in all stages of osteogenesis.

A study on the safety and efficacy of rhFGF-2 in fracture, suggested a beneficial effect of rhFGF-2 on bone repair. However, none of the clinical studies has demonstrated any significant improvement in the healing rates compared with the controls[28].

**Stem cells**

A stem cell is a cell that has two essential characters: and ability to differentiate into a particular cell type and self-renew[29]. Adult stem cells are pluripotent. They participate in physiologic remodeling/turnover of normal tissues and repair of the injured tissue[30].

Bone marrow is the most intensively studied source of stem cells for bone repair. However, stem cells have been harvested from other tissues, including muscle, periosteum, adipose tissue, vascular pericytes, dermis, and peripheral blood[31]. Fat-derived stem cells still on debate[32].

Quarto et al[33] demonstrated successful healing of large bone defects (average of 5 cm) in three patients with bone marrow-derived mesenchymal stem cells (MSCs) seeded on a ceramic scaffold.

Marcacci et al[34] used bone marrow-derived MSCs seeded on a ceramic scaffold to treat four diaphyseal bone defects which were stabilized with external fixators. All bone defects demonstrated complete healing at an average of 6 mo with no recorded complications.

Novel techniques of MSCs harvesting, in vitro expansion are encouraging[35]. MSCs in vitro expansion done by growing them in an osteogenic differentiation media prior to transplantation in the host[36]. But these approaches add costs and risks of viral or bacterial contamination, besides time consuming since they require a two-stage surgery[37].

The use of MSCs in fracture healing is still in the beginnings, mainly due to a lack of studies into the MSCs in vivo biology in the fracture environment[38].

**Tissue engineering**

Bone tissue-engineering is a strategy combines the principles of orthopaedics with biology, physics, materials science and engineering, to generate cell-driven, functional tissues[39].

It combines progenitor cells which are seeded in biocompatible scaffolds with appropriate growth factors, in order to form hybrid constructs to generate and maintain bone, especially for the management of large bone defects[40].

Seven human studies have been done using these hybrid constructs for bone defects healing[41]. They are heterogeneous studies and drawing conclusive evidence from them is complicated[41].

Bone-tissue engineering is still starting, and there are many concerns of efficacy, safety and cost should be addressed before being clinically applied[42].

**Gene therapy**

It involves the transfer of genetic material into the target cell genome. Genetic material can be done using viral (transfection) or non-viral (transduction) vectors, and by either an in vivo or ex vivo gene-transfer strategy[43].

Delivery of growth factors, particularly BMPs, using
this technique for bone healing produced encouraging results in animal studies but the issues of cost, efficacy and safety still under concern[44,45].

Systemic enhancement of bone regeneration
The use of systemic agents, including growth hormone[46] and parathyroid hormone (PTH)[47] for acceleration of bone-regeneration process is under extensive research. There are multiple trials conducted that these biologic agents can be administered systemically to enhance bone repair[48]. The major advantage of these systemic drugs is that healing can be stimulated for a prolonged period of time besides being non-invasive procedures. Recombinant PTH is available clinically, but two more agents; sclerostin antibody and anti-Dkk-1 (anti-Dickkopf antibody), are currently being developed for enhancing bone repair in humans. In preclinical fracture studies, sclerostin antibody systemic administration significantly increases the bone mass and callus[49].

Future directions
A strong need of clinical results is required to further progress in cell therapy. Launched trials will hopefully provide this information in the near future. If clinical results are positive, far greater challenges may be raised by the development of more complex tissue engineering techniques, and this may allow the treatment of large bone defects and unsolved situations. A multidisciplinary approach will be required to improve implanted cell survival and to ensure prompt vessel ingrowth into the biomaterial via careful selection of structure and shape. The development of new combinations (hydrogel-based, bioceramic-based, or other) that could eventually craft solutions for supplying cells and biomaterials percutaneously is expected in the near future. The immunosuppressive properties of MSCs may allow the transplantation of allogeneic MSCs in various orthopedic conditions, with the establishment of cell banks for regenerative medicine. Early trials evaluating allogeneic MSCs in delayed unions are already under way. And last but not least, a future step that may help to further define and spread these therapies is a careful cost–benefit assessment and a broad economic evaluation to clarify the best indications of bone repair cell therapy as a standard procedure, if confirmation of safety and efficacy is clearly derived from current trials[50].

CONCLUSION
Successful fracture healing requires mechanical stability and a viable biologic microenvironment. Fractures with compromised biology will benefit from treatment options that can augment the biologic potential at the site of bone repair. An ideal bone graft should be osteoinductive, osteoconductive, osteogenic, and angiogenic. Furthermore, an ideal bone graft should provide mechanical support and promote physiologic healing without any significant adverse effects.

Regenerative strategies like the use of bone morphogenic proteins, plateleth rich plasma, stem cells and anabolic agents are promising in the treatment of fractures either acute or fracture non-union.

However, large bone defects with compromised biology may not be amenable to simple regenerative strategies and will require polytherapy, which incorporates all of the critical components that are required for bone regeneration.

In future, use of these therapies in the bone regeneration under specific indications and with safety roles will simulates the normal bone formation cascade with reduced morbidity and cost in the long term.

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