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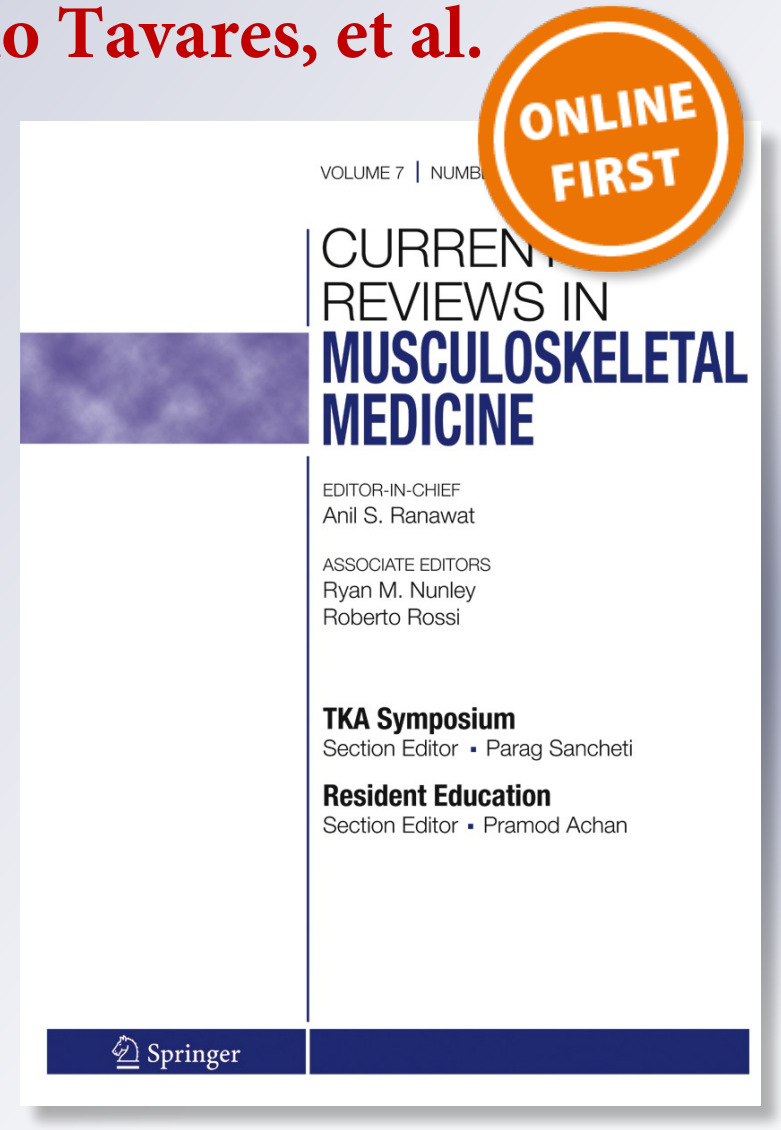
Current Reviews in Musculoskeletal Medicine

ISSN 1935-973X

Curr Rev Musculoskelet Med
DOI 10.1007/s12178-014-9225-z



Springer

Curr Rev Musculoskelet Med
DOI 10.1007/s12178-014-9225-z

KNEE: STEM CELLS (M FERRETTI, SECTION EDITOR)

Updates in biological therapies for knee injuries: bone

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Abstract Bone is a unique tissue because of its mechanical properties, ability for self-repair, and enrollment in different metabolic processes such as calcium homeostasis and hematopoietic cell production. Bone barely tolerates deformation and tends to fail when overloaded. Fracture healing is a complex process that in particular cases is impaired. Osteoprogenitor cells proliferation, growth factors, and a sound tridimensional scaffold at fracture site are key elements for new bone formation and deposition. Mechanical stability and ample vascularity are also of great importance on providing a proper environment for bone healing. From mesenchymal stem cells delivery to custom-made synthetic scaffolds, many are the biological attempts to enhance bone healing. Impaired fracture healing represents a real burden to contemporary society. Sound basic science knowledge has contributed to newer approaches aimed to accelerate and improve the quality of bone healing.

Keywords Bone fracture · Fracture healing · Angiogenesis · Callus · Endochondral · Intramembranous · Periosteal · Bone morphogenetic protein · Cancellous voids · Bone defects · Allograft · Bone substitutes · Scaffolds · Bone graft · Growth factors · Bone regeneration · Gene therapy · Physical stimulation · Ultrasound · Osteochondral graft · Knee injuries · Biological therapies

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Introduction

Bone is a specialized tissue able to regenerate [1, 2]. This special property assures that bone heals without connective tissue scar [3]. Fracture repair is a complex staged process that depends on mechanical and biological environment [4, 5]. Blood supply and a certain degree of stability at the fracture site are requisites for bone healing [6, 7]. Bone is only formed on a solid scaffold. Intramembranous ossification and chondral ossification are the 2 ways by which bone develops [8]. In the first one, a base of connective tissue serves as a scaffold into which bone is deposited. In the latter, a base of mineralized cartilage is replaced by bone. Mechanical environment dictates the modality of bone healing. There are 2 basic processes of bone healing [9]. Natural healing, also denominated secondary osseous repair, implies in callus formation and is observed when the blood supply is ample and a limited degree of motion exists at fracture site. This healing pattern is observed in fracture treatments adopting casts, intramedullary nails, and bridging plates. In rare conditions, where the fracture site is reduced anatomically and maintained under compression, bone heals without callus formation. In this process, where absolute stability is provided, osteonal units cross-fracture the site directly. This process is denominated as primary or direct healing. Healing is, however, not the only aim of fracture treatment. Adequate restoration of limb function is also an ultimate goal. Therefore, uneventful bone healing associated with limb malalignment and joint incongruence, a condition described as malunion, should be avoided. Malunions are generally avoided by the adoption of proper principles of fracture care, with adequate fracture reduction, stable fixation, and early joint motion [10]. Malunions are not associated with healing impairment but with the inability to restore former bone anatomy. Impaired

Published online: 17 July 2014

fracture healing, despite the natural bone ability to regenerate, is expected in a group of patients. A number of risk factors are associated with impaired bone healing like systemic diseases, chronic use of anti-inflammatory drugs, degree of associated soft tissue injury, fracture pattern, and treatment modality [11]. A condition where the healing process ceases to the extent that complementary surgical means are needed to achieve a complete fracture repair is denominated nonunion. In the United States roughly 10% of the 8 million fractures sustained every year are associated with varying degrees of healing impairment [12•]. Impending vascular supply, mechanical instability, existence of large defects, and the proliferation of competing tissues at the fracture site are the main causes of nonunions. Malunions and nonunions around the knee promote loss of function, multiple surgical interventions, morbidity to the patient, and a burden to socioeconomic costs. Different strategies have been proposed to enhance bone healing or to deal with bone defects. Biological principles associated with those strategies involve osteogenic transfer, osteoconduction, and osteoinduction. Gold standard method of osteogenic transfer is autologous cancellous graft, where bone cells and matrix are transferred to the fracture site to enhance its healing ability. Isolated cell transfer to a void is less effective than to a scaffold with architecture very similar to the bone. A material that provides a tridimensional base for bone formation is named osteoconductive. Growth factors are able to produce bone tissue even in places where bone normally does not occur. This effect is known as osteoinduction and has been extensively adopted in the management of nonunions. Although autologous bone graft is the only method that comprises osteogenic, osteoconductive, and osteoinductive properties, a number of interesting approaches have been developed to promote or even augment bone healing. The scope of this manuscript is the description of biological approaches aimed at bone regeneration and whenever possible correlate them to fractures and nonunions around the knee.

Improving bone healing

The diamond concept has advocated that 4 prerequisites are essential for bone healing [9]. First, the existence of a population of multipotent mesenchymal cells able to originate osteoblasts at fracture site. Second, concentration of growth factors and signaling molecules at fracture hematoma enabling cell interactions. Third, the constitution of an extracellular matrix acting as a scaffold for cells deposition and interaction. Fourth, mechanical stability at the fracture site, which is mandatory for either callus formation or direct bone healing. Two other elements are considered crucial for fracture repair, namely the vascularity at fracture site and host comorbidities and genetic predisposition [13]. Strategies for enhancing bone healing shall address at least 1 of the prerequisites depicted by diamond concept.

Pluripotent mesenchymal cells

Mesenchymal stem cells (MSCs) are considered a key element in bone regeneration [14, 15]. These cells are precursors of different cell lineages including osteoprogenitors. Currently, there is a lack of knowledge on specific markers for MSCs that makes the prospective purification of native bone marrow mesenchymal cells (BM MSCs) extremely difficult [16••]. One of the methods of isolation of BM MSCs consists on aspiration of the marrow from the anterior iliac crest. Bone marrow that is filtered does eliminate fat and debris. Bone marrow cells are successively filtered until mononuclear cells are isolated and concentrated in a fluid that is ready for the in vivo injection [17, 18]. Experimental studies as well as pilot clinical trials have demonstrated satisfactory outcomes. Tissue engineering developments are scaffolds seeded with MSCs or even composites of pluripotent cells. In a laboratory setting, the association of bone morphogenetic proteins and MSCs has enhanced osteogenesis. Beyond their ability to generate osteoprogenitors and osteoblasts, MSCs are able to recruit new cells to fracture cells, particularly attracting host vasculature, which would be an indirect path for enhancing bone repair [19].

Growth factor

A number of growth factors associated with bone healing have been used as local biological enhancing therapies.

Bone morphogenetic proteins

Almost 50 years ago, it was demonstrated that bone extracellular matrix contains substances that can generate new bone formation [20]. Since then, more than 15 substances have been isolated and denominated bone morphogenetic proteins (BMPs). These proteins are involved in different stages of the bone healing cascade, regulating cell recruitment and differentiation [21, 22]. Experimental and clinical data has been retrieved from the use of different types of BMPs showing equivalent results once compared with bone autograft [23–27]. The main advantage of BMPs should be the ability to enhance bone healing without the potential complications associated with bone graft harvesting. A very discouraging argument for clinical use of BMPs is its cost-effectiveness, due to high costs associated with this therapy and clinical results that do not compare with those observed in animal models [28].

Moreover, BMP-specific complications in the field of lumbar spine fusions, which include the heterotopic ossification within the epidural space or neuroforamina, postoperative

radiculitis, and endplate osteolysis with interbody device subsidence, have been described recently arguing for the need of proper designed clinical studies to define the use of BMPs in spine surgery [29, 30].

Vascular endothelial growth factor

Ample blood supply is a requisite for bone healing. Therefore, angiogenesis at fracture site is of paramount importance. Vascular endothelial growth factor (VEGF) is directly involved with new blood vessels proliferation and differentiation [31, 32]. Moreover, it has demonstrated its positive effects on osteoprogenitor cells, especially when combined with BMPs [33]. Nevertheless, VEGF is under preclinical evaluation, since it's very expensive to produce and very fragile to manipulate in vivo [34].

Platelet derived growth factor

Platelet Derived Growth Factor (PDGF) is involved not only on angiogenesis but also on osteoblast proliferation [35, 36]. Thus, it enhances bone healing through improvement of vascularity and increase of osteogenesis. PDGF as well as other angiogenic factors are produced at fracture hematoma at the moment that platelets degranulate. PDGF is available in the recombinant form and its use in orthopedics is limited to pilot trials. Platelet-rich plasma (PRP) is also a method of delivering angiogenic growth factors to fracture sites. Laboratory data on the use of PRP advocates that it promotes cell proliferation and increases the extracellular matrix [35]. Clinical data is still controversial and based on case series where PRP is used either alone or associated with bone grafts or bone substitutes [37, 38]. To date, there is no evidence sustaining the use of PRP as a supporting aid in bone regeneration [39–41].

Scaffold

Temporary platforms with a solid architecture are a requisite for depositing new bone. In case of severe bone loss or bone defects, gaps need to be filled up to allow bone healing.

Biological grafts

Bone autograft is considered the gold standard method for enhancing bone healing due to its osteogenic, osteoinductive, and osteoconductive properties [42••]. Autograft harvesting, however, is associated with a considerable complication rate at the donor site and also with the limited availability. An alternative source to scaffolds is allogeneic bone. The main advantage of allografts is its unlimited availability associated with no need for additional harm to the patient. The main advantage of using allografts is its unlimited availability associated with no need for additional harm to the patient related to autologous bone graft harvesting. Nevertheless, the risk of disease transmissions and infection are drawbacks related to the use of allografts. Demineralized bone matrix (DBM) is considered a sort of bone allograft without viable cells. It has osteoconductive and osteoinductive properties [43••]. DBM retains many proteins and growth factors native to bone and is available as putty, paste, or flexible pieces. There is a huge inconsistency among different DBM composites with variability in bone regeneration observed in clinical and experimental series [44–46]. DBM is considered void filler that obviate the need to harvest autograft or even extend the volume of an autograft. Nevertheless, as an alloimplant it carries out the risk of disease transmission and its use alone in sites subjected to high compressive loads is not advisable, due to its lack of structural rigidity [43••].

Artificial grafts

Synthetic scaffolds have been developed with the aim of overcoming limitations of biological grafts. Although synthetic scaffolds can be customized according to the needs of the patient regarding size, porosity, rigidity, and format, they are mainly osteoconductive and osteoinductive platforms [42••]. That means they act as a tridimensional frame for cell adhesion and for allowing the proliferation of bone regeneration. Different raw materials can be used for producing synthetic scaffolds. Calcium phosphate ceramics, like hydroxyapatite and tricalcium phosphate, have been largely used as scaffolds, once their physical properties can be tailored according to specific needs, especially regarding porosity, which can be adjusted for ideal osseous ingrowth [47]. Bioactive glasses are hard materials made of varying proportions of sodium oxide, calcium oxide, and silicon dioxide. Like calcium phosphate ceramics, bioactive glasses form a bone-like apatite layer on their surfaces in the living body, and bond to bone through this apatite layer [48]. Ceramic scaffolds are associated with brittleness and low mechanical strength when used as a weight-bearing component. Polymers are organic materials also used for producing scaffolds. They are molecules with very good processability and biocompatibility. Polyesters are the most used polymers in orthopedics. As polymers have converse mechanical properties to ceramics, interesting composites have been proposed based on the association of both [49]. Synthetic scaffolds can be coated with extracellular bone matrix proteins as well as seeded with mesenchymal stem cells. These associations aim to improve osteogenic properties in a base that is primarily osteoconductive and osteoinductive [50]. Synthetic scaffolds constitute a promising research field in bone regeneration. To date, bone autograft remains the gold standard technique while ceramic scaffolds are the closest synthetic frames to resemble bone architecture.

Gene therapy

Gene therapy is a very interesting approach in bone regeneration, where, instead of delivering a limited pharmaceutical dose of growth factors, gene delivery induces the production of physiological amounts of those proteins over time [51•]. Genes can be delivered in vivo directly into recipient cells at the fracture site or can be transferred in vitro into stem cells that will be delivered at the fracture site. The DNA transfer will ultimately result in the expression of proteins that will be involved in bone regeneration. Critical-size bone defect experimental models are used to assess the potential of gene therapy. There are, to date, no clinical trials on gene therapy aimed at bone regeneration. Hurdles involve the risks of immune responses while using viral vectors, limitations on transferring expected outcomes from small animals to humans, and the control of the amount and shape of new generated bone [52].

Systemic pharmacologic agents

A number of drugs have been used in the management of osteoporosis aiming to improve bone formation reducing the risks of fragility fractures. Some of these drugs are considered not only to be efficient on fracture prevention but also on improving fracture healing [53••].

Parathyroid hormone (PTH)

Teriparatide, a human derivative of parathyroid hormone, has shown anabolic effects on osteoblast proliferation in osteoporotic patients. It reduces fracture risks by increasing bone mass [54]. Laboratory studies have demonstrated that teriparatide increases the volume of callus formations as well as bone strength [55]. Clinical trials are still ongoing. The only prospective randomized trial using teriparatide for accelerating bone healing in distal radius fractures showed accelerated fracture healing [56•].

Antiresorptive agents

Bisphosphonates are widely used agents to treat osteoporosis. They promote a bond of hydroxyapatite crystals, thus, inhibiting bone resorption by osteoclasts and decreasing the rate of bone remodeling. Different studies have confirmed that bisphosphonates treatment does not improve fracture healing [57, 58]. This happens because bisphosphonate use interferes with the maturation of cartilaginous callus delaying its remodeling to woven bone and, subsequently, to lamellar bone.

Strontium ranelate effect on fracture repair has been also evaluated in experimental studies. While in normal rats the administration of strontium ranelate did not show any difference in fracture healing behavior, in ovariectomized rats its use has been beneficial and comparable with PTH [59, 60].

Wnt signaling proteins

Secreted proteins Dickkopf 1 (Dkk1) and sclerostin are inhibitors of Wnt signaling, an important pathway in the development of osteoblasts from mesenchymal cells [61]. Hence, the use their antibodies has been studied regarding the ability of inducing new bone formation. Administration of Dkk1 antibodies has been beneficial in the day of the fracture but not a few days after the injury in animal models [61, 62]. Sclerostin antibodies have also shown capacity to increase bone formation in rat models [63]. The clinical use of these 2 antibodies is foreseen as an adjuvant therapy for enhancing bone healing.

Physical stimulation therapies

Bone healing is dependable on the biological and biomechanical environment. A number of physical stimulation therapies have been proposed in order to accelerate bone healing [64].

Low-intensity pulsed ultrasound (LIPUS)

LIPUS is transmitted into connective tissues as an acoustic wave. The intensities vary from 0.1 to 0.3 W/cm^2 and shall not be higher than 1W/cm^2. Average treatment time is 4–6 months with daily sessions of 20 minutes. Clinical and experimental studies have confirmed that LIPUS stimulates callus formation and radiographic bone healing in fresh fractures [65]. LIPUS is a safe treatment in acute fractures and nonunions and contraindicated in cases of substantial bone loss [66].

Electromagnetic field stimulation

Electromagnetic effects on bone cells and the relationship between electricity and callus formation has been already reported [67]. The results of clinical trials on the use of electromagnetic stimulation aimed at bone healing have been mixed. A recent meta-analysis did not depict a significant impact of electromagnetic stimulation on delayed unions. Authors, nevertheless, appointed that the role of electromagnetic stimulation is uncertain since more appropriate studies with reproducible methods and appropriate sample sizes are needed [68].

Bone healing in knee surgery

Trauma around the knee is a common condition in the contemporary society. While in high-energy trauma challenges are mostly related to the extent of soft tissues damage and bone comminution, in low-energy fragility fractures bone fixation and healing are the demanding issues. Each of the bones that constitute the knee joint has particularities that can affect its healing process once fractured or osteotomized.

Proximal tibia

Proximal tibia has a very thin soft tissues envelope and a close relationship with popliteal vessels. High-energy trauma in proximal tibia is frequently associated with a compromise of soft tissues envelope or even compartment syndrome. As proximal tibia is mainly constituted of cancellous bone, nonunions are rarely associated with fractures at this level. Malunions however, are very likely, especially if fractures were not treated according to principles of articular fractures management. Fractures of the tibial plateau often involve depression of the articular fragments. Elevating the depressed articular fragments often reveals a metaphyseal void. Anatomic reduction of articular surface normally requires either rafting or grafting of the subchondral bone. Bone autograft is the preferred method for filling up the resulting void in metaphyseal bone, although it does not allow full weight bearing until the fracture heals. Recent studies have demonstrated that injectable calcium phosphate cement is able to fill up the voids allowing full weight bearing within 6 weeks after surgery, without the hazards associated with bone autograft harvesting [69, 70]. Bone cements are, nevertheless, only fillers that are not replaced by bone tissue. This can be an issue in late arthroplastic reconstructions, due to resulting bone defects at the tibial plateau. A recent review on the literature has pointed out that use of bone substitutes in tibial plateau fractures is associated with shorter total operative time, greater tolerance of early weight bearing, and improved early functional outcomes within the first year of postsurgery. The authors have concluded that, despite a lack of good quality randomized clinical trials, there is sufficient evidence supporting the use of bone substitutes for dealing with depressed tibial plateau fractures [71•]. Proximal tibia is also a site for corrective osteotomies around the knee. Open wedge proximal tibia osteotomy is aimed to correct knee varus alignment. This technique generates a space at the osteotomy site and some authors defend the use of bone autograft or bone substitutes for filling up this void. The development of more stable implant constructs, as locking plates, made the use of grafts at opening wedge techniques questionable. Although some articles have reported satisfactory outcomes with the use of bone substitutes augmenting osteotomy gaps, it has been shown that augmentation is not always needed [72].

Patella

Patella is a subcutaneous bone that leverages knee extensor mechanism. Patellar fractures commonly compromises the ability of stretching the knee. Nonunions are rare and may be associated with knee stiffness, pain, and loss of function. Patellar fractures must follow principles of articular fracture management, which involves anatomic reduction, stable fixation, and early motion. In cases of nonunions, attempts to reestablish a complete range of motion may involve internal fixation or even partial or total resection of this bone [73]. There is a paucity of data depicting the use of biological therapies on enhancing patellar bone healing.

Distal femur

Distal femur is characterized by its articular surface and relationships with proximal tibia and patella. Distal femur fractures can be either intra-articular or extra-articular, but they always affect knee function. Distal femur metaphyseal bone loss is sometimes challengeable and asks for reconstructive alternatives. Bone grafting is frequently needed in cases of distal femur nonunion or segmental loss. Reamer-irrigator-aspirator (RIA) is an alternative technique to retrieve bone autograft from femoral medullary canal. Medullary debris is qualitatively comparable with iliac crest, and, in some cases, great amounts of graft are available [74•]. RIA grafts are not structured and, therefore, its use alone is not advocated in cases of large segment loss. In those cases, where metaphyseal defects are present, the use of RIA grafts associated with structured scaffolds, like metal cages, seems to be a promising alternative.

Osteochondral allografts in knee malunions

Current basic science and clinical data support the safety of fresh osteochondral allografts in the management of malunions around the knee [75]. Osteochondral allograft (OCA) is a single-stage technique that allows the management of post-traumatic articular malunion, by means of a biologic arthroplasty [76, 77]. Chondrocyte viability is a key success element of OCA transplantation. Therefore, improvement in storage methods is essential for keeping highest rates of viable chondrocytes for longer periods of time. OCA use in proximal tibial malunion has been reported in small case series with a survivorship rate of 95% and 65% reported, respectively, at 5 and 15 years [78•].

Conclusions

Bone injuries around the knee require anatomic restoration, stable fixation, and early motion. A number of biologic strategies for enhancing bone healing, from mesenchymal stem cells transfer to provision of osteoconductive scaffolds and osteoinductive growth factors have been used in experimental and clinical set. Gene therapy seems to be a promising alternative once it targets the continuous physiological production of growth factors. Systemic drug therapies as well as physical stimulation with low-intensity ultrasound proved to be effective in stimulating callus formation. Biological therapies on optimizing bone repair result from basic science approaches and clinical needs aiming to improve patient care. Orthopedic trauma is associated with morbidity and high socio-economic costs. Development strategies that allow faster and solid bone healing represent a turning point to the injured patient and to the whole society where this patient is inserted.

Compliance with Ethics Guidelines

Conflict of Interest M. Kfuri Jr, R. Lara de Freitas, B. B. Batista, R. Salim, M. T. Castiglia, R. A. Tavares, and P. H. Araújo declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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