World Journal of Orthopedics

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5312/wjo.v6.i8.623

World J Orthop 2015 September 18; 6(8): 623-628
ISSN 2218-5836 (online)

Recent biological trends in management of fracture non-union

Khaled M Emara, Ramy Ahmed Diab, Ahmed Khaled Emara

Khaled M Emara, Ramy Ahmed Diab, Department of Orthopaedic Surgery, Ain Shams University, Cairo 11511, Egypt

Ahmed Khaled Emara, Faculty of Medicine, Ain Shams University, Cairo 11511, Egypt

Author contributions: Emara KM designed and wrote up the research; Diab RA collected the data and wrote up the research; Emara AK collected the data and wrote up the research.

Conflict-of-interest statement: None.

Correspondence to: Dr. Khaled M Emara, Professor, Department of Orthopaedic Surgery, Ain Shams University, 13 B Kornish elNile, Agha Khan, Cairo 11511, Egypt. kmemara@hotmail.com
Telephone: +20-2-22055661
Fax: +20-2-22055662

Received: February 13, 2015
Peer-review started: February 13, 2015
First decision: May 13, 2015
Revised: May 31, 2015
Accepted: July 16, 2015
Article in press: July 17, 2015
Published online: September 18, 2015

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

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 morphogenic proteins, platelet rich plasma, stem cells and anabolic agents are promising in the treatment of fractures either acute or fracture non-union.

Emara KM, Diab RA, Emara AK. Recent biological trends in management of fracture non-union. *World J Orthop* 2015; 6(8): 623-628 Available from: URL: http://www.wjgnet.com/2218-5836/full/v6/i8/623.htm DOI: http://dx.doi.org/10.5312/wjo.v6.i8.623

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 (osteoconductive 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

Autologus 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%[4].

The iliac crest is the commonly used donor sites. But harvesting has its complications and needs an additional surgical procedure[5].

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[6]. 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[7].

Bone-graft substitutes are alternatives to autologous or allogeneic bone grafts. They are composed of scaffolds, such as collagen, hydroxyapatite, b-tricalcium phosphate[8] that enhance the proliferation of bone cells for bone regeneration[6].

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 aveaged 6 mo after injection. In a retrospective study involving 60 atrophic tibial nonunions Hernigou *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 × 10^5/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[13].

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[14].

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 rhBMP-7 or autogenous bone graft. 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 are 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 osteoblast gene expression and apoptosis[27].

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-renewal[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[7]. Fat-derived stem cells still on debate[31].

Quarto *et al*[32] 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*[33] 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[34]. MSCs *in vitro* expansion done by growing them in an osteogenic differentiation media prior to transplantation in the host[35]. But these approaches add costs and risks of viral or bacterial contamination, besides time consuming since they require a two-stage surgery[36].

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[37].

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[38].

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[39].

Seven human studies have been done using these hybrid constructs for bone defects healing[40]. 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-Dickopff 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, platelet 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 regenneration.

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.

REFERENCES

1 **Childs SG**. Stimulators of bone healing. Biologic and biomechanical. *Orthop Nurs* 2003; **22**: 421-428 [PMID: 14705472 DOI: 10.1097/00006416-200311000-00010]

2 **Heckman JD**, Sarasohn-Kahn J. The economics of treating tibia fractures. The cost of delayed unions. *Bull Hosp Jt Dis* 1997; **56**: 63-72 [PMID: 9063607]

3 **Carofino BC**, Lieberman JR. Gene therapy applications for fracture-healing. *J Bone Joint Surg Am* 2008; **90** Suppl 1: 99-110 [PMID: 18292364 DOI: 10.2106/JBJS.G.01546]

4 **Zimmermann G**, Müller U, Löffler C, Wentzensen A, Moghaddam A. [Therapeutic outcome in tibial pseudarthrosis: bone morphogenetic protein 7 (BMP-7) versus autologous bone grafting for tibial fractures]. *Unfallchirurg* 2007; **110**: 931-938 [PMID: 17989951 DOI: 10.1007/s00113-007-1347-y]

5 **Bhargava R**, Sankhla S, Gupta A, Changani R, Gagal K. Percutaneous autologus bone marrow injection in the treatment of delayed or nonunion. *Indian J Orthop* 2007; **41**: 67-71 [PMID: 21124686 DOI: 10.4103/0019-5413.30529]

6 **Kettunen J**, Mäkelä EA, Turunen V, Suomalainen O, Partanen K. Percutaneous bone grafting in the treatment of the delayed union and non-union of tibial fractures. *Injury* 2002; **33**: 239-245 [PMID: 12084640 DOI: 10.1016/S0020-1383(01)00075-4]

7 **Giordano A**, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. *J Cell Physiol* 2007; **211**: 27-35 [PMID: 17226788 DOI: 10.1002/jcp.20959]

8 **Bajada S**, Harrison PE, Ashton BA, Cassar-Pullicino VN, Ashammakhi N, Richardson JB. Successful treatment of refractory tibial nonunion using calcium sulphate and bone marrow stromal cell implantation. *J Bone Joint Surg Br* 2007; **89**: 1382-1386 [PMID: 17957083 DOI: 10.1302/0301-620X.89B10.19103]

9 **Sim R**, Liang TS, Tay BK. Autologous marrow injection in the treatment of delayed and non-union in long bones. *Singapore Med J* 1993; **34**: 412-417 [PMID: 8153688]

10 **Connolly JF**, Guse R, Tiedeman J, Dehne R. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. *Clin Orthop Relat Res* 1991; **(266)**: 259-270 [PMID: 2019059 DOI: 10.1097/00003086-199105000-00038]

11 **Hernigou P**, Poignard A, Beaujean F, Rouard H. Percutaneous autologous bone-marrow grafting for nonunions. Influence of the number and concentration of progenitor cells. *J Bone Joint Surg Am* 2005; **87**: 1430-1437 [PMID: 15995108 DOI: 10.2106/JBJS.D.02215]

12 **Galois L**, Bensoussan D, Diligent J, Pinzano A, Henrionnet C, Choufani E, Stoltz JF, Mainard D. Autologous bone marrow graft and treatment of delayed and non-unions of long bones: technical aspects. *Biomed Mater Eng* 2009; **19**: 277-281 [PMID: 20042794]

13 **Alsousou J**, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: a review of the literature. *J Bone Joint Surg Br* 2009; **91**: 987-996 [PMID: 19651823 DOI: 10.1302/0301-620X.91

B8.22546]
14 **Sánchez M**, Anitua E, Azofra J, Andía I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *Am J Sports Med* 2007; **35**: 245-251 [PMID: 17099241 DOI: 10.1177/0363546506294078]
15 **Hakimi M**, Jungbluth P, Sager M, Betsch M, Herten M, Becker J, Windolf J, Wild M. Combined use of platelet-rich plasma and autologous bone grafts in the treatment of long bone defects in mini-pigs. *Injury* 2010; **41**: 717-723 [PMID: 20097341 DOI: 10.1016/j.injury.2009.12.005]
16 **Yamada Y,** Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T. Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: tissue-engineered bone regeneration. *Tissue Eng* 2004; **10**: 955-964 [PMID: 15265313 DOI: 10.1089/1076327041348284]
17 **Sánchez AR,** Sheridan PJ, Kupp LI. Is platelet-rich plasma the perfect enhancement factor? A current review. *Int J Oral Maxillofac Implants* 2003; **18**: 93-103 [PMID: 12608674]
18 **Lieberman JR**, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. *J Bone Joint Surg Am* 2002; **84-A**: 1032-1044 [PMID: 12063342]
19 **Bragdon B**, Moseychuk O, Saldanha S, King D, Julian J, Nohe A. Bone morphogenetic proteins: a critical review. *Cell Signal* 2011; **23**: 609-620 [PMID: 20959140 DOI: 10.1016/j.cellsig.2010.10.003]
20 **Govender S**, Csimma C, Genant HK, Valentin-Opran A, Amit Y, Arbel R, Aro H, Atar D, Bishay M, Börner MG, Chiron P, Choong P, Cinats J, Courtenay B, Feibel R, Geulette B, Gravel C, Haas N, Raschke M, Hammacher E, van der Velde D, Hardy P, Holt M, Josten C, Ketterl RL, Lindeque B, Lob G, Mathevon H, McCoy G, Marsh D, Miller R, Munting E, Oevre S, Nordsletten L, Patel A, Pohl A, Rennie W, Reynders P, Rommens PM, Rondia J, Rossouw WC, Daneel PJ, Ruff S, Rüter A, Santavirta S, Schildhauer TA, Gekle C, Schnettler R, Segal D, Seiler H, Snowdowne RB, Stapert J, Taglang G, Verdonk R, Vogels L, Weckbach A, Wentzensen A, Wisniewski T. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J Bone Joint Surg Am* 2002; **84-A**: 2123-2134 [PMID: 12473698]
21 **Jones AL**, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, Pollak AN, Golden JD, Valentin-Opran A. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. *J Bone Joint Surg Am* 2006; **88**: 1431-1441 [PMID: 16818967 DOI: 10.2106/JBJS.E.00381]
22 **Friedlaender GE**, Perry CR, Cole JD, Cook SD, Cierny G, Muschler GF, Zych GA, Calhoun JH, LaForte AJ, Yin S. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am* 2001; **83-A** Suppl 1: S151-S158 [PMID: 11314793]
23 **Ekrol I**, Hajducka C, Court-Brown C, McQueen MM. A comparison of RhBMP-7 (OP-1) and autogenous graft for metaphyseal defects after osteotomy of the distal radius. *Injury* 2008; **39** Suppl 2: S73-S82 [PMID: 18804577 DOI: 10.1016/S0020-1383(08)70018-4]
24 **Seeherman HJ**, Li XJ, Bouxsein ML, Wozney JM. rhBMP-2 induces transient bone resorption followed by bone formation in a nonhuman primate core-defect model. *J Bone Joint Surg Am* 2010; **92**: 411-426 [PMID: 20124069 DOI: 10.2106/JBJS.H.01732]
25 **Carragee EJ**, Hurwitz EL, Weiner BK. A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. *Spine J* 2011; **11**: 471-491 [PMID: 21729796 DOI: 10.1016/j.spinee.2011.04.023]
26 **Shields LB**, Raque GH, Glassman SD, Campbell M, Vitaz T, Harpring J, Shields CB. Adverse effects associated with high-dose recombinant human bone morphogenetic protein-2 use in anterior cervical spine fusion. *Spine* (Phila Pa 1976) 2006; **31**: 542-547 [PMID: 16508549 DOI: 10.1097/01.brs.0000201424.27509.72]
27 **Kawaguchi H**. [Bone fracture and the healing mechanisms. Fibroblast growth factor-2 and fracture healing]. *Clin Calcium* 2009; **19**: 653-659 [PMID: 19398832]
28 **Kawaguchi H**, Jingushi S, Izumi T, Fukunaga M, Matsushita T, Nakamura T, Mizuno K, Nakamura T, Nakamura K. Local application of recombinant human fibroblast growth factor-2 on bone repair: a dose-escalation prospective trial on patients with osteotomy. *J Orthop Res* 2007; **25**: 480-487 [PMID: 17205557 DOI: 10.1002/jor.20315]
29 **Ehnert S**, Glanemann M, Schmitt A, Vogt S, Shanny N, Nussler NC, Stöckle U, Nussler A. The possible use of stem cells in regenerative medicine: dream or reality? *Langenbecks Arch Surg* 2009; **394**: 985-997 [PMID: 19644703]
30 **Khosla S**, Westendorf JJ, Mödder UI. Concise review: Insights from normal bone remodeling and stem cell-based therapies for bone repair. *Stem Cells* 2010; **28**: 2124-2128 [PMID: 20960512 DOI: 10.1002/stem.546]
31 **Niemeyer P**, Fechner K, Milz S, Richter W, Suedkamp NP, Mehlhorn AT, Pearce S, Kasten P. Comparison of mesenchymal stem cells from bone marrow and adipose tissue for bone regeneration in a critical size defect of the sheep tibia and the influence of platelet-rich plasma. *Biomaterials* 2010; **31**: 3572-3579 [PMID: 20153047 DOI: 10.1016/j.biomaterials.2010.01.085]
32 **Quarto R**, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 2001; **344**: 385-386 [PMID: 11195802 DOI: 10.1056/NEJM200102013440516]
33 **Marcacci M**, Kon E, Moukhachev V, Lavroukov A, Kutepov S, Quarto R, Mastrogiacomo M, Cancedda R. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Eng* 2007; **13**: 947-955 [PMID: 17484701 DOI: 10.1089/ten.2006.0271]
34 **Jäger M**, Herten M, Fochtmann U, Fischer J, Hernigou P, Zilkens C, Hendrich C, Krauspe R. Bridging the gap: bone marrow aspiration concentrate reduces autologous bone grafting in osseous defects. *J Orthop Res* 2011; **29**: 173-180 [PMID: 20740672 DOI: 10.1002/jor.21230]
35 **Kim SJ**, Shin YW, Yang KH, Kim SB, Yoo MJ, Han SK, Im SA, Won YD, Sung YB, Jeon TS, Chang CH, Jang JD, Lee SB, Kim HC, Lee SY. A multi-center, randomized, clinical study to compare the effect and safety of autologous cultured osteoblast(Ossron) injection to treat fractures. *BMC Musculoskelet Disord* 2009; **10**: 20 [PMID: 19216734 DOI: 10.1186/1471-2474-10-20]
36 **McGonagle D,** English A, Jones EA. The relevance of mesenchymal stem cells in vivo for future orthopaedic strategies aimed at fracture repair. *Curr Orthop* 2007; **21**: 262-267 [DOI: 10.1016/j.cuor.2007.07.004]
37 **Jones E**, English A, Churchman SM, Kouroupis D, Boxall SA, Kinsey S, Giannoudis PG, Emery P, McGonagle D. Large-scale extraction and characterization of CD271+ multipotential stromal cells from trabecular bone in health and osteoarthritis: implications for bone regeneration strategies based on uncultured or minimally cultured multipotential stromal cells. *Arthritis Rheum* 2010; **62**: 1944-1954 [PMID: 20222109]
38 **Salgado AJ**, Coutinho OP, Reis RL. Bone tissue engineering: state of the art and future trends. *Macromol Biosci* 2004; **4**: 743-765 [PMID: 15468269 DOI: 10.1002/mabi.200400026]
39 **Giannoudis PV**, Dinopoulos H, Tsiridis E. Bone substitutes: an update. *Injury* 2005; **36** Suppl 3: S20-S27 [PMID: 16188545 DOI: 10.1016/j.injury.2005.07.029]
40 **Chatterjea A**, Meijer G, van Blitterswijk C, de Boer J. Clinical application of human mesenchymal stromal cells for bone tissue engineering. *Stem Cells Int* 2010; **2010**: 215625 [PMID: 21113294 DOI: 10.4061/2010/215625]
41 **Ohgushi H**, Kotobuki N, Funaoka H, Machida H, Hirose M, Tanaka Y, Takakura Y. Tissue engineered ceramic artificial joint--ex vivo osteogenic differentiation of patient mesenchymal cells on total ankle joints for treatment of osteoarthritis. *Biomaterials* 2005; **26**: 4654-4661 [PMID: 15722135 DOI: 10.1016/j.biomaterials.2004.11.055]
42 **Patterson TE**, Kumagai K, Griffith L, Muschler GF. Cellular

strategies for enhancement of fracture repair. *J Bone Joint Surg Am* 2008; **90** Suppl 1: 111-119 [PMID: 18292365 DOI: 10.2106/JBJS.G.01572]

43 **Chen Y**. Orthopedic applications of gene therapy. *J Orthop Sci* 2001; **6**: 199-207 [PMID: 11484110 DOI: 10.1007/s007760100072]

44 **Calori GM**, Donati D, Di Bella C, Tagliabue L. Bone morphogenetic proteins and tissue engineering: future directions. *Injury* 2009; **40** Suppl 3: S67-S76 [PMID: 20082795 DOI: 10.1016/S0020-1383(09)70015-4]

45 **Tang Y**, Tang W, Lin Y, Long J, Wang H, Liu L, Tian W. Combination of bone tissue engineering and BMP-2 gene transfection promotes bone healing in osteoporotic rats. *Cell Biol Int* 2008; **32**: 1150-1157 [PMID: 18638562 DOI: 10.1016/j.cellbi.2008.06.005]

46 **Tran GT**, Pagkalos J, Tsiridis E, Narvani AA, Heliotis M, Mantalaris A, Tsiridis E. Growth hormone: does it have a therapeutic role in fracture healing? *Expert Opin Investig Drugs* 2009; **18**: 887-911 [PMID: 19480608 DOI: 10.1517/13543780902893069]

47 **Tzioupis CC**, Giannoudis PV. The Safety and Efficacy of Parathyroid Hormone (PTH) as a Biological Response Modifier for the Enhancement of Bone Regeneration. *Curr Drug Saf* 2006; **1**: 189-203 [PMID: 18690930 DOI: 10.2174/157488606776930571]

48 **van Bezooijen RL**, ten Dijke P, Papapoulos SE, Löwik CW. SOST/sclerostin, an osteocyte-derived negative regulator of bone formation. *Cytokine Growth Factor Rev* 2005; **16**: 319-327 [PMID: 15869900 DOI: 10.1016/j.cytogfr.2005.02.005]

49 **ten Dijke P**, Krause C, de Gorter DJ, Löwik CW, van Bezooijen RL. Osteocyte-derived sclerostin inhibits bone formation: its role in bone morphogenetic protein and Wnt signaling. *J Bone Joint Surg Am* 2008; **90** Suppl 1: 31-35 [PMID: 18292354 DOI: 10.2106/JBJS.G.01183]

50 **Petite H**, Vandamme K, Monfoulet L, Logeart-Avramoglou D. Strategies for improving the efficacy of bioengineered bone constructs: a perspective. *Osteoporos Int* 2011; **22**: 2017-2021 [PMID: 21523397 DOI: 10.1007/s00198-011-1614-1]

P- Reviewer: Patra SR, Teli MGA
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com