Review Article

Insights in biology and physiology of bone and bone healing in critical-sized bone defects: A brief review

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ABSTRACT

Bone, a mineralized connective tissue that is highly dynamic, complex and vascularized, is unlike other tissues; when injured, such tissue does not form scar; instead, it heals and remodels throughout life. However, bone has limited regenerative capacity; bone can only repair itself when the injury is not extensive. When the defect is too large, bone may not be able to repair itself without treatment. In this review, we discuss the biology and physiology of bone and bone healing.

ABSTRAK

Tulang, jaringan ikat termineralisasi yang sangat dinamis, kompleks, dan tervaskularisasi, tidak memiliki sifat seperti jaringan lainnya; ketika mengalami cedera, tulang tidak membentuk jaringan parut, namun sembuh dan mengalami pembentukan kembali (remodeling*) sepanjang hidup. Meskipun demikian, tulang memiliki kapasitas regenerasi yang terbatas; tulang hanya dapat memperbaiki diri ketika cedera tidak bersifat ekstensif. Ketika defek terlalu besar, tulang kemungkinan tidak mampu memperbaiki diri tanpa tatalaksana. Pada review ini, kami mendiskusikan biologi dan fisiologi tulang dan penyembuhan tulang.*

Keywords: bone, bone healing

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INTRODUCTION

Bone is a mineralized connective tissue that is highly dynamic, complex and vascularized.[1–3] Unlike other tissues, when injured, bone does not form scar[4]; instead, it heals and remodels throughout life.[1] However, the regenerative capacity of bone is limited. Several circumstances including necrosis, traumatic injury, pathological fracture, osteoporotic conditions, malignancies (e.g., osteosarcoma), and prior implant failures, may cause severe damage, or a large enough defect that proper repair of bone is not possible.[5–7] In other words, bone can only repair itself when the injury is not extensive.[6,8] Large bony defects, also referred to as critical-sized bone defects (CSBDs), remain a great challenge for orthopaedic surgeons as bone cannot heal by itself naturally when such conditions are present.[4,9] These defects should be filled by means of bone grafts, which could be retrieved either from the patients themselves (autograft) or from a donor (allograft), or bone tissue substitutes.[10,11] However, autologous and allogeneic bone grafts have several drawbacks, such as donor-site morbidity and rejection issues, respectively, whereas bone substitutes often fail to provide a desired clinical outcome. This indicates the need for a new effective therapy.

When considering treatment modalities for CSBDs, the mechanism of bone formation should be taken into consideration. The bone could regenerate by three mechanisms: by providing a scaffold or matrix that stimulates bone cells to grow on its surface (osteoconduction), inducing mesenchymal stem cells (MSCs) from surrounding tissues to differentiate into bone cells (osteoinduction), and providing living osteoprogenitor cells (osteogenesis).[12–15]

Osteogenicity can be artificially recreated by pre-seeding osteogenic cells onto the material before implantation; whereas, osteoinductivity requires the construct to be able to induce cell differentiation and therefore, is more difficult to re-create.[16] In this review, we elaborate the biology and physiology of bone and bone healing.

Bone composition

Bone performs several important functions in the body, such as providing a framework for attachment of muscles and other tissues; enabling body movements; providing protection of internal organs from injury; in addition to the production of blood cells; calcium homeostasis; and acid/base buffering.17 This mineralized dense connective tissue consists mainly of inorganic and organic matrices. The inorganic bone matrix accounts for 99% of the body's storage of calcium, 85% of the phosphorus, and 40 to 60% of the magnesium and sodium stores. Inorganic matrix predominantly consists of hydroxyapatite, in which its crystals are embedded between individual collagen molecules.[18,19] This matrix provides the majority of bone strength, stiffness, and resistance to a compressive force. Removal of the inorganic matrix will result in soft, malleable, and spongy bone; an example is osteomalacia or rickets secondary to vitamin D deficiency.[19]

The organic matrix, which is secreted by osteoblasts, is predominantly composed of type I collagen (90%) with less type III collagen.[18] Other than collagen, this matrix also contains proteoglycans, glycoproteins, and growth factors.[19] One of the most critical components of bone matrix is the BMPs, which are described in 1965 by Urist.[20] BMPs are members of the TGF-β superfamily; there are 20 known to exist. These proteins have critical osteoinductive properties in postnatal bone formation and healing.[19] BMPs, together with other transforming growth factor (TGF)-β family factors, interleukin (IL)-1, IL-6, osteocalcin, osteonectin, and bone sialoprotein play critical roles in the osteogenesis, mineralization, and remodeling of bone. Organic matrix gives bone its form and provides resistance to tensile forces.[19]

Bone development

Embryonic bone formation occurs only by two common routes: intramembranous and endochondral ossification[18,21,22] Both types of formation mirror the two types of bone healing that occurs in adults; therefore, it is critical to understand both processes.

The first type, intramembranous ossification, occurs when MSCs directly differentiate into osteoblasts, which further lay down the mineralized matrix.[18,22] As osteoprogenitor cells directly differentiate into osteoblasts, there is no cartilage intermediary phase, thereby no callus is formed.1[9,23] This process occurs during flat bone formation, primary bone healing, and distraction osteogenesis (DO) process.[19]

Endochondral ossification occurs via a cartilaginous intermediate that is eventually replaced by bone.[21] This mechanism is the main process involved in long bone formation and is also an essential process in longitudinal

growth of long bones at the physeal plate and in the natural bone healing cascade. This ossification begins with chondrocytes proliferating into a hyaline cartilage framework with four distinct zones: the resting, proliferative, zone 3, and calcification zone. The resting zone is furthest from the site of bone formation and contains normal chondrocytes. As the chondrocytes actively replicate themselves in the proliferative zone, they subsequently enlarge in the hypertrophic zone and release alkaline phosphatase, which eventually results in chondrocyte apoptosis and release of angiogenic factors including VEGF. The zone of dead chondrocytes (calcification zone) creates a barren matrix that promotes capillary ingrowth and migration of osteoprogenitor cells. The osteoprogenitor cells then differentiate into osteoblasts and produce bone.[19]

The process of bone healing

The process of bone healing is a complex biological process consisting of inflammatory, reparative, and remodeling phases that involves many intracellular signaling pathways.[8] Unlike other tissues, bone injuries and fractures heal without scar formation.[24] However, despite its self-repair ability, bone has limited capacity. In cases where large and massive bone defects occur, self-regeneration of bone often fails. These conditions often result from necrosis, congenital deformities, degenerative disease, pathological fractures, and fractures resulting from high-energy trauma.[8,17,18,24] When this healing process fails, several complications including malunions, delayed unions, nonunions, and osteomyelitis could develop, and these may impair a patient's quality of life.

In the inflammatory phase, a fracture leads to rupture of blood vessels inside bone and in the surrounding soft tissues, thereby initiating inflammatory cascade and promoting the healing process. Subsequently, soft tissues adjacent to the fracture become acutely inflamed, which characterized by vasodilatation and exudation of plasma and leukocytes. The ends of the broken bones die-off to a variable distance from the fracture depending on the degree of trauma, and within the fracture gap, fibrinogen is converted into fibrin, leading to the formation of hematoma.[25] This phase is regulated by numerous growth factors, including TGF-β, FGF, PDGF, IGF-1, ILs, VEGF, and BMPs. These factors, released during bone healing process, help MSCs to migrate, be recruited, and proliferate into osteoblasts, chondrocytes, adipocytes, and endothelial cells.[25,26] The inflammatory phase results in the formation of a primitive callus, which is further organized during the proliferative, or fibroplasia, phase. During the proliferative phase, a periosteal response occurs; therefore, the primitive callus is replaced by woven bone through intramembranous or endochondral ossification. During the final phase of bone repair, the immature bone becomes lamellar bone. This process consists of mineralized callus replacement with mature mineralized bone and bone remodeling back to its original shape and size. The end product of bone repair is an area of bone that has biomechanical property similar to its pre-injured state.[26]

Current limitations of available therapy

Numerous treatment modalities for regenerating bone provide relatively satisfactory results; however, they are often limited with various drawbacks and limitations. Moreover, most of the available bone substitutes have inferior biological or mechanical properties. This necessitates the development of new treatments for regenerating bone to overcome these limitations.[27]

Currently, the gold standard and the most effective method for treating bone defects is autologous bone graft (ABG), as it has all the characteristics necessary for the growth of new bone: osteoconduction, osteoinduction, and osteogenesis.[4,24,28–31] Furthermore, such graft has already been studied extensively[32], and some studies showed that it is histocompatible and non-immunogenic, as it is derived from the patient's own tissue.[27,33,34] Moreover, a study by Mazock *et al*[33] and Jakse *et al*[34] indicated that autologous bone graft provides an ideal environment for new blood vessel formation. However, it is not without drawbacks. To treat a large bone defect, a considerable portion of bone should be grafted from other parts of the body, creating inevitable donor-site morbidity.[4] Further, a study by Oryan *et al*[24] showed that the harvesting process is also fraught with risk of major vessel or visceral injuries. Moreover, such graft is associated with pain, the need to undergo two operative procedures, a limited availability[35,36], and uncontrollable resorption rates.[37,38] These pitfalls have attracted investigators to develop alternative treatments for CSBDs that could overcome the limitations of autogenous bone graft.[36] One alternative is homogenous or allograft, which is a graft derived from human cadavers or living donors.[2,27] It is the second most common type of graft used nowadays, and it is widely available as a bone source due to its availability in bone banks.[2] Moreover, it is relatively inexpensive[26], and Lu *et al*[39] stated that it provides a good, natural and bony scaf-

fold. Moreover, unlike autogenous bone graft, allogenous bone graft is not associated with donor-site morbidity or prolonged surgery in the harvesting process of autogenous bone graft. Allogenous bone grafts are available in numerous preparations, such as demineralized bone matrix (DBM), morcellised and cancellous chips, corticocancellous and cortical grafts, and osteochondral and whole-bone segments, depending on the recipient site requirements. Such grafts also have drawbacks; despite various biological properties, Dimitriou *et al*[27] and Campana *et al*[40] showed that allogenous bone grafts generally possess weak osteoinductive properties (some grafts, depending on their processing, may still have growth factors) and no cellular component, as donor grafts are devitalized via irradiation or freeze-drying processing. They are also associated with the risk of infection due to sterilization-associated toxicities[41], disease transmission[42], as well as variable host immune responses (e.g. rejection).[43] Another type of bone graft is heterogeneous or xenograft, which is a graft harvested from an animal donor, such as bovine bone. This type of graft needs to be sterilized, and its protein has to be deactivated, leaving only its mineral matrix.[2] Diker *et al*[44] stated that the use of xenograft provides bone formation and vascularization. However, such graft carries the risks of transmission of zoonotic diseases; moreover, humans tend to reject this graft more likely and aggressively.[24] Despite all the advantages of CSBD therapy, both homogenous and heterogenous grafts have only osteoconductive properties; therefore, they are often combined with the patient's own MSCs or growth factors. This means that osteogenic, such as bone marrow aspirate, or osteoinductive compounds as BMPs or platelet-rich plasma (PRP) must also be used in the procedures[2], making the procedure not at ease. Moreover, Osugi *et al*[32] found that both grafts are difficult to shape into the desired shapes.

Alternatively, studies have been focusing on finding safer, less expensive and easier to use synthetic bone grafts. However, despite the new emerging bone grafting substitutes in recent years, there is no single ideal replacement grafting material.[45] Such substitutes can be developed from biomaterials such as hydroxyapatite (HA), tricalcium phosphate (TCP), biphasic calcium phosphate bioactive glass, ceramics, inorganic and organic matrices (e.g., deproteinized bovine bone[46] and demineralized bone collagen[47]), and synthetic polymers.[2,48] These grafts are available in order to limit the drawbacks of autogenous bone, which include donor site morbidity, limited source, and prolonged surgery.[48] Despite their unlimited available volume, some studies found that these types of graft often lack sufficient osteoinductive and osteogenic properties while providing not always optimal osteoconductive matrix, resorption times, and biomechanical assets, especially for the treatment of CSBDs.[2,49,50,39] Moreover, these materials also bring a risk of infection.[32]

In situations in which osteoconduction is not the primary concern, but osteoinduction is required, BMPs are available.[51] These proteins are potent recombinant osteoinductive proteins that can induce bone formation, even at ectopic locations.[52,53] Despite having great osteoinductive properties, Osugi *et al*[32] and Kawasaki *et al*[54] found that super-physiological doses of BMPs are required to be used for bone regeneration. This may induce severe inflammatory responses and excessive bone formation, making the use of it restricted to certain situations.[55–58] Moreover, due to the high doses needed for its clinical use, the costs of such treatment is expensive. Due to these reasons, great efforts are being made to search for alternative treatments or ways to reduce the amounts of BMPs needed.

Table 1. Comparison of endochondral and intramembranous ossification

Ossification type	Endochondral ossification	Intramembranous ossification
Process	Formation through a cartilaginous phase	Direct bone formation
Location	Embryogenesis of long bones	Embryogenesis of flat bones
	Longitudinal bone growth	Growth of flat bones
	Fracture healing	Fracture healing
		Predominant process during distraction osteogenesis

The role of mesenchymal stem cells in bone healing

A stem cell is an unspecialized cell from various organs and tissues that have the ability to proliferate and differentiate into several lineages. Due to its proliferative capacity, stem cell is suitable for various diseases.[59,60] In fact, it has been considered as the main cell source for bone regeneration. Stem cell-based approach for bone repair largely emulates autologous bone grafting, which

provides osteogenic cells as well as key osteogenic and angiogenic growth factors and templates to recruit host cells that actively lay down bone matrix and vascularize the bone construct.[4]

Generally, two types of stem cells exist, which are embryonic stem cells (ESCs) and adult stem cells. The use of ESCs, which are derived from embryonic or fetal tissues, has many ethical issues, whereas the use of the adult ones is generally well accepted by society. An adult stem cell is an undifferentiated cell found in various tissues, which has self-renewal capacity and ability to differentiate into specialized cell types. Their progeny

Table 2. Comparison of current available therapy for CSBD

Treatment methods	Properties	Advantages	Disadvantages
Autogenous bone graft	Osteoconductive, osteoinductive, osteogenic	Has been studied extensively Carries no risk of immunologic reaction or disease transmission Provides an ideal environment for new blood vessel formation	Donor-site morbidity Painful Prolonged surgery Limited availability Uncontrollable resorption rates
Allogenous bone graft	Osteoconductive and osteoinductive	Wide availability Provides a good, natural, and bony scaffold	Lack of osteogenic properties Fraught with risk of infection sterilisation-associated toxicities and disease transmission Variable host immune responses (e.g. rejection) Costly
Xenograft	Osteoconductive and osteoinductive	Low cost High availability	Lack of osteogenic properties Risk of immunogenicity Risk of transmission of infectious and zoonotic diseases Poor outcome
Synthetic bone graft	Osteoconductive	Unlimited availability Circumvent donor site morbidity and additional surgical time and costs	Lack of sufficient osteoinductive and osteogenic properties The osteoconductive property is not always optimal
Bone morphogenetic protein	Osteoinductive	Able to promote bone formation, even at ectopic locations	Require very high doses to regenerate bone May induce severe inflammatory responses and excessive bone formation Very high cost

include both new stem cells and committed progenitors with limited differentiation potential. These progenitor cells, in turn, give rise to more specialized cell types.[60] Given their multipotentiality, adult stem cells serve as internal repair system in many tissues. When stem cells divide, they uniquely have the ability to divide to make more stem cells, a property known as "self-renewal", and the ability to produce more differentiated progeny.[59,61] Compared to differentiated cells, stem cells proliferate and regenerate better.[60] However, MSCs have disadvantages of limited availability and proliferation, a decrease in regenerative properties with extended expansion[62] and the increasing age of an individual.[63] These factors limit the use of autologous MSCs in older population who represent a major portion of patients in need of bone replacement.[4]

In order for bone to regenerate, specific MSCs have to be recruited, proliferate and differentiate into osteoblasts and chondrocytes.[64] This regeneration process involves osteogenesis and angiogenesis, and it is of utmost importance, particularly for difficult nonunion fractures caused by trauma, ischemia, etc.[65,66] This process is initiated by MSCs with the formation of soft and hard calluses.65 MSCs can migrate to the injured sites and facilitate bone regeneration, but the fundamental mechanisms beyond this process remain unclear. The ability of MSCs to form bone cell have led them to be used in regenerative medicine for bone repair.[26,65]

In CSBD, nonunion would occur if one of the three reasons below happen: (1) MSC does not migrate into the defect site, (2) the number of osteoblast progenitor cells in CSBD is insufficient, and (3) MSC fails to differentiate into osteoblasts. As the first step of the chain in the treatment of non-unions, many investigators have attempted to recruit MSCs to the defect site. The exact mechanism of MSC homing to the injured area remains unclear; however, chemotactic factors released at the defect site must play an essential role in MSC homing and recruitment.[65]

Initially, it was thought that MSC treatment of musculoskeletal injuries was due to their multipotentiality.[67–69] In particular, it is generally assumed that when implanting MSCs, the cells would colonize and differentiate into various lineages at the injured area, thereby replacing cells in such site and eventually leading to tissue regeneration.[70,71] However, studies have found that MSCs only live for a short period of time and their engraftment has remained surprisingly low.[72–76] Due to this reason, it is suggested that MSCs mainly act by providing regenerative microenvironment (paracrine signaling) by means of activating or empowering other local cells to facilitate tissue regeneration, rather than direct incorporation into the injured site. In this mechanism, specific bioactive factors exerting beneficial effects on the surrounding cells and tissues were secreted.[59,66,77–79] This proposed theory regarding paracrine stimulation is supported by preclinical studies which demonstrated numerous cells to respond to paracrine signaling from MSC resulting in several cellular responses, including survival, proliferation, migration and gene expression.[80]

CONCLUSION

Fundamental knowledge regarding the biology and physiology of bone and bone healing is important to be understood. More comprehensive reviews are required to provide a detailed picture of the complex biological pathways through which bone is regenerated.

REFERENCES

1. Ratnayake J, Mucalo M, Dias G. Substituted hydroxyapatites for bone regeneration: A review of current trends. J Biomed Mater Res - Part B Appl Biomater. 2017;105(5):1285–99.
2. Martin V, Bettencourt A. Bone regeneration: Biomaterials as local delivery systems with improved osteoinductive properties. Materials Science and Engineering C. 2018.
3. Florencio-Silva R, Sasso GRDS, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: Structure, function, and factors that influence bone cells. Biomed Res Int. 2015;2015:421746.
4. Im G Il. Stem cells for reutilization in bone regeneration. J Cell Biochem. 2015;116(4):487–93.
5. Lewallen EA, Riester SM, Bonin CA, Kremers HM, Dudakovic A, Kakar S, et al. Biological strategies for improved osseointegration and osteoinduction of porous metal orthopedic implants. Tissue Eng Part B Rev. 2015;21(2):218–30.
6. Zomorodian E, Baghaban Eslaminejad M. Mesenchymal stem cells as a potent cell source for bone regeneration. Stem Cells Int. 2012;2012:980353.
7. Durão SF, Gomes PS, Colaço BJ, Silva JC, Fonseca HM, Duarte JR, et al. The biomaterial-mediated healing of critical size bone defects in the ovariectomized rat. Osteoporos Int. 2014;25(5):1535–45.
8. Oryan A, Kamali A, Moshirib A, Eslaminejad MB. Role

of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? Cells Tissues Organs. 2017.
9. Zhang X, Liu T, Huang Y, Wismeijer D, Liu Y. Icariin: Does it have an osteoinductive potential for bone tissue engineering? Phyther Res. 2014;28(4):498–509.
10. Stanovici J, Le Nail LR, Brennan MA, Vidal L, Trichet V, Rosset P, et al. Bone regeneration strategies with bone marrow stromal cells in orthopaedic surgery. Current Research in Translational Medicine. 2016.
11. Sponer P, Kucera T, Diaz-Garcia D, Filip S. The role of mesenchymal stem cells in bone repair and regeneration. Eur J Orthop Surg Traumatol. 2014 Apr;24(3):257–62.
12. Park H-C, Son Y-B, Lee S-L, Rho G-J, Kang Y-H, Park B-W, et al. Effects of Osteogenic-Conditioned Medium from Human Periosteum-Derived Cells on Osteoclast Differentiation. Int J Med Sci. 2017;14(13):1389–401.
13. Giannoudis P V., Dinopoulos H, Tsiridis E. Bone substitutes: An update. Injury. 2005;
14. Kraus KH, Kirker-Head C. Mesenchymal stem cells and bone regeneration. Vet Surg. 2006;
15. Polo-Corrales L, Latorre-Esteves M, Ramirez-Vick JE. Scaffold Design for Bone Regeneration. J Nanosci Nanotechnol. 2014;
16. Bracey DN, Jinnah AH, Whitlock P, Hutchinson I, Seyler T, Willey JS, et al. Proving Osteoinductive Potential of a Decellularized Xenograft Bone Substitute. bioRxiv. 2018;
17. El-Rashidy AA, Roether JA, Harhaus L, Kneser U, Boccaccini AR. Regenerating bone with bioactive glass scaffolds: A review of in vivo studies in bone defect models. Acta Biomaterialia. 2017.
18. Walmsley GG, Ransom RC, Zielins ER, Leavitt T, Flacco JS, Hu MS, et al. Stem Cells in Bone Regeneration. Stem Cell Reviews and Reports. 2016.
19. Buck DW, Dumanian GA. Bone biology and physiology: Part I. the fundamentals. Plastic and Reconstructive Surgery. 2012.
20. Urist MR. Bone: formation by autoinduction. 1965. Clin Orthop Relat Res. 2002;
21. Evans CH. Advances in regenerative orthopedics. In: Mayo Clinic Proceedings. 2013.
22. Ono N, Kronenberg HM. Bone repair and stem cells. Current Opinion in Genetics and Development. 2016.
23. Grosso A, Burger MG, Lunger A, Schaefer DJ, Banfi A, Di Maggio N. It Takes Two to Tango: Coupling of Angiogenesis and Osteogenesis for Bone Regeneration. Front Bioeng Biotechnol. 2017;5:68.
24. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: Classic options, novel strategies, and future directions. Journal of Orthopaedic Surgery and Research. 2014.
25. Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nature Reviews Rheumatology. 2012.
26. Fillingham Y, Jacobs J. Bone grafts and their substitutes. Bone Jt J. 2016;
27. Dimitriou R, Jones E, McGonagle D, Giannoudis P V. Bone regeneration: Current concepts and future directions. BMC Medicine. 2011.
28. García-Gareta E, Coathup MJ, Blunn GW. Osteoinduction of bone grafting materials for bone repair and regeneration. Bone. 2015.
29. Oliveira HL, Da Rosa WLO, Cuevas-Suárez CE, Carreño NLV, da Silva AF, Guim TN, et al. Histological Evaluation of Bone Repair with Hydroxyapatite: A Systematic Review. Calcified Tissue International. 2017.
30. Miller CP, Chiodo CP. Autologous Bone Graft in Foot and Ankle Surgery. Foot and Ankle Clinics. 2016.
31. Marx RE. Bone and Bone Graft Healing. Oral and Maxillofacial Surgery Clinics of North America. 2007.
32. Osugi M, Katagiri W, Yoshimi R, Inukai T, Hibi H, Ueda M. Conditioned Media from Mesenchymal Stem Cells Enhanced Bone Regeneration in Rat Calvarial Bone Defects. Tissue Eng Part A. 2012;
33. Mazock JB, Schow SR, Triplett RG. Proximal tibia bone harvest: Review of technique, complications, and use in maxillofacial surgery. Int J Oral Maxillofac Implant. 2004;
34. Jakse N, Seibert FJ, Lorenzoni M, Eskici A, Pertl C. A modified technique of harvesting tibial cancellous bone and its use for sinus grafting. Clin Oral Implants Res. 2001;
35. Kretlow JD, Mikos AG. Review: Mineralization of Synthetic Polymer Scaffolds for Bone Tissue Engineering. Tissue Eng. 2007;
36. Burdette AJ, Guda T, Thompson ME, Banas R, Sheppard F. A Novel Secretome Biotherapeutic Influences Regeneration in Critical Size Bone Defects. J Craniofac Surg. 2018;
37. Smith JD, Abramson M. Membranous vs Endochondral Bone Autografts. Arch Otolaryngol. 1974;
38. Vuyk HD, Adamson PA. Biomaterials in rhinoplasty. Clinical Otolaryngology and Allied Sciences. 1998.
39. Lu H, Liu Y, Guo J, Wu H, Wang J, Wu G. Biomaterials with antibacterial and osteoinductive properties to repair infected bone defects. International Journal of Molecular Sciences. 2016.
40. Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, et al. Bone substitutes in orthopaedic surgery: from basic science to clinical practice. J Mater Sci Mater Med. 2014;

41. Moreau MF, Gallois Y, Baslé MF, Chappard D. Gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds for osteoblast-like cells. Biomaterials. 2000;
42. Buck BE, Malinin TI, Brown MD. Bone transplantation and human immunodeficiency virus. An estimate of risk of acquired immunodeficiency syndrome (AIDS). Clin Orthop Relat Res. 1989;
43. Lewandrowski KU, Rebmann V, Päßler M, Schollmeier G, Ekkernkamp A, Grosse-Wilde H, et al. Immune response to perforated and partially demineralized bone allografts. J Orthop Sci. 2001;
44. Diker, N., Sarican, H., Cumbul, A., & Kilic, E. (2018). Effects of Systemic Erythropoietin Treatment and Heterogeneous Xenograft in Combination on Bone Regeneration of a Critical-Size Defect in an Experimental Model. Journal of Cranio-Maxillofacial Surgery. doi:10.1016/j.jcms.2018.09.015
45. Jensen SS, Bornstein MM, Dard M, Bosshardt DD, Buser D. Comparative study of biphasic calcium phosphates with different HA/TCP ratios in mandibular bone defects. A long-term histomorphometric study in minipigs. J Biomed Mater Res - Part B Appl Biomater. 2009;
46. Jung RE, Ha CHF, Sailer HF, Weber FE. Effect of rh-BMP-2 on guided bone regeneration in humans. Clin Oral Implants Res. 2003;
47. Milovancev M, Muir P, Manley PA, Seeherman HJ, Schaefer S. Clinical application of recombinant human bone morphogenetic protein-2 in 4 dogs. Vet Surg. 2007 Feb;36(2):132–40.
48. Zhang Y, Yang S, Zhou W, Fu H, Qian L, Miron RJ. Addition of a Synthetically Fabricated Osteoinductive Biphasic Calcium Phosphate Bone Graft to BMP2 Improves New Bone Formation. Clin Implant Dent Relat Res. 2016;
49. Barba M, Cicione C, Bernardini C, Michetti F, Lattanzi W. Adipose-derived mesenchymal cells for bone regereneration: State of the art. Biomed Res Int. 2013;
50. Miron RJ, Zhang Q, Sculean A, Buser D, Pippenger BE, Dard M, et al. Osteoinductive potential of 4 commonly employed bone grafts. Clin Oral Investig. 2016;
51. Egol KA, Nauth A, Lee M, Pape HC, Watson JT, Borrelli J. Bone grafting: Sourcing, timing, strategies, and alternatives. J Orthop Trauma. 2015;
52. Courvoisier A, Sailhan F, Laffenêtre O, Obert L. Bone morphogenetic protein and orthopaedic surgery: Can we legitimate its off-label use? International Orthopaedics. 2014.
53. Visser R, Bodnarova K, Arrabal PM, Cifuentes M, Becerra J. Combining bone morphogenetic proteins-2 and -6 has additive effects on osteoblastic differentiation in vitro and accelerates bone formation in vivo. J Biomed Mater Res A. 2016 Jan;104(1):178–85.
54. Kawasaki K, Aihara M, Honmo J, Sakurai S, Fujimaki Y, Sakamoto K, et al. Effects of recombinant human bone morphogenetic protein-2 on differentiation of cells isolated from human bone, muscle, and skin. Bone. 1998;
55. Joseph V, Rampersaud YR. Heterotopic bone formation with the use of rhBMP2 in posterior minimal access interbody fusion: A CT analysis. Spine (Phila Pa 1976). 2007;
56. MacDonald KM, Swanstrom MM, McCarthy JJ, Nemeth BA, Guliani TA, Noonan KJ. Exaggerated inflammatory response after use of recombinant bone morphogenetic protein in recurrent unicameral bone cysts. J Pediatr Orthop. 2010;
57. Robin BN, Chaput CD, Zeitouni S, Rahm MD, Zerris VA, Sampson HW. Cytokine-mediated inflammatory reaction following posterior cervical decompression and fusion associated with recombinant human bone morphogenetic protein-2: A case study. Spine (Phila Pa 1976). 2010;
58. Shah RK, Moncayo VM, Smitson RD, Pierre-Jerome C, Terk MR. Recombinant human bone morphogenetic protein 2-induced heterotopic ossification of the retroperitoneum, psoas muscle, pelvis and abdominal wall following lumbar spinal fusion. Skeletal Radiol. 2010;
59. Bollini S, Gentili C, Tasso R, Cancedda R. The Regenerative Role of the Fetal and Adult Stem Cell Secretome. J Clin Med. 2013;
60. Khan WS, Rayan F, Dhinsa BS, Marsh D. An osteoconductive, osteoinductive, and osteogenic tissue-engineered product for trauma and orthopaedic surgery: How far are we? Stem Cells International. 2012.
61. He S, Nakada D, Morrison SJ. Mechanisms of stem cell self-renewal. Annu Rev Cell Dev Biol. 2009;25:377–406.
62. Both SK, van Apeldoorn AA, Jukes JM, Englund MCO, Hyllner J, van Blitterswijk CA, et al. Differential bone-forming capacity of osteogenic cells from either embryonic stem cells or bone marrow-derived mesenchymal stem cells. J Tissue Eng Regen Med. 2011 Mar;5(3):180–90.
63. Fan M, Chen W, Liu W, Du G-Q, Jiang S-L, Tian W-C, et al. The Effect of Age on the Efficacy of Human Mesenchymal Stem Cell Transplantation after a Myocardial Infarction. Rejuvenation Res. 2010;
64. Marsell R, Einhorn TA. The biology of fracture healing. Injury. 2011.
65. Maltman DJ, Hardy SA, Przyborski SA. Role of mesenchymal stem cells in neurogenesis and nervous system repair. Neurochem Int. 2011;59(3):347–56.
66. Lin W, Xu L, Zwingenberger S, Gibon E, Goodman SB, Li G. Mesenchymal stem cells homing to improve bone healing. Journal of Orthopaedic Translation. 2017.

67. Salem HK, Thiemermann C. Mesenchymal stromal cells: Current understanding and clinical status. Stem Cells. 2010;28(3):585–96.
68. Chatterjea A, Meijer G, Van Blitterswijk C, De Boer J. Clinical application of human mesenchymal stromal cells for bone tissue engineering. Stem Cells Int [Internet]. 2010; Available from: http://dx.doi.org/10.4061/2010/215625
69. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood. 2003;101(9):3722–9.
70. Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. Stem Cell Research and Therapy. 2016.
71. Linero I, Chaparro O. Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration. PLoS One. 2014;9(9):e107001.
72. Iso Y, Spees JL, Serrano C, Bakondi B, Pochampally R, Song YH, et al. Multipotent human stromal cells improve cardiac function after myocardial infarction in mice without long-term engraftment. Biochem Biophys Res Commun. 2007;
73. Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc Natl Acad Sci. 2002;
74. Lee RH, Seo MJ, Pulin AA, Gregory CA, Ylostalo J, Prockop DJ. The CD34-like protein PODXL and {alpha}6-integrin (CD49f) identify early progenitor MSCs with increased clonogenicity and migration to infarcted heart in mice. Blood. 2009;
75. Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs Improve Myocardial Infarction in Mice because Cells Embolized in Lung Are Activated to Secrete the Anti-inflammatory Protein TSG-6. Cell Stem Cell. 2009;
76. Dai W, Hale SL, Martin BJ, Kuang JQ, Dow JS, Wold LE, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: Short- and long-term effects. Circulation. 2005;
77. Horie M, Choi H, Lee RH, Reger RL, Ylostalo J, Muneta T, et al. Intra-articular injection of human mesenchymal stem cells (MSCs) promote rat meniscal regeneration by being activated to express Indian hedgehog that enhances expression of type II collagen. Osteoarthr Cartil. 2012;
78. Horwitz EM, Prockop DJ, Gordon PL, Koo WWK, Fitzpatrick LA, Neel MD, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. Blood. 2001;
79. Amin HD, Brady MA, St-Pierre J-P, Stevens MM, Overby DR, Ethier CR. Stimulation of Chondrogenic Differentiation of Adult Human Bone Marrow-Derived Stromal Cells by a Moderate-Strength Static Magnetic Field. Tissue Eng Part A. 2014;
80. Hocking AM, Gibran NS. Mesenchymal stem cells: Paracrine signaling and differentiation during cutaneous wound repair. Exp Cell Res. 2010;316(14):2213–9.