Review Article ISSN 2639-944X

Journal of Medical - Clinical Research & Reviews

# Mesenchymal Stem Cell Therapies for Bone After Damage from Osteosarcomas

**Austin Malchiodi and Vincent S. Gallicchio***

*Department of Biological Sciences, College of Science, Clemson University, Clemson, SC 29671, US.*

***Correspondence:**
Vincent S. Gallicchio, Department of Biological Sciences, College of Science, Clemson University, Clemson, SC 29671, US.

**Received:** 13 January 2020; **Accepted:** 30 January 2020

**Citation:** Austin Malchiodi, Vincent S. Gallicchio. Mesenchymal Stem Cell Therapies for Bone After Damage from Osteosarcomas. J Med - Clin Res & Rev. 2020; 4(1): 1-6.

## ABSTRACT

*Osteosarcoma is a rare type of cancer characterized by immature bone growth and proliferation that only produces primary bone. Because of the stagnant advancement in treatment methods and high rates of body rejection, other treatment options are being pursued. One such option is the implantation of mesenchymal stem cells in regenerating bone tissue. This review covers a description of the structure of the bone, how bone is remodeled, and the current mesenchymal stem cell sources being studied. It also evaluates the progress of clinical and animal trials and what future advances can be made.*

## Keywords

Mesenchymal Stem Cells, Osteosarcoma, Bone regeneration, Tissue therapy.

## Abbreviations and Symbols

ADSCs: Adipose Tissues Stem Cells, AF-MSCs: Amniotic Fluid Tissue Mesenchymal Stem Cells, AM-MSCs: Amniotic Membrane Mesenchymal Stem Cells, BM-MSCs: Bone Marrow Mesenchymal Stem Cells, CM-MSCs: Chorionic Membrane Mesenchymal Stem Cells, GPCRs: G-Protein Coupled Receptors, MSCs: Mesenchymal Stem Cells, OB: Osteoblast, OC: Osteoclast, OS: Osteosarcoma, (PCL–TCP): Polycaprolactone–Tricalcium Phosphate, ROS: Reactive Oxygen Species, UC: MSCs: Umbilical Cord Mesenchymal Stem Cells.

## Introduction

The skeleton is comprised of 213 bones listed into four categories; long, short, irregular, and flat [1]. The different shapes and sizes of bones directly correlate to their function. Functions of the bone include structural support, protection of internal organs, locomotion, reservoirs for calcium and phosphate deposition, and hematopoiesis in bone marrow.

Inside of the bone there are two types cortical and trabecular that help with the functions of bone [1]. Cortical bone is what we typically think of when we think of bone. It is the hard, mineralized tissue that constitutes structure and support. It contains two layers, the periosteal layer on the outside and the endosteal layer on the inside. Most of the activity in the periosteal layer is important for appositional growth and fracture repair [1]. Also, there is a higher rate of bone formation versus bone resorption as observed on the surface. The endosteal surface undergoes much greater strain from mechanical stress than periosteal surface. For this reason, a higher rate of bone resorption than formation is seen in this surface, as constant remodeling is necessary. Trabecular bone, also referred to as 'spongy' bone, makes up the inside of the bone. As the name implies, it is porous and softer. It provides a lighter interior of skeleton, so it does not become too heavy and bulky for the body to handle. Additionally, it provides the bone with stability for multidirectional support. The human skeleton, as a whole, is made up of roughly 80 percent cortical bone and 20 percent trabecular bone [2].

The smallest functional unit of both cortical and trabecular bone is the osten, which may have different names when associated with bone [1]. In cortical bone, the osteon is known as the Haversian System [2]. It is cylindrical in shape and forms a network within the bone that helps with its rigidity [2]. The Haversian system is organized in lamellae, which are rings that surround the central canal. In the lamellae, there are individual lacunae, each which incorporates an osteocyte. The central canal is the Haversian System's blood supply, which is central to the entire Haversian

System. Trabecular bone's osteons are known as 'packets', which produce plates and rods that provide structural support. Unlike cortical osteons, they are semilunar in shape.

## Bone Growth and Remodeling

All bones must go under growth during the course of life [1]. Longitudinal growth, sometimes known as endochondral ossification, is generally done during early development and adolescence. Endochondral ossification occurs by a process in which the growth (epiphyseal) plate creates hyaline cartilage in between the plate and the metaphyseal area, which is then mineralized and ossified. Over time, the epiphyseal plate does not keep up production of cartilage with the speed in which the cartilage is ossified. Over time, epiphyseal plate is then ossified itself, resulting in no more longitudinal growth. This is what is known as your "growth plates closing."

Modeling is a constant process in which bone is broken down and replaced with new bone to properly deal with the stresses of mechanical loads as well as help with mineral homeostasis. The body is constantly remodeling the skeleton to adjust to the current needs of the body. In remodeling, trabecular packets are continuously removed and renewed with a proteinaceous matrix. From there, the matrix is mineralized which forms the new bone [1].

Osteoclasts (OCs) and osteoblasts (OBs) are the cells responsible for bone resorption and deposition. First, recruitment of OCs is necessary. This is done through a mechanism in which a monocyte-macrophage is activated in circulation [3], which form preOCsts. The preOCss bind to the bone matrix and forms a seal around the bone-resorbing compartments to protect them. OCs are then formed, activated, and regulated through a variety of activators and hormones [4], including parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D. Hydrogen ions are secreted using an ATPase pump that lowers the pH within the bone-resorbing compartment. The lower pH stabilizes the bone mineral [5]. Resorption is completed when various secretions digest the organic matrix, which result in lacunae on trabecular bone and haversian canal in cortical bone. Resorption is then transitioned to formation, utilizing monocytes, osteocytes and pre-osteoblasts, all recruited from various areas. The coupling signals for this seemingly smooth transition are unknown. Deposition of bone can take anywhere from four to six months. OBs lay down new organic matrix and regulate mineralization by releasing vesicles that contain phosphate and calcium, as well as enzymatically destroy mineralization inhibitors [6]. After deposition is complete, anywhere between 30%-50% OBs differentiate into osteocytes or bone lining cells that assist with efflux and influx of mineral ions. The rest of the OBs undergo apoptosis [1]. The end-product of bone remodeling is a new osteon. The process is the same for both cortical and trabecular bone.

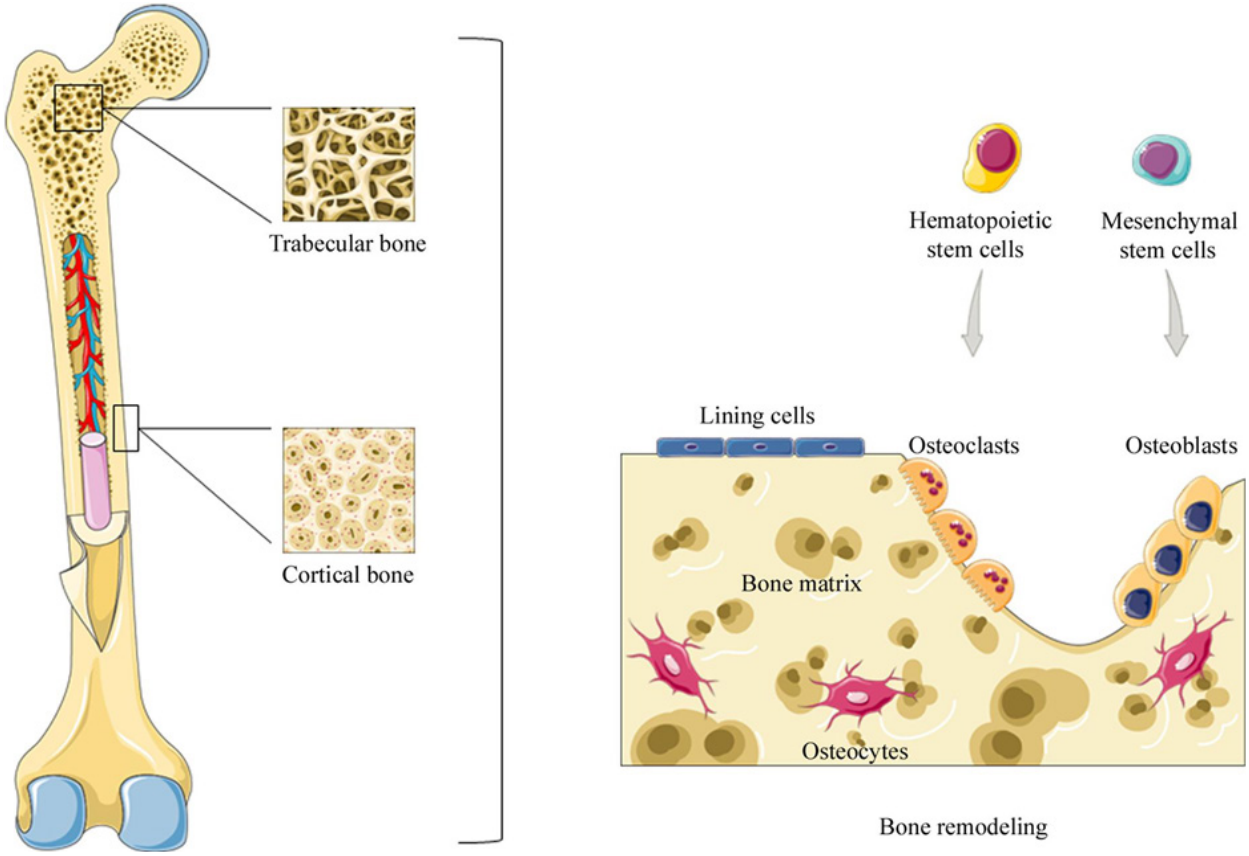


**Figure 1:** Representation of bone structure and bone regeneration. Two types of bone tissue can be seen: Cortical and trabecular. During bone remodeling, OCs, derived from hematopoietic stem cells, resorb bone, which osteoblasts, derived from MSCs, lay down new bone after old bone has been removed [6].

## Osteosarcomas

An osteosarcoma (OS) is a malignant tumor characterized by the formation of immature bone or by tumor cells [7]. They frequently occur in the long bones of the appendicular skeleton. The femur, tibia, and humerus account for roughly 85% of all cases [7]. Though the etiology can very, there is a high number of OSs that occur in the pubertal growth spurt of young adults. This suggests a correlation between rapid bone growth and proliferation and the likelihood of an OS forming [8]. They occur in the metaphysis of the bone, where bone has likely just been mineralized in a growing adult or being remodeled in an adult.

The exact causes of an OS are still relatively unknown. A viral origin has been suggested by showing an OS can be induced from cell-free extracts [9]. Additionally, ionizing radiation has shown to be a cause [9], as well as a genetic predisposition to these types of tumors as several families have multiple members that have been affected [10].

G-protein Coupled Receptors (GPCRs) are the largest type of cell-surface molecules that are involved in many physiological processes [11]. Activation of a GPCR initiates a signal transduction pathway, that could result in many processes such as physiological changes, gene activation, cell division, and tumor growth [11]. Recently, a family of GCPRs, known as the Adhesion Family, has been shown to be involved with various bone abnormalities and dysfunctions [12,13].

Specifically, GPR56 and GPR110 have both been shown to have an effect on the rate of OS proliferation. High GPR56 expression showed an increase in OS proliferation and invasion [12], while low GPR110 expression has shown a decrease in OS proliferation and invasion [13]. This further shows that OS growth and proliferation may be due a genetic effect, and likely has to do with GPCRs that affect OB and OC activity.

## Current Treatment

Treatment for OSs are limited and nature, and most times require one of two different types of surgical procedures: Limb salvage and amputation. Limb salvage is removal of the area with disease and regrowth of the bone [14]. Amputation is the removal of the limb so it cannot spread to the rest of the body. Though rare, chemotherapy is also sometimes an option.

Limb salvage, which includes up to 90% of current treatments for Oss, involves two steps: resection and reconstruction [14]. Resection is the removal of the tumor on the bone and all tracts within a 2 cm margin of the area [15]. Most times, this is a

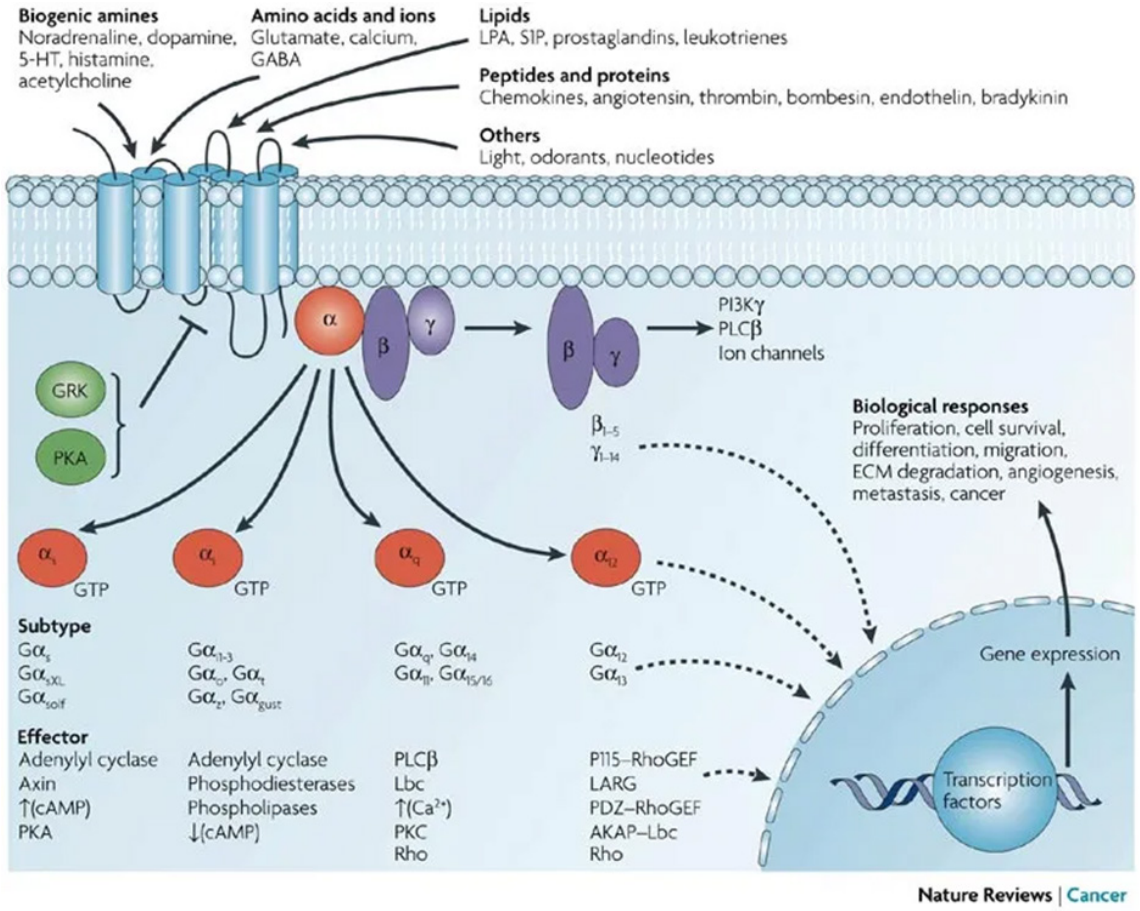


**Figure 2:** Normal Function of a GPCR. The Ligand binds to the GPCR, and GDP is phosphorylated to GTP. After phosphorylation, the alpha subunit and GTP breaks off from the complex and activates the signal transduction pathway. GDP then attaches to the alpha subunit, and the complex binds together again [11].

relatively simple procedure. However, when the joint becomes involved, it varies on a case by case basis. If the OS is found in the joint, some advocate for amputation [15] while others advocate to preserve the joint through resections through the growth plate [16]. If resection is possible, the next step involved is reconstruction. Reconstruction is only necessary in weight-bearing bones, as in bones like the proximal fibula and ulna, excision through bone does not cause an abnormality in function [17]. For weight-bearing bones, the two ways to reconstruct the bone are replacement with tissues or endoprosthetic replacement [15].

Replacement with tissues, also known as biological replacement, is the use of autografts and allografts to rebuild the bone. The effectiveness of allografts and autografts has been brought into question. In as study of 92 patients who had massive allograft reconstruction conducted by Donati et al., it was determined that 45% and 29% had an "excellent" and "good" outcome, while 15% of all of the allografts completely failed [18]. Though the allograft and autograft replacement are the more popular option of the two, the comparison of treatment options all showed low local recurrence rate (<15%) in three separate clinics [19].

Amputation is the removal of an entire limb or bone and is mostly reserved to soft tissue or neuromuscular junction contamination [15]. Previous studies have shown that limb salvage is the preferred treatment compared to amputation, as 5-year survival rates and better functional outcomes concluded [20,21].

## Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are undifferentiated cells that have the capabilities to proliferate, provide self-maintenance, repair damaged tissue, and develop into various cell types needed throughout the body [22]. They are defined as non-blood adult stem cells that are pluripotent. Pluripotency refers to the ability of the cell to differentiate into many different types of tissue, including cartilage, tendon, bone, adipose tissue, and muscle. They regenerate via a process known as self-renewal [23]. The process of self-renewal is not exactly known, and theories of how it occurs vary between the different types of stem cells [23].

MSCs have the ability to develop into a wide range of cell types, including chondroblasts, lipoblasts, and OBs. Additionally, they aid in cell to cell communication involved with cytokines, growth factors, and cell signaling pathways [24]. For this reason, MSCs are being investigated for their use in stem cell therapy [25].

Clinically, MSCs must be subject to minimum criteria to be used safely, as established by the International Society for Cell Therapy [26]. They must be characterized as multipotent cells capable of plasticity and differentiation. Additionally, they must express cell surface markers CD73, CD90, and CD105 [27], and have an absence of monocytes and lymphocytes.

The ability of MSC's to differentiate into OBs led to their interest and use in methods for bone regeneration and repair [25]. After injury, response from platelets, macrophages, and inflammatory cells promotes migration of these MSC's that can then differentiate once they arrive. With this method, studies have been shown to increase bone regeneration with MSC utilize scaffolds as carriers for the cells [28,29].

The osteogenic differentiation capacity of MSCs, including placental tissue, amniotic membrane MSCs (AM-MSCs), amniotic fluid tissues (AF-MSCs), bone marrow MSCs (BM-MSCs), Adipose tissues (ADSCs), chorionic membrane MSCs (CM-MSCs) and umbilical cord MSCs (UC-MSCs) [30,31]. These data shows that the osteogenic differentiation ability of UC-MSCs and AM-MSCs are good sources for bone regeneration. Other studies have shown that UC-MSCs and ADSCs each show higher differentiation ability than the other when derived from Wharton's Jelly [32,33].

## Mesenchymal Stem Cell Therapy

Cell-based therapies using MSCs can provide many potential solutions to bones that have diseases, including OSs [34]. When bone is subjected to inflammatory stimuli, a cascade of regenerative events are induced for bone repair and regrowth [35]. This process is known to initiate MSCs to differentiate into osteoblasts and chondrocytes, which enables bone formation at the site of the injury. This is a pivotal step in allowing for bone regrowth after injury in disease [36]. Exogenous MSC migration directly following injury to bone leads to a decrease in immune responses [34], which would suppress the growth of bone. Intermediate periods that can last from days to weeks showed the opposite effect when treated with exogenous MSC migration, as it gave these cells time to differentiate into chondrocytes and Osteoblasts, which in turn will aid in bone growth [34].

## *In-Vitro* Expansion

Cell dosage and viability are usually determined from in vitro data or prior experience using MSCs [34]. In clinical applications in which a bone graft is necessary, it is impossible to know the correct number of cells necessary as direct mechanisms of cell-mediated bone regeneration are not fully understood [34]. However, preclinical models of bone repair are a good source to provide potential models for the future [37]. Culturing cells ex vivo can expose them to hyperoxic conditions [34], which could lead to high intracellular reactive oxygen species (ROS) production, which is detrimental to cell viability [34]. Additionally, genetic mutations may occur in cell cultures, as rapid cell division may be cause unchecked cell division and growth [34].

## *In Vivo*

Choi et al. conducted a study in which MSCs were used to regenerate bone tissue in rat calvarial defect models [38]. To help with their in-vivo differentiation ability, the cells were treated with resveratrol (RSV) before they were implanted. When they were implanted, the MSCs with RSV significantly improved bone regeneration at eight weeks post-surgery [38]. However, exact viability of cells was not tested, so it is inconclusive how successful this method may be, as the total number of cells actually alive is unknown [38].

Long-bone defects in larger animals likely provides a better model for future clinical applications in humans. Effective treatment has been demonstrated using 6-7 year old sheep with 3 cm full thickness defects with polycaprolactone–tricalcium phosphate (PCL–TCP) scaffolds as well as MSCs [39]. Bone defects only saw a 38% bridging in both PCL-TCP scaffolds and the PCL-TCP scaffolds with the MSCs [39]. Addition of MSCs showed no enhancement of bone growth as previous studies had shown, but the viability of cells was again not tested [39]. Because of this, no animal has been shown to be the 'gold standard' of preclinical models, and more research must be conducted on a variety of animals to evaluate the effectiveness of MSCs [34].

## Clinical Trials

Gjerde et al. used MSCs to repair mandibular ridge resorption [40]. Bone volume was measured before surgery and 4-6 months after surgery, with 11 of 13 patients showing successful ridge augmentation and an adequate amount of bone for dental implants [40]. In this study, viability was tested using the Trypan Viability test. All cell cultures showed an 87%-90% viability rate before they were used [40]. Unfortunately, many clinical trials involving MSCs and bone repair either do not have published data and/or do not provide enough information involving their protocol, so the studies cannot be replicated in other labs [41].

## Conclusion

MSCs are stem cells that have the proliferative and differential ability to be used in clinical settings to help repair bone damage following OSs. Although there has been significant progress in stem cell biology, what still remains to be proven is safety and proper stem cell, especially to determine their limitations. The first limitation is immune rejection, as the stem cell populations must be absent of monocytes and lymphocytes in order to be properly administered. The second limitation is the lack of research, both on humans and animal models. Animals models have shown promising results; however we do not know is the full extent to which they can be used and how effective they are in a clinical setting. The future of research with OSs lies heavily upon the ability to overcome these two challenges. These challenges may be able to be overcome by checking cell viability, as that is a true benchmark for how effective the MSCs are in therapy. Regardless of the exact method utilized, testing for cell viability before implantation has shown tremendous tissue growth and proliferation and is extremely beneficial to the overall quality of tissue growth method. The evidence that MSCs can provide bone tissue growth and regeneration provides the rationale and relevance for their use in regenerative medicine.

## References


1. Clarke B. Normal Bone Anatomy and Physiology. Clinical Journal of the American Society of Nephrology. 2008; 3: 131-139.
2. Eriksen EF, Axelrod DW, Melsen F. Bone Histomorphometry, New York, Raven Press. 1994; 1-12.
3. Roodman GD. Cell biology of the osteoclast. Exp Hematol. 1999; 27: 1229-1241.
4. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003; 423: 337-342.
5. Silver IA, Murrills RJ, Etherington DJ. Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. Exp Cell Res. 1988; 175: 266-276.
6. Anderson HC. Matrix vesicles and calcification. Curr Rheumatol Rep. 2003; 5: 222-226.
7. Picci P. Orphanet J Rare Dis. 2007; 2: 6.
8. https://doi.org/10.1093/annonc/mdq276
9. Finkel MP, Reilly CA, Biskis BO. Pathogenesis of radiation and virus-induced bone tumors. Recent Results Cancer Res. 1976; 92-103.
10. Swaney JJ. Familial osteogenic sarcoma. Clin Orthop Relat Res. 1973; 64-68.
11. Dorsam R, Gutkind J. G-protein-coupled receptors and cancer. Nat Rev Cancer. 2007; 7: 79-94.
12. Chen ZG, Gao PJ, Li ZP. Expression of G protein-coupled receptor 56 is an unfavorable prognostic factor in osteosarcoma patients. Tohoku J. Exp. Med. 2016; 239: 203-211.
13. Liu ZQ, Zhang GR, Zhao CL, et al. Clinical significance of G protein-coupled receptor 110 GPR110 as a novel prognostic biomarker in osteosarcoma. Med. Sci. Monit. 2018; 24: 5216-5224.
14. Bacci G, Ferrari S, Longhi A, et al. Neoadjuvant chemotherapy for high grade osteosarcoma of the extremities: long-term results for patients treated according to the Rizzoli IOR/OS-3b protocol. J Chemother. 2001; 13: 93-99.
15. Misaghi A, Goldin A, Awad M, et al. Osteosarcoma: a comprehensive review. SICOT J. 2018; 4: 12.
16. Betz M, Dumont CE, Fuchs B, et al. Physeal distraction for joint preservation in malignant metaphyseal bone tumors in children. Clin Orthop Relat Res. 2012; 470: 1749-1754.
17. Watts HG. Introduction to resection of musculoskeletal sarcomas. Clin Orthop Relat Res. 1980; 153: 31-38.
18. Donati D, Di Liddo M, Zavatta M, et al. Massive bone allograft reconstruction in high-grade osteosarcoma. Clin Orthop Relat Res. 2000; 377: 186-194.
19. Grimer RJ, Taminiau AM, Cannon SR. Surgical outcomes in osteosarcoma. J Bone Joint Surg Br. 2002; 84: 395-400.
20. Stevenson J, Tsagkozis P, Grimer R. Functional and quality of life outcomes in bone sarcoma following amputation, rotationplasty or limb-salvage. Expert Review of Quality of Life in Cancer Care. 2016; 1: 303-312.
21. Han G, Bi WZ, Xu M, et al. Amputation versus limb-salvage surgery in patients with osteosarcoma a meta-analysis. World J Surg. 2016; 40: 2016-2027.
22. Potten CS, Loeffler M. Stem cells attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development. 1990; 110: 1001-1020.
23. Slack J. Origin of stem cells in organogenesis. Science. 2008; 322: 1498-1501.
24. Ling L, Nurcombe V, Cool SM. Wnt signaling controls the fate of mesenchymal stem cells. Gene. 2009; 433: 1-7.
25. Šponer P, Kučera T, Diaz-Garcia D, et al. Eur J Orthop Surg Traumatol. 2014; 24: 257.
26. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria

for defining multipotent mesenchymal stromal cells. The International Society for cellular therapy position statement. Cytotherapy. 2006; 8: 315-317.
27. Liu L, Sun Z, Chen B, et al. Ex vivo expansion and in vivo infusion of bone marrow-derived FIk-1+ CD31− CD34− mesenchymal stem cells: feasibility and safety from monkey to human. Stem Cells Dev. 2006; 15: 349-357.
28. Warren S, Nacamuli RK, Song HJ, et al. Tissue-engineered bone using mesenchymal stem cells and a biodegradable scaffold. J Craniofac Surg. 2004; 15: 34-37.
29. Viateau V, Guillemin G, Bousson V, et al. Long-bone critical-size defects treated with tissue-engineered grafts a study on Wheel. J Orthop Res. 2007; 25: 741-749.
30. Liu J, Yu F, Sun Y, et al. Concise reviews characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells. 2015; 33: 627-638.
31. Shen C, Yang C, Xu S, et al. Comparison of osteogenic differentiation capacity in mesenchymal stem cells derived from human amniotic membrane AM, umbilical cord UC, chorionic membrane CM, and decidua DC. Cell Biosci. 2019; 9: 17.
32. Mennan C, Wright K, Bhattacharjee A, et al. Isolation and characterisation of mesenchymal stem cells from different regions of the human umbilical cord. Biomed. Res. Int. 2013.
33. Zajdel A, Kałucka M, Kokoszka-Mikołaj E, et al. Osteogenic differentiation of human mesenchymal stem cells from adipose tissue and Wharton's jelly of the umbilical cord. Acta Biochim. 2017; 64: 365-369.
34. Grayson WL, Bunnell BA, Martin E, et al. Stromal cells and stem cells in clinical bone regeneration. Nat. Rev. Endocrinol. 2015; 11: 140-150.
35. Loi F, Córdova LA, Pajarinen J, et al. Inflammation, fracture and bone repair. Bone. 2016; 86: 119-130.
36. Su P, Tian Y, Yang C, et al. Mesenchymal stem cell migration during bone formation and bone diseases therapy. Int. J. Mol. Sci. 2018; 19: E2343.
37. Carlier A, Liesbet Geris, Katie Bentley, et al. Mosaic: a multiscale model of osteogenesis and sprouting angiogenesis with lateral inhibition of endothelial cells. PLoS Comput Biol. 2012; 8: e10022724.
38. Choi Y, Yoon DS, Lee KM, et al. Enhancement of Mesenchymal Stem Cell-Driven Bone Regeneration by Resveratrol-Mediated SOX2 Regulation. Aging Dis. 2019; 10: 818-833.
39. Reichert JC, Cipitria A, Epari DR, et al. A tissue engineering solution for segmental defect regeneration in load-bearing long bones. Sci Transl Med. 2012; 4: 141ra93.
40. Gjerde C, Mustafa K, Hellem S, et al. Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. Stem Cell Research & Therapy. 2018; 9.
41. Iaquinta MR, Mazzoni E, Bononi I, et al. Adult Stem Cells for Bone Regeneration and Repair. Frontiers in Cell and Developmental Biology. 2019; 7: 268.