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**[Review Article]**

# Regenerative Therapy for Treatment of Critical-size Bone Defects

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**Abstract**

CRITICAL-SIZED bone defects, as well as delayed and non-union fractures, are unable to heal naturally without targeted intervention. Bone graft was the gold method in treating such conditions with potential deleterious disadvantages including immune rejection, persons needed for graft harvesting, increased pain and morbidity, and failure of healing in large bony lesions. Recently, the regenerative therapeutic strategies involving stem cells, microvesicles, and biological scaffolds have shown promising results in bone healing even in large-sized defects in experimental and clinical studies. Mesenchymal stem cells and microvesicles can secrete growth factors that aid vascularization and osteoblast differentiation. This systematic review represents an overview that investigates the advantages, disadvantages, and outcomes of using mesenchymal stem cells, exosomes, and biological scaffolds to manage critical-sized bone defects. In recent years, regenerative therapy for the treatment of critical-size bone defects was considered a major issue in bone tissue engineering and received extensive attention. Stem cells, EVs, and scaffolds played a crucial role in the improvement of the rate and quality of osteogenic differentiation, mechanical strength, and osteogenic conductivity. Studies on EVs and composite biomaterials showed promising results for the future of bone tissue engineering.

**Keywords:** critical-sized bone defect, stem cell, microvesicle, scaffold, Bone fracture.

## Introduction

Critical size defect (CSD) is one of the most challenging issues in orthopedic practice. It is defined as the smallest size of the intraosseous wound in a particular bone and species of animal which shows less than 10% spontaneous healing during the animal's lifetime [1]. Bone tissue has the potential for spontaneous healing after injuries. The regenerative capacity of bone tissue is limited by many factors, such as age, type of fracture, and genetic bone disorder [2]. The reconstruction of large CSD remains a major problem for orthopedic surgeons.

Bone grafts or biomaterial substitutes are commonly used as therapeutic strategies for filling and reconstructing large bone defects. Autologous bone grafts (ABGs) were considered a gold therapy to support and accelerate bone regeneration [3]. However, major disadvantages included immune-mediated rejection, persons required for graft harvesting, graft quantity, and additional anaesthetic time [3,4,5]. Stem cell therapy is a promising therapeutic strategy for repairing CSD. Mesenchymal stem cell (MSC) tissue engineering avoids some drawbacks associated with other treatment methods such as the limited number of grafts/scaffolds, requirements of microvascular surgeries, and long treatment duration [6].

Giannoudis et al and Dilogo et al reported that osteogenic cells must work together with osteoconductive/osteoinductive scaffold and mechanical environment to achieve excellent fracture healing. Mechanical stability and solid fixation are often required to facilitate and accelerate bone reconstruction. [7,8]. Bone tissue engineering (BTE) therapeutics primarily focuses on the development of biomaterials that facilitate and support bone regeneration in a physiologically appropriate manner [9]. BTE includes stem cells, microvesicles, and exosomes, scaffolds, microenvironments that support

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cell differentiation and adequate vascularization [10]. The aim of this systematic review was to investigate the regenerative capability of mesenchymal stem cells, exosomes, and biological scaffolds for the management of critical-sized bone defects.

*Stem cells*

Stem cell therapy is a potential strategy for bone regeneration. Mesenchymal stem cells (MSCS) can be harvested from different body sources such as adipose tissue, bone marrow, dental pulp, amniotic membrane, and umbilical cord. MSCS are multi-potent cells characterized by self-renewal capacity, fast proliferation and long-term viability. They are classified according to their progressive stage and capacity for dissimilarity [11,12].

The valuable impact of mesenchymal stem cells (MSCs) is mainly due to their capacity to enhance natural regeneration and liberate some biomolecules, such as cytokines, chemokines, and exosomes [13,14,15].

MSCs have a particular systemic anti-inflammatory effect on cytokines generated after fracture. They had a marked effect on reducing IL-6, tumor necrosis factor α (TNF-α), and IL-1β levels on the 3$^{rd}$ day after fracture which resulted in reduce tissue damage and stop fibrosis formation [15]. They express CD105, CD73, and CD90, and are not able to express CD45, CD34, CD14, or CD11b, CD79α or CD19 antigens. In addition, many studies confirmed that MSCs are useful for fracture repair by differentiation into osteoblasts [1,10,11]. Another study found that transplanted MSCs support the bone fracture healing by expressing BMP-2 which induces osteogenesis [16]. It's unknown how MSC homing processes to bone fracture occurred. Moreover, the precise functions of MSCs in fracture healing are still unclear [17].

The origin of MSCs has a crucial influence on their characteristics. The anatomical source and donor-specific characteristics like age and health are among the elements that contribute to MSC variability, which makes the therapeutic translation of these cells challenging [18]. Bone marrow-derived mesenchymal stem cells (BM-MSCs) were considered the most utilized source of mesenchymal stem cells due to their high regenerative capacity [19]. Chuang et al reported rejection of (BM-MSC) xenotransplantation after one week, while, adipose-derived stem cells (ADSCs) scaffolds were able to avoid rejection and support the bone-healing process [20]. Han et al found that BM-MSCs are more efficient than (ADSCs) in differentiating into osteoblasts [21]. On the contrary, recent studies found that ADSCs showed a higher proliferation capacity, angiogenesis, and Vasculogenesis than BM-MSCs [18,22]. Stromal vascular fraction (SVF) is a component of adipose tissue that is considered a rich source of inflammatory cells, preadipocytes, MSCs, and cytokines. SVF is essential for tissue healing because it promotes the growth, differentiation, and viability of ADMSCs [22]. Both dental pulp-derived stem cells (DPSCs) and ADSCs are easily harvested with minimally invasive approaches compared to BM-MSCs. They showed a high proliferation rate. Moreover, ADSCs showed faster bone regeneration than DPSCs in the treatment of mandibular defects in a rat model [23]. Platelet-rich plasma (PRP) significantly enhanced bone growth and improved the quality of bone healing when added to bone grafts by producing platelet lysate (PL) and growth factors [24,25].

Mesenchymal stem cells (MSCs) that were grown in a PL medium displayed faster proliferation and the greatest potential for bone repair [26]. The PL improves vascularization and continuous releases of growth factors that result in increased bone volume and osteoclasts when combined with MSCs [27].

The use of serum-free media supports MSCs to maintain their phenotype (higher genetic stability) and to enhance their osteogenic ability with reduced incidence of immune rejection [28].

MSCs have been used alone or added to scaffolds to designate three-dimensional structures planning to reinforce bone restoration [29,30]. However, the effectiveness of these approaches may be limited by variable regulatory considerations [31,32].

Stem cells cannot support healing in a less vascularized environment, inadequate nutrition, and massive tissue damage. Moreover, the use of MSC therapy included the risk of in vivo unintended differentiation, phenotypic changes, immune rejection, the tendency to stimulate tumor growth, and the variable cell survival rate that restricts its clinical applications [33,34].

*Extracellular vesicles (EVs)*

The favorable biological effects of stem cells on tissue regeneration are mediated through the paracrine effect of nanostructured extracellular vesicles (EVs). EVs overcome the limitations of stem cell treatments [35,36]. Extracellular vesicles (EVs) can be classified into three types, exosomes, microvesicles (MVs), and apoptotic bodies [37].

Exosomes (Microvesicles) are cell-free, phospholipid bilayer nanostructures (with diameters ranging approximately from thirty to one hundred and fifty Nanometres). They function to transport a wide range of active proteins, lipids, and abundant nucleic acids. It has the same functions as their mesenchymal stem cells [13,38,39].

Previous studies have shown that these microvesicles have pro-regenerative, anti-inflammatory, anti-fibrotic, and anti-apoptotic pathways [40].

Exosomes are stable (can overcome proteolytic degradation), and have a minor risk of immune

rejection and no risk of aneuploidy, so, they provide a promising alternative remedy in different medical fields [41,42].

Previous studies showed the major regenerative capability of exosomes in tendon and tendon-bone healing [43,44], treatment of osteoarthritis [40,45], and improving bone regeneration via promoting bone homeostasis, osteogenesis, angiogenesis, and inflammatory modulation [46,47]. Although much research focused on EVs for the repair of critical-size bone defects, the specific mechanisms remain unclear.

The intra-articular injection of exosomes played a crucial role in delayed OA progression and repair of critical-sized osteochondral defects [48,49]. Bioactive material-loaded EVs have the potential to efficiently heal critical-size bone defects [50].

Exosomes can be harvested from various sources such as adipose tissue, bone marrow, embryonic stem cells, umbilical cord, or induced pluripotent stem cells. The selection of a suitable source of parent cells of EVs has a great influence on the rate and quality of bone regeneration [51]. EVs formed by MSCs accelerate bone repair via similar mechanisms to their parent cells [52]. The commonly used parent MSCs of EVs mainly include umbilical cord mesenchymal stem cells [53], adipose-derived stem cells (ADSCs) [54], and bone marrow [55]. ADSCs are considered the favorable and most applicable parent cells for EVs because they are easily harvested, rapidly proliferated, widely distributed in the animal body, and decrease susceptibility to aging [56,57]. The exosomes formed by MSCs can prevent osteocytes from apoptosis in a hypoxia/serum deprivation model and an induced osteonecrosis model [58]. In addition, MSC-derived exosomes have been shown to have a significant role in fracture repair in addition to osteoporosis [59].

MSCs exosomes could enhance bone formation at every step of bone repair, suppress bone loss, share in bone rehabilitation through immune regulation, and prevent osteoporosis [54,60,61,62]. Application of EVs alone could not guide bioactive molecules to reconstruct the CSD and this could be attributed to the clearance of the reticuloendothelial system that leads to a quick loss of EVs and failure to reach the effective therapeutic concentration locally [63]. Thus, some bioactive scaffolds are needed in EVs-based BTE applications to ensure the sustained release of EVs [64].

*Biological scaffolds*

Biological scaffolds, also known as bioactive materials can be used in conjunction with mesenchymal stem cells (MSCs) and their released biomolecules to restore critical-size bone lesions. These scaffolds maintain the shape of critical-size bone defects and also help stem cells and EVs for better osteogenesis through different mechanisms [65]. Scaffold permits the formation of new bone along a predictable pattern determined by the biology of the graft and the mechanical environment of the host-graft interface [66].

The ideal biomaterial should have high osteoinductive and angiogenic potentials, biological safety, low patient morbidity, high volumetric stability, easy market availability, long shelf life, and reasonable production costs [67,68].

Hydrogels were considered the ideal carrier material for delivering EVs to bone defects with controlled production and suitable biocompatibility [69]. EVs loaded in hydrogels showed a significant increase in both bone mineral density and volume [70]. Hydrogel can be fully absorbed with osteogenic induction and accelerated bone remodelling [62]. Engineered modified EVs loaded with sodium alginate hydrogels were used for the continuous and controllable release of EVs to repair critical-size skull defects [71].

Hydroxyapatite (HA) has a similar composition to bones and teeth. HA synthetic grafts result in excellent stability and bone regeneration as they stimulate bone regeneration and are gradually replaced by bone [72,73]. Injectable hydrogel including hydroxyapatite, hyaluronic acid, and alginate had an effective role in accelerating osteoblast differentiation [62].

Porous bioceramics are now the material of choice for scaffolds for transplanting stem cells or EVs. They are characterized by mechanical strength, biocompatibility, and biodegradability [74]. Bioceramics were classified as absorbable bioceramics, such as β-TCP (tricalcium phosphate), and non-absorbable bioceramics, such as alumina and zirconia.

Porous-silicated calcium phosphate biomaterials were found to facilitate functional bone production at the defect site. Their use in bone regeneration is limited due to their stiffness and low osteoinductivity [75].

Hydroxyapatite (HA) glass ceramics exhibit favorable biological activities. BMMSCs EVs loaded hydroxyapatite/TCP bioceramics showed better osteogenesis [76]. HA biomaterials seeded with bone marrow stroma showed complete fusion between the implant and host bone with good implant integration [77].

Osteoconductive β-TCP acts as an osteoconductive scaffold those releases hiPSC-MSC-derived exosomes in a controlled manner, which promotes osteogenesis by proliferation, migration, and differentiation of hBMMSCs [78].

Adding minerals such as strontium to bioceramics significantly improved the rate and quality of osteogenic differentiation of EVs [79]. Decreased porous diameters of the bioceramics create a suitable

microenvironment for EVs for better osteogenesis, angiogenesis, and antimicrobial activities [80].

Bioceramics can be used only in low-loading orthopedic applications due to their less sufficient strength and low toughness [81].

Synthetic polylactic-glycolic acid (PLGA), PLGA polyethylene glycol triblock, and Polycaprolactone polymers were considered effective delivery carriers of EVs because of their ability to release controlled and adjustable number of exosomes resulted in the improvement of bone regeneration [54,82,83]. Polymer composites have an elastic modulus comparable to that of bone tissues, although not have the same biological activity as hydrogels or bioceramics [64].

Polycaprolactone is the most commonly used polymer in bone tissue engineering. It is characterized by its thermal stability, high biocompatibility, high permeability, and ability to maintain its mechanical properties for 6 months [84,85].

Biodegradable polymers showed weak mechanical properties and failure of strong bone integration due to their poor adhesion and lack of bioactivity [86].

Titanium and its alloys are the only metals that have osseointegration characteristics. They are widely used in BTE due to their optimal porosity, biocompatibility, and high mechanical strength and corrosion resistance of these metals [87]. Modified titanium alloy had a better ability to repair critical-size bone defects [88].

Composite biomaterials formed by a combination of polymers and ceramics resulted in improved bioactivity, biocompatibility, and mechanical strength with reduced creep-induced failure [89]. Metals added to composites increased the strength and osteogenesis. Fielding et al., found increased bone density, mechanical strength, and rapid cell proliferation of tricalcium phosphate by adding silica. [90].

The combination of more than one polymer with calculated ratios in one scaffold (composite) improves the rate and quality of osteogenesis. Combination of hydroxyapatite and poly lactic acid (HA/PLA), Combination of tri calcium phosphate and poly lactic glycolic acid (TCP/PLGA), phosphate glass fiber/PLA composite, chitosan- β-tricalcium phosphate composite, PEGylated poly (glycerol sebacate) (PEGS)/hydroxyapatite composite and calcium phosphate-zirconia scaffold demonstrated effective osteogenesis, vascularization and mechanical strength [91,92,93,94,95,96]. Moreover, the ratio of biomaterials forming the composite affects both osteogenesis and biodegradation. Composite biomaterials of PGA/beta-TCP biomaterials in a 1:3 ratio resulted in better bone mineral density and superior biodegradability than PGA/beta-TCP biomaterials in a 1:1 ratio for repair of CSD in a rat model [97].

Carbonated hydroxyapatite (CHA)-gelatin was effective in coating biomaterials when added to poly (ε-caprolactone)-tri calcium phosphate (PCL/TCP) composite.

Arafat et al. found that (PCL/TCP) composite coated with (CHA)-gelatin resulted in a significant increase in proliferation rate and osteogenic differentiation of cultured porcine (BM-MSCs) than non-coated CHA-coated composites. [98].

## Conclusion

In recent years, regenerative therapy for the treatment of critical-size bone defects was considered a major issue in bone tissue engineering and received extensive attention. Stem cells, EVs, and scaffolds played a crucial role in the improvement of the rate and quality of osteogenic differentiation, mechanical strength, and osteogenic conductivity. Studies on EVs and composite biomaterials showed promising results for the future of bone tissue engineering.

*Author contribution*

AAS and EGH planned and designed the review. AHA and AIA collected the data. AAS and ANA reviewed the manuscript. All authors wrote the manuscript and approved the final version of the manuscript.

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*Declaration of Conflict of Interest*

The authors declare that there is no conflict of interest.

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## العلاج التجديدي لعلاج عيوب العظام ذات الحجم الحرج – بحث مرجعي

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**الملخص**

إن كسور العظام ذات الحجم الحرج، وكذلك الكسور المتأخرة والغير ملتحمة غير قادرة على الشفاء بشكل طبيعي دون تدخل مستهدف. كان التطعيم العظمي هو الطريقة الذهبية في علاج مثل هذه الحالات مع عيوب ضارة محتملة بما في ذلك الرفض المناعي، واحتياج المتبرعين لجمع الطعم عظمى، وزيادة الألم والمضاعفات ، وفشل الشفاء في الإصابات العظمية الكبيرة. في الآونة الأخيرة، أظهرت الاستراتيجيات العلاجية التجديدية والتي تشمل استخدام الخلايا الجذعية والحويصلات الدقيقة والسقالات البيولوجية نتائج واعدة في شفاء العظام حتى في العيوب الكبيرة الحجم في الدراسات التجريبية والسريرية. يمكن للخلايا الجذعية الميزانشيمية والحويصلات الدقيقة أن تفرز عوامل النمو التي تساعد في تكوين الأوعية الدموية وتمايز الخلايا العظمية. تهدف هذه المراجعة المنهجية إلى تقديم نظرة عامة تبحث في مزايا وعيوب ونتائج استخدام الخلايا الجذعية الوسيطة، والأكسوزومات(الحويصلات الدقيقة )، والسقالات البيولوجية لإدارة عيوب العظام ذات الحجم الحرج. في السنوات الأخيرة، اعتُبرت العلاجات التجديدية لعلاج العيوب العظمية الكبيرة قضية رئيسية في هندسة الأنسجة العظمية وحظيت باهتمام واسع. حيث لعبت الخلايا الجذعية، والحويصلات الدقيقة(EVs) ، والهياكل الداعمة دوراً حاسماً في تحسين معدل وجودة التمايز العظمي، والقوة الميكانيكية، والتوصيل العظمي. كما أظهرت الدراسات حول الحويصلات الدقيقة والعناصر البيومادية المركبة نتائج واعدة لمستقبل هندسة الأنسجة العظمية.

**الكلمات الدالة:** عيوب العظام الحرجه الحجم، الخلايا الجذعية، الحويصلات الدقيقة، السقالات البيولوحيه، التجدد، الكسور.