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REVIEW PAPER

Novel bone graft substitutes in bone tissue engineering

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ABSTRACT

Objective(s): Globally, the prevalence of bone illnesses and diseases has been significantly rising. Bone tissue engineering (BTE), which can be produced continuously and doesn't transmit disease, has been suggested as a possible alternative to the traditional use of bone grafts. However, a number of limitations or challenges have prevented the further development of BTE techniques for clinical application. In order to promote bone regeneration, BTE uses a synergistic combination of biomaterials, cells and therapeutic components. Tissue engineering and bone tissue engineering (BTE) are rapidly expanding fields with increasing clinical applications. However, there are still challenges and limitations that need to be addressed, including incomplete knowledge of the biomaterials and their interactions with cells. With this in mind, we focused on the most recent researches to find what new strategies are being used to overcome obstacles in bone tissue engineering and which ones have the most potentials based on their results for future investigations.

Materials and Methods: To gather information for this article, we conducted a thorough search using PubMed, Scopus and Web of Science search engines. We used relevant keywords such as Bone tissue engineering, scaffolds, bioactive glass-ceramic, and hydrogel. From the initial search results, we selected 92 of the most recent and relevant articles based on their creativity in methods, novelty, and relevance to the subject.

Results: Biocompatibility, osteoconductivity, osteogenicity and osteointegration are the main important properties of the bone mimetic scaffold platforms used in bone tissue engineering. Development of scaffolds with sufficient mechanical properties, porous structure, appropriate surface topography is a challenging process. In this regard, a combination of various types of biomaterials such as bioactive glass-ceramic, different biodegradable polymers and even stem cells/ autologous cells are required.

Conclusion: Even though many BTE procedures have been numbered, only a few of them have so far been given clinical approval. The majority of these methods use a single component and fill deficiencies with cells, substances or materials. In order for BTE to become a widely used clinical reality, it must combine the most recent technologies that make use of all the required components.

Keywords: *Biomaterials, Bone tissue engineering, Hydrogels, Scaffold*

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INTRODUCTION

Bone tissue engineering (BTE) is a field that aims to surpass current treatments by offering potential alternatives to present bone regeneration procedures. The goal of BTE is to use a combination of reparative cells, signaling chemicals, and scaffolds to induce new tissue repair and regeneration [1, 2]. BTE is fast becoming a key instrument in the treatment of bone disorders like osteoporosis [3], osteosarcoma [4] and osteonecrosis [5]. Bone tissue disorders impose significant socio-economic costs on governments each year; Hence, many studies have been formed to solve this problem [6].

Several materials designs and methods are used for BTE like [7]: nanoparticles [1, 8],

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bioceramics [9], metals [8], polymers [10], stem cells [11, 12] and finite element method (FEM) [13]. The method and materials design are selected according to the type of bone tissue disorder and the individual and the properties of the materials [7]. These advances seek to harness stem cells, novel scaffolds, and biological factors to create robust, reproducible, and boosted bone formation strategies to enhance the quality of life for an aging population.

BTE has been around for about three decades [14]. To attain the ultimate aim of developing bone grafts that improve bone repair and regeneration, BTE requires the collaboration of scientists, engineers, and surgeons [15].

The purpose of this paper is to review recent researches about BTE and discuss different materials such as stem cells, nanoparticles, biomaterials, and hydrogel.

Bone structure and related diseases

Osteoblasts, bone lining cells, osteocytes, and osteoclasts are the four types of cells that form the bone [16]. Locomotion, support, and protection of soft tissues, calcium and phosphate storage, and bone marrow harboring are all significant roles of bone in the body [17]. Osteocytes have been shown to operate as mechanosensors and orchestrators in the bone remodeling process [18, 19]. Although the function of bone lining cells is unknown, they appear to play a significant role in coupling bone resorption and creation [20].

Bone remodeling is a complicated process in which old bone is replaced by new bone in a three-phase cycle: 1) initiation of bone resorption by osteoclasts, 2) the transition (or reversal period) from resorption to new bone creation, and 3) initiation of bone formation by osteoblast [21]. This occurs as a result of the coordinated efforts of osteoclasts, osteoblasts, osteocytes, and bone-lining cells [22]. Fig. 1 shows the bone structure and the cells associated with the bone remodeling process [23].

Different bone diseases such as osteoporosis, osteosarcoma, and osteonecrosis are major public health problems.

Osteoporosis is a polygenetic, environmentally changeable illness that leads to vertebral fragility fractures, hip and radius fractures. The clinical demand for bone regeneration applications, such as systemic or in situ directed bone regeneration and BTE, is a challenge to healthcare system. Aside from in situ directed tissue regeneration, traditional *ex vivo* BTE still has a long way to reach the level of routine clinical application, despite the development of many scaffolds and growth factors. [24, 25].

The rise in osteoporotic fractures around the world is driving BTE research to develop new and better biomaterials for bone scaffolds. However, the researchers faced a significant problem in reconstructing bone abnormalities in patients with osteoporosis. [3, 26].

Osteosarcoma is a prevalent bone cancer

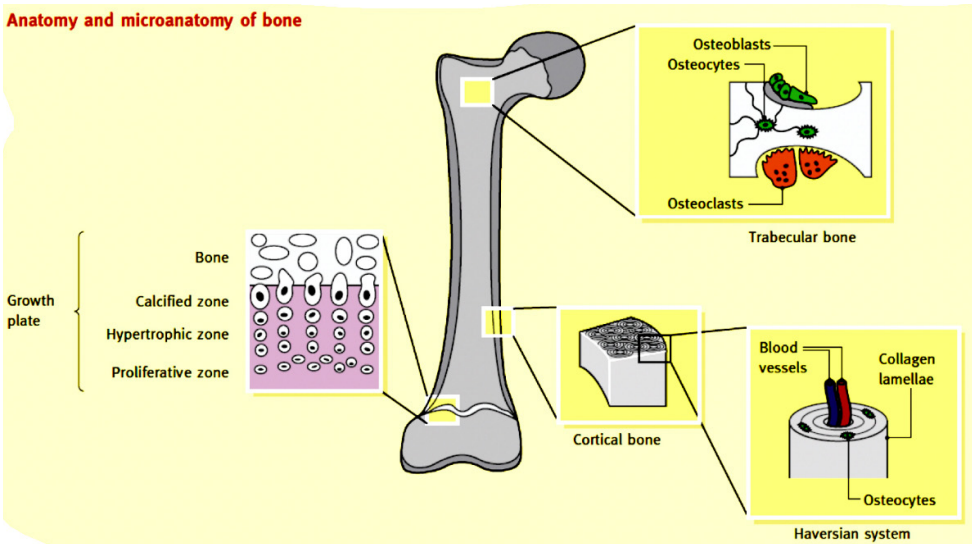

Fig. 1. Anatomy and microanatomy of bone (23) with permission from Elsevier (License Number 5395211320861), 2022

that affects people of all ages. Surgical and chemotherapy treatments have failed to slow tumor development. Tissue engineering and personalized medicine are both interested in developing effective patient-specific therapy methods for osteosarcoma. Tissue engineering scaffolds can also be used to replace missing bones, trap cancer cells, and distribute immune cells [27].

Osteonecrosis is one of the most serious illnesses in clinical orthopedics, caused by trauma, glucocorticoid misuse, or alcoholism. Enhancing bone regeneration is an important management technique in osteonecrosis treatment. BTE, based on constructed three-dimensional (3D) scaffolds with appropriate architecture and osteoconductive activity, has been developed to improve bone regeneration in osteonecrosis [5].

Bone tissue engineering (BTE)

History

It is important to know where we have been to make logical predictions as to where we are headed [28]. The term "tissue engineering" was invented in 1987. It is the use of a combination of multidisciplinary approaches to strengthen or replace biological tissues [29, 30]. With the rapid development of tissue engineering technology in recent years, BTE has become a promising approach to bone defect repair [29]. This field was begun around three decades ago, and interest and advances in the BTE field have grown tremendously over the years [14, 31, 32]. In recent years, autologous and allogeneic transplants have been widely used for bone diseases [33]. In the 1960s to solve the limitations of bone replacement materials which had made the clinical progress complex, costly, and restricted, scientists developed biomaterials that were sufficient for bone repair and replacement. These biomaterials have three generations and each of these generations is designed to achieve different goals [34]. The first generation which appeared in the 1960s is aimed to have the least toxic reaction to the host. They are generally bioinert and they have minimal interaction with the surrounding tissues. Metals , synthetic polymers, and ceramics are some examples of this generation. The second generation has a bioactive nature which means some of them could be biodegradable. The third generation of biomaterials aims to induce specific beneficial biological responses by adding instructive substances that are based on the second-generation biomaterials with superior properties and/or new biomaterials with outstanding performance [29].

Principles

Tissue engineering has emerged to provide a fundamental understanding of changes in the structure and function of pathological tissue and to develop biological alternatives for repairing damaged tissue [35]. The Benefits of using synthetic bone scaffolds include eliminating the risk of disease transmission, reducing the risk of surgical procedures, and abundant availability of synthetic scaffolding material [36].

In tissue engineering cells, extracellular matrix (ECM), blood vessels, nerves, intercellular communication, and cell-matrix interaction are only some "ingredients" to grow new tissues in vivo. These single components have to be combined in a well-coordinated spatial and time-dependent fashion [37]. Rather than the above-mentioned "ingredients", well elaborated surgical concepts are a prerequisite for the successful in vivo application [38].

Stem cells are being used for cell proliferation and differentiation. Stem cells are unique for their ability to proliferate through multiple generations and their potential to differentiate into a variety of cell types [1, 39]. The conventional method of BTE is to inoculate osteogenic functional cells on a three-dimensional scaffold material in vitro and implant them into the body after a culture period to repair the bone-deficient tissue. Another way is repairing a bone defect in vivo, which uses active growth factors or osteogenic functional materials to induce bone formation in autologous tissue (periosteum or mesenchymal cells) [35].

Tissue engineering strategies fall into three general categories: cell-based; matrix-based and growth-factor based. However, tissue engineering strategies for BTE combine two or more of these strategies for a solution. Two major tissue engineering techniques have appeared as the most promising approaches. 1) Before implantation, Mesenchymal stem cells (MSCs) are isolated (autograft or allograft), proliferated ex vivo, seeded on synthetic scaffolds and ECM is generated on the scaffolds under controlled culture conditions and finally placed on the patient's bone defect. 2) Implantation of cell-free scaffolding immediately after injury/bone removal [36].

In the field of BTE, these strategies need interaction between osteogenic, osteoinductive, and osteoconductive components. Osteogenic elements include cells that are capable of producing bone such as osteoprogenitor cells or differentiated osteoblasts. Osteoinductive factors include bioactive chemicals that actuate recruitment, differentiation, and proliferation of the proper cell types at damage. A fabric that bolsters bone development on itself illustrates osteoconductivity. Bone conduction scaffolds can provide sites for mechanical support, cell adhesion, and neovascularization, as well as delivering media for transplanted growth factors and cells [36]. These growth factors induce the differentiation, proper recruitment, and proliferation of suitable cell types at the injured site [3]. A perfect biomaterial scaffold should provide mechanical support to the defect site and also deliver cells and growth factors into an injured site to encourage tissue growth [36].

The goals

The field of BTE aims to design materials that are superior to autologous and allogeneic bone implants [7]. This field follows some goals such as fabricating three-dimensional (3D) scaffolds with interconnected pores for the reconstruction and regeneration of damaged or deformed tissues or organs and designing delivery methods for skeletal stem/progenitor cells for bone repair or replacement [40, 41].

The ultimate goal is to prepare artificial biodegradable bone substitutes that can be inserted into a bone defect and remodeled by the recipient's cells. These materials are mostly assembled in the form of scaffolds that act as supportive structures for cell adhesion and mineralized matrix deposition [7, 42]

Scaffold design considerations

Given the impressive range of materials available for tissue engineering, it is possible to design matrices with specific properties. These properties include 1) Biocompatibility: lack of immunogenic reaction. Because of its biocompatibility and similarity to the composition of real bone minerals, synthetic hydroxyapatite (HA) has been the most thoroughly investigated of the CaP ceramic phases. HA is a biocompatible, non-immunogenic substance that has been widely used as a scaffold or to modify the surface of biomaterials and implants. 2) Osteoconductivity: The quality of the porous interconnected structure that allows new cells to proliferate, attach, and migrate within the structure, it also permits the exchange of nutrient waste and the infiltration of new blood vessels; 3) Osteoinductivity: it possesses the essential proteins and growth factors which can induce the progression of MSCS and other osteoprogenitor cells to the osteoblast lineage; 4) Osteogenicity: the osteoblasts which are at the site of generating the new bone are capable of producing minerals for calcifying the collagen matrix which can form the substrate for new bone; and 5) osteointegration: the newly formed mineralized tissue have to be capable of forming a close bond with the implant material [43]. For example, it has been figured out that nanophase titania/ poly D,L-lactic-co-glycolic acid (PLGA) composites have great cytocompatibility properties crucial for designing better scaffold materials [4, 44, 45].

The mechanical properties of materials should preferably match (instead of greatly exceed) those of native bone, to prevent the stress shielding phenomenon, which is observed with conventional metallic bone-fixation systems. If the modulus elasticity of the implant is higher than the modulus elasticity of the surrounding tissue, most of the loading force will be carried by the metal rather than the surrounding bone. Furthermore, in bone-tissue-regeneration strategies using biomaterial scaffolds, the implanted materials not only aim to mechanically support the growing bone tissue but are also aimed to undergo biodegradation. Different materials that are being used in BTE are natural polymers (Collagen or gelatin, Silk, Alginate), synthetic polymers (Poly (lactic-co-glycolic acid) Poly (propylene fumarate) Poly(ε-caprolactone)), bioceramics (Hydroxyapatite, β-Tricalcium phosphate, Bioactive glasses (such as 45S5 composition), biodegradable metals (Magnesium and its alloys), carbon-based nanomaterials (Carbon nanotubes, Graphene or graphene oxide) [7].

Rather than the mentioned essentials, other design considerations can change the suitability of the matrix for its application, such as:

- Biofunctionality: Scaffold should be capable of meeting the functional requirements that was designed for, to restore the functions of the replaced tissue.
- Bioresorbability or biodegradability: The

scaffold's capacity to break down gradually over time *in vivo* or *in vitro* settings at a regulated rate to create room for growth of new tissue. This means that the scaffold voids should develop as long as the cells are multiplying, and the pace of material degradation should match the rate of healing or regeneration process [46].

- Mechanical properties: Mechanical properties such as tensile strength, elastic modulus, fracture toughness, and elongation percentage have to be very close to the replaced tissue.
- Pore size and porosity: A three-dimensional design affects the placement and location of cells, nutrients, and oxygen, thus affecting the viability of the newly formed tissue. Porous scaffolds promote cell migration and proliferation, providing a microenvironment suitable for cell proliferation and differentiation, and allow mass moving of nutrients, oxygen, and metabolic waste products within the structure. Scaffolds must have a huge internal surface area due to pore size. Pore sizes used in BTE scaffolds must be between 100 and 900 µm. Pore sizes larger than 300 µm are preferred by osteoblast cells (10–50 µm) because they facilitate the passage of nutrients and waste products as well as the penetration of biomolecules. It has also been demonstrated that increasing pore sizes decreases the water contact angle, allowing for greater fluid entry when implanted within the body [47, 48].

Materials used in the structure of the ideal scaffold platform

Bioactive glass-ceramic

The ideal biomaterial is three-dimensional, resorbable, biocompatible, porous, and strong enough to withstand mechanical forces. Due to their higher biocompatibility and osteoinductive capabilities, bioactive glasses have emerged as a possible alternative; however, their poor mechanical properties have generally limited their applicability [49]. The structure of a bioactive glass-ceramic (GS) scaffold produced by gel-cast foaming is shown in Fig. 2. By dispersing a gas in the form of bubbles into a ceramic suspension or colloidal sols, followed by solidification, it is possible to create highly porous ceramics with pores as small as 20 nm and as large as 1-2 mm.

The living tissue for growing into the pores of the biomaterial and maintaining its viability needs to have adequate large open pore size. It is estimated that the smallest size of open pores that allow the formation of a biological link between the implant and the bone is 100 µm. Osteon formation within the bone implant is possible if the pores reach 200 µm [50].

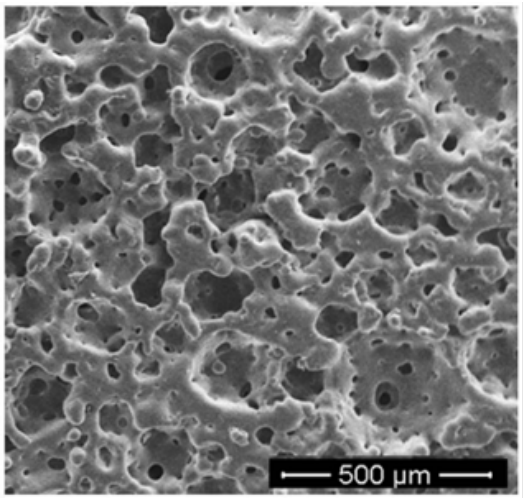

Fig. 2. This scaffold was obtained by gel-cast foaming, freeze-drying, and sintering (51).

In the process of in situ polymerization of an organic monomer (or gel-cast foaming), an organic monomer that is soluble in water (for example, acrylates) is needed. Thereafter, other materials such as an initiator and a catalyst to supply the in-situ polymerization are added to a high-solid-load aqueous ceramic suspension. The two latter ingredients are required to control the polymerization reaction's actual onset (also known as the induction time), which must occur during casting when processing porous ceramics [51].

The compositional and structural analysis of a three-dimensional composite scaffold made up of polylactic acid and spray-dried glass-ceramic microparticles (SGCMs) revealed that the spray-dried powder developed as glass-ceramic with a completely linked porosity structure [52].

Bioactive glass-ceramics are termed "Smart" materials because they have exceptional biocompatibility and osteoconductivity and can make direct chemical bonds with human bones [53]. PLA-GC (polylactic acid matrix SGCMs) composite ink has a sharper shear-thinning tendency, as well as a higher loss and storage modulus than pure PLA. The findings also suggest that 3D scaffolds had a very well-interconnected porosity and uniform distribution of glass-ceramic particles and that compression strength is reliant on the presence of SGCMs and scaffold

porosity. The PLA-GC scaffolds had a superior biomineralization capacity, as larger and denser sediments developed on the PLA-GC scaffolds after 7- and 14-day soaking. The right mix of biomaterials/methods for fabricating 3D porous constructions, as well as their bioactivity and biocompatibility, both of which are critical for BTE applications [52, 54].

Combining synthetic and natural polymers strives to maximize the benefits of both materials, including the ease of processing, ability to add specific functional groups, low cost, and high biocompatibility of natural polymers [55].

To repair critical-sized bone defects in rabbit mandibles, researchers developed a new form of bioactive glass-ceramic (AP40mod) as a scaffold including Endothelial progenitor cells (EPCs) and bone marrow-derived mesenchymal stem cell (BMSCs). For in vitro experiments, AP40mod was generated using a Digital light processing (DLP) system, and the ideal EPC/BMSC ratio was determined by assessing cell proliferation and alkaline phosphatase (ALP) activity and the influence of genes involved in osteogenesis and angiogenesis, using direct injection into the scaffolds. The use of AP40mod/EPCs/BMSCs to repair and rebuild a critical-sized mandible defect in a rabbit (following 7 days of in vitro spin culture) revealed that all scaffolds were successfully and precisely transplanted into the defect location. The AP40mod combination of EPCs/BMSCs is a potential method for repairing and reconstructing massive load-bearing bone defects, according to the findings [53].

Biosilicate® is a revolutionary bioactive glass-ceramic with two crystalline phases (BioS-2P) that was created to improve the mechanical properties of glass-ceramics without losing their biocompatibility. BioS-2P has osteoinductive and osteogenic characteristics, meaning it helps MSCs differentiate into osteoblasts and boosts osteoblast activity *in vitro*. BioS-2P is also a biocompatible substance with osteoconductive capabilities, resulting in significant bone growth, according to *in vivo* evidence. [49].

For BTE applications, macroporous composite scaffolds made of gelatin scaffold with variable glass-ceramic composition can be constructed by using the lyophilization process. Gelatin is a desirable scaffold material due to its affordability, accessibility, and simplicity of dispersion in aqueous solutions [56]. They are not poisonous and are simple to apply in the form of thin coats. However, they lack bioactivity, have unpredictable degradation in aqueous environments, and have unsatisfactory mechanical qualities. Making a composite material with bioactive glass and glass-ceramics and adding it to gelatin as a biomaterial is one way to improve the many gelatin limitations. [57].

Kwon JW et al. in a 2024 clinical trial concluded that there was no apparent difference in the radiological and clinical results between anterior cervical discectomy and fusion (ACDF) cases treated with bioactive glass-ceramic cages and polyether ether ketone (PEEK) cages. On the other hand, compared to the PEEK group, the bioactive glass-ceramic group's operation time was significantly lower. In conclusion, with an autologous iliac bone graft in single-level ACDF, a non-window-type bioactive glass-ceramic cage is a workable replacement for a PEEK cage so this material can be used instead of the old methods [58].

Biodegradable materials

To improve the repair of bone defects by promoting cell attachment, proliferation, and vascularization during new bone formation, a biocompatible and biodegradable scaffold with load-bearing capabilities is required. However, maintaining porosity and biodegradability while maintaining mechanical qualities (particularly compressive strength) is difficult [59].

- Microspheres of polytrimethylene carbonate with tricalcium phosphate form a biocompatible and biodegradable scaffold:

A scaffold of composite microsphere was constructed using polytrimethylene carbonate (PTMC) microspheres loaded with vancomycin hydrochloride (VH). A microsphere scaffold with three-dimensional oleic acid-modified tricalcium phosphate (PTMC-OA-TCP)/PTMC-VH was made with a thermal system.

In terms of bone regeneration in vivo, the PTMC-OA-TCP scaffold performed similarly to the bone cement. The produced bioactive scaffolds, which showed good mechanical characteristics and aided osteogenesis, could be a promising BTE alternative to bone cement [60].

- BTE using a new chitooligosaccharide / gelatin/demineralized bone matrix composite scaffold and periosteum-derived MSCs:

Gelatin (G), chitooligosaccharide (COS), and

demineralized bone matrix (DBM) are three biodegradable scaffolds that could be useful in BTE. Using a lyophilization approach, three-dimensional scaffolds made of G, COS, and DBM were constructed into three groups: G, G/COS (G/C), and G/COS/DBM (GCD). For four weeks, the scaffolds were cultured with MSCs in GCD scaffolds and showed more osteogenic differentiation than those in G and G/C scaffolds. In a rat model, the G, G/C, and GCD scaffolds were also found to stimulate *in vivo* ectopic bone growth. When compared to other scaffolds, the GCD scaffolds had the highest osteoinductive activity [61].

- Biodegradable poly (caprolactone) (PCL) – poly (glycolic acid) (PGA) - beta tricalcium phosphate (beta TCP) load-bearing scaffolds for bone tissue regeneration:

With most polymeric materials, the compressive strength of the implant is lower than native bone tissue, which renders most polymers unsuitable for some orthopedic applications. During the process of making a Load-bearing biodegradable PCL-PGA-beta TCP scaffold, the sample was compressed to 50% of the initial size which resulted in an increased compressive strength. The samples were reheated to relieve the stress generation due to compression, followed by cooling. Compression molding was used to create a biodegradable composite structure of PCL with different amounts of PGA (25, 50, 75 weight (wt)%) and a set amount (20 wt%) of beta TCP. With an increase in the PGA amount in PCL, the rate of dissolution and weight loss both increased. The PCL-PGA scaffold with beta TCP can be a qualified candidate for BTE applications [59].

- Photo-cross-linked bioactive polycaprolactone-based osteoconductive biocomposite

A photo-cross-linkable poly (propylene fumarate) (PPF)-co- PCL tri-block copolymer was used to make a light cross-linkable biocomposite scaffold. TCP bioceramic was used to further modify the developed biodegradable scaffold.

The results of the characterization validated the creation of a biodegradable and photocrosslinkable PCL-based biocomposite reinforced with TCP bioceramic. The biocompatibility and mineralization capability of the produced bioceramics were established in vitro. Overall, the findings of this study indicated that the photocrosslinkable PCL-PPF-PCL tri-block copolymer reinforced with TCP is a potential biocomposite for BTE [62].

- Silicon-Phosphorus-Nanosheets-Integrated 3D-Printable Hydrogel

Silicon phosphorus (SiP) was investigated as a new type of bioactive and biodegradable nanomaterial with great angiogenesis and osteogenesis properties. In this regard, biohybrid hydrogel of methacrylate gelatin (GelMA) and polyethylene glycol diacrylate (PEGDA) integrated with photocrosslinkable SiP-nanosheet (GelMA-PEGDA/SiPAC) was printed. The GelMA-PEGDA/SiPAC showed great biocompatibility and biodegradability, as well as the ability to release Si and P elements over time and can improve MSCs osteogenesis and tubular networking in human umbilical vascular endothelial cells in comparison with biohybrid hydrogel scaffolds with black phosphorus nanosheets (Fig. 3)[63].

- Radially patterned transplantable biodegradable polycaprolactone polymer scaffolds

This transplantable scaffold for bone regeneration was proposed in a 2021 study using biodegradable PCL polymer and defined by capillary force lithography technology and a polydimethylsiloxane (PDMS) mold was used.

Because of its beneficial properties, such as biocompatibility, high rigidity and flexibility, and manageable biodegradability between one to several years, depending on the molecular weight, degree of crystallinity of the polymer, and degradation conditions, PCL is one of the most widely used polymers for biomedical applications.

The radially oriented design considerably aided host cell recruitment and osteoblast migration into the defect region. Furthermore, by promoting cell migration and differentiation, the transplantable scaffolds facilitated the regeneration of critical-sized bone lesions [64].

- Nanohybrid biodegradable scaffolds releasing Transforming growth factor-β3 (TGF-β3)

TGF-β3 is one of the most commonly used growth factors (GFs) for BTE since it is crucial in attracting stem/progenitor cells to the process of tissue regeneration and remodeling. The injured spinal disc has shown higher proteoglycan content deposition following direct TGF-β3 injection.

In a study, a 3D PLGA/ PCL nanohybrid scaffold was designed and embedded with PLGA macroparticles (MPs) coupled with TGF-β3. TGF- β3 conjugation was increased with over 80% loading efficiency and sustained release in

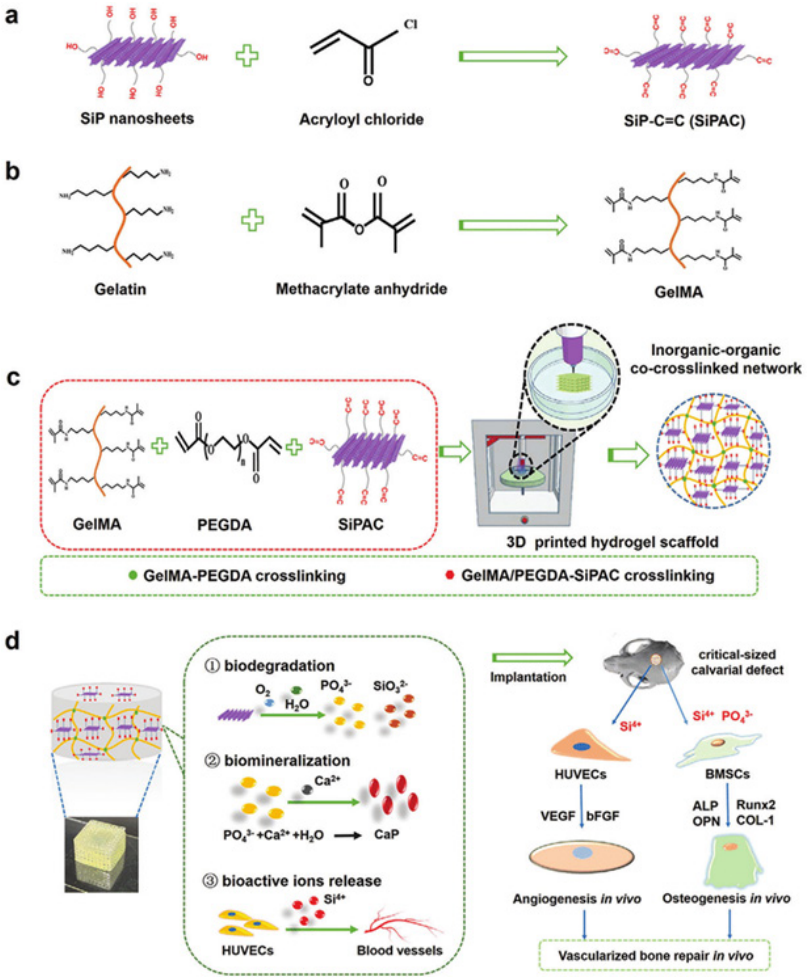

Fig. 3. Schematic representation of the creation of 3D printable hydrogel scaffolds using SiP nanosheets and their use in the regeneration of vascularized bone (63) with permission from Wiley (License Number: 5593590649991), 2023

PLGA MPs, using a microfluidic-based technique. Replica molding was used in the design and construction of the microfluidic device. As a result, the nanohybrid scaffold of PLGA-TGF- β3 MPs/PCL has great potential for cartilage regeneration and other regenerative medicine applications (Fig. 4) [65].

- Glass Microfibers Wrapped in Aligned Polymer Nanofibers

A modified air-gap electrospinning technique was used in a study to continually wrap highly aligned polycaprolactone polymer nanofibers around individual bioactive glass microfibers, resulting in a synthesized structure that resembles osteons. The Haversian canals, which contain the blood and nerve vessels, are surrounded by osteons, hollow cylindrical structures. Osteogenic cells which are present throughout the osteons constantly repair the bone structure. Using a high voltage to extrude a polymeric solution, results in the creation of highly porous, nano-sized fibers through the process of electrospinning. Because of the high voltage's instability-causing effects, the solvent evaporates before being deposited on the substrate due to electrostatic repulsion. This procedure often yields fibers that are randomly arranged and imitate the ECM, enabling better cellular incorporation into the scaffold [66].

- Copolymer scaffolds with altered surface hydrophilicity

Recent research suggests that both poly(l-lactide-co-1,5-dioxepan-2-one) (or poly (LLA-co-DXO) and poly(l-lactide-co—caprolactone) (or poly (LLA-co-CL) porous scaffolds are good candidates for use as biodegradable scaffold materials in tissue

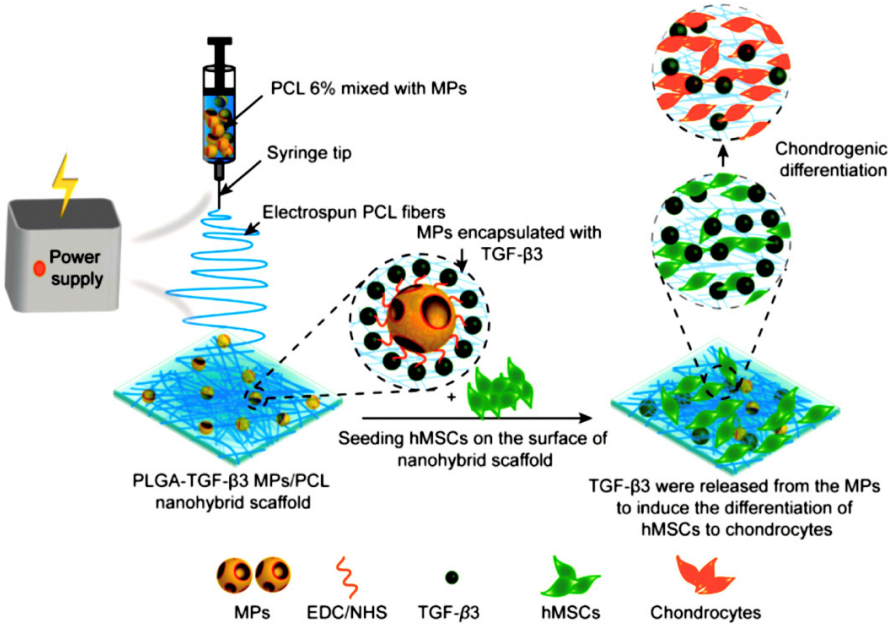

Fig. 4. The creation of the PLGA-TGF-3/PCL nanohybrid scaffold and its use in promoting human MSC growth and chondrogenic differentiation are shown schematically. (65) with permission from Elsevier (License Number: 5593580000230), 2023

engineering; however, their surface characteristics, such as hydrophilicity, still need to be enhanced. In a 2020 study, Xing Z et al. used various doses of the surfactant Tween 80 to control the hydrophilicity of both materials. Three different weight ratios of 3 percent, 10 percent, and 20 percent Tween 80 were added to the dissolved polymer solution and mixed overnight. Furthermore, the alteration was applied to a porous scaffold as well as a solid scaffold as a film. The addition of Tween 80 significantly increased wettability, according to the data, and the human MSCs displayed delayed attachment and dissemination. According to PCR results, the 3% Tween 80 group had numerous osteogenesis-related genes up-regulated and human MSC development was enhanced. The results showed, a high hydrophilic scaffold may accelerate osteogenic differentiation [67].

- Multi-functional and time-Sequential 3D printed MgO_2 /PLGA scaffold

A novel approach to treating osteosarcoma-associated bone defects is multifunctional bioactive scaffolds with time-sequential functionalities of encouraging bone defect repair, limiting bacterial infection, and avoiding tumor recurrence. By using low-temperature 3D printing, a nanocomposite scaffold composed of magnesium peroxide (MgO_2) and poly (lactide-co-glycolide) is created, allowing for the time-sequential release of magnesium ions (Mg^{2+}) and reactive oxygen species. The hydrogen peroxide that is released triggers chemo-dynamic treatment, which causes tumor cells to undergo apoptosis and ferroptosis. Additionally, it activates the anticancer immune microenvironment by causing macrophages to become M1 polarized. Following the release of Mg^{2+}, the Wnt3a/GSK-3β/β-catenin signaling pathway is activated, which promotes osteogenic differentiation of bone marrow mesenchymal stem cells and the creation of an osteo-promotive immunological milieu via macrophage M2 polarization. This ultimately improves bone regeneration [68].

- TFRD-loaded HA/CMCS/PDA scaffold

Natural biomaterials (chitosan), synthetic inorganic materials (hydroxyapatite), and polymers (polydopamine (PDA)) are frequently employed in the construction of biomaterial scaffolds, depending on the specifications of the biomaterial scaffold. Lin H et al. created a unique 3D-printed scaffold with anti-infection properties, biodegradability, and angiogenesis induction, utilizing hydroxyapatite (HA), carboxymethyl chitosan (CMCS), PDA, and total flavonoids of Rhizoma Drynariae (TFRD). The scaffold performed well in terms of drug release and degradation. The scaffold extract increased mineral deposition and bone-related gene/protein expression in BMSCs, which encouraged osteogenesis in

vitro. Additionally, by upregulating particular genes and proteins linked to cell migration and tube formation, it increased endothelial cell migration and encouraged angiogenesis. This could be explained by the PI3k/AKT/HIF-1α pathway being activated, which promotes osteogenesis and angiogenesis [69].

- Neuro-vascularized bone regeneration with GelMA/GeP@Cu

Using copper ion-modified germanium-phosphorus (GeP) nanosheets is a novel biohybrid biodegradable hydrogel (gelatin methacrylate, GelMA) with antibacterial and neuro-vascular regeneration properties. The copper ion modification procedure provides a platform for the continuous release of bioactive ions while also strengthening the stability of the GeP nanosheets.

The integrated hydrogel can upregulate proteins linked to neural differentiation in neural stem cells in vitro, promote angiogenesis in human umbilical vein endothelial cells, and dramatically increase the osteogenic differentiation of bone marrow mesenchymal stem cells [70].

Sun X et al. researched how bone regeneration was affected by a cell-free bone tissue engineering system like exosomes made from human umbilical cord mesenchymal stem cells (hUCMSC-Exos). In vitro, hUCMSC-Exos induced Human Umbilical Vein Endothelial Cells (HUVECs) migration, proliferation, and tube formation; the impact grew as exosome concentrations rose. Through the enhancement of angiogenesis and osteogenesis, the combination of hUCMSC-Exos and 3D-printed silk fibroin/collagen I/nano-hydroxyapatite (SF/COL-I/nHA) scaffolds improved alveolar bone defect repair *in vivo* [71].

Hydrogels

A considerable amount of water can be absorbed and maintained by hydrogels, which are 3D structures made from hydrophilic polymers and cross-linked with the right methods. For BTE applications, hydrogels' inflated state and cross-linked polymer chains offer a 3D and porous structure. The porosity architecture is essential for cell migration and infiltration, as well as for the turnover of nutrients and waste [72]. Hydrogels are deemed ideal and appealing materials for the attachment, proliferation, migration, and differentiation of loaded cells [73]. In Table 1 a summary of recent studies on hydrogel-based scaffolds used in bone tissue engineering is shown.

Three important elements, Hydrogel scaffold, cells, and growth factors, are shown in Fig. 5.

Hydrogel preparation methods should be integrated with synthetic materials with good qualities and novel technologies in various domains to better control drug release in time and orientation, making hydrogel more suitable for the local treatment of bone disorders [74].

Any tissue engineering approach that uses collected or grown adherent cells must include the

Table 1. A summary of recent studies on hydrogel-based scaffolds used in bone tissue engineering

Materials	The preparation method	Outcomes	Ref.
Gellan gum and Xanthan gum, Chitosan and Pentasodium tripolyphosphate (TPP)	9:1 Gellan: Xanthan ratio for hydrogel preparation with basic fibroblast growth factor (bFGF) and bone morphogenetic protein 7 (BMP7) as a dual growth factor delivery system	Due to the prolonged release of growth factors, cell proliferation and differentiation were greatly improved. Additionally, Gellan/Xanthan gels had antimicrobial properties	(75)
Peptide gel solution (Arginine-Alanine- Aspartic Acid) and PLGA/ nano HA macroporous matrices	The gel solution was injected into the porous matrix (with the ratio of 4g PLGA to 1 g nHA)	Preosteoblastic cell proliferation was boosted by the hybrid scaffold system	(76)
Bacterial Polyglucuronic Acid (PGU), Alginate(Alg), Carbon Nanofibers (CNFs) and Hydrogel Nanocomposite	----------	The generated bacterial PGU/Alg/CNFs hydrogel nanocomposite demonstrated suitable properties and can be regarded as a novel biomaterial for BTE scaffolds	(72)
poly (ethylene glycol)- poly (D, L-lactide) (PLEL), resveratrol (Res) and dexamethasone (DEX)	----------	Experiments conducted in vitro demonstrated that the hydrogel efficiently increased mesenchymal stem cell osteogenic development, eliminated extraneous reactive oxygen species (ROS) within the cell, and controlled macrophage polarization to lessen inflammatory reactions. The hydrogel was found to alter immune responses and promote osteoporotic bone defect healing *in-vivo*	(77)
Exosomes (Exos) and Hydrogel microparticles (HMPs)	A microfluidic approach is used to create the HMP@Exo system using Exos containing Hyperbranched Poly Ethylene Glycol Diacrylate (HB-PEGDA) and Sulfhydryl-Modified Hyaluronic Acid (SH-HA). Arginine, Glycine, and Aspartic Acid peptides are added to the HMP surface to improve cell adherence	The system showed excellent injectability, remarkable compatibility, exceptional cell adhesion properties, and a slow degradation capacity. Additionally, the prolonged release of Agomir-29a-Exos from HMPs boosted the migration and proliferation of HUVECs and BMSCs while encouraging osteogenesis and angiogenesis	(78)

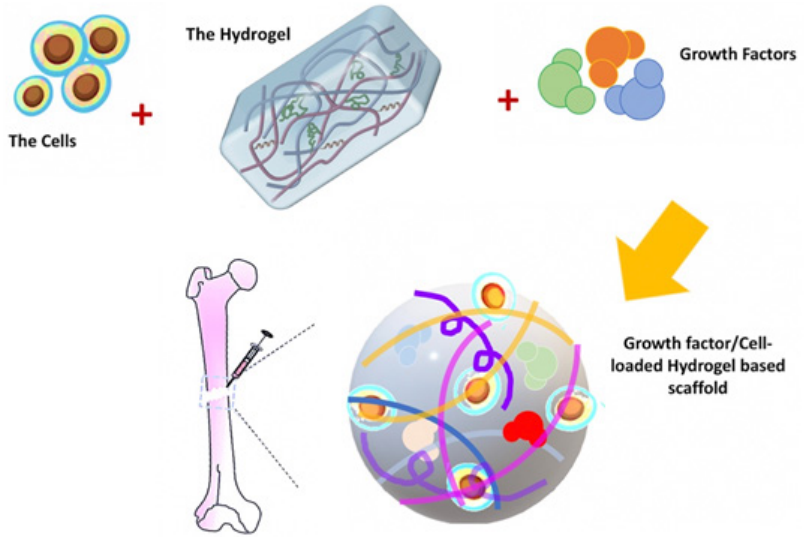

Fig. 5. Schematic representing the development of cell/growth factor-loaded scaffolds used for bone tissue engineering

seeding of cells onto scaffolds. There are various ways for cell seeding, depending on the intended purpose. When properly prepared, hydrogels offer a thick web of fibers for cellular adhesion and encapsulation, establishing a barrier that keeps cells from being removed by the medium. Kotlarz M et al. conducted a comparison between traditional manual cell seeding in media and cell seeding using a bio-printed hydrogel. To do this, they employed the reactive jet impingement (ReJI) bioprinting technique to apply high cell density, cell-laden hydrogels onto the surface of the scaffolds and a binder jet 3D printed bioceramic scaffold as a model system for bone tissue synthesis. Significant and similar levels of mineralization and cell migration were observed for the two seeding techniques. However, in situ, cell seeding into implants, which is desired in clinical tissue engineering procedures, may be accomplished with bio-printed hydrogels. This approach eliminates the need to maintain a specific orientation until attachment happens and the time it takes for cells to attach from media [79].

Cell types used in the BTE platform

To facilitate artificial bone construct integration into a person's body, scaffolds are enriched with different cells.

Stem cells

Due to their unique biological capacity to differentiate into osteogenic lineages, a variety of stem cells, including embryonic stem cells (ESCs), BMSCs, Umbilical cord blood mesenchymal stem cell (UCB-MSCs), adipose tissue-derived stem cells (ADSCs), muscle-derived stem cells (MDSCs), and dental pulp stem cells (DPSCs), have recently attracted significant attention in this field. It is necessary to induce *in vitro* differentiation of

Table 2. Some examples of gene therapeutics used in BTE

Gene name	Targeted cell	Results	Study type	Ref.
transforming growth factor β1 (TGF-β1) and vascular endothelial growth factor (VEGF-A)	pre-osteoblast MC3T3-E1 cells	A flexible method for encouraging bone repair with a synergistic effect is offered by the combination of tunable dual-crosslinked hydrogel and multi-gene activation using non-viral CRISPRa system	*In vivo*	(89)
HOTAIRM1	Human dental follicle stem cells (hDFSCs)	To improve the osteogenesis of hDFSCs, HOTAIRM1 elevated KDM6A/B and inhibited EZH2 in a way that was dependent on HIF-1α	*In vivo*	(90)
Wnt10b and Foxc2	BMSCs	encouraged osteogenesis while inhibiting adipogenesis *in vitro*. BMSCs increased osteogenic differentiation of BMSCs and decreased adipogenic differentiation	*In vitro* and *in vivo*	(91)

these stem cells into osteoblasts to use them for BTE [80].

MSCs have been the focus of numerous studies in recent years. The majority of investigations have concentrated on adult BMSCs because of their innate capacity to develop into diverse cell types and high osteogenic profile [81].

Using stem cells has been known as a promising approach in regenerative medicine; but there are limitations like the migration, uncontrollable proliferation, and differentiation of the stem cells but fortunately these features could be improved after stem cells were encapsulated in the hydrogel [73].

Mesenchymal stem cell sheets have been demonstrated in recent research to improve bone repair in various animal models. Mito K et al. studied the effectiveness of BMSC sheets implanted without a scaffold on a defected bone. Male Sprague-Dawley rats that were 5–6 weeks old had their femora removed, and BMSCs were separated from it. Cell sheets were then created on temperature-responsive culture plates, and the sheets were inserted into the bone defect. When compared to the control, they discovered a significant rise in the volume proportion of new bone production. A higher percentage of freshly produced bone and a higher overall histological score were found by histomorphometry analysis [82].

A 2024 study used 3D co-culture of STRO1+ Gingival Mesenchymal Stem Cells (sGMSCs) (sGMSC spheroids, GS) and HUVECs (sGMSC/HUVEC spheroids, GHS) to create a novel stem cell spheroid and results showed high osteogenic and angiogenic potential [83].

Some factors enable the development of autologous bone grafts in vitro like bioreactor culture systems and bone scaffolds. They can be used for bone repair applications in vivo and are shown in Fig. 6 [84].

Autologous blood clots can safely act as a scaffold for bone repair in critical-sized bone lesions. If using stem cells, fibrin sealant is more advantageous and leads to more bone formation without the negative effects on the donor and host cells [85].

Genetically modified cells

Though the previously described delivery systems are capable of achieving sustained release of growth factors, the high dosage requirements for bone applications are still of great concern. A novel approach to this problem is to genetically modify cells to produce osteo-inductive growth factors and seed these cells into a scaffold [86].

The ability to accelerate regenerative processes by combining cell and gene therapy approaches has been demonstrated by the in vivo implantation of stem cells, genetically modified to carry osteogenic genes. Importantly, the quantity of exogenous cells that need to be implanted may be lowered as a result of the recruitment of host cells. Adult stem cells have been modified to express bone morphogenetic protein family genes, such as Bone morphogenetic protein 2 (BMP2), BMP4, and BMP7, in numerous searches. The PI3K-Akt signaling pathway is essential for the osteogenic

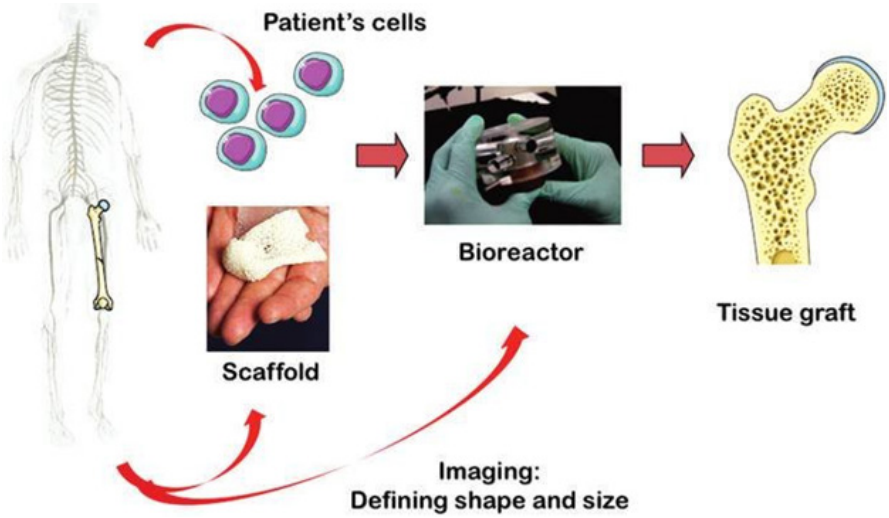

Fig. 6. BTE model with autologous stem cells (84)

differentiation of MSCs. Additional relevant genes include those that encode transcription factors that are necessary for osteoblast differentiation (such as core binding factor 1 (Cbfa1) and Osterix), as well as proteins that promote angiogenesis (for example, noggin) for an additional level of control over bone formation [87, 88].

Utilizing the Sendai virus vector has recently increased the effectiveness of creating Induced pluripotent stem cells (iPSCs) and made it simpler to create them from a variety of somatic cell types [92].

The role of non-coding (nc)-RNAs in the regulation of osteogenic differentiation in BMSCs has recently received a lot of attention. The ncRNAs could be used as biomarkers for bone disorders. Endonucleases called Drosha and Dicer play a role in the production of microRNA and are directly associated with the osteogenic differentiation of BMSCs. [93]. The ability of different types of RNA like ribosomal RNAs, small nuclear RNAs, and small nucleolar RNAs to regulate osteogenic differentiation is still unknown and should be revealed. [94].

CONCLUSION

The study of tissue engineering specifically BTE, is expanding quickly. Products based on BTE are starting to be used in clinical settings. In the coming years, patients can hope to have access to even more BTE technologies based on the existing success. While current efforts are concentrated on creating effective BTE methods, we expect that in the future, the discussion will shift to identifying the BTE strategies that are the most economical. Although the race to make BTE a clinical reality is highly justified, there are still a lot of challenges and restrictions in this area.

The development of biomaterials for BTE applications has come a long way, but there are still many unmet demands and difficulties that prevent the clinical deployment of biomaterials-based techniques. One such barrier is our incomplete knowledge of how most biomaterials work and how cells react, so systematic studies are required to address this need.

Even though numerous BTE techniques have been developed, only a few numbers have so far received clinical approval. Most of these techniques use a single component and involve cells, growth factors, or materials that fill defects. BTE needs to incorporate the most recent technologies that make use of all the necessary components to become a widely used clinical reality. Therefore, more work needs to be done to develop effective intraoperative cell seeding techniques and enhance bone tissue regeneration *in vivo*.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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