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REVIEW

Application of biomimetic three-dimensional scaffolds in bone tissue repairing

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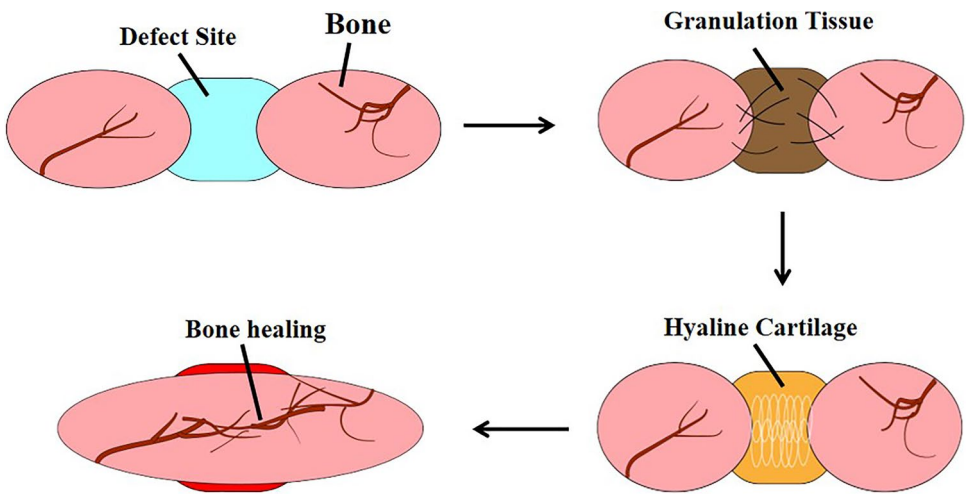
Abstract

Bone defects and fractures represent common health concerns, with bone repair posing a challenging physiological process. This reparative process is often complicated with the presence of bacterial toxins, inflammation, and oxidative stress. Furthermore, bone tissue, being highly metabolic, requires a substantial amount of nutrients during the healing process. These factors collectively contribute to the difficulty in spontaneous or timely bone tissue regeneration. Currently, the conventional approach to facilitate bone defect healing involves surgically implanting the patient's autologous tissue graft at the defect sites. However, this method necessitates surgical intervention, presents challenges in deformity correction, exhibits limited plasticity, and has constrained availability, thus increasing the likelihood of associated complications. Clinically, an ideal scaffolds should exhibit attributes such as cost-effectiveness, ease of preparation, minimal invasiveness, and compatibility with the surrounding bone tissue to facilitate nutrient transportation and the formation of blood vessels. This review critically examines the merits and demerits of the two most widely employed three-dimensional (3D) biomimetic scaffolds for bone tissue repairing. Furthermore, it delves into the fundamental prerequisites and prospective advancements of 3D biomimetic porous scaffolds, emphasizing their potential future development trends.

Graphical abstract

Schematic of the process of bone fracture healing process.



Keywords Bone defects · Three-dimensional biomimetic scaffolds · Bone tissue repairing

Extended author information available on the last page of the article

Abbreviations

3D	Three dimensional
ALP	Alkaline phosphatase
BG-XLS/GelMA-DFO	GelMA coated onto the surface of BG-XLS-DFO scaffold
BG-XLS-DFO	DFO was loaded on BG-XLS bracket
BMSCs	Bone mesenchymal stem cells
β-TCP	$Ca_3(PO_4)_2$ scaffold
BMD	Bone mineral density
CMS	Methacrylate gelatin freeze-gel microspheres
DFO	Deferoxamine
ECM	Extracellular matrix
GC	Glycol chitosan
GelMA	Gelatin methacrylamide
GMPT	Ti-6/Al-4/V porous scaffolds
GelMA	Gelatin methacrylamide
hMSCs	Human adipose-derived mesenchymal stem cells
hBMSCs	Human bone stenotic stromal cells
MeGC	Methacrylated chitosan
NC	Nanoclay
NoBS	NC-organic bone sealant
PGC	Phytochemical-conjugated glycol chitosan
SAG	Smoothened agonist
SAG-NC	SAG-loaded NC
TMSPMA	3-(Trimethoxysilyl) propyl methacrylate)

1 Introduction

Bone tumors, traumatic injuries, and congenital diseases give rise to significant health concerns by inflicting damage upon the skeletal system [1, 2]. The repair and regeneration of bone tissues are intricate processes encompassing various physiological responses, including acute inflammation, angiogenesis, recruitment of mesenchymal stem cells to foster cartilage callus formation and calcification, and ultimately goal is to promote bone tissue reconstruction (Fig. 1) [3, 4]. As a highly metabolically active tissue, bone necessitates an extensive vascular network to facilitate the transportation of osteoblasts and hematopoietic cells to defect sites [5]. These cells differentiate into bone and bone marrow, respectively, with local osteoblasts eventually maturing into functional bone-forming cells, thereby promoting bone healing. Of paramount importance within this complex network of physiological conditions is the synchronized development of nutrient-supplying vessels and mineralized bone, both being pivotal factors in bone tissue repairing [6]. Angiogenesis plays a crucial role in the process of new bone formation, not only for fulfilling the tissue's requirements for nutrients, waste elimination, and ion transport, but also for facilitating the migration of chondrocytes, inflammatory cells, and bone progenitor cells to the defect sites, thus

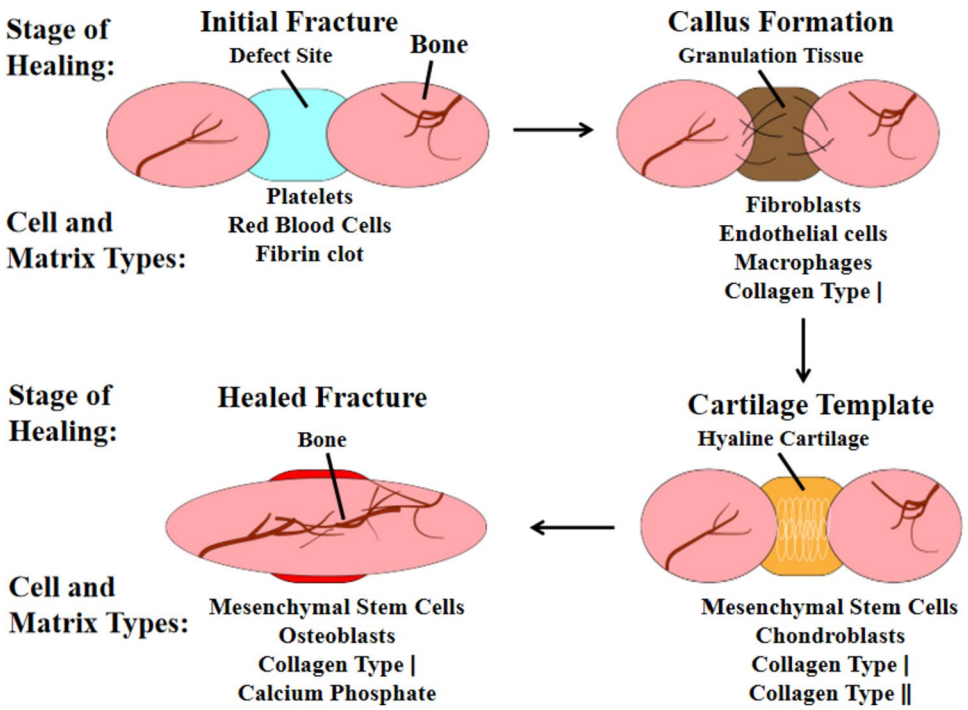


Fig. 1 Schematic of the process of bone fracture healing, showing the major cells and matrix types involved at each stage. Copyright 2013 Elsevier B.V

creating a conducive microenvironment for bone growth and remodeling [7]. However, when bone tissues are subjected to external insults, such as infection, trauma, tumor resection, or skeletal abnormalities, extensive surface damage can impede bone tissues' ability to unite or heal promptly [8, 9]. Moreover, the reparative process of bone tissues generates elevated levels of reactive oxygen species (ROS), resulting in an oxidative stress microenvironment [10, 11]. This, in turn, inhibits the osteogenic differentiation of mesenchymal cells, induces cellular damage, and promotes adipose tissue formation, ultimately hindering the formation of new bone tissue.

Currently, the promotion of bone defect healing involves the application of autografts, allografts, and xenografts [12]. However, these methods are associated with complications such as donor site morbidity, disease transmission, and immune rejection [13]. Consequently, the gold standard for enhancing the healing of substantial bone defects remains the utilization of autologous tissue grafts from patients [14]. These grafts encompass crucial cell types, stroma, and vascular systems essential for bone regeneration within the injured area [15]. Nonetheless, the practical application of autologous grafts faces significant challenges due to multiple interventions, limited plasticity, difficulties in correction, and unstable connections resulting from inaccurate positioning and a scarcity of bone donors [16]. These hindrances severely limit their practicality. Obtaining allografts from genetically identical species offers a solution to the donor shortage issue, however, these grafts are susceptible to immune rejection, and their long-term use heightens the risk of disease transmission and associated complications [17]. Consequently, addressing large bone defects remains a central challenge in clinical practice. In the pursuit of more effective bone tissue repair, researchers have introduced an innovative approach involving the implantation of bone grafting devices into defect sites through surgical means [18]. Nevertheless, the bioactivity and mechanical properties of these graft devices often prove unsatisfactory, potentially impeding effective bone tissue healing. Additionally, surgical interventions, while promising, are frequently accompanied by significant trauma and necessitate repeated operations, imposing substantial economic and psychological burdens upon patients [19, 20]. Consequently, the establishment of a stable and perfused blood vessel network emerges as a necessary step to enhance the potential for bone regeneration and achieve robust bone repair capabilities.

In the context of the rapidly advancing field of biomedicine, there has been a notable proliferation of bone tissue repair scaffolds constructed from biological materials [21]. These biomimetic scaffolds, when judiciously introduced into defect tissues with minimal invasiveness, exhibiting a multifaceted capacity. They not only aptly emulate the intricate tissue structure, enabling the sustained release of therapeutic agents, but also offer a conducive milieu for cellular adhesion, fostering an environment conducive to cellular growth. Furthermore, they furnish vital pathways for nutritional support, thereby facilitating tissue growth, differentiation, nutrient transport, and metabolic processes [22, 23]. Consequently, biomaterials-based bionic scaffolds playing a pivotal and integral role in the realm of bone tissue regeneration engineering. However, a common challenge associated with most biomaterials-based scaffolds lies in their relatively low mechanical strength, predisposing them to fragmentation upon implantation into defect areas. This fragility can lead to the premature release of therapeutic agents, and the persistence of non-degraded fragments within the tissues [24, 25]. Therefore, an ideal three-dimensional (3D) bionic scaffolds should possess a set of paramount attributes: (1) the ability to maintain the bioactivity of drugs and biomolecules, (2) the capacity for sustained and on-demand drug release, (3) minimal invasiveness, superior biocompatibility, controlled biodegradability, appropriate mechanical integrity, and support for cellular adhesion and the induction of seed cells, and (4) the ability to seamlessly integrate with the surrounding tissue of the bone defect, thus fostering a coordinated inter-organizational development that, in turn, promotes the regeneration and repair of bone tissues [26, 27]. This comprehensive review aims to delve into the applications of select multifunctional 3D bionic scaffolds possessing outstanding mechanical attributes, antibacterial properties, osteogenic potential, and angiogenic capabilities in the domain of bone tissue repair.

2 Hydrogel-assisted bone tissue repairing

Hydrogel, recognized as exemplary 3D scaffolds, offers distinct advantages such as minimal invasiveness, pliable morphological characteristics, and an interconnected pore network. These attributes playing a pivotal role in facilitating intercellular communication while enhancing the diffusion of oxygen, nutrients, and the excretion of metabolites. Consequently, hydrogel found extensive utility in diverse domains encompassing wound healing, tissue repair, and beyond [28]. However, a notable limitation of hydrogel lies in their relatively modest mechanical properties, rendering them ill-suited to withstand the high-frequency mechanical stresses associated with bone tissue. This shortfall, in turn, restricts their applicability in the context of bone tissue repair [29]. In recent years, researchers have sought to address this limitation by introducing a variety of organic and inorganic materials as crosslinking agents or additives into hydrogel-based 3D scaffolds, thereby enhancing their mechanical robustness and physical attributes [30]. Among the range of organic and inorganic materials explored, nanoclay (NC) emerges as a noteworthy option. As both

a physical crosslinking agent and a filler within polymer matrices, NC not only serves to promote the adhesion, proliferation, and differentiation of osteoblasts following implantation into bone tissue defect sites, but also contributes to the augmentation of bone density and strength [31]. Furthermore, the degradation products of NC provide essential support and protection for bone formation and regeneration, extending their beneficial influence to the renewal and remodeling of bone tissue, as well as potentially other tissues and organs within the body [32]. Inspired by these unparalleled properties, Lee and his staffs utilized nanoclay (LAPONITE; A trademark of BYK Additives Ltd.) as the basic substrate to construct NC-organic hydrogel bone sealant (NoBS) with caffeic acid via physical and chemical crosslinking [33]. NC are composed with naturally derived polymers and various synthetic and has larger surface areas, which can lead smoothened agonist (SAG) intercalated into the inner space of the NC (SAG-NC) via cation exchange between the cationic form of SAG and the Na^+ ion within NC [34, 35]. Caffeic acid has antibacterial, antioxidant and anti-inflammatory properties that can effectively relieve the inflammatory response during the bone healing process [36]. Through the carbodiimide chemical reaction between the amino group of ethylene glycol chitosan (GC) and the carboxyl group of caffeic acid, it was successfully grafted to GC, and the caffeic acid grafted chitosan (PGC) was obtained. NoBS hydrogel was obtained by the self-assembly by PGC and NC through multiple noncovalent and covalent interactions (Fig. 2) [37]. The obtained porous NoBS hydrogel system can fill the irregular bone defect area with minimal invasion model, and effectively support cells adhesion, proliferation and differentiation in tissue engineering. Moreover, the positively charged protonated amino groups of GC and the hydroxyl groups of caffeine acid can form a barrier on the surface of bacterial that can prevent nutrients entry into the bacterial and leads the destruction of the bacterial [38]. After NoBS hydrogel was treated with mouse skull defect model for 8 weeks, the new bone was regenerated in the original defect areas. Compared with NoBS without NC and SAG, the bone healing areas of NoBS with SAG can be significantly increased, which was attributed to the fact that the released SAG can activate hedgehog signal and regulate the Wnt/β-catenin pathway, thus promoting bone regeneration and robust bone repairing [39]. Above results indicated that this NC-inspired multifunctional NoBS hydrogel provided favorable microenvironment for cells survival, proliferation and differentiation and their superior mechanical property can meet the highly frequency movement of bone tissue during bone repairing process.

The fundamental requirement for bone healing is to facilitate the optimal aggregation of bone healing-related cells within the bone defect sites [40]. Key bone cells, including bone progenitor cells, osteoblasts, osteoclasts, and osteocytes, playing a pivotal role in repairing and maintaining the functionality of bone tissue. This is primarily attributed to their role as mechanical sensing cells in bone defect repair, capable of producing and secreting pertinent proteins and forming a new extracellular matrix, consequently regulating

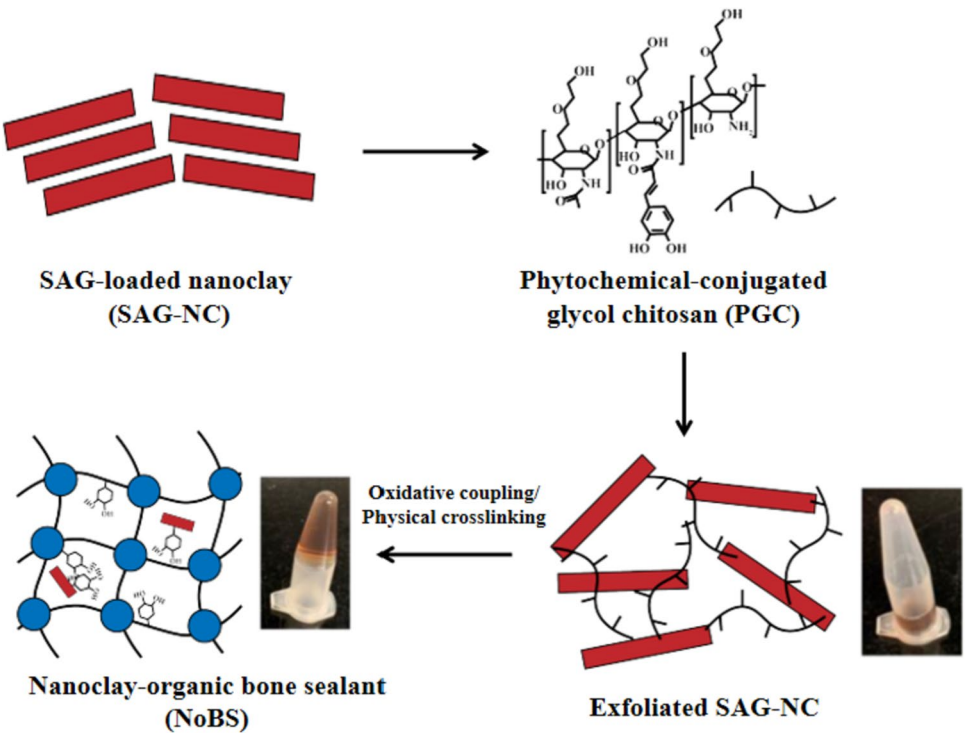


Fig. 2 Preparation of SAG-NC via intercalation of SAG between NC layers. Exfoliation of SAG-NC by electrostatic interactions between PGC and SAG-NC. The mixture of PGC and SAG-NC was cross-linking to develop the NoBS via oxidative coupling and physical cross-linking upon the addition of oxidizer. Copyright 2020 Wiley–VCH GmbH

the functions of local osteoblasts and osteoclasts [41]. Osteoblasts are recruited to the fracture site during bone mineralization, obtaining the appropriate geometry necessary to fill the defect and secreting non-collagenous proteins like osteocalcin, osteopontin, and osteopontin. These proteins effectively promote quicker, more efficient, and more thorough healing of challenging bone defects [42]. Thus, cells-based therapy assumes a crucial role in preserving bone function, especially in the context of bone growth, remodeling, and repairing. However, a prerequisite for cells-based bone tissue healing is the effective delivery of cells [43]. Currently, the primary method of delivering cells to damaged tissues relies on direct or intravenous injection, potentially resulting in a low cells retention rate within the damaged area. This, in turn, diminishes the long-term efficacy of cells. Furthermore, frequent injections may mechanically damage the cells, consequently further impeding bone tissue healing and undermining the positive therapeutic effects [44]. Recently, diverse biomaterial-assisted cells delivery system have emerged to address these challenges [45]. Injectable hydrogel, among them, exhibit the ability to accommodate complex tissue structures. Their 3D network structure promotes the diffusion of oxygen, proteins, and nutrients, fostering favorable cell behavior and enhancing the retention and survival rates of cells within tissues [46]. Moreover, encapsulated cells within these hydrogel are effectively shielded, preserving the activity and biological properties of the cells during freezing storage and injection [47].

As mentioned previously, a 3D hydrogel-based drug delivery system offers not only enhanced cells survival and precise drug delivery but also the potential for sustained drug effects. This, in turn, leads to accelerated and more robust blood vessel and bone tissue formation. In view of this, Mooney and his staffs developed an environment-friendly methacrylate-alginate porous frozen gel cells carrier, which significantly promoting cells implantation, reduced cells damage, enhanced the retention of cells at the diseased site, and had high mechanical stability and shape memory characteristics, thus achieving a breakthrough in injectable hydrogel cells delivery system [48]. Cryogel, as a subclass of hydrogel, has porous sponge-like scaffolds, which is prepared by hydrogel precursor solution at sub-zero temperature [49]. Inspired by these incomparable advantages, Yuan et al. developed biocompatible and injectable methacrylate gelatin freeze-gel microspheres (CMS) by adopting the emulsification technology of gradient cooling cryogelation (Fig. 3) [50]. Different from the traditional established methacrylate-alginate porous frozen gel, CMS contains the targeting sequences of matrix metalloproteinases, which is very similar to the natural ECM, so as to effectively promote cells proliferation and spread. Interesting, the size of CMS can be controlled by freezing time, which was attributed to that longer freezing time can lead to excessive growth of ice crystals, but prolonging freezing time can lead to uneven distribution of micropores and high pore/particle ratio on CMS surface [51]. The porous structure of CMS can promote cells adhesion, provide more surface areas for cells proliferation, have a 7-day high stem cells levels, and reduce cells collision and pressures during the injection. In addition, CMS can promote the expression of type 1 collagen and alkaline phosphatase in human bone stenotic stromal cells (hBMSCs), and provide nucleation site for mineral deposition. The biomineralization process can promote the bone induction differentiation of hBMSCs by promoting the production of calcium and other bioactive ions from bone induction media. Therefore, CMS provides a favorable environment for cells transport in bone repairing. Generally speaking, compared with traditional cells delivery system, CMS shown stronger cells affinity without any surface modification, and its shape memory ability and adjustable pore size can enhance the retention of cells in the diseased sites, thus promoting tissue regeneration, and at the same time, it is minimally invasive. In the future, CMS cells delivery system can not only effectively improve the survival rates of cells, but also prevent cells spread into surrounding tissues thereby promote cells accumulation at diseased tissues. Hence, the cryogel transfer system holds significant promise in the realm of cells delivery for precision disease treatment.

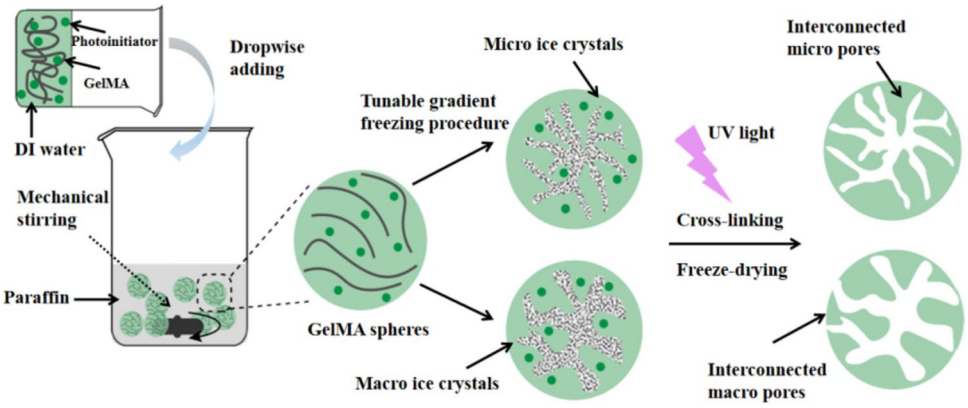


Fig. 3 Scheme of the fabrication of CMS via a combination of an emulsion technique with gradient-cooling cryogelation. Cells morphology observed under SEM for hBMSCs. Copyright 2021Wiley-VCH GmbH

Leveraging cells to assist in bone tissue healing has proven to be a valuable approach in enhancing the process; however, the in vitro proliferation capacity of these cells is inherently limited, thereby hindering the acquisition of a sufficient cells population for effective bone tissue regeneration. Moreover, the injection of allogeneic cells into the body often triggers host immune responses, albeit manageable through immunosuppressive drugs, which, regrettably, entail a multitude of unavoidable toxic side effects. Consequently, the cultivation of cells in vitro with low immunogenicity stands as a compelling strategy to advance cells therapy.

The 3D hydrogel network, serving as a bone tissue repair scaffolds, exhibits the capability to seamlessly incorporate diverse inorganic and organic components for bone tissue regeneration, offering significant advantages. Nonetheless, its intricate design, suboptimal mechanical characteristics, and limitations in large-scale production have, in turn, posed challenges to its clinical implementation.

3 Three-dimensional mineral scaffolds-assisted bone tissue repairing

The precarious conditions surrounding defective bone tissue, coupled with insufficient nutrient supply, pose considerable challenges, often leading to non-unions or delayed unions [52]. Consequently, establishing a robust vascular network serves not only to facilitate the migration of crucial elements, such as proteins, vitamins, and calcium, essential for bone tissue regeneration, but also to efficiently eliminate metabolic by-products produced during the intricate process of bone tissue repair or reconstruction [53]. Moreover, vascularizing bone tissue contributes to upholding the stability of the bone microenvironment, thereby fostering the differentiation of bone marrow mesenchymal stem cells and creating an optimal milieu for bone remodeling [54]. In recent years, the rapid advancements in bone tissue engineering have spurred the emergence of a myriad of bone tissue repair materials. Among these, 3D mineral scaffolds material have garnered attention for their ability to provide a conducive microenvironment for cellular growth, proliferation, and the formation of blood vessels [55]. Leveraging the analogous hardness and interconnected pore structure shared by the 3D mineral scaffolds and natural bone tissue, these materials effectively facilitate the formation of new blood vessels, establishing innate conditions for successful bone tissue healing [56].

At present, a variety of 3D mineral scaffolds based on metals, ceramics and bioactive glass are widely applied in clinical practice because of their accurate internal structure and personalized customization that integrates internal blood vessels growth, osteogenesis-inductive activity, and recruiting bone marrow mesenchymal to promote bone tissues repairing as the goal [57]. However, these 3D mineral scaffolds required permanent implantation, and long-term use can easily cause 3D mineral scaffolds fatigue [58]. To prolong the service life of these 3D mineral scaffolds, promote bone resorption, and enhance bone bonding performance, Ma and his colleagues provided Ti-6/Al-4/V porous scaffolds (GMPT) infiltrated with "soft" gelatin methacrylamide (GelMA) hydrogel matrix, which can simulate the extracellular matrix (ECM) of bone to promote the interaction with osteoblast lineage and osteoclasts, which is based on the heterogeneous microstructure and mechanical properties of bone tissues (Fig. 4a) [59]. 3-(Trimethoxysilyl) propyl methacrylate) (TMSPMA) as a linker can obviously improve the mechanical stability of GMPT scaffolds compared with physical cross-linking alone. Multiporous structure of GMPT not only can promote cells adhesion, proliferation and differentiation and improve the expression of osteogenic-related genes, but also can improve ALP (early osteogenic marker) expression and ECM mineralization (a marker of osteogenic differentiation in the late), to promote bone tissues integration. GMPT scaffolds have demonstrated a notable capacity to enhance the development of additional vascular segments and to induce a heightened expression

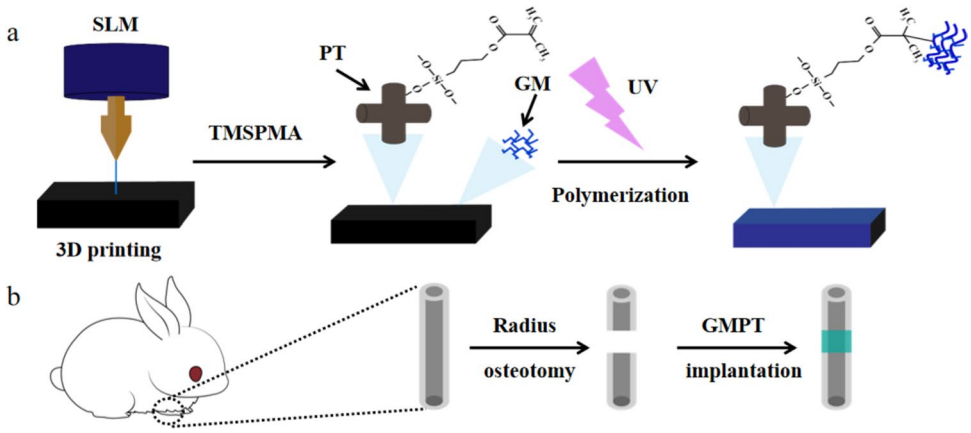


Fig. 4 **a** Schematic illustration of the fabrication of GMPT. TMSPMA was used as a linker to immobilize GelMA hydrogel onto the surface of 3D printed PT, thus generating hard-soft hybrid 3D scaffolds. **b** Schematic illustration of GMPT in vivo implantation. Copyright 2021 Elsevier B.V

of genes related to antigenicity within bone tissue defect locations. These findings align with prior studies that have affirmed GelMA's ability to stimulate antigenic processes [60]. After GMPT scaffolds were implanted into the rabbit radius defect model, the new bone around the GMPT scaffolds increased, and the bone trabecula became thicker and more regular after 12 weeks treatment, which was ascribed to GelMA scaffolds have good surface hardness, that can promote cells adhesion, proliferation and differentiation, regulates the activity of metalloproteinases, and promotes osteogenesis and angiogenesis (Fig. 4b). By optimizing the GelMA concentration, 10% GelMA concentration in GMPT scaffolds can simulate the heterogeneous microstructure and mechanical property of bone tissues, and provide a favorable platform for cells attachment and differentiation. These results indicated that the hard metal implants with suitable bioactive stiffness surfaces can obtain better osseointegration results. Nonetheless, the extent to which the implantation of the metallic scaffolds can achieve optimal compatibility with the neighboring bone tissue remains a critical factor influencing the vascularization of the bone defect sites. Besides, the application of the hard metal implants into the body has the risk of metal poisoning, so its safety performance should be considered before clinical application.

The utilization of bioceramic layers for surface modification of 3D mineral scaffolds serves to address not only the concern of potential metal ion poisoning but also provides a superior compatibility with the surrounding bone defect tissue. This enhanced compatibility can be attributed to the fact that bioceramics possess a Ca/P ratio analogous to that of human bone, in addition to displaying remarkable mechanical properties and biocompatibility. As a result, they effectively facilitate the rejuvenation and remodeling of the microenvironment within the bone tissue. This phenomenon has been well-documented [61]. However, how to construct a bioceramic scaffolds that meets osteo-inductive activity, internal growth of blood vessels, and recruiting bone marrow mesenchymal stem cells at the same time is still the current major challenge. To solve the above problems, Han and his collaborators were inspired by the unique biological structure of the “Lotus seedpod” with the concept of internal vascularization. Injectable hydrogel microspheres loaded with DFO-liposome were used as “lotus seeds” and directly injected into the 3D scaffolds to manufacture the internally vascularized “lotus seed” bioceramic 3D printing scaffolds (TGL) to promote the repair and neovascularization of defective bone (Fig. 5a) [62]. The obtained TGL scaffolds have the adequate micropore diameter and porosity, and their porous structure was evenly filled with DFO-liposome loaded injectable hydrogel microspheres (Fig. 5b). TGL drugs delivery system not only has superior biocompatibility to promote bone mesenchymal stem cells (BMSCs) adhesion, but also can improve the expression of HIF 1a and VEGF to induce blood vessels rapidly formatted inside the TGL scaffolds. As we all know, in the process of osteoblast differentiation, the basic biological behaviors of bone matrix synthesis, secretion and mineralized nodule maturation are considered as the signs of osteoblast differentiation and maturation [63]. Besides, TGL scaffolds can also promote the mineralization properties of rat BMSCs at OIC medium. After TGL scaffolds were implanted into the femoral defect model of rats in vivo, it was completely consistent with the postoperative femoral ring defect. Furthermore, an increasing proportion of newly formed bone displayed a progressive healing pattern, commencing from the periphery and advancing towards the central region, as

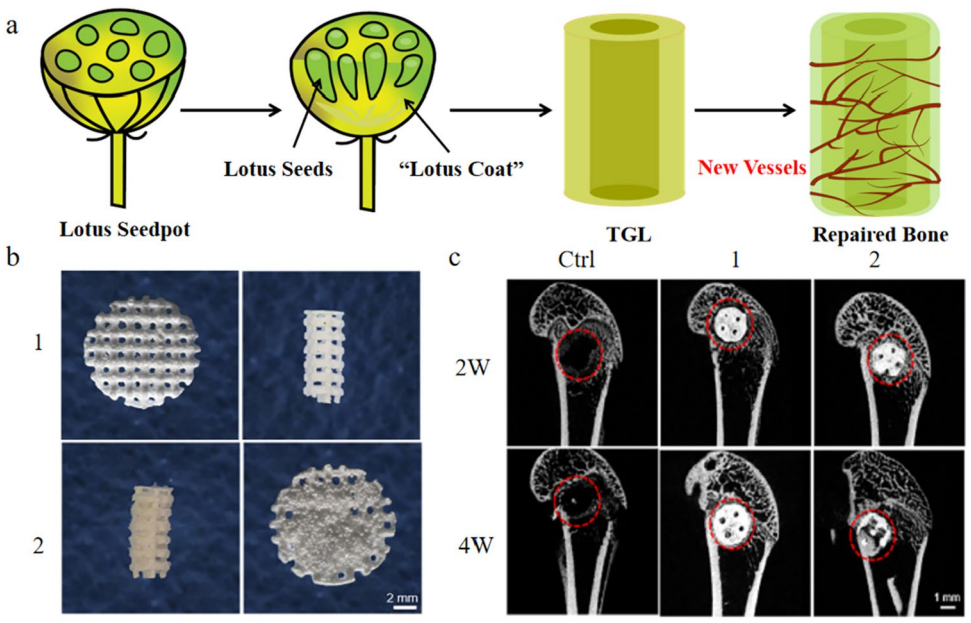


Fig. 5 **a** The unique biological structure of lotus seedpod and inspirational. **b** Digital camera photos of $Ca_3(PO_4)_2$ scaffold (β-TCP) and TGL scaffolds. **c** Micro-CT images of bone defect. The red circle is the site of bone defect constructed by an electric drill. 1: β-TCP scaffold, 2: TGL scaffold, Ctrl: control. Copyright 2020 Elsevier B.V

well as several regular channels formed between the newly bone tissues, which indicated that TGL scaffolds provided a positive support for bone tissues repairing via its interconnected channels (Fig. 5c). Therefore, the combination of 3D TGL scaffolds and DFO can not only effectively increase the blood vessel density and bone volume, but also promote the exchange of nutrient and oxygen at the fracture sites, and make cells evenly distributed and migrated. And this drug delivery system with "Lotus seedpod" structure can effectively control the on-demand release and local drugs concentration of DFO, and keep its biological activity, so as to construct a suitable microenvironment for promoting internal blood vessels regeneration and provide a suitable Ca/P ratio in bone tissue repair.

Bioglass (BG), as another 3D mineral scaffolds, has excellent biological activity, good bonding ability with living bone tissues and bone conductivity, and plays an important role in bone tissues repairing [64]. However, to solve the problem of poor bone induction, brittleness and low fracture toughness of BG in the process of bone tissue repair. Zheng and his staffs introduced NC into BG (BG-XLS) to improve its mechanical property, osteogenic differentiation and alkaline phosphatase (ALP) expression of human adipose-derived mesenchymal stem cells (hMSCs) [65]. As shown in Fig. 6a, the obtained BG-XLS scaffolds have macroporous and microporous structure, which can effectively increase its surface area, and is conducive to cells adhesion, proliferation, differentiation and angiogenesis. It should be noted that the compressive modulus increases with the increase of XLS concentration, but the porosity of scaffolds decreases, and the BG-XLS scaffolds becomes contracted. From the point of pore size, morphology and mechanical property, XLS with concentrations of about 1% and 3% was selected as the appropriate dosage for bone tissues repairing. As hypoxia simulator and iron generator, deferoxamine (DFO) can promote the expression of vascular endothelial growth factor and vascular endothelial growth factor in human umbilical vein endothelial cells, thus activating endogenous angiogenesis and promoting new bone formation [66]. DFO was loaded on BG-XLS bracket (BG-XLS-DFO) by simple physical absorption. However, DFO loading gelation nanofibrous can inhibit the osteogenic differentiation of hMSCs in vitro cells assays as previously reported [65]. The compressive modulus of gelatin methacrylamide (GelMA) is close to the appropriate rigidity of osteogenesis, and it can simulate the ECM of bone to help BG-XLS-DFO scaffolds interact with osteoclasts and osteoblast lineage cells [67]. Thus, GelMA coated onto the surface of BG-XLS-DFO scaffold (BG-XLS/GelMA-DFO) can promote ECM mineralization for osteogenesis, and prolong the DFO sustained release, reduce DFO toxicity, protect DFO degraded. Figure 6b shown, BG-XLS/GelMA-DFO scaffolds were implanted into skull defect of Sprague Dawlye rats. 3D micro-CT results showed that all groups had new bone formation, but the new bone mass in BG-XLS Gelma-DFOND BGBG-XLS-BMP (positive group) treatment group was higher than that in BG-XLS treatment group. Therefore, the NC-based mineral scaffolds can not only improve the stiffness of biomaterials, but also

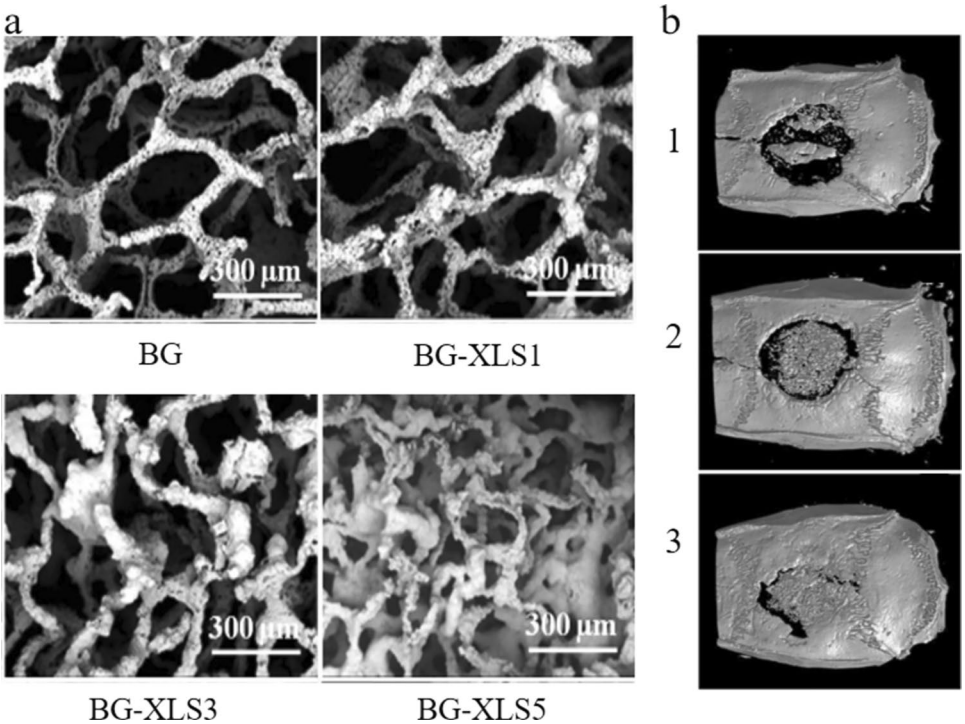


Fig. 6 Scanning electron microscope images of **a** BG scaffolds, BG-XLS1 scaffolds, BG-XLS3 scaffolds, BG-XLS5 scaffolds, certain amount of XLS (1 wt%, 3 wt%, 5 wt%). **b** Micro-CT images of (1) BG-XLS, (2) BG-XLS/GelMA-DFO, (3) BG-XLS-BMP2 scaffolds at 8 weeks post-operation. Copyright 2021Bioact Mater

improve the combination of biomaterials and bone tissue, thus enhancing the osseointegration. NC-based biomaterials exhibited unparalleled advantages during the bone tissue repairing process, but their tedious preparation process limited their application. Therefore, before clinical transformation, we need to simplify their preparation process.

While 3D mineral scaffolds have the capacity to precisely regulate the overall performance of biological materials, accurately replicate the porosity of cancellous bone, and facilitate bone tissue repairing, they have a significant drawback in that their implantation often results in substantial surgical wounds and a limited capacity for in vivo degradation. Consequently, this can lead to a decline in bone density at the defect sites and adversely impact tissue vascularization. This presents a substantial impediment to effective bone tissue remodeling and healing, potentially necessitating repeated surgical interventions in the later stages. Hence, it is imperative for researchers to devise an innovative 3D biomimetic scaffolds that can be implanted with minimal invasiveness into bone defects and seamlessly integrate with the surrounding tissue in these areas. This would significantly enhance cells aggregation and angiogenesis, ultimately achieving comprehensive bone healing, remodeling, and controlled degradation of the 3D biomimetic scaffolds.

4 Perspectives

Conditions associated with bone tissue injuries, such as tumors, fractures, and trauma, not only result in severe bone tissue defects but also impede the supply of blood, nutrients, and oxygen during the bone tissue healing process. Consequently, most bone defects struggle to undergo spontaneous healing. Currently, the primary clinical approach for treating bone defects involves the implantation of bone scaffolds. These scaffolds facilitate osteoblast differentiation, aiding in the formation of an extracellular bone matrix and promoting the development of new blood vessels. As such, neovascularization and the reconstruction of the bone matrix represent two pivotal elements in bone injury recovery. Nonetheless, the implantation of bone scaffolds is associated with the potential for substantial surgical wounds, which impose significant physical and psychological burdens on patients.

In the past decade, with the rapid development of biomedicine, various biomaterials have been widely developed and applied in bone tissue repairing. Importantly, these biomaterials exhibited distinct biological functions. Upon implantation at defect sites, they demonstrated exceptional biocompatibility and offer appropriate mechanical strength. These characteristics playing a crucial role in fostering osteoblast adhesion and differentiation, as well as creating a conducive environment for the formation of new blood vessels, ultimately facilitating bone healing. Among these biomaterials, 3D scaffolds closely resemble the extracellular matrix. They not only seamlessly conform to bone defect sites but also possess a 3D microstructure that facilitates tissue growth, differentiation, nutrient transport, and metabolism. Furthermore, they boast sufficient mechanical strength to support the movement of bone tissue. Additionally, different 3D biomimetic scaffolds exhibit varying biological properties that effectively modulate the body's biological performance, including the promotion of cells adhesion and angiogenesis, culminating in enhanced bone tissue repairing. Differing from the bone tissue repair scaffolds commonly used in clinical practice, the 3D biomimetic scaffolds presented in this paper offer distinct advantages, including reduced invasiveness, cost-effectiveness, and highly favorable economic outcomes (Table 1). However, most single-function 3D bionic scaffolds have limited physiological regulation characteristics, which limit the tissue repair, immune regulation and metabolic regulation, thus affecting bone tissue healing. Therefore, integrating different biomaterials or cytokines with different functions into the 3D bionic scaffolds can achieve “integration” functions, such as enhancing cells adhesion, proliferation and differentiation, reducing the immune response and promoting angiogenesis. In addition, the designed multifunctional 3D bionic scaffolds have a porous structure, which can promote cells communication

Table 1 The advantages and disadvantages of hydrogel-based 3D scaffolds and 3D mineral scaffolds presented in this review

3D biomimetic scaffolds	Advantages	Disadvantages
Hydrogel-based 3D scaffolds	Minimal invasion Pliable morphological characteristics An interconnected pore network can support cells adhesion, proliferation and differentiation	Intricate design Suboptimal mechanical characteristics Limitations in large-scale production
3D mineral scaffolds	Accurate internal structure Excellent mechanical properties Its structurally similar to the extracellular mechanism	There is a risk of metal ion poisoning Poor bone induction Complex preparation process It is easy to cause large wounds in the process of transplantation

and physiological response of the body, and promote the repair of bone tissue.

Currently, 3D biomimetic scaffolds, engineered from diverse biomaterials, offer varying biological functionalities. Nonetheless, the enhancement of osteoblast adhesion to these 3D biomimetic scaffolds primarily relies on surface modifications or the incorporation of cytokines. These strategies are aimed at fostering efficient bone defect healing. However, the practical and economic aspects bring into focus several challenges that hinder the successful transition of these scaffolds from experimental stages to clinical application. These challenges encompass intricate design, labor-intensive manufacturing processes, difficulties in scaling up production, and cost-effectiveness considerations. Therefore, the overarching objective for future research is the development of 3D bionic scaffolds characterized by stable performance, ease of accessibility, simple preparation, cost-effectiveness, and a structural and functional resemblance to human bone. These advancements are essential to achieve superior biocompatibility, controlled degradation, and the ability to facilitate osteoblast adhesion, ultimately contributing to the promotion of bone tissue healing.

5 Conclusion

In summary, this paper has presented an array of 3D biomimetic scaffolds designed to enhance cellular diffusion, facilitate nutrient transportation, and expedite the clearance of metabolic byproducts. These scaffolds playing a crucial role in orchestrating the simultaneous advancement of bone mineralization and neovascularization, showcasing unparalleled advantages within the realm of bone tissue repair. Notably, these 3D biomimetic scaffolds exhibit exceptional attributes, including high mechanical strength, wear resistance, fatigue resistance, deformation resistance, and favorable biological inertia. Furthermore, these scaffolds, functioning as local drug delivery systems, possess the capability to integrate various drugs. This feature offers benefits such as minimally invasive administration, prolonged release duration, and effective mitigation of challenges related to low intrinsic osteogenic induction ability and insufficient angiogenic potential. Additionally, leveraging these scaffolds helps in extending service life and reducing the incidence of associated complications. Hence, 3D biomimetic scaffolds have demonstrated their potential to drive bone tissue repair by leveraging both mechanical and biological properties. Furthermore, they provided a promising avenue for future development, aiming to enhance biocompatibility and responsiveness to human physiological needs in 3D biomimetic scaffold design.

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Availability of data and material The data used in this paper have been marked under the figure, and corresponding links are provided if necessary.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethical approval and consent to participate All animal experiments were performed according to the protocol approved by the Institutional Animal Care and Use Committee of Nantong University.

Consent for publication All authors proved.

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