# 3D Hydrogel/ Bioactive Glass Scaffolds in bone Tissue Engineering: Status and Future Opportunities

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## Abstract

Repairing significant bone defects remains a critical challenge, raising the clinical demand to design novel bone biomaterials that incorporate osteogenic and angiogenic properties to support the regeneration of vascularized bone. Bioactive glass scaffolds can stimulate angiogenesis and osteogenesis. In addition, natural or synthetic polymers exhibit structural similarity with extracellular matrix (ECM) components and have superior biocompatibility and biodegradability. Thus, there is a need to prepare composite scaffolds of hydrogels for vascularized bone, which incorporates bioactive glass to improve the mechanical properties and bioactivity of natural polymers. In addition, those composites' 3-dimensional (3D) form offers regenerative benefits such as direct doping of the scaffold with ions. This review presents a comprehensive discussion of composite scaffolds incorporated with BaG, focusing on their effects on osteo-inductivity and angiogenic properties. Moreover, the adaptation of the ion-doped hydrogel composite scaffold into a 3D scaffold for the generation of vascularized bone tissue is exposed. Finally, we highlight the future challenges of manufacturing such biomaterials.

***Key words:*** Bioactive glasses; ion-doped hydrogel scaffold; 3D scaffold; vascularized bone

## 1. Introduction

Treatment of significant bone defects resulting from trauma, infections, tumors, congenital malformations, or skeletal diseases represents a significant challenge for clinicians worldwide [2]. All bone grafts, either as autografts or allografts, are associated with common limitations, which mostly include limited availability of donors, morbidity at the donor site, disease transmission, and a high incidence of non-union healing [3, 4].

The bone matrix is comprised of organic (mainly collagen) and inorganic (mainly hydroxyapatite) components. Different natural and synthetic materials have been developed to prepare an ideal scaffold for bone repair; however, these materials have their shortcoming in inducing bio-mineralization, inadequate mechanical properties, and flexibility [3, 5]. Moreover, various natural and synthetic materials, such as metals [6], ceramics, and hydrogel, have been applied in bone tissue engineering [7]. However, the lack of rapid vascularization after implantation remains a critical hurdle in bone tissue engineering. Vascularization is needed to supply the implanted construct, providing oxygen and nutrients to osteoblasts and removing waste products [9]. In the case of significant bone defects, designing bone biomimetic materials, which incorporates both inorganic and organic components to simulate the typical structure and mechanical support of natural bone matrix, remains a critical challenge for complete repair [8].

Recent developments in bone tissue engineering have attempted to tackle this problem by designing materials that benefit bone components and structure [9]. In this context, efforts have been focused on incorporating mineral components such as bioactive glasses [1] into organic scaffolds [10, 11]. In the 1970s, Hench and coworkers fabricated the first bioactive glasses with the first composition that is known as 45S5 Bioglass® (BG), which was composed of four components 45 $SiO_2$–24.5 CaO–24.5 $Na_2O$–6 $P_2O_5$ (weight %) [10]. Bioactive glasses (BaG) being utilized for bone tissue engineering belong to a group of surface reactive amorphous materials identified with good biocompatibility, biodegradation properties, and the property of stimulating the osteogenic

differentiation of stem cells [11]. Modification of BaG could change the biological and chemical structure. In bone regeneration, bioactive glass can bind firmly to the bone tissue, consequently inducing angiogenesis and osteogenesis. However, due to the fast dissolution behavior and fragility of BaG, they are applied for coatings of implants and composite polymers [12, 13]. Relatedly, BG-based scaffolds were shown to have angiogenic properties that promote neo-vascularization and vascular ingrowth within the bone tissue [13-15], fulfilling the osteogenic and angiogenic properties required for bone repair [14].

Indeed, the result of adding inorganic ions dissolved from S53P4 bioactive glass into cell. In addition, silicate or phosphate-based BGs, are inorganic bioactive biomaterials that have been used as scaffolds for bone tissue engineering [15]. These glasses have a high level of bioactivity, and biological fixation enables bonding with soft and hard tissues. BGs are composed of elements such as silica (Si), calcium (Ca), phosphorus (P), and sodium (Na) that presents naturally in the body. The surface of a Bioglass implant is mineralized to a carbonated hydroxyapatite layer when subjected to body fluids, thus induced differentiation of osteoblasts and deposit new bone [16]. Different metal ions such as strontium (Sr) [17], zinc (Zn) [18], fluorine (F)[19], magnesium [20] [21], tantalum (Ta)[22] silver (Ag), and boron (B) [20] have been doped within bioactive glasses to induce specific biological responses by changing intracellular ionic concentrations [1]. Recently, Bioglass scaffold with doping potentially therapeutic elements such strontium and silicon has shown to enhance the ability to stimulate osteogenesis and induce angiogenesis [23]

Culture media promoted both endothelial and osteogenic processes, supporting the simultaneous formation of vascular-like structures and mineralization. Furthermore, it has been also indicated that the angiogenic effects of BaG, such an increased secretion of vascular endothelial growth factor (VEGF) in fibroblasts, endothelial cells proliferation and tubules formation [24]. Moreover, a model of 3D co-culture in the same medium indicated the formation of functional vessel-like structures characterized by the presence of an internal lumen [25-27],

4

showing that BaGs can induce vascularization and promote osteogenesis in co-culture systems [26]. Besides its osteogenic and angiogenic abilities in co-culture systems, combination of BaG with bioactive nanoparticles (NPs), or its incorporation with synthetic polymers enhanced the mechanical and biological properties of BaG composite scaffolds [3, 28]. For example, when BaG and NPs are combined for bone repair, they can induce osteogenesis by releasing active Si and Ca ions [29]. Ions such as Si, Ca, and Cu combined with BaG that improved osteogenic and angiogenic properties [30]. This review aims to highlight the recent progress in research on the use of BaGs to stimulate vascularization and osteogenic properties of bone-engineered constructs, particularly the application of BaGs in 3D co-culture hydrogel-based scaffolds for the induction of angiogenesis and osteogenesis.

## 2. Angiogenesis and osteogenic properties of bioactive glass

The mesoporous bioactive glass (Cu-MBG) has attracted enormous attention for bone regeneration. In comparison to traditional Bioglass, synthesized mesoporous Bioglass (MBG) with $CaO-P_2O_5-SiO_2$ system, has been suggested to exhibit superior osteogenic bioactivity, degradation and enhanced drug loading[32]. However, the angiogenesis property of MBGs seem to be not prominent, therefore requires combining of additional angiogenesis properties. It can be seen from various studies that incorporation of some ions, including Co, Rb greatly promot angiogenesis and osteogenesis capacities [33, 34]. He *et al.* investigated the effect of Rb doped MBG on proliferation of hBMSCs, angiogenesis, and osteogenesis, which was further characterized by the analyzing of the subsequent, genes of bone formation (alkaline phosphatase (ALP), COL-1), angiogenesis (VEGF, HIF-1α), and Wnt/β-catenin related-signaling pathway [33]. This study indicated that incorporating Rb could increase the expression of ALP and HIF-1α, the secretion of VEGF, and Coli hBMSCs through the Wnt/ß-catenin signal pathway. In this context, Rb-MBG was probably restrain GSK3β activity, which makes it possible for β-catenin to aggregate in cytoplasm, thus activated Wnt pathway [33]. Furthermore, the application of ions such as

5

$Cu^{2+}$ has been proposed as a potential strategy to enhance neovascularization, especially stimulating the proliferation of endothelial cells, and VEGF expression by stabilizing HIF-1a expression. Hypoxic condition is critical in cell recruitment, cell differentiation, and blood vessel formation, thus linking osteogenesis to angiogenesis.

Moreover, Hypoxia can be artificially mimicked by applying $Cu^{2+}$, which leads to stabilized HIF-1a expression [35]. In a study conducted by Wu et.al, the addition of $Cu^{2+}$ into MBG scaffolds significantly promoted hypoxia-like tissue reaction and consequently led to the coupling of angiogenesis and osteogenesis. This study indicates that Cu-MBG scaffolds could induce hypoxia-inducible factor [36]-1a and VEGF expression in human bone marrow stromal cells (hBMSCs)[34]. Moreover, boron-containing MBG (B-MBG) is an excellent scaffold with significantly enhanced osteogenic properties. Boron can facilitate the release of osteoinductive growth factors and cytokines. Chen et.al indicated that a composite scaffold composed of novel p(N-isopropylacrylamide-co-butyl methylacrylate) (PIB) nanogels combined with p(N-isopropylacrylamide-co-butyl methylacrylate) (PIB) nanogels, able to treat defects of irregular shapes as an injectable, thermoresponsive hydrogel that undergoes rapid thermal gelation once body temperature is reached [37].

## 3. Hydrogel -based Scaffold incorporated with BaG

Since bone consists of inorganic and organic components, e.g., calcium phosphate and collagen, bioactive inorganic materials and naturally-derived polymers are considered a suitable choice as the scaffolding matrices for bone tissue engineering (BTE) [37]. Natural polymers have attracted much attention in bone tissue engineering due to their specific properties, such as biocompatibility, biodegradability, and non-toxicity [3]. However, they exhibit low mechanical characteristics and limited bio-mineralization, which are critically needed for bone repair [5]. In this regard, fabrication of multifunctional composite scaffolds composed of hydrogel combined with inorganic BaG and encapsulated with cells and growth factors could enhance bone

6

regeneration by promoting osteogenesis and angiogenesis. In addition, BG dispersed in natural polymer scaffold were shown to improve mechanical properties, and osteogenic induction, and stimulate the production of growth factors, proliferation, and angiogenesis in bone tissue repair [38]., Previous studies have shown the multifunctional properties of scaffolds consisting of BG nanoparticles and alginate cross-linked with various cations in bone tissue engineering [39, 40]. Study has shown that BG incorporated in an oxidized alginate-gelatin hydrogel (ADA-GEL) had a great impact on the covalent crosslinking between ADA and GEL that was displayed by the higher degree of crosslinking and shorter gelation time of the fabricated hydrogels and further increasing mechanical properties of the resulted scaffold [41].

In addition to the benefits mentioned above of hydrogel/ BG composites, an injectable hydrogel has gel properties at physiological temperatures without requiring any chemical and environmental treatment. This allows the gel to fully repaired critical-size bone defects *in vivo* [42, 43]. Among injectable hydrogels, chitosan (CH)/ glycerophosphate [44] hydrogel with thermal sensitivity has been used in combination with other materials such as collagen, gelatin, silk fibroin, and BaG to obtain better performance of critical-size injured bone tissues [29, 45-47]. For example, Wu *et al.* fabricated an injectable nanocomposite hydrogel of (CH/ silk fibroin (SF)/ GP (CH/SF/ GP gel) incorporated with Cu-BG. This nanocomposite hydrogel exhibited control release of Si, Ca, and Cu ions when serving as a cell-free injectable scaffold at a critical-size calvarias bone defect in a rat model, supporting the neovascularization and full repair of the defective area.

Moreover, *in vitro* experiments of cell seeded-nanocomposite hydrogel scaffold showed apatite formation and upregulation of expression of angiogenic and osteogenic genes. In addition, Cu-BG/CH/SF/GP gel exhibited complete restoration of the bone defect with the formation of vascularized bone tissue and mineralized collagen deposition during 8 weeks without including any cells and growth factors [14]. Bioactive glass nanowhisker (BGnW) composed of 58% $SiO_2$,

33% CaO, and 9% P2O5 (based on mol%) displayed excellent binding to hard and soft tissues. Thus it could be combined with a hydrogel-based scaffold to increase osteogenic differentiation. Azizipour et.al. incorporated BGnW into 3D porous hydrogel nanocomposite scaffold, which consisted of gelatin-glutaraldehyde-collagen (Gel-Glu-Co). The result of the study showed the hydrophilic properties of Gel-Glu-Col/BGnW hydrogel scaffold, which increase the viability and proliferation of hMSCs seeded on the scaffold. In addition, MSCs cultured on the scaffold showed osteoblastic differentiation that upregulated the expression of type 1 collagen, Runx-2, and ALP and the protein expression of osteocalcin and osteopontin (Figure 1 a-d) [48].
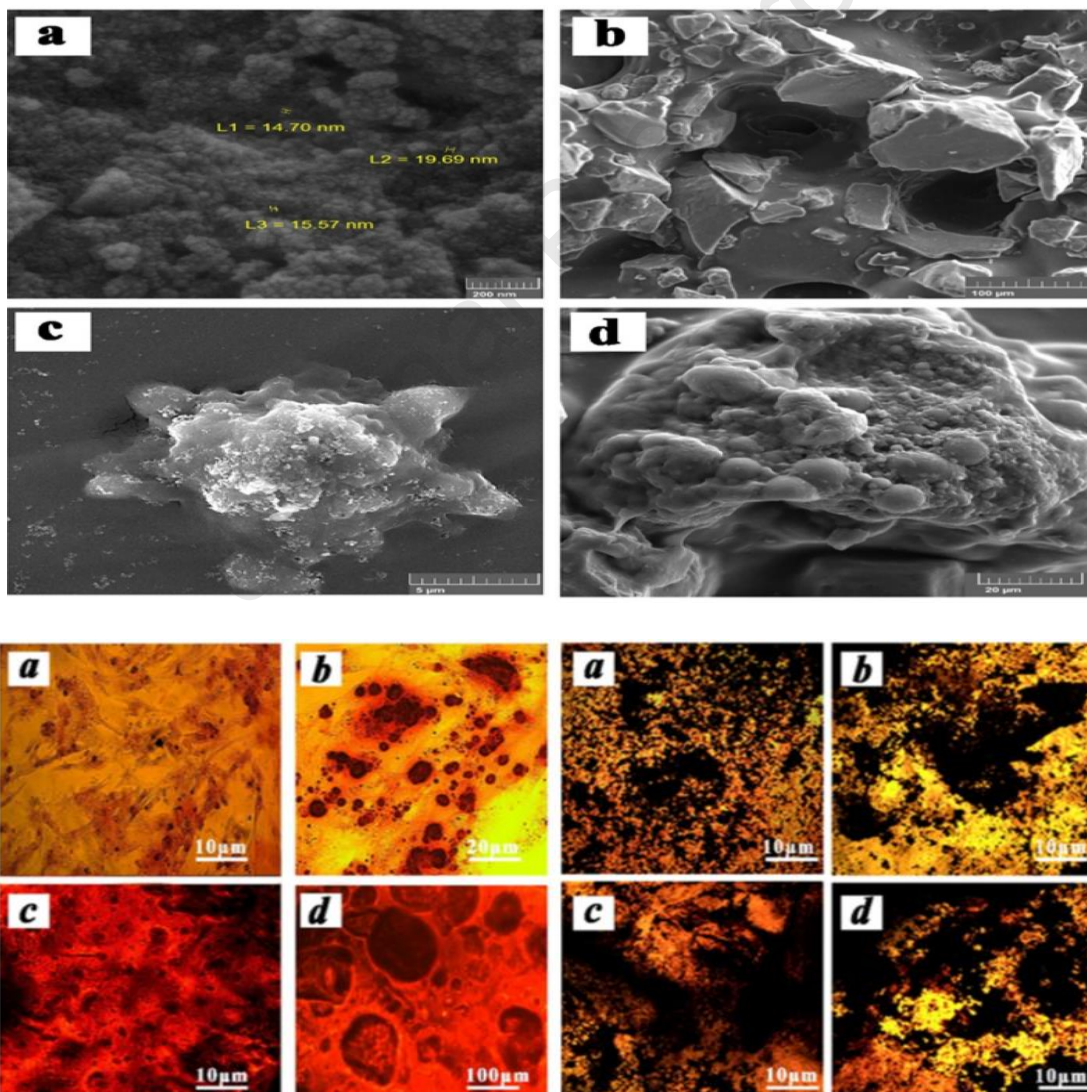
**(Figure 1. Top panel showed Scanning electron microscopy images. a) BGnW, b) Gel-Glu-Col/BGnW, c) Gel-Glu-Col (pure), d) Gel-Glu-Col/BGnW. Bottom panel showed Alizarin red staining to confirm the osteogenic differentiation of hMSCs. Cells were cultured in presence of osteogenic induction media for 14 days: TCPS (a), BGnW100 µg/ml (b), Gel-Glu-Col (c), Gel-Glu-Col/BGnW1% (d). b) von Kossa staining to confirm the osteogenic differentiation of hMSCs during 14 days clturing in presence of osteogenic induction media: TCPS (a), BGnW100 µg/ml (b), Gel-Glu-Col (c), Gel-Glu-Col/BGnW1% (d)).**

## 2.1 Proteins

### 2.1.1 Gelatin-BGs

Gelatin, a partial collagen derivative, has been used as a nanocomposite scaffold in combination with bioactive glasses. Embedding BG within gelatin increased its bioactivity and mechanical properties, facilitating their application for bone tissue engineering [49, 50]. Moreover, gelatin promotes cell attachment, improving tissue regeneration in vivo. Zare jalize et.al. fabricated strontium-delivering BGs in a $SiO_2$-CaO-SrO-$P_2O_5$ structure, in combination with gelatin as a composite scaffold. This composite scaffold increased exhibited enhanced compressive strength and elastic modulus. In addition,, this strontium-enriched BG improved neovascularization and enhanced osteoblast cell viability and differentiation [1]. Zhou *et al.* synthesized self-cross-linking hybrid gelatin /oxidized chondroitin sulfate (OCS) hydrogels that incorporated mesoporous bioactive glass nanoparticles (MBGNs) (**Figure 2**). The addition of MBGNs enhanced crosslinking and the gelation process.

Moreover, the storage modulus and compressive strength increased after including MBGNs. Further analyzing the biological activity of Gel-OCS/MBGN hydrogels revealed osteogenic differentiation of bone marrow mesenchymal stem cell (BMSCs) in vitro as well as effective bone regeneration in vivo compared with Gel-CS hydrogels without MBGNs (Figure 3 a-d) [51]. Apart from that, the polymer coating may reduce the bioactivity and osteogenesis activity of the scaffold presence of coating may reduce the bioactivity and osteogenesis activity of the scaffolds. To overcome this issue, the researcher incorporated BaGs in gelatin-coated scaffolds. In this context,

Zheng e.al; prepared a gelatin-coated scaffold with the addition of Cu-containing bioactive glass nanoparticles (Cu-BGN: $95SiO_2$-$2.5CaO$-$2.5CuO$, in mol %), as bioactive fillers. The Cu-BGN/gelatin nanocomposite-coated BGS revealed high bioactivity, appropriate mechanical properties, and osteogenic potential. In addition, the results showed that the cells could attach to the BG scaffolds and the incorporation of Cu-BGN in the coating did not significantly affect cell attachment (**Figure 3a-c**) [52].
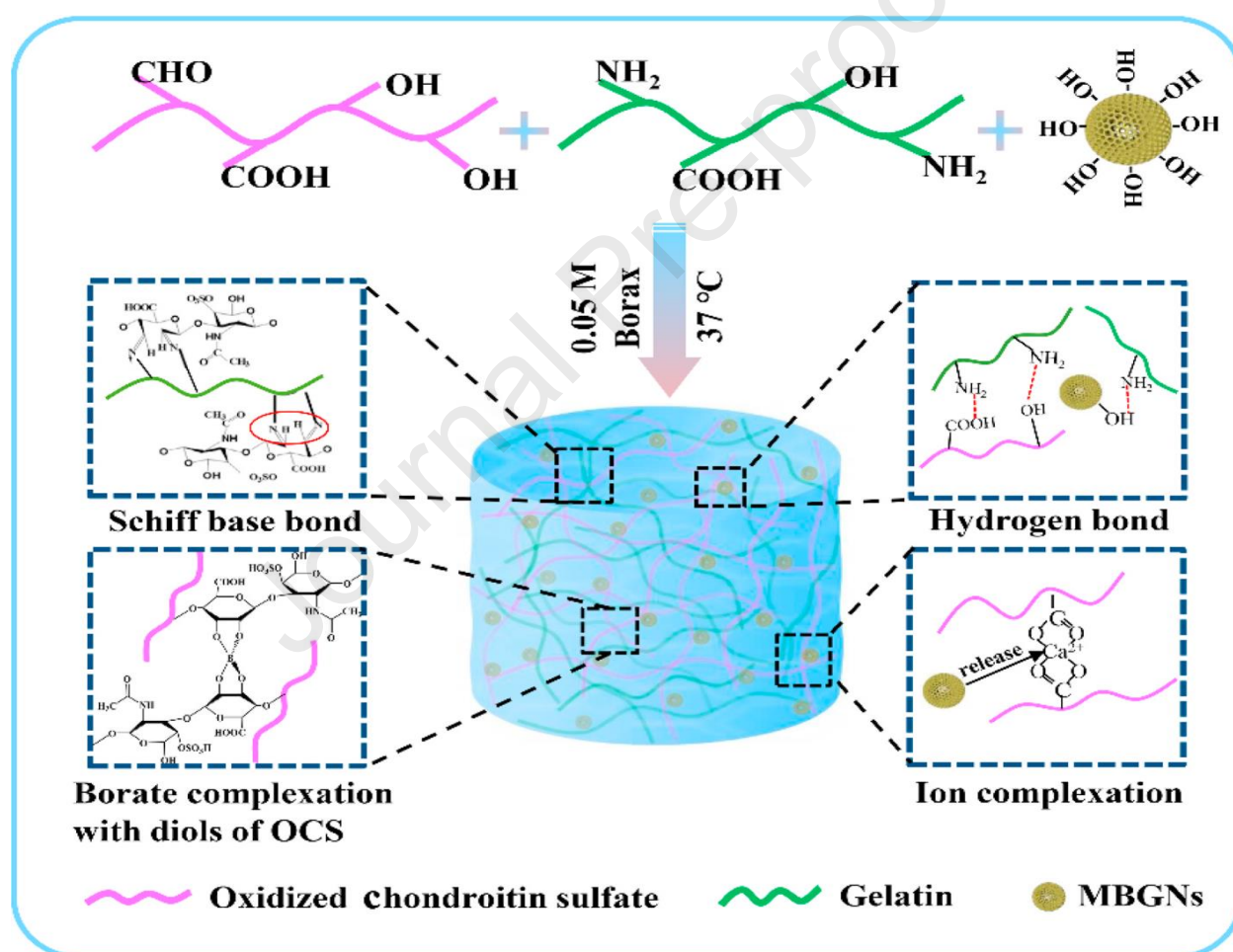


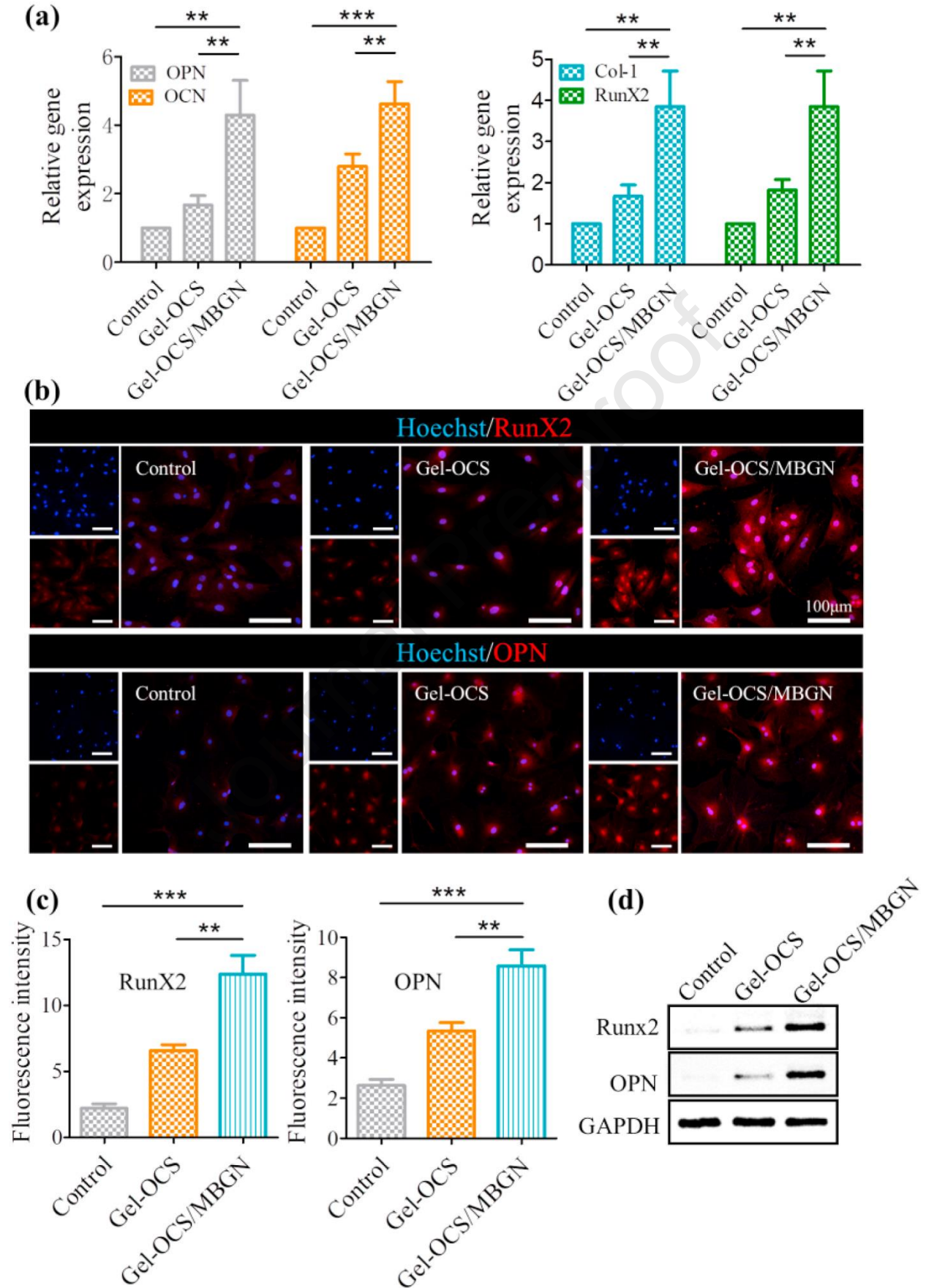(Figure 2. Schematic depicted mechanisms involved in the gelation of hybrid Gel-OCS/MBGN hydrogels)

(a)

(b)

(c)

(d)

**Figure 3.** Showed osteoblast-related gene and protein expressions of BMSCs cultured on the hydrogel surfaces on day 14. (a) RT-PCR analysis of osteogenic-related markers such as OPN, OCN, RunX2, and Col-1. (b) immunofluorescent images of osteogenic-associated proteins RunX2 and OPN in different groups. RunX2 and OPN are marked by red fluorescence, and the cell nuclei were stained blue by the Hoechst. (c) Quantitative analysis of RunX-2 and OPN fluorescence intensity. (d) Protein expression of RunX2 in BMSCs and GAPDH

## 2.2. Polysaccharides

### 2.2.1 Alginate-BGs

Alginate is a natural polysaccharide polymer widely used in foods, industry, and tissue engineering materials. It can be cross-linked in the presence of certain divalent cations, e.g., $Ca^{2+}$, $Sr^{2+}$, and $Ba^{2+}$, leading to the formation of a hydrogel [53]. Alginate with Ca2+ crosslinks has been used as a polymer matrix to compensate for the lack of Ca ions in Zn- and Mg-doped bioactive glasses. Although alginate is used in bone tissue engineering, it has the disadvantages of inadequate mechanical properties and the lack of bio-mineralization and flexibility, which are required for bone regeneration [4]. In this regard, Zamani *et al.* fabricated an alginate scaffold incorporated with BGs composed of Zn and Mg ions. This scaffold exhibited antibacterial effects, enhanced the mechanical properties and the bioactivity of the Alginate/BG composite. In addition, the antibacterial activity was related to the released Zn and Mg ions, restricting the growth of both *S. aureus* and *E. coli* [3].

Alginate lacks a cell-binding ligand, attributing it with inferior cell adhesion properties. Hence, alginate and a hydrogel improved vascular cell adhesion and proliferation *in vitro* [54]. In addition, alginate has an intrinsic non-degradable nature in mammalian tissues, because of the lack of alginase enzyme excreted from the body. The lack of mammalian enzymes that degrade alginate has its shortcoming. In this context, Alginate/gelatin scaffolds combined with BG showed immediate release at ~1 h by the process in which oxidation of alginate generates reactive

12

aldehyde groups in the backbone of alginate, allowing *in vivo* degradation of the hydrogel by making covalent bonds with ε-amino groups of lysine or hydroxylysine of gelatin [55, 56]. A study conducted by Rottensteiner-Brandl *et.al.* showed that the combination of gelatin and oxidized alginate (alginate dialdehyde, ADA) with BG had beneficial effects on cell survival and angiogenesis of bone marrow-derived MSCs encapsulated within the composite hydrogel. Further, *in vivo* implantation of the composite hydrogel revealed the recruitment of endothelial cells and a consequent increase in angiogenesis [55]. Bioglass/Alg composites are generated using sol-gel and freeze-drying, 3D printing [57], and surfactant foaming [58]. The addition of Zirconia (Zr2+) as one of the strongest nanoparticles in hard and soft tissue enhanced biocompatibility. Ramya *et al.* fabricated a freeze-dried nanosheet of a BG (45S5 Bioglass®)/Alg composite doped with Zr in the scaffold. The inclusion of Zr in the hybrid hydrogel promoted the growth rate of spheroid when scaffold was co-cultured with HDF and KB-3-1 cell lines by increasing surface roughness and changing porosity. This study concluded that alg-BG/alg-nBG-Zr could be used as hemostats, soft and hard tissue grafts, and a composite scaffold for organotyping [59].

### 2.2.2 Dextran-BG

Dextran, a hydrophilic carbohydrate biopolymer showed good degradation properties in certain physical environments without any effect on the cell viability. Cells proliferated in clumps on dextran rather than spreading, thus inorganic materials such as hydroxyapatite has been incorporated with dextran to increase the bioactivity and mechanical properties [60]. Bioactive glass ceramics (BGC) containing $SiO_2–CaO–P_2O_5$ networks have drawn much attention among inorganic materials due in part to their biocompatibility, bioactivity and osteoconductive properties, which bonds to both hard and soft tissues through the formation of surface hydroxy carbonate apatite layer. Nikpour *et al.* synthesized Cross-linked dextran hydrogels (CDH)-bioactive glass ceramic nanoparticles (nBGC) for bone tissue engineering (Figure 4 a-c). The concentration of nBGC nanoparticles affects their distribution in the composite scaffold in which

13

nBGC at low contents (2 wt%) is dispersed homogenously within the Dex matrix. However, nBGC nanoparticles at higher concentrations revealed agglomeration and increased water uptake. The composite scaffold supported the growth and improved ALP[61] activity of human osteoblasts (HOBs) at a concentration up to 16 (wt%) over two weeks (Figure 5 a-c) [62].
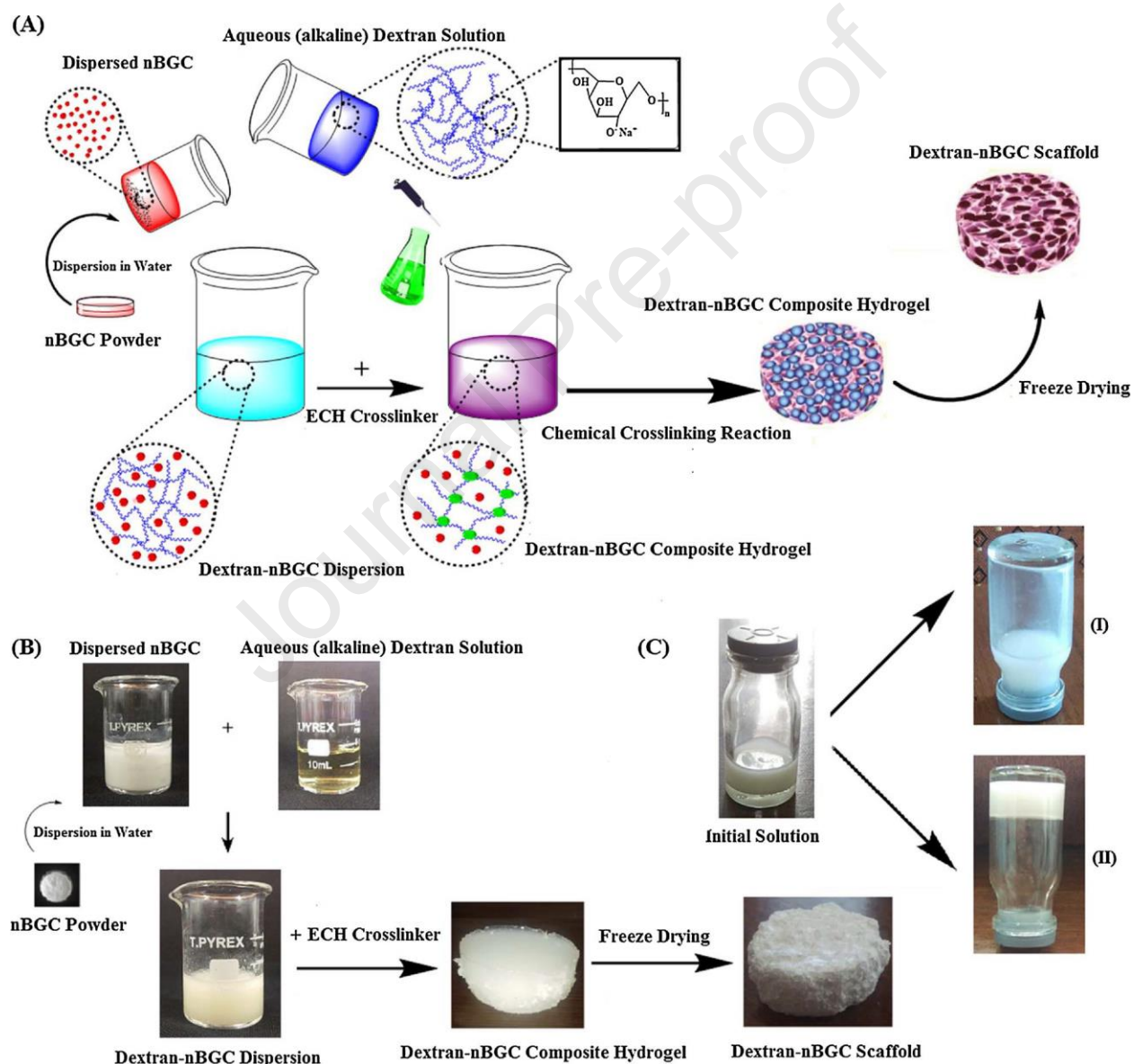


Figure 4. Schematic illustration of synthesized composite scaffolds (A), fabrication process of CDH-nBGC composite scaffolds (B) CDH-nBGC composite hydrogels before (I) and after (II) gelation
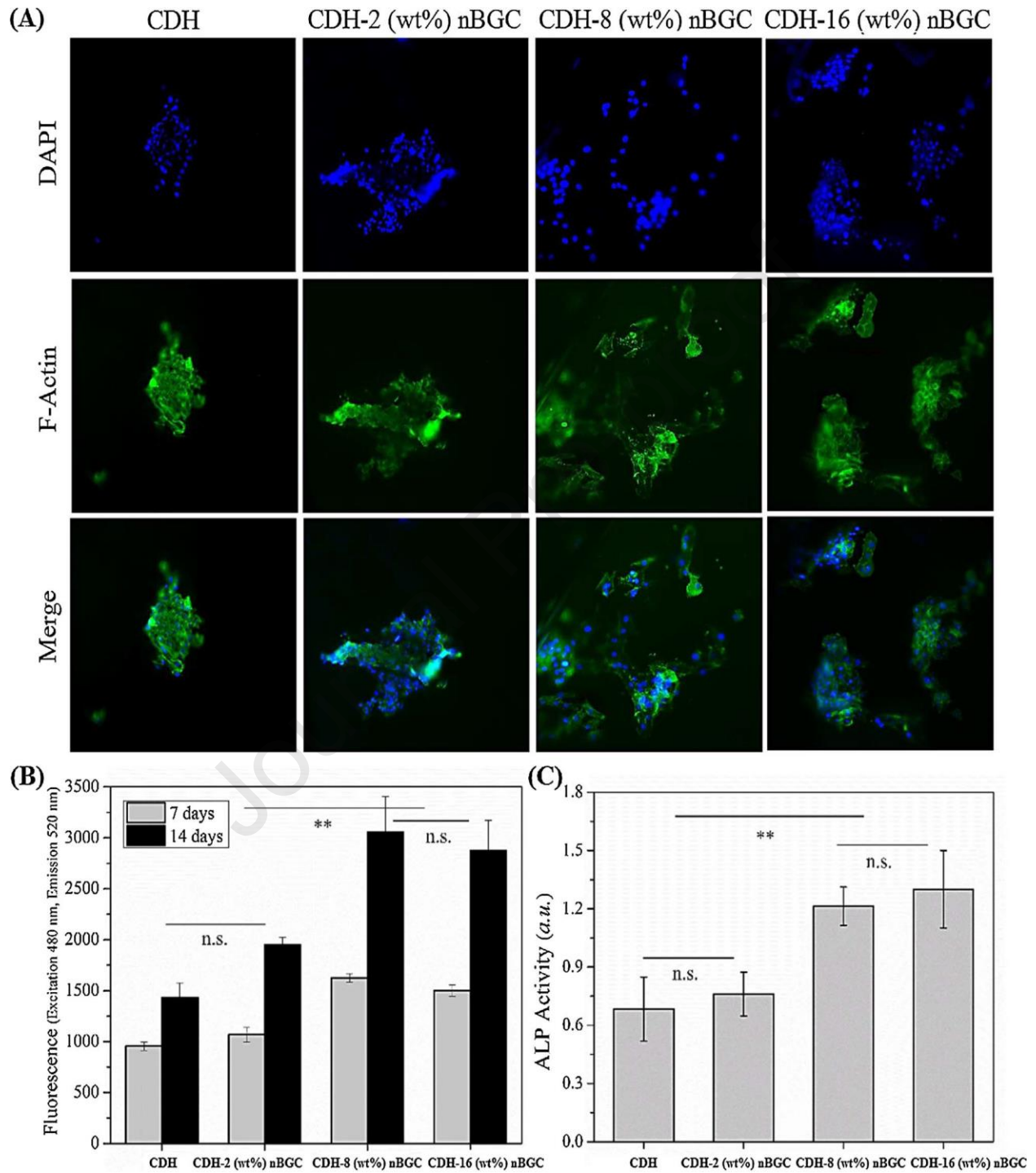
Figure 5. (A) Immunofluorescence images of seeded HOB cells on nanocomposite scaffolds, (B) the PrestoBlue viability of HOB cells on composite scaffold over two weeks and (C) Alkaline phosphatase [61] activity of HOB cells at day 14.)

## 2.3. Biodegradable polymers

### 2.3.1 Poly D, L-lactide (PDLLA) doped-BGs

Biodegradable thermoplastic polymers, especially poly D, L-lactide (PDLLA) has been used for bone regeneration. Bejarano *et al*. fabricated a biodegradable PDLLA scaffold incorporated with sol-gel BG of chemical composition 60 $SiO_2$; 25 CaO; 11 $Na_2O$; and 4 $P_2O_5$ (mol %) doped with 1 CuO or ZnO (1 mol %). This study indicated that PDLLA scaffolds with Cu-doped BG increased angiogenic potential confirmed by VEGF secretion, while scaffolds with Zn-doped BG showed the higher potential of osteogenic properties confirmed by enhancing ALP[61] expression. In addition, scaffolds prepared with co-dopping of both ions revealed enhanced osteogenic and angiogenic properties, and antibacterial activity against methicillin-resistant *S. aureus* bacteria [63]. In another study, Meretoja et.al. fabricated e-caprolactone/D, L-lactide-based scaffolds in combination with BG filler (70/30 caprolactone/lactide ratio and related composites with < 45 µm BaG filler size). When implanted in rats, the scaffold enhanced osteogenic response and in-growth vascularization within the macroporous scaffold [64].

### 2.3.2 β-tricalcium phosphate (β-TCP)-doped BGs

Synthetic β-tricalcium phosphate (β-TCP; commercially available as Vitoss), which have been used as the most common bone substitutes, are limited by their inadequate stimulation of angiogenesis and osteogenic differentiation and insufficient filling of the bone defect due to imbalance between resorption and osseous regeneration [65, 66]. Studies have shown that the fabrication of composite scaffold of β-TCPs and BGs nanoparticles was able to overcome these obstacles and improve the properties of β-TCP scaffolds; for example, by enhancing osseointegration, osteogenic differentiation *in vitro*, bone formation within implants *in vivo*. These composite scaffolds were also able to enhance the mechanical characteristics of nanoparticles of β-TCPs-incorporated BG. In this context, the BG nanoparticles in the Vitoss-

based scaffold promoted osteogenic differentiation of MSCs [67]. Westhauser *et al.* described a stimulatory effect of 45S5-BG particles in Vitoss BA on vascularization. Furthermore, Tartrate-Resistant Acid Phosphatase-Positive (TRAP+) cells in Vitoss BA scaffolds were responsible for the maturation of the osteoid [68].

## 4. Bioactive Glass Inorganic Ions in Co-culture System

Three-dimensional (3D) models are superior to 2D models as they have been demonstrated to be vital to simulate the native tissue environment [69]. 3D cell cultures incorporate the additional spatial dimension to simulate the tissue microenvironment, enhancing cell-cell andcell–matrix interactions [70]. BGs are stable in harsh condition involved in scaffold preparation, no effect of toxicity has been observed with their application compared to the application of solid growth factors, and the application of inorganic ions is more cost-effective than the additive of a small number of growth factors [71]. Alginate scaffolds have been commonly used as a 3D matrix in biomedicine, drug discovery, and tissue engineering studies [72]. Recently, Bargavi *et al.* have used the sol-gel process to fabricate a nano-membrane of a BG (45S5 Bioglass®)/Alginate composite, and zirconium (Zr) ions were doped were introduced into the BG in the composite scaffold. This 3D scaffold exhibited enhanced bioactivity and cell adhesion efficiency (**Figure 6**). In vitro 3D co-culture of HDF (human dermal fibroblast cell lines) and KB-3-1 cell line (human epithelial cells) cultures revealed that 3D nBG/Alg and nBG-Zr/Alg hydrogel membrane served a suitable matrix for cells resulting ingrown spheroids over the composite hydrogel membrane (**Figure 7**). Toldrà *et al.* exploited the osteogenic and angiogenic properties of S53P4 BG in a co-culture system in which dental pulp pluripotent-like stem cells were cultured in S53P4 BG-conditioned media containing different concentrations of inorganic ions dissolved from S53P4 BG. Vascular-like structures and osteogenesis were induced under the stimulatory effects of BG ions from the S53P4 BG-conditioned media [26]. In another study, Rath *et al.* fabricated copper ions-doped 45S5 BG scaffolds co-cultured with the MSCs and human dermal microvascular

17

endothelial cells (HDMECs). In this system, MSCs secreted VEGF into the culture media in the presence of 1% $Cu^{2+}$, which in turn induced HDMECs to display endothelial phenotype; thus, the $Cu^{2+}$- doped BGs acted indirectly as angiogenic growth factor delivery system, suggesting a potential stimulatory effect of $Cu^{2+}$ on MSCs in the MSC- HDMECs co-culture system (**Figure 8**). Deb et.al. fabricated Bioglass derived porous scaffolds made of Bioglass 45S5 with polyvinyl alcohol (PVA) as the porogen. The scaffolds were derived from 4:1 and 3:1 glass-polymer compositions, which referred as BG1 and BG2, respectively. In this study, the proliferation of human umbilical vein endothelial cells (HUVECS) and human osteoblasts (HOBS) were investigated in both co-culture and mono-culture conditions and compared with a commercial HA (SynHApor HA scaffolds), demonstrated that a porous scaffold prepared from 45S5 Bioglass supported the proliferation responses of HUVECS cultured with human osteoblasts in both co-culture and mono-culture system [73].
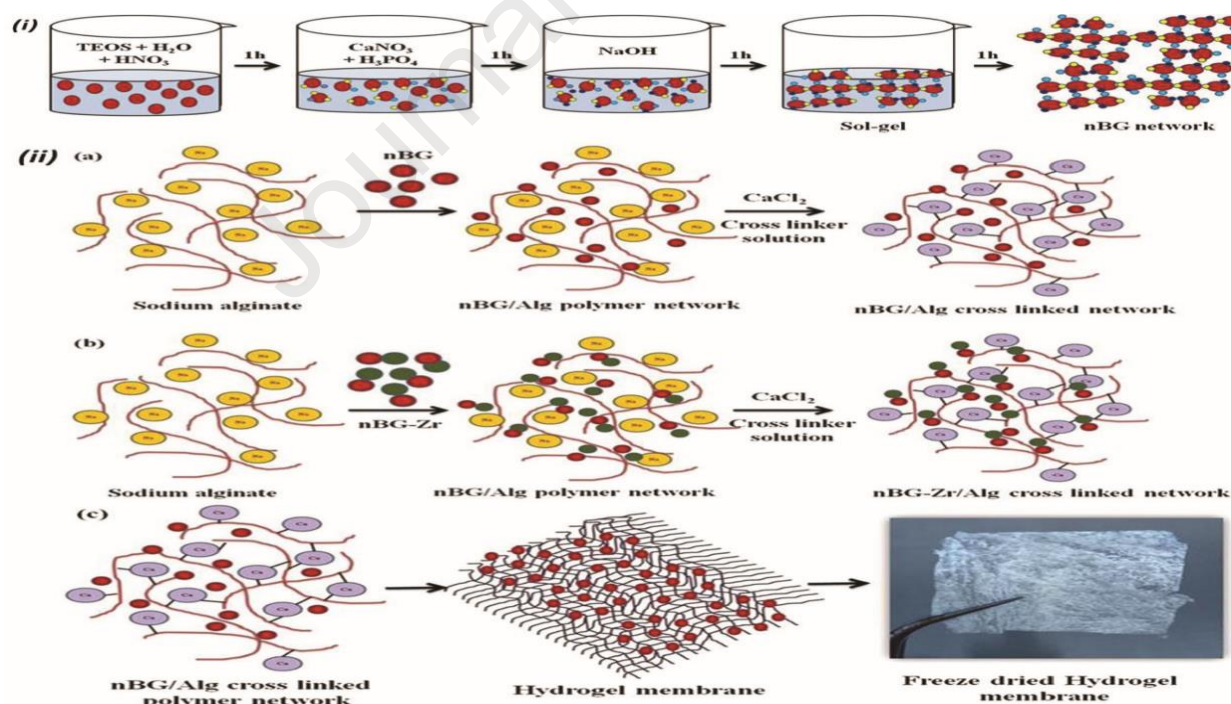


**Figure 6**. Bioactive glass nano-particles synthesized by a sol-gel process. (a–c) the network formation of nano-bioactive glass (nBG)- (alginate) Alg and nBG-(zirconium) Zr/Alg composite hydrogel membranes
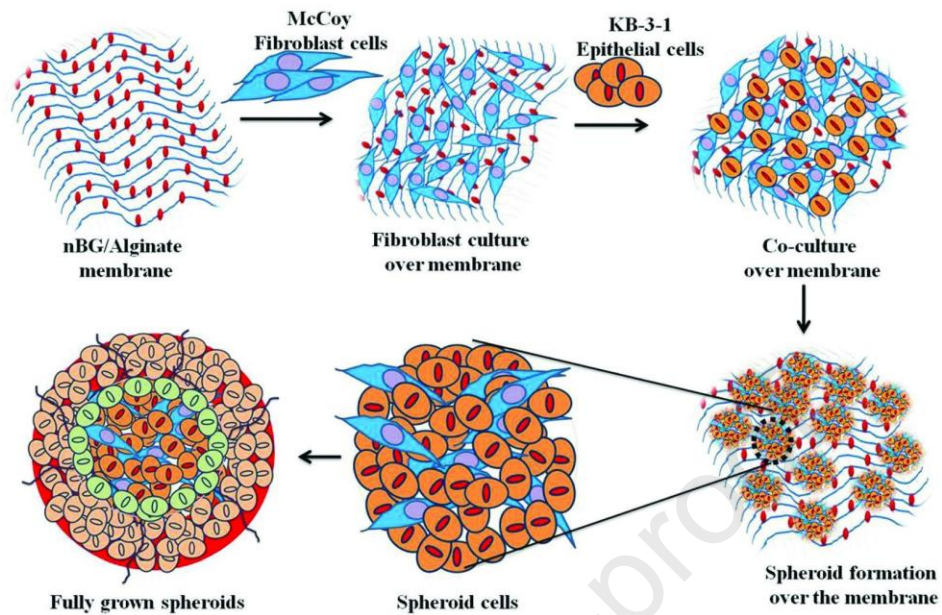
**Figure. 7.** A schematic illustrating 3D spheroid formation of a co-culture of HDF cells (human dermal fibroblast cells lines) and KB-3-1 cells (human epithelial cell lines)
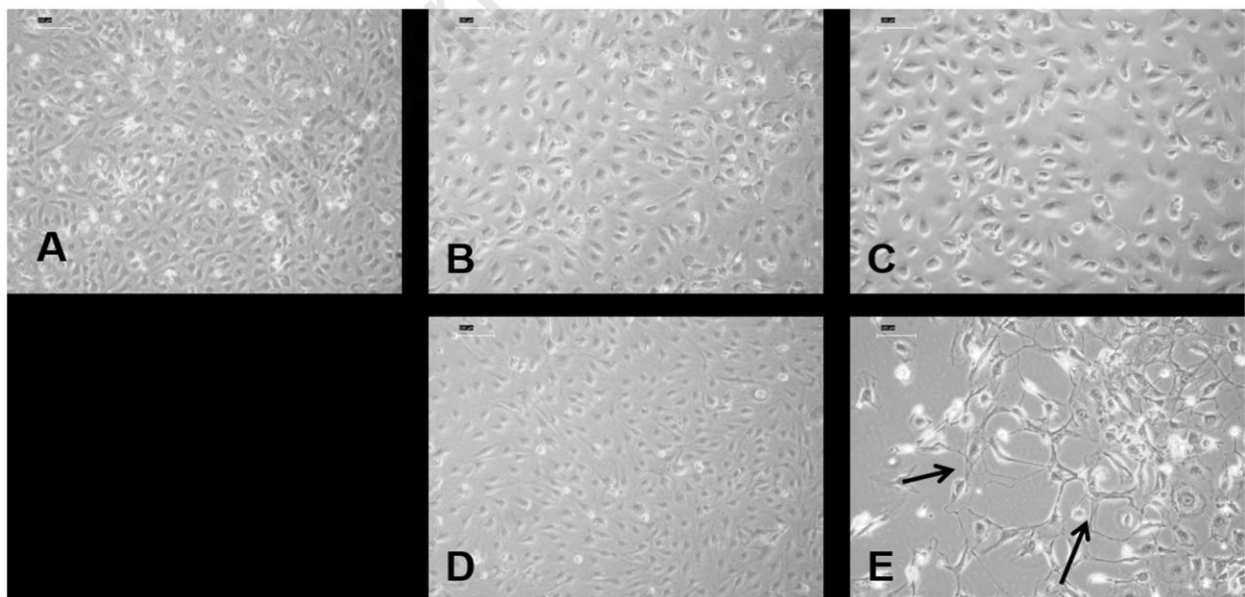


**Figure 8.** Light microscopic images showing tube formation of human dermal microvascular endothelial cells (HDMECs) cells cultured in the presence of both MSCs and

19

Cu2+-doped bioglass (arrows). (A) Group I, (B) group II, (C) group III, (D) group IV, and (E) group V

## 5. Conclusion and Future Challenge

The paper reported in this review highlights the incorporation of bioactive glass particles into natural and biodegradable polymers-based composite scaffold and their effects on closely related osteogenesis and angiogenesis in bone tissue engineering. Natural polymers including collagen, gelatin, and alginate, are biocompatible and non-cytotoxic, applied for medical devices for soft and hard tissues. However, such natural polymers suffer from low bioactivity and poor mechanical properties that limit their applications. To tackle these drawbacks, natural polymers can be combined with bioactive glass (BG) nanoparticles and microparticles to produce composites scaffolds. More importantly, incorporating BGs improves the mechanical properties of the resulted scaffold and its bioactivity and osteogenic differentiation compared to pure scaffolds without BG. Additionally, 3D co-culture of hydrogel-based BGs provides the additional spatial dimensions required to mimic a tissue microenvironment and enhance hierarchical cell-to-cell and cell–matrix interactions. Such a 3D structure serves as an ideal substrate for cell adhesion and proliferation. In the 3D structure, a synergistic effect of ions (released from BaG) promoted vascularization, which could inhibit the cost and potential disadvantage of growth factor dependent models. Hence, the accurate methods of 3D co-culture system using natural-based BaG scaffold to investigate the formation of vascular-like structures under the effect of BaG is of paramount importance. Further research is required to determine the most advantageous combination of natural polymers and BG to obtain good mechanical with specific biological performance. Moreover, the response of specific natural polymers should be investigated to avoid an undesired reaction after implantation.

**Conflict of interest**

Authros declared that there is n conflict of interest

**Table of Abbreviations**

| | |
|---|---|
| ALP | Alkaline phosphatase |
| BG | Bioactive glass |
| BGC | Bioactive glass ceramics |
| BMSC | Bone marrow mesenchymal stem cell |
| CDH | Cross-linked dextran hydrogels |
| DPPSC | Dental pulp pluripotent-like stem cells |
| HCA | Hydroxy carbonate apatite |
| HDMEC | Human dermal microvascular endothelial cells |
| HUVECS | Human umbilical vein endothelial cells |
| ICBME | Iranian conference on biomedical engineering |
| MBG | Mesoporous bioactive glass |
| MBGN | Mesoporous bioactive glass nanoparticles |
| OCS | Oxidized chondroitin sulfate |
| VEGF | Vascular endothelial growth factor |

**List of Figures legends:**

**Figure 1.** Top panel showed Scanning electron microscopy images. a) BGnW, b) Gel-Glu-Col/BGnW, c) Gel-Glu-Col (pure), d) Gel-Glu-Col/BGnW. Bottom panel showed Alizarin red staining to confirm the osteogenic differentiation of hMSCs. Cells were cultured in presence of osteogenic induction media for 14 days: TCPS (a), BGnW100 μg/ml (b), Gel-Glu-Col (c), Gel-Glu-Col/BGnW1% (d). b) von Kossa staining to confirm the osteogenic differentiation of hMSCs during 14 days clturing in presence of osteogenic induction media: TCPS (a), BGnW100 μg/ml (b), Gel-Glu-Col (c), Gel-Glu-Col/BGnW1% (d).[48].

**Figure 2.** Schematic depicted mechanisms involved in the gelation of hybrid Gel-OCS/MBGN hydrogels [51].

**Figure 3.** Showed osteoblast-related gene and protein expressions of BMSCs cultured on the hydrogel surfaces on day 14. (a) RT-PCR analysis of osteogenic-related markers such as OPN, OCN, RunX2, and Col-1. (b) immunofluorescent images of osteogenic-associated proteins RunX2 and OPN in different groups. RunX2 and OPN are marked by red fluorescence, and the cell nuclei were stained blue by the Hoechst. (c) Quantitative analysis of RunX-2 and OPN fluorescence intensity. (d) Protein expression of RunX2 in BMSCs and GAPDH was used as a reference. [51].

**Figure 4.** Schematic illustration of synthesized composite scaffolds (A), fabrication process of CDH-nBGC composite scaffolds (B) CDH-nBGC composite hydrogels before (I) and after [74] gelation [62].

**Figure 5**. (A) Immunofluorescence images of seeded HOB cells on nanocomposite scaffolds, (B) the PrestoBlue viability of HOB cells on composite scaffold over two weeks and (C) Alkaline phosphatase [61] activity of HOB cells at day 14. [62].

**Figure 6**. Bioactive glass nanoparticles synthesized by a sol-gel process. (a–c) the network formation of nano-bioactive glass (nBG)- (alginate) Alg and nBG-(zirconium) Zr/Alg composite hydrogel membranes [59].

**Figure. 7.** A schematic illustrating 3D spheroid formation of a co-culture of HDF cells (human dermal fibroblast cells lines) and KB-3-1 cells (human epithelial cell lines) [59].

**Figure 8.** Light microscopic images showing tube formation of human dermal microvascular endothelial cells (HDMECs) cells cultured in the presence of both MSCs and Cu2+-doped Bioglass (arrows). (A) Group I, (B) group II, (C) group III, (D) group IV, and (E) group V [75].

22

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**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: