Running title: 3D Ion-doped hydrogel bioactive glass in bone regeneration

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

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Abstract

The repair of significant bone defects remains a critical challenge, raising the clinical demand to design novel bone biomaterials incorporating osteogenic and angiogenic properties to support the regeneration of vascularized bone. Bioactive glass scaffolds have the ability to stimulate angiogenesis and osteogenesis. In addition, natural or synthetic polymers are 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, incorporating bioactive glass to improve natural polymers' mechanical properties and bioactivity. 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 (Moses, Nandi, & Mandal, 2018). All bone grafts, either as autograph or allograft, are associated with common limitations, which mainly include limited availability of donors, morbidity at the donor site, disease transmission, and a high incidence of non-union healing (Sheikh, et al., 2015; Zamani, Moztarzadeh, & Bizari, 2019).

The bone matrix is comprised of organic (mainly collagen) and inorganic (mainly hydroxyapatite) components. It has developed different natural and synthetic materials to prepare an ideal scaffold for bone repair; however, these materials have their shortcoming in inducing bio-mineralization, and inadequate mechanical properties and flexibility (Hajiali, Tajbakhsh, & Shojaei, 2018; Zamani, et al., 2019). A variety of materials such as metals (Chiu, Chang, Yang, & Wang, 2015), ceramics, and polymers, including natural and synthetic, have been applied (Deux, et al., 2002), and proteins (Jonker, Löwik, & Van Hest, 2012). Moreover, the lack of rapid vascularization after implantation remains a critical hurdle to bone tissue engineering. We need vascularization to supply the implanted construct, providing oxygen and nutrients to osteoblasts and removing waste products (Quinlan, et al., 2015). In the case of significant bone defects, the design of bone bio-mimetic materials incorporating both inorganic and organic components to simulate the typical structure and mechanical support of the natural bone matrix remains a critical challenge for complete repair(Jonker, et al., 2012).

Recent developments in bone tissue engineering have attempted to tackle this problem by designing materials using components and bone-mimetic architectures through various approaches [9]. In this context, it has focused efforts on incorporating mineral components, such as bioactive glasses (Jalise, Baheiraei, & Bagheri) into organic scaffolds [10, 11]. BaGs belong to a group of surface reactive amorphous materials identified with good biocompatibility, biodegradation properties, and stimulate the osteogenic differentiation of stem cells (Baino, Hamzehlou, & Kargozar, 2018). In bone regeneration, bioactive glass can bind firmly to the bone tissue and consequently induce angiogenesis and osteogenesis. However, due to the fast dissolution behavior and fragility of BaG, they are applied for coatings of implants and composite polymers (Badr-Mohammadi, Hesaraki, & Zamanian, 2014; Zamani, Razmjooee, Moztarzadeh, & Bizari, 2017).

In the 1970s, Hench and coworkers fabricated the first bioactive glasses with the first composition that is known as 45S5 Bioglass® (BG), composed of four components 45 SiO_2–24.5 CaO–24.5 Na_2O–6 P_2O_5 (weight%) (Hench, 2006). Relatedly, BG-based scaffolds demonstrated to have angiogenic properties that promote neo-vascularization and vascular ingrowth within the bone tissue (Gorustovich, Roether, & Boccaccini, 2010; Mahmood, et al., 2001; Nandi, Kundu, Datta, De, & Basu, 2009), fulfilling the osteogenic and angiogenic properties required for bone repair (J. Wu, et al., 2019). Indeed, the result of the addition of inorganic ions dissolved from S53P4 bioactive glass into cell culture media promoted both endothelial and osteogenic processes, supporting the simultaneous formation of vascular-like structures and mineralization. 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 (Coelho, Cabral, & Fernandes, 2000; Núñez-Toldrà, et al., 2019; Shahabipour, et al., 2020), showing that BaGs can induce vascularization and promote osteogenesis in co-culture systems (Núñez-Toldrà, et al., 2019). Besides its osteogenic and angiogenic abilities in co-culture systems, a combination of BaG with bioactive nanoparticles (NPs), or its incorporation with synthetic polymers, enhanced the mechanical and biological properties of BaG composite scaffolds (Z. Wu, et al., 2020; Zamani, et al., 2019). For example, when BaG and NPs are combined for bone repair, they can induce osteogenesis by releasing active Si and Ca ions (Moreira, Carvalho, Mansur, & Pereira, 2016). Ions such as Si, Ca, and Cu combined with BaG improved osteogenic and angiogenic properties (Zheng, et al., 2017).

This review aims to highlight the recent progress in research on using 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. Hydrogel/Polymer-based Scaffold Incorporated With BaG

Natural polymers have attracted much attention in bone tissue engineering partly due to their specific properties, such as biocompatibility, biodegradability, and non-toxicity (Zamani, et al., 2019). However, they exhibit low mechanical characteristics and limited bio-mineralization, which are critically needed for bone repair (Hajiali, et al., 2018). In this regard, the fabrication of multifunctional composite scaffolds composed of hydrogel combined with inorganic BaG, encapsulated with cells and/or growth factors, can enhance the bone regeneration by promoting osteogenesis and angiogenesis. Indeed, previous studies have

indicated the multifunctional properties of scaffolds comprising bioactive glass nanoparticles and alginate crosslinked with various cations in bone tissue engineering (Cattalini, et al., 2015; Erol, et al., 2012). In addition, BG dispersed in a polymer scaffold improved mechanical properties, osteogenic induction, and stimulated growth factors, proliferation, and angiogenesis in bone tissue repair (Vichery & Nedelec, 2016).

Besides the above-mentioned benefits of hydrogel/ BG composites, an injectable hydrogel has gel properties at physiological temperature without requiring any chemical and environmental treatment when injected at the defect site. This allows the gel to fully repair critical-size bone defects *in vivo* (Liu, et al., 2017; Reakasame & Boccaccini, 2018). Among injectable hydrogels, chitosan (CH)/ glycerophosphate (Li, et al.) 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 (Kondiah, et al., 2016; Moreira, et al., 2016; J. Wu, Liu, Shi, & Wan, 2016; H. Y. Zhou, Jiang, Cao, Li, & Chen, 2015). 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 controlled 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 the expression of angiogenic and osteogenic genes. In addition, Cu-BG/CH/SF/GP gel exhibited full restoration of the bone defect with the formation of vascularized bone tissue and mineralized collagen deposition during 8 weeks including no cells and growth factors (J. Wu, et al., 2019). Bioactive glass nanowhisker (BGnW) composed of 58% SiO2, 33% CaO, and 9% P2O5 (based on mol%) displayed excellent binding to hard and soft tissues. Thus it could be used combined with a hydrogel-based scaffold to increase osteogenic differentiation. Azizipour et.al. incorporated BGnW into 3D porous hydrogel nanocomposite scaffold 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, Mesenchymal stem cells (MSCs) cultured on the scaffold indicated osteoblastic differentiation that upregulated the expression of type 1 collagen, Runx-2, and alkaline phosphatase, as well as the protein expression of osteocalcin and osteopontin (**Figure 1**) (Azizipour, et al., 2021)

Figure 1. proposed location

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 in bone tissue engineering (Nadeem, Kiamehr, Yang, & Su, 2013; Peter, et al., 2010). Moreover, gelatin promotes cell attachment, improving tissue regeneration in vivo. Zare jalise et.al. fabricated strontium-delivering BGs in a SiO2-CaO-SrO-P2O5 structure, in combination with gelatin as a composite scaffold. This composite scaffold increased exhibited enhanced compressive strength and elastic modulus. Moreover, this strontium-enriched BG improved neovascularization and enhanced osteoblast cell viability and differentiation (Jalise, et al., 2018). Zhou *et al.* synthesized self-crosslinking 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 cells (BMSCs) in vitro as well as effective bone regeneration in vivo compared with Gel-CS hydrogels without MBGNs (Figure 3) (L. Zhou, et al., 2021).

Figure 2 proposed location

Figure 3 proposed location

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 crosslinked in the presence of certain divalent cations, e.g., Ca^{2+}, Sr^{2+}, and Ba^{2+}, leading to the formation of a hydrogel (Rottensteiner, et al., 2014). 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 [5]. In this regard, Zamani *et al.* fabricated an alginate scaffold incorporated with BGs composed of Zn and Mg ions. This scaffold exhibited antibacterial effects and enhanced the mechanical properties and the bioactivity of the Alginate/BG composite. In addition, it related the antibacterial activity to the released Zn and Mg ions, restricting the growth of both *S. aureus* and *E. coli* (Zamani, et al., 2019).

Alginate lacks a cell-binding ligand, attributing it to inferior cell adhesion properties. Hence, alginate, in combination with a hydrogel, improved vascular cell adhesion and proliferation *in vitro* (Singh, et al., 2016). 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 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 (Jeong, et al., 2011; Sarker, et al., 2014). 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 (Sarker, et al., 2014). Bioglass/Alg composites are generated using different approaches including sol-gel and freeze-drying, 3D printing (Luo, Wu, Lode, & Gelinsky, 2012), and surfactant foaming (Mishra, Basu, & Kumar, 2009). 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, which was 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 Human Dermal Fibroblasts (HDF) and KB-3-1 cell lines by increasing surface roughness and changing in 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 (Bargavi, et al., 2020).

2.2.2 Dextran-BG

Dextran, a hydrophilic carbohydrate biopolymer, showed good degradation properties in specific physical environments without affecting cell viability. Cells proliferated in clumps on dextran rather than spreading, thus, inorganic materials such as hydroxyapatite have been incorporated with dextran to increase the bioactivity and mechanical properties (Varoni, et al., 2010). Bioactive glass ceramics (BGC) containing SiO2–CaO–P2O5 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 (HCA) layer. Nikpour *et al.* synthesized Crosslinked dextran hydrogels (CDH)- bioactive glass ceramic nanoparticles (nBGC) for bone tissue engineering (Figure 4). The concentration of nBGC nanoparticles affects their distribution in the composite scaffold in which nBGC at low contents (2 wt%) dispersed homogenous within the Dex matrix. However, nBGC nanoparticles at higher concentration revealed agglomeration and a consequent increasing water uptake. The composite scaffold supported growth and improved alkaline phosphatase (ALP) activity of human osteoblasts (HOBs) at concentration up to 16 (wt%) over two weeks (Figure 5) (Nikpour, et al., 2018).

Figure 4 proposed location

Figure 5 proposed location

2.3. Biodegradable polymers

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

Biodegradable thermoplastic polymers, especially poly D, L-lactide (PDLLA) have 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 one cupric oxide (Bertrand, Criscuolo, Faivre, & Sorci) or ZnO (1 mol %). This study indicated that PDLLA scaffolds with Cu-doped BG increased angiogenic potential confirmed by vascular endothelial growth factor (VEGF) secretion, while scaffolds with Zn-doped BG showed a higher potential of osteogenic properties confirmed by enhancing ALP 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

(Bejarano, Detsch, Boccaccini, & Palza, 2017). 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 ingrowth vascularization within the 9icroporous scaffold (Meretoja, Tirri, Malin, Seppälä, & Närhi, 2014).

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 (Bellucci, Sola, & Cannillo, 2016; Karadjian, et al., 2019). Studies have shown that the fabrication of composite scaffolds of β-TCPs and BGs nanoparticles were 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 could also enhance the mechanical characteristics of nanoparticles of β-TCPs-incorporated BG. In this context, the BG nanoparticles in the Vitoss-based scaffold promoted the osteogenic differentiation of MSCs (Westhauser, Karadjian, et al., 2019). 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 (Westhauser, Essers, et al., 2019).

3. 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 models to simulate the native tissue environment (Pampaloni, Reynaud, & Stelzer, 2007). 3D cell cultures incorporate the additional spatial dimension to simulate the tissue microenvironment, enhancing cell-cell and cell-matrix interactions (Simian & Bissell, 2017). BGs are stable in a 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 few growth factors (Jakubietz, et al., 2019). We have commonly used alginate scaffolds as a 3D matrix in biomedicine, drug discovery, and tissue engineering studies (Liao, et al., 2017). 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 (DPPSC) 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 (Núñez-Toldrà, et al., 2019). In another study, Rath *et al.* fabricated copper ions-doped 45S5 BG scaffolds co-cultured with the MSCs and human dermal microvascular endothelial cells (HDMECs). In this system, MSCs secreted VEGF into the culture media in the presence of 1% Cu^{2+}, which induced HDMECs to display endothelial phenotype; thus, the Cu^{2+}- doped BGs acted indirectly as an 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 (Deb, Mandegaran, & Di Silvio, 2010).

Figure 6 proposed location

Figure 7 proposed location

Figure 8 proposed location

4. Conclusion and Future Directions

In this review, we summarized the bioactive glasses (BGs)-incorporated within bone scaffolds to induce vascularization in bone tissue implants. Our discussed approaches included using co-culture systems, BG nanoparticles, and hydrogel-doped BG scaffolds. Co-culture of endothelial and osteogenic cells appears to be a promising approach to stimulate vascularization and osteogenesis of bone tissue scaffolds. Hydrogels embedded with cells displayed favorable properties in bone tissue engineering. However, they have limitations in terms of application. Some of these limitations can be solved by hydrogel scaffolds combined with BGs, which promote the formation of vessel-like structures, vessel-like structures, and osteogenesis formation. This enhancement could be attributed to ions released by BGs such as BG-released Cu2+, which could induce VEGF secretion from the MSCs. Therefore, the hydrogels embedded with ions-doped BGs with suitable cell co-culture systems could create a multifunctional composite scaffold. However, understanding the mechanisms regulating angiogenesis in bone tissue engineering requires further investigation. A fabricated scaffold that can mimic the native bone structure, including its cellular organization, scaffold composition, and three-dimensional (3D) architecture, could be helpful to achieving this goal. Overall, fabricated scaffolds with ions-doped BGs represented a promising strategy in bone regeneration mainly because of their cost-effective, timesaving processing of cells and eliminated applying expensive growth factors.

Conflict of interest

The authors declare that there is no conflict of interest.

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Abbreviation

ALP	Alkaline phosphatase
BGC	Bioactive glass ceramics
BMSC	Bone marrow mesenchymal stem cells
CDH	Crosslinked dextran hydrogels

DPPSC	Dental pulp pluripotent-like stem cells
ECM	Extracellular matrix
HCA	Hydroxy carbonate apatite
HDMEC	Human dermal microvascular endothelial cells
HUVECS	Human umbilical vein endothelial cells
ICBME	Iranian conference on biomedical engineering
MBGN	Mesoporous bioactive glass nanoparticles
OCS	Oxidized chondroitin sulfate

Figure's legends

Figure 1. The top demonstrated Scanning electron microscopy images. A) BGnW, b) Gel-Glu-Col/BGnW, c) Gel-Glu-Col (pure), d) Gel-Glu-Col/BGnW. The bottom panel showed Alizarin red staining to confirm the osteogenic differentiation of hMSCs. Cells were cultured in the 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 of culturing in the presence of osteogenic induction media: TCPS (a), BGnW100 µg/ml (b), Gel-Glu-Col (c), Gel-Glu-Col/BGnW1% (d).(Azizipour, et al., 2021)

Figure 2. Schematic depicted mechanisms involved in the gelation of hybrid Gel-OCS/MBGN hydrogels (L. Zhou, et al., 2021).

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 osteopontin (OPN), fibronectin, osteocalcin (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 Hoechst stained the cell nuclei blue. (c) Quantitative analysis of RunX-2 and OPN fluorescence intensity. (d) Protein expression of RunX2 in BMSCs and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference. (L. Zhou, et al., 2021).

Figure 4. Schematic illustration of synthesized composite scaffolds (A), the fabrication process of CDH-nBGC composite scaffolds (B) CDH-nBGC composite hydrogels before (I) and after (II) gelation (Nikpour, et al., 2018).

Figure 5. (A) Immunofluorescence images of seeded HOB cells on nanocomposite scaffolds, (B) the PrestoBlue viability of HOB cells on a composite scaffold over two weeks, and (C) ALP activity of HOB cells at day 14. (Nikpour, et al., 2018).

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 (Bargavi, et al., 2020).

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) (Bargavi, et al., 2020).

Figure 8. Light microscopic demonstrating 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 (Rath, et al., 2014).

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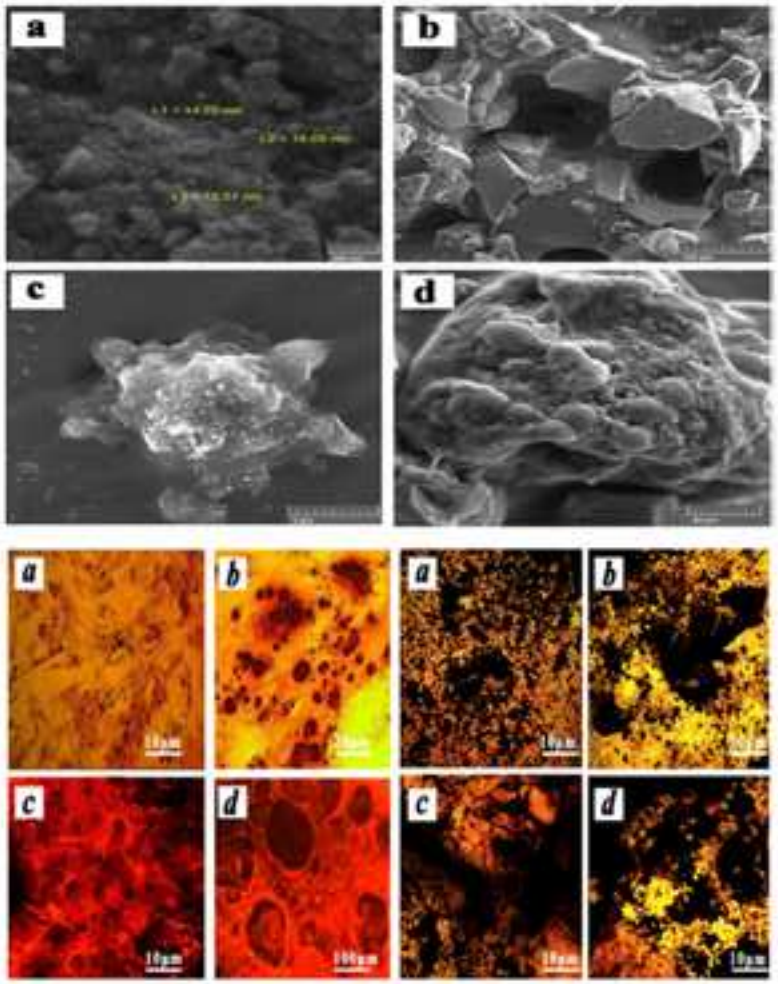

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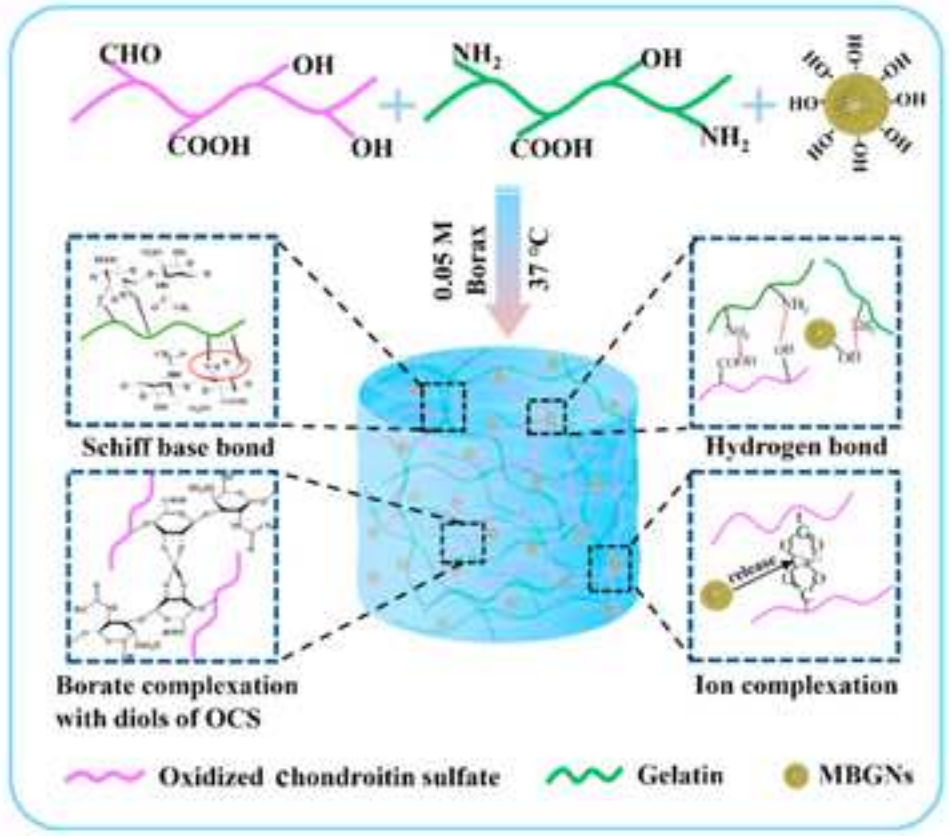

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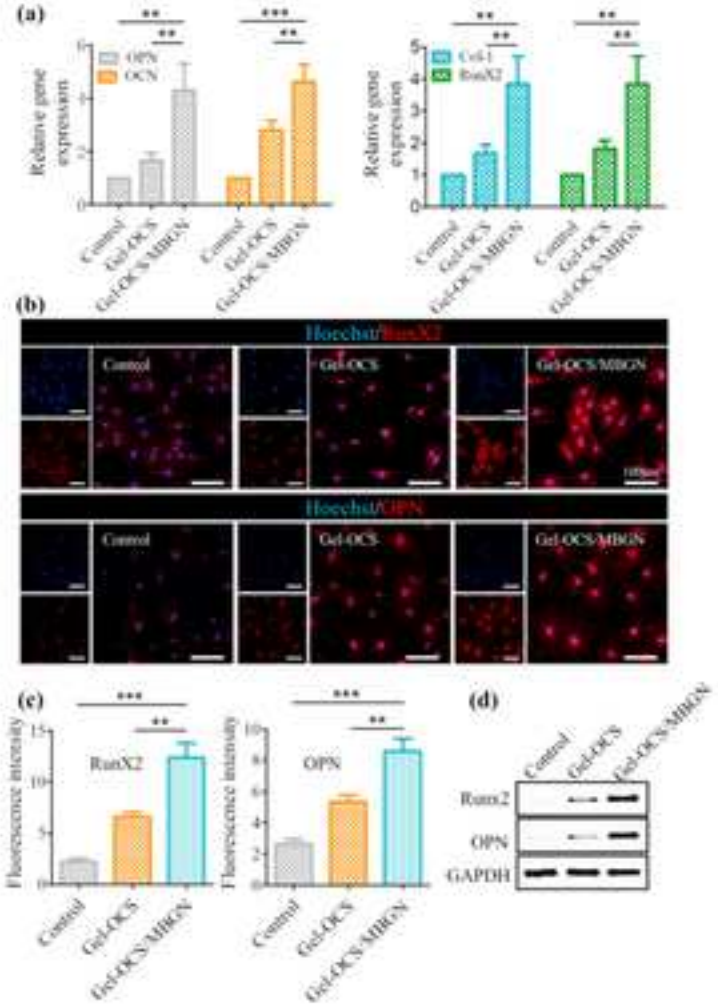

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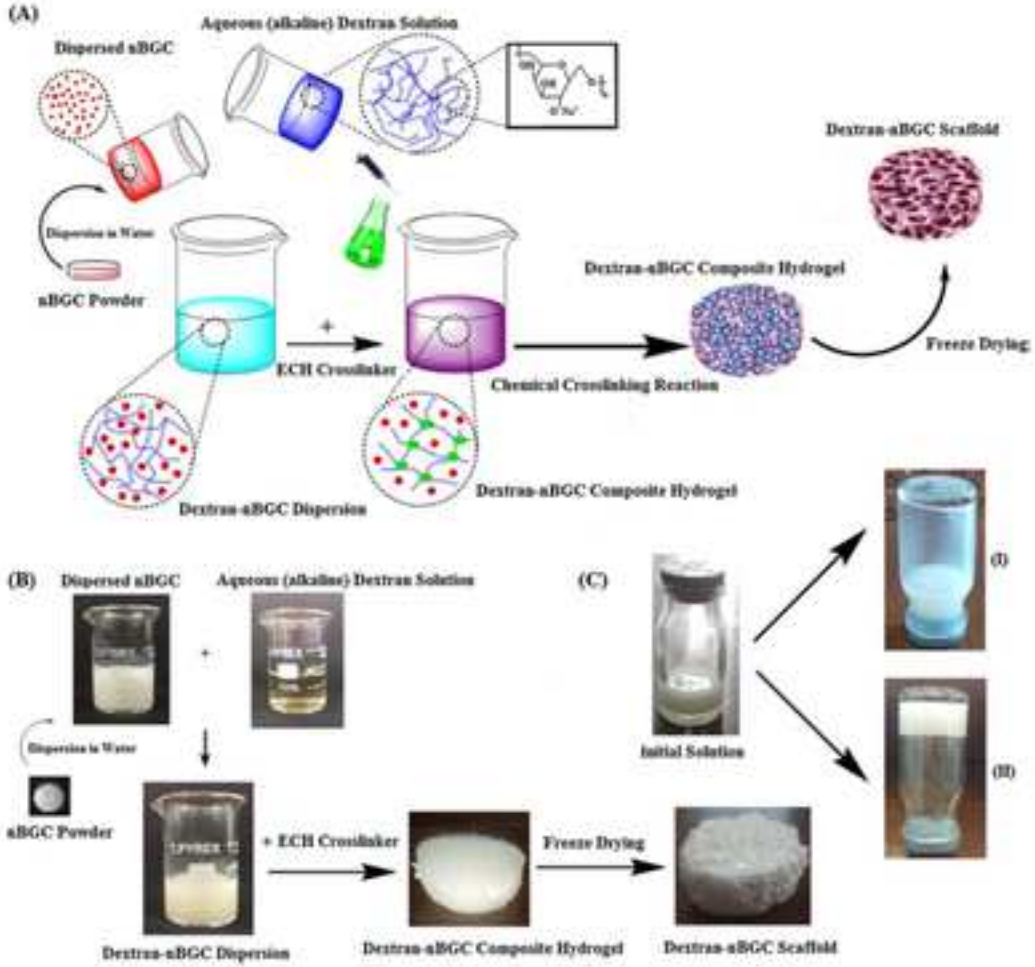

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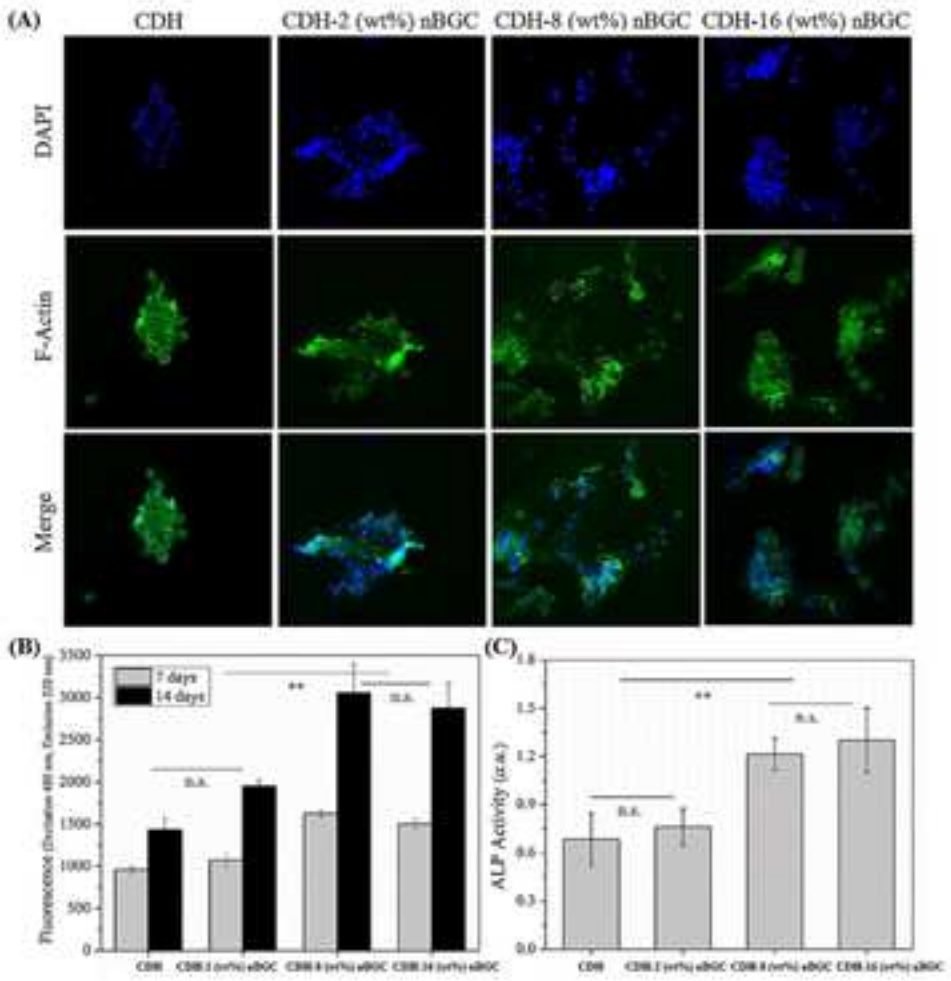

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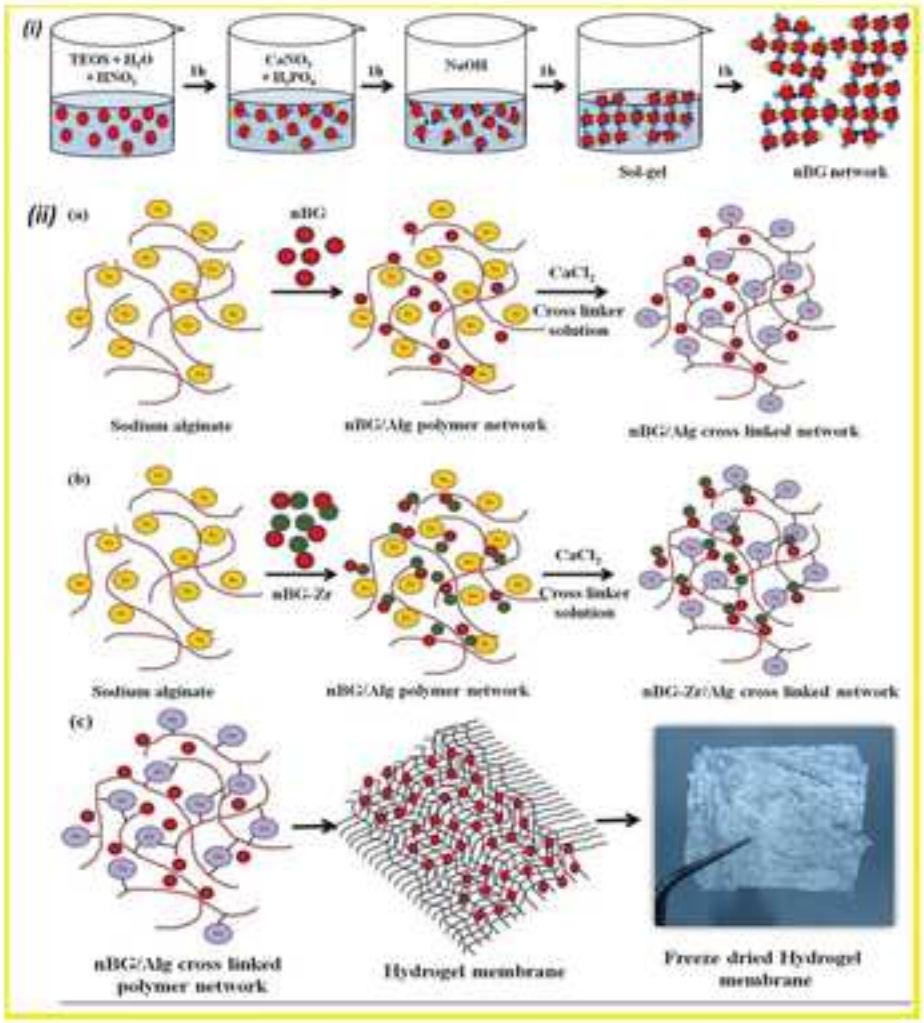

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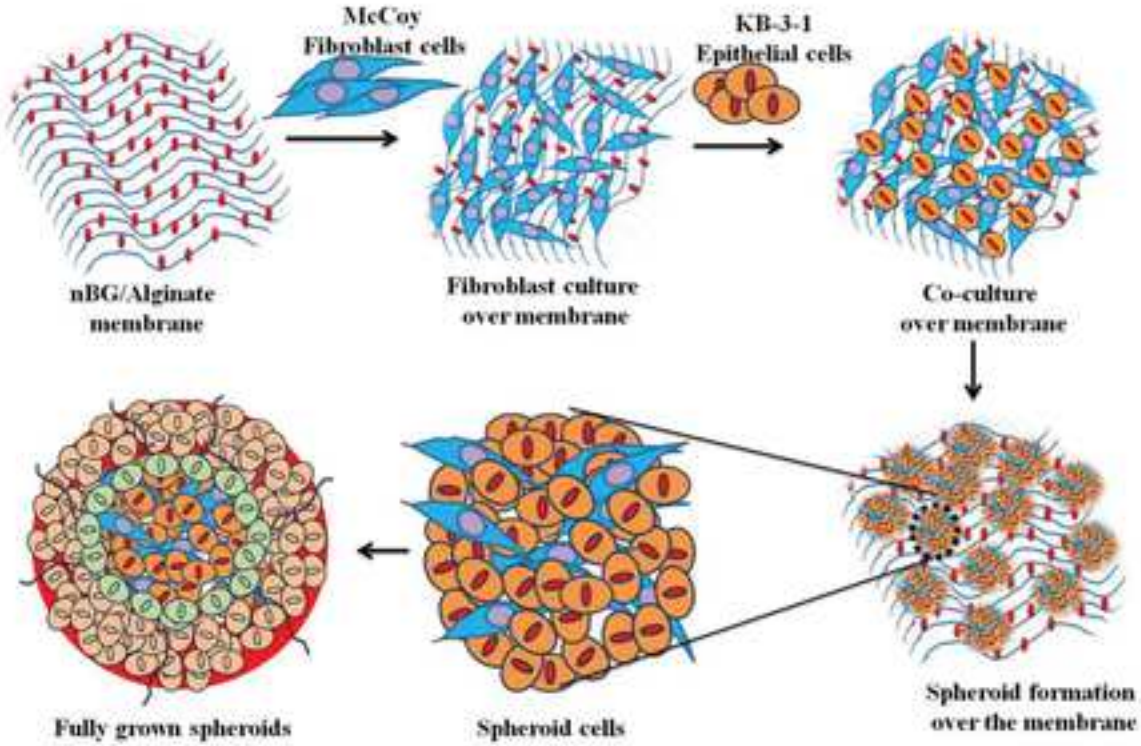

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