Iranian Journal of Chemical Engineering

Journal Homepage: www.ijche.com

pISSN: 1735-5397
eISSN: 2008-2355

Special Issue Article

Developing Collagen Hydrogel Including Bioactive Glass Nanoparticles for being used in Bone Tissue Engineering

M. Takapoui, M. Saadatmand*, F. Ghobadi

Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran.

ARTICLE INFO

Article history:
Received: 2024-01-09
Accepted: 2024-02-06
Available online: 2024-02-06

Keywords:
Osteoporosis,
Bone tissue engineering,
Collagen,
Bioactive glass,
Nanoparticles,
Elastic modulus

ABSTRACT

Numerous bone disorders and injuries, such as osteoporosis, are among the most spreading types of human tissue injuries worldwide, and the available treatments for these injuries are often insufficient and inefficient. Nowadays a lot of attention has been paid to the regenerative medicine, specifically tissue engineering because of its unique features. The extracellular matrix is a key component in tissue engineering because it must have specific properties to support cell survival and proliferation. Natural and synthetic polymeric hydrogels are among the materials commonly employed in tissue engineering. Because the extracellular matrix of bone is particularly mineralized and has a high elasticity, various nanoparticles are commonly utilized to improve the mechanical properties of polymeric hydrogels. In this study, first we extracted the collagen type I from rat tail and characterized it with FTIR spectrum and self-assembly, second we synthesised the bioactive glass nanoparticles and characterized them with XRD and EDAX. Then we developed a polymeric collagen hydrogel (3 mg/ml) scaffold, including bioactive glass nanoparticles (3 %w/v) which increase the mechanical properties of the scaffold (10^3 pa elastic modulus) in comparison to those of the collagen scaffold (0% w/v nanoparticles), that can be used for bone tissue engineering applications.

DOI: 10.22034/ijche.2024.434522.1511 URL: https://www.ijche.com/article_189841.html

1. Introduction

Bone is a highly mineralized tissue that plays important roles in the body, including facilitating movement, supporting soft tissues, regulating hormones, and serving as the supplier of bone marrow [1]. Additionally, bone is referred to as a dynamic organ because it undergoes a process known as "remodeling" throughout the lifespan in which osteoblast and osteoclast cells respectively form new bone and resorb aged bone during growth and

*Corresponding author: m.saadatmand@sharif.edu (M.Saadatmand)

injury [2]. Osteoporosis, which typically affects women over the age of 50, is one of the most frequent disorders caused by an imbalance in the remodeling process [3]. According to available statistics, this condition will cause around three million bone fractures and a total yearly cost of $25.3 billion by 2025 [4]. Bone is the third most common location of cancer metastasis and is the most common site for breast, prostate, and lung cancer metastases. Other malignancies, such as thyroid and kidney cancers and skin melanoma, can also spread to the bone [5]. To reconstruct and mimic the bone physiological microenvironment, biomimetic hydrogels, including type 1 collagen hydrogel, which makes up 90% of the bone matrix, can be combined with different nanoparticles or other natural and synthetic polymers to create proper scaffolds. In some studies, type I collagen has been used as a hydrogel scaffold for osteogenesis. In 2021, Simorgh et al. isolated type I collagen from rat tail and synthesized collagen hydrogels with varying concentrations as the scaffolds to study osteogenesis and implanted the scaffolds in the mouse [6].

Nanoparticles such as hydroxyapatite [7], bioactive glass [8], gold [9] and graphene oxide [10] have been used to improve the mechanical properties and strength of hydrogels, and for better mimicking the bone microenvironment. Bioactive glass (BG) is an amorphous silicate-based material that is compatible with the human body and can bond with bone and stimulate osteogenesis through its dissolution over time [11]. The bioactive glass was first discovered in 1969 by Larry Hench and colleagues at the University of Florida, and it was the first material that had the ability to be grafted on to the host's bone [12]. The percentage composition of the first biological glass was: 46.1% SiO_2, 24.4% NaO, 26.9% Cao and 2.6% P_2O_5 (molar percentage), and that glass became known as 45S5 biological glass which is still known by the same name in the world markets today. The discovery of Hench and his colleagues has opened a new window to the clinical uses of ceramics and bioactive glasses since 1985. Subsequent studies in the field of biological glasses showed that by changing the constituents of glasses, they can be used for soft tissues as well [13]. Currently, researchers are focusing on improving the properties of these biomaterials in osteogenesis and their potential in developing different structures to stimulate the body's spontaneous repair mechanism [14]. In general, the bioactive glass has a great ability to stimulate osteogenesis and generally stimulate the spontaneous repair of other body tissues through two mechanisms. Its first and most important mechanism is through the immersion of these particles in an aqueous environment which can cause the formation of hydroxyapatite on their surface that can mineralize the ECM (mainly the aqueous environment of the body) [15]. The second mechanism, through which the bioactive glass has the ability to effectively stimulate osteogenesis and repair other body tissues such as doing angiogenesis, is the mechanism of releasing various ions. For example, strontium ion for the bone differentiation [16], copper ion for influencing endothelial cells and angiogenesis [17], cobalt ion for angiogenesis [18] and silver ion for antibacterial properties [19] are used. In 2015 Solgi et al. used the sol-gel process to synthesize the bioactive glass that contained a four-element blend of silicon, calcium, phosphorus, and strontium. In this work, bioactive glasses were synthesized in three distinct experimental groups using strontium ions with the concentrations of 0, 5, and 10 mole percent. In this work they aim to examine how various concentrations of strontium ions

affect the activity of osteoblast-secreted alkaline phosphatase and cell proliferation. The results of this study showed that the glass with the concentration of 5% of strontium had the best and most optimal effect on the proliferation of osteoblast cells and increasing the activity of alkaline phosphatase [20]. In 2012 Wu et al. synthesized the biocompatible nanoparticles of the mesoporous bioactive glass with copper, silicon, calcium, and phosphorus. These nanoparticles have been produced using varying quantities of copper ions, namely 0, 1, 2, and 5 mole percent. The aim in this study was to investigate the impact of varying concentrations of copper ions on the stem cell differentiation, proliferation, VEGF secretion, HIF-α factor expression, and bone factor expression. At the end of this study, it was reported that copper ions with a concentration of 5 mol% inside the bioactive glass structure has the most optimal concentration to achieve the predetermined goals, and the authors state that the use of this combination of nanoparticles has a very good potential for treating bone injuries [21].

Collagen and the bioactive glass have both utilized hydrogel and nanoparticle thus far in a variety of studies, particularly those focused on bone treatments. However, there hasn't been any research done on using these two materials together for the bone tissue engineering.

In this study, for the first time, to mimic the bone ECM, we used collagen type I which is the most common protein of the bone matrix (almost 90% of the bone matrix) and we added bioactive glass nanoparticles with a distinct composition (Sr and Cu together in the glass) to the collagen and obtained the hydrogel scaffold with proper mechanical and biological properties for their application in bone tissue engineering.

2. Materials and methods

2.1. Synthesis of bioactive glasses

The composition of elements considered for the synthesis of the bioactive glass by the sol-gel method, for being used in this study and in order to create the better osteogenesis and angiogenesis, is shown in Table1 as follows:

Table1. Percentage of the elemental compounds of the bioactive glass along with the precursors and their amounts [22].

Composition	Percentage in total	Precursor	Brand	Amount of precursor
SiO_2	58	TEOS	Merck	11.24 ml
CaO	26	$CaNO_3.4H_2O$	Merck	2.65 gr
P_2O_5	9	TEP	Merck	5.31 ml
SrO	5	$SrNO_3.2H_2O$	Merck	0.91 gr
CuO	2	$CuNO_3.3H_2O$	Merck	0.39 gr

First, we poured 15 ml of pure ethanol and the amount of TEOS as mentioned in Table 1, 10 ml of distilled water and 5 ml of a 2 M nitric acid into a beaker and allowed 45 minutes for the mixture to mix well on a magnetic stirrer. After 45 minutes, in the second step, we added the amount of TEP as mentioned in Table 1 and then waited for another 45 minutes as the mixing time. After 45 minutes of mixing in the second stage, we added the amount of $CuNO_3.3H_2O$ as mentioned in Table 1 to the mixture and mixed it again for 45 minutes. In the same way, we added other precursors, namely $SrNO_3.2H2O$ and $CuNO_3.3H2O$, with their amounts as mentioned in Table 1, and we waited for 45 minutes of the mixing time for each step. After 45 minutes from the last step of mixing the precursors, a mixture is obtained, which is called a sol. We neutralized the sol by

adding a 1 M ammonia solution drop by drop until a gel is formed. We put the created gel in an oven at 120 °C for two days to evaporate the existing solvents. After two days, we took out the dried gel from the oven and placed it in another oven at 600 °C for two hours, so that the remaining solvents will evaporate, and to have a completely dry product. After taking it out of the furnace, the glass powder is poured into the mortar and pounded until the bioactive glass powder is obtained, and at the last stage, the final powder is passed through the mesh number 325 to obtain the required fine particles. For the particle characterization, the XRD and EDAX analyses were taken.

2.2. Collagen extraction

To extract type 1 collagen, 12 rat tails (obtained from Iran university of medical science, Tehran, Iran) were used and all procedures were performed on ice. In this way, the tails were washed with DI water and then the tendons were separated from the rat's tail with forceps and scissors. The tendons were washed three times in PBS(1X) and then placed in acetone then in ethanol for five minutes. Finally, the tendons were removed from the ethanol and poured into a 0.02 M acetic acid and placed on a stirrer in the refrigerator for 24 hours to dissolve the tendons in acetic acid. After 24 hours, a viscous gel solution was obtained, and to completely dissolve the tendons, the viscous solution was stirred for half an hour using a homogenizer to obtain a homogeneous solution. Then, the solution was passed through the dialysis bag with Kataf 12000. The dialysis operation was performed for 5 days and dialysis fluid was removed each day and after that the gel was removed from the dialysis bag and finally a collagen sponge was obtained by gel lyophilization [23]. For the collagen characterization, the FTIR spectrum, SEM and self-assembly analyses were performed.

2.3. Preparation of hydrogel

In order to investigate the effect of BG nanoparticles, as shown in Table 2, there were two different experimental groups. To prepare the collagen hydrogel with a concentration of 3 mg/ml, first, 9 mg of freeze-dried collagen was poured in 1300 µl of a 0.5 M acetic acid and mixed for 18 hours on a magnetic stirrer at 4°C until a clear solution was obtained. Then it was neutralized with 560 µl of a 1 M NaOH alkaline solution and its pH was adjusted to approximately 7.4. It should be noted that all neutralization steps were performed on ice in order not to damage the collagen structure. The volume of the final neutral solution was 3 ml with PBS (10X) and it was incubated for two hours in the incubator at 37 °C. For preparing the second group including BG, before incubation, BG nanoparticles powder was added to the solution. For the hydrogel characterization, the rheology and porosity analyses were performed.

Table2. Experimental groups of hydrogel with/without the bioactive glass

	Coll3.BG0	Coll3.BG3
BGs	0	3 % w/v
Collagen	3 mg/ml	3 mg/ml

3. Results and discussion

3.1. Bioactive glass XRD

The X-ray diffraction of nanoparticles was done in the range of 3-80 degrees. As it can be seen in Figure1, the peaks relate to the formation of bioactive glass particles are scattered and do not have very sharp diffraction, which indicates the shapeless or amorphous nature of the particles. The peak in the range ($2\theta=30$-40) shows the

typical characteristic of an amorphous silicate glass [24].

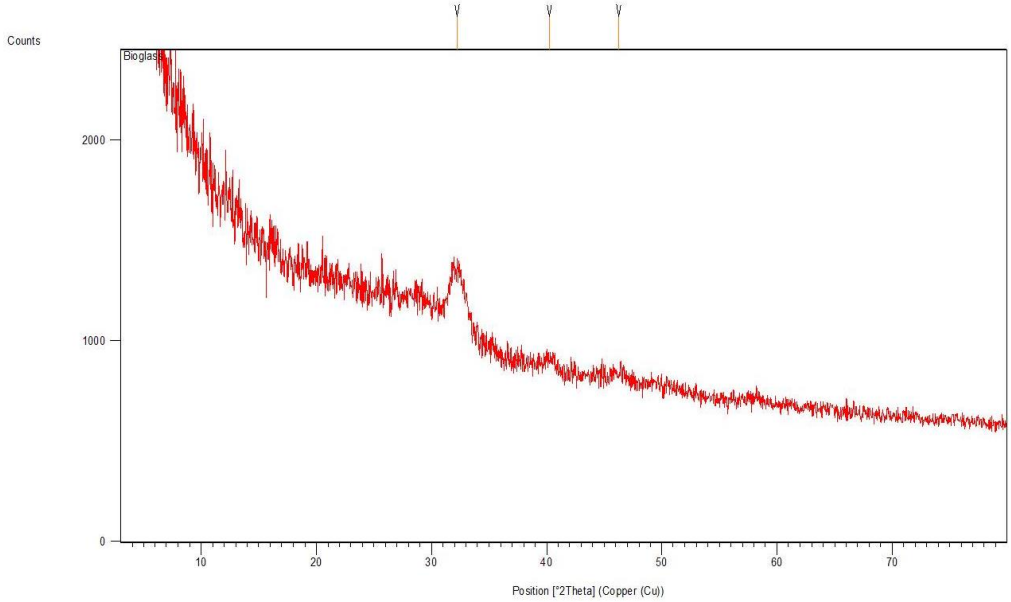

Figure 1. Analysis of the bioactive glass by the X-ray diffraction

3.2. Bioactive glass EDAX

According to the percentage composition that was stated in the bioactive glass synthesis section (Table1), the presence of bioactive glass constituents is confirmed by the EDAX analysis. As it can be seen in the Figure 2, the elements of silicon, calcium, phosphorus, strontium and copper are present in the structure of our synthesized bioactive glass.

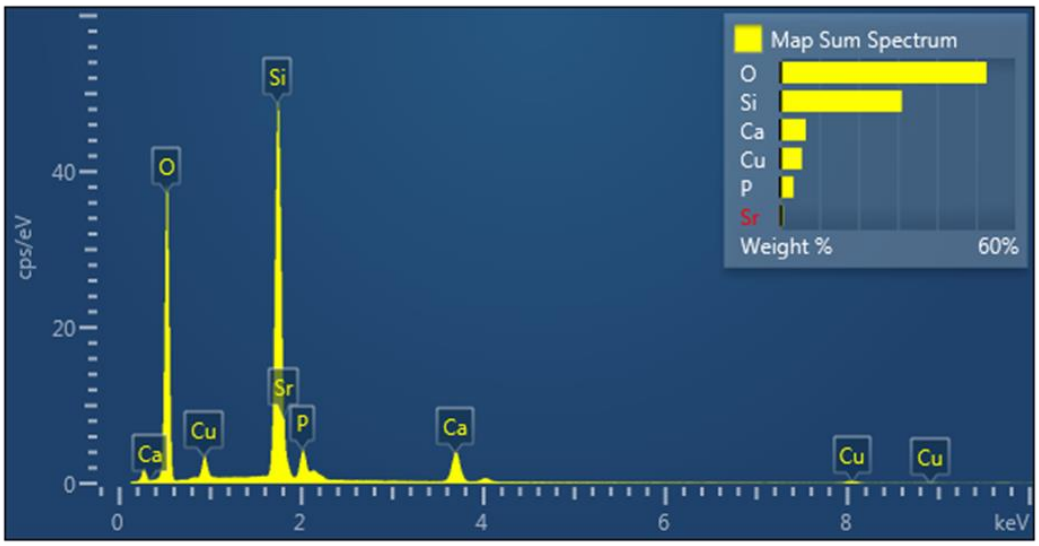

Figure 2. EDAX elemental analysis of the synthesized bioactive glass.

3.3. Collagen FTIR

An FTIR spectrum of the isolated collagen is shown in Figure 3 which shows the typical bands associated with type I collagen: amide A, amide B, amide I, amide II, and amide III. In all collagen samples, amide A bands are observed between 3320 and 3430 cm^{-1} and amide B bands between 2920 and 2960 cm^{-1}. The bands around 1638, 1531, and 1377 cm^{-1} are also found to be related to various amides I, II, and III [25].

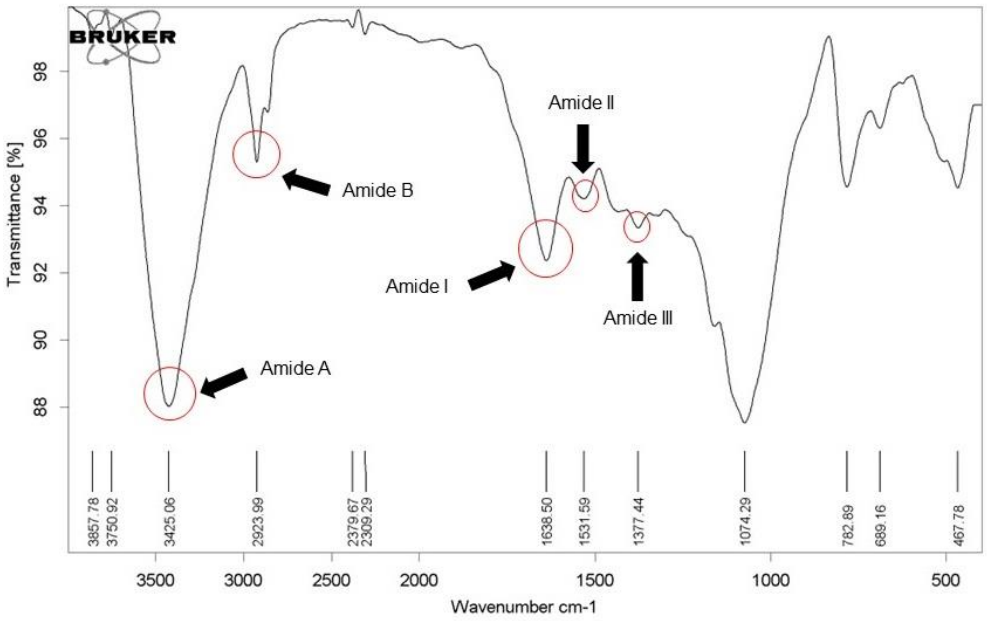

Figure 3. Collagen bands resulted from the FTIR spectrum

3.4. Collagen self-assembly

Figure 4 shows the collagen self-assembly test. When we dissolved the dried collagen in acetic acid and then neutralized it, by placing the solution (3 mg/ml) in a place with the temperature of 37 °C, the collagen solution was crosslinked and the collagen hydrogel was formed, which shows that the collagen structure is fine.

Figure 4. Collagen self-assembly test by forming the crosslinked hydrogel in 37 °C

1.1. Porosity and SEM of the collagen hydrogel

The porosity of collagen hydrogel (3 mg/ml) was measured by the liquid displacement method [26]. Ethanol was used as the displacement liquid as it permeates through collagen scaffolds without shrinking or swelling the matrix. A lyophilized collagen was immersed in a graduated cylinder containing a known volume (V_1) of ethanol. During keeping the sample in the ethanol for 7 minutes, a series of quick evacuation and depressurization cycles were carried out to drive the ethanol into the scaffold's pores. After that, (V_2) represents the total volume of ethanol and the ethanol-impregnated scaffold. After removing the ethanol-impregnated

scaffold from the cylinder, the remaining ethanol volume (V_3) was measured. The porosity of the scaffold (ε) was obtained by equation1:

$$(\varepsilon)\% = \frac{(V_1 - V_3)}{(V_2 - V_3)} \times 100 \qquad (1)$$

The mean porosity of the collagen scaffold was measured five times and obtained as 88 ± 2% which means the high porosity hydrogel has been obtained. As shown in Figure 5, the SEM pictures from dried collagen hydrogel can confirm the mean porosity that has been obtained by equation 1.

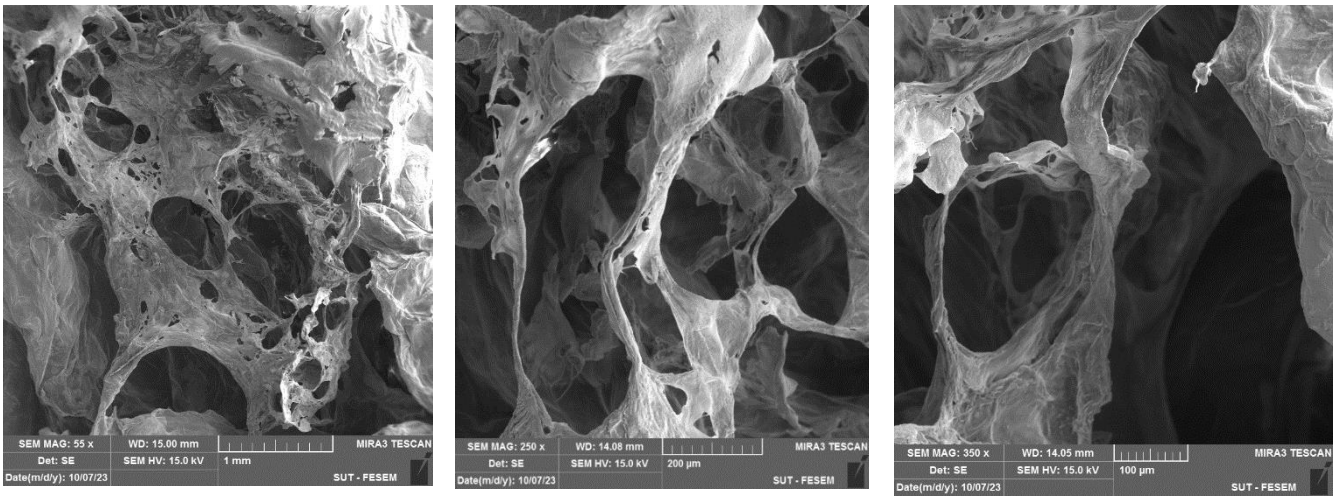

Figure 5. SEM pictures of the dried porous collagen hydrogel

1.2. Rheological behavior of hydrogels

Under tensile strain circumstances, mechanical properties such as storage and loss modulus are dictated by the rheology of the gel, and the point at which the storage modulus and loss modulus meet can be used to calculate the percentage of the gelation loss [27]. At the point where both graphs G' and G" intersect, the material's properties tend to range from elastic to viscous. Additionally, the higher G' indicates better mechanical properties. As it can be seen in Figure 6, the Coll3.BG0 with the elastic modulus of 10^2 pa has poor mechanical properties but when bioactive glass nanoparticles are added, the scaffold Coll3.BG3 shows better mechanical properties. As shown in Figure 5 (B), the addition of 3%w/v of bioactive glass nanoparticles causes a significant increase in the elastic modulus and increases it to 10^3 pa which means 10 times higher than that of Coll3.BG0 and the scaffold Coll3.BG3 loses its gelation property at the higher tensile strain circumstances than that of Coll3.BG0 which means the Coll3.BG3 has more resistance to strain and can keep the gel shape.

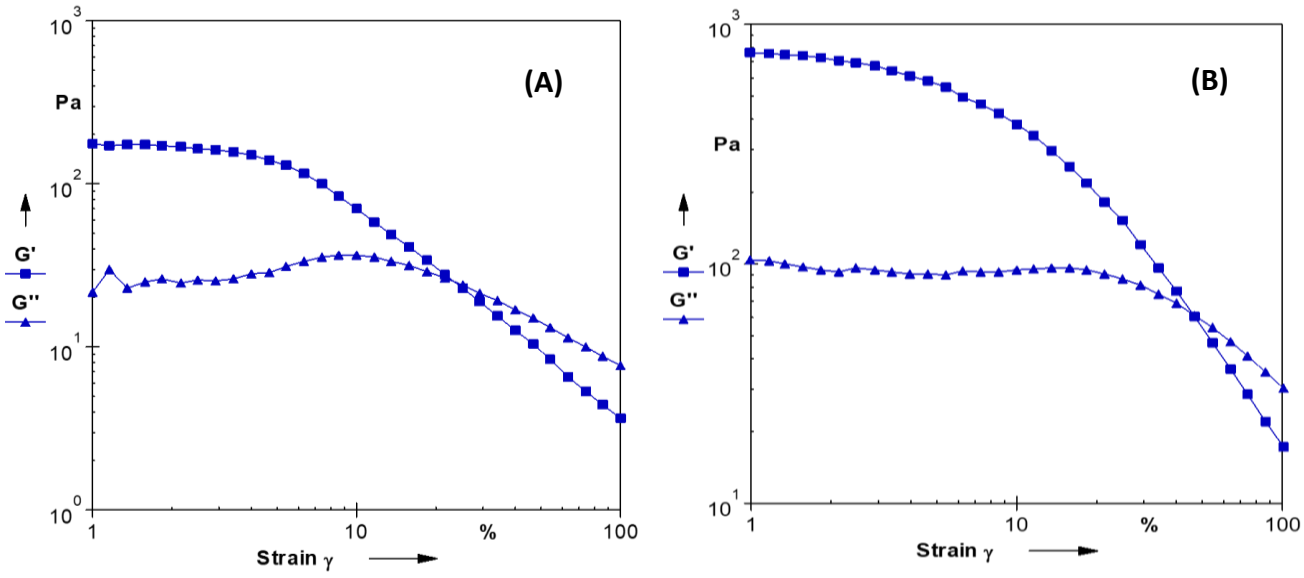

Figure 6. Rheological analysis of the collagen hydrogel scaffold; loss(G") and storage(G') moduli curves according to the strain: (A)Coll3.BG0 (B)Coll3.BG3

2. Conclusion

The extracellular matrix (ECM) is one of the major and influential factors in tissue engineering. Therefore, the hydrogels should play the ECM role in body and provide the necessary conditions for the better cell survival and proliferation. In this study, a collagen hydrogel scaffold with a high porosity (almost 90%) was obtained. The scaffold's high porosity allows the cells to fit snugly inside of it, and a successful mass transfer procedure ensures that the cells receive the nutrients they need. But the porous scaffold usually has poor mechanical properties, and since the ECM of bone is highly elastic, the scaffold with high mechanical properties and stiffness is needed. For this purpose, with the addition of bioactive glass nanoparticles, not only the elastic modulus and mechanical properties of the pure collagen scaffold increase significantly, but the composition of the bioactive glass, which includes copper, strontium, calcium, and phosphorus ions, may increase the osteogenic properties of the scaffold. The pure collagen hydrogel characterization shows 88 $\pm$ 2% porosity and the Coll3.BG3 group (collagen 3 mg/ml + BG 3%w/v) has 10^3 pa elasticity which means 10 times higher than what Coll3.BG0 (pure collagen hydrogel) has and also the hydrogel loses its gel property at higher strain values.

Acknowledgements

The authors thank Dr. Sara Simorgh for supplying the rat tails required.

References

[1] R. Florencio-Silva, G. R. D. S. Sasso, E. Sasso-Cerri, M. J. Simões, and P. S. Cerri, "Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells," *BioMed Research International*, vol. 2015. Hindawi Publishing Corporation, 2015. doi: 10.1155/2015/421746.

[2] B. Clarke, "Normal bone anatomy and physiology.," *Clinical journal of the American Society of Nephrology :*

CJASN, vol. 3 Suppl 3. 2008. doi: 10.2215/CJN.04151206.

[3] B. Health, "A Report of the Surgeon General." [Online]. Available: http://www.surgeongeneral.gov/library

[4] J. J. W. Roche, R. T. Wenn, O. Sahota, and C. G. Moran, "Effect of comorbidities and postoperative complications on mortality after hip fracture in elderly people: Prospective observational cohort study," *Br Med J*, vol. 331, no. 7529, pp. 1374–1376, Dec. 2005, doi: 10.1136/bmj.38643.663843.55.

[5] F. Macedo *et al.*, "Bone metastases: An overview," *Oncol Rev*, vol. 11, no. 1, 2017, doi: 10.4081/oncol.2017.321.

[6] S. Simorgh *et al.*, "Human olfactory mucosa stem cells delivery using a collagen hydrogel: As a potential candidate for bone tissue engineering," *Materials*, vol. 14, no. 14, Jul. 2021, doi: 10.3390/ma14143909.

[7] S. K. Chemical and Biological Microsystems Society (Gyeongju, *MicroTAS 2015*. CBM Society, 2015.

[8] G. Kaur, O. P. Pandey, K. Singh, D. Homa, B. Scott, and G. Pickrell, "A review of bioactive glasses: Their structure, properties, fabrication and apatite formation," *Journal of Biomedical Materials Research - Part A*, vol. 102, no. 1. John Wiley and Sons Inc., pp. 254–274, 2014. doi: 10.1002/jbm.a.34690.

[9] H. Hashemzadeh *et al.*, "Gold nanowires/fibrin nanostructure as microfluidics platforms for enhancing stem cell differentiation: Bio-AFM study," *Micromachines (Basel)*, vol. 11, no. 1, Jan. 2020, doi: 10.3390/mi11010050.

[10] G. Li, T. Zhou, S. Lin, S. Shi, and Y. Lin, "Nanomaterials for Craniofacial and Dental Tissue Engineering," *Journal of Dental Research*, vol. 96, no. 7. SAGE Publications Inc., pp. 725–732, Jul. 01, 2017. doi: 10.1177/0022034517706678.

[11] P. N. Gunawidjaja *et al.*, "Biomimetic apatite mineralization mechanisms of mesoporous bioactive glasses as probed by multinuclear31P,29Si,23Na and13C solid-state NMR," *Journal of Physical Chemistry C*, vol. 114, no. 45, pp. 19345–19356, Nov. 2010, doi: 10.1021/jp105408c.

[12] G. Piotrowski, "Mechanical Studies of the Bone Bioglass Interfacial Bond," 1975.

[13] M. Vallet-Regi and A. J. Salinas, "Mesoporous bioactive glasses for regenerative medicine," *Materials Today Bio*, vol. 11. Elsevier B.V., Jun. 01, 2021. doi: 10.1016/j.mtbio.2021.100121.

[14] L. L. Hench, "The story of Bioglass®," in *Journal of Materials Science: Materials in Medicine*, Nov. 2006, pp. 967–978. doi: 10.1007/s10856-006-0432-z.

[15] F. Westhauser, S. Wilkesmann, Q. Nawaz, S. I. Schmitz, A. Moghaddam, and A. R. Boccaccini, "Osteogenic properties of manganese-doped mesoporous bioactive glass nanoparticles," *J Biomed Mater Res A*, vol. 108, no. 9, pp. 1806–1815, Sep. 2020, doi: 10.1002/jbm.a.36945.

[16] P. Naruphontjirakul, A. E. Porter, and J. R. Jones, "In vitro osteogenesis by intracellular uptake of strontium containing bioactive glass nanoparticles," *Acta Biomater*, vol. 66, pp. 67–80, Jan. 2018, doi: 10.1016/j.actbio.2017.11.008.

[17] C. Stähli, M. James-Bhasin, A. Hoppe, A. R. Boccaccini, and S. N. Nazhat,

"Effect of ion release from Cu-doped 45S5 Bioglass® on 3D endothelial cell morphogenesis," *Acta Biomater*, vol. 19, pp. 15–22, Jun. 2015, doi: 10.1016/j.actbio.2015.03.009.

[18] S. Kargozar *et al.*, "Strontium- and cobalt-substituted bioactive glasses seeded with human umbilical cord perivascular cells to promote bone regeneration via enhanced osteogenic and angiogenic activities," *Acta Biomater*, vol. 58, pp. 502–514, Aug. 2017, doi: 10.1016/j.actbio.2017.06.021.

[19] S. Bano *et al.*, "Synthesis and characterization of silver-strontium (Ag-Sr)-doped mesoporous bioactive glass nanoparticles," *Gels*, vol. 7, no. 2, Jun. 2021, doi: 10.3390/gels7020034.

[20] S. Solgi *et al.*, "Synthesis, Characterization and In Vitro Biological Evaluation of Sol-gel Derived Sr-containing Nano Bioactive Glass," *Silicon*, vol. 9, no. 4, pp. 535–542, Jul. 2017, doi: 10.1007/s12633-015-9291-x.

[21] C. Wu *et al.*, "Copper-containing mesoporous bioactive glass scaffolds with multifunctional properties of angiogenesis capacity, osteostimulation and antibacterial activity," *Biomaterials*, vol. 34, no. 2, pp. 422–433, Jan. 2013, doi: 10.1016/j.biomaterials.2012.09.066.

[22] W. Xia and J. Chang, "Preparation and characterization of nano-bioactive-glasses (NBG) by a quick alkali-mediated sol-gel method," *Mater Lett*, vol. 61, no. 14–15, pp. 3251–3253, Jun. 2007, doi: 10.1016/j.matlet.2006.11.048.

[23] N. Rajan, J. Habermehl, M. F. Coté, C. J. Doillon, and D. Mantovani, "Preparation of ready-to-use, storable and reconstituted type I collagen from rat tail tendon for tissue engineering applications," *Nat Protoc*, vol. 1, no. 6, pp. 2753–2758, Jan. 2007, doi: 10.1038/nprot.2006.430.

[24] J. Chen, L. Zeng, X. Chen, T. Liao, and J. Zheng, "Preparation and characterization of bioactive glass tablets and evaluation of bioactivity and cytotoxicity in vitro," *Bioact Mater*, vol. 3, no. 3, pp. 315–321, Sep. 2018, doi: 10.1016/j.bioactmat.2017.11.004.

[25] P. Noohi, S. S. Mahdavi, M. J. Abdekhodaie, M. H. Nekoofar, and A. Baradaran-Rafii, "Photoreactive Hydrogels Based on Type I Collagen Extracted from Different Sources as Scaffolds for Tissue Engineering Applications: A Comparative Study," *Materialia (Oxf)*, vol. 27, Mar. 2023, doi: 10.1016/j.mtla.2022.101651.

[26] R. Nazarov, H. J. Jin, and D. L. Kaplan, "Porous 3-D scaffolds from regenerated silk fibroin," *Biomacromolecules*, vol. 5, no. 3, pp. 718–726, May 2004, doi: 10.1021/bm034327e.

[27] J. Ahn *et al.*, "3D microfluidic bone tumor microenvironment comprised of hydroxyapatite/fibrin comp," *Front Bioeng Biotechnol*, vol. 7, no. JUL, 2019, doi: 10.3389/fbioe.2019.00168.