Egypt. J. Chem. **Vol. 66**, No. 11 pp. 43-52 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

The Effect of Processing Physical Parameters on the Mechanical, Microstructure and Porosity of Gelatin-HA Scaffolds Produced by Freeze-Drying Technique for Biomedical Applications

Aya Elhelw[1*], Mohamed EL-Henawey[1], Raafat Mandour[2], Maher El-Tonsy[1]

[1] Faculty of Science, Physics Department, Mansoura University, 35516 Mansoura, Egypt

[2]Toxicology Department, emergency hospital, Faculty of Medicine, Mansoura University, 35516 Mansoura, Egypt

Abstract

Scaffolds made for bone tissue engineering are the most preferred way of addressing the complex challenges of bone defect healing. Natural polymers are extensively explored in this area because of their features (Biodegradability, biocompatibility, natural abundance, and high processing ability). Hydroxyapatite (HA) has been employed as a restorative material because of its bone compatibility following implantation. However, the brittleness of HA, which limits its usage, is typical of ceramic materials. As a result, a gelatin layer was applied to the surface of the HA scaffold to provide hardness. This study uses the Freeze-Dryer technique to create porous HA scaffolds doped with gelatin. The scaffolds' microstructures, porosity, and mechanical characteristics were studied. XRD and FTIR measurements prove the presence of HA in the prepared gelatin-HA Scaffolds. The mechanical properties of the studied scaffolds have high value after adding chitosan and starch to the prepared gelatin-HA scaffolds. The porosity of the composite scaffolds was enhanced depending on the strategies used to create them and the cross-linked agent added to the manufactured scaffolds. The results confirm the excellent agreement between the porosity and mechanical properties of the scaffolds, in addition to the excellent agreement between gelatin and hydroxyapatite.

Keywords: Gelatin, Hydroxyapatite, Scaffolds, Bone tissue engineering.

1. Introduction

Natural bone is an organic-inorganic nanoparticle-based bio-composite material. Porous hydroxyapatite (HA) has attracted much interest as a bone substitute material[1] because of its crystallographic and chemical similarities with other calcified tissues in vertebrates. Three-dimensional scaffolds come in various shapes and sizes, and they're frequently employed in tissue engineering for various applications[1, 2].

Scaffolds play an important role in tissue engineering because they may maintain and enhance cell or tissue growth[3, 4]. An ideal scaffold should have the following desirable properties: non-toxicity, biocompatibility, suitable mechanical strength, biodegradation rate that matches tissue regeneration pace, and biodegradable chemicals that do not negatively impact surrounding tissues and organs [6].

Biopolymers containing proteins like collagen, gelatin, fibrin, and silk and polysaccharides like chitosan, starch, hyaluronan, and alginate have been regarded as viable scaffold materials because they resemble the organic component of bone tissue and/or great biological capabilities[5]. Hybrid ceramic/polymer composites, in which bioactive ceramic particles are incorporated in a biodegradable polymer matrix, have received much attention[6-10]. The use of hydroxyapatite (HA) as a filler material in composites is planned to reinforce the polymer, particularly by increasing its stiffness, may turn a non-bioactive polymer into a bone-bonding composite and improve the materials' bone-bonding characteristics, which are critical for attaining early bone in-growth and implant stabilization by bone tissues[7].

Gelatin, a hydrolysis product of collagen (a crucial organic molecule in bone, extracellular matrix (ECM)), is less antigenic than its progenitor but includes part of the arginine–glycine–aspartic acid (RGD) sequence present in collagen, which promotes cellular adhesion, proliferation, and differentiation

Corresponding author e-mail: ayakadri@mans.edu.eg

Receive Date: 27 June 2022, Revise Date: 15 September 2022, Accept Date: 25 September 2022

DOI: 10.21608/EJCHEM.2022.147414.6392

[11].gelatin is a water-soluble natural substance, so it is biocompatible, biodegradable, and inexpensive. It has physical flaws such as low flexibility and form stability [12, 13]. Many techniques of cross-linking, combining it with other biopolymers, or creating composites with inorganic components like hydroxyapatite or bioactive glasses have been proposed to address these issues. Because of these characteristics, gelatin is an excellent candidate for long-term medicine delivery and tissue engineering [14]. In their combined state, chitosan and gelatin interact electrostatically and via hydrogen bonds[15]. Electrostatic interactions occur between the amino groups of chitosan and the carboxyl groups of gelatin. At the same time, hydrogen bonds are formed within and between polymer chains containing carbonyl, hydroxyl, and amino groups[16]. This method relies heavily on porous three-dimensional temporary scaffolding to change cellular activities. Porous structures in tissue engineering scaffolds can be achieved using various methods [14, 17]. Al_2O_3, SiO_2, and TiO_2 are the most widely used aligned porous materials produced using the unidirectional freeze-drying approach.

Aim of the current work: The findings suggest that gelatin is an excellent biomaterial for making aligned porous scaffolds for tissue engineering and a balance between the constructed scaffold's mechanical qualities and porosity. In the current work, the effect of the amount of solvent in the mixture of HA/polymer just before freezing on the physical and morphological properties of the produced scaffold. The amount of solvent in the samples was adjusted by different methods to prevent undesirable factors' effects. Also, the effects of using polymer blend rather than homo-polymer on the porosity as well as the final mechanical strength will be studied.

2. Experimental

Tianjin Chemical Reagent Company supplied all other reagents, while Sigma supplied gelatin powder from bovine skin (Bloom 225, average molecular weight 50,000) and lysozyme (grade III; from chicken egg white). All of the chemicals were used exactly as they were delivered.

2.1. Preparation of Gelatin Scaffolds

10 g gelatin was dissolved in 50 ml distilled water in a flask and kept at 60 ºC until completely swollen, then spun at 350 rpm to generate a homogenous 20: 100 gelatin solution. Then 4 g of HA was added to the gelatin solution and subjected to strong (40 watts) ultrasonic waves for 15 minutes to get a homogenous mixture; hence it was poured into stainless steel molds. The water percentage in these samples should be managed to make a difference in the porosity of these scaffolds, so 4 physical processes were applied to 7 samples of gelatin-HA composite to control the water percentage in each sample. One sample was added to the freeze dryer immediately, two samples were left in the open air for different times, hot water was added to the other two samples with different amounts, starch was added to one sample, and chitosan was added to the last sample. Then the molds were placed in a deep freezer for two days at –18ºC to fix the amount of water until processing the whole samples.

The molds were put in a freeze dryer "lyophiliser" (Martin Christ alpha 1-2 LD plus, Germany) for further two days. The full drying process passes through three phases. During the first, the sample was frozen at (- 40ºC, vacuum 6.4 mbar) for 10 minutes. During the warm-up vacuum pump phase, the sample was exposed to (- 15ºC, vacuum 1.4 mbar) for 20 minutes. Finally, the samples were exposed to a temperature of 30°C and a vacuum of 0.98 mbar for three days which is the main drying phase. The obtained gelatin scaffolds are listed in Table 1.

2.2. Characterization of Gelatin/HA Scaffolds

2.2.1. Density and Porosity

The density and porosity of the scaffolds were determined using the liquid displacement technique. The displacement liquid was hexane, as described by Rina Nazarov[18]. The procedure was as follows: A sample of weight W was submerged in a known volume (V_1) of hexane in a graduated cylinder. Hexane was used to cover the sample for 5 minutes. The hexane and hexane-impregnated scaffold are discussed in volume V_2. The hexane-impregnated scaffold was removed from the cylinder, with the leftover hexane volume recorded as V_3. The density (d) of the scaffold was determined using the formula $d = W/ (V_2 - V_3)$, and the porosity (P) of the scaffold was calculated using the formula $P = 100 *(V_1 - V3) / (V_2 - V_3)$ %

2.2.2. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR absorption spectra in the range 400-4000 cm^{-1} were acquired using the KBr pellets method at room temperature using a Mattson 5000 FTIR spectrometer with a 2 cm^{-1} spectral resolution. A thin disc was created by combining 0.002g of the produced material powder with 0.2g of KBr. At least three samples of each material were tested. The spectrum of each sample is collected through a collection of 20 scans. Different functional groups in the produced samples were identified using FTIR spectra.

Table 1: Weights of processed Gelatin scaffolds before and after the freeze-drying.

Sample name	Wt. of as-prepared samples	time of exposure to air	hot water added	starch added	chitosan added	Wt. of samples after freeze-drying
0	19.28	-	-	-	-	10.35
1	19	2 hr	-	-	-	10.42
2	18.48	4 hr	-	-	-	10.4
3	22.21	-	2 ml	-		10.18
4	23.86	-	4 ml	-	-	10.26
5	22.2	-	-	-	1 gm	10.11
6	21.48	-	-	2 gm	-	10.48

2.2.3. X-Ray Diffraction (XRD)

The X-ray spectroscopy technique is used to detect the crystallinity of the material as well as the type and nature of present crystals. XRD diffraction patterns were recorded using the Brucker Axs-D8 Advance and a CuK radiation source (CuK=0.1540600 nm). Data was collected in step mode with 0.02° intervals throughout a two-dimensional range of 10°-90° with a dwell period of 0.4 seconds. The experimental patterns were compared to a set of standards by a joint committee.

2.2.4. Scanning Electron Microscope (SEM)

Scaffold surface topography, morphology, and microstructure characteristics were explored using a scanning electron microscope (SEM). The JEOL-JSM-6510 LV was used to record the scanned micrographs. The microscope had a 25 kV accelerating potential and a magnification range of 300,000 X. all samples were coated with a thin layer of gold (JCPDS) by a sputtering unit to avoid charging at the sample surface on powder diffraction and standards.

2.2.5. Micro Hardness Measurements (*Hv*)

At room temperature, the produced samples' hardness value (Hv) was evaluated using the SHIMADZU-HMV-G20S microhardness tester (Shimadzu, Kyoto, Japan). At various locations on the surface, ten indentations have been loaded. A high-resolution microscope was used to measure the length of the indentation imprint diagonals, ensuring that the

measurement was accurate. The micro-hardness (Hv) values were calibrated using the formula below:

$$H_V = 1.854\ \frac{F}{d^2}$$

Where Hv is the Vickers hardness in kilograms per millimeter squared, F is the applied force in newtons, and d is the mean diagonal length of the indentation in meters.

3. Results and Discussion

The researchers investigated a newly discovered freeze-drying process for manufacturing porous gelatin scaffolds for tissue engineering applications. The effects of six freeze-drying method process variables on the mechanical characteristics and porosity of the scaffolds were examined. After preparation, the porosity of the prepared scaffolds was adjusted by varying the amount of water in the prepared samples utilizing various effects, such as:
Sample Name. 0: which was placed in the freezer dryer immediately after preparation.
Sample Name. 1: exposure for 2 hours in the air before entering the freeze dryer.
Sample Name. 2: exposure for 4 hours in the air before entering the freeze dryer.
Sample Name. 3: 2 ml hot distilled water was added to the sample before entering it in a freeze dryer
Sample Name. 4: 4 ml hot distilled water was added to the sample before entering it into the freeze dryer.
Sample Name. 5: 1 gram of chitosan was added to the sample before entering it in the freeze dryer.

Sample Name. 6: 2 grams of starch was added to the sample before entering it in the freeze dryer.

Successfully a correlation has been established to quantify the influence of these process variables on the mechanical properties and porosity of the scaffolds.

3.1. FTIR Measurement

The first step in FTIR characterization of Gelatin scaffolds was to characterize the crystal structure and prove the presence of HA (Fig. 1 (a)). Phosphate vibration peaks at 1030–1034 cm^{-1} and phosphate n4 bending vibration peaks at 565 and 604 cm^{-1} are visible in the HA spectra[19].

Gelatin and its composites with HA are shown in (Fig. 1 (b)). As a result of the presence of glycine, proline, and hydroxyproline in the gelatin backbone, pure gelatin had amide (1240, 1540, and 1650 cm^{-1}) and carboxyl (1300–1450 cm^{-1}) bands[20].

With the addition of HA, vibrational bands for OH (630 cm^{-1}) and PO_4 (570, 600, 960, and 1030-1090 cm^{-1}) also formed alongside gelatin bands[21].

Despite different effects being given to the scaffolds as prepared, there was no variation in the gelatin band structure with HA composites, proving that there was minimal chemical interaction between the HA and the gelatin in the composites (see fig.2). The functional groups of gelatin and HA were discovered and marked in this FTIR investigation (Fig. 2). O-H stretch is defined by Lawrie et al., 2007 as a broad band ranging from 3700 cm^{-1} to 3000 cm^{-1}. Peaks at 650 cm^{-1} are also attributed to the O-H stretch. O-H is linked to the presence of HA[22].

The presence of gelatin can also be detected using FTIR. The peak around 1630 cm^{-1} to 1695cm^{-1} is matched with Amide I, confirming the presence of gelatin[23].

The gelatin structure is also represented by COO^- peaks with a band range of 1596 cm^{-1} to 1412 cm^{-1} [24]. As a result, it's proven that the scaffold was made from HA impregnated with gelatin.

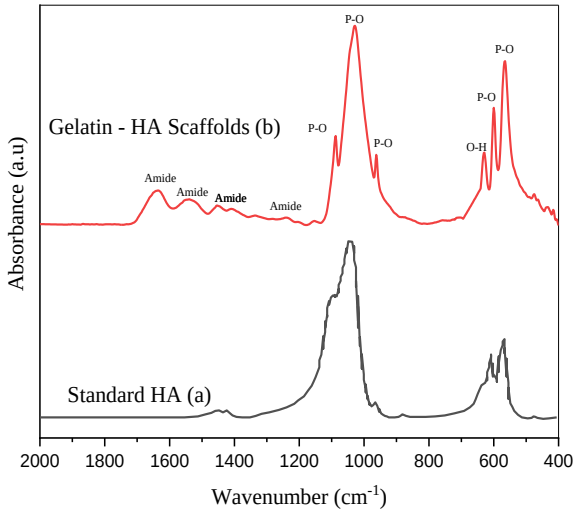

Fig. 1: FTIR spectrographs of the HA and gelatin-HA composites

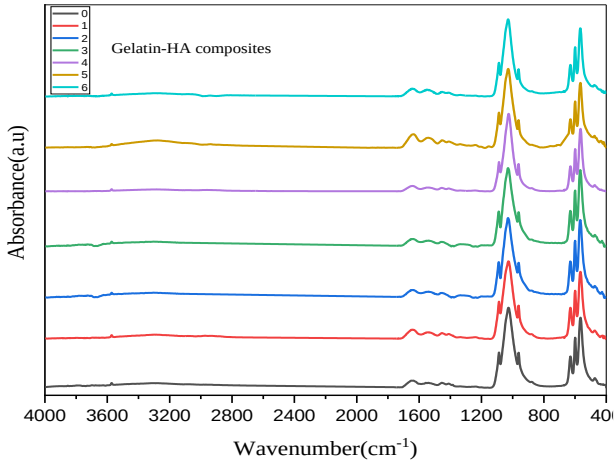

Fig.2: FTIR spectrographs of different samples of gelatin-HA composites

3.2. X-Ray Measurement

XRD patterns were used to determine the phase of the gelatin and gelatin-HA composite scaffolds, as shown in Fig. 3.

A prominent peak at 20° was seen in pure gelatin, which is typical of gelatin[25]. The presence of characteristic HA peaks after HA addition suggests that the solid phase was primarily microcrystalline or disordered, similar to natural bone[26]. The structure of the Gelatin-HA composites does not change when exposed to varied influences, as demonstrated in fig.2, and fig.4 shows the x-ray of different samples of Gelatin-HA composites that are the same and agree

with FTIR fig.2.

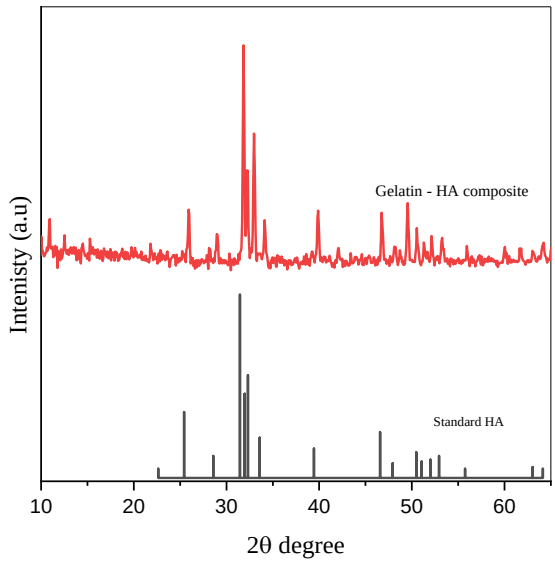

Fig.3: XRD patterns of the Standard HA and selected sample of gelatin- HAcomposite

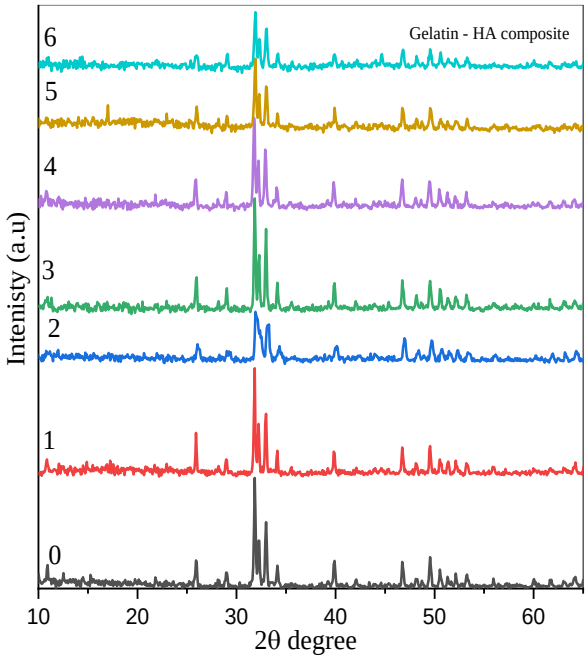

Fig. 4: XRD patterns of different samples of gelatin-HA composite.

3.3. Porosity Measurement

Morphological results reported that the freeze-drying process resulted in an interconnected porous network. This porosity helps cells infiltrate and develop, while smaller pores allow nutrients and bodily fluids to pass through.

The average porosity of Gelatin/Hydroxyapatite composite scaffolds manufactured using various physical treatments yielded varying results.

Fig.5 shows gelatin scaffolds' porosity; all the gelatin scaffolds have high porosity.

Differences in pore size between manufactured scaffolds at different physical processes can be described in terms of ice crystallization due to water in the system, and the amount of cross-linking agent has a practical impact on the porosity and mechanical properties of gelatin scaffolds.

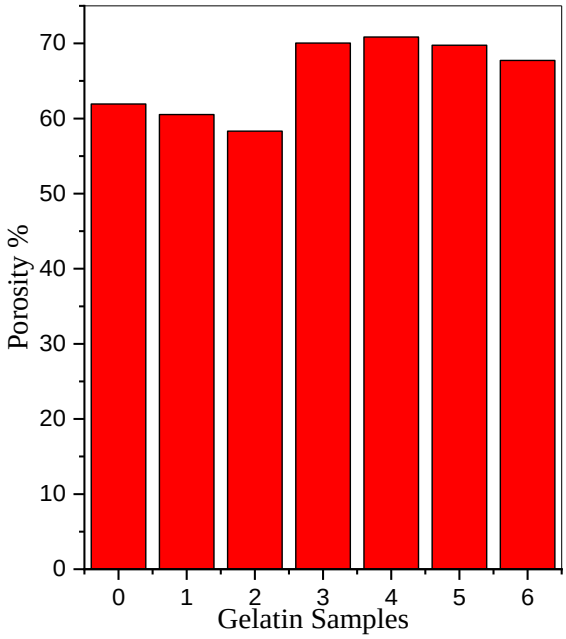

Fig.5. The porosity of gelatin-HA scaffolds.

3.4. Mechanical Properties

The load was applied to the scaffold until it cracked. For each composite scaffold, a test was carried out. Results from the Vickers Hardness test and porosity are summarized in Table 2.

Fig. 6 shows the Hardness values of the as-prepared composite scaffolds. As mentioned previously, they reported the effect on the scaffold's mechanical behavior by the different processes applied to the prepared scaffolds.

Gelatin should be investigated from the perspective of higher mechanical qualities, such as strength and stiffness, obtained from the rigidity of HA particles, as these traits are required for hard tissue applications. By using the gelatin component's form availability and flexibility, the osteoconductivity and bioactivity of HA may also be delivered more effectively[27]. The mechanical information from the samples under static stress is essential for understanding how composite foams evolve. Since recovery and viscoelastic characteristics are required to comprehend the hydrogel features of the composites, such as water absorption and swelling capacity, research into the

mechanical responses of the foams under dynamic loading is also in progress. Although a high percentage of porosity and a large pore size promote bone ingrowth, the mechanical strength of the scaffold is weakened, reducing its mechanical strength. According to Barralet et al., increased porosity contributed to decreased compressive strength (from 37000kPa to 430kPa) and lower Young's Modulus (from 29000MPa to 37MPa)[28].
The results of the composite scaffold's porosity and mechanical characteristics are presented below. The table shows that using a polymer blend from two biopolymers can satisfy the requirements for an ideal scaffold that simultaneously possesses high porosity and good mechanical strength.

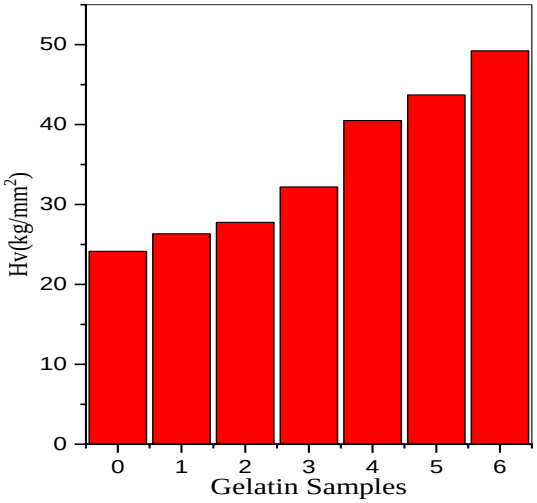

Fig .6 Hardness Values of prepared scaffolds.

Table 2: The porosity percentage and mechanical properties of the composite scaffolds.

Sample	Vickers hardness	Tensile strength MPa	Porosity %
0	24.13	76.98533	61.9310
1	26.33	84.03	60.5212
2	27.76	88.57	58.3170
3	32.2	102.7	70.0364
4	40.5	129.19	70.8459
5	43.7		69.7607
6	49.23	157.05	67.7278

3.5. Analysis of the Scaffold Structure by SEM

Cell proliferation, function, and migration are all important challenges in tissue engineering, and the microstructure of a scaffold has a significant impact on them.
SEM was used to evaluate the morphology and microstructure of composite scaffolds. Figure 7 shows the size of the pores in the composite scaffolds.
SEM cross-sections of porous scaffolds manufactured under four distinct process conditions were used to explore the effects of physical process parameters on the microstructure (Fig. 7).

Pure gelatin had a clean, smooth stem surface with well-developed pores that ranged in size from 400 to 500 µm[28]. When the amount of cross-linking agent was increased, the pore size decreased and became random. Except for the one depicted in instances (5) and (6), all scaffolds had the same pore size[29]. A high viscosity gelatin solution prevented ice crystals from growing in one direction during the freezing process, giving the porous structure an irregular appearance and making the walls of the pores appear thinner. This causes the porous structure to appear more irregular when a high amount of cross-linking agent is used.

Open-pore structure with a significant degree of interconnectivity can be seen in samples (5) and (6). The arranged platelets structure shown in fig. 7. (5) may be responsible for the high mechanical strength of the scaffold, and for the same reason in fig. 7. (6).
The samples' average porosity was assessed to be 70%. Gelatin-HA scaffolds doped with chitosan have ordered pore sizes with about 69.7607 percent porosity. As can be seen, adding chitosan to gelatin increased both pore aperture and porosity of the scaffolds; chitosan chains include a large number of amino groups that appear to strengthen fibers by anchoring them in situ and acting as a cross-link to improve overall matrix integrity.

Recently, biodegradable starch and chitosan-based polymers were presented as having significant potential for various biological applications. When reinforced with bioactive, bone-like ceramic fillers like HA, they can be a viable option for bone tissue regeneration [30]. Adding starch and chitosan to the HA scaffolds might make them less brittle. This is owing to the helical shape of amylose in starch, which, when stretched, forms an open network structure. Starch's recognition significantly influenced the medical profession since it enforced favorable conditions such as biocompatibility, cheap cost, good biodegradability, and non-toxicity. Starch-based polymers have excellent potential to be used in biomedical applications such as bone replacement, bone cement, and bone tissue engineering. Starch has undergone several chemical changes to meet various applications' needs and obtain useful qualities[30, 31]. Chitosan chains include many amino groups that appear to strengthen fibers by anchoring them in situ and acting as a cross-link to improve overall matrix integrity.

4. Conclusion

Gelatin-HA composite scaffolds were made using the Freeze-drying method. The porosity obtained is appropriate for human osteoblast cell growth. The scaffold has the most significant porosity, with noticeable pores under SEM. Pore size, porosity, swelling, degradation, and mechanical strength are variables for suitable manufacturing scaffolds. HA was then utilized to construct bone tissue engineering scaffolds. The scaffolds developed have improved mechanical properties and porosity. The scaffold composition and cross-linking affected the Physico-chemical, morphological, and mechanical properties.
Finally, the research highlights gelatin-doped chitosan and starch composite as promising biomedical scaffold materials.

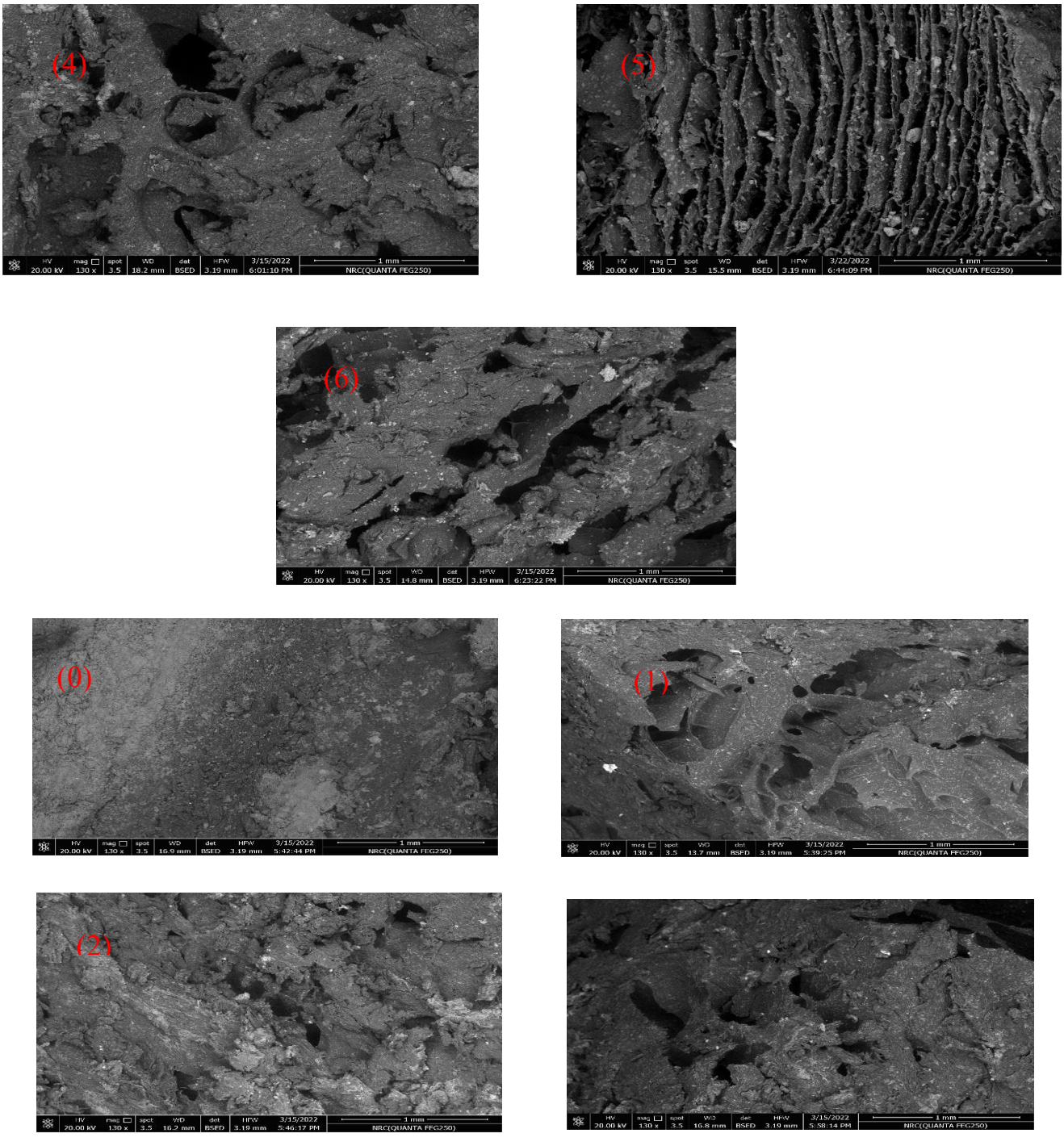

Fig.7 (0–6) shows the SEM morphologies of gelatin-HA composites.

5. References

[1] H.M. Tang, *Porous melt-derived bioactive glass scaffolds for bone regeneration via gel-cast foaming*, (2013).

[2] E. Ahern, T. Doody, K. Ryan, *Bioinspired nanomaterials for bone tissue engineering*, Bioengineered nanomaterials. Boca Raton; London, (2013) 369-412.

[3] Y. Li, Y. Liu, R. Li, H. Bai, Z. Zhu, L. Zhu, C. Zhu, Z. Che, H. Liu, J. Wang, *Collagen-based biomaterials for bone tissue engineering*, Materials & Design, **210** (2021) 110049.

[4] D.T. Dixon, C.T. Gomillion, *Conductive Scaffolds for Bone Tissue Engineering: Current State and Future Outlook*, Journal of Functional Biomaterials, **13** (2021) 1.

[5] S. Pina, R.L. Reis, J.M. Oliveira, *Natural polymeric biomaterials for tissue engineering*, in: Tissue Engineering Using Ceramics and Polymers, Elsevier, 2022, pp. 75-110.

[6] M.C. Biswas, B. Jony, P.K. Nandy, R.A. Chowdhury, S. Halder, D. Kumar, S. Ramakrishna, M. Hassan, M.A. Ahsan, M.E. Hoque, *Recent Advancement of Biopolymers and Their Potential Biomedical Applications*, Journal of Polymers and the Environment, (2021) 1-24.

[7] M. Swetha, K. Sahithi, A. Moorthi, N. Srinivasan, K. Ramasamy, N. Selvamurugan, *Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering*, International journal of biological macromolecules, **47** (2010) 1-4.

[8] G.E. Damrawi, R.M. Ramadan, M.E. Baiomy, *Structural Role of Strontium Oxide in Modified Silicate Glasses*, Silicon, **14** (2022) 4879-4885.

[9] G. El Damrawi, R.M. Ramadan, M. El Baiomy, *Effect of SrO on the Structure of Apatite and Wollastonite Phases of Na2O-CaO-SiO2-P2O5 Glass System*, New Journal of Glass and Ceramics, **11** (2021) 45-56.

[10] G. El Damrawi, M. El Baiomy, R.M. Ramadan, *Structural Characterization of Strontium Silicate Glasses and Glass Ceramics Applied as Glass Ionomer Cements (GICs)*, Silicon, (2022) 1-11.

[11] D. Qin, N. Wang, X.-G. You, A.-D. Zhang, X.-G. Chen, Y. Liu, *Collagen-based biocomposites inspired by bone hierarchical structures for advanced bone regeneration: Ongoing research and perspectives*, Biomaterials Science, (2022).

[12] A.B. Bello, D. Kim, D. Kim, H. Park, S.-H. Lee, *Engineering and functionalization of gelatin biomaterials: From cell culture to medical applications*, Tissue Engineering Part B: Reviews, **26** (2020) 164-180.

[13] M. Maisani, D. Pezzoli, O. Chassande, D. Mantovani, *Cellularizing hydrogel-based scaffolds to repair bone tissue: How to create a physiologically relevant micro-environment?*, Journal of Tissue Engineering, **8** (2017) 2041731417712073.

[14] M.N. Collins, G. Ren, K. Young, S. Pina, R.L. Reis, J.M. Oliveira, *Scaffold fabrication technologies and structure/function properties in bone tissue engineering*, Advanced Functional Materials, **31** (2021) 2010609.

[15] D. Li, H. Cui, K. Hayat, X. Zhang, C.-T. Ho, *Superior environmental stability of gelatin/CMC complex coacervated microcapsules via chitosan electrostatic modification*, Food Hydrocolloids, **124** (2022) 107341.

[16] C.D. Moreira, S.M. Carvalho, R.G. Sousa, H.S. Mansur, M.M. Pereira, *Nanostructured chitosan/gelatin/bioactive glass in situ forming hydrogel composites as a potential injectable matrix for bone tissue engineering*, Materials Chemistry and Physics, **218** (2018) 304-316.

[17] A. Haider, S. Haider, M.R. Kummara, T. Kamal, A.-A.A. Alghyamah, F.J. Iftikhar, B. Bano, N. Khan, M.A. Afridi, S.S. Han, *Advances in the scaffolds fabrication techniques using biocompatible polymers and their biomedical application: A technical and statistical review*, Journal of saudi chemical society, **24** (2020) 186-215.

[18] Q. Lv, Q. Feng, *Preparation of 3-D regenerated fibroin scaffolds with freeze drying method and freeze drying/foaming technique*, Journal of Materials Science: Materials in Medicine, **17** (2006) 1349-1356.

[19] F. Alhamoudi, H. Akhtar, Y. Almoshawah, A. Talari, A. Chaudhry, G. Reilly, I. Rehman, *Bioactive Nano Hydroxyapatites for Orbital Floor Repair and Regeneration*, (2021).

[20] H.W. Kim, J.C. Knowles, H.E. Kim, *Hydroxyapatite and gelatin composite foams processed via novel freeze-drying and cross-linking for use as temporary hard tissue scaffolds*, Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, **72** (2005) 136-145.

[21] M.K. Mintu, *Comparison of Physical and Chemical Properties of Five Different*

Alloplastic Bone Graft Materials, in, Sree Mookambika Institute of Dental Sciences, Kulasekharam, 2013.

[22] V. Máková, *Hybrid organosilane materials and its application in material engineering*, (2021).

[23] T. Riaz, R. Zeeshan, F. Zarif, K. Ilyas, N. Muhammad, S.Z. Safi, A. Rahim, S.A. Rizvi, I.U. Rehman, *FTIR analysis of natural and synthetic collagen*, Applied Spectroscopy Reviews, **53** (2018) 703-746.

[24] S. FARAJI, *Activated Carbon Nano-Fiber from Polymers*, (2014).

[25] S. Pawde, K. Deshmukh, *Characterization of polyvinyl alcohol/gelatin blend hydrogel films for biomedical applications*, Journal of applied polymer science, **109** (2008) 3431-3437.

[26] P. Ma, W. Wu, Y. Wei, L. Ren, S. Lin, J. Wu, *Biomimetic gelatin/chitosan/polyvinyl alcohol/nano-hydroxyapatite scaffolds for bone tissue engineering*, Materials & Design, **207** (2021) 109865.

[27] M. C Echave, L. S Burgo, J. L Pedraz, G. Orive, *Gelatin as biomaterial for tissue engineering*, Current pharmaceutical design, **23** (2017) 3567-3584.

[28] K. Razali, N.M. Nasir, E. Cheng, N. Mamat, M. Mazalan, Y. Wahab, M.M. Roslan, *The effect of gelatin and hydroxyapatite ratios on the scaffolds' porosity and mechanical properties*, in: 2014 IEEE Conference on Biomedical Engineering and Sciences (IECBES), IEEE, 2014, pp. 256-259.

[29] S. Priya, U. Batra, R. Samshritha, S. Sharma, A. Chaurasiya, G. Singhvi, *Polysaccharide-based nanofibers for pharmaceutical and biomedical applications: A review*, International Journal of Biological Macromolecules, (2022).

[30] J. Sundaram, T.D. Durance, R. Wang, *Porous scaffold of gelatin–starch with nanohydroxyapatite composite processed via novel microwave vacuum drying*, Acta biomaterialia, **4** (2008) 932-942.

[31] D. Puppi, F. Chiellini, A.M. Piras, E. Chiellini, *Polymeric materials for bone and cartilage repair*, Progress in polymer Science, **35** (2010) 403-440.