RESEARCH PAPER

Fabrication of Porous Biologic Hydroxyapatite Scaffold Reinforced with Polymer Coating for Bone Tissue Engineering Candidate

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Abstract: *Hydroxyapatite (HA) scaffold is commonly used in the applications of bone tissue engineering due to its bioactivity and equivalent chemical composition to the inorganic constituents of human bone. The present study focused on the fabrication of porous 3D hydroxyapatite scaffold which was modified by polymer coating as a successful strategy to improve the mechanical properties. A 3D porous hydroxyapatite scaffolds were fabricated by gel-casting method by using freshly extracted egg yolk (EY) with (50 and 60) wt% of HA powder. To enhance the mechanical properties, composite PVA/HA scaffolds were produced by using dip coating in Polyvinyl alcohol (PVA). Fourier transform infrared spectroscopy (FTIR) was used to recognize the functional group associated with the hydroxyapatite scaffolds before and after PVA coating. The physical (density and porosity) and mechanical (compressive strength and elastic modulus) properties were investigated before and after coating. SEM was used to inspect the surface morphology and pore modification of the scaffolds. Wettability was determined by using a water contact angle to analyze the scaffold hydrophobicity. Surface roughness was studied by atomic force microscopy (AFM). It was revealed that the scaffold porosity decreased with increased solid loading of HA powder in the gel and after PVA coating. The findings showed that PVA coating improved mechanical strength of scaffold to be double by covering the small pores and filling microcracks sited on the scaffold strut surfaces, inducing a crack bridging mechanism. The scaffolds' strength was in the range of trabecular bone strength. This indicates non-load bearing applications.*

Keywords: *Biologic hydroxyapatite, Gel-casting, Egg yolk, Polyvinyl alcohol, 3D porous scaffold.*

1. INTRODUCTION

A porous scaffold is considered an important requirement for tissue and organ regeneration. There are several factors that must be taken into consideration when fabricating scaffold that reflects the role successful performance for bone reconstruction and tissue engineering. Hence, porosity and pores interconnectivity control the scaffold biodegradability. They also enhance cell attachment, growth, and metabolic activity. Furthermore, the scaffold should provide suitable mechanical properties that mimic the damaged tissues and support the regeneration process [1]. Therefore, many researchers focused on finding suitable materials and designs to create scaffolds that have biological and mechanical properties as well as provide cell penetration and capillary ingrowth.

Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$] is a bioceramic material that has structural and functional similarities to the inorganic constituents of human teeth and bones. Thus, it represents a suitable choice for orthopedic, dental, and maxillofacial applications because it can support bone tissue regeneration [2]. HA can be produced from natural and synthetic sources, natural resources such as animal bones, eggshells and marine mollusc shells could be used as, it is believed that, they offer more biocompatible and biologically safe [3, 4]. Many studies prepared 3D porous HA scaffolds for bone substitute materials using numerous processing techniques, i.e., replica, sacrificial template, and direct foaming [5]. Nowadays, researchers attempt to discover simple, low cost and environmental-friendly methods.

Gel-Casting is a promising technique to produce a 3D complex shape which was developed to overcome some of the limitations of other complex-shape production techniques [6]. It can produce 3D porous solid structures of ceramic with relatively acceptable green mechanical strengths and low shrinkage at high solid loading conditions resulting in good handling properties with few defects [7]. The achievement of this method depends on the concentration of powder solid-loading which is preferred to be more than

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50 Vol% as well as on the low suspension viscosity and suitable fluidity that is necessary for the casting and shaping process[8]. The idea of employing egg white and yolk as excellent binder materials as well as organic pore creating agents to achieve the desired porosity was used by previous research [9-12]. Those researches demonstrated that proteins in egg white and yolk coagulate and solidify during heating to 60-70°C. This fact encouraged using egg yolk to be a favorite binder for holding elements together. Moreover, egg yolk offers a moisture film between particles that facilitates the forming process [10]. Many studies consistently showed that HA is osteogenic and osteoconductive, but its brittleness and non-elastic nature make it difficult to be manufactured into different shapes and sizes [13]. Sometimes, for porous ceramic scaffolds, the use of a polymeric coating on the scaffold struts improves the mechanical strength and fracture toughness by making the structure more elastic and tough, increasing the scaffold's performance and reliability [14, 15].

Accordingly, HA scaffold structure was reinforced by combination with an elastic component such as a biodegradable polymer coating, reducing the fragility and improving the compressive strength [16]. The biopolymers coating process can be done by physical or chemical techniques.

The strategy of polymer physical coating, is a direct deposition of the biopolymer onto the substrate using different techniques, e.g., spin coating, dip coating, electrospinning, and vapor transport [17]. In this study, 3D porous scaffold from biologic HA was synthesized via gel-casting using egg yolk as a binder and organic pore forming agent materials. HA powder of (50, 60) Wt.% was used to fabricate scaffolds. A dip coating by Polyvinyl alcohol (PVA) was applied to prepare composite scaffolds. The effect of HA wt.% solid loading and PVA reinforcing was investigated by using Fourier Transformed Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) techniques. The physical properties (density, porosity, and scaffold wettability) as well as the mechanical properties (Compressive Strength and Elastic Modulus) were studied. Surface topography in terms of surface area and roughness was investigated by Atomic Force Microscopy (AFM).

2. EXPERIMENTAL PROCEDURES

2.1. Fabrication of Hydroxyapatite Porous Scaffold

The preparation and X-Ray characterization to confirm the purity of biological hydroxyapatite powder from bovine bone was described extensively in the previous paper [18]. The final particle size (D_{50}) was 1.19 µm. Porous HA scaffold was fabricated by using the gel-casting technique as per the following procedure: a fresh egg yolk (EY) was used in a beaker and stirred on a magnetic stirrer for 5 minutes. The stirring speeds were manageable to avoid any foaming of EY. DarvanC (as a dispersant) was utilized in the ratio [(1 ml) of DarvanC per (10 gm) of the HA powder].

The hydroxyapatite powder was combined with the yolk to form slurry and then the slurry was stirred for 5 minutes. After that, the slurry was cast in the molds and dried in the oven at 70°C for 1 hr. Two different solid loading percentages (50, 60 wt./vol.%) of HA powder were used.

The solid loadings were selected after many rough experiments and calculations to get castable gel at the specified casting temperature. The green sample was left to age at room temperature for 48 hrs before de-moulding. Sintering processes included two steps: calcination of the green sample at 600°C to burn out the yolk according to [9], then sintering the sample at 1000°C.

2.2. Polyvinyl Alcohol (PVA) Film Coating

The biopolymers coating process requires preparing an aqueous solution. The properties of the prepared solution in terms of solubility and viscosity depend on the molecular weight of the biopolymer [17]. PVA was supplied from CDH (Central Drug House).

It has Mw (Molecular weight) of 125000 g/mol. The solution was prepared by dissolving (5) g of PVA powder in 100 mL distilled water using a stirrer and heating to 50°C. After cooling the solution to room temperature, the two groups of sintered HA scaffolds were immersed for 10 min in the PVA solution (100 mL) using an ultrasonic path to get homogeneous coatings. Then, the scaffolds were picked up and dried in a Furness at 80°C for 3 hr. The scaffold groups are named in Table 1:

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Table 1. Scaffold groups parameters

Scaffold parameters	Scaffold name
50% HA solid loading	A
50% HA solid loading +PVA coating	AC
60% HA solid loading	B
60% HA solid loading +PVA coating	BC

2.3. Characterization

2.3.1. Density and porosity

Scaffolds density and apparent porosity before and after PVA coating were calculated by Archimedes method according to ASTM C20 [19], using the equations below:

$$\text{Density} = W_D/(W_S - W_I) \quad \text{(Eq.1)}$$

$$\%\text{Apparent Porosity} = (W_S - W_D / W_S - W_I) \times 100. \quad \text{(Eq. 2)}$$

Where: (W_D) weight of dry scaffold, (W_S) scaffold soaked weight and (W_I) scaffold immersed weight.

2.3.2. Mechanical properties

The mechanical properties of the fabricated scaffolds with and without PVA coating were estimated by testing maximum compressive strength and elastic modulus, (Microcomputer controlled electronic Universal Testing Machine-CHINA). A mechanical tester with 0.1 mm/sec crosshead speed was used; three samples, at least, were tested to get the average strength value.

2.3.3. Scanning electron microscopy (SEM)

The morphology of all scaffold groups was described by using Scanning Electron Microscopy (SEM) (TESCAN, Performance in nanospace, type VEGA 3 SBU) after sputter coating with a thin layer of gold (for 30 sec).

2.3.4. Roughness measurement

Atomic Force Microscopy (AFM) was used to characterize topography features for scaffold's surface before and after PVA coating. The principle of AFM was based on mechanical contact between the surface of the scaffold and tip. The test was carried out by tapping mode AFM (AA3000). The results were represented by measuring scaffold's surface roughness denoted as average roughness (Ra), and surface area

2.3.5. Fourier transformed infrared spectroscopy (FTIR)

The functional groups present in the scaffolds before and after PVA coating were recognized by FTIR (Fourier Transform Infrared Spectrophotometer, SHIMADZU). The absorbance mode in the range of 4000 and 400 cm^{-1} was used to record spectra. Scaffolds were pulverized and mixed with KBr.Then the powder mixture was pressed into pellets.

2.3.6. Contact angle

It has been stated that many biological properties of materials like protein adsorption and cell response including (attachment, migration, and spreading) significantly are related to surface morphology and hydrophilicity [20-22]. For this reason, wettability of the scaffolds was inspected by measuring the contact angle with the sessile drop of distilled water which was put on the surface of the scaffold before and after coating by using optical contact angle and interface tension meter (USA KINO Industry Co. Ltd). A drop of distal water (2 µl) was deposited onto the surface of the scaffolds and the measurement was done after 5 sec for all samples. The reported data is a result of five measurements.

3. RESULTS AND DISCUSSION

3.1. Physical and Mechanical Properties

The porous structure is an essential requirement for bone tissue engineering. Many researchers identified that porous scaffolds are considered important materials for biological applications. According to (Fig. 1) the density of the scaffolds increased with increasing HA solid loading before and after PVA polymer coating. (Fig. 2) confirmed the relation between density and porosity. The apparent porosity of the scaffolds was found to decrease from (61%) for scaffold A to (36%) for scaffold B in terms of increasing HA solid loading and decreasing the amount of binder (yolk) that fired and evaporated during sintering. So, both scaffolds were in the range of the required porosity (40-90%) for bone tissue engineering scaffold that encourages osteointegration and bonding with surrounding tissue [23]. However, porosity calculations showed a reduction in apparent porosity for coated scaffolds (AC and BC) compared to uncoated ones (A and B). The SEM images about

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PVA polymer coating effect indicated that the polymer layer clogged the small pores and enclosed the large pores, leading to a reduction in pore volume and porosity.

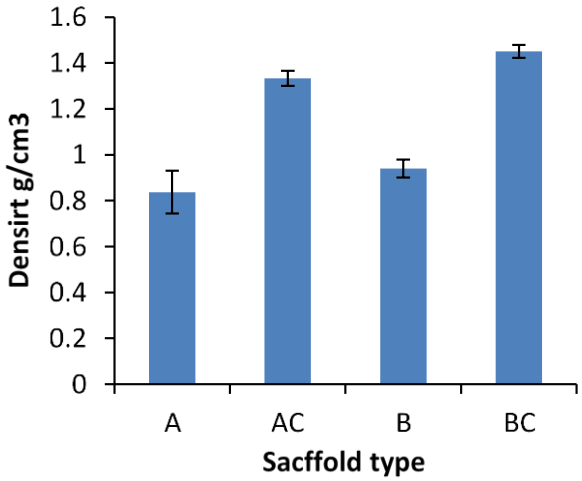

Fig. 1. Density of scaffolds before and after PVA coating

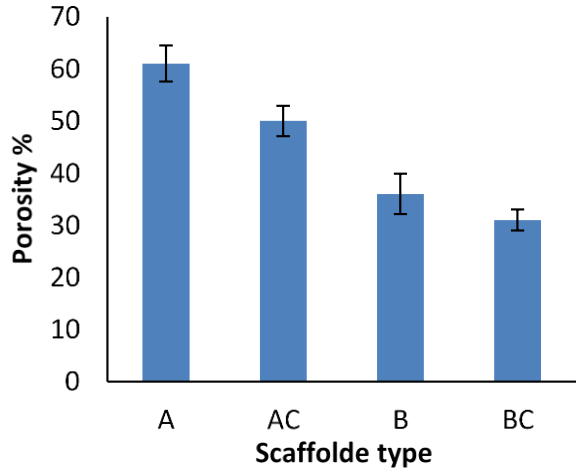

Fig. 2. Porosity of scaffolds before and after PVA coating

It has been stated that poor mechanical properties of porous ceramic scaffold are related to brittleness of solid volume and high void space of pores. Surface modification by bio-polymer dip-coating is an attractive strategy to overcome the brittleness and improve mechanical strength with a slight reduction in porosity and crack blocking. (Fig.3) and (Fig.4) show the Max. compressive strength and Elastic Modulus for all scaffolds, there is an increment in compressive strength (up to 1.23 and 1.51)MPa for coated scaffolds (AC and BC) respectively, with improvement of Elastic modulus as compared to uncoated scaffolds A and B.

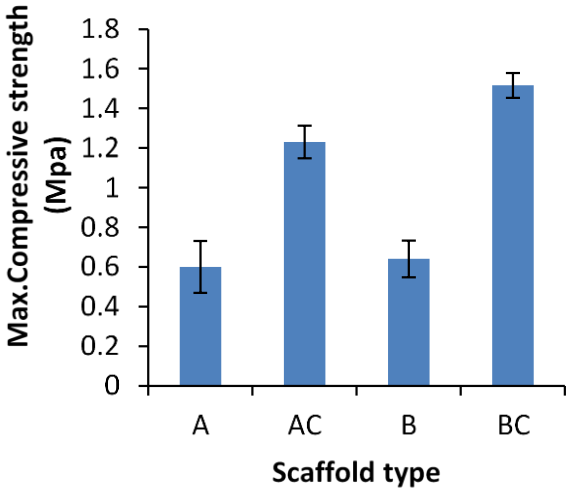

Fig. 3. Max. Compressive strength for uncoated and coated scaffolds

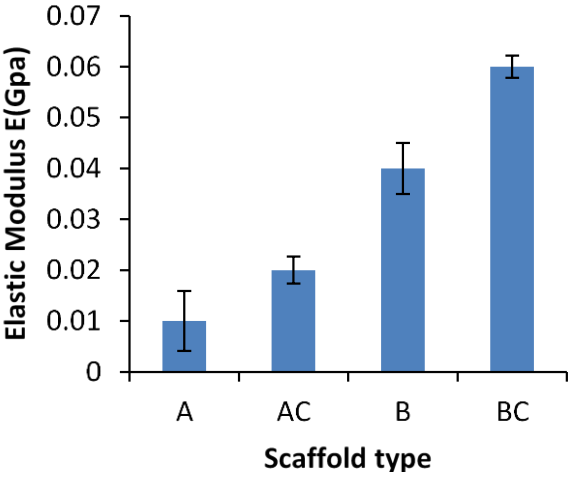

Fig. 4. Elastic modulus for uncoated and coated scaffolds

(Fig. 5) shows the stress-strain behavior for all scaffolds. A possible reason for this performance is the strengthening and toughening effects of polymer layer which covers the small pores and fills microcracks sited on the scaffold strut surfaces that induced a crack bridging mechanism. It is similar to the collagen fiber's role in natural bone [24, 25].

These results are similar to that reported by Pezzotti et al. [26] who toughened hydroxyapatite by the crack-bridging mechanism using poly (methyl methacrylate) and Liverani et.al. [27], Fu et al. [28] got an enhancement in mechanical performance by applying polymer coating to a bioglass scaffold. The physical and mechanical properties of all scaffolds are summarized in Table 2.

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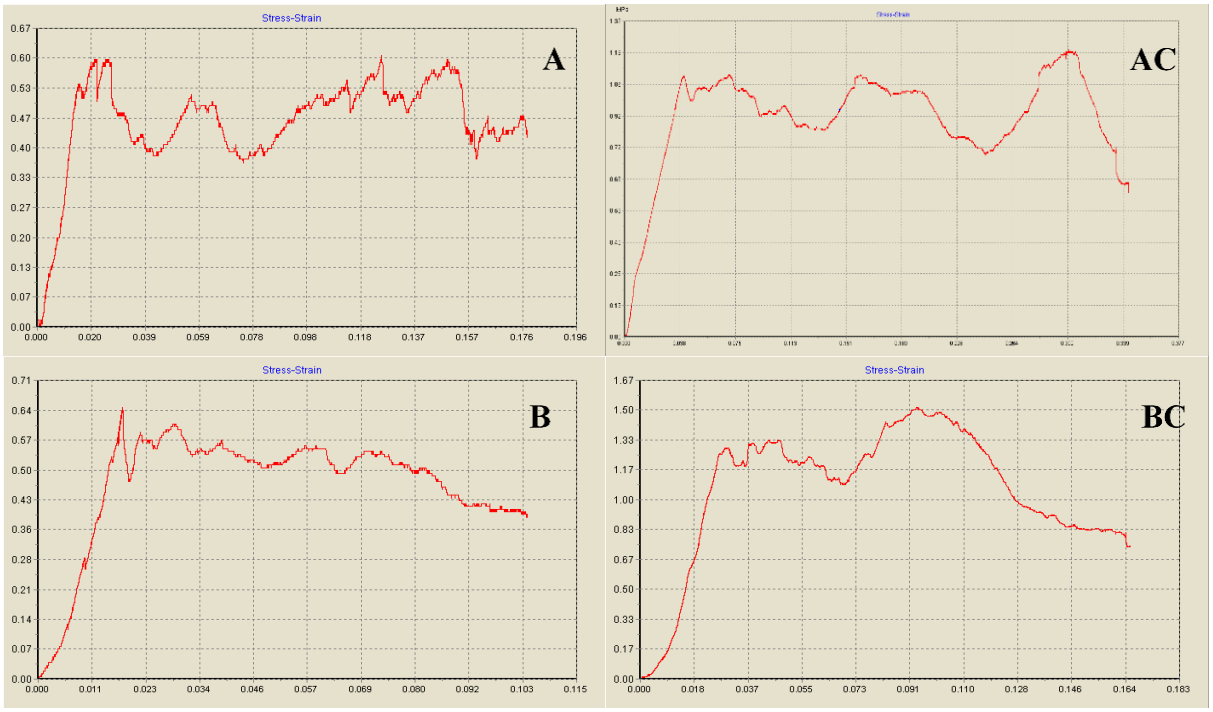

Fig. 5. Typical stress–strain curves for A, B (uncoated scaffolds), and AC, BC (coated scaffolds)

Table 2. Physical and mechanical properties for scaffolds before and after PVA coating

Scaffold type	Density (g/cm^3)	Porosity%	Compressive strength (MPa)	Elastic modulus(GPa)
A	0.84±0.09	61±3.4	0.6±0.13	0.01±0.005
AC	1.33±0.03	50±2.9	1.23±0.08	0.02±0.003
B	0.94±0.04	36±3.8	0.64±0.09	0.04±.004
BC	1.45±0.02	31±2	1.516±0.06	0.06±.002

3.2. FTIR Analysis

(Fig. 6) represents FTIR spectra of hydroxyapatite scaffolds before and after coating by PVA (A and AC) respectively. The main peaks related to hydroxyl groups were observed at (3572, 3425, 2368.5, 2368, 2075, 1998, 972) cm^{-1}, while peaks at (2939, 1458) cm^{-1} assigned to stretching C-H, and peaks at (586, 540) cm^{-1} linked to =C-H. FTIR results indicate that all coated scaffolds exhibit the characteristic bands of PVA polymer. The stretching vibration bands of a phosphate group (PO_4^{-3}) in hydroxyapatite were detected at (571, 987.55, and 1072.42) cm^{-1} respectively. The weak band around 1620 cm^{-1} corresponds to H_2O absorption. The peaks at about 1411 and 1458.1 cm^{-1} were attributed to asymmetric stretching and bending vibrations of the carbonate group.

3.3. Microstructure Characterization

SEM images of the (50 wt%) HA scaffolds are shown in (Fig. 7 (a and b)). The uncoated scaffold (A), exhibits high porosity (61%) with micro to nano pores which is stated to be appropriate for the applications of bone tissue engineering. It is well known that Micropores (< 20 µm) encourage the surface for protein adsorption and cell attachment. Also, Nanopores (5-50 nm) are important for the crystallization of the apatite bonding layer [29, 30]. After the coating process, (Fig. 7 (b)) shows that most of the open pores were maintained. However, the small pores were nearly closed by the PVA layer explaining the reduction of porosity to (50%). While the surface images for the (60 wt%) HA scaffold as shown in (Fig. 8(a)) confirm the lower porosity (36%) and smaller pore size for scaffold B than scaffold A. Also, the PVA coating blocked the small pores leading to reduce the porosity to (31%) as it is clear in (Fig. 8(b)). It has been stated that scaffold topography has a large effect on cell adhesion and mechanical properties. Thus it is important to control the scaffold design by paying close attention to the porosity and pore size [31].

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(Fig. 9) shows a 3D surface topography for all scaffolds using AFM. The measurements performed by image quantitative analysis as in Table 3 demonstrates that surface roughness for PVA coated scaffolds (AC and BC) is smaller than for uncoated scaffolds (A and B). These results are supported by SEM images as the PVA coating layer blocked the small pores and minimized the large pores. It is worth pointing out that the roughness of the A and AC scaffolds (58.9 and 49.8) nm are higher than B and BC scaffolds (31.7 and 11.8) nm respectively.

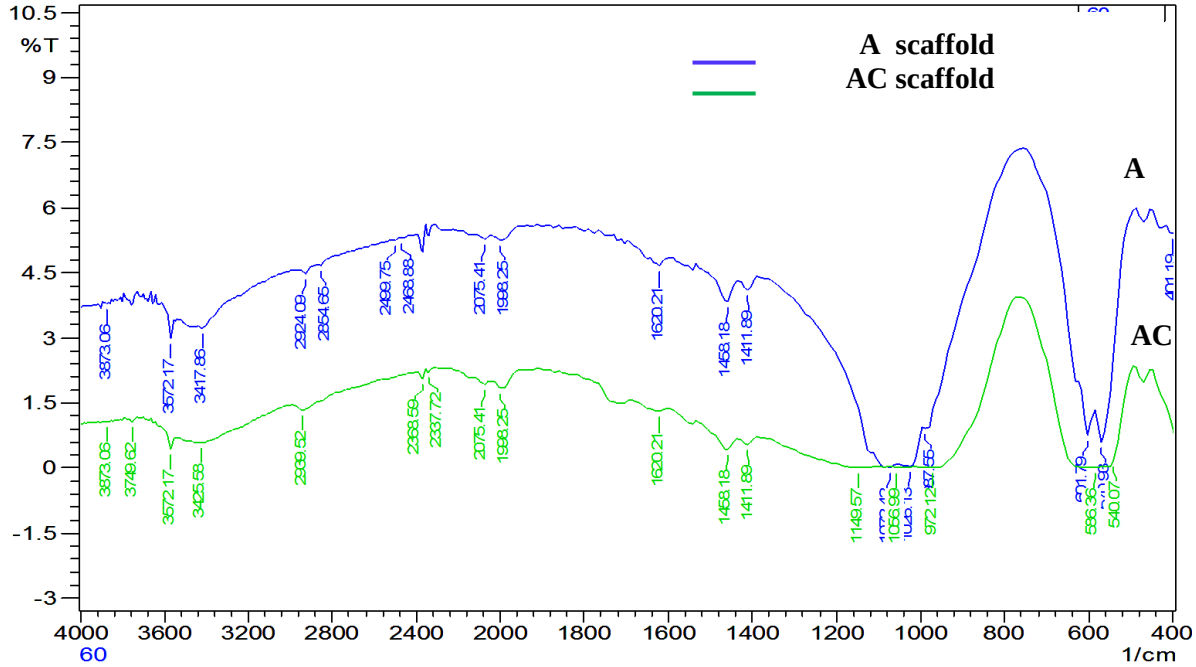

Fig. 6. FTIR spectrogram of scaffold (A) before coating and Scaffold (AC) after coating

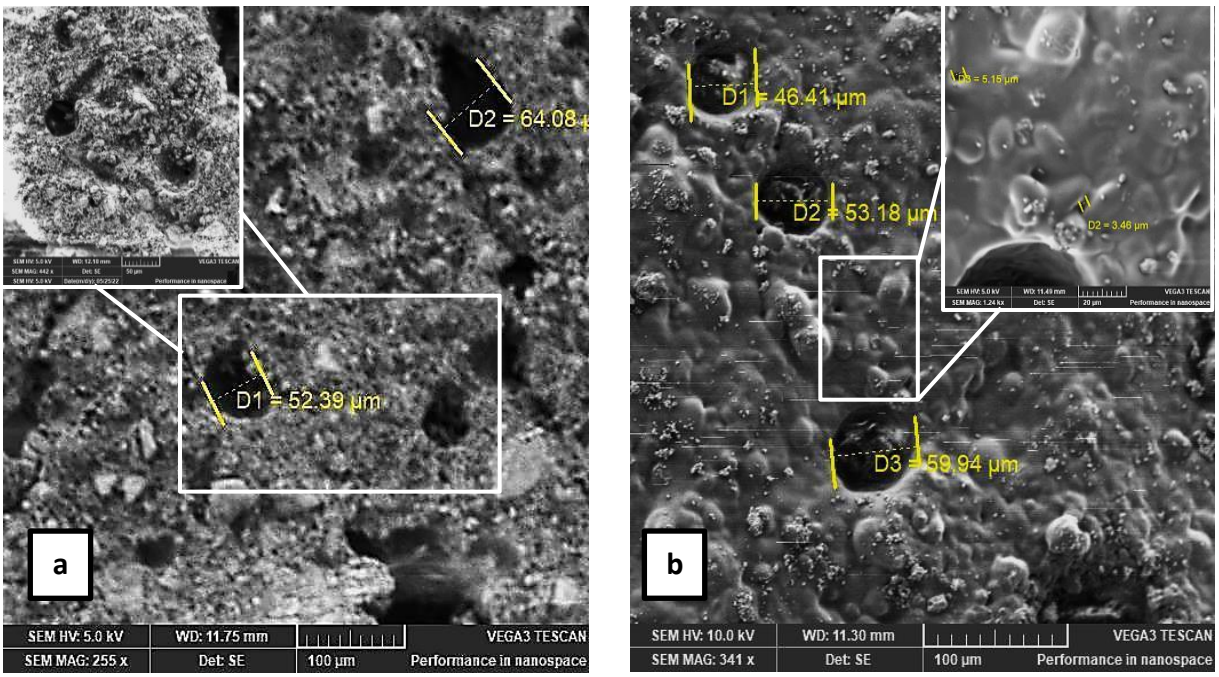

Fig. 7. The microstructure of 50 wt.% scaffold surfaces: a) scaffold A (before coating), b) scaffold AC (after coating)

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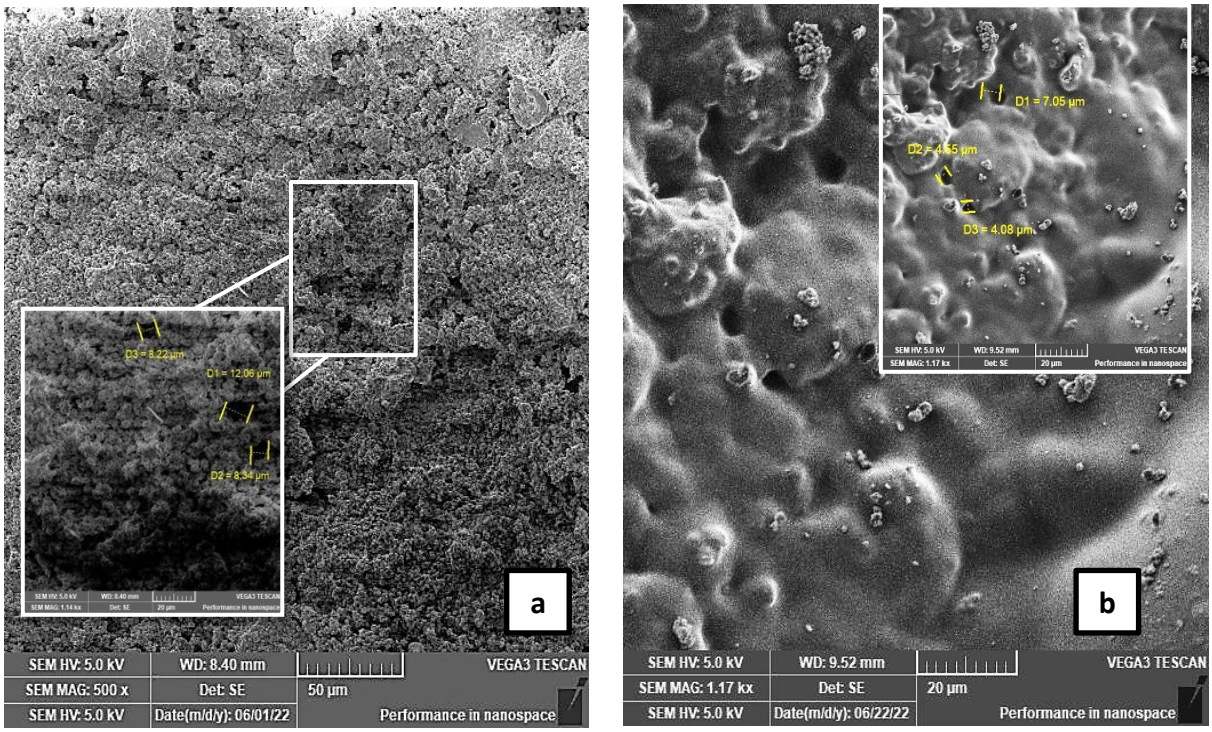

Fig. 8. The microstructure of 60 wt.% scaffold surfaces a) scaffold B (before coating), b) scaffold BC (after coating)

Table 3. The surface roughness of coated and uncoated scaffolds

Surface properties / Scaffolds	Roughness average (mm)	Surface area ratio	Root mean square (mm)
A	58.9	4.9	75.9
AC	49.8	3.7	68.6
B	31.7	3.1	38.3
BC	11.8	1.2	16.7

3.4. Scaffolds Wettability (Contact angle) Measurement

Table 4 shows the wettability of the coated scaffolds AC and BC by using images and measuring water contact angle values. As observed, the contact angle of the BC scaffold is greater than AC scaffold- although they were both coated by a hydrophobic PVA layer. The variation in contact angle demonstrates the relation between surface roughness, porosity, and pore size with scaffold wettability. The higher porosity of the AC scaffold produces more surface roughness leading to more surface wettability and hydrophlicity as compared with BC. This agrees with the results indicated by Liverani et al. [27]. The measuring contact angle for both uncoated scaffolds (A and B) is zero degrees which is assigned to the completely hydrophilic surface related to the nature of porous ceramic surfaces.

4. CONCLUSIONS

The gel-casting technique was effectively applied to fabricate a porous HA scaffold using freshly extracted egg yolk (EY) followed by Polyvinyl Alcohol (PVA) film coating. The results achieved that the coating process led to reinforced scaffolds by doubling the compressive strength as a result of decreasing porosity to the rate of 18% and cracks blocking. At the same time, there was a reduction in surface roughness to the rate of (15, 61)% and wettability for coated scaffolds. This means that it is important to modify surface topography by tailoring the scaffold design and coating process to get the required properties for specific applications. In summary, all scaffolds which were fabricated for the present study have compressive strength in the range of trabecular bone strength. This reflects a good suitability for non-load bearing applications.

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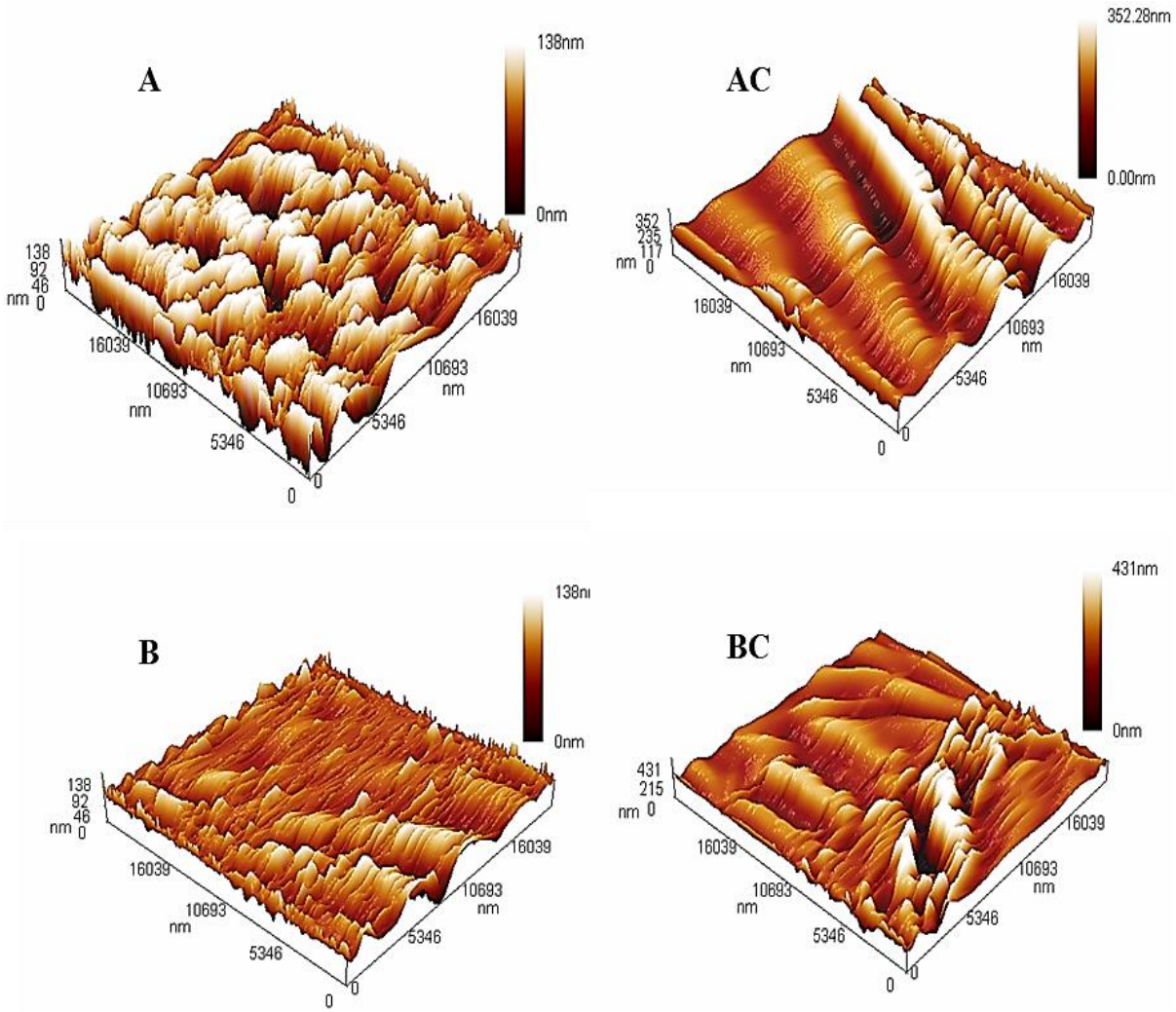
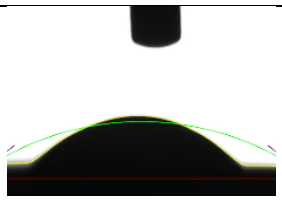
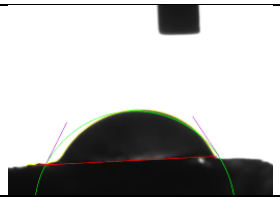


Fig. 9. Typical AFM images recorded for coated and uncoated scaffolds

Table 4. The wettability angle of PVA coated scaffolds

Scaffold type		Water contact angle
AC		51.924°±2.3
BC		70.030°±1.9

REFERENCES

[1]. Sultana N., Mokhtar M., Hassan M. I., Mad Jin R., Roozbahani F., and Khan T.H., "Chitosan based nanocomposite scaffolds for tissue engineering applications", Materials and Manufacturing Processes, 2015, 30(3):273-278.

[2]. Suresh G. and Girija VM., "One step method to synthesize flower-like

[DOI: 10.22068/ijmse.3118]

hydroxyapatite architecture using mussel shell bio-waste as a calcium source", Ceram. Int., 2017, 43:3457–61.

[3]. López-Álvarez M., Rodríguez-Valencia C., Serra J., and González P., "Bio-inspired ceramics: promising scaffolds for bone tissue engineering", Procedia Engineer., 2013, 59, 51-58.

[4]. Sari M. and Yusuf Y., "Synthesis and characterization of hydroxyapatite based on green mussel shells (Perna viridis) with calcination temperature variation using the precipitation method", Int. J. Nanoelectronics Material, 2018, 11(3):357–70.

[5]. Zhihong Y., Nan C., and Xiaome Q., "Fabrication of Porous Al_2O_3 Ceramics with Submicron-Sized Pores Using a Water-Based Gelcasting Method", Materials; 2018, 11, 1784.

[6]. Chalkia V., Marathoniti E., Vourdas N., and Gypakis A., "Application of gel-casting method in ceramics shaping", Proceedings of the 10th Panhellenic Conference of Chemical Engineering, At: Patra, Greece. 2015.

[7]. Gilissen R., Erauw J.P., Smolders A., answijgenhoven E.V., and Luyten J., "Gel-casting, a Near Net Shape Technique", Materials and Design, 2000, 21(4):251-257.

[8]. Padilla S., Sanchez-Salcedo S., and Vallet-Regi M., "Bioactive glass as precursor of designed-architecture scaffolds for tissue engineering", J. Biomedical Materials Research, 2006, 81(1) A: 224-232.

[9]. Sopyan I., and Fadli A., "Porous alumina through protein foaming-consolidation method: effect of dispersant concentration on the physical properties", Asia-Pacific Journal of Chemical Engineering, 2011, Vol. 6, issue 6, 863-869.

[10]. Farizaa A.R., Zuraidab A., and Sopyan I., "Egg Yolk as Pore Creating Agent to Produce Porous Tri-calcium Phosphate for Bone Implant Application", Advanced Materials Research, 2011, Vols. 264-265, 760-764.

[11]. Lis S., Suryanto S., Ahmad F., and Ramesh S., "Protein Foaming-consolidation Method for Fabrication of High Performance Porous Bioceramics", Advanced Materials Research, 2013, Vol. 622-623, 1759-1763.

[12]. Batool A.A., "Synthesis of porous bioglass-ceramic (SiO_2-MgO-Na_2O-P_2O_4-CaO) by compaction forming and gel-casting", Thesis, 2019, University of Babylon, College of materials engineering, 2019, 10.

[13]. Pertici G., Carinci F., Carusi G., Epistatus D., Villa T., Crivelli F., Rossi F., and Perale G., "Composite polymer-coated mineral scaffolds for bone regeneration: from Material characterization to human studies", Journal of Biological Regulators & Homeostatic Agents, 2015, Vol. 29, no. 3 (S1), 136-148.

[14]. Elisa F., Jacopo B., Enrica V., and Francesco B., "Bioactive Glasses: From Parent 45S5 Composition to Scaffold-Assisted Tissue-Healing Therapies", J Funct Biomater, 2018, 9(1): 24.

[15]. Kamalan K. A. M., Ashok R. C., Arish D, Amirhossein P., and Dušan G., "Recent Advancements in Materials and Coatings for Biomedical Implants", Gels, 2022, 8(5), 323.

[16]. Ryan K. R., Gabriel L. C., Robert J. K., and Weimin Y., "Hydroxyapatite-Reinforced Polymer Biocomposites for Synthetic Bone Substitutes", Biological Materials Science, JOM, 2008, 38-45.

[17]. Song J., Winkeljann B., and Lieleg O., "Biopolymer-Based Coatings: Promising Strategies to Improve the Biocompatibility and Functionality of Materials Used in Biomedical Engineering", Adv. Mater. Interfaces, 2020, 7.

[18]. Israa S., Ola M., "Characterization, Mechanical, and In Vitro Bioactivity Properties of Hydroxyapatite/Bioactive Glass Composite", Journal of Babylon University/Engineering Sciences, 2016, No. (4), Vol. (24), 820-831.

[19]. ASTM International, Designation: C20−00 (Reapproved 2010), Standard Test Methods for Apparent Porosity, Water Absorption, Apparent Specific Gravity, and Bulk Density of Burned Refractory Brick and Shapes by Boiling Water.

[20]. Sharon K. J. U. and Naznin S., "Contact angle, Conductivity and Mechanical Properties of Polycaprolactone/

[DOI: 10.22068/ijmse.3118]

Hydroxyapatite/ Polypyrrole scaffolds using Freeze-Drying Technique", ARPN Journal of Engineering and Applied Sciences, 2016, VOL. 11, NO. 23, ISSN 1819-6608.

[21]. Brien F.J. O., Harley B.A., Waller M.A., Yannas I.V., Gibson L.J., and Prendergast P.J., "The effect of pore size on permeability and cell attachment in collagen scaffolds for tissue engineering", Technol Health Care, 2007, 15(1):3-17.

[22]. Smith J. R., Lamprou D. A., Larson C., and Upso S.J., "Biomedical applications of polymer and ceramic coatings: a review of recent developments", The International Journal of Surface Engineering and Coatings, 2022, Volume 100, Issue 1., 25-35.

[23]. Karageorgiou V., and Kaplan D., "Porosity of 3D biomaterial scaffolds and osteogenesis, Biomaterials", 2005, Vol. 26, 5474-5491.

[24]. Philippart A., Boccaccini AR., Fleck C., Schubert D.W., and Roether J.A., "Toughening and functionalization of bioactive ceramic and glass bone scaffolds by biopolymer coatings and infiltration: a review of the last 5 years", Expert Review of Medical Devices, 2015, 12:93-111.

[25]. Peroglio M., Gremillard L., Chevalier J., Chazeau L., Gauthier C., and Hamaide T., "Toughening of bio-ceramics scaffolds by polymer coating", Journal of the European Ceramic Society, 2007, 27:2679-85.

[26]. Pezzotti G., and Asmus S.M.F., "Fracture behavior of hydroxyapatite/ polymer interpenetrating network composites prepared by in situ polymerization process", Materials Science and Engineering: A, 2001, 316:231-237.

[27].Wei L., "45S5 Bioactive Glass-Based Composite Scaffolds with Polymer Coatings for Bone Tissue Engineering Therapeutics", PhD Thesis, University of Erlangen-Nuremberg, 2015.

[28]. Fu Q., Jia W., Lau G.Y., and Tomsia A.P., "Strength, toughness, and reliability of a porous glass/biopolymer composite scaffold", J. Biomed. Mater. Res. B Appl. Biomater., 2018, 106, 1209–1217.

[29]. Woodard J.R., Hilldore A.J., Lan S.K., Park C.J, Morgan A.W., Eurell J.A.C., Clark S.G., Wheeler M.B., Russell D.J., and Amy W.J., "The Mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity", Biomaterials, 2007, 28, 45-54.

[30]. Almeida R.M., Gama A., and Vueva Y., "Bioactive sol-gel scaffolds with dual porosity for tissue engineering", J. Sol-Gel Science and Technology, 2011, Vol. 57, 3, 336- 342.

[31]. Hollister S.J., "Porous scaffold design for tissue engineering", Nature Materials, 2005, Vol. 4, 518-524.

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