Original Article

The cross-linked polyvinyl alcohol/hydroxyapatite nanocomposite foam

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ABSTRACT

Polymeric foams are widely used for medical applications especially in terms of bio-scaffolds. This article tries to indicate a new methodology in fabrication of PVA-based composite scaffolds using esterification and formalizing methods simultaneously to improve scaffolds’ water resistivity and their relative mechanical (up to 6.5 MPa in plateau stress) and biomedical properties. Hydroxyapatite nanoparticles are also added as a secondary agent to improve scaffolds’ water absorbance (in range of 58–71%), water uptake (from 5.9 to 9.1 times of initial weight) and bioactivity. Fourier Transform Infrared spectroscopy was used to prove the formation of new chemical bonds between the chains of PVA and HA nanoparticles. In addition, scanning electron microscope images were employed to investigate the pore size of fabricated scaffolds in addition to using liquid absorbance method to measure the volume fraction of pores in scaffolds. SEM images also showed that biodegradability has taken place due to degradation of scaffolds under body simulated liquids in 37 °C. The presence of HA nanoparticles in scaffolds’ structure caused an improvement in their bioactivity by showing the formation of new HA contents on polymeric matrix.

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1. Introduction

Nowadays the development of medical science needs the scientists to replace or amend some damaged body tissues by proper materials. Biomaterials play an important role in medical and biomedical science while they can be categorized into three major sections: metals, ceramics and polymers which their combination can pursue different aims for wide range of applications [1]. These materials are shaped into variety of designs to replace soft and hard tissues or ones in contact with blood [2].

In each day thousands of surgical operations are done to repair or even replace the damaged tissue. For this purpose the field of tissue engineering (TE) is very useful to regenerate damaged tissues and lead them to grow as a new tissue [3–5]. Indeed tissue engineering exploits both cellular-molecular biology and material science to reach a proper contact between implanted materials and physiological mediums [6].

Tissue engineering makes attempt to help the growth of cells and proliferation on particular matrices which are known as scaffolds. The properties of scaffold depend not only on

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its material, but also on the size and distribution of pores, biocompatibility, biodegradability and bioactivity [7,8].

Different methods are employed to produce an appropriate scaffold with considered properties while their variety can be constrained due to the materials and special characterizations [9]. Electro-spinning [10,11], gas foaming [9,12], solvent casting and particulate leaching [13,14], liquid molding [15,16] and thermally induced phase separation [17,18] are more important methods to fabricate the scaffolds.

Another key point in the fabrication of scaffold is choosing the proper materials. Besides the conventional materials the synthetic and biodegradable polymers can be used for making three-dimensional scaffolds because of their vast properties [19–22]. Poly-vinyl alcohol (PVA) is one of the most famous and low price synthetic polymers which can easily solve in water due to its alcohol functional group (Fig. 1) [23,24]. Cross-linking methods such as freeze-thawing, irradiation, acetalizing and esterification can be used to reduce the water solubility of PVA to reach a water resistant structure [23,25].

Perhaps one of most main limitation of scaffold is lack of compatibility between the interface and bone tissue. To solve this problem adding ceramic as a bioactive agent to scaffold is very common [26–31]. Hydroxyapatite with chemical composition of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is one of the most famous forms of calcium phosphates and it can improve biocompatibility and bioactivity of the main structure [27,28].

Based on the literature survey done by other authors, there are some articles concentrated on the PVA based scaffolds. It seems that the articles have focused on different fabrication methods and additional secondary agents to improve their mechanical and biological properties. For example Abdul Wahab et al. tried to form PVA hydrogel using freeze–thaw (FT) method understood that the stiffness of PVA could be controlled by manipulating the concentration of PVA and number of FT cycles [29]. Three-dimensional magnetic graphene oxide-magnetite polyvinyl alcohol (3D-GO/PVA/Fe$_3$O$_4$) nanocomposites were successfully prepared [30]. In 2008, Mansur et al. fabricated scaffolds synthesized from PVA blended with bioactive glass and chitosan via sol–gel precursor solution [32]. Furthermore, Thomas used citric acid as a reactant agent and P-toluene sulphonic acid as catalyst to cross-link PVA chains for vascular tissue engineering goals [33]. Asran et al. employed electro-spinning method to fabricate a rectangle shape of a PVA mixture with collagen and hydroxyapatite nanoparticles [34]. Poursamar et al. used freeze–thawing method to improve PVA/HA nano-particles composite, mechanical properties while other properties were examined under bio-simulated conditions [35]. Hsieh et al. used acetalization method to improve the water resistivity of PVA/Starch scaffolds by using formaldehyde and sulfuric acid as a catalyst [23]. On the other hand, Chang et al. made foam shape PVF (poly-vinyl formal) without pore-foaming agent. The idea of this experiment is borrowed from the combination of mentioned methods and materials to reach an appropriate structure for biomedical aims [36]. Chen et al. fabricated poly(vinyl alcohol)/β-tricalcium phosphate (PVA/β-TCP) composite scaffolds using Fused deposition modeling (FDM) [37].

Although there are some articles concentrated on the fabrication of PVA/HA but most of them concentrated on the freeze thawing or electrospinning methods. To the best of our knowledge fabrication of PVA/HA using combination of formalizing and leaching methods has not been under more attention. For this purpose, in this research, a combination of methods and materials were used to reach an appropriate foam structure which can be practical in different medical approaches. PVA as a matrix were prepared by using esterification and acetalizing (formalizing) method simultaneously while hydroxyapatite was employed as a secondary agent to improve their biomedical properties. Foams were examined under variety of physical, mechanical and biomedical situations to characterize their properties. SEM, FTIR, compression, biodegradability and bioactivity tests are performed to figure out their characterization for further biomedical applications. Using PVA and hydroxyapatite as a composite foam leads to exploit an inexpensive, biodegradable and existing material to create a 3-dimensional scaffolds which can be beneficial in regenerative medicine.

2. Experimental procedure

2.1. Preparation of cross-linked PVA/HA composite scaffold

PVA with average molecular weight of 72,000 and hydrolysis degree of more than 98.5% was obtained from Merck Company. A viscous solution was prepared by adding 2 g of PVA to 10 ml deionized water. Hydroxyapatite nanoparticles obtained from Merck Company were added to the solution with ratio of 0, 0.05, 0.1 and 0.25 with respect to the weight of PVA which are named PHF0, PHF1, PHF2 and PHF3 for better recognition (Table 1).

| Table 1 – Composition of fabricated PVA/HA nanocomposite. |
|-----------------|----|----|-------------|
| Name            | PVA (g) | HA (g) | Weight ratio |
| PHF0            | 2    | 0    | 0           |
| PHF1            | 2    | 0.1  | 0.05        |
| PHF2            | 2    | 0.2  | 0.1         |
| PHF3            | 2    | 0.5  | 0.25        |
4 ml of sulfuric acid as a catalyst and 12 g of citric acid in order to create esterification bonding obtained from Kimia Mavad were added to the solution. Then 12 g of edible salt (NaCl sized from 100 to 400 μm) were added to the solution. Finally 3 ml of formaldehyde obtained from Merck Company were added to the solution to initialize the formalizing process. The solution injected to the cylindrical molds and cured in 85 °C for 40 min (Fig. 2).

2.2. FT-IR analysis

FT-IR (Shimidzu, Japan) was used for analyzing the structure of scaffolds and to see whether binding have taken place or not. The changes in the functional groups were identified by recording the spectrum intensity at 400–4000 cm⁻¹ which was resulted by cross-linking procedure.

2.3. Microscopic evaluation

A scanning electron microscope (Cambridge-S360, England) were employed to evaluate the surface morphology and pores size of different specimens. Beforehand, air-dried scaffolds were plated with gold layer in a vacuum deposition machine (Polaron SC7640, England) by an accelerating voltage of 20 kV at a 9–12 nm working distance. Moreover, received images were processed under IMAGEJ program to measure pores aspects by changing them to a binary image.

2.4. Porosity and water uptake

Liquid displacement method was employed to calculate the porosity and water uptake value of the composite foams. The samples were air-dried under room temperature and weighed after 72 h (w_i). Their initial volumes were measured by submerging them in a certain volume of water (V_f) and the secondary were recorded after the immersion in water for 30 min (V_s) which their subtraction shows the exact volume of each foam after gaining water. By using Eqs. (1)–(4) the volume of foam, water uptake, pore volume and porosity percent can be obtained.

\[ V_{\text{foam}} = V_s - V_f \]  
\[ \text{Water uptake} = \frac{w_f - w_i}{w_i} \]  
\[ \text{Pore volume} = \frac{w_f - w_i}{\rho_{\text{water}}} \]  
\[ \text{Porosity} \% = \frac{\text{Pore volume}}{\text{Foams' final volume}} \times 100 \]

2.5. Compression test

Compression behavior of cross-linked foams was measured by compression test (Santam STM-150) under 1 mm/min cross head speed rate. Three different samples were prepared for further examinations with height to diameter ratios of 1.5–2 according to ASTM D695 standard.

2.6. Biodegradability

Biodegradability was assessed under phosphate buffer saline (PBS) and Ringer’s solution at 37 °C. Samples were prepared after releasing from the buffer solution by washing with water and drying under room temperature for 48 h. Scanning electron microscope was employed to evaluate the surface changes of the scaffolds resulted from the biodegradation process under mentioned situation.

2.7. Bioactivity and biocompatibility

The bioactivity and biocompatibility of specimens were examined by immersing them in Ringer’s solution for 28 days under 37 °C condition. Then, they were removed from solutions and were dried in room temperature. Specimens were observed under scanning electron microscope to evaluate whether hydroxyapatite nucleation took place or not. X-ray diffraction (Bruker Model D8 Advance, Germany) with Cu Kα (Lambda = 1.54 Å) at 40 kV and 40 mA in the 2θ range of 5–90° with scan speed of 0.05°/s were also employed to prove the presence of hydroxyapatite as a sign of bioactivity.

3. Results and discussion

3.1. FTIR

Investigations showed that cross-linking of polyvinyl alcohol has taken place through acetalizing and esterification
methods simultaneously. Acetalizing or formalizing can be characterized with data resulted from FTIR test which are shown in Fig. 2. In resulted FTIR spectra, C-H relevant stretching bands of formal can be seen in absorption peaks of 2960, 2860, 2790 and 2660 cm\(^{-1}\). Also, new bands appeared at 1470, 1428, 1400, 1390 and 1360 cm\(^{-1}\) due to C–H vibration bonding. Nevertheless, stretching vibration of C–O–C–O–C can be relative to the bands at 1180, 1135, 1070 and 1020 cm\(^{-1}\). The residual acetate group forms C=O which causes 1735 cm\(^{-1}\) band in FTIR spectra [24].

Esterification bonding were observed at band 1715 cm\(^{-1}\) due to C=O bonding while the bands at 1084 and 1208 indicate the presence of C–O bonding. These bands can prove that ester linkages were formed during the curing process [33].

The other issue which should be emphasized on is the presence of proper bonding between hydroxyapatite particles and the polymeric matrix. To prove this, a composite specimen was examined under FTIR test and the result can be seen in Fig. 3. The FTIR spectra are affected by the phosphate ions, especially PO\(_4^{3-}\), as a principal molecular component in hydroxyapatite at the 550–1200 cm\(^{-1}\) band region. The characteristic peaks at 972 and 1093 are due to the stretching vibrations of PO\(_4^{3-}\) while the 543 cm\(^{-1}\) peak is resulted from the deformation vibrations of mentioned component. OH— is also another major component in each phase which causes some peaks between about 2500 cm\(^{-1}\) and 3700 cm\(^{-1}\) [38]. According to different data extracted from FTIR patterns, Table 2 summarizes them in a more comprehensive manner.

### 3.2. Micro and macro-morphology (SEM)

Observation of morphological properties of fabricated foams was attained by using SEM to prove that pore sizes are appropriate for further biomedical applications. As a result, images indicated that the size and the distribution of the pores in the 3D foams (Fig. 4), are in an appropriate range and also they are interconnected due to the presence of micro-pores available in the foam structure.

![FTIR pattern before and after the chemical reaction.](image)

**Fig. 3 –** FTIR pattern before and after the chemical reaction.

<table>
<thead>
<tr>
<th>Bonding</th>
<th>Wave length (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetalization [31]</td>
<td>2790, 2860, 2660, 2960</td>
</tr>
<tr>
<td>C=H</td>
<td>1400</td>
</tr>
<tr>
<td>C–O–C–O–C</td>
<td>1022, 1068, 1132, 1181</td>
</tr>
<tr>
<td>C=O</td>
<td>1735</td>
</tr>
<tr>
<td>Esterification</td>
<td>1068</td>
</tr>
<tr>
<td>RCO–OH carboxilic acid (2931 and 1068)</td>
<td>1737,</td>
</tr>
<tr>
<td>RCOOR’ esters (1737 and 1068)</td>
<td>2931</td>
</tr>
<tr>
<td>Phosphine</td>
<td>606,</td>
</tr>
<tr>
<td>Phosphine oxide</td>
<td>803,</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1137,</td>
</tr>
<tr>
<td></td>
<td>1022</td>
</tr>
</tbody>
</table>

**Table 2 –** Data extraction from FTIR spectra relative to different bonds.

![SEM micrographs of fabricated foams: (a) PHF0; (b) PHF1; (c) PHF2.](image)

**Fig. 4 –** SEM micrographs of fabricated foams: (a) PHF0; (b) PHF1; (c) PHF2.
Table 3 – Pore sizes of specimens according measurements received from SEM images.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Maximum pore size (μm)</th>
<th>Minimum pore size (μm)</th>
<th>Mean pore size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHF0</td>
<td>210</td>
<td>48</td>
<td>111</td>
</tr>
<tr>
<td>PHF1</td>
<td>220</td>
<td>59</td>
<td>117</td>
</tr>
<tr>
<td>PHF2</td>
<td>228</td>
<td>31</td>
<td>104</td>
</tr>
<tr>
<td>PHF3</td>
<td>215</td>
<td>42</td>
<td>108</td>
</tr>
</tbody>
</table>

Table 4 – Value of porosity volume fraction and water uptake extracted from water immersion data.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Porosity volume fraction (%)</th>
<th>Water uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHF0</td>
<td>58</td>
<td>590</td>
</tr>
<tr>
<td>PHF1</td>
<td>63</td>
<td>870</td>
</tr>
<tr>
<td>PHF2</td>
<td>68</td>
<td>890</td>
</tr>
<tr>
<td>PHF3</td>
<td>71</td>
<td>910</td>
</tr>
</tbody>
</table>

Image] software was used to evaluate the pores’ size of produced foams by examination of 20 surface pores under SEM observation for each of them (volume defects were neglected). The results showed that most of them are in a proper range from 100 to 200 μm which is an appropriate range for bone regeneration [39]. The results of distribution of pores is summarized in Table 3 while the data shows that changes of hydroxyapatite content has no significant effect on micro pores’ size because of employed pore-forming methods. The micro pores are formed by salt leaching and phase separation method which is independent from presence of hydroxyapatite nano-particles.

3.3. Porosity

Porosity and swelling ratio are two essential factors which should be examined to indicate that fabricated foams can absorb enough water for special applications such as blood absorbance in scaffolds. Porosities’ size should be interconnected and in a certain range to maximize their ability in transferring nutrients to intended cells.

The porosity value of fabricated foams was increased by increasing the hydroxyapatite content which might be the result of producing microspores in the foam structure [26]. From Table 4, it is obvious that the porosity volume fraction has increased from 58% in HPF0 to 71% in HPF3 while the foams’ water absorption has changed from 5.9 to 9.1 times their own weight due to water penetration and their physical bonding with water molecules resulted from hydroxyapatite and unreacted hydrophilicity of chains of PVA. It is obvious that more water absorption can improve their ability for further medical applications because of blood’s hydrophilic nature which can lead to improve the scaffolds’ drug and nutrients delivery to cultured cells.

3.4. Compression behavior

Mechanical properties of fabricated foams were identified by using air-dried specimens under compression test at the room temperature. As mentioned before, specimens were prepared with certain aspect ratio depend on ASTM standard. Each group of tests was carried out three times and their average values were plotted in Fig. 5 to evaluate their behavior. It is obvious that each of them includes three different sections and they are: linear elasticity caused by cell walls bend, plateau or collapse region resulted from cell walls buckle, yield or fracture and finally the densification of foam as a result of cell walls crushing together.

The information of different areas was extracted from Fig. 5 which is available in Table 5. Plateau stress was obtained from the average stress between 0.2 and 0.3 strain values and the densification was started from 1.3 times of plateau stress. The densification strain was also achieved from the corresponding point of densification stress.

The results show that using hydroxyapatite particles as a secondary agent, although can improve foams bioactivity and their biodegradability; they may cause some defects in foams’ structure which finally lead to a plateau stress reduction from 5 MPa in PHF0 to 3.37 MPa in PHF3. Hydroxyapatite particles may produce some micro size air bulbs in their surrounding and this fact can be concluded by comparing their porosity values.

On the other hand their densification stress also has decreased from 6.5 MPa in PHF-0 to 4.38 MPa in PHF-3 and their relative strain have reduced from 0.42 to 0.37 respectively due to hydroxyapatite particles aggregation in the structure of foams resulted from compression test. With respect to similar works which have been done on similar materials, the maximum strength of dried scaffolds was much lower than present project [40,41]. As an example, Nie et al. works on PVA/BPC [41] could only reach to 0.24 MPa while the endeavors of Mansur et al. [32] lead to an increase from 0.4 to 1.8 due to addition of secondary phases (Chitosan and Bioactive Glass) to the PVA matrix. However, the present results showed that the strength of the scaffolds is in the range between 3.37 and 5 MPa which
3.5. **Biodegradability**

Biodegradability of foam was another essential factor to characterize their behavior under biological situations. Phosphate buffer saline solution was used to prevent from further hydroxyapatite formation during the test while PHF0 in Ringer’s solution showed similar attitude due to lack of HA nanoparticles. The SEM images in Fig. 6 shows different scales of degradation of fabricated scaffolds under body simulated situations while in a closer look in Fig. 7, the microstructure of scaffolds has become more open due to walls destruction in PBS solution.

3.6. **Bioactivity and XRD**

Bioactivity test was evaluated by immersing fabricated specimens in Ringer’s Solution for 28 days under 37 °C temperature while SEM and XRD were used to prove the formation of further hydroxyapatite. SEM results in PHF-0 samples showed that although the structure of foam was destructed under biological situation, no additional structure was added to the primary foam. However, by investigating the PHF3 samples under SEM, it was obvious that the formation of another phase have taken place during the biological test. This phenomenon was caused due to presence of hydroxyapatite as a secondary content in the polymer matrix and the results comparison can be seen in Figs. 8 and 9.

On the other hand, XRD was employed to characterize the phase changes by comparing PHF-3 foam’s spectra pattern before and after immersion in Ringer’s solution [42] the results showed that the XRD pattern of foams with higher content of hydroxyapatite have changed considerably after immersing in Ringer’s solution during 21 days. Index peaks of hydroxyapatite at 45.3° and 31.6° showed a magnificent growth during the test while the peaks at 25.1° and 33.4° also are relevant to the presence of hydroxyapatite (Fig. 10).
4. Conclusions

The cross-linked PVA/hydroxyapatite composite 3D foams depict that a proper structure for scaffold aimed applications the results are remarked as follows:

- The FTIR analysis proves that mentioned reactions (esterification and formalization) are successfully took place in addition to chemical bonding between hydroxyapatite and polymer matrix.
- Scaffolds due to their high percent of porosity up to 71% (in an appropriate size range) and water uptake ratio up to 910% can absorb large amounts of water to itself.
- Compression tests demonstrate that although the plateau stress drops from 5 to 3.37 MPa due to increasing of hydroxyapatite content, their strength are significantly more than other relevant tests performed in other researches.
• Biodegradability of foams is also examined under blood simulated conditions and the SEM observation results showed that the structure of scaffolds has changed during the 28 days after immersion in Ringer's solution.

Conflicts of interest

The authors declare no conflicts of interest.

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