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Research Paper

The Study of Brown Rice Starch Effect On Hydroxyapatite Composites

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Abstract

The fabrication of starch-hydroxyapatite (HA) scaffolds was conducted previously by using corn, tapioca and rice. Here, local brown rice was chosen as the source of starch since different type of rice may give different outcome of in term of the scaffold's materials characteristics. The main aim of this study is to obtain a brown rice starch-hydroxyapatite (HA) composite scaffolds that could imitate the structure and the characteristics of a natural bone. The fabrication process involved solvent casting and particulate leaching method which using NaCl as a porogen agent. Four ratios of starch-HA were fabricated with concentration of starch 50wt%, 60wt%, 70wt% and 80wt%. Afterward, the effects of the brown rice starch on the scaffolds were investigated by water absorption test and Scanning Electron Microscope (SEM). Here, only 50wt% and 60wt% ratio of starch-HA can be used to fabricate tissue scaffolds using solvent casting and particulate leaching method. Hence, the 60wt% ratio scaffolds has the highest water absorption of all and the pore's size observed through SEM corresponded to this. The FTIR also shows there are more interactions between Brown Rice starch and HA for the 60wt% ratio.

Keywords: *Brown rice, Starch, Hydroxyapatite, Bone tissue scaffold*

1. Introduction

Starch is a natural and low cost biopolymer that can be applied in biomedical field. It can be a supporting component that is able to improve the properties of a developed material[1], [2]. There are many types of starches which can be found and these different types of starch may give different level of strength, water absorption and other material properties for fabricated materials[3]–[6]. Here, the brown rice starch has been chosen for further study. The composition of the hydroxyapatite (HA) is almost similar with calcium phosphate of the human hard tissues. The properties found in hydroxyapatite are biocompatible, bioactive osteoconductive, non-toxic and has non-inflammation nature[7], which are very significant in producing scaffold for repair, regeneration and reconstruction of damage bone tissue. The fabrication process can be conducted by using several techniques. For instance, gas foaming method, electrospinning method, solvent casting and particulate leaching method, phase separation and microwave vacuum drying method[8]. The method that will be used in this project is solvent casting and particulate leaching. This method casts mixtures in a mold to produce a scaffold. When the solvent evaporates, it creates a structure of composite material (starch-HA) consisting of the particles together with the polymer. The starch-HA is then placed in a solution which dissolves the particles, leaving behind a porous structure[9]. The characteristic of the molded mixture/scaffolds will be characterized by using water absorption test, scanning electron microscopy (SEM) and Fourier transform infrared (FTIR). Surface morphology, pore shape and pore size distribution of starch-HA scaffold will be studied by SEM[10].

2. Material and Method

The brown rice used was a produce of EcoBrown Sdn. Bhd. from Kedah, Malaysia. Salt or sodium chloride (NaCl) used in this study was supplied by Kasihku Marketing Sdn. Bhd. Glutaraldehyde 25% and ethanol 70% was supplied by Merck.

2.1 Scaffolds Fabrication

Figure 1 shows the step to fabricate the brown rice starch-hydroxyapatite (HA) scaffold. Four different ratio of HA:starch 50:50(wt%), 40:60(wt%), 30:70(wt%) and 20:80(wt%) were fabricated respectively. The brown rice starch solution was continuously stirred until the temperature reached 35˚C. At this temperature, the NaCl was poured and the mixture was stirred again until the temperature was at 65 ˚C then only the HA solution was poured into it and it was stirred again until the solvent are evaporated and the homogeneous solution was obtained approximately at 80°C.This method is called double boiling method. After that, the HA-Starch composite with NaCl were poured into the prepared mold. Next step was to dry the molded composites in an oven for 48 hours with the temperature of 80°C. After 24 hours, the samples were taken out of the mold and placed in the disk and put them back into the oven. After drying them in the oven, they were allowed to dry under room temperature (22-25°C) for 24 hours. They were then cross-linked with GA solution for 4 hours. Then, the samples were immersed in the distilled water for 72 hours in order to leach out all the salt and the GA[11]. The samples would be again immersed into 180ml of ethanol 70% solution for 10 hours followed by drying at room temperature for another 24 hours and then incubated at 80°C for 48 hours for complete removal of moisture.

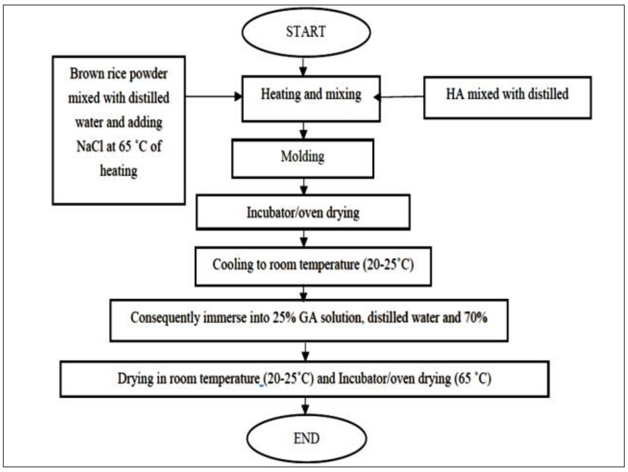


Figure 1. Flowchart of the fabrication of a brown rice starch-HA scaffolds

2.2 Water Absorption Test

For this test, the scaffolds were soaked in the water in order to investigate the water uptake percentage of the scaffolds. The data was recorded each day until there were no significant changes in weight. The water uptake is generally dependent on the porosity and hydrophilicity of the materials[12]. This test should show a high rate of diffusion coefficients in the porous scaffold rather than the nonporous scaffolds[12], [13].The percentage of water absorption can be calculated using the equation as shown below:

$$W_A = [(W_f - W_0)/W_0] \text{ x } 100 \tag{1}$$

Where,
W_A =Percentage of water absorption
W_0 = Initial weight of the scaffold in gram
W_f = Final weight of the scaffold in gram

2.3 Scanning Electron Microscopy (SEM)

The scanning electronic microscopy used in this project was HITACHI TM3000 with the acceleration voltage of 5 or 15kV, tungsten source, X15 to X3000 of magnification and 30nm resolution[14]. SEM is used to analyze the pores' shape, pore size distribution[15], surface morphology, microstructural characterization[7], [16] and interconnectivity of the fabricated porous scaffold samples.

2.4 Fourier Transform Infrared (FTIR)

The wavelength[17] of Brown rice starch incorporated with HA for all different ratios were obtained from Perkin Elmer FTIR spectrometer which was performed on a range of 500 to 4000 cm^{-1}. During the fabrication process, 50% (w/w) of starch in this scaffold are successfully been fabricated. During solvent casting and particulate leaching process, out of five samples of scaffold with 60% (w/w) starch proportion, only one sample was successfully been fabricated. The rest were dispersed in GA solution. However, the samples with 70% and 80% (w/w) starch were not able to maintain their structure during the solvent casting and particulate leaching process.

3. Results and Discussion

3.1 Water Absorption Test

The results of the water uptake for each of the scaffolds are shown in Figure 2. According to Figure 2, the non-porous scaffolds with 50% (w/w) starch had the least percentage water absorption which is 83.0% in comparison to the other two porous scaffolds. This low percentage is due to the non-porous morphology of the scaffold. Meanwhile, the other porous scaffolds with the same proportion of starch had taken up more water percentage which was 106.6%. Nevertheless, the highest water absorption percentage was the scaffolds with 60% (w/w) of starch with 129.5%. This is expected since the scaffolds had the highest porosity compared to the other two scaffold samples.

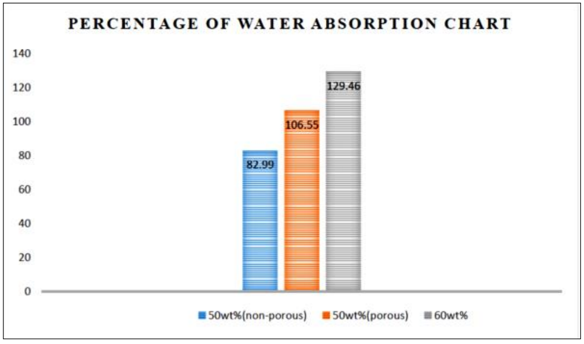
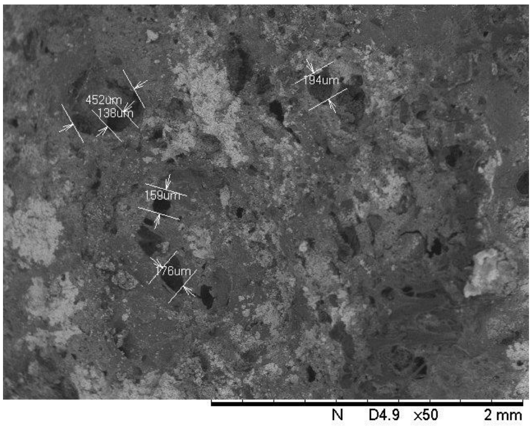


Figure 2. Percentage of water absorption of scaffold for non-porous 50% (w/w) starch, porous 50% (w/w) starch and 60% (w/w) starch

Therefore, the water uptake of the scaffolds was dependent on the presence of the porous microstructure, the porosity of the scaffold and hydrophilicity of the scaffold materials. Moreover, the water absorption characteristic of the scaffolds also depends on the surface to volume ratio of scaffolds[12]. Thus, it can be utilized to describe the suitability of biomaterial for tissue engineering application[11].

3.2 Scanning Electron Microscopy (SEM)

The scaffold with the starch percentage of 50% (w/w), 60% (w/w) and non-porous 50% (w/w) were analyzed under SEM. Each of the samples was cut into half to measure the pore size of the inner part of the samples without disturbing the pore structure of the scaffolds. Figure 3 shows the results of SEM on three different scaffolds.



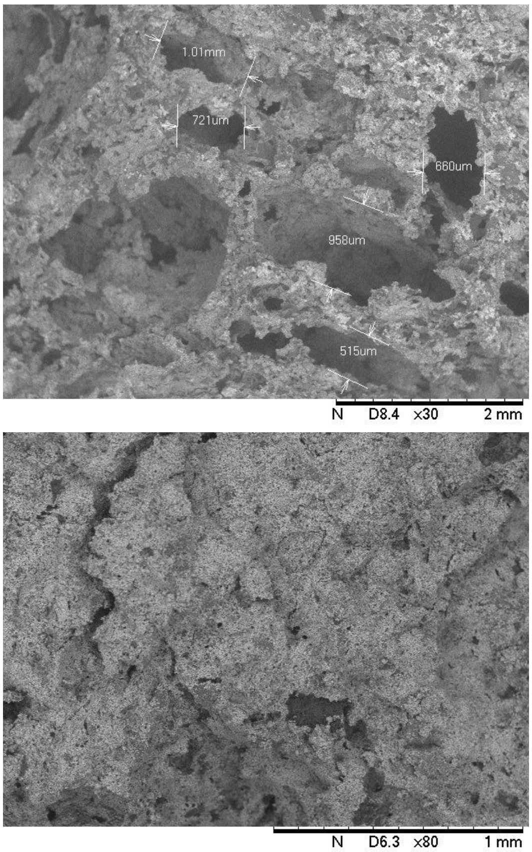


Fig. 3. Scanning Electron Microscopy **(**SEM) image; (a) 50% (w/w), (b) 60% (w/w), (c) 50% (w/w) starch scaffold (without solvent casting process)

Figure 3(a) show the morphology and pore size distribution of scaffold with 50% (w/w) of starch. The pore sizes were between 138µm to 452µm. However, the pore sizes of 60% (w/w) starch scaffold were in the range of 515µm to 1.01mm as shown in Figure 3(b). The results showed that the pore size of 60% (w/w) starch scaffold was larger in size compared to the scaffold with 50% (w/w) of starch. The porosity and the pore size will increase as the starch concentration increase because it was controlled by the amount of porogen used. Despite of this, the scaffold without any porogen did not show any pore as shown in Figure 3(c) above. Besides, the mean pores size between 20µm to 1500µm is desirable for tissue engineering purpose[18]. But, most of the studies recommend that a pore size larger than 300 µm is suitable for the formation of new bone and vascularization within the scaffold since this size could facilitate capillary formation and able to guide direct osteogenesis[19]. At the same time, stimulation of osteochondral ossification might occur at the pore size smaller than 300µm. Cell seeding occur at average pore size with diameter of 20-125µm[20].

3.3 Fourier Transform Infrared (FTIR)

Below are the FTIR spectrum for the four different starch percentage scaffolds (50%(w/w), 60%(w/w), 70%(w/w), 80%(w/w)) and one HA powder wavelength spectrum. Based on Figure 4, an O-H stretching band was located around 3280cm^{-1} to 3382cm^{-1}, which was the hydroxyl group of the starch[21]. This range of O-H stretching band had exhibited overlapping of O-H stretching band of HA with O-H stretching band of amylopectin in brown rice starch[3], [22]. Besides, pure HA had the higher O-H stretching band compared to other four scaffolds since O-H stretching band was originated from pure HA powder. The appearance of O-H stretching confirmed that there was interaction between the starch and HA[3]. Furthermore, 2922cm^{-1} to 2933cm^{-1} were assigned as asymmetric stretching of C-H of starch bond[3], [21], [22]. According to infrared spectroscopy absorption table, 2175-2140cm^{-1} was S-CΞN stretching where the band in the Figure 4 shows 2168 to 2170cm^{-1} which was located within the range of S-C≡N stretching. Next, Figure 4 shows the wavelength spectrum were between 2025-2027cm^{-1} which was within the range of N=C=S stretching in the infrared spectroscopy that was 2140-1990 cm^{-1}[23]. The band between 1639cm^{-1} and 1653cm^{-1} represents H-O-H bending and H2O absorption[22], [24]. The band around 1413 cm^{-1} and 1436cm^{-1} were indicated as the hydroxyapatite carbonate group (CO_3^{2-}) substitution. The presence of hydroxyapatite carbonate allows the regeneration of bone tissue[25]. This band also appeared only in the FTIR result of the pure HA and 50% (w/w) of brown rice starch scaffold due to large concentration of HA. Likewise, 1355cm^{-1} to 1359cm^{-1} band indicates the angular deformation of C-H[22]. The peak at 1009cm^{-1} to 1016 cm^{-1} represents the phosphate (PO_4^{3-}) vibration[7], [15], [24]. The analysis of hydroxyl group (OH-) band, phosphate (PO_4^{3-}) vibration band, hydroxyapatite carbonate group (CO_3^{2-}) and H_2O absorption proved that the FTIR spectra recorded correspond with the porosity of the scaffold. On the other hand, as seen in Figure 4, there was an absence of the aldehyde groups of glutaraldehyde in the samples. If the glutaraldehyde trace was there, this should be detected at 1720 to 1740 cm^{-1}[20].

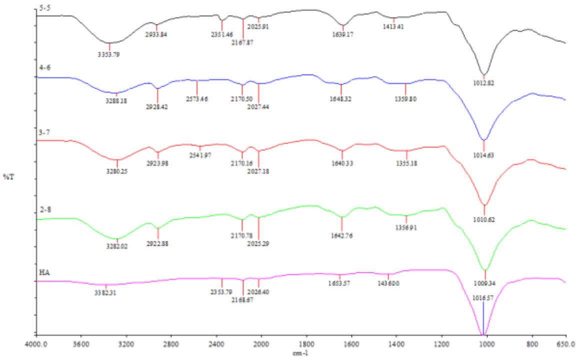


Figure 4: FTIR spectrum for the scaffolds and pure HA

4. Conclusion

The different amount of starches such as 50wt%, 60wt%, 70wt% and 80wt% had influenced the data analysis of water absorption test, SEM and FTIR. As brown rice starch increased, the porosity also increased respectively. Scaffold with 60wt% of starch had the highest percentage of water absorption test due to its higher porosity. The water absorption percentage may indicate that this composite scaffold is able to support the cell regeneration for tissue engineering because it shows the ability to absorb fluids from the medium. Fourier Transform Infrared Spectroscopy (FTIR) analysis show the existence of functional group found in the composite scaffold. Therefore, it is confirmed that there are interactions formed between the brown rice starches and HA.

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