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Fabrication and Characterisation of Gelatine/Hydroxyapatite Porous Scaffold

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Abstract. Hydroxyapatite (HAp) has been used as bone substitute materials due to its bone compatibility after implantation. However, major drawbacks of HAp typical of ceramic materials are its brittleness which limits its applications. Therefore, to introduce toughness in HAp scaffold, gelatine layer was coated on the surface of the HAp scaffold. In this research, porous HAp scaffolds coated with gelatine are fabricated via polymer reticulate method using polyurethane foam (PU) template. Initially, HAp powders were calcined at 1100°C to reduce its viscosity and agglomeration effect when formed into slurry. Preparation of HAp slurry with 40wt% of calcined HAp powders and 3 wt% of polyvinyl alcohol (PVA) powder were prepared before immersing the PU foams. The HAp coated PU foams were then heat treated at 650°C for an hour to burnout all PU, then sintering was done at 1100°C. Finally, the sintered HAp scaffold was coated with 10, 15 and 20wt% of gelatine. Additionally, the gelatine was crosslinked at 130°C and 160°C. Compressive strength analysis shows that as the wt% of gelatine increases the compressive strength of the gelatine/HAp scaffold increases. Highest compressive strength of 71.0 kPa was obtained for 20wt% of gelatine coated on the HAp scaffold. Higher crosslinking temperature also increases the compressive strength. The average compressive strength for gelatine/HAp scaffold crosslinked at 130 °C and 160 °C are 66.4 kPa and 72.3 kPa respectively. XRD analysis shows that 66% of HAp transformed into beta-tricalcium phosphate (β -TCP) after calcination while 71% transformed into β -TCP after sintering. FTIR and EDX analysis showed that the higher amount of gelatine content found in scaffolds with higher crosslinking temperature correlate to the increased in compressive strength. SEM analysis shows that the increased in the compressive strength is attributed to the covering of cracks on the strut of the HAp scaffolds by the gelatine.

1. Introduction

Bone scaffolds are intended to be used for implantation in critical size bone defects[1]. Traditionally, autografts and allografts has been used to correct bone defects or fracture problem. However, autografts and allografts has its disadvantages such as limited quantity of bone to harvest, massive blood sepsis, extra surgery, donor-site morbidity and risk of disease transmission[2]. Therefore, 3-D synthetic bone substitute scaffold is an alternative for bone remodelling. To mimic a scaffold, a number of key considerations are important when designing or determining the suitability of a scaffold for use in tissue



engineering. Those considerations include biocompatibility, biodegradability, mechanical properties, scaffold architecture and its manufacturing technology[3]. Successful scaffold design should stimulate new bone growth leaving only native bone tissue with no trace of the implanted scaffold at end state of success bone implantation process[4]. Scaffolds play an important character in new tissue regeneration and solving bone defects problems. Thus, this research focuses on the fabrication of the scaffold with enhancement of natural polymer which is gelatine.

2. Experimental study

2.1. Preparation of HAp slurry

HAp slurry was prepared by mixing 40wt% pre-calcined (1100 °C) HAp powder, 60wt% of water and additional of 3wt% of PVA as a binder. PVA binder is first dissolve in water at around 40°C using stirring hotplate and a magnetic bar. HAp powders are then added into the PVA solution and the stirring continued at 500 rpm for 4 hours using Mechanical Stirrer (RW20D, MAXIMA, IKA, Germany).

2.2. Impregnation of HAp slurry onto PU foam

HAp scaffolds were prepared by polymer reticulate method using polyurethane (PU) foam template cut into dimension of 1.5cm x 1.5cm x 2.54cm. PU foam were then squeezed to remove air trapped by fingers before immersing into the HAp slurry. HAp slurry should cover the whole PU foam to allow better impregnation of HAp slurry. PU foam is then taken out and rolled for 5 times using corrugating machine. The same method of immersion of foam into slurry and rolling were repeated twice to ensure full penetration. Air bubbles created during rolling and excess HAp slurry were removed by using air pressure generated by air gun.

2.3. Heat Treatment

The HAp impregnated PU foams are then put into oven (Memmert, UN30) at 80°C for 24 hours. Then the coated HAp/PU foam are sintered at 650 °C for one hour soaking time for PU foam burnout follow by sintering of the scaffold at 1100°C for one hour with temperature increment of 5 °C.

2.4. Gelatine coating process

10 wt%, 15 wt% and 20 wt% of gelatine concentrations were prepared by dissolving and stirring gelatine in water Beaker containing the gelatine solution were covered with aluminium foil to prevent excessive water loss through evaporation. Once a clear solution of gelatine is obtained, the sintered HAp was immersed into the solution at 35°C to enable good flow ability of gelatine to completely impregnate the sintered HAp scaffold. Next excessive gelatine was allowed to flow out by leaving in an oven at 80 °C for one minute. Lastly, the scaffolds were placed into vacuum drying oven at 130°C and 160°C for 24 hours.

2.5. Characterization and analytical techniques

The starting material and finished scaffolds were characterized by X-ray diffraction (XRD) analysis and Fourier transform infrared spectroscopy (FTIR) analysis. The XRD pattern were recorded with a diffractometer system (X-ray Diffractometer D8 Advance BRUKER-binary V3) using CuK α (λ of K-A1 = 1.54060 Å) radiation generated at 40 kV. The FTIR spectra were measured using a FTIR spectrometer (PERKIN ELMER Inc., MA, USA) in the wavelength range of 450-4000 cm⁻¹.

Universal Testing Machine (Series 5982, Instron, USA) was used to determine the mechanical properties of HAp scaffold incorporated with gelatine using 5 kN load cell with a cross head speed of 1 mm per min. Five samples for each experimental group were tested and their mean value was calculated.

Morphology of the scaffold were observed using Scanning Electron Microscope (SEM) (Hitachi TM3000, Japan).

3. Results and discussion

3.1. X-Ray Diffraction (XRD) and Fourier Transform Infra-Red (FTIR) Analysis

Figure 1 shows the XRD pattern of as received commercial HAp powder, calcined HAp powder at 1100 °C and sintered HAp scaffold at 1100 °C. The as-received raw HAp gives diffuse intensities indicated that as-received raw HAp has lower crystallinity than the standard reference which has higher intensity resembling its high crystallinity HAp due to the processing route used. The XRD peak of calcined and sintered HAp in Figure 1 shown significantly different characteristics of sharper and narrow diffraction peaks as compared to as-received raw HAp powders before the heat treatment process. This changed revealed that calcination and sintering process also altered the crystal size of HAp powder. This finding is parallel with statement claimed by Ahmed et al. (2015) that the physical properties of HAp powder are largely influenced by the heat treatment process[5]. The crystallinity of the HAp powder was found to be largely enhanced with the increase in calcination temperatures.

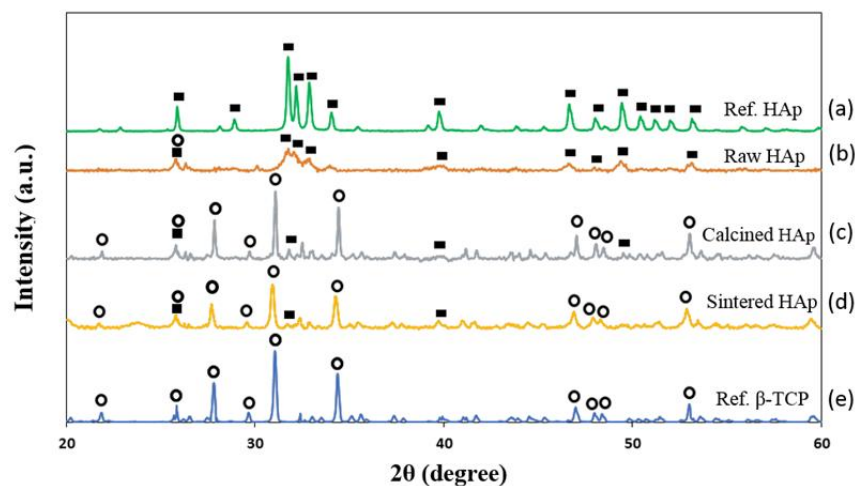


Figure 1. XRD patterns of (a) standard reference of commercial HAp; (b) raw commercial HAp used; (c) calcined HAp at 1100°C; (d) sintered HAp at 1100°C; (e) standard reference of commercial β -TCP

Nonetheless, all the XRD peaks of raw commercial HAp powders are found to match with the XRD peaks of the reference standard of commercial HAp as shown in the 1. This matching is done by using X'Pert High Score Plus software and the standard ICDD code number 98-004-0506. From the XRD analysis, it is found that HAp phase changes to β -TCP phase after calcination and sintering. Most of the HAp have been transformed into β -TCP as shown in Figure 1 with peaks under rectangular box represent HAp while circle representing β -TCP. Percentage of HAp being transformed during calcination and sintering are 66 % and 71 % This is supported by Rao et. al that stated above 600 °C, HAp structure starts to transfer to β -TCP explaining the XRD results obtained in this study[6].

Figure 2 shows the FTIR spectra obtained for HAp scaffold, with 10 wt%, 15 wt% and 20 wt% gelatine concentration coated. In this FTIR analysis, the functional group of gelatine and HAp are found and labelled in the Figure 2. According to Lawrie et al., 2007, broad band that ranges from 3700 cm^{-1} to 3000 cm^{-1} is assigned to O-H stretch[7]. Besides, peaks appear at 650 cm^{-1} are assigned to the O-H stretch[6]. Presence of O-H can be predicted to the presence of HAp. This has been confirmed with the XRD analysis shown in Figure 1. FTIR are also used to detect the presence of gelatine. Amide I with band range from 1630 cm^{-1} to 1695 cm^{-1} are matched with the peak detected in 2 confirms the existence of gelatine. Peaks detected as COO- with band range from 1596 cm^{-1} to 1412 cm^{-1} also represents the gelatine structure[7]. Thus, it is confirmed that the scaffold fabricated consist of HAp impregnated with gelatine.

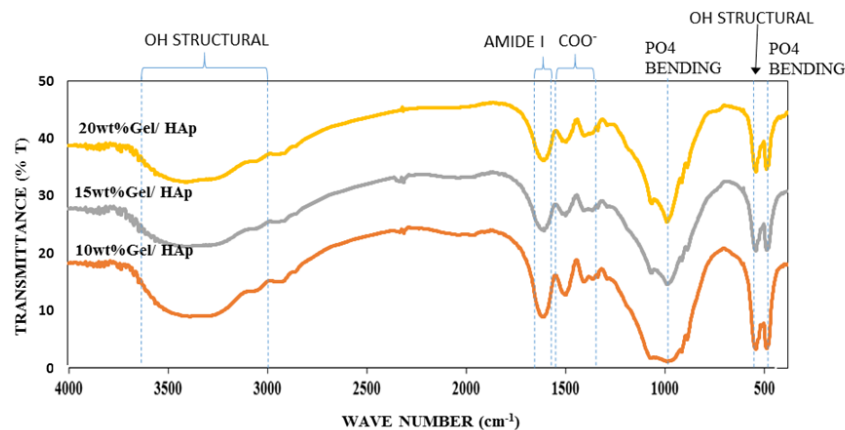


Figure 2. FTIR spectra of HAp scaffold with different concentration of gelatine coated.

3.2. Compressive Strength Analysis

The results from Figure 3 shows the trend of increasing compressive strength as the gelatine concentration increases. HAp coated with 20wt% of gelatine concentration has the highest compressive strength of 71.0 kPa followed by 22.8 kPa and 7.8 kPa for HAp coated with 15 wt% and 10 wt% gelatine concentrations respectively. The crosslinked gelatine after de-hydrothermal (DHT) process act as a strong adhesive to join the sphere-like shape HAp particles together from spreading away easily under stress thus increasing the toughness of this brittle HAp scaffold[8]. The effect of crosslinking temperature on the compressive strength is relatively not significant as compared to the effect of gelatine concentration on the compressive strength as shown in Figure 3. The compressive strength of pure HAp scaffold without gelatine coating are not shown in graph due to its brittleness characteristics.

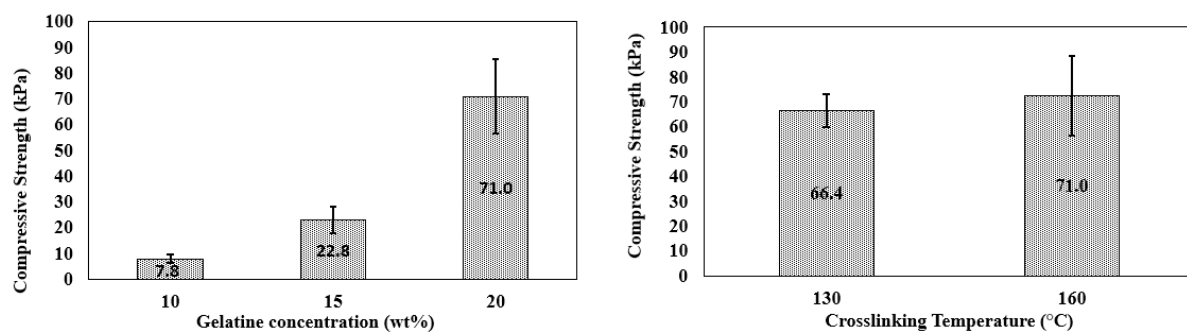


Figure 3. Compressive strength of 10 wt%, 15 wt% and 20 wt% of gelatine/HAp scaffold and compressive strength of gelatine/HAp scaffold coated under two crosslinking temperature.

3.3. Scanning Electron Microscope (SEM) Analysis

The morphology of the sintered HAp scaffolds is shown in Figure 4 (a) – (d). From these figures it can be observed the existence of missing part of strut as the supporting structure. Thus, this explains the brittleness of HAp scaffold which lowers the compressive strength of the HAp scaffold to undetected value during the compression testing. Moreover, Figure 4 (b) shows the pores existed on the strut. The reason of regular pores presented are unknown. Figure 4 (c) shows a relatively deep scratch line found on strut which may due to the organic type of impurities burn out during the high temperature firing leaving a structure look similar to scratch line. Figure 4 (d) shows the empty core of the scaffold strut. This is believed to be due to the PU foam burn out at 650 °C leaving this empty core behind. All these contribute towards the low compressive strength of HAp scaffold. Figure 4 (e) shows the cracking in

strut linked to the pores with approximate 150 μm long in length. The initial point of cracking is unknown. However, the reason of crack presented was believe to be due to the volumetric expansion behaviour of unit cells during transformation of HAp to β -TCP[9].

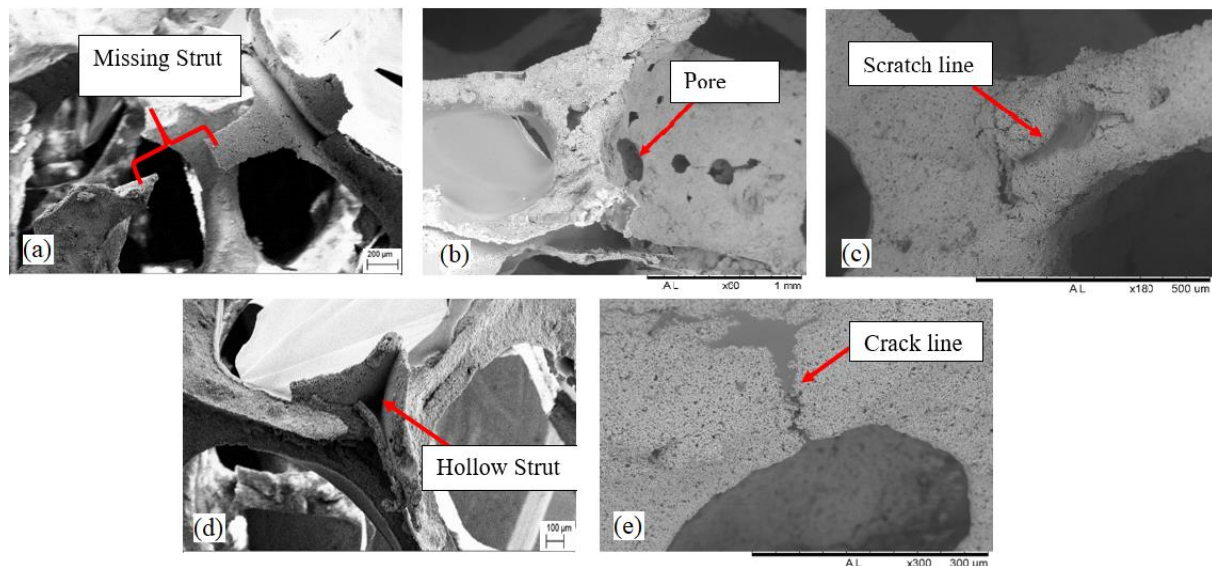


Figure 4 (a) – (e). Morphology of fabricated uncoated HAp porous scaffold.

Coating of the gelatine was found to contribute to the increases in compressive strength of HAp scaffold as the gelatine penetrates and closes the cracks and micropores on the surface of the HAp scaffold. Figure 5 (a) (b) shows a distinct gelatine layer at concentration 10 wt% and 15 wt% coated on HAp scaffold which was successfully observed and captured using SEM Tabletop machine. It can be observed that the gelatine layer is around 10 μm and 20 μm for 10 wt% and 15 wt% gelatine/HAp scaffold. The increment in gelatine concentration was proven to increase the strength of the scaffold as shown in Figure 3. This is because as the gelatine concentration increased, less water content is lost during the DHT crosslinking process. The higher gelatine concentration resembles the more bonding and crosslink achievable and thus strengthen the scaffold structure and avoiding it from collapsing during service. Gelatine not only shows excellent biocompatibility and biodegradability, but also exhibits superior plasticity and adhesiveness that is essential for developing mechanical strength in the scaffold. Besides, gelatine also acts as a binding agent in medical application because it is derived from collagen and contains biological functional groups, such as amino acids within its backbone which can enhance cell growth and proliferation[8].

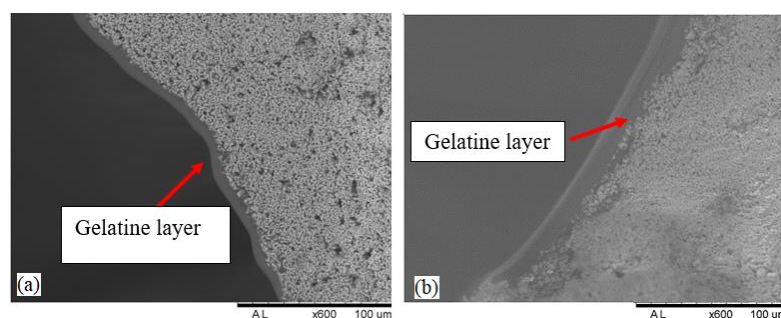


Figure 5. Morphology of fabricated gelatine/HAp porous scaffold at (a)10 wt% and (b)15 wt%.

4. Conclusion

Gelatine/HAp porous scaffold with 40 wt% of solid loading via polymer reticulate method was successfully fabricated in this study. The calcination treatment on HAp powder was proven to facilitate the slurry infiltration process. The HAp powder calcined at 1100 °C introduces more negative charges on the HAp particle surface to enable a well-stabilized HAp suspension of high solid loading with constantly low viscosity being prepared. In this study, the gelatine/HAp scaffold with average compressive strength of 7.8 kPa, 22.8 kPa and 71.0 kPa was achieved for HAp coated with 10 wt%, 15 wt% and 20 wt% gelatine concentrations respectively. On the other hand, the average compressive strength of scaffolds underwent crosslinking at temperature of 130 °C and 160 °C recorded as 66.4 kPa and 71.0 kPa. Increase in gelatine concentration and crosslinking temperature will increase the HAp scaffold compressive strength. However, the effect of crosslinking temperature on compressive strength is less significant compared to the gelatine concentration.

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