EFFECT OF ALGINATE CONCENTRATION ON THE COMPRESSIVE STRENGTH OF HYDROXYAPATITE SCAFFOLD

Ong XQ and Nurazreena A*
Faculty of Mechanical and Manufacturing Engineering,
Universiti Tun Hussein Onn Malaysia(UTHM),
86400 Parit Raja, Batu Pahat, Johor, Malaysia

The effect of alginate concentration on the mechanical properties of hydroxyapatite (HAp) scaffold was investigated in this study. Sodium alginate solution with concentration 1%, 3% and 5% was prepared through the neutral extraction of cultivated Sargassumpolycystum brown seaweed. HAp scaffold was prepared via polymer reticulate method and sintered at 1250℃. HAp scaffolds were then immersed in sodium alginate solution at 1%, 3% and 5% concentration under vacuum. FTIR spectra show that the extracted sodium alginate has the same functional groups as in commercial sodium alginate. Compressive strength increased as the sodium alginate concentration increased. Morphology observation shows that the micro cracks on the struts were covered by alginate. The increase in the compressive strength is due to the coverage of the cracks on the struts by sodium alginate.

Keywords: alginate, hydroxyapatite scaffold, compressive strength

INTRODUCTION

Worldwide incidences of bone disorders and conditions such as genetic conditions, bone infections, bone tumors and bone loss by trauma have trended steeply upwards and are expected to double by year 2020 [1]. Traditionally, bone grafts have been used to restore damaged bone.However, due to the drawbacks of bone grafts such as limited bone supply, the need for second surgery, donor site morbidity, disease transmission and cost have encouraged an alternative approach. Now, engineered bone tissue has been viewed as a potential alternative to the conventional use of bone grafts, due to their limitless supply and no disease transmission. Bone scaffolds which have the potential to allow new bone tissue ingrowth and mechanical properties to match that of natural bone are extensively studied. An ideal scaffolding materials requires biocompatibility, sufficient mechanical strength and optimum pore size as well as bioresorbability [2].

Hydroxyapatite (HAp; Ca10(PO4)6(OH2)) has been used most extensively as bone replacement materials. However, typical of ceramics materials, HAp is brittle which limited its application in non-load bearing applications [3]. To overcome this, composite of HAp with synthetic biodegradable polymers such as polylactic acid (PLA) and polylactic acid-co-glycolic acid (PLGA) are fabricated to overcome the brittleness and gives the scaffold partial elasticity. However, the surfaces of synthetic polymers are hydrophobic which limits cell adhesion and growth in 3D architecture. Lacking of functional groups on the surface of these polymers also limits the possibility for modification [4]. Therefore, instead of synthetic biodegradable polymers, alginate, a natural polysaccharide was chosen in this study as the polymeric phase. Alginate shows excellent biocompatibility, biodegradability,

* Corresponding author: Tel: +6 04 599 6117
E-mail: nurazreena@usm.my

non-antigenicity and chelating ability which makes alginate promising biomaterial.

In this study, the feasibility of sodium alginate solution at different concentration to be used to enhance the compressive strength of HAp scaffold will be evaluated.

MATERIALS AND METHODS

HAp Scaffold Preparation

HAp scaffolds are prepared via a polymer reticulate method. Briefly, HAp powder (Sigma Aldrich, United Kingdom) were mixed with 60 wt% of water and 40 wt% of polyvinyl alcohol (PVA; Merck, Germany). The slurry was then mixed for 4 hours using a mechanical stirrer. Commercially available polyurethane foam (PU; Wansern Technology Sdn. Bhd., Malaysia) with pore size of 1 mm were cut into the dimension of 2.5 cm x 2.5 cm x 2.5 cm. The cut PU foams were then immersed in the HAp slurry for impregnation. Then the foams were rolled using corrugating machine to remove excess slurry. The immersion and rolling processes were repeated for 5 times to ensure full penetration of the HAp slurry into the PU foams. The coated foam was then dried at 80℃ for 24 hours in a drying oven. After drying, the foams were sintered at 1250℃ for 1 hour using a four stage schedule: (i) heating from room temperature to 500℃ at a heating rate of 5℃/ min to burn out the PU foam and prevent thermal shock; (ii) further heating from 500℃ to 1250℃ at a heating rate of 5℃/ min to allow sintering of the HAp slurry; (iii) a hold at 1250℃ for 1 hour; (iv) cooling down to room temperature in furnace.

Extraction of Sodium Alginate from Cultivated Sargassum Polycystum Brown Seaweed

Sodium alginate was extracted via a neutral extraction method. Firstly, 10g of blended seaweed were immersed in 0.2M of hydrochloric acid (HCl; Merck, Germany) at pH 4 and stirred for one hour at room temperature. The residual solution then was drained and the algae were washed with distilled water. 1, 3 and 5% of sodium carbonate (Na2CO3; Fluka) was poured onto the seaweed until pH 10 was reached and stirred for 2 hours. The paste obtained was put into a centrifuge machine at 3000rpm for 30 minutes. The undissolved cellulose was removed. The dissolved cellulose was then added with 10% of calcium chloride (CaCl2; R&M Chemical, UK) solution, using the ratio of 2.2:1 parts of calcium chloride to alginate in the algal raw material. The alginate fibers obtained were then washed with distilled water. HCl acid was added to the fibrous residue and the alginic acid was separated by filtration and dried in air followed by drying in an oven at 40°C until a constant weight was obtained. In order to obtain sodium alginate, Na2CO3 was once again added to the solid alginic acid until pH 8 was achieved and stirred for 2 hours. An equal volume of ethanol (C2H6O; Merck, UK) was added to the sodium alginate solution for precipitation of alginate. The precipitate was then dried in an oven at 40°C.

Preparation of Alginate/ HAp Scaffold

The obtained HAp scaffold then was immersed into the extracted sodium alginate at different concentration (1, 3 and 5% concentration). The HAp scaffold were immersed completely in the solution and the conical flasks containing the HAp scaffold and alginate solution were then vacuum pumped to ensure full penetration of sodium alginate into the HAp scaffold. The conical flasks were put under vacuum until no bubbles were observed. Then the alginate/ HAp scaffold were removed from the solution and allowed to dry before characterization.

X-Ray Diffraction Analysis

For compositional analysis, the HAp scaffold were characterized by means of X-ray

diffraction, (XRD, Bruker AXS D8 Advance, Karlsruhe, Germany) using monochromatic CuKα radiation generated at 40 kV and 40 mA.

Fourier Transform Infrared Spectroscopy

Initial characterization of the extracted sodium alginate was done by using Fourier transform infrared spectroscopy (FTIR; Perkin Elmer One Spectrometer, Shelton, USA). Solid sodium alginate was deposited on the glass slides and the specimens were scanned at the wavelength in the range of 450–4000 cm-1.

Compressive Strength Measurement

Instron Universal Testing Machine (Model TTC, Instron Corp., Canton, USA) was used to measure the compressive strength of the alginate/ HAp scaffold. The specimens were positioned at the center of the testing machine and were subjected to compression testing at a cross head speed of 1 mm/min using 100 kN load cell. Compressive strength was determined using Eq. 1.

$$\sigma = F/A \qquad (1)$$

Where σ is the compressive strength, F is load at failure and A is the specimen cross sectional area.

Scanning Electron Microscope Analysis

For morphological analysis, scanning electron microscope (SEM; Hitachi TM300, Japan) was used at an accelerating voltage of 10 kV. The surface was coated with gold prior to observation.

RESULTS AND DISCUSSION

Fig. 1 shows the XRD pattern of HApbefore and after sintering at 1250℃. Fig. 1(a) shows the XRD pattern of HAp powder before sintering. This XRD pattern matches the HAp pattern with ICDD code number 98-007-7628 for pure HAp. However, after sintering at 1250℃, as shown in Fig. 1(b) the peaks obtained match the standard ICDD code number 98-008-2569 for pure β-tricalcium phosphate (β-TCP). The presence of β-TCP peaks indicates that HAp has started to decompose at 1250℃. The decomposition started off with the formation of intermediate phase, oxyapatite that forms through gradual loss of the radical OH^- (dehydroxylation) in the matrix when HAp is sintered at temperature above 1200℃ [5].

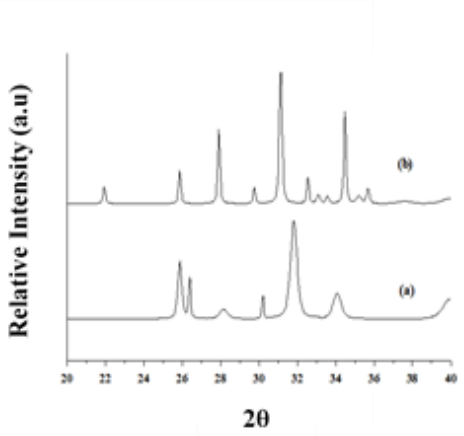


Fig.1. *XRD pattern of (a) before (b) after sintering HAp powder at 1250℃.*

FTIR spectra of commercially available sodium alginate and the extracted sodium alginate of different concentrations are shown in Fig. 2. From this figure, it can be observed that the vibration bands of the extracted sodium alginate of different concentration match the vibration bands of the commercially available sodium alginate.

Fig. 3 shows the compressive strength of alginate/HAp scaffold immersed in sodium alginate at different concentration. From this figure it can be seen that there is a significant increased in the compressive strength of the alginate/HAp scaffold compared to the HAp scaffold. As the concentration of the sodium alginate increases the compressive strength also increases steadily. Alginate/HAp scaffold immersed in 5% concentration has a compressive strength of 86.9 kPa compared

to 2.8 kPa of the uncoated HAp scaffold. The increase in the compression strength can be explained by the crosslinking between calcium ion (Ca2+) from HAp and the carboxyl groups (COO^-) of glucuronic acid in sodium alginate. The G blocks of alginate correspond to high affinities for divalent ions such as Ca2+ and easily replaceable Na+ ions in sodium alginate. Therefore, Ca2+ from HApcrosslinked with sodium alginate and thus increases the mechanical properties of alginate/HAp scaffold [6]. Hence, the higher the concentration, the more Ca2+ ions on the HAp surface that will cross-linked with the carboxyl group alginate as Ca2+ easily replaces Na+ ions in sodium alginate [7].

Fig.4 shows the microstructures of the HAp scaffold struts before and after immersion in 1, 3 and 5% of sodium alginate concentration. From Fig. 4(a) it can be seen that the strut microstructure of HAp scaffold before immersion in sodium alginate concentration has many small, as well as large cracks. These cracks contribute to the brittleness of the HAp scaffold. However, after immersion in sodium alginate solution, it can be seen that the struts are thicker and the surface of the struts are smoother even though the cracks are still visible. It is well established that HAp-polymer coatings increases the thickness of the strut and thus contribute to the increase in the compressive strength [7]. Immersing HAp scaffold in the sodium alginate solution allows the solution to fill the microcracks on the strut and improves the mechanical stability of the scaffold. Thus, this explains the increase in the compressive strength of alginate/HAp scaffold when compared to the monolithic HAp scaffold.

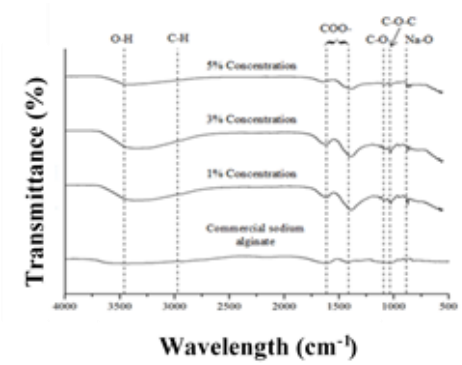


Fig.2. *FTIR spectra of commercially available sodium alginate and the extracted sodium alginate of 1, 3 and 5% concentration.*

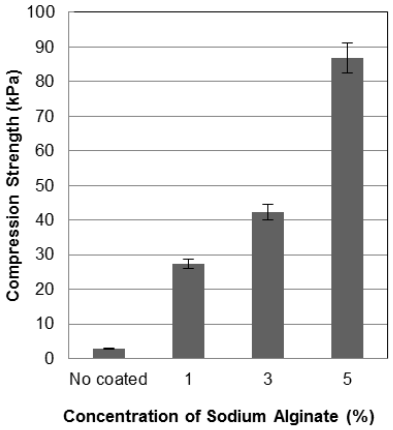
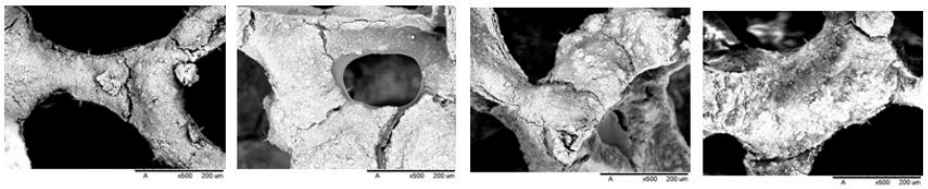


Fig.3. *Compressive strength of alginate/Hap scaffold immersed in different sodium alginate concentration.*



(a) *(b)* *(c)* *(d)*

Fig.4. *Scanning electron micrograph of HAp scaffolds (a) before immersion in sodium alginate solution (b) after immersion in 1% concentration (c) after immersion in 3% concentration and (d) after immersion in 5% concentration of sodium alginate.*

CONCLUSION

Alginate/Hap scaffold was successfully prepared. An increase in sodium alginate concentration increases the compressive strength of the alginate/HAp scaffold. This is due to the crosslinking between the Ca2+ ions and the Na+ ions and also due to the coverage of the cracks on the struts by the polymeric layer of sodium alginate. Therefore, sodium alginate can be used to enhance the mechanical properties of HAp scaffolds.

ACKNOWLEDGEMENT

This work is supported by Transdisciplinary Research Grant No: 203/PBAHAN/6761003

REFERENCES

[1] Amini, A. R., Laurencin, C. T., Nukavarapu, S. P. (2012). Bone Tissue Engineering: Recent advances and challenges, *Critical ReviewsTM in Biomed*. Eng. 40 363-408.

[2] Bose, S., Roy, M., Bandyopadhyay, A. (2012). Recent advances bone tissue engineering scaffolds. *Trends in Biotechnology*. 30 546-554.

[3] Noro, T., Ito, K. (1999). Biomechanical behavior of hydroxyapatite as bone substitute material in a loaded implant model: On the surface strain measurement and the maximum compression strength determination of material crash, *Bio-Med. Mater*. Eng. 9 319-324.

[4] Cao, N. (2012). Fabrication of alginate hydrogel scaffolds and cell viability in calcium crosslinked alginate hydrogel, PhD Thesis, University of Saskatchewan.

[5] Muralithran, G., Ramesh, S. (2000). The effects of sintering temperature on the properties of hydroxyapatite, Ceram. Int. 26 221-230.

[6] Zhang, Z., Zhang, S., Shang, L. (2013). Apatite coated polymer template for implant and drug delivery, S. Zhang (Eds.) Hydroxyapatite coating for Biomedical Application. 239.

[7] Han, J., Zhou, Z., Yin, R., Yang, D., Nie, J. (2010). Alginate-chitosan/hydroxyapatite polyelectrolyte complex porous scaffolds: Preparations and characterization, *Int. J. Bio. Macro*. 46 199-205.