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Cell Evaluation on Alginate/Hydroxyapatite Block for Biomedical Application

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Abstract

Initial cell evaluation on alginate/hydroxyapatite block was investigated. Sodium alginate with 1, 3 and 5% concentration was obtained via neutral extraction of locally obtained brown seaweed, Sargassumpolycystum. Commercially available hydroxyapatite (HAp) powder was pressed uniaxially at 3 MPa to obtain the HAp block. The HAp block was then sintered at 900℃. The sintered HAp block was then immersed in the sodium alginate solution at different concentration for 24 hours under vacuum condition. Morphological observations show that normal cell growth was observed on alginate/HAp blockafter post treatment for day 1 and 2. However, the cell starts to show some distinct morphological changes when compared to the control cells for day 5 and 7. Cell viability assay results shows that a consistent cell growth was obtained with HAp block incorporated with 3 and 5% sodium alginate. While HAp block without the incorporation of sodium alginate and HAp block incorporated with 1% sodium alginate concentration shows inconsistent cell growth. Initial cell evaluation results suggest that alginate/HAp block shows no toxicity on cell attachment and proliferation.

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1. Introduction

Damage and functional disorder of bone have become a global health care problem. However, current medical treatments which focuses on replacing the lost bone with autogenous and allogenic bone grafts induces many disadvantages such as limited bone supply, risk of diseases transmission, donor site morbidity and cost[1]. Therefore, artificial bone scaffolds which have the potential to allow new bone tissue ingrowths and mechanical properties to match that of natural bone are in high demand. Hydroxyapatite (HAp; $Ca_{10}(PO_4)_6(OH_2)$) has been used extensively in bone implantation to repair damage bone due to its similarity in chemical composition with the human bone[2]. However, sintered HAp often associated with its brittleness which limits its application in nonload bearing applications. Therefore, to overcome this drawback, HAp is often incorporated with biodegradable polymers to overcome its brittleness. Composite of HAp with biodegradable polymers are thought to improve the mechanical properties of HAp scaffold since it mimic the human bone which is a composite of organic and inorganic phase. The combination of these phases gives the bone superior strength and partial elasticity[3].

Alginate, naturally derived polymers are of interest to be incorporated into HAp scaffold due to its high biocompatibility, low toxicity, relatively economical and widely used in the food and pharmaceutical industry[4]. In addition, the hydrophilic nature of the alginate allows simple and rapid cell seeding that facilitate the nutrition transport and cell growth inside the scaffold[5].These properties of alginate makes it a promising material when compared to synthetic biodegradable polymers such as polylactic acid (PLA) and polylactic acid-co-glycolic (PLGA) to be incorporated into HAp scaffold because these synthetic biodegradable polymers are hydrophobic which limits cell adhesion and once implanted in vivo, the degradation products of synthetic biodegradable polymers can invoke chronic immune reaction[6].

Taking into account the advantages of alginate and HAp, a composite of alginate/HAp block was fabricated in this study and the initial cell evaluation on the alginate/HAp block was evaluated.

2. Materials and Methods

2.1. Preparation of alginate/HAp block

Sodium alginate was extracted from seaweed, Sargassumpolycystum harvested from Semporna, Sabah, Malaysia via neutral extraction method. Briefly, 10 g of blended seaweed were immersed in 0.2M of hydrochloric acid (HCl; Merck, Germany) at pH4 and stir for one hour at room temperature. The residual solution was then drained and the algae were washed with distilled water. Then, 1, 3 and 5% of sodium carbonate (Na_2CO_3; Fluka, Germany) was pour to the seaweed until pH 10 was reached and stirred for 2 hours. The paste obtained was put into a centrifuge machine at 3000 rpm for 30 minutes. The undissolved cellulose was removed. The dissolved cellulose was then added with 10% of calcium chloride ($CaCl_2$; 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. Next, HCl acid was added to the fibrous residue. 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. To get sodium alginate, $Na_2CO_3$was once again added to the solid alginic acid until pH 8 was achieved and stirred for 2 hours. An equal volume of ethanol (C_2H_6O; Merck, UK) was added to the sodium alginate solution for precipitation of alginate. The precipitate was then dried in an oven at 40°C.

For the preparation of HAp block, commercially available hydroxyapatite (HAp; Sigma Aldrich, UK) powders were put in a stainless steel mould and pressed uniaxially at 3 MPa to obtain a block of dimension 10 x 5 mm in diameter and thickness using a hydraulic hand press. The compacted samples were then sintered in a furnace at 900°C with heating rate of 5°C/min.

The prepared HAp blocks were then immersed into a glass bottles containing sodium alginate solution at different concentration. The bottles were then placed in vacuum desiccators for 24 hours to allow full penetration.