

## The Mechanical Characterization of *Dioscorea Alata* and *Plectranthus Rotundifolius* Starch-HA Composite

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### ABSTRACT

*The scaffolds made of starch-hydroxyapatite (HA) were fabricated by using solvent casting method and particulate leaching technique. The starches originated from Malaysian tubers which were Dioscorea alata (purple yam) and Plectranthus rotundifolius (Chinese potato). A composite were prepared by adding different weight of HA and of starch powder together with 20 ml of distilled water. The 50%, 60%, and 70% concentrations of starch-HA were prepared in this study. After the fabrication process, the effect of the starch-HA composites were investigated. The microstructures and the morphology of the composite scaffold were observed using SEM and the mechanical properties (Young's modulus) of the scaffold is obtained by performing compressive test on the scaffolds. The results shows that the mechanical strength of Plectranthus rotundifolius is stronger compared to Dioscorea alata with the Young's modulus of (70% starch in the composite) is 15.55 MPa for Dioscorea alata and 18.81 MPa for Plectranthus rotundifolius.*

**Keywords :** Scaffold, Starch, Fabrication and Mechanical Strength.

### I. INTRODUCTION

Tissue engineering is an interdisciplinary field which applies the principles and the methods of engineering and life sciences towards the fundamental understanding of structural and functional relationship in normal and pathological tissue and the development of biological substitutes [1]. Scaffold is a main component for tissue engineering. Scaffold is usually made from materials that are biocompatible, biodegradable and safe as implant. Scaffold is used for cell attachment and tissue developing, typically made of polymeric biomaterials; this will provide the structural support for cell attachment and subsequently, tissue development [2].

Scaffold should has the extracellular matrix tissue same like a native tissue but it is too difficult to mimic the same extracellular matrix tissue like native tissue [3]. This is because the native tissue has multiple functions and the dynamic nature of extracellular matrix tissue [4]. Thus, the basic concept for scaffolding is actually to mimic at least certain partially functions of the extracellular matrix of native tissue. Therefore, it is important that the scaffolds should have suitable morphology, microstructure and mechanical strength that will able to support cell or tissue growth.

Starch is a natural biopolymer which is known to be safe and biocompatible to man. However, it is difficult to produce a scaffold with adequate mechanical properties based on the strength, the elasticity, and the absorption and its chemical degradation. The starches that were used in this study are from *Dioscorea alata* and *Plectranthus rotundifolius* which is a native tuber of Malaysia. Starch is very sensitive. Therefore, the application of starch alone is not suitable for bone tissue scaffold fabrication [5-6]. In this study, the scaffold is fabricated using

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(Hydroxyapatite) HA- starch for the future bone tissue scaffold application based on native starches.

The morphology and the microstructures of the scaffolds were examined by Scanning Electron of Microscopy (SEM) and the mechanical strength of the scaffold will be determined by using Instron Universal Testing Machine. It is expected that the properties of the scaffold may be improved especially in terms of their morphology and mechanical strength. In this study, solvent casting and particulate leaching would be used for the scaffold fabrications [7-9].

## 2. MATERIALS AND METHODS

*Dioscorea alata* and *Plectranthus rotundifolius* were obtained from local market in Pasir Mas, Kelantan (Malaysia). Sodium Chloride (NaCl) lab grade was purchased from Sigma-Aldrich. Glutaraldehyde (25 v/v%) was supplied by Merck.

### 2.1 Scaffold Fabrication

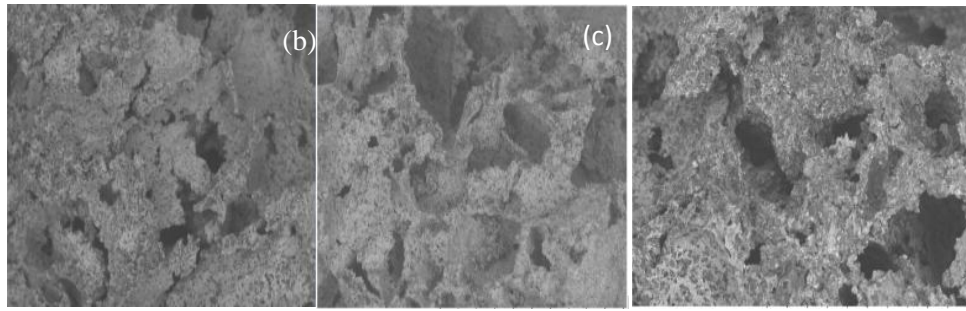
Here, starches from the Malaysian tubers were used together with HA to fabricate tissue engineering scaffolds and the samples were used for SEM and mechanical testing. Firstly, the tubers were peeled off and were thin sliced. After that, all the tubers were cleaned up and dried at 65°C for 24 hours and were mashed up into starch powder. The starch powder was added with HA powder and 20ml distilled water. The amount of the starch powder and the HA powder mixed is in accordance to the concentration of the starch solution. In this experiment, three concentrations of the starch solution were prepared (50%, 60% and 70%). The starch, HA and 20 ml distilled water were added together and the mixture was stirred for 10 minutes. Then, the mixture was continued to be stirred using double boiling technique up to 35°C.

At 35°C, NaCl particles were added into the mixture. The function of NaCl is to form the pores of the scaffold. The mixture was stirred until the temperature went up to 75°C. Then, the mixture was poured into a Teflon mold. Then, the mixtures were dried at 70°C in 48 hours. After that, the samples were left at room temperature for 5 hours. Then, the dried samples were immersed with 25% of glutaraldehyde (GA) for 10 hours. After that, the samples were immersed with 70% ethanol for 10 hours. Salt leaching technique was used here where the samples were immersed in distilled water to remove GA and to dissolve the salt. This process was performed for 72 hours and the distilled water was changed every day. After that, the samples were dried at room temperature for one hour and were dried again at 80°C for 5 hours. These samples would be used for SEM and mechanical testing.

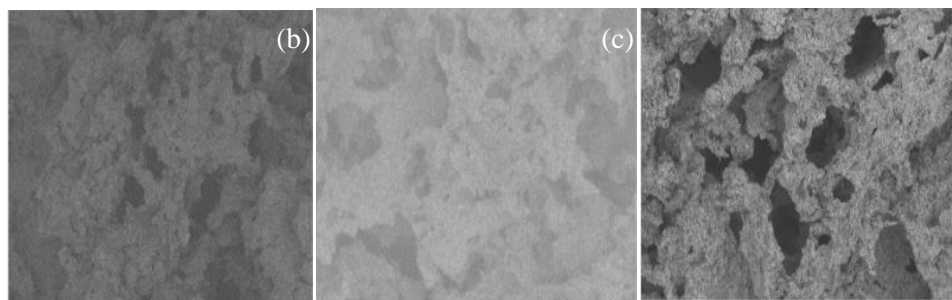
## 3. RESULTS AND DISCUSSION

### 3.1 Scanning Electron Microscope (SEM)

Here, different type of starch gives a different microstructure of the scaffold. For each ratio of starch-HA scaffold samples, the differences of each surface images were observed. The ratio of the starch-HA are 5:5, 6:4, and 7:3 for each of different tubers.



**Figure 1.** The morphology and microstructure for (a) 50%, (b) 60% and (c) 70% concentration of *Dioscorea alata*.



**Figure 2.** The morphology and microstructure for (a) 50% , (b) 60% and (c) 70% concentration of *Plectranthus rotundifolius*.

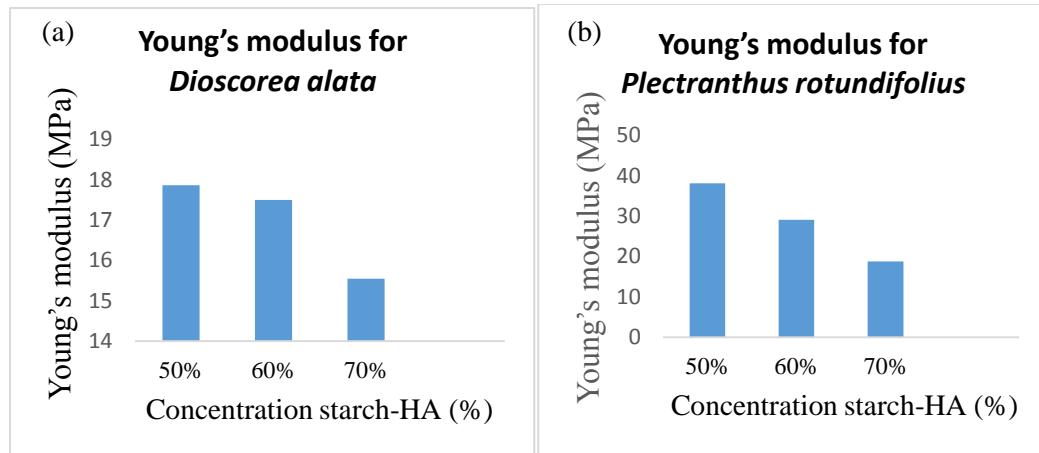
Figure 1 shows the Scanning Electron Microscope (SEM) result for 50%, 60% and 70% starch-HA composite scaffold for *Dioscorea alata*. For 50% concentration starch-HA, the pore size were in the range of 127 – 333  $\mu\text{m}$ . For 60% concentration starch-HA, the pore size were in the range of 179 – 550  $\mu\text{m}$  and for 70% concentration starch-HA, the pore size were in the range of 185 – 530  $\mu\text{m}$ . The scaffolds showed good pore structures but relatively poor distribution. The pore size had also increased up to 550  $\mu\text{m}$  for the scaffold.

Figure 2 shows the Scanning Electron Microscope (SEM) results for 50%, 60% and 70% starch-HA composite scaffolds for *Plectranthus rotundifolius*. For 50% concentration starch-HA, the pore size were in the range of 230 - 315 $\mu\text{m}$ . For 60% concentration starch-HA, the pore size were in the range of 265 - 452  $\mu\text{m}$  and for 70% concentration starch-HA, the pore size were in the range of 352 - 458  $\mu\text{m}$ . The porosity seems to improve as the starch increased. The pore size had also increased up to 458  $\mu\text{m}$ . From the results, most of the pores size that obtained were preferable for cell growth and suitable for bone tissue development. From the previous researches, it shows that the range of pores that are suitable for bone tissue engineering are in the range of 200  $\mu\text{m}$  to 500  $\mu\text{m}$  [10].

### 3.2 Mechanical Testing

Figure 3 (a) shows the result of the Young's modulus for three different samples from the *Dioscorea alata* starch-HA scaffolds with the 50%, 60% and 70% concentration of starch. The Young's modulus for 50% starch-HA concentraton is 17.86 MPa. The Young's modulus for 60% and 70% starch-HA concentration are 17.50 MPa and 15.55 MPa respectively. Figure 3 (b) shows the result of the Young's modulus for the three different samples from the *Plectranthus rotundifolius* with the 50%, 60% and 70% concentration of starch. The Young's modulus for

50% starch-HA concentraton is 38.14 MPa. The Young's modulus for 60% and 70% starch-HA concentrations are 29.17 MPa and 18.81 MPa respectively.



**Figure 3.** Young's modulus for (a) *Dioscorea alata*, (b) *Plectranthus rotundifolius*.

From the results of the mechanical testings, it is concluded that, when the starch concentration increases, the strength of the scaffolds had decreased. The strength also depend on the porosity. The higher porosity will decrease the value of the Young's modulus [11]. The porosity will increase when the starch ratio increase and this had affected the strength of the scaffold. Thus, the scaffolds could be used for bone tissue applications since the mechanical properties of the human bones varies. The Young's modulus for *Plectranthus rotundifolius* also can be predicted since it is directly propotional with the percentage of starch cocentration.

#### 4. CONCLUSION

The starches from Malaysian tubers such as *Dioscorea alata* and *Plectranthus rotundifolius* were successfully used to fabricate tissue scaffold using solvent casting and particulate leaching. The pore size that were observed using Scanning Electron Microscope (SEM) is suitable for bone tissue application since the range of the pores are between 127 – 550  $\mu\text{m}$ . In addition, the mechanical strength is depended on the porosity of the scaffold. When the porosity increased, the mechanical strength of the scaffold will also decrease. The porosity increased when the starch ratio increased. Thus, the pore size plays an important role in influencing mechanical performance [12]. Here, the composites made from *Plectranthus rotundifolius* showed a higher strength in comparison to the *Dioscorea alata* composites. The strength of the scaffolds made from *Plectranthus rotundifolius* was more proportional to the amount of the starch added to the composites in comparison to the scaffolds made from *Dioscorea alata*. From the Young's modulus results obtained, the scaffolds may have potential to be used for tissue engineering applications [13-15].

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