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Preliminary Study of Trong Clay – HA Bone Scaffold Fabrication Using Solvent Casting/Particulate Leaching Method and Indirect 3D-Printing Technique

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ABSTRACT

This paper is about the feasibility of using the combination of Trong clay and hydroxyapatite for the fabrication of bone tissue scaffold. Trong clay is one of Perak's (a state in Malaysia) natural resources which can be potentially used as a biomaterial. Two methods were involved in the scaffold fabrication: the solvent casting/particulate leaching method and the indirect three-dimensional printing method. It is found that the solvent casting/particulate leaching method was not able to produce tissue scaffold as intended. However, tissue scaffolds made using indirect three-dimensional printing were able to be fabricated using the 9:1 Trong clay-HA ratio. It was found also that the scaffolds with lower percentage of Trong clay were more fragile and brittle. In this study, the scaffolds produced using this technique have an interconnected porous structure with approximately 500µm pores diameter observed using Field Emission Scanning Electron Microscope (FESEM).

Keywords: Trong Clay, Bone Scaffold, Hydroxyapatite, Indirect 3D Printing.

1. INTRODUCTION

Malaysia is rich with her biodiversity and natural resources which can be potentially used as biomedical materials. Natural materials derived from seashells [1, 2], native rice [3-5] and tubers [6] which were found abundantly in Malaysia can be potentially used as a bone tissue scaffolds' materials. Apart from these biological resources, Malaysia is also known for her richness in ceramic materials such as clay which is now found its place as a new potential commodity [7, 8]. Historically, clay of Perak origin is used to produce popular traditional Malaysian made potteries such as Labu Sayong which was used in the old days to store water for drinking [9, 10]. Recently, clays from Perak including the variant from Trong, Larut Matang and Selama, Perak is used for traditional ceramic construction and mechanical products [7, 8], potteries [11] and in "halal industry" such as a "samak" clay [12].

Here, the potential of Trong clay as a scaffold material is investigated. Scaffold is defined as a framework or structural element that hold cells or tissues together [13]. The ideal characteristics for bone scaffold tissue engineering application would be much dependent on its biocompatibility, biodegradability, high porosity, adequate pore size (100-500 µm), interconnected pores and sufficient mechanical properties [14].

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Traditional materials to produce scaffold is usually made of hydroxyapatite (HA) which has similar natural properties as in human bone[15], [16]. However, hydroxyapatite has several weaknesses due to its brittleness, low toughness and low flexural strength [17]. Thus, in this study, clay may improve the properties of the bone scaffold especially in term of the mechanical strength [18]. For this study, Trong clay is selected as a material which can be used along with HA for bone scaffold fabrication.

Thus, to fabricate bone scaffold, two methods were studied to observe their feasibility. First, the solvent casting and particulate leaching which is a common preliminary methods used to fabricate bone scaffold [19]. Here, the materials of interests would be dissolved in a solvent and the other components of the composites would be added. Hence, the slurry would be casted into a mold and it would be allowed to evaporate in order to create the scaffold of interest [20, 21].

The second method would be the three-dimensional printing method [22]. There are two types of three-dimensional printing method, which are the direct and the indirect 3D printing methods. The direct 3D printing involves direct manufacturing of the scaffold as a final product. Usually, this procedure requires post processing stages such as curing and cleaning before obtaining the final product. However, the materials that could be used directly using this method is limited [23].

Therefore, another alternative to solve this issue is to apply the indirect 3D printing method. The indirect 3D printing method involves the fabrication of a mold using computer aided design solution by using desired materials which will fixed the final shape of our scaffold. Later, the mold will be removed, leaving the final construct with the desired pores [22].

It is hoped that a tissue engineering bone scaffold made from Trong Clay-HA composites can be fabricated using the solvent casting/ particulate leaching method or the indirect 3D printing method. Later, the successful bone scaffolds developed would be characterized using Field Emission Scanning Electron Microscopy (FESEM) to observe their porosity.

2. METHODOLOGY

2.1 Solvent Casting / Particulate Leaching

Trong clay (was obtained from Natural Intention Sdn. Bhd.), HA (Merck) and sodium chloride (Merck) as the porogen were weighted as per ratio needed for each sample. Then, Trong clay and HA in the form of powder were mixed by using these ratios, 9:1, 8:2, 7:3, 6:4 and 5:5. The slurry of Trong clay-HA mixtures were made by adding distilled water and they were stirred in the water bath. Later, sodium chloride was added into the solution. The mixtures were poured into Teflon molds and then, placed in the incubator for about 48 hours at 65ºC which was supposed to be able to produce tissue scaffolds as intended. The newly fabricated samples were then immersed in 25% glutaraldehyde solution for 5 hours to cross-link both Trong clay and HA [4]. This procedure should continue with the immersion of the samples in distilled water for approximately 4 days for salt leaching [3], [4], [6] and this should contribute to the pore formation of the Trong clay-HA bone tissue scaffolds.

2.2 Indirect Three-Dimensional Printing

For the PLA (polylactic acid) template printing, the template was designed using software CATIA V5 and was later saved in the STL format. The scaffold was designed to be 15mm×25mm×15mm with the size of the pore to be approximately 0.5mm×0.5mm

(500μm×500μm). The STL format of the inverse template was then used to generate G-Code by using Slic3r software (Figure 1 (a)). The G-Code was then exported to Pronterface software. Hence, the PLA inverse template was printed (Figure 1 (b)).

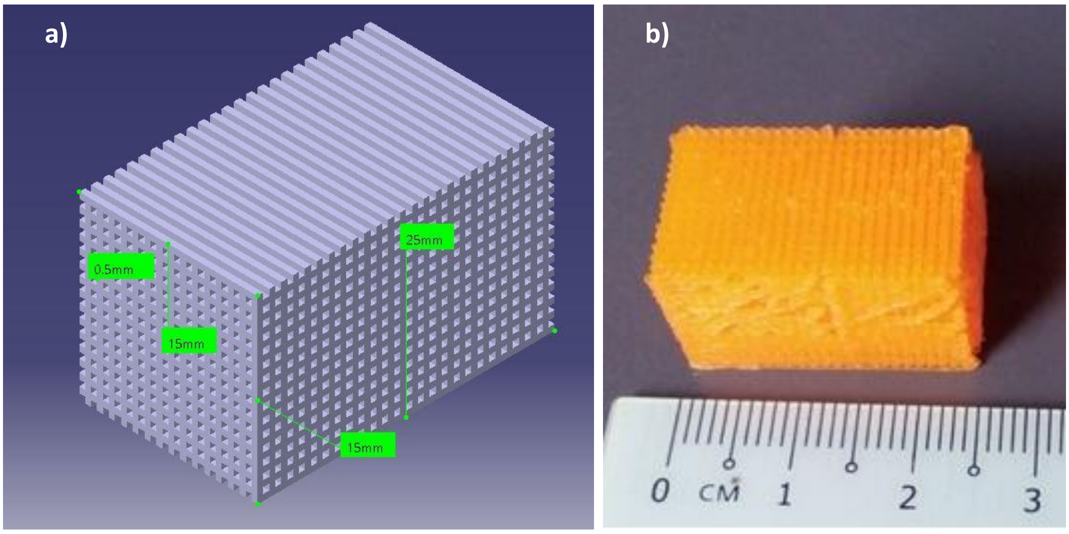

Figure 1. (a) Three-dimensional inverse template design and **(b)** the actual 3D PLA template.

During the sample fabrication, the Trong clay and the HA were weighted as per ratio (as in the solving casting and salt leaching section previously) needed for each sample. Both materials were mixed using distilled water as the solvent. The PLA templates were later immersed into the mixtures for 12 hours to make sure the slurry would occupy the pores of the bone scaffold templates.

The samples were then left to dry for about 3 hours at the room temperature and they were later heat treated in the furnace. The soak temperature chosen here was 388ºC for 3 hours in order to remove the PLA template and the solvent completely as shown in Figure 2.

The samples were analysed through Field Emission of Scanning Electron Microscope (FESEM) with 30 kV of acceleration voltage and they were scanned under vacuum. The samples were coated with a thin layer of platinum prior to observation under FESEM to study the morphological structures of the newly fabricated Trong clay-HA bone scaffolds.

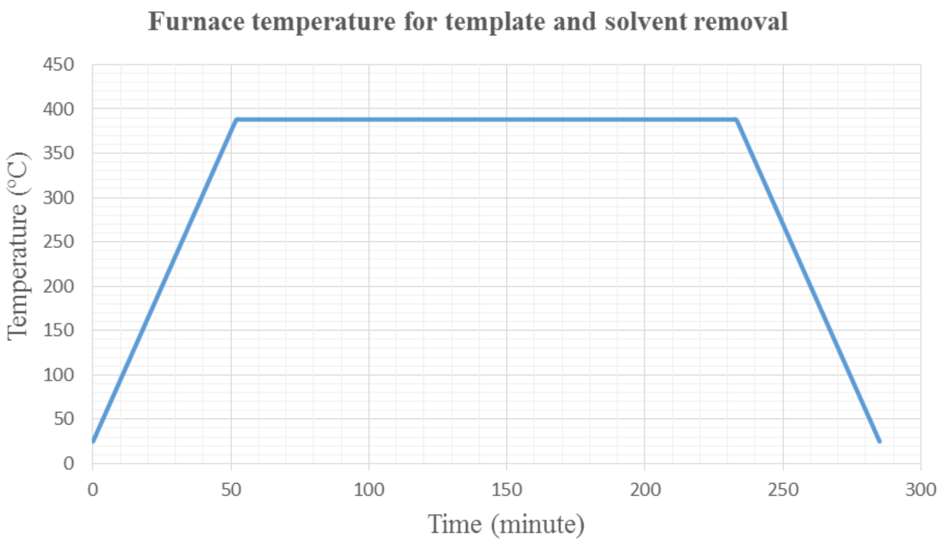

Figure 2. Furnace temperature setting for template and solvent removal.

3. RESULTS AND DISCUSSION

3.1 Solvent Casting and Particulate Leaching

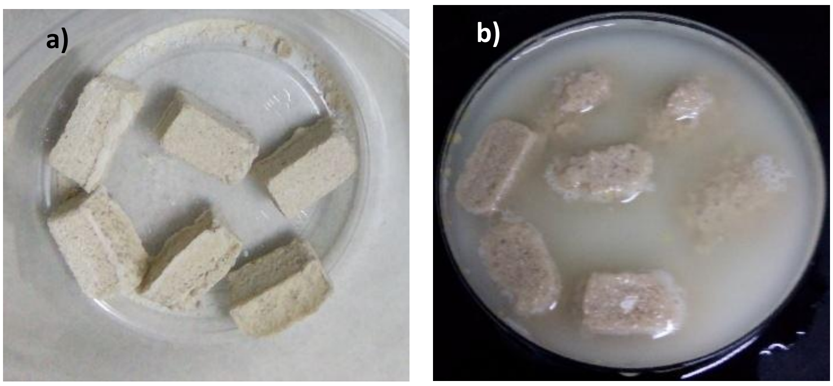

Figure 3. (a) shows the condition of the scaffold before the GA solution and (**b)** rightly after immersing the scaffold into the GA solution.

The samples were immersed in 25% Glutaraldehyde (GA) for 5 hours [4] to crosslink Trong clay and HA in order to improve the scaffold's mechanical strength . However, the scaffolds had failed to maintain their structures as they had slowly dissolved into the distilled water after immersion.

Later, the oven temperature used to dry the bone scaffold was increased to 80ºC which was intended to remove the moisture efficiently prior to GA immersion. However, there was no improvement in term of structural stability observed during the scaffolds' immersion in the 25 % GA solution as intended.

Then, the samples were immersed in 50% GA solution where the concentration of the GA was increased in comparison to the first and the second batch. The result shows that the samples with 90 wt% of Trong clay were able to retain their structure for 1 hour at the GA immersion stage. However, the samples had degraded after 1 hour as shown in Figure 3 (b). Meanwhile, other samples were dissolved several minutes after their immersion. Thus, this shows that the solvent casting/ particulate leaching technique is not suitable to be used for Trong clay-HA tissue scaffold application.

3.2 Indirect Three-Dimensional Printing

Figure 4. A Trong clay-HA bone scaffold.

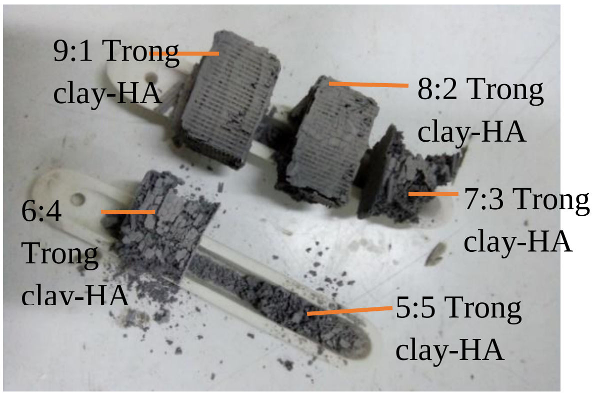

Figure 5. Scaffolds after template and solvent removal.

For the heat treated indirect three-dimensional printing tissue scaffolds, only samples with 9:1 Trong clay-HA were able to maintain their bone scaffold structure. The samples with 8:2 Trong clay-HA were more fragile compared to the samples with 9:1 Trong clay-HA. Meanwhile, for samples with 7:3 and 6:4 Trong clay-HA, they were partially collapsed during the template and solvent removal process. However, for the samples with 5:5 Trong clay-HA, all of them were totally broken during the heat treatment (Figure 5).

3.3 Field Emission Scanning Electron Microscopy

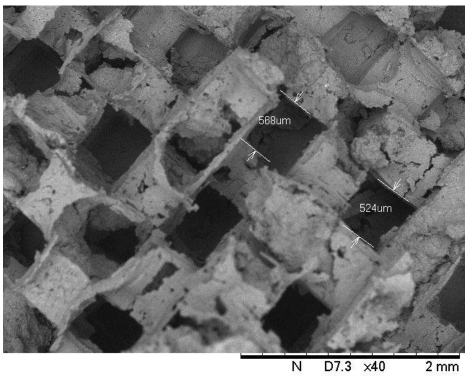

Figure 6. FESEM micrograph of a 9:1 Trong clay-HA bone scaffold at ×40 magnification.

The tissue scaffolds with 9:1 Trong clay-HA had maintained their pore size at 500μm. From the FESEM micrograph, it is observed that the indirect three-dimensional printing technique had able not only to satisfactorily produced Trong clay-HA scaffolds but also can control the size of the pores as intended [24]. Thus, with the correct ratio of Trong clay-HA mixture together with the indirect three-dimensional printing, a bone scaffold with an interconnected pore and customized porosity was produced successfully [22].

In this study, the indirect three-dimensional printing was able to fabricate the Trong clay-HA scaffold using the 9:1 Trong clay-HA ratio. Here, the solvent casting/particulate leaching technique had failed to produce a stable scaffold although the moisture content of the scaffold was reduced before GA immersion and the GA concentration was increased in order to promote the integrity of the scaffold. Although solvent casting/ particulate leaching is well known for its simplicity and robustness [3], [4], [6], [25] to produce bone tissue scaffold, the technique was not able to facilitate the formation of Trong clay-HA bone scaffold even before salt leaching process.

It is also found that the suitable mixture for Trong clay-HA scaffold contains higher amount of clay. This is showed by the stability of the 9:1 Trong clay-HA scaffold after the solvent and PLA template removal at 388°C. Other scaffolds with lower amount of clay were less able to maintain their structure.

Here, the superiority of the indirect three-dimensional printing was observed through this technique ability to precisely control the size of the pores. The pores' sizes were similar to the PLA template produced and this is shown by the FESEM micrograph (Figure 6). Thus, it can be concluded that the application of the indirect three-dimensional printing for the clay based bone scaffolds is promising.

4. CONCLUSIONS

Trong clay is a potential material used to fabricate the bone tissue scaffold. Several ranges of Trong clay-HA ratios such as 5:5, 6:4, 7:3, 8:2 and 9:1 were prepared. The best ratio observed was 9:1 with 90wt% of Trong clay and 10wt% of HA. However, the scaffolds could not maintain its structural integrity where, in just 1 hour, all the samples produced by this technique and ratio were dissolved in the solution. For the scaffolds fabricated using indirect 3D printing, the 9:1 Trong clay-HA ratio had maintained their structure without collapsing after being heat treated at 388°C. The scaffolds could be characterized using FESEM without any issues and the pore size obtained is approximately 500μm. Thus, it is suggested that the indirect 3D printing method is a promising technique to fabricate a 3D bone tissue scaffold produced from Trong clay-HA composites.

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