The Characterization Of Bario Rice Starch-nanoHA Scaffolds using SEM and Dielectric Measurement

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Abstract. Bone tissue scaffold had been ventured for over the decades as a solution for bone failure and trauma. Starch is the most common natural polymer that has been used as a biomaterials and a good candidate for scaffold fabrication as there are many resources of starch in Malaysia. Native starches may contribute differently in their structure in terms of the amylose content, interactions between granules, swelling ability and solubility in which those differences can be mainly due to the botanical origin. In Malaysia, there are various resources of rice starches. Here, the Bario rice starch from Sarawak was used to fabricate the scaffold via solvent casting and salt leaching technique. Bario rice is usually collected from various sources and variety which may contribute to different physical affect in comparison of using other type of rice. From the study, the morphologies and microstructures of the scaffold were evaluated using Scanning Electron Microscopic (SEM) and this showed no trend of pore size increment when starch percentage varied. Then, the dielectric properties were obtained via the values of dielectric constant and dielectric loss which are another type of indirect measurement to study the porosity of the scaffolds. The porosity has the value of the dielectric constant and the loss air matrix whereby this air matrix is represented by the pore in the scaffold. Here, the results were consistent with the observation made through SEM. Thus, the relationship between the porosity and the dielectric properties of the Bario Rice Starch-nHA tissue scaffolds had been established and there were no particular pattern could be concluded by using Bario rice for this scaffold.

1. Introduction

Hydroxyapatite is known to have similar chemical composition, mineral phase, and crystallinity of natural bone [1] which majorly exhibit high osteoconduct and osteoinduction when implanted in the human body [2]. Unfortunately, the nature of the hydroxyapatite is brittle and low in mechanical strengths which limit its application [3]. Besides that, hydroxyapatite alone is not mechanically competent to support various applications in biomedical engineering [3], thus various types of materials were used to fabricate tissue engineering scaffolds [4-7].

The starch is recognized to have favourable criteria such as biocompatible, low in cost, good biodegradable term, and non-toxic [8, 9]. Biopolymers formulated from starch have been widely applied in biomedical application such as for bone replacement, bone cement, and bone tissue engineering [9, 10]. Charoenrein et al. stated that different starches would exhibit different criteria and in order to improve the textural qualities of starch, the incorporation of starch with different botanical sources had been performed [11]. The differences in starch behaviour is mainly due to the concentration, mixing ratio, amylose content, interactions between granules, swelling power and solubility, and granule characteristics [12]. Considering the Sarawakian rice has numerous varieties and differs in term of botanical resource, it had been used as reinforcement to hydroxyapatite for bone tissue scaffold. Bario rice which is well known originated from Sarawak was chosen to be used in this study. The properties
of the newly fabricated scaffolds were characterized in detail including their mechanical and electrical properties. Few dielectric properties studies were conducted on various types of starches including tapioca, corn, wheat, rice, waxy maize, amylo maize and also basmati rice but none are performed especially on the scaffold fabricated from starch [13]. Thus, it is hope that by venturing the dielectric properties of scaffold to analyze its porosity may contribute to the new method in examining the porosity in scaffolds.

2. Materials and Methods
The Bario rice used was purchased from a market in Sarawak. Hydroxyapatite and 50% of glutaraldehyde was supplied by Sigma Aldrich and sodium chloride used was also purchased from a local market.

2.1. Scaffold Fabrications
Figure 1 shows the step to fabricate the Bario rice starch-hydroxyapatite (HA) scaffold. Five different ratio of Bario rice starch-nHA scaffolds were prepared which are 50:50(wt%), 60:40(wt%), 70:30(wt%), 80:20(wt%) and 90:10(wt%) were fabricated respectively. At first, the starch powder was mixed with distilled water to form the starch solution. Then, the porogen of NaCl was added into the starch solution followed by the addition of hydroxyapatite solution and stirred for 3 minutes. The slurry solution obtained was then casted onto the Teflon mold with dimension of 25mm x 15mm x 15mm. The next process was drying the slurry in the mold for 48 hours at 60°C. After the drying process, the scaffolds were soaked in 25% glutaraldehyde for 5 hours to enhance the structure of the scaffold and followed by immersion in distilled water to remove the glutaraldehyde. Lastly, the scaffolds were dried again at room temperature before characterizations proceed.

2.2. Scanning Electron Microscopy (SEM)
The samples for Scanning Electron Microscopy (SEM) were cut symmetrically to observe the scaffold’s porosity at the inner part and coated with platinum. The SEM model used was the Table Top TM3000, Hitachi with the excitation voltage of 15kV which was bombarded upon the samples. The magnifications used were about 100X to 300X.
2.3. Dielectric Measurement

The measurement of the dielectric properties was based on the transmission line method by using Agilent E8362B Performance Network Analyzer (PNA) to obtain the dielectric constant $\varepsilon'$ and dielectric loss $\varepsilon''$. The samples were prepared with the dimension of 8 mm x 16 mm x 5 mm which was basically based on the dimension of WR62 waveguide dielectric holder. After setting up the measurement, the frequency was set in the range of 12.4GHz to 18.0GHz. Then, the open air condition measurement was taken without the sample. Only then, the sample was placed in the WR62 waveguide holder with the wave guide adapter and the measurements were taken in triplicate.

3. Results and Discussion

3.1. Scanning Electron Microscopy (SEM)

For the SEM analysis, the pore size obtained from the 50wt%, 60wt%, and 70wt% of Bario rice starch-nHA composite scaffolds are 246µm, 282µm, and 163µm respectively as shown in Figure 2. This trend was observed for all the Bario rice starch-nHA composite scaffolds. Usually for other starch based scaffolds, the pore size would increase by increasing the starch percentages [7]. However, the Bario rice starch-nHA composite scaffolds did not show this particular trend. The high amount of starch contributes to the ruptures in scaffolds’ structure where Ahmed et al., claimed that such high starch percentages would lead to scaffold’s cracking and rupture [13].

![Figure 2: a) 50wt% Bario rice starch, b) 60wt% Bario rice starch, c) 70wt% Bario rice starch scaffolds](image-url)
Collectively, the pores’ size from 50wt% to 70wt% of starch percentage for Bario rice starch-nHA scaffolds would impose the range of pore size in between 10µm to 400µm which suitable to furnish the nutrient and osteoblast cellular infusion while preserving the structure of the scaffolds [14]. Besides, the mean pores size between 20µm to 1500µm is desirable for tissue engineering purpose [15]. But, most of the studies recommend that a pore size larger than 300 µm is suitable for the formation of new bone and vascularization within the scaffold since this size could facilitate capillary formation and able to guide direct osteogenesis [16]. At the same time, stimulation of osteochondral ossification might occur at the pore size smaller than 300µm. Cell seeding occur at average pore size with diameter of 20-125µm [17].

3.2. Dielectric Measurement
The measurement of the dielectric properties was based on transmission line method by using Performance Network Analyzer (PNA) to obtained the dielectric constant \( \varepsilon' \) and dielectric loss \( \varepsilon'' \). Figure 3 shows the graphs of dielectric constant for 50wt%, 60wt%, and 70wt% Bario rice starch percentages.

![Dielectric constant for Bario rice starch scaffold](image)

Figure 3: Dielectric constant

Figure 4 shows the graphs of dielectric loss for 50wt%, 60wt%, and 70wt% Bario rice starch percentages. The value of dielectric constant and dielectric loss remained constant but at high frequency the dielectric constant and the dielectric loss is not stable and drastically dropped. This phenomenon is due to the energy absorption caused by phase lag between the dipole rotation and the field [18].

From the Figure 3, it is noticed that at the rice starch percentage 50wt% and 60wt% of Bario rice starch-nHA scaffolds has the highest dielectric constant the dielectric constant are reduced as increasing the rice starch percentage 50wt% to 70wt%. Ahmed et al. also reported that the dielectric constant and the dielectric loss will be reduced while increasing the concentration of the Indian Basmati rice flour slurry [19]. Besides, Ndife et al. agreed that by increasing the starch concentration, the dielectric constant and the dielectric loss value will be diminished [20]. However, this trend is not observed with the Bario rice starch-nHA scaffolds. The dielectric constant is highly affected by the water content or the moisture content [21] as water is a main polar component liable for any changes in the dielectric properties of a material [21].
Figure 4: Dielectric loss

Higher water content or moisture content may increase the mobility of the water molecule or the polar molecule that couple the electromagnetic energy portrayed by the larger value of the dielectric properties. Consequently, the 50wt% has a higher dielectric constant and dielectric loss compared to the 60wt% and the 70wt% as increasing starch percentages may reduce the water content [19], thereby contributing to lesser mobility of water molecules in the system. It is suggested that the higher starch percentages may induced higher porosity and this should be indicated by the dielectric properties of the air matrix [22] where the dielectric constant for air is 1 and the dielectric loss is 0 [23] but this is not shown here in the results.

Nevertheless, there is inconsistency trend in the dielectric loss value for the Bario rice starch-nHA scaffolds in correspondent to the porosity as the starch percentages increased. This might due to the structural shrinkage of the scaffolds. It is suggested that this could affect the fixation of the scaffold into the waveguide holder during the experimental set up, therefore influencing the dielectric loss value. As mentioned earlier, the resource of Bario rice starch which might come from several type of rice which might affect the results. Hence, the porosity of the Bario rice starch scaffold cannot be controlled and thus, affected the dielectric properties as well.

4. Conclusion

Native rice starch is a potential material to fabricate the bone tissue scaffold. Here, dielectric properties characterizations were used as indirect measurement techniques to determine the porosity of the scaffolds. The characterization of dielectric for porosity analysis shows that the porosity behave as dielectric properties of air matrix whereby, initially the porosity is claimed as the air matrix. Thereby, dielectric measurement for porosity analysis may lay new preliminary study for porosity in bone tissue scaffold. For the SEM analysis, it is showed that by increasing the rice starch percentage, the porosity distributed on the scaffold will increase but this did not occur for the 70wt% starch percentage. It is realised that the Bario rice starch-nHA scaffolds, there is an increment from 50wt% to 60wt% starch percentages but decrement started to occur thereafter. Thus, it is interesting to discover this peculiarity which could be explained further, perhaps by understanding the biochemistry interactions happened at the molecular level of these scaffolds composition.
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6. References

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