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FABRICATION AND CHARACTERIZATION OF CHITOSAN COATED AND UNCOATED PCL/HA/PPY COMPOSITE SCAFFOLDSUSING FREEZE DRYING TECHNIQUE

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ABSTRACT

Chitosan is an abundantly common, naturally occurring, polysaccharide biopolymer. In this study, chitosan was used to coat previously fabricated conductive Polycaprolactone/Hydroxyapatite/Polypyrrole (PCL/HA/PPY) composite scaffold and the properties of the coated and non-coated scaffolds were investigated and compared. The morphology of the chitosan coated and non-coated scaffolds were characterized using a scanning electron microscope (SEM). The wettability was determined using a water contact angle measuring system. Furthermore, water uptake was determined by measuring the water absorption of each sample before and after coated with chitosan. Water contact angle result revealed an increase in wettability of the scaffolds ranging from 108º ± 4.2 down to 59.4º ± 0.7. On the other hand, the coated sample showed a higher water uptake than the non-coated sample. The results indicated that coating with chitosan was important to increase water absorption of composite scaffold, rendering it more hydrophilic.

Keywords: chitosan, coated, 3D scaffolds, bone tissue engineering.

1. INTRODUCTION

The use of absorbable orthopedic implants in recent decades has been on the upsurge, which in turn has prompted extensive research in the field of bone tissue engineering. In orthopedic applications, tissue engineering approach is based on the fabrication of three-dimensional (3D) scaffolds that support, strengthen, and consolidate the regenerating tissue. The scaffold utilized in bone tissue engineering manipulates the functions of osteoblasts and guides new bone development in the desired forms[1].

A perfect scaffold material must fulfill a few conditions and possesses some qualities. The scaffold must be capable of being fabricated in precise shapes. It must possess a measured porous architecture to permit cell penetration. Also, the scaffold material must aid cell attachment, development, tissue restoration, and vascularization. It must have good mechanical properties, particularly at large deformations, to protect structural reliability during culture and utilization [2-4]. Furthermore, the scaffolds must be osteoconductive. Chitosan, being one of the extensively studied polymers and promising for use as scaffold material in bone tissue engineering application, is the copolymer of D-glucosamine and N-acetyl-Dglucosamine [5]. It is biodegradable, biocompatible, non-toxic and also possesses antibacterial properties. In this study, chitosan because of its several advantages was used in coating already fabricated conductive PCL/HA/PPY composite scaffold which was fabricated using freeze drying technique.

2. MATERIALS AND METHODS

The materials used were already fabricated scaffolds Polycaprolactone/ Hydroxyapatite/ Polypyrrole (PCL/HA/PPY), 0.2g chitosan, 50ml distilled water and 1ml of 2% acetic acid.

2.1 Method: Coating of scaffolds with chitosan: To prepare chitosan solution, 0.2g chitosan was dissolved in 50ml distilled water containing 1ml of 2% acetic acid with the aid of magnetic stirrer. After the chitosan solution was prepared, the scaffold, 10% PCL/HA/PPY was selected and dipped inside the chitosan solution for 5 minutes before it wasfreezed at low temperature for 6 hours.

2.2 Characterization of samples

2.2.1 Scanning electron microscope and energy dispersive x-ray (EDX) analysis: The surface morphology of all the fabricated scaffolds were examined with a scanning electron microscope (SEM, Table Top TM3000) and Field Emission Scanning Electron Microscope (FESEM, SU8020, Hitachi) to study their morphology and structures.

2.2.2 Contact angle measurement: The wettability of composite scaffolds was determined by measuring water contact angle using video contact angle system (VCA Optima, AST Product, Inc). The measurements were done after 5 seconds of dropping deionized water of 1µl size onto the composite surface for each sample. Contact angle values of the water droplet at the left and right side were measured and average values were calculated. Five measurements were performed for each sample at different locations of the scaffold surface and was analyzed using VCA Optima software. Contact angle values reported were the average of three measurements taken at different locations of the fibers.

2.2.3 Water uptake: Water uptake was also determined by measuring the water absorption of each sample before and after coated with chitosan. Each of the samples are weighted and then immersed in distilled water for 5 minutes. After that, the sample was weighted again after removing the excess water. The water uptake for each sample was calculated using the equation:

$$\text{Water uptake } (\%) = (W_w - W_d)/W_d \times 100$$

Where W_w and W_d are the sample weight after and before soaked in distilled water respectively. The data is then tabulated and results of the coated scaffolds were compared with the uncoated ones.

3. RESULTS AND DISCUSSIONS

Surface modification is the act of modifying the surface of a material which results in emergence of new physical, chemical or biological characteristics than the original one. In this study surface modification was done by coating the already fabricated scaffolds with chitosan andwe investigated the effect of chitosan coating on the composite scaffolds.

3.1 Morphological characterization: The SEM images of coated samples showed a clear surface indicating the presence of chitosan as being disclosed on the surface morphology. Figure-1 shows the SEM images of the samples comparing the morphology of the non-coated with the coated samples. From SEM image the results, the pores tends to reduce after coating with chitosan when compared with uncoated scaffold.

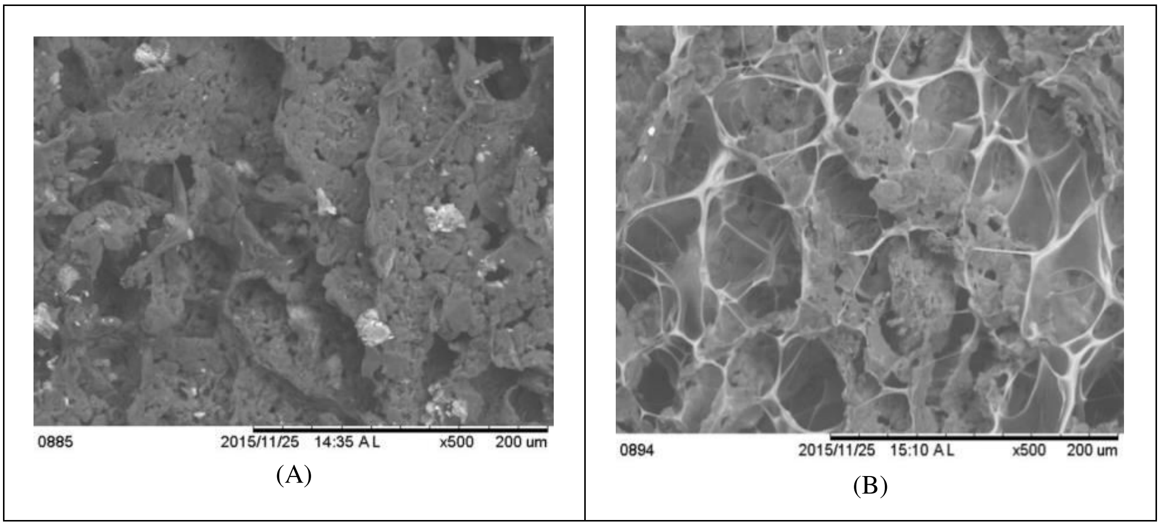
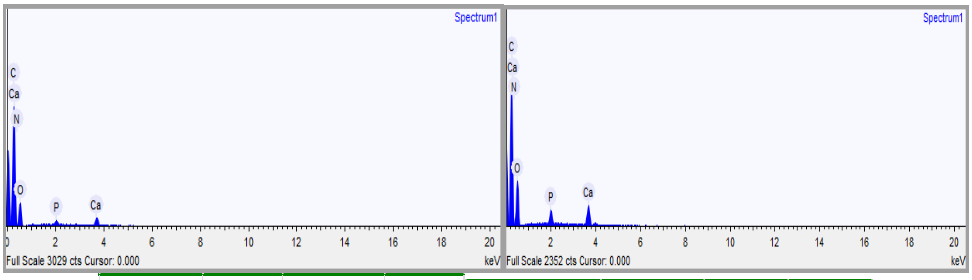


Figure-1. SEM micrograph of fabricated scaffolds comparing the morphology of non- coated with the chitosan coated scaffolds; (A) non-coated PCL/HA/PPY, and (B) coated PCL/HA/PPY at × 500 magnifications.

EDX analysis of PCL/HA/PPY scaffolds are shown in Figure-2. The presence of compound for coated and non-coated can be differentiated by determining the amount of energy absorbed by the elements present in the scaffold. By comparing the theoretical compound structure of the scaffolds with the scaffolds obtained in this study, it was observed that the elements on the scaffold recorded on the graph are nearly the same with the existing theoretical elements.



Element	Weight %	Weight % σ	Atomic %
Carbon	62.936	0.863	70.455
Nitrogen	0	0	0
Oxygen	33.713	0.874	28.333
Phosphorus	0.885	0.106	0.384
Calcium	2.466	0.157	0.827

Element	Weight %	Weight % σ	Atomic %
Carbon	56.02	0.802	64.773
Nitrogen	0	0	0
Oxygen	37.947	0.816	32.939
Phosphorus	1.94	0.118	0.87
Calcium	4.093	0.172	1.418

Figure-2. EDX analysis of (A) non-coated PCL/HA/PPY and (B) coated PCL/HA/PPY scaffolds.

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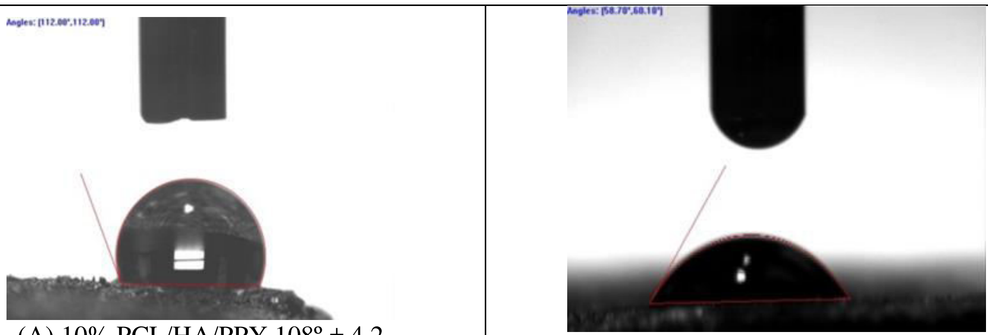
In PCL, the compositions are carbon (C) and oxygen (O_2). The compositions of hydroxyapatite are phosphorus (P) and calcium (Ca). With addition of polypyrrole (PPY), the composition present is nitrogen (N). While for chitosan elements, the composition present is nitrogen (N). The entire scaffolds that were coated with chitosan are supposed to have nitrogen (N) elements in their structure. However, element N could not be detected. This could be because of the higher composition of Carbon element than other elements.

3.2 Contact angle: The wettability of the coated samples was measured using video contact angle system (VCA Optima, AST Product, Inc.). Wettability also known as hydrophilicity is one of the requirements for tissue engineering scaffolds [6]. Water contact angle measurement is the measurement technique used to determine the surface wettability of scaffolds. Properties that affects wettability measurement, includes;(1) chemical bonding, (2) surface morphology and (3) surface roughness [7]. Figure-3 shows the water contact angle images and values of the scaffolds. Water contact angle value that is more than 90º is considered hydrophobic. As shown in the figure, the surface of the coated samples were hydrophilic with contact angle value ranging from 59.4º ± 0.7 for PCL/HA/PPY as compared with the non-coated samples with 108º ± 4.2 for PCL/HA/PPY. The wettability of the coated scaffolds increased greatly because chitosan is a known hydrophilic polymer. Scaffolds with hydrophilic surfaces are more favorable for cell attachment and proliferation as well as cell migration [8-11].



(A) 10% PCL/HA/PPY 108º ± 4.2

(B) 10% PCL/HA/PPY 59.4º ± 0.7

Figure-3. Water contact angle comparing the non -coated and the coated composite scaffolds, (A) non-coated WCA of PCL/HA/PPY (B) PCL/HA/PPY scaffolds coated with chitosan.

Water Uptake: Water absorption is crucial for several metabolic activities. Water uptake measurement was carried out on both the coated and non- coated samples. Their weight before and after being immersed for 5 minutes in mineral water was measured. Table-2 shows weight of the samples, and their corresponding water uptake (%).

Table-1. Weight and water uptake of the samples.

Sample		**Weight** (g)	**Weight** (g)	**Water uptake** (%) $(W_w - W_d)/W_d \times 100$
		Before W_d	**After** W_w	
Non-coated	10%PCL/HA/PPY	0.0581	0.0850	46.30
Coated	10%PCL/HA/PPY	0.0642	0.1800	180.37

From the results obtained, it was observed that for every sample used, the coated sample showed a higher water uptake than the uncoated sample. As seen in the result, between coated PCL/HA/PPY and non-coated PCL/HA/PPY, there was a significant difference in the coated and uncoated composite scaffold. Coated samples showed higher water uptake. Nevertheless, the overall result indicates that coating with chitosan is essential to increase water absorption of composite scaffold, rendering it more biocompatible for cell proliferation. This on the other hand enhances bone regeneration.

4. CONCLUSIONS

Chitosan was successfully used to coat PCL/HA/PPY composite scaffold. Results of the coated scaffolds were compared with uncoated scaffolds. Results showed that coating of the scaffolds helped in enhancing the surface properties of the scaffold. There was a significant increase in wettability of the scaffolds.

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