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# Fabrication of Alginate and Hydroxyapatite Biocomposite Film for Tissue Regeneration Application

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**Abstract:** One of the most promising techniques for bone repair and regeneration of tissue that is difficult to mend on its own is tissue engineering. This research is focusing on the fabrication of alginate hydroxyapatite biocomposite film to enhance tissue regeneration. The objectives for this study are to fabricate alginate hydroxyapatite biocomposite film via solution casting method and to analyse its physicochemical also mechanical properties that is shown via FTIR, AFM and contact angle. The composition of hydroxyapatite was applied at different weight percentage which are from 0.2 wt. %, 0.4 wt. %, 0.6 wt. %, 0.8 wt. % up to 1.0 wt. %. It was revealed that the thickness of fabricated biocomposite films were found between 0.157 mm and 0.232 mm. The viscosity decreased as the weight percentage of the hydroxyapatite increased. For FTIR, the characteristic peaks of $PO_4^{-3}$ and $OH^-$ groups which corresponding to hydroxyapatite are existed in the films. For contact angle measure, hydroxyapatite influenced the contact angle measurement and the values decreased compared to the sample with alginate only. AFM shows hydroxyapatite contributes to the rougher surface of the film which this led to the attachment of film to the bone surfaces. Also, for swelling studies, the ability of films to swell is also depending on the hydroxyapatite composition. It can be concluded that the alginate and hydroxyapatite biocomposite film has potential in a tissue regeneration application.

**Keywords:** Alginate, Hydroxyapatite, Tissue Regeneration

## 1. Introduction

Every year, many people are involved in accidents and illnesses that result in tissue damage and organ malfunction. Tissue engineering is one of the promising techniques for repairing damaged tissues and organs. For tissue engineering technology, the role is to artificially recreate a specific biochemical and physical functions of certain cells. For smaller sized of injuries, it is the best to treat it by surgical reconstruction which it will be used the ability of tissue to regenerate spontaneously with time. However, for the larger damage, a scaffold or substrate is required to promote cell proliferation and facilitate cell-repair process. Currently, there are two methods to treat broken tissue which are by using

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autograft and allograft. However, there are few drawbacks of those methods such as the unavailability of matching donor tissue, higher risk of disease transmission and immune rejection [1].

The mechanical and structural properties need to be investigated properly as it is a man-made thing that requires so much knowledge. Sodium alginate is a natural polysaccharide that is obtained from sea algae and helps in treating the lose bone as well as defect bone. This natural polymer has been investigated and widely used for many fields such as biomedical application because of its biocompatibility, relatively low cost, low toxicity and mild gelation when adding divalent cations [2]. It is biodegradable because it dissolved slowly in the body when the crosslinking agents in the alginate release and exchange reaction with monovalent cations found in the body fluids. However, it has some drawbacks such as low mechanical strength and lack of interaction [3]. On the other hand, hydroxyapatite is naturally occurring mineral form of calcium apatite which then makes calcium phosphates and its derivatives ae of great interest to various field of science [4]. It is biocompatible, bioactive and thermodynamically stable in the body fluid which makes it become one of the suitable material to be used in biomedical field [5].

The objectives of this research are to fabricate alginate and hydroxyapatite biocomposite film via solution casting method, to investigate the physiochemical and mechanical properties of alginate and hydroxyapatite biocomposite film, and also to study the swelling activities of the biocomposite film.

## 2. Materials and Methods

### 2.1 Materials

Sodium alginate ($NaC_6H_7O_6$), hydroxyapatite and calcium chloride ($CaCl_2$) were obtained from Sigma-Aldrich.

### 2.2 Methods

To prepare the sample, solution casting method was done. 1 g of sodium alginate was added to 100 ml of deionized water and they were dissolved by using magnetic stirrer. The mixed solution of sodium alginate with hydroxyapatite was prepared in appropriate composition based on Table 1. Then, the mixture was stirred until hydroxyapatite fully dissolved in sodium alginate solution. After that, 20 ml of film-forming solution was poured into petri dish and left at room temperature for 24 hours. The thin film then was immersed in 200 mL of calcium chloride, CaCl2 for crosslinking for 60 minutes. Finally, thin film was washed with deionized water to remove excess Ca2+ and left to dry at room temperature for 48 hours.

**Table 1: Composition of alginate and hydroxyapatite**

| No. | Alginate, ml | Hydroxyapatite, g | Label |
|---|---|---|---|
| 1 | 100 | - | SA |
| 2 | 100 | 0.2 | SH0.2 |
| 3 | 100 | 0.4 | SH0.4 |
| 4 | 100 | 0.6 | SH0.6 |
| 5 | 100 | 0.8 | SH0.8 |
| 6 | 100 | 1.0 | SH1.0 |

Swelling test, viscosity test and thickness measurement are the three types of physical testing that have been performed. For characterization of the film, another 3 types of tests have been done which are contact angle measurement, surface roughness by using AFM and FTIR analysis. Viscosity test is done by dipped Viscolite700 into the solution. Meanwhile, thickness of the films was measured with

using digital micrometer and swelling test was done by soaked the samples in superabsorbent polymer for 30 minutes, 60 minutes and 120 minutes. The functional group, molecular and chemical structure of alginate/hydroxyapatite film were identified by using FTIR spectrometer with a wavelength of 4000-600 $cm^{-1}$. The next testing is contact angle measurement which is used to investigate the wettability of the film. 1µl of water was injected into the sample. AFM (XE-100) was used to examine the surface roughness of biocomposite film.

## 3. Results and Discussion

### 3.1 Fabrication of alginate hydroxyapatite biocomposite film

After they were fabricated, the biofilms were left for 24 hours in room condition to let it stable before crosslink with calcium chloride, CaCl so that chapped sample would not be produced. When the crosslinking process done, the samples left in the room temperature for 48 hours and the perfect, firm thin biofilms produced as in Figure 1. During crosslinking process, when sodium alginate exposed to calcium ions, it form ionic bond between alginate chains which resulting in physical gel with highly tunable mechanical properties [6].

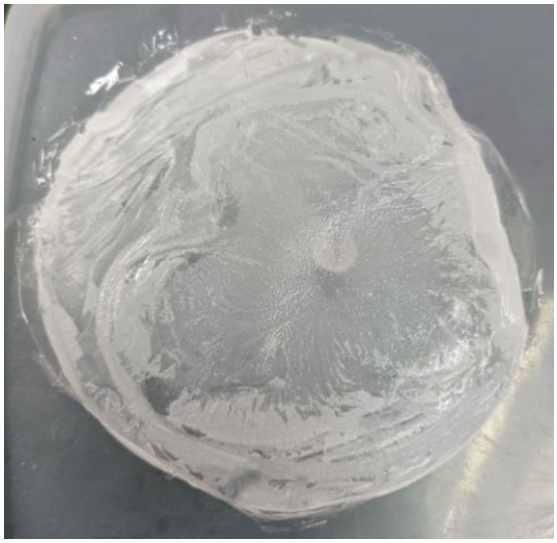

**Figure 1: Fabricated alginate/hydroxyapatite biocomposite film**

### 3.2 Viscosity test

It can be seen that the viscosity of Alg/HAP decreased when the content of hydroxyapatite increased from 0.2 wt. %, 0.4 wt. %, 0.6 wt. %, 0.8 wt. % and 1.0 wt. %. The higher the concentration of hydroxyapatite, the lower the viscosity. A higher molecular weight should, in theory, resulting in more binding between chains. It is important to note that when the molecular weight of the solution decreases, the viscosity of the solution increases substantially, making it easier to distribute a concentration of alginate in a given volume of distilled water. When cells are grown in a medium with high viscosity, their morphology is immediately altered, as cell length [7].

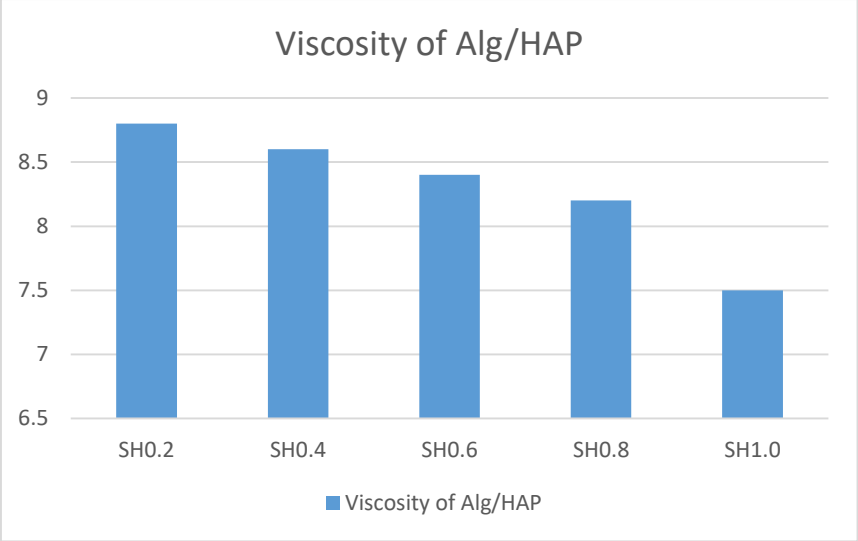


**Figure 2: Viscosity of Alg/HAP**

3.2 Thickness measurement

Each samples' thickness were measured for three time and then the average is calculated to get more accurate data. Table 2 shows the thickness of the samples. Thickness will likely influence various aspects of biofilm architecture, including density, shape and porosity, as well as the redox gradient and hence the local biofilm environment in general [8].

**Table 2: Thickness of thin film**

| Sample | Thickness at trial 1, mm | Thickness at trial 2, mm | Thickness at trial 3, mm | Average thickness | Standard deviation |
|---|---|---|---|---|---|
| SH0.2 | 0.291 | 0.153 | 0.231 | 0.225 | 0.474 |
| SH0.4 | 0.146 | 0.174 | 0.152 | 0.232 | 0.482 |
| SH0.6 | 0.144 | 0.212 | 0.129 | 0.192 | 0.438 |
| SH0.8 | 0.208 | 0.201 | 0.247 | 0.218 | 0.467 |
| SH1.0 | 0.154 | 0.150 | 0.167 | 0.157 | 0.396 |

3.2 Swelling studies

The swelling degrees of Alg/HAP biofilms with different concentration of hydroxyapatite are as shown in Table 3. As can be seen, both samples for SH0.6, it reached the maximum within the first 30 minutes and then burst. This was most likely due to the polymer network collapsing as a result of the increased water absorption. Incorporation of HA into the film formulation also resulted to decrease in the swelling degree. Higher concentrations of HA in the biofilms led to much lower swelling degrees, probably owing to increase in the crosslinking density caused by alginate-HA interaction. In this research, SH0.8 has higher average swelling compared to SH1.0. Overall, the swelling percentage is almost double at every time they are being weighed. It is important to note that differences in HA concentration had a considerable impact on the swelling behaviour of the samples. It can be determined that HA powders do not remain homogenously dispersed in the polymer solution [9].

**Table 3: Swelling data of Alg/HAP**

| Sample | Trial | Swelling percentage, % | | | Average swelling, % | | |
|---|---|---|---|---|---|---|---|
| | | 30 mins | 60 mins | 120 mins | 30 mins | 60 mins | 120 mins |
| SH0.6 | 1 | Burst | - | - | Burst | - | - |
| | 2 | | | | | | |
| SH0.8 | 1 | 66 | 137 | Burst | 73 | 125 | Burst |
| | 2 | 80 | 113 | | | | |
| SH1.0 | 1 | 19 | 43 | Burst | 50 | 112 | Burst |
| | 2 | 82 | 180 | | | | |

3.2 Fourier Transform Infrared Spectroscopy Analysis (FTIR)

Figure 3 exhibits the broad peak at 3372 $cm^{-1}$ which are representing the hydroxyl group. Peaks at 1628 $cm^{-1}$ and 1418 $cm^{-1}$ show asymmetric and symmetric stretching of the carboxyl group in sodium alginate. The characteristic peaks of $PO_4^{-3}$ and $OH^-$ groups which corresponding to hydroxyapatite were existed in the synthesized powders. For $OH^-$ groups, the peaks locations are around 940-962 $cm^{-1}$ which corresponding to bending an at peaks 1628 - 1599 $cm^{-1}$ is the stretching of $COO^-$ of carboxylic appear to be shifted. The main reasons are due to the interaction of hydroxyapatite with Ca atoms and oxygen from sodium alginate [10,11]. $CO_3^{2-}$ is produced from $CO_2$ during synthesis process. $PO_4^{-3}$ stretching bands at 1021 - 1010 $cm^{-1}$ are become sharp and narrow due to the rise in the percentage of sodium alginate concentration while bands at 625 - 623 $cm^{-1}$ are referring to phosphate bending vibration.

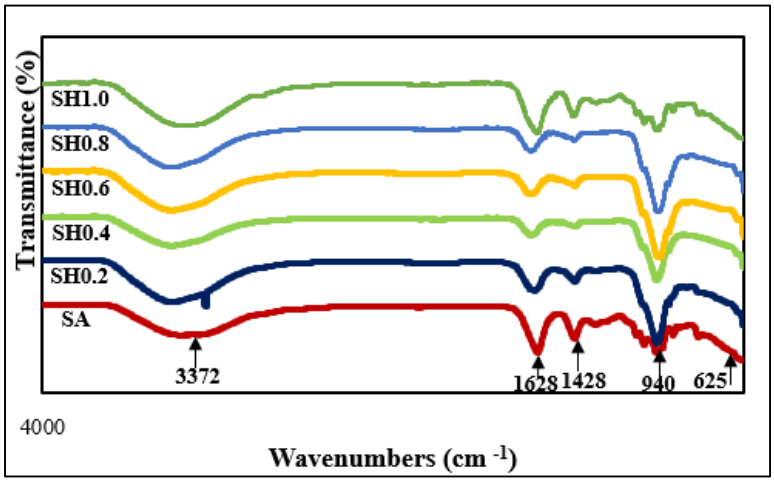


**Figure 3: FTIR of alginate only and Alg/HAP**

3.2 Contact angle analysis

For this research, the value of contact angle fluctuates and does not show constant trend as the concentration of hydroxyapatite increases as shown in Figure 4 and Table 4. However, all samples show hydrophilic properties as the contact angle recorded are less than 90º. The liquid drop spread out on the solid surface if the liquid molecules are strongly attracted to the solid molecules, resulting in a low contact angle. Overall, hydroxyapatite influenced the contact angle measurement and the values decreased as compared to sample of alginate only. This shows that the film with hydroxyapatite can easily attached to the bone and led to faster recovery on the defect surfaces.

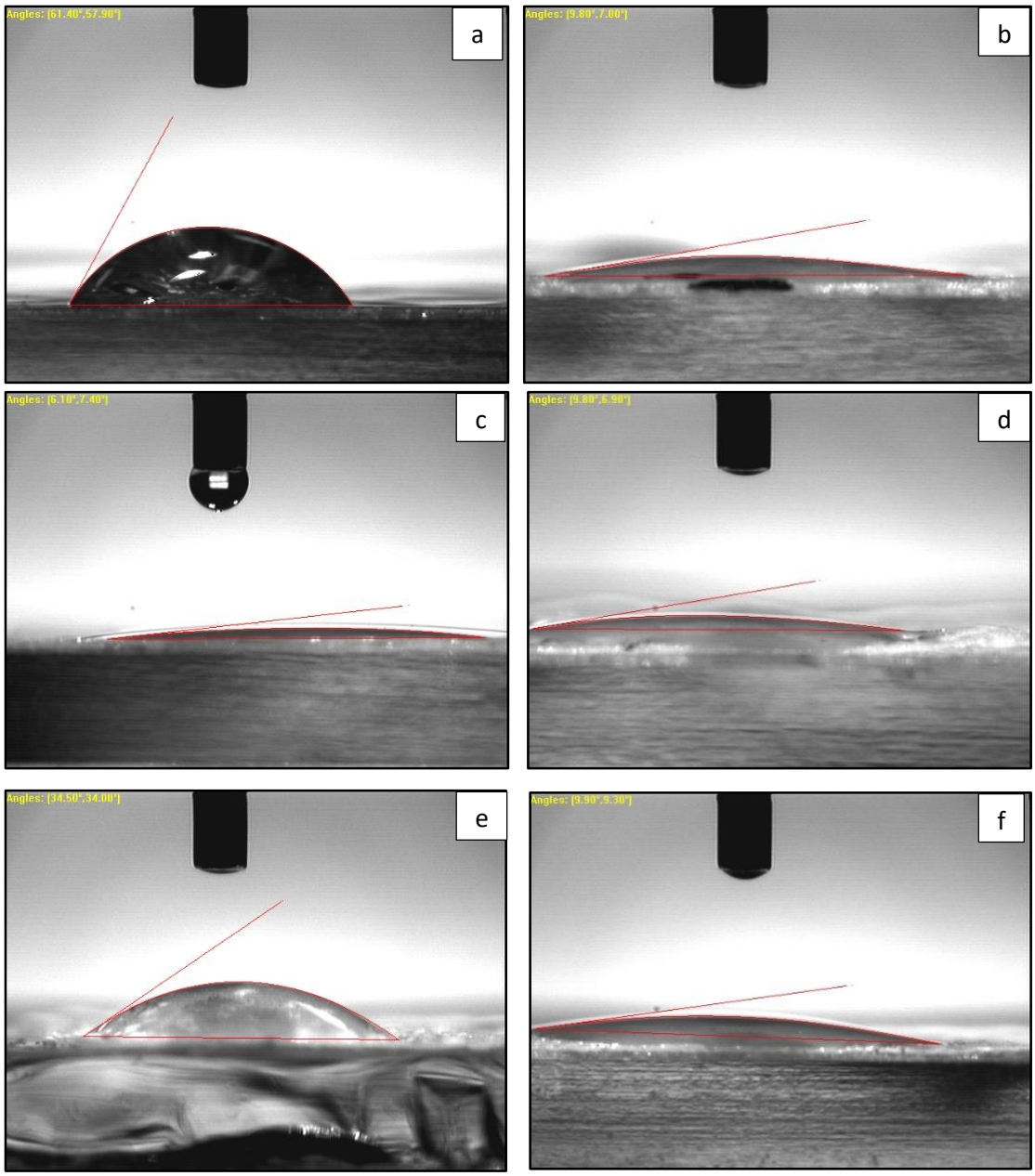


**Figure 4: Contact angle of SA and Alg/HAP: a) SA, b) SH0.2, c) SH0.4, d) SH0.6, e) SH0.8 and f) SH1.0**

**Table 4: Contact angle of thin film**

| Sample | Contact angle, º |
|---|---|
| SA | 61.4 |
| SH0.2 | 9.8 |
| SH0.4 | 6.1 |
| SH0.6 | 9.8 |
| SH0.8 | 34.5 |
| SH1.0 | 9.9 |

3.2 Atomic Force Microscope analysis (AFM)

It was found that the average surface roughness, $R_A$ of Alg/HAP biofilms at concentration of hydroxyapatite 0.2 wt. %, 0.4 wt. %, 0.6 wt. % and 1.0 wt. % were 3.232 nm, 37.616 nm, 10.198 nm

and 43.544 nm, respectively as can be seen in Figure 5. The lowest $R_A$ was for SH0.2 which is 3.232 nm while the highest value was for SH1.0 which is 43.544 nm. Based on Figure 6, he topography texture of the films are different based on the concentration of hydroxyapatite. It can be seen significant trend as hydroxyapatite contributes to the rougher surface of the film. This rougher surface will assist the attachment of the film to the bone surfaces.

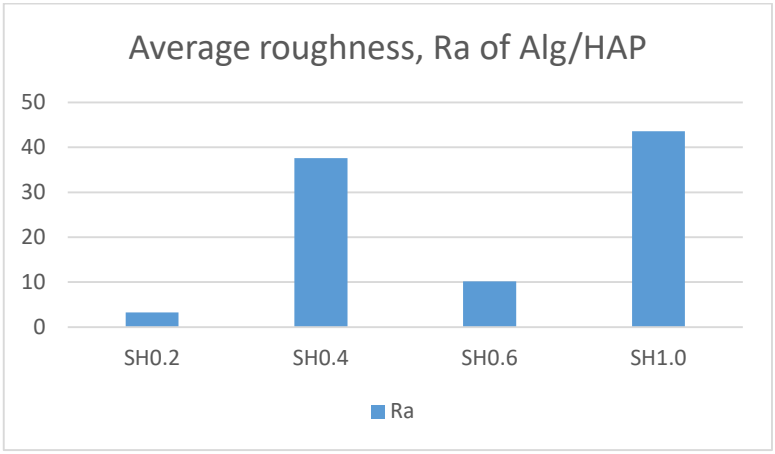


**Figure 5: Average roughness of Alg/HAP**

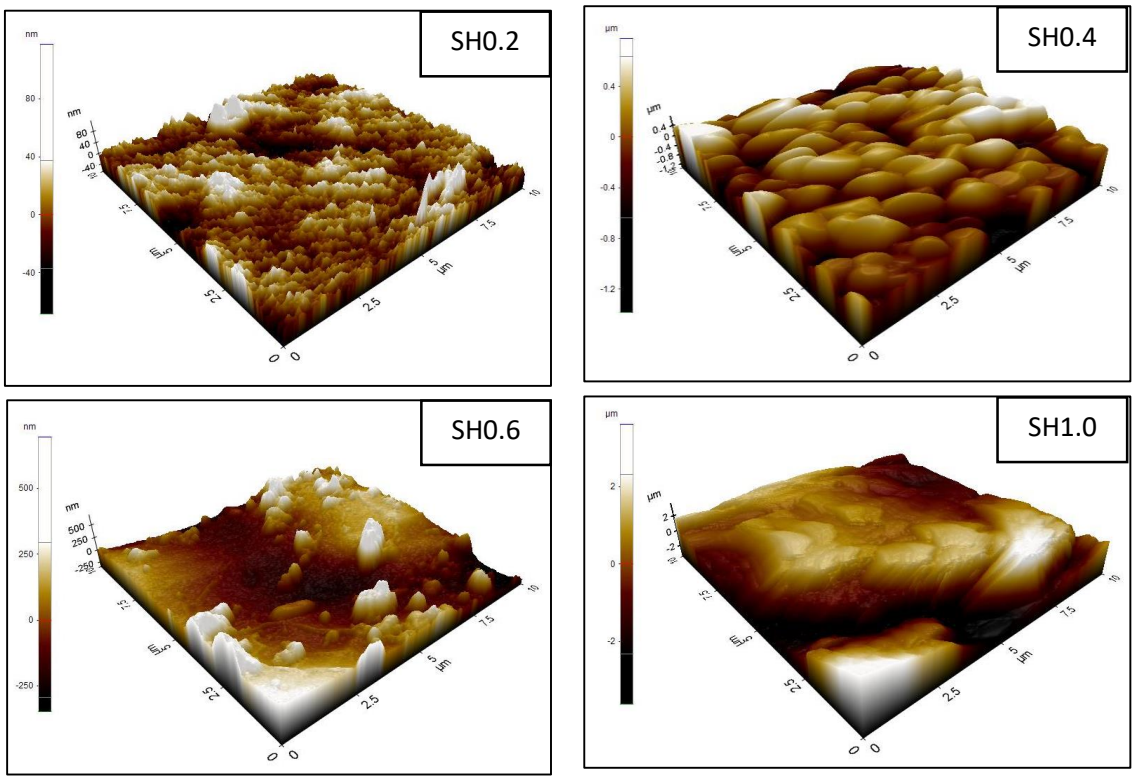


**Figure 6: AFM images of Alg/HAP**

## 4. Conclusion

As conclusion, alginate hydroxyapatite has been successfully fabricated with calcium chloride, $CaCl_2$ as crosslinking agent. To get the firm film, crosslinking rate must be rate so hydroxyapatite can fully bind with Ca atom and oxygen from sodium alginate. Moreover, the thickness of biofilms are within the range, so it able to function efficiently. Thickness of the biofilms are important as it linked

with water flow and development age. The viscosity analysis displays that as the concentration of hydroxyapatite increases, the viscosity decreases. From FTIR analysis, it shows that sodium alginate and hydroxyapatite has been successfully mixed by using solution casting method. For the surface wettability, it does not show constant trend as the contact angle value fluctuate as the concentration of hydroxyapatite increases. It goes the same for surface roughness as well. Average roughness of Alg/HAP fluctuated as the concentration of hydroxyapatite increases so the roughness of the film depends on the composition of hydroxyapatite. The swelling test has been done for SH0.6, SH0.8 and SH1.0. SH0.6 burst after 30 minutes while SH0.8 and SH1.0 burst after 120 minutes. The average swelling percentage of SH0.8 is higher than SH1.0. Hence, alginate/hydroxyapatite biocomposite film has a potential to be used for tissue regeneration.

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