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SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE-GELATIN COMPOSITES WITH *IN-SITU* AND *EX-SITU* WET PRECIPITATION

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ABSTRACT

Bone damage such as fractures is increasing from year to year. Therefore, the need of synthetic bone implants is also increasing. The bone implant can be made of ceramic such hydroxyapatite (HAp). HAp was synthesized using wet precipitation method. The procedures consisted of calcination, hydration, HAp synthesize, and thermal treatment. Shells of garden snail (*Bellamya javanica*) were to $CaCO_3$ calcinated to obtain CaO. Then allowed to react with air to produce $Ca(OH)_2$, which was used as the raw material to synthesize HAp. The synthesized HAp was mixed with $(NH_4)_2HPO_4$ and $Ca(OH)_2$ using two different methods: *in-situ* and *ex-situ*, with various gelatin concentrations: 10, 20 and 30%. The HAp-gelatin composites were analyzed by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectrophotometer, and scanning electron microscope (SEM) were used to determine the best HAp-gelatin composite. The best composite was HAp with 30% gelatin and synthesized under *in-situ* method, based on its highest crystalinity dan small homogeneous granules showed by SEM analysis.

Keywords: *Bellamya javanica, ex-situ*, gelatin, hydroxyapatite, *in-situ*

INTRODUCTION

Bone damage such as fractures is increasing from year to year. Fractures due to accidents has increased six times for the last 20 years. Therefore, the need of bone implants is also increasing. So people develop the synthetic biomaterial to replace the original bone. The biomaterial can include metals, ceramics, polymers, or composites. One of synthetic biomaterial that is commonly used is hydroxyapatite (HAp). HAp is a ceramic biomaterial with molecular formula $Ca_{10}(PO_4)_6(OH)_2$ belong to the group of calcium phosphate, it has mineral composition similar to bone tissue of vertebrates. In addition to accelerating the formation of new bone, HAp is also directly bound chemically to bone tissue through the formation of apatite layer, and biologically through the interaction interface. HAp can be used as bone implants because it is highly biocompatible, bioactive, no immune response, no inflammatory response, and osteoconductive[1]. Osteoconductive is the ability of a substance to stimulate osteoblasts to bone mineralization. There are two ways to synthesize HAp, by dry method and wet method. Dry method means Ca and P as precursors are mixed in the solid fase. The wet method involve by first dissolving the precursors, then mixed the precursors. Wet method consists of three types, that is precipitation, hydrothermal, and hydrolysis[2]. In this study, wet precipitation method was selected because, the use of low working temperature, high percentages of pure product, and in-expensive equipment requirement. The characteristic of HAp can be influenced by the variation of pH, stirring speed, temperature, time, and concentration, so that these factors can be used to increase the quantity and quality of HAp[3]. Ca precursors can be derived from the shells of garden snails due to its high concentration of Ca. Gelatin is added into HAp to decrease pore size and strengthen HAp bond by shorten bond distance[4]. In this study, gelatin was added in 2 ways, through *in-situ* and *ex-situ* methods. The difference between the two is in the time of process of adding porosifier. In the *in-situ method,* the mineral apatite formed in a matrix of gelatin, while in the *ex-situ* method, gelatin is added after the precipitation process is completed. The results from these two methods were identified to determine the best composite HAp-

gelatin. This study aims to determine the method of synthesis and optimum concentration that will result the best HAp-gelatin composite.

EXPERIMENTAL

Material and Methods

The reagents used in this study were garden snail shells (*Bellamya javanica*) from Pasar Anyar Bogor, $CaCO_3$ standard from Wako, HNO_3, gelatin, and $(NH_4)_2HPO_4$ from Merck.

General procedure

Garden snail shells were cleaned using water and then boiled for at least 1 hour. After that, the shells were separated from the flesh. Garden snail shells were dried under the sun and milled into powders, then sieved to obtain the powder size in range of 100 mesh. The powders were calcined at 1100 °C for 3 hours, flattened on the container and allowed to interact with the air (hydrated) for a week, made into 0.5 M solution, and then reacted with $(NH_4)_2HPO_4$ 0.3 M as follows:

$10\ Ca(OH)_2 + 6\ (NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6\ H_2O + 12\ NH_4OH$

In the *in-situ* method, $(NH_4)_2HPO_4$ 0.3 M was dropped into $Ca(OH)_2$ 0.5 M at 40 ± 2 °C with a flow rate of 1.3 mL per minute for about 1 hour while added a solution of gelatin with various concentration of 10%, 20% and 30%, while constantly stirring with a magnetic stirrer. This reaction produces NH_4OH, so the pH should be checked every minute and set to 10. Then the mixture was decanted for 24 hours and sonicated for 6 hours. After that, the solution was centrifuged for 15 minutes. The pellets was filtered and rinsed with aquadest. Then it was dried in an oven at 105 °C for 3 hours, heated in a furnace at 1100 °C for 2 hours. HAp-gelatin powder that has been formed is removed and allowed to cool at room temperature before sieved with 100 mesh sieve. In the *ex-situ* method, $(NH_4)_2HPO_4$ 0.3 M was dropped into $Ca(OH)_2$ 0.5 M at 40 ± 2 °C with a flow rate of 1.3 mL per minute for about 1 hour without adding gelatin. After that, the solution was decanted, sonicated, centrifuged, filtered, rinsed, dried, and sieved (same with *in-situ*). The powders were diluted with aquadest, then added with a solution of gelatin with various concentration of 10%, 20% and 30% while constantly stirring with a magnetic stirrer. The mixture was dried again in an oven at 105 °C and sieved.

Characterization Techniques

All of HAp-gelatin composites analyzed by XRD and FTIR spectrophotometer. The best and the worst composites were analyzed by SEM.

RESULTS AND DISCUSSION

X-Ray Diffractogram of HAp-Gelatin

The result of HAp powder analysis with XRD can be seen in Fig-1. The main peaks were observed at 2θ 31.80, 33.06, and 34.08. HAp characteristic peaks appear at 2θ approximately 31.80°-34.00°[5]. The result of HAp-gelatin powder analysis can be seen in Fig-2. The main peaks were observed at 2θ 31.82, 32.24, and 32.98 (HAp+gelatin 10% *in-situ*), 31.84, 32.26, and 33.04 (20%), 31.82, 32.24, and 33.00 (30%), 31.86, 32.26, ans 33.02 (HAp+gelatin 10% *ex-situ*), 31.82, 32.24, and 33.10 (20%), 31.84, 32.20, and 32.94 (30%). The addition of gelatin doesnot change the characteristic of HAp shown by fewshifts of the angle of 2θ.

Crystallinity of HAp-Gelatin Composite

Data from the diffractogram were analyzed to derive the value of the degree of crystallinity. The degree of crystallinity of HAp-gelatin composites was calculated using the formula[6]:

$$X_c = 1 - \frac{I112 - I300}{I300} \cdot 100\%;$$

with X_c is% crystallinity, *I112-I300* is the intensity of hollow between 112 at 2θ 32.19° and 300 at 2θ 32.90° diffraction peak of HAp (based on the data JCPDS of HAp), and *I300* is the intensity of 300

diffraction peak. The degree of crystallinity of HAp was 66.02%, HAp+gelatin *in-situ* 83.83% (10%), 84.14% (20%), 84.62% (30%), *ex-situ* 84.09% (10%), 28.99 (20%), 22.06 (30%). The best composite was HAp-gelatin 30% due to its crystallinity.

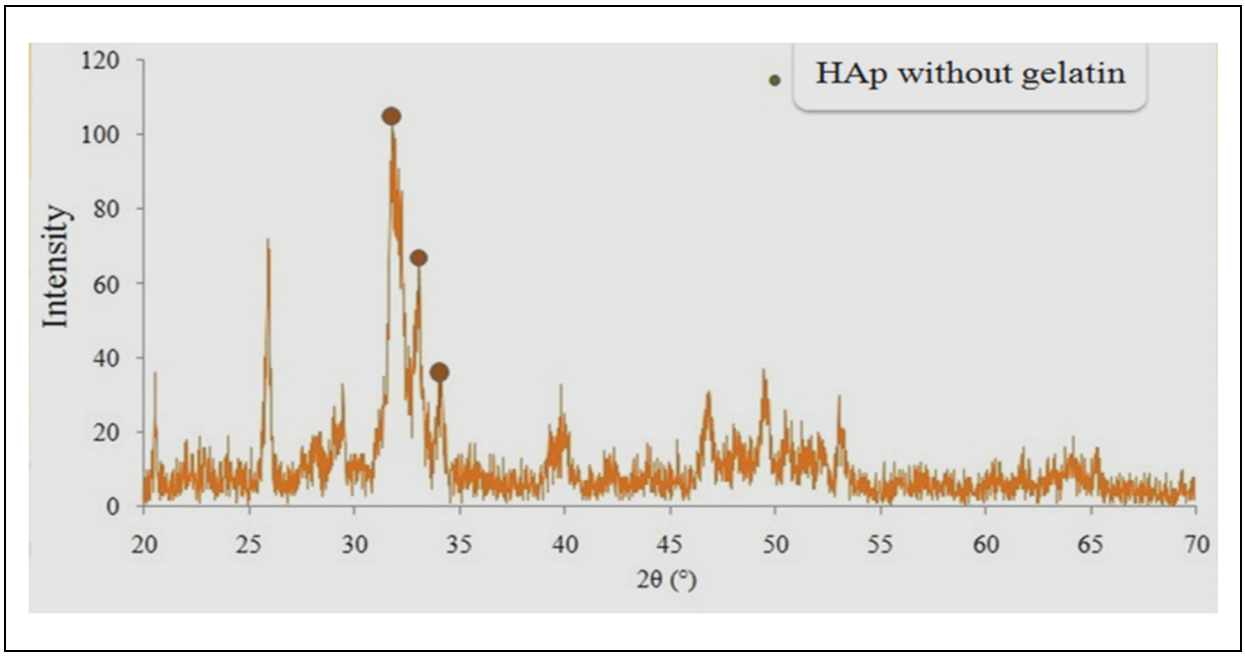

Fig-1: X-Ray diffractogram of HAp from garden snail shell

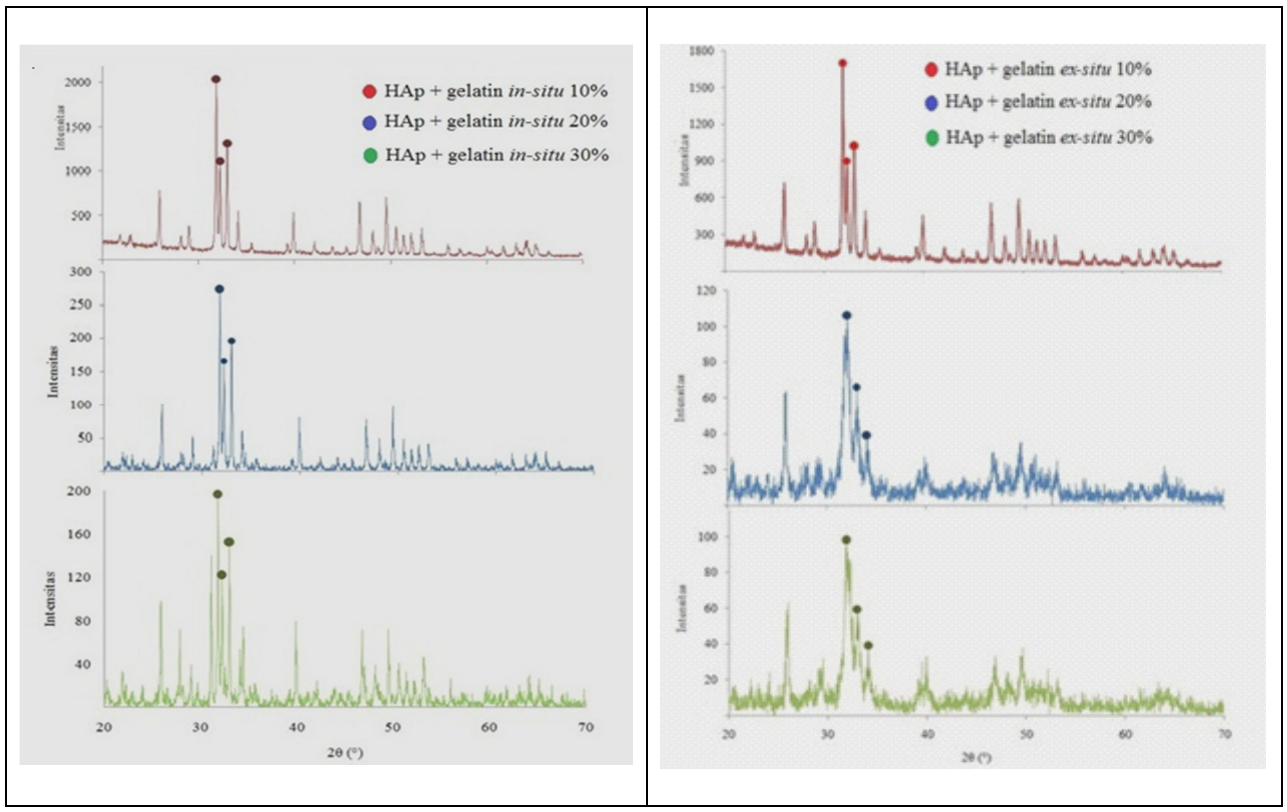

Fig-2: X-Ray diffractogram of HAp-gelatin

Analysis of Functional Groups of HAp-Gelatin Composite

The functional groups of HAp without gelatin and the HAp-gelatin composites that has been synthesized were analyzed by FTIR. Characteristics peak of HAp can be observed in the wavenumber (cm^{-1}) about 600, about 962, and about 1049 to 1030, showing the vibrational modes of stretching and bending vibration of P-O at phosphate group, and around 3569 that shows stretching vibration hydroxyl group. Absorption band is also observed in 1300-1650 showing the stretching and bending vibration of the C-O ions (CO_3^{2-}). Vibrational modes of PO_4^{3-} and OH^- is the characteristic absorption of HAp[7].FTIR spectrum of HAp (Fig.-3) shows the vibration absorption band at wavenumber (cm^{-1}): 3790.02 showing the stretching vibration OH^-, 1031.88 showing the asymmetry stretching vibration PO_4^{3-}, 962.45 which shows the symmetry stretching vibration PO_4^{3-}, 603.70 and 601.77 which shows the asymmetry bending vibration PO_4^{3-}, and 1425.35 showing the CO_3^{2-} vibration.Vibration of CO_3^{2-} shows that $CaCO_3$ was remaining in the HAp powder. CO_3^{2-} can substitute PO_4^{3-} in the HAp lattice forming carbonate apatite type A and B or AKA and AKB.

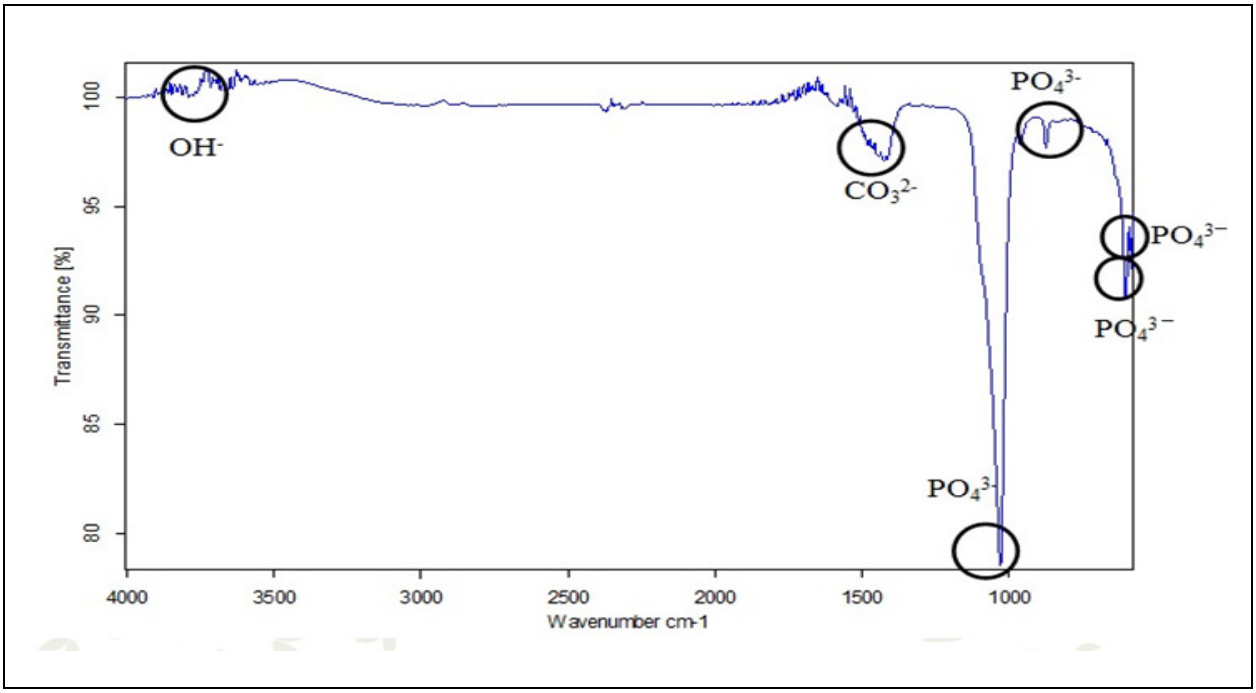

Fig-3: FTIR spectrum of HAp from garden snail shell

FTIR spectrum of HAp + gelatin *in-situ* in Fig-4 shows the vibration absorption band at wavenumber (cm^{-1}) are overlapping: 3568.21 showing the stretching vibration OH^-, 1031.88 showing the asymmetry stretching vibration PO_4^{3-}, 962.45 which shows the symmetry stretching vibration PO_4^{3-}, 603.70 and 601.77 which shows the asymmetry bending vibration PO_4^{3-}, and 1419.57 showing the CO_3^{2-} vibration.FTIR spectrum of HAp+ gelatin *ex-situ* shows the vibration absorption band at wavenumber (cm^{-1}) are overlapping: 3597.14 at 10% (red line), 3595.21 at 20% (blue line), and 3626.07 at 30% (green line) showing the stretching vibration OH^- and N-H; 1030.39 at 10% (red line), 1031.11 at 20% (blue line), and 1033.16 at 30% (green line) showing the asymmetry stretching vibration PO_4^{3-}; 961.08 at 10% (red line), 955.82 and 869.38 at 20% (blue line), and 963.85 and 866.81 at 30% (green line) which shows the symmetry stretching vibration PO_4^{3-}; 636.70 and 600.66 at 10% (red line), 598.91 in 20% (blue line), and 628.38 and 603.43 at 30% (green line) which shows the asymmetry bending vibration PO_4^{3-}, and 1413.11 at 20% (blue line) and 1418.53 at 30% (green line) showing the CO_3^{2-} vibration.Gelatin concentration does not shift the wavenumber of spectrum too much, indicated that gelatin does not affect the absorption of HAp. The FTIR spectrum of HAp-gelatin *ex-situ*also shows absorption band of

vibration of polipeptide with the main functional groups C=O and N-H.Absorption of N-H overlaps with OH^- shown by the lower intensity of the transmittans of OH^- stretching vibration than *in-situ*. Absorption band of C=O stretching vibration observed at wavenumber (cm^{-1}) 1651.42 at 10% (red line), 1651.02 at 20% (blue line), and 1651.02 at 30%(green line). The presence of C=O and N-H show that gelatin is not lost in the process of *ex-situ* synthesis. Gelatin is not lost because when it was added to HAp, the solution is no longer calcinated so that the gelatin does not evaporated.

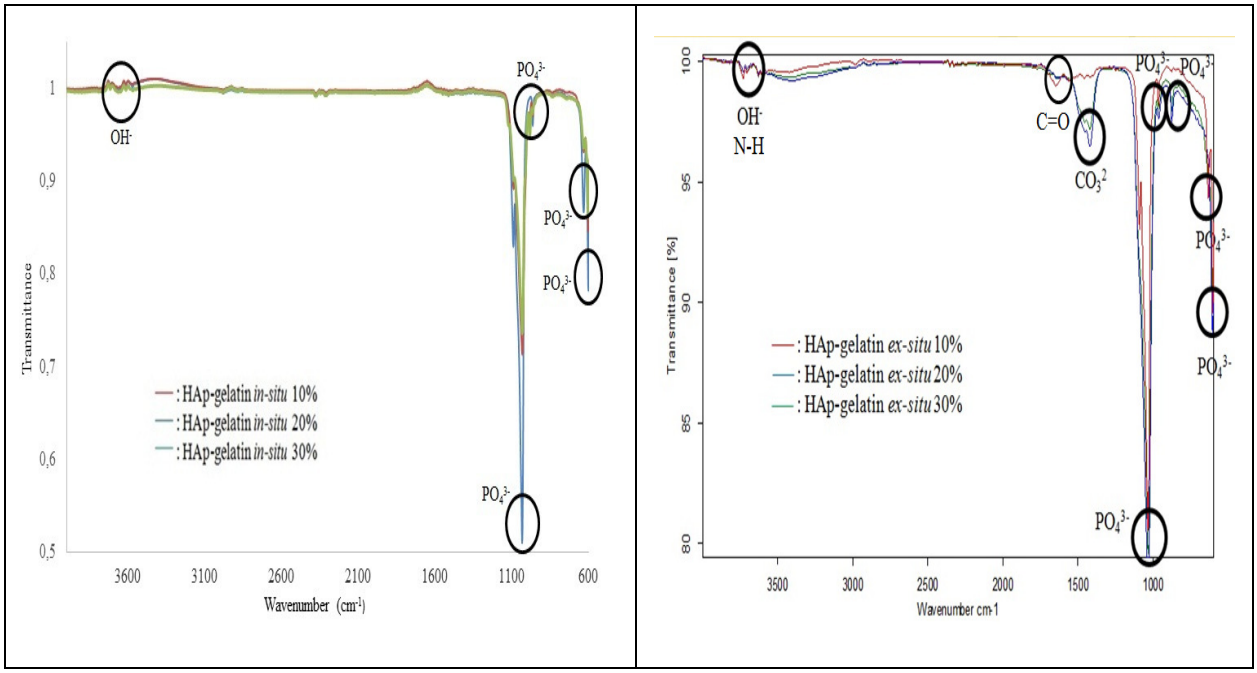

Fig-4: FTIR spectrum of HAp-gelatin

Morphology Analysis of Composite HAp-Gelatin

HAp-gelatin composites with the largest and the smallest of degree of crystallinity, the HAp-gelatin 30% were synthesized by the method of *in-situ* and *ex-situ*, were analyzed by SEM, to compare the morphology of the best and the worst of HAp-gelatin composites.The shape of HAp without gelatin granules are larger and less homogenous (Fig-5 a, b, c) when compared with HAp-gelatin *in-situ* 30% (Fig-5 d, e, f). The shape of HAp-gelatin *in-situ* 30% granules are smaller and more homogeneous due to the presence of gelatin strengthen the bonds of HAp so that the molecules of HAp become more regular, relatively have the same size, and more homogeneous. HAp is formed in the gelatin matrix so that interaction with gelatin HAp is stronger and increase the regularity of HAp. SEM image of HAp-gelatin *ex-situ* 30% (Fig.-5 g, h, i) shows that the gelatin does not bind completely with HAp but spread like patches of uneven.

This happens because the process of formation of HAp is not done in a gelatin matrix, so the interactions between HAp and gelatin only occured in the surface of HAp crystal.The addition of gelatin concentration will cause the gelatin spread in the HAp surface and make it more amorphous.This is why HAp-gelatin *ex-situ* 30% has the lowest crystallinity degree. When measured through its SEM image, HAp-gelatin *in-situ* 30% has a small size that is equal to 0.2 µm.

CONCLUSION

The best composite was HAp-gelatin 30% and synthesized by *in-situ* method. Due to its highest crystalinitydan small homogeneous granules by SEM. HAp-gelatin with *in-situ* method is more crystalline than the *ex-situ* method, from the value of the degree of crystallinity, but the synthesis of the *ex-situ* method does not cause the gelatin becomes lost. The value of the degree of crystallinity of HAp

was increased with the addition of gelatin *in-situ* and continues with the addition of gelatin concentration because HAp formed inside the gelatin matrix to form a strong interaction between the group C=O gelatin with a Ca^{2+} from HAp and makes the HAp intermolecular distance shorten. HAp-gelatin *ex-situ* 30% has the lowest crystallinity because the gelatin spread in the HAp surface and make it more amorphous.

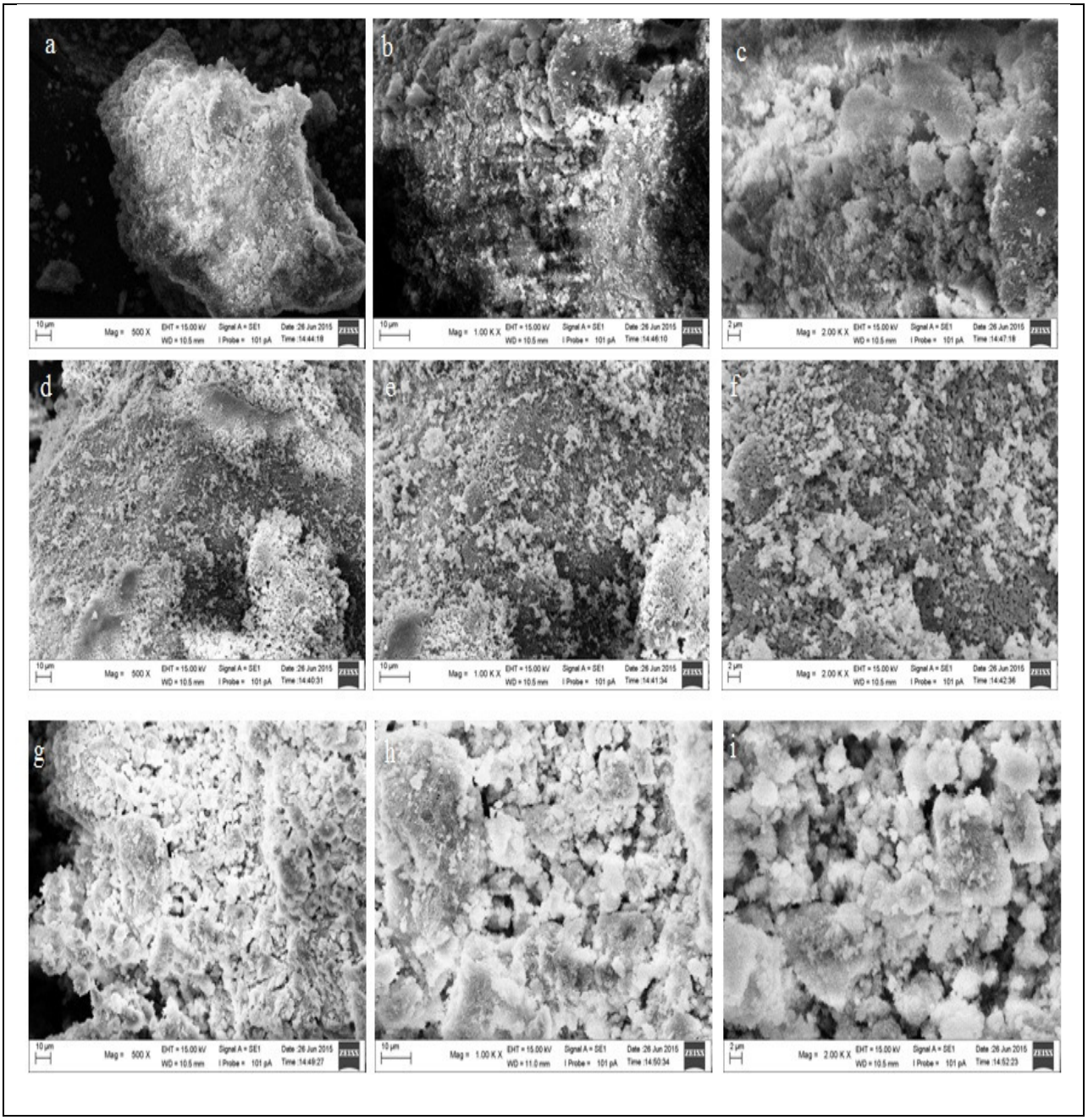

Fig-5: Morphology of SEM of HAp with 500x (a), 1000x (b), 2000x (c) magnification, HAp-gelatin *in-situ* 30% with 500x (d), 1000x (e), 2000x (f) magnification, and HAp-gelatin *ex-situ* 30% with 500x (g), 1000x (h), 2000x (i) magnification

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