NANOFIBROUS BIO-INORGANIC HYBRID STRUCTURES FORMED THROUGH SELF-ASSEMBLED PEPTIDE (FEFEFRFR)

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Abstract— **The purpose of this research is to investigate the morphology of nanofibrous hybrid formed through self-assembled peptide (FEFEFRFR). It is also carried out to synthesize the HAp and characterize its properties of nanoparticles. HAp is used because of its crystallographic structure that commonly found in natural bond as an inorganic compounds with excellent biocompatibility, bioactivity and osteoconductivity. The peptide used is a sequence of Phenylalanine, Glutamic Acid and Arginine. This combination is made by considering the advantages of diversity in the body of amino acid residues to have a peptide with the special characteristics to be able to combine with HAp nanoparticles to form nanofibrous with large surface area and stronger structure. The methods to synthesize HAp known as precipitation method. The experimental work is carried out at first by synthesize of HAp before making it into supersaturated of HAp then the supersaturated HAp nanoparticles is mineralized with self-assembled peptide (FEFEFRFR). Then, the mineralized nanofibres is tuned by using three different media: distilled water, sodium perchlorate salt and sodium iodide salt. There are several equipment's will be used to characterize the nanoparticles for the HAp nanoparticles without mineralization with peptides and with the mineralization with peptides namely Powder X-Ray Diffraction (XRD) to identify the phase of crystalline material which resulted in the high degree of the crystallinity, Fourier Transform Infrared Spectroscopy (FT-IR) to characterize the functional group of HAp nanoparticles showed the most characteristic chemical groups which are PO_4^{3-}, OH-, and CO_3^{2-} , Inductive Coupling Plasma (ICP) to detect the presence of [Ca2+] ion with concentration of 4mM and Geology microscope to observe the surface morphology. The structure of elongated fibre is observed in distilled water, sodium perchlorate salt while amourphous structure in sodium iodide salt.**

Keywords— Hydroxyapatite, Self-assembly peptide, supersaturated HAp, precipitation method

1. INTRODUCTION

Nanofibrous Bio-inorganic is a hybrid structures with combination of biomaterials of organic components which is peptide for ordering and supporting the functional inorganic materials such as HAp nanoparticles to align within them into higher-order structures. These hybrid structures are one of the advance nanotechnology that has many applications been found in electronics, photonics, catalysis, and tissue engineering. In bone tissue engineering, this nanofibrous recently used as an artificially designed scaffolds which provide an extracellular matrices to multiple criteria or mimics one of the natural ECM a biomimetic scaffold for facilitate cell recruiting/seeding, adhesion, proliferation, differentiation and neo tissue genesis for tissue formation in biomedical applications (Ma, 2008) .

In recent years, nanostructured biomaterials can be made the by several preparation of fabrication methods that can be in form of 3D scaffolds in bone tissue engineering. The commonly used techniques include melt-plotting, template synthesis, phase separation, electrospraying, electrospinning, and the newly methods have been used called as electrohydrodynamic printing. A variety of structures is applicable to produce via these methods that can be random or ordered form. For instance, by using fibers that can be obtain from electrospinning electrospraying or fibers obtained from electrospinning can produce particles to rise to non-ordered structures with random orientation which is not favourable for clinical applications. However, for certain properties need to have a good biomaterials control, such as porosity and other mechanical properties, it is applicable to use this type of ordered structures owing to its possibility that can interfere the cells behaviour. This make it possible when using electrospinning or electrohydrodynamic printing that have aligned structures (Gardin Chiara, 2012).

This study is carried out to identify the great potential of Nanofibrous Bio-inorganic with the special characteristics of peptide by using the body structure of amino acid residues which has positive or negative charge along with hydrophobic or hydrophilic properties with the combination of HAp. The peptide use is a sequence of Phenylalanine and Glutamic Acid. This study can guide us to identify the principles in terms of roles of charge, size and hydrophobicity for self-assembly into specific structures. By using the selected equipment's to characterize this nanoparticles, the observation on how this nanoparticles morphology formed and others characteristics through FEFEFRFR self-assembled peptide can be made.

This research is conducted to synthesize the HAp and characterize its properties of nanoparticles, to synthesize nanofibrous hybrid structures formed through self-assembled peptide (FEFEFRFR) by using HAp as a model of inorganic material component and to study the morphology of nanofibrous hybrid formed through self-assembled peptide (FEFEFRFR).

2. OVERVIEW OF INORGANIC HAP NANOPARTICLES

Hydroxyapatite is one of the main mineral element which have high levels of type I Col and several non-collagenous proteins (such as osteopontin, bone sialoprotein and osteocalcin) found in the calcified tissues (bone and teeth). The bone mineral can be vary in size but commonly in size of 50nm in length, 25nm in width and 2–5nm in thickness. The orientation of the crystals in the HAp

followed to each other with long crystallographic *c*-axis parallel form an alliance with Col tropocollagen molecules (J. Venugopal *et al*, 2010).

The most broad used for the implantation material in bone substitute is a synthetic HA ($Ca_{10}(PO_4)_6(OH)_2$) due to its excellent as having osteoinductive properties. HAp also have the potential to improve surface characteristics (for instance the enlargement of surface area and the increasing of charge and the ability to promote cell response and proliferation by modification of the adsorption of chemical species so it be able to trigger the mineralization in bone tissue engineering (J. Venugopal *et al*, 2010). A main inorganic mineral component of bone is hydroxyapatite which can be used to act as a structural template for the bone mineral phase and commonly it also contains the good level of bioactivity and biocompatibility that can be used as a substitution in the bioceramic filler in polymer-based bone. Besides that, HAp have the same features of inorganic compounds found in natural bone that expressed the structure of crystallographic structure.(J. Venugopal *et al*, 2010).

3. OVERVIEW OF AMINO ACIDS

Amino acids are the sub units that make up peptides and proteins. It have a group of organic compounds consists of one or more amino groups,NH2, and one or more carboxyl grou ps,-COOH. The alpha amino acids $RCH(NH_2)COOH$ consists of R group at that can be categorized as hydrogen or an organic group component molecules of proteins, at which can be synthesized in the body or may need to be inserted in diet because it cannot produce naturally in body (Collins English Dictionary). There are 20 different types of amino acid that can come by naturally. They are indeed starting from relatively simple molecules being able to trigger into a high molecular complexity, being at the molecular basis of the living world. Owing to its high versatility in functions cause modularity of amino acids and provide the potential to driving self-assembly and self-organization. Amino acids can be functioning in both way either as single molecules and when inserted in peptides. Furthermore, the functional groups on the side chains and at the N- and C-terminus can be tailored to make it potentially to enlarge endlessly their molecular variability.

The classification of amino acids into several types is based on their R groups which can be divided into hydrophobic amino acids, aliphatic amino acids, and aromatic residues. Hydrophobic amino acids composed of either an aliphatic or aromatic side group. Whereas the example of aliphatic amino acids are alanine, valine leucine isoleucine and methionine. The last is aromatic residue which can formed π-π stacking interactions that is crucial for the self-assembly process in hydrophobic medium (R. V. Ulijn *et al*, 2008). These amino acids include phenylalanine, tryptophan, and tyrosine. Another amino acids also existed as in the charged form which can be classified as positively charged, which are lysine and arginine and negatively charged, which are aspartic acid and glutamic acid. The position of peptide sequence and charged amino acids can affect the self-assembly process of different peptides.

Besides the charged amino acids there are also the neutral but polar amino acids serine, threonine, asparginine, and glutamine which are capable of forming hydrogen bonds by their hydroxyl or amide groups. Lastly, there are the amino acids cysteine, proline and glycine, which are all capable of imparting their own unique properties on a peptide. The properties of cysteine that have a thiol group make it having the ability to form a disulphide bond which can stabilise protein structure. This ability to form this bond also been explored in order to control the self-assembly of peptide systems (J. D. Hartgerink *et al*, 2002). The inflexible structure given by the covalent bonding of its side chain to the amino terminus of Proline usually disintegrate helices and β-sheet structures in proteins. It is also found in β-turn structures, and as such has been exploited by Pochan, Schneider and colleagues in the self-assembly of their MAX series of peptides (J. P. Schneider *et al*, 2002). Finally the glycine side group which only consists of a hydrogen atom that can permits the residue of unique flexibility due to the lack of steric hindrance that is usually given by the R group (R. V. Ulijn *et al*, 2008).

4. APPLICATIONS OF SELF-ASSEMBLED PEPTIDE

4.1 Drug Delivery

One of the vital key to cure treatments such as gene therapy is by using the targeted drug delivery. However there are some problems regarding to the transportation of the drugs to reach on the targeted spot due to the existed barriers such as cell membranes (P. Chen, 2005) and the low solubility of the drugs that need to take into account since it is important to have sufficient amount of the drugs. Many drugs require a vehicle to be transported to improve solubility in aqueous environments and also to prevent disintegration of the drug by reaction with enzymes (P. Chen, 2005).

The ideal cell penetration display an ideal characteristics via Viral based drug delivery systems instead of Non-viral systems such as polymeric carriers and liposomes. However, the safety of a viral system and effectiveness need to take into consideration because it is shown that non-viral systems do not cause any defects in safety issues but they are less effective at cell penetration (P. Chen, 2005).

It has been seen that ionic-complementary peptides can assemble around hydrophobic compounds, with the peptide hydrophilic face on the outside of structure, and the hydrophobic face encapsulating the compound. This increases the compound's solubility in aqueous environments (P. Chen, 2005).

The amino acid sequence and chain length can be adjusted accordingly to improve the cells penetration by the peptide's ability. Surface charge and amphiphilicity are known to manipulate the penetration of biological barriers (P. Chen, 2005). The loading and release capacity of the drug based delivery system can be increase by the hydrophobic and hydrophilic residues controlling (P. Chen, 2005).

Nagai et al. have investigated the use of RADA16 as a drug release system. Phenol red, bromophenol blue, 8-hydroxypyrene-1,3,6-trisulfonic acid sodium salt (3-PSA), 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (4-PSA) and Coomassie Brilliant Blue G-250 (CBBG) dyes were chosen as drug models. Electrostatic interactions between the peptide nanofibres and the dyes controlled release rate can be presented by the Differences in the diffusivity of the dyes from the peptide hydrogel. The concentration of peptide used is another factor to indicate the diffusivity by means the higher concentration has lower rate of diffusion. By this, it can be said that by controlling the charged amino acids and concentration of the peptide sequences can be a sign of the release rates of drugs (Y. Nagai *et al*, 2006).

4.2 Tissue Engineering

The ability of self-assembling peptides to form fibrous hydrogels, composed of more than 99% water has led them to be investigated in the field of tissue engineering (C. A. E. Hauser., 2010). One of the important factor includes the nanoscale size of the fibres and pores formed in the fibre matrix, surface interactions, and mechanical properties of the fibrils. The structure of the hydrogel emulates that of the ECM, meaning they are potentially good environments for tissue engineering (S. G. Zhang, 2005).

The peptides can form nanofibre matrix to be use as a scaffold for the attachment of cells, upon which the cells can adhere together and form functional tissues (S. G. Zhang, 2003). The peptides scaffolds can act as substrates for cell growth, differentiation and biological function due to their potential to use many cell types, and for the formation of higher tissue architectures.

Chen defined the criteria that would lead to an ideal biological scaffold for cell attachment (P. Chen, 2005):

- The components should be from biological sources.
- The basic units should be easily designed and modified for specific needs.
- Biodegradable.

- No cytotoxicity.
- Reasonable cell-substrate interactions.
- Elicit minimal immune response and inflammation.
- Simple material production, purification and processing.
- Readily transportable.
- Chemically compatible with aqueous solution and physiological conditions.

Gelain *et al* added by stated that the cirteria must be able to integrate with other materials in the body (F. Gelain *et al*, 2007). The use of salts such as NaCl and KCl and the incorporation of specific functional motifs can lead to the potential to control nanofibre and scaffold formation. Thus, designation of the peptides for specific applications, enhancing their performance in terms of cell-materials interaction, cell proliferation, migration, differentiation and performing their biological function can be made (S. G. Zhang *et al*, 2005). Several bio-active sequences found in nature have been added to traditional peptide structures. Different motifs can be incorporated in the same self-assembled structure by mixing ratios of different peptide.

5. MATERIALS AND METHODS

4.1 Synthesis of HAp nanoparticles

14.17 gram of Calcium Nitrate Tetrahydrate (0.2 /mol) and 5.62 gram of Sodium Hydrogen Disphosphate (0.12/mol) will be add in the 300mL deionized water. The solution will be stir vigorously while heat up at 85˚C using the heating plate. Then 300 ml of concentrated ammonium hydroxide solution (28–30% concentrate) will be quickly added to the solution, which immediately induced the nanoparticle precipitation. The mixture will maintain at 85˚C for approximately 24 h to ensure a complete conversion. After that, the mixture will cool down to room temperature (range 25-28˚C) The solid material form after the heating will be settle in the container and excess liquid is remove. Fresh deionized water will be add with the solid material. The mixture then will stir briefly before allowing the solid to settle and decanting once again. The dilution and decanting process was repeated until the pH value of the mixture was below 9. In this study, these as-fabricated HAp nanoparticles without surface modification are referred to as untreated HAp nanoparticles.

4.2 Preparation of Supersaturated Hydroxylapatite (HAp) Solution

5.1839 gram of HAp powder will dissolve in 100mL of 100 mM hydrochloric acid and had a final concentration of 50 mM of calcium. 40 mL of the stock solution will pipette into a clean polythene container. The volume of the stock solution will increased to 450 mL by adding with distilled water. The pH value will adjusted to 7.01 with 0.05 M potassium hydroxide. 10mL Sodium chloride will be add on to the solution to reach a final concentration of 200 mM. Then the final volume will be adjust to 500 mL with distilled water. The concentration of the resultant HAp-supersaturated solution is [Ca2+] = 4 mM.

4.3 Mineralization of Supersaturated Hydroxylapatite (HAp) with Peptide

200 µL solution of peptide KAAAAAK with pH ~7.5 and 200 µL supersaturated HAp solution pH ~7.01 will mix together in a glass tube to form a clear solution. The medium will be use are salts (Sodium Perchlorate) and distilled water. The solution will let in an incubator at 36.7 °C to vaporize the solvent water slowly. Then the mixture will undergo centrifugation. The solid materials obtained will be suspended in water.

4.4 Synthesis of Nanofibrous Bio-inorganic with salts

200 µL solution of Nanofibrous Bio-inorganic is immersed in each 200 µL distilled water, Sodium Perchlorate and Sodium Iodide solution then these combination solution is stirred vigorously an left for a day to completely react with each other. The solution is then undergo centrifuge. The solid materials obtained will be suspended in the solution.

6. RESULTS AND DISCUSSION

6.1 X-Ray Diffraction of HAp powder

X-ray diffraction (XRD) is used to characterize the phase composition and degree of crystallinity of the produced biological HAp. Considering the crystallography of HAp, it occurs as a hexagonally packed crystal belonging to $P6_3/m$ space group. The unit cell consists of Ca^{2+}, PO_4^{2-}, and OH^- groups closely packed together in a hexagonal arrangement. The OH^- group serves as the backbone for HAp. The six phosphates, PO_4, are in a helical arrangement around the *c*-axis. These phosphate groups form a skeletal frame structural network which provides the stability of HAp. On X-ray diffraction, the crystallite size can be described by the diffracting plane of HAp at 2θ=25.50° for the reason that this miller index correspond to the *c*-axis length. As HAp becomes more crystalline, the *c*-axis length increases which can be verified by the diffraction peak for becoming narrower. The ratio [Ca]/[P] = 1.67 is used in this work because when the [Ca]/[P] ratio approaches the value of 1.67, for stoichiometric HAp, the degree of crystallinity increases. The HAp sample with [Ca]/[P] ratio closest to 1.67 has the highest degree of crystallinity. However, as [Ca]/[P] ratio goes beyond 1.67, a decrease on the degree of crystallinity is observed.

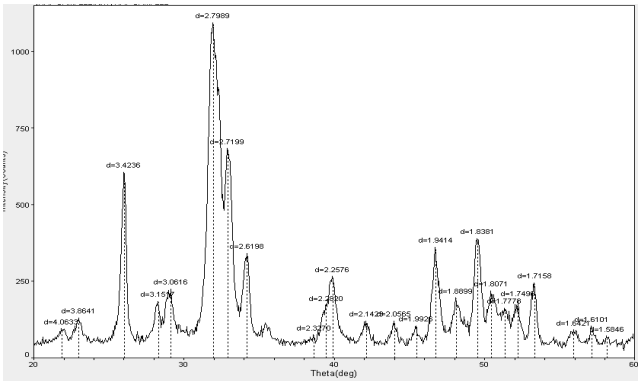

Figure 6.1: X-Ray Diffraction of Powder Hydroxylapatite

XRD can also be used to detail information about peptide secondary structure, and also fibril structure. Samples that display an ordered repeat distance, such as in a β-sheet, can diffract an X-ray beam, resulting in a Bragg peak. Here samples were performed within a 2θ regions range of 20º-60º on a Cintag Pad V x-ray diffractometer with Cu Kα radiation and Ni filter. This is intended to study the crystallinity structure of the HAp powder. The XRD patterns in Figure 6.1 indicate that nanoparticles have the characteristic peak at 2θ regions of 26º,28º,29º,32º,33º,-34º,40º,47º,53º, which are consistent with the HAp phase. This results suggested that the selected reactions are on the HAp surface and the intrinsic properties of HAp especially structure are maintain.

6.2 FTIR Spectrum on HAp powder

Fourier transform infrared spectroscopy (FTIR) is one of the methods which, systematically monitoring variations of structural characteristic groups and vibrations bonds, it also able to provide an indirect evaluation of the synthesized Ca/P implant materials and contents of peptide in these materials. Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is dominating and the most significant mineral phase in the solid tissues of the vertebrates. It consists of the same ions that form mineral part of teeth and bones. A biological HAp usually has a calcium deficient; it is always substituted with a carbonate. Two types of carbonate substitution are possible: (1) direct substitution of OH^- with CO_3^{2-}(A-type substitution $(CO_3)^{2-} \leftrightarrow 2OH^-$) and (2) necessity after charge compensation, PO_4^{3-} substituting a tetrahedral group with CO_3^{2-} (B-type substitution).

Substitution groups may provoke characteristic changes in the lattice parameters, crystallinity, crystal symmetry, thermal stability, morphology, and solubility, physical, chemical and biological characteristics (Shi, 2006). The most characteristic chemical groups in the FTIR spectrum of synthesized HAp are PO_4^{3-}, OH-, and CO_3^{2-}.

Based on Figure 6.2, the bands at 1340.16 cm^{-1} is referred to out of plane bending mode of CO_3^{2-} . This can be seen when that the synthesized material is formed with an apatitic HAp structure with a slight Ca deficient, which is proven by presence of the CO_3^{2-} group, however this amount is constantly reducing along with reaction approaching its end. The bands at 1090.01 cm^{-1} is NO^{3-} that appears as the synthesis residue that will disappears during the calcifying process (Borodajenko, 2012). The bands at 1022.4 cm^{-1} and 962.30 cm^{-1} are ascribed to the stretching mode of phosphate while the bands at 601.09 cm^{-1} and 560.12 cm^{-1} are ascribed to the bending mode of phosphate. The absorption bend at 629.89 cm^{-1} represents the stretching vibration mode of lattice OH^-, this also proves the presence of HAp. These are all the major characteristic bands of HAp. While, the absorption band at 3217.35 cm^{-1} indicates the bending mode of adsorbed water in the samples. The absorption band of adsorbed water is relatively wide at range of 3600-2600 cm^{-1} but it will becomes narrower under influence of thermal treatment because it will affected the behaviour of CaP in terms of various factors, like atmosphere of sintering, ratio of Ca/P, method and conditions of powder synthesis, type and amount of impurities, sample size, particle size, etc. In order to avoid such issues, the experimental procedure is work within the allowable temperature which is in range of room temperature till 100°C during the dehydration process of HAp.

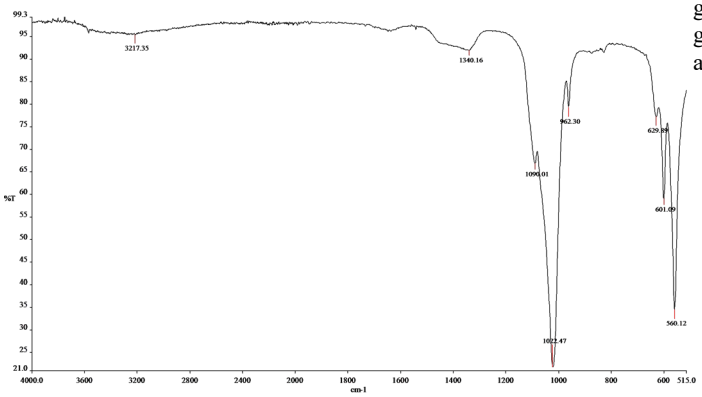

Figure 6.2: FTIR Spectrum on HAp

Wavelength (cm^{-1})	Stretching mode	Functional Group
3217.35	Bending mode of absorbed water	Adsorbed water
1340.16	Out of plane bending mode	CO_3^{2-}
1090.01	Synthesis residue that disappears during the calcifying	NO^{3-}
1022.4	Stretching mode of phosphate	PO_4^{3-}
962.30	Stretching mode of phosphate	PO_4^{3-}-
629.89	Proves the presence of HAp	OH^-
601.09	Bending mode of phosphate	PO_4^{3-}
560.12	Bending mode of phosphate	PO_4^{3-}

Table 6.2: Characteristic chemical groups of FTIR absorption band

6.3 Inductive Coupling Plasma (ICP) on Supersaturated HAp

This analysis is conducted in 4 samples to choose the supersaturated HAp with calcium concentration $[Ca2^{+}]$ =4.0mM. The concentration of calcium at 4mM is preferable because this concentration is approaching to the concentration of the Ca^{2+} concentration in body fluid and it remain stable in room temperature. The $[Ca^{2+}]$ =4 mM in solution also lead to the high nucleation rate at many sites on the inner membrane to form amorphous calcium phosphate before mineralize with peptide (Q.L. Feng, 1997) . Based on the analysis of ICP, it shows that Sample 2 is close to 4mM concentration of Ca^{2+} which is used to be mineralize with peptide.

Sample	Concentration (ppm)	Concentration (mM)
1	136.00	3.40
2	167.28	4.17
3	115.63	2.90
4	210.53	5.25

Table 6.3: Analysis of ICP on supersaturated HAp

6.4 Geology microscope on Nanofibrous Bio-inorganic

The purpose of this study is to see the morphology of nanofibrous bio-inorganic structure formed through self-assembled peptide (FEFEFRFR). In addition, it also conducted to see the morphology when this nanofibrous is tuned by using chaotropic anions I^- and ClO_4^{2-}. This peptide-based biomaterials is able to form 2 and 3-D structures. This feature is crucial for proper cellular differentiation and tissue formation, especially the ability to form different structures as respond to environmental change. The ability to control the formation of inorganic crystals using natural peptides would be a valuable tool in tissue engineering for the restoration and regeneration of hard tissues. Proteins which is peptide play a significant role in crystal nucleation, growth and morphology and enable the process of biomineralization to occur under ambient temperature and pressure conditions. Therefore, biological template mineralization could be employed to synthesize bone mimetic materials. This is done by mineralized the sequence of peptide (FEFEFRFR) which consists of aromatics hydrophobic neutral of phenylalanine, polar hydrophilic negative charge of glutamic acid and polar hydrophilic positive charge of arginine groups of peptides with calcium ions in HAp and aligning them in an orientation that matches the crystal structure of HAp.

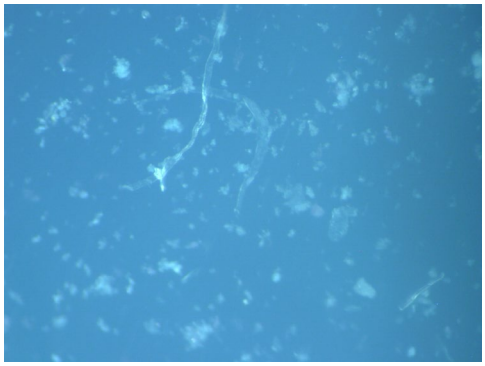

Figure 6.4.1: Geology microscope image of Nanofibrous Bio-inorganic in distilled water

The Geology microscope images showed the size distribution and morphology of Nanofibrous Bio-inorganic in three different medium. The functionality of nanofibrous is determine based on the structure of its order assembled architectures. Tunable peptides that response to the external stimuli (I^- or $ClO4^{2-}$) due to the screening effect that form the needle-like structure is the desired structure to be used for further developed as bone implant material due to its biocompatibility. Based on Figure 6.4.1, the morphology of the nanofibrous shows the structure of elongated fibre formation in distilled water. This structure still can be use in bone application with some modification. In Figure 6.4.2, the morphology under influence of Sodium Perchlorate also have the structure of elongated fibre. However, the images shows in Figure 6.4.3, the morphology is amorphous structure in the presence of Sodium Iodide which is cannot be used for any applications in medical fields. These analysis shows the incapable of this nanofibrous to self-associate into dendritic, needle-like structure in solutions containing chaotropic anions I^- and ClO_4^{2-}.

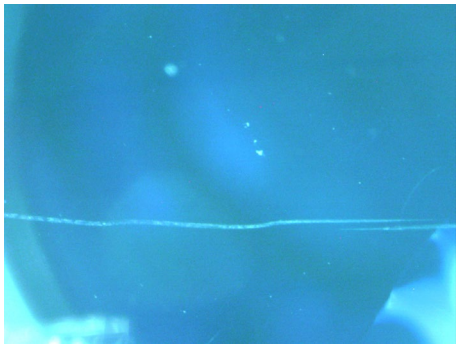

Figure 6.4.2: Geology microscope image of Nanofibrous Bio-inorganic in Sodium Perchlorate

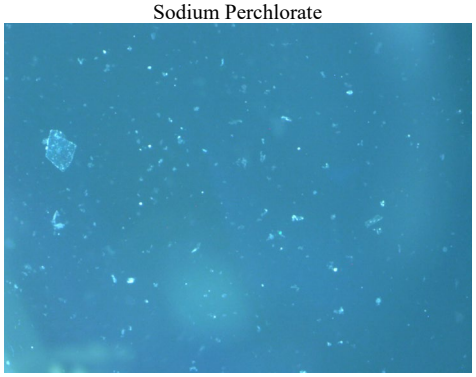

Figure 6.4.3: Geology microscope image of Nanofibrous Bio-inorganic in Sodium Iodide

7. Conclusion

In summary, HAp nanoparticles is synthesized by precipitation method is then undergoes process to become supersaturated of HAp before the mineralization with self-assembled peptide (FEFEFRFR). The analysis is conducted by using several equipment to analyze the characteristics of the HAp, supersaturated HAp and nanofibrous bio-inorganic hybrid structures formed through self-assembled peptide. Based on the analysis results by using X-Ray Diffraction (XRD) it shows that the synthesis of powder HAp have the characteristic peak at 2θ regions of 26°,28°,29°,32°,33°,-34°,40°,47°,53°, which are consistent with the HAp phase. This shows the high ccrystalinity of the powder is achieved in this research before it can be proceed to be supersaturated HAp.While, the usage of FTIR in HAp powder resulted in producing 3217.35 cm^{-1}, 1340.16 cm^{-1}, 1090.01 cm^{-1}, 1022.4 cm^{-1}, 962.30 cm^{-1}, 629.89 cm^{-1}, 601.09 cm^{-1} and 560.12 cm^{-1} indicated the existence of the most characteristic chemical groups in the FTIR spectrum of synthesized HAp which are PO_4^{3-}, OH-, and CO_3^{2-} . The analysis of supersaturated HAp resulted in calcium concentration $[Ca^{2+}]$ = 4.17mM in sample 2 which is most close to the desired concentration of 4mM. The mineralization of supersaturated HAp with self-assembled peptide similar morphology for distilled water and sodium perchlorate which is elongated fibre structure but different morphology in sodium iodide which is amorphous structure. This analysis shows that the nanofibrous formed through this self-assembled peptide (FEFEFRFR) is not reliable to be use in bone implant materials because it is unable to self-associate into dendritic needle-like structure in solutions containing chaotropic anions I^- and ClO_4^{2-}. These results provide understanding of the influence of self-assembled peptide when hybrid with the HAp nanofibre in terms of functional properties of nanoparticles to be used in biomedical applications.

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References

[1] Dindyal Mandala, A. N. S. a. K. P., 2014. Self-Assembly of Peptides to Nanostructures. Org Biomol Chem, p. 3544–3561.

[2] Gardin Chiara, F. L. F. L. S. E. S. D., 2012. Nanostructured Biomaterials for Tissue Engineered Bone. International Journal of Molecular Science, pp. 737-757.

[3] J. Venugopal, M. P. R. Y. Z. S. L. A. T. C. a. S. R., 2010. Biomimetic hydroxyapatite-containing composite nanofibrous substrates for bone tissue engineering. Phil. Trans. R. Soc. A, p. 2065–2081.

[4] Liang Chen, J. M. M. J. C.-M. L. a. H. L., 2011. The role of surface charge on the uptake and biocompatibility of hydroxyapatite nanoparticles with osteoblast cells. Nanotechnology 22.

[5] Ma, P. X., 2008. Biomimetic materials for tissue engineering. Advanced Drug Delivery Reviews, p. 184–198.

[6] Tao He, G. A. B. C. a. C. M., 2010 . Nanofibrous Bio-inorganic Hybrid Structures Formed Through Self-Assembly and Oriented Mineralization of Genetically Engineered Phage Nanofibers. Small, p. 2230–2235.

[7] Santos MH, de Oliveira M, de Freitas Souza P, Mansur HS, Vasconcelos WL. Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process. Mater Res. 2004; 7(4): 625-630.

[8] Bouyer E, Gitzhofer F, Boulos MI. Morphological study of hydroxyapatite nanocrystal suspension. J Mater Sci: Mater Med. 2000; 11: 523-531.

[9] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Enhanced functions of osteoblasts on nanophase ceramics. Biomaterials. 2000; 21: 1803- 1810.

[10] Balasundaram, G.; Webster, T.J. An overview of nano-polymers for orthopedic applications.

[11] *Macromol. Biosci.* 2007, *7*, 635–642.

[12] amino acid. (n.d.). Dictionary.com Unabridged. Retrieved November 29, 2016 from Dictionary.com website http://www.dictionary.com/browse/amino-acid

[13] Amino Acid Guide (2010, November 29). Retrieved from http://www.aminoacidsguide.com/

[14] G.M. Whitesides, J.P. Mathias, C.T. Seto Science, 254 (1991), pp. 1312–1319

[15] Letsinger et al., *J. Am. Chem. Soc*. 1998, *120*, 1959.

[16] amino acid. (n.d.). Collins English Dictionary - Complete & Unabridged 10th Edition. Retrieved December 13, 2016 from Dictionary.com website http://www.dictionary.com/browse/amino-acid

[17] R. V. Ulijn, A. M. Smith, *Chemical Society Reviews* 2008, *37*, 664-675.

[18] J. D. Hartgerink, E. Beniash, S. I. Stupp, Proceedings of the National Academy of Sciences of the United States of America 2002, 99, 5133-5138.

[19] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* 2001, *294*, 1684-1688.

[20] C. J. Bowerman, B. L. Nilsson, *Journal of the American Chemical Society* 2010, *132*, 9526-9527.

[21] J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis, J. Kretsinger, Journal of the American Chemical Society 2002, 124, 15030-15037.

[22] P. Chen, Colloids and Surfaces a-Physicochemical and Engineering Aspects 2005, 261, 3-24.

[23] Y. Nagai, L. D. Unsworth, S. Koutsopoulos, S. Zhang, Journal of Controlled Release 2006, 115, 18-25.

[24] C. Keyes-Baig, J. Duhamel, S. Y. Fung, J. Bezaire, P. Chen, *Journal of the American Chemical Socitey* 2004, 126, Chap. Chapter, pp. 7522-7532.

[25] K. Rajagopal, M. S. Lamm, L. A. Haines-Butterick, D. J. Pochan, J. P. Schneider, Biomacromolecules 2009, 10, 2619-2625.

[26] S. Sutton, N. L. Campbell, A. I. Cooper, M. Kirkland, W. J. Frith, D. J. Adams, Langmuir 2009, 25, 10285-10291.

[27] C. A. E. Hauser, S. Zhang, Chemical Society Reviews 2010, 39, 2780-2790.

[28] S. G. Zhang, Nature Biotechnology 2003, 21, 1171-1178.

[29] P. Chen, Colloids and Surfaces a-Physicochemical and Engineering Aspects 2005, 26

[30] F. Gelain, A. Horii, S. G. Zhang, Macromolecular Bioscience 2007, 7, 544-551. 1, 3-24.

[31] S. G. Zhang, F. Gelain, X. J. Zhao, Seminars in Cancer Biology 2005, 15, 413-420

[32] A. Horii, PLoS ONE 2007, 2, e190.

[33] K. M. Galler, R. N. D'Souza, J. D. Hartgerink, G. Schmalz, Advances in Dental Research 2011, 23, 333-339.

[34] L. A. M. Horton, K.G. Scrimgeour, M.D.Perry, J.D.Rawn, Principles of Biochemistry. 4 ed., Editor, Pearson Education, New Jersey, 2005.

[35] N. Sewald, J. H-D., Peptides: Chemistry and Biology. Editor, Wiley-VCH, Germany, 2002, pp. 1-54.

[36] Martin CR, Kohli P. Nat. Rev. Drug discovery. 2003;2:29–37.

[37] Reches M, Gazit E. Nano Lett. 2004;4:581–585.

[38] Ghadiri MR, Granja JR, Milligan RA, McRee DE, Khazanovich N. Nature. 1993;366:324–327

[39] Cui H, Webber MJ, Stupp SI. Biopolymers. 2010;94:1–18.

[40] Santoso S, Hwang W, Hartman H, Zhang S. Nano Lett.2002;2:687–691

[41] Behanna HA, Donners JJ, Gordon AC, Stupp SI J Am Chem Soc. 2005 Feb 2; 127(4):1193-200

[42] Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI Science. 2004 Feb 27; 303(5662):1352-5.

[43] Tovar JD, Claussen RC, Stupp SI J Am Chem Soc. 2005 May 25; 127(20):7337-45.

[44] Hartgerink JD, Beniash E, Stupp SI Science. 2001 Nov 23; 294(5547):1684-8.

[45] Bull SR, Palmer LC, Fry NJ, Greenfield MA, Messmore BW, Meade TJ, Stupp SI
J Am Chem Soc. 2008 Mar 5; 130(9):2742-3.

[46] Hamley IW, Dehsorkhi A, Castelletto V, Seitsonen J, Ruokolainen J, Iatrou H. Soft Matter. 2013;9:4794–4801.

[47] Castelletto V, Cheng G, Greenland BW, Hamley IW. Langmuir. 2011;27:2980–2988.

[48] Balamurugan A, Michel J, Faure J, Benhayoune H, Wortham L, Sockalingum G, Banchet V, Bouthors S, Laurent-Maquin D, Balossier G. Synthesis and structural analysis of sol gel derived stoicheometric monophasic hydroxyapatite. Ceramics-Silikaty. 2006; 50(1): 27-31.

[49] Masuda Y, Matubara K, Sakka S. S ynthesis of hydroxyapatite from metal alkoxides through sol gel technique. J Ceram Soc, Jpn. 1990; 98: 1266- 1277

[50] Li P, de Groot K. Better bioactive ceramics through sol-gel process. J Sol-gel Sci Technol. 1994; 2: 797-801. 32. Brendel T, Engel

[51] Brendel T, Engel A, Russel C. Hydroxyapatite coating by polymeric route. J Mater Sci: Mater Med 1992; 3: 175-179.

[52] Suchanek WL, Riman RE. Hydrothermal synthesis of advanced ceramic powders. Adv Sci Technol. 2006; 45: 184-193.

[53] Li P, de Groot K. Better bioactive ceramics through sol-gel process. J Sol-gel Sci Technol. 1994; 2: 797-801. 32. Brendel T, Engel

[54] Kimura I. Synthesis of hydroxyapatite by interfacial reaction in a multiple emulsion. Res Lett Mater Sci. 2007; Article ID 71284: 1-4.

[55] Castelletto V, Cheng G, Stain C, Connon CJ, Hamley IW. Langmuir. 2012;28:11599–11608.

[56] Dong H, Paramonov SE, Aulisa L, Bakota EL, Hartgerink JD J Am Chem Soc. 2007 Oct 17; 129(41):12468-72.

[57] Marini DM, Hwang W, Lauffenburger DA, Zhang S, Kamm RD. Nano Lett. 2002;2:295–299.

[58] Self-assembly of giant peptide nanobelts. Cui H, Muraoka T, Cheetham AG, Stupp SINano Lett. 2009 Mar; 9(3):945-51.

[59] Artificial peptide-nanospheres self-assembled from three-way junctions of beta-sheet-forming peptides. Matsuura K, Murasato K, Kimizuka N J Am Chem Soc. 2005 Jul 27; 127(29):10148-9.

[60] myscope, 2013. *Australian Microscopy & Microanalysis Research Facility.* [Online]
Available at:
http://www.ammrf.org.au/myscope/tem/introduction/
[Accessed 16 December 2016].

[61] W.L.O, W. A. A. J., 2011. OVERVIEW OF AN INDUCTIVELY COUPLED PLASMA (ICP) SYSTEM. *International Journal of Chemical Research,* 3(2), pp. 41-48.

[62] Borodajenko, L. B.-C. a. N., 2012. Research of Calcium Phosphates Using Fourier Transform Infrared Spectroscopy. Infrared Spectroscopy – Materials Science, Engineering and Technology.

[63] Q.L. Feng, Q. C. H. W. F. C., 1997. Inßuence of concentration of calcium ion on controlled precipitation of calcium phosphate within unilamellar lipid vesicles. Journal of Crystal Growth, Volume 186, pp. 245-250..