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BIOACTIVITY OF STARCH-ADDED NANO CALCIUM PHOSPHATE CEMENT

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ABSTRACT

Injectable nano-size particle calcium phosphate cements (CPC) added starch from rice (RS) had been studied for its bioactivity. CPC added with 4wt% of RC was selected as it shows the best properties. For further study, bioactivity of starch-added CPC was investigated by soaking in simulated body fluid (SBF) for a period of 1 - 35 days. From a study using SEM, apatite growth was observed after 1 day soaking in SBF. The apatite increased its growth with the increasing of soaking days. The XRD patterns show that the calcium deficient hydroxyapatite (CDHA) was formed on the samples after soaking in SBF. Besides that, the FTIR spectrum shows the reduction of phosphate (PO_4^-), carbonate (CO_3^-) and hydroxyl (OH^-) ions due to the formation of apatite on the samples.

INTRODUCTION

The needs for biomaterials has become more popular as the increasing of world population. Biomaterials based on calcium phosphate (CaP) ceramics is one of the materials that have gained clinical acceptance for bone defect repairs and substitution, as it consists the same ions as the mineral in natural bone [1]. Due to its excellent bioactive, bioinert and biomimetic and mechanical properties, calcium phosphate cements (CPCs) has been choosen as a good materials for bone defect repair in orthopedic and dental surgery. Bioinert implantation with bone like CPCs beneficially bond to native bone because its biomimetic provides a preferred substrate for cell attachment [2].

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, commonly referred as HA is a class of calcium phosphate based bioceramic. It is frequently used in the biomedical field because of its mineral components that are similar to natural bone. It is also known as one of most important implantable materials due to its biocompatibility, bioactivity, and osteoconductivity that are useful for bone defect repairs and human hard tissue

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substitution material. However, the clinical use of HA as a bone substitute materials has proven problematic [3-5]. For example, it takes a long time for self-setting and to be degrading in the body. To overcome this problems, beta tricalcium phosphate (β-TCP) and calcium sulphate dehydrate (CSD) are added to from better biodegradability [6] and its self setting.

The starch from rice had been selected as its gelatinizing temperature, processing stabilities, viscosities, and particles size which is smaller than other starch. Besides that, it also shows good mechanical properties as a composite reinforcement. Starch is also biodegradable, low cost, renewable and non toxic material [7, 8].

METHODOLOGY

Samples preparation

The bone cement was produced by mixing the sintered hydroxyapatite (HA), beta tricalcium phosphate (β-TCP), and calcium sulphate dihydrate (CSD), with a specific ratio. The mixing was done by using ball milling process (*RETSCH Pulvarisette*) for a short period between 30 – 60 minutes at medium rotational speed, until the powders were mixed homogenously. Then, the CPC was aged for 7 days (a week) to stabilize it phase. The samples were produced after a week ageing period is finished. The CPC and RS were mixed together by using agate and mortar. The paste formed from the mixture was putted in the syringe and injected into the 6mm x 12mm cylindrical shape mold. The setting time was measured from the initial mixing until the surface of the paste in the mold cannot be penetrated by Gilmore Needle's tip. Mean while, the injectability of paste was also measured. The samples were left in incubator at human body temperature, 37°C, for 7 days ageing, and characterizations were handled after the ageing was finished.

Simulated Body Fluid (SBF) preparation

The SBF was prepared by dissolving reagent NaCl, $NaHCO_3$, KCL, K_2HPO_4, $MgCl_2.6H_2O$, $CaCl_2$, and Na_2SO_4 one by one in 1 liter of deionized water and stirred. The SBF was buffered at pH 7.4 by using HCL and trishydromethylaminomethane at 37°C. The samples then were soaked in the SBF for 1, 3, 7, 14, 21, and 35 days. The SBF was changed every 3 days of soaking to ensure that the rate of ion exchange is comparable with in vivo [9].

Characterization

The samples were characterized by using X-Ray Diffraction (XRD, *Siemens D-5000*), Fourier Transmission Infra Red Spectroscopy (FTIR, *Perkin Elmer*) and Scanning Electron Microscopy (SEM, *LEO model 1450 VP*).

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RESULTS AND DISCUSSION

Morphology

The soaked samples for 1 until 35 days of soaking were viewed under SEM to determine the growth of apatite on the CPC added 4wt% starch. The micrographs for the samples had shown as below.

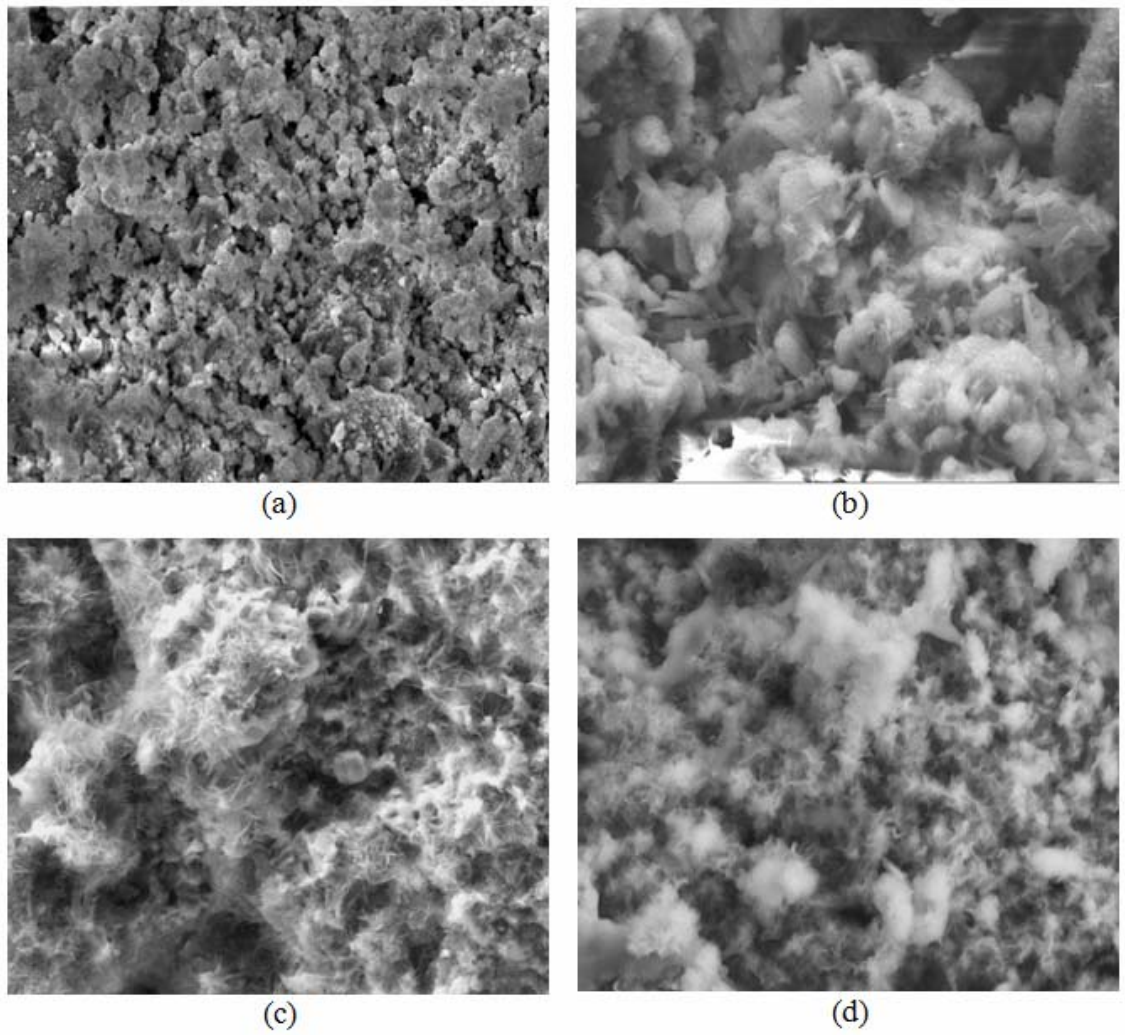

Figure 1: Micrographs of samples (a) before soaking, (b) 1 day, (c) 7 days and (d) 35 days soaked

The micrographs show that there were no reaction and growth of apatite occurs on the non soaked sample. After a day of soaking in SBF (b), the apatite was starting to growth on the sample but with a small coverage spot. The needle like shape of apatite was also start to form, but not so clear in the image. During 7 days of soaking, the needle like shape was clearly viewed in the micrographs. The surface area of its growth also increased on the sample. After 35 days, the apatite growth rapidly covering the sample surface area. The size of needle shape also increased. This shows that the samples were

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successfully reacted with the ions of surrounding SBF.

The morphology of precipitation is quite similar among the bioceramic surfaces. Precipitation starts at individual tiny needles gradually grow together to form a dense layer on the specimen surface and covering microspores [10]. In the stage of soaking time, the dissolution precipitation process was dominated by ion exchange. The apatite layer was formed by the reaction between release of calcium (Ca^{2+}) ions from the sample and phosphate (PO_4^-) ions in the solution [11]. Furthermore, the apatite growth was also affected by the days of soaking in SBF, beside the concentrations of Ca^{2+} and PO_4^- ions [12].

Fourier Transform Infra Red Spectroscopy (FTIR)

The samples were characterized by using FTIR to measure the functional groups that exhibit before and after soaked in the SBF. The FTIR was done to support that dissolution precipitation reaction was happened on the samples during the immersion in the SBF and caused the carbonate apatite layer formed. The FTIR spectrum was collected as showed in Figure 2.

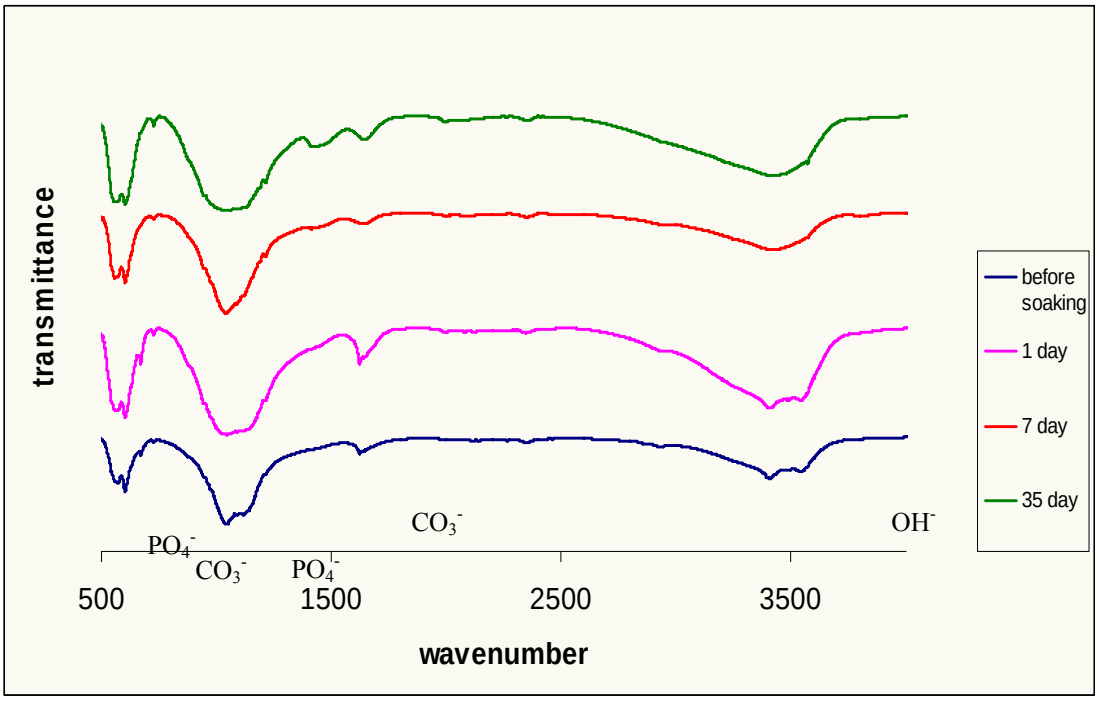

Figure 2: FTIR spectrum of CPC added 4wt% RS before and after soaking in SBF

The FTIR spectrum shows that phosphate (PO_4^-) functional groups exhibit at 602.91 cm^{-1} and 1088.78 cm^{-1}, while the carbonate (CO_3^-) groups at 1044.89 cm^{-1} and 1640.60 cm^{-1}. The hydroxyl (OH^-) group is also observed at 3541.07 cm^{-1}. As the sample soaked in the SBF for 35 days, PO_4^- ions were reduced to form an apatite layer. These ions are needed in forming the apatite layer and only being supplied by the SBF. Mean while,

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the OH^- band has broadened and decreased in the transmittance intensity due to the formation of calcium deficient HA (CDHA).

X-Ray Diffraction (XRD) Analysis

The samples were characterized by XRD to analyze crystalline composition that exhibit in the samples, both before and after 35 days of soaking. The patterns observed from the analysis showen in Figure 3.

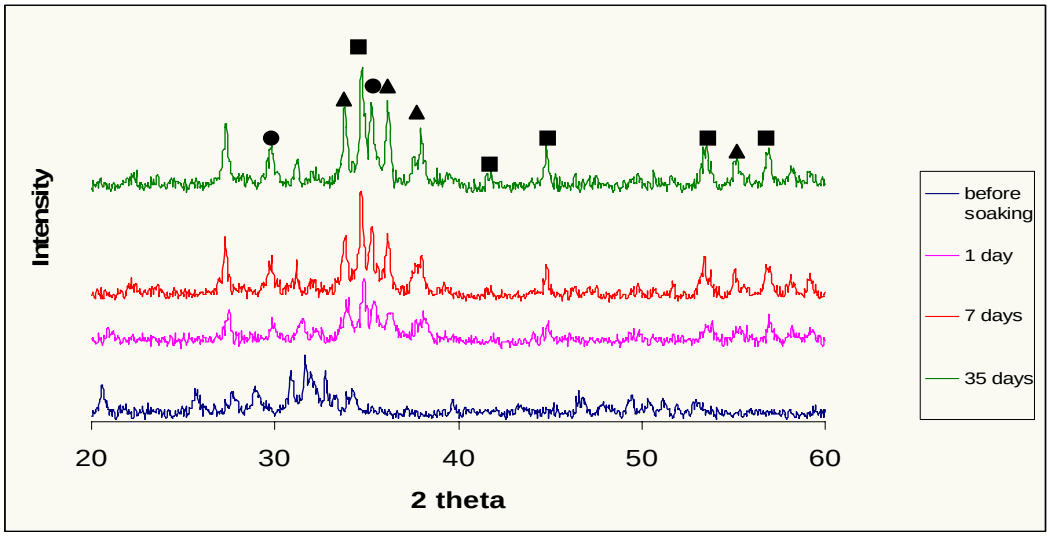

Figure 3: XRD patterns of CPC before and after soaking in the SBF; (■) HA, (▲) β-TCP, (●) CSD

From Figure 3, it can be seen that the samples contained a mixture of HA, β-TCP, and CSD. As the starch from rice is not in the XRD standard data base, the peak of its existence cannot be shown in the pattern. From the patterns, the HA peaks were increased within the soaking time. This situation occurred due to the development of CDHA. At the same time, the β-TCP also shows high peaks even after 35 days of soaking although it should be lower due to its high biodegradability property [6]. This means that β-TCP in the sample was degraded and dissolute but with a slower rate. The degradation was occurred on β-TCP although it took a long time than the usual condition.

CONCLUSION

Bioactivity of nano calcium phosphate cements were analyzed by soaking in simulated body fluid (SBF) solution. The used of starch is to improve the properties of the cement samples which proved that the calcium phosphate cements were successfully fabricated by using the mixture formulation. In order to form apatite layer, it needs the PO_4^- group which was supplied from the SBF solution. FTIR spectra have shown the reductionof the PO_4^- group whereas intensity of the XRD increased after long period of soaking

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meant that the apatite has been formed. However higher intensity of β-TCP peaks show that the degradation of the cement samples are low.

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