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Crystallization, characterization, & application of hydroxyl apatite on hydroxyl ethyl cellulose in the present of stimulated body fluid solution (SBF)

Hassanal Haris, Fathima Shahitha Jahir Husin, Kumaran Kadirgama, Mashitah Mohd Yusoff

Faculty of Science, Industry and Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.

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ABSTRACT

Background: From the experiment we discovered that the hydroxyapatite can be synthesis by the crystallization process on hydroxyethylene sponges. The sponges of hydroxyethylene was fabricated successfully. The liquid-solid hydroxyethylene was placed in the freeze dryer for the sponges formation. The sponges let the samples to have larger surface area compared when it was in liquid-solid form. The larger surface area result the larger area for the formation of crystal. At the end of the study we discovered that the crystal was successfully growth on the sponges.

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INTRODUCTION

Hydroxyethyl cellulose is a water soluble cellulose ether, a non-ionic carbohydrate polymer and a biodegradable polymer with a typical polysaccharide structure. Hydroxyapatite is a chemical compound with a formula of $Ca_{10}(OH)_2(PO4)_6$. It has a non-toxic, biocompatibility, good osteoinductive and osteoconductive capabilities. Furthermore, without result any infection to human body, hydroxyapatite can enhance bone growth. Normally the stimulated body fluid (SBF) have been used in together to produce a very beautiful apatite on the surface of biomaterial. Tissue engineering is a very paramount field that consists of sundry type of biological study. The study of biological structure amends the construal of the structure for the utilization of human being in instauration and amends the tissue function. As the tissue engineering have been concerned, the scaffold become one of the main angle to be discovered more and the vacuum area of research that need to be consummate. This is because the injunctive authorizations of scaffold that act as artificial extracellular matrix (ECM) have been tremendously incremented. Some of them are fabricated by using the electrospinning with a present of electrical field (Reneker &Chun, 1996; Taylor, Fang, & Reneker, 2006; Yarin, Koombhongse, & Reneker, 2001). But the fabrication of scaffold is not limited by using electrospinning, there are a lot of methods have been introduce such template methods, sponges, and drawing methods (Kumbar, Nukavarapu, James, Nair, &Laurencin, 2008). The extracellular matrix will fortify the affixment of cell and promote the systematic incipient tissue formation. So that’s why the best scaffold is the one that has virtually 100% kindred properties with the natural extracellular matrix. The homogeneous properties eschew any possibility of functional failure because the scaffold will circumvent by sensitive biological tissues. The communication and biological interaction between the scaffold and natural tissue environment will affect the rate of incipient cell magnification.

The interaction customarily comes in the form of biological signal where the signal is very consequential for cell proliferation, migration, regeneration and adhesion. Extracellular matrix must be good in mechanical properties and have an ability to stand in high pressure accordingly to the mundane biological circumventing area. There are many methods have been used to fabricate scaffold such as electrospinning (Huang, Zhang, Kotaki, & Ramakrishna, 2003) and sponges but in this research we will use sponges. We will used the HEC as a main ingredient for the scaffold because most best scaffold fabricated nowdays have been made from natural and synthetic polymers (Beumer, van Blitterswijk, Bakker,& Ponec, 1993; Dai, Williamson, Khammo, Adams, & Coombe). This research will increase the use of scaffold in healing

Corresponding Author: Yayasan Dakwah Islamiah Malaysia (YADIM), Kompleks Pusat Islam, Jalan Perdana, 50480, Kuala Lumpur, Malaysia.
Tel.:+60-01125492490; E-mail: hassanalharis@hotmail.com

human. Scaffold have been use recently and overcome many of limition in healing human being (Groeber, Holeiter, Hampel, Hinderer,& Schenke-Layland, 2011; Macneil, 2008). Nowadays, people get involved in the field of nano-biomaterials. They utilized the nano-biomaterials for many purport and one of the purport is to become a vigorous scaffold for tissue. They are many methods have been go through so that the nano-biomaterials may be engendered. Among the methods are by utilizing phase disseverment, and electrospinning. (Kumbar, Nukavarapu, James, Nair, &Laurencin, 2008). However, the most famous techniques among all the techniques are the electrospinning. It is because the electrospinning can synthesize variety polymer fiber in nano size (Huang, Zhang, Kotaki, & Ramakrishna, 2003). There are several reasons why many researchers relish fixating on electrospinning. Most of them like electrospining because it only utilized the electrospinner to synthesize the nanofiber. The only constraint for this technique is the technique takes an extravagant quantity of time for the observation so that the electrospinner can fabricate a high quality nanofiber. On the other hand the electrospinning technique requires high electric field. All these inhibition let us to go further in nano-biomaterials and we discovered that there is a high potential for other method like crystallization method on a polymer sponges to engender nanostructure biomaterial in lieu of utilizing electrospinning.

The main advantage of sponge technique is, it need only scintilla of electric consumption to synthesize of nano-biomaterials and no tube block quandary (Reneker &Chun, 1996; Taylor, Fang, & Reneker, 2006; Yarin, Koombhongse, & Reneker, 2001). Hydroxylethyl cellulose is cellulose that consists of carbon atoms, hydrogen atoms, oxygen and atoms. It withal kenned with the abbreviation of HEC. There are many intriguing characteristics of HEC in which it is a non-ionic chemical. The non-ionic material will not react to any cations and anions so that it will not additionally have tencency to compose another salt. However it is very soluble in dihydrogen monoxide or in other words it is dihydrogen monoxide-soluble. Albeit it has constraint but this constraint of dihydrogen monoxide-soluble could be overcome by crosslinking method. This method can transmute the properties of HEC to another HEC that insoluble in dihydrogen monoxide. Moreover, HEC has a good surface activity and the crystallization, esterification, etherification and acetal reaction can transpire facilely on the surface of HEC.

The physical appearance of HEC is very unique as it is always in white to yellowish in colour, insipid, and ordourless. The coalescence of HEC's powder with dihydrogen monoxide will compose a very slurry liquid and viscous solution. One researcher by the designation of Tadic had discovered this method of fabrication. He discovered by commixing the dihydrogen monoxide soluble polymers with calcium phosphate and salt crystals may engender porous hydroxyl apatite. The present of dihydrogen monoxide soluble polymer and crystals act as an agent to develops the diminutive pores. The progens are very soluble in dihydrogen monoxide so that it will not remain and can vanish after an abundance of times. In addition to that, the hydroxyl apatite pores have good interconnectivity and the pores diameter varies from 250 micrometer to 450 micrometer. Pore-creating volatile particles is a technique of fabrication of scaffold with small tinny pores.

This methods required volatile particle like carbon, naphthalene, paraffin, starch, flour, hydrogen peroxide, and some synthetic polymers such as polyvinyl butyral. All of them will be then integrate up to the hydroxyl apatite slurries or hydroxyl apatite powder. The pores will appear as the volatile particle evaporates. So we can control the morphology, size and distribution of pores by adjusting the amount of volatile particle/ pre making agents. The size of pores customarily lay between 0.2 micrometers until 50000 micrometers. Ceramic foaming technique is also among the techniques that have been used to fabricate scaffold with pores. Foam technique additionally kenned as foam-gel method. Foam technique is a technique that involve foam agent such as hydrogen peroxide, baking powder, and carbonate salt. The foaming agent is co-mixed with the hydroxyl apatite slurry. Then the stirrer was applying to stir the coalescence. The process engenders foam before it undergoes polymerization and sintering process. The pores of hydroxyl apatite from this method are varies from 40 micrometers to 660 micrometers. This technique needs cross-linking of polymer. The most immensely colossal failure of this technique is it result a non-uniform pores distribution, and has poor interconnection between pores. Sponges also could produce good scaffold. Polymeric sponges are a technique used to fabricate hydroxyl apatite porous structure. This method consists of fabrication of polymer sponge and then the hydroxyl apatite will replicate the polymer sponge in which the sponge structure is plenary of minute pores. The best characteristic is the shape and the size of the pore can be control facilely compare to other methods. In integration to that, this method engender good quality pores of hydroxyl apatite as it meet good osteoconduction limit bench mark. The properties of the porous hydroxyl apatite can facilely control by transmuting the ceramic powder concentration. By transmuting the concentration of the powder the characteristic of crystallization additionally can be controlled. Coversion of marine coral skeleton and natural bone also coul produce scaffold. This is the most fascinating technique compare to others. It is a technique that use marine coral skeleton. Unbelievable when researcher discovered that the coral can be utilize as a pore making agent. The process commences when the marine coral undergo hydrothermal exchange. The hydrothermal exchange will let the marine coral to emit the hydroxyl apatite. But the hydroxyl apatite will only engender if the phosphate ions there. The phosphate ions customarily come when the di-ammonium hydrogen phosphate presents. The porosity of the hydroxyl apatite engendered are vary from 55%-65%. The pores structures are varies from 250 micrometers until 550 micrometers. Albeit this methods is the most intriguing methods compare to other methods, it additionally has its own constraint. The circumscription is the amount of marine coral. This is the main obstruction to utilize the method to engender porous hyroxy apatite at the same time we require to preserve the coralline species that have been decremented tremendously nowadays. If people keep harvesting coralline species it will damage the fish habitats and cause imbalance interaction between macrocosm species. Electrospining also could produce scaffold. By utilizing electrospinning we can synthesize a very thins nano fiber by utilizing solution of polymer, ceramic, and composite. In integration to that, the morphology of fiber engendered, diameter of fiber, and composition can be controlled. However it is very arduous to control all of these properties because of many circumscription of electrospining technique. One of the main quandary in utilizing electrospinning is the solution may stuck in the needle sometimes because some polymer will transmute to solid in the ambient temperature. This situation eschew the solution to emit from the needle and avert the formation of fiber on the target plate accordingly to the electrical energy that have been supply to the electrospinner.

Criteria of hydroxylethyl cellulose for teeth tissues scaffold

The deep research of bone supersession is referring to the development of macroporous and microporous of mineral phase of authentic living bone and teeth. The research made recently discovered that the macro and microporous bioactive ceramics comport with high performance of osteogenesis and it has astronomically immense surface area. So it eschews any impediment in connection and stays vigorous for long term. There are some paramount criteria and characteristics of good porous hydroxyl apatite for the supersession. The pore-size distribution, pore orientation, pore morphology and the degree of porosity are the criteria need to take into consideration. All the criteria relate to expedition of bone magnification so that it is very paramount to take it into researcher consideration. Bone perforation is predicated on the percent of connection with pore, pore size and distribution, porosity and morphology. The connectivity between pore withal very paramount because it let the body to apportion and transfer the alimentation, body fluid, and ion diffusion, vascularization and osteoblast cell perforation.

Hydroxylapatite is form by crystallization. In order to develop incipient inorganic-organic film, HAp particles that represent the inorganic phase was commixed well with hydroxyethyl cellulose acetate (HECA), which representing the organic phase and then the inorganic-organic films were fabricated by evaporating the solvent. The simulated body fluid (SBF) was utilized as source of HAp in lieu of utilizing human blood. The fibers were covered with HAp crystals after being marinated in the SBF solution for 7 days.

Before undergo crystallization, the sample need to undergo crosslinking process first. Every gas, liquid and solid has its own solubility. Solubility is the quantification of rate of solute that dissolves in certain quantity of solvent. The physical and chemical properties will affect the value of solubility. PH, pressure and temperature additionally factors that affect solubility of substances. Mundanely the solvent come in the form of liquid. Weather the liquid is an amalgamation or pristine substance. There are substance that plenary soluble in solution, partially soluble and insoluble. For example ethanol is illimitable soluble in dihydrogen monoxide. The word insoluble refers to the deportment of the substance that has very low ability to dissolve in the solvent. People may misunderstand the different between solubility and the ability of solute to dissolve. Both are not same, because the formation of solution can be weather in the present of chemical reaction or without the present of chemical reaction. The formation of solution with chemical reaction can be visually perceived when a zinc chloride is com mixed up with hydrochloric acid. The zinc chloride will dissolved in the hydrochloric acid solvent in the present of chemical reaction between hydrogen gas and zinc chloride. The rate of will become more expeditious if the solute is in more minuscule form. These transpire because if the more minute the solute the more immensely colossal surface area expose to the solvent. When we optically discern the comportment of hydroxyl ethyl cellulose in dihydrogen monoxide, we can agnize that the solubility show that hydroxyl ethyl cellulose is very soluble in dihydrogen monoxide. If we put hydroxyl ethyl cellulose in dihydrogen monoxide directly, it will plenarily dissolves in a few second due it properties that soluble in dihydrogen monoxide. This limits the formation of crystal because the crystallization needs the hydroxyl ethyl cellulose porous structure so that the quality of crystal form is high. So in order to transform the hydroxyl ethyl cellulose to insoluble material in dihydrogen monoxide its must undergo crosslinking. Different polymers can connect together and the connection between the polymers kenned as cross-link. The connection could be either in ionic bond or covalent bond. Researcher have been utilize this term in their researches. As we ken it is very paramount to manipulate the physical properties and characteristics of polymer in doing research. So the technique of connecting between polymers could sometimes transmute remotely the physical properties without transmuting to much the chemical properties. Cross-linking will let the polymers involved change into harder polymer. The boiling point will additionally increase as well. If the harness become higher that mean the polymer's flexibility will become lower as compared with the polymer that do not undergo cross-link. Mundanely pressure, incensement in temperature, variation of pH and radiation will cause minuscule chemical reaction and enhance the cross-link between the polymer. The chemical used to initiate the cross-link called as Cross-linking reagent. We can visually perceive facilely the cross-linking transpired on thermoplastic material. The cross-linking customarily transpired when the thermoplastic material expose to the radiation of electron beam/UV light/ or gamma-radiation. So in short we can verbally express that cross-linking is a method use to solve solubility quandary. In this research we ken that the hydroxyl ethyl cellulose is very soluble in dihydrogen monoxide. So if we let it immerse in dihydrogen monoxide without undergo cross-linking first it will dissolve facilely in dihydrogen monoxide and the crystallization of hydroxyl apatite could not transpired. If the number of cross-link is increase the hardness of material will be additionally increase. Figure below illustrate the cognation between concentration of cross-link and the hardness of polymers composed.

1.1 Scope of Study:

To introduce a new novel in organic biomaterial for tissue engineering. (The focal point of the study is the crystallization of hydroxyapatite on the biocompatible polymer of hydroxyethyl cellulose sponges)

1.2 Objectives:

To study the processing steps in order to produce a novel hybrid material of inorganic-organic material using a biocompatible polymer. To study the crystallization of hydroxyapatite on difference concerntration of HEC sponges and in difference concentration of HEC sponges and in different concentration os SBF solution. To study the morphologies and characteristics of both sponges and crystallization products being produced in the research.

Methodology:

The 2-hydroxyethyl cellulose, 2-HEC with molecular weight 250,000 is used in this study with the brand name Sigma was obtained from US. This HEC is in powder form. The phosphoric acid, H_3PO_4 was obtained from R&M Chemical located in UK. The acetone was from Syarikat Perniagaan Kimia, Malaysia and glutaraldehyde chemical was obtained from Merck Schuchaidt OHG, Germany. As for the SBF solution making, the chemicals that being used are, calcium chloride dehydrate, $CaCl_2.2H_2O$ potassium chloride, KCl, sodium chloride, NaCl and magnesium chloride hexahydrate, $MgCl_2.6H_2O$. Next, sodium dihydrogen phosphate with molecular formula, NaH_2PO_4 was used instead of $NaH_2PO_4.H_2O$ since that is the only chemical that can be obtained from the laboratory. Last, sodium dihydrogen phosphate, $NaHCO_3$ was used. All the chemicals for the SBF solution preparation are obtained from the FIST Laboratory. The deionizer ultra-pure water is an additional material that being used throughout the experiment for preparing all the solutions regarding the experiment. All the glassware is cleaned by using nitric acid and ultrapure water. Figure below show the HEC solution prepared.

2.1 Preparation of HEC sponges:

HEC solution of 5wt% was prepared by dissolving 5 g of HEC powder in 100mL deionized water in a beaker. The solution is left stirred until it is completely dissolved and is allow for continuous stirring for overnight in order to obtain a homogenous solution. The preparation of the HEC sponge is then continued with the freeze-dry process. The solution (5 wt %) is allowed to be keep in the freezer in order to let the sample to freeze and form solid sample. The solution is left there for about 4 days in order to ensure the solution completely be in solid form. After 4 days, the solid HEC is taken out and is kept in drier for 3 days to completely dried it and become a sponge.

2.2 Cross-linking studies:

Crosslinking could increase the mechanical, thermal and chemical properties (Mansur, Oréfice, & Mansur, 2004).The HEC sponges then were peeled and cut into 4 cm pieces and kept in Petri dishes with diameter of 90 mm. The cross-linking study of HEC sponges was carried out with glutaraldehyde as a cross-linker to fabricate water insoluble sponges. Acteone, glutaraldehyde followed by some phosphoric acid with specific amount was put in the petridish contained of sponges for about 48 hours. The detail are mentioned as follow: Glutaraldehyde: 600uL; Acetone: 4mL; Phosphoric acid: 5-8 drops

The sponges should be fully immersed in the solution. The water resistance of the sponges was evaluated by immersing it in distilled water.

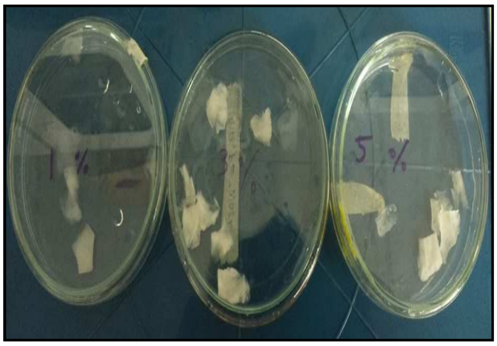

Fig. 2.1: Sample undergo crosslinking

2.3 Crystallization in SBF and Characterization:

The hydroxyethyl cellulose sponges then were immersed in the prepared stimulated body fluid. The hydroxyethyl cellulose were immersed 7 days in the stimulated body fluid. Then the hydroxyethyl cellulose sponges were taken out and washed for characterization. Several sets of samples were taken for characterizations as shown below. At the end of the characterization all the experiment procedures were repeated by using different weight concentration of HEC (3wt% and 1 wt%) . The morphology of the hydroxyapatite crystal obtained on the hydroxyethyl cellulose was observed by a Field Emission Scanning Electron Microscopy (FESEM) (JEOL JSM 7800F) at expediting voltage of 10kV. The elemental compositions were analyzed utilizing Energy Dispersive X-Ray, EDX.

RESULT AND DISCUSSION

The bio mineralization process that was done on HEC based polymer lead to the formation of unique, ordered and refined shape inorganic-crystal. The formation of hydroxyapatite crystals were dine by the immersion of different weight percentages of HEC polymer on different concentration of SBF solution for 1 week and 2 weeks.

The observation of crystals structure were done by using an optical microscope and to further confirmed the crystal formation, a characterization under FESEM-EDS was carried out. Figure 3.1 (a), (b) shown the crystals formed on 5%wt HEC sponges in 10 x SBF solution for 2 weeks. Most crystal that was formed in 2 weeks immersion having shape like an arrowhead twins and fernlike stellar dendrites and few cubic crystals shape. The amount of cubic crystals shape increased with increasing in period of crytallization. The crystals with fernlike shape starting to grow bigger forming the arrowhead twins crystals and clearly seen like the one formed in the 5 wt% of HEC sponge as shown in figure 3.1 (b). (Mei *et al*.,2012)

The crystals formation starting to grow further forming the rhombohedral shape of crystals which formed as cubic crystals stacked together and surrounded by fernlike crystals after 2 weeks as presented in figure 3.1 (a), (b)

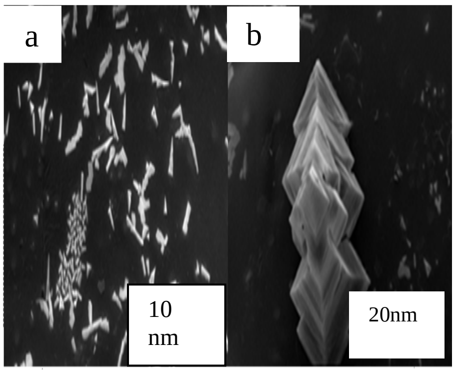

Fig. 3.1: (a) Clusters formed on 5wt% HEC sponge for 2 weeks immersion in 10xSBF (10 nm), (b) Crystals formed on 5wt% HEC sponge for 2 weeks immersion in 10xSBF (20 nm),

Conclusion:

Crystallization is the (natural or artificial) process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas. Crystallization is also a chemical solid–liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs. In chemical engineering crystallization occurs in a crystallizer. Crystallization is therefore an aspect of precipitation, obtained through a variation of the solubility conditions of the solute in the solvent, as compared to precipitation due to chemical reaction.

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