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Biomaterials of hydroxyapatite reinforced with SiO_2 shell

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ABSTRACT

In this study, orthopedic biomaterials were synthesized by the hydrolysis of Calcium Hydrogen Phosphate ($CaHPO_4•2H_2O$) in alkaline solution with close monitoring of temperature and time. These biomaterials synthesized were called hydroxyapatite (HA), also known as artificial bones. Porous HA was produced by varying ethanol ratio or adding Cetrimonium Bromide (CTAB) into the solution during the hydrolysis of Calcium Hydrogen Phosphate. A coat of inorganic silicate was then applied to the porous HA through sol-gel reaction of Tetraethylorthosilicate (TEOS). By adjusting the pH value and the amount of TEOS in the silica shell, the shell was able to cover more surface of the porous HA. The mechanical strength and the biocompatiblity of HA were also improved through the change of pH value and amount of TEOS in the silica shell. Finally, we compared the growth of the T75 cultured osteoblast in the original (uncoated SiO2) HA and in the modified HA with surface coating of SiO_2 .

Keywords：sol-gel reaction、hydroxyapatite、Calcium Hydrogen Phosphate、artificial bones。

1. INTRODUCTION

The ultimate goal of bone tissue engineering is to aid the study of anatomy and physiology in understanding more about how to produce a more compatible and accurate bone healing response (Hsieh, 2001). Clinically, the artificial bone must be able to integrate with the original bone in the surrounding in addition to provide sufficient mechanical support (Chang, 2002). There are several important factors in tissue engineering and they are the following (Yeh, 2009). Firstly, there must be cells that can produce bone in the process of bone tissue engineering. Second of all, there must be scaffolding materials that can carry osteoblasts and be able to assist osteoblasts with adhesion, migration, differentiation and reproduction on the bone after filling in the defected parts. This property that these material possesses is called "bone conduction".

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Thirdly, besides the support from the aforementioned bone conducting materials, there must be some bioactive factors, such as growth factors, to further aid the bone cells in reproduction, differentiation, and maintenance of the bone phenotypes. The property that these factors possesses is called “bone induction”. Lastly, there must be a bioreactor that provides an environment suitable for the tissues to grow quickly by giving it necessary physical stimulation.

Hydroxyapatite (HA), a calcium phosphate bone similar to our biological bones, is used to construct artificial bone tissues. It is often used to repair bone defects since it is biocompatible, resulting in very good compatibility with human bones. It also has a special "osteo-inductive" nature, which can lead to bone cell regeneration and repair.

The purpose of creating composite nano-particles that help with repairing defective bone tissues can be divided into four aspects. First, the nanoparticles are capable of generating characteristics that are different from the original’s. Secondly, by adjusting the surface properties of nanoparticles, its surface charge density, functionality, reactivity, biocompatibility, stability or dispersion can be changed. Thirdly, using the core like nanoparticles for templates, hollow nanoparticles can be produced. Lastly, creating versatile nanoparticles is of much importance.

Based on the four points mentioned above, we found that silicon dioxide is the most promising material to coat the main body of the artificial bone. SiO_2 can also tolerate against strong acid and base, block electricity, and stabilize active chemicals. Its formidable rigidity can withstand abrasion and washing. In addition, it can act as a very good adsorption material, which helps to improve biocompatibility (de Groot, 1983).

This experiment reacts hydroxyapatite with SiO_2 to synthesize materials (Layrolle, 1998; Jillavenkatesa, 1998) that can be helpful for the engineering of the artificial bone. This experiment also further explores the material’s biocompatibility and hydrophilic properties. By combining these inorganic materials with biological molecules, the surface rigidity and its ability to tolerate abrasion can be enhanced. We hope by adding the materials, the synthesized bones can become more durable.

〔1〕 The chemical principles of sol-gel

A typical sol-gel production contains a series of chemical reactions. It can turn organic metals into ceramics at a lower temperature (25 ℃ ~ 60 ℃). The organic metals include tetraethoxysilane (TEOS, $C_8H_{20}O_4Si$) or tetramethoxysilane (TMOS, $C_4H_{12}O_4Si$); they are easily catalyzed by acid or base in a sol-gel reaction. Either acidic or basic catalysts has a great influence on the final structure and kinetics of the product. The product has fast hydrolysis and slow condensation under acidic conditions. The single chain silicate will form a long bond, resulting in a low degree of crosslinking network. It has a greater density and a smaller pore size and surface area. It has a slow hydrolysis but fast condensation under basic conditions. The monomers will grow into branched chains, forming a less uniform but better crosslinked colloid particles. It has bigger pores but smaller density. Its functionality can be improved by adjusting its pH. Specifically, by performing sol-gel reaction under basic conditions while heating, organic polymers can be removed, resulting in a porous sieve. Under acidic conditions, SiO_2 can have enhanced flexibility.

〔 2 〕 Production of organic and inorganic hybrid using sol-gel

The inorganic components in organic and inorganic hybrid polymer include Si, A1, Ti, Zr , etc. Powdered silicone is not small enough to the required molecular size, but through the sol-gel reaction, the molecules can be dispersed into smaller size (Lee, 2005). By hydrolyzing silicate and performing condensation on the resulting hydrolysis product, silanol, three dimensional repeating structures of -Si-O- can be generated. Advantages of the sol-gel reaction include that it can synthesize glass and exquisite ceramics at a low temperature (Chen, 2006), that it can keep the products' consistency and good dispersion, that it is easy to control the composition ratio, and that it can synthesize polycrystalline ceramics from homogeneous particles (Cheng, 2001; Lin, 2002).

Synthesis can happen under room temperature. When organic materials or biological molecules are added, the organic structure will not be destroyed. Instead, it will gain better mechanical properties and chemical stability. As a result, this method is widely used.

2. Methods

There are four parts to the experiment. The first part consists of hydrolysis of dicalcium phosphate dihydrate (DCPD) in 2.5M NaOH solution while maintaining the temperature at 75 ° C for an hour. Then, after cooling it at 0 ° C cold water, HA powder is filtered out using filter paper until NaOH is not visible on HA's surface anymore. The second part consists of using the sol-gel reaction to coat a thin layer of inorganic SiO_2 shell on the HA powder. The third part consists of burning HA powder at different temperatures and making specimens tested under different parameters of the synthesis of the HA (Shih, 2007). The fourth part consists of synthesizing the ideal HA powder and using both the synthesized and unprocessed HA powder to culture osteoblast and analyze its growth.

3. Results and discussion

HA morphology at different pH values. In the sol-gel reaction, by adjusting the pH value, HA's surface roughness patterns can be changed. Fig. 1 to Fig. 6 are SEM images of the pore size change under pH values from 5 to 10.

Surface morphology of HA after adding CTAB. Cetyl trimethyl ammonium bromide (CTAB) induces a porous HA surface structure, which is shown in Fig. 7.

Comparison of HA surface morphology between with and without sol-gel reaction. The experimental results have shown that without sol-gel reaction, HA surface appears to be smoother (Fig. 8), but with sol-gel reaction, HA surface appears to have a more porous structure (Fig. 9).

HA's surface morphology when changing the added amount of TEOS. By fixing the amount of HA and changing the amount of added TEOS, HA appears to have different surface roughness patterns. When the amount of added TEOS is much greater than the amount of HA, HA particles with good dispersion can be produced.

HA's surface morphology under different proportion of HA. The experimental results have shown that HA powder synthesized with 0.86g DCPD / 5ml TEOS hydrolysis and with 2.00g HDCPD / 9ml TEOS hydrolysis both appear to shape like a ball. However, the average diameter of the particles under the different proportions of ingredients differ in 3 times the length, as shown in Table 1.

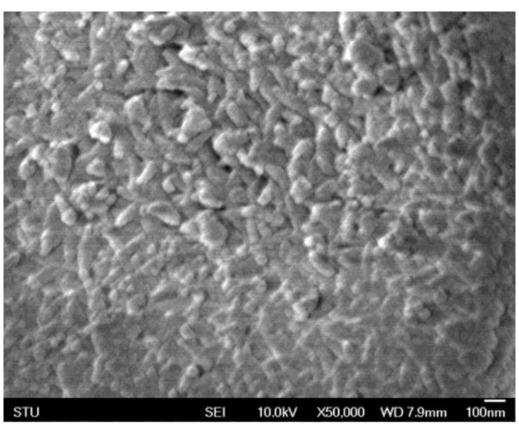

Fig. 1 SEM image of HA at pH 5

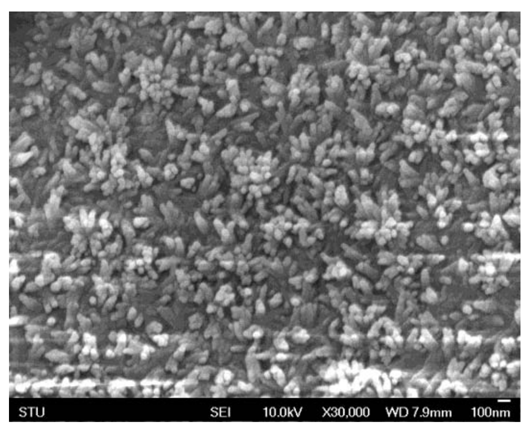

Fig. 2 SEM image of HA at pH 6

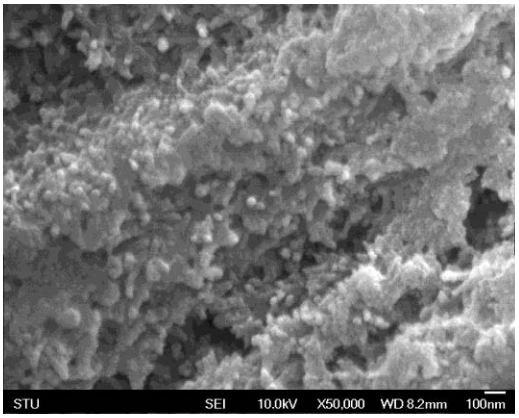

Fig. 3 SEM image of HA at pH 7

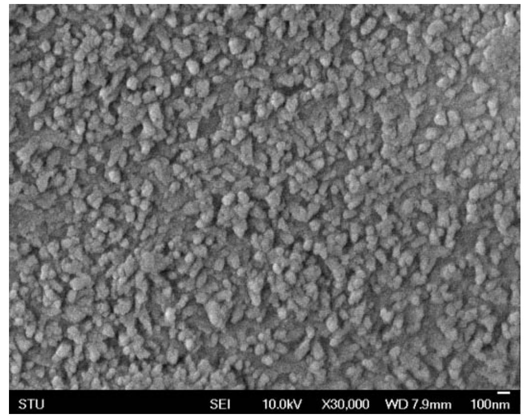

Fig. 4 SEM image of HA at pH 8

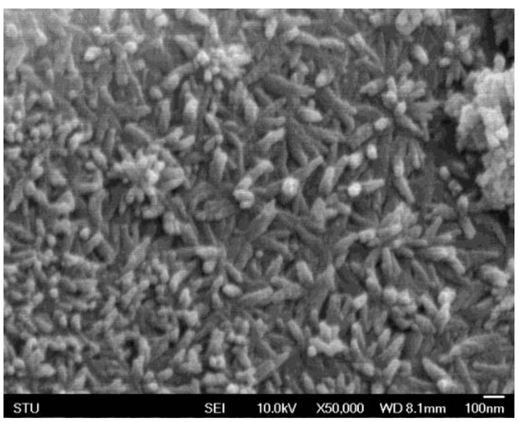

Fig. 5 SEM image of HA at pH 9

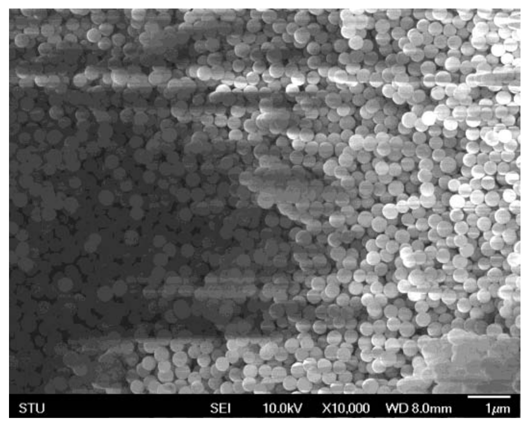

Fig. 6 SEM image of HA at pH 10

Table 1、 Diameter of HA ball shaped particles with different DCPD/TEOS proportions

DCPD(g) / TEOS(ml)	Average particle size (nm)
0.86g / 5ml	300nm
2.00g / 9ml	100nm

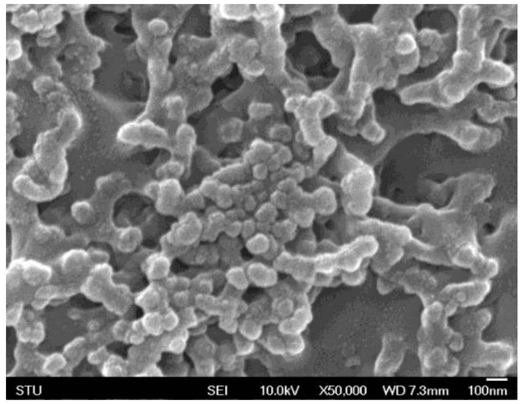

Fig. 7 SEM image of HA after adding CTAB

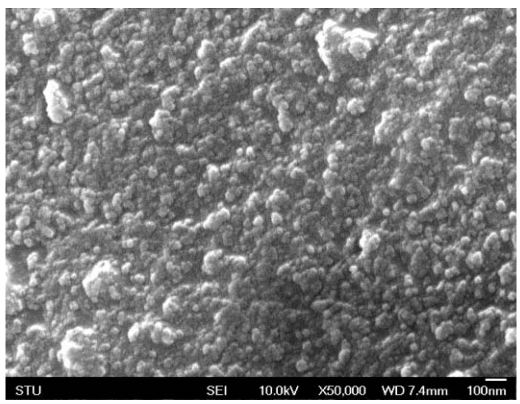

Fig. 8 SEM image of HA surface morphology without sol-gel reaction

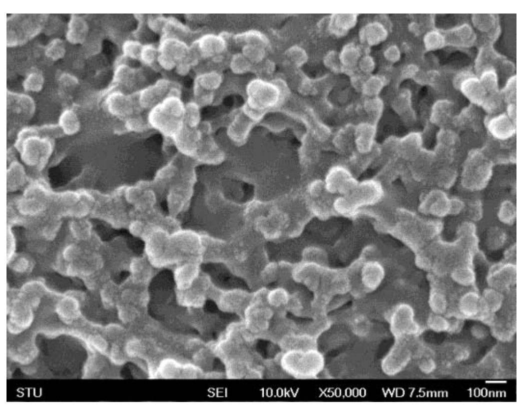

Fig. 9 SEM image of HA rough surface morphology with sol-gel reaction

3. CONCLUSIONS

In the course of the experiment , we can produce a variety of HA powder by adjusting the parameters. After several trials, the experiment can be discussed through two perspectives. First of all, HA powder does not appear to have completely irregular shapes, instead, we can find the HA surface morphology becomes ball shaped with increasing pH value. Secondly, HA powder also becomes ball shaped by increasing amount of TEOS. However, its diameter is smaller than the HA powder produced by adjusting the pH values. SEM confirms that the average difference between the diameter is about 2.5 to 3 times.

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