行政院國家科學委員會專題研究計畫　成果報告

溶膠凝膠法製備無機有機混合鈣磷矽鋅/幾丁聚醣仿生複合材

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計畫主持人：丁信智

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Organic-inorganic hybrid Ca-P-Si-Zn/chitosan biomimetic composites prepared by sol-gel method

計畫編號：NSC 94-2320-B-040-013

執行期限：94 年 8 月 1 日至 95 年 7 月 31 日

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一、中文摘要

具骨引導性質的氫氧基磷灰石應用於骨移植取代與修復。鋅元素具有刺激骨母細胞分化和增生，及促進骨質成長的功能。天然高分子幾丁聚醣有良好的生物性質如生物可降解性，可作為生醫材料如藥物制放微粒、牙周膜修復、細胞支架等。本研究以溶膠凝膠法合成磷灰石，並在溶膠溶液中調整pH值，後續並添加氧化鋅加以燒結處理，以冷凍乾燥技術滲覆幾丁聚醣於含氧化鋅之磷灰石以製作出無機-有機複合塊材，進一步分析基本性質及浸泡行為，以期作為骨缺損修補材。結果發現X光繞射顯示出以溶膠凝膠法製作的原料需要經過 500°C的熱處理才能得到磷灰石晶相結構，但溶膠酸鹼值調至 9 時則可以 300°C低溫合成磷灰石。低pH值可得較高結晶性。未調整pH的磷灰石塊材的徑向拉伸強度為 3.8 MPa，明顯低於pH 9 的塊材（5.3 MPa）。氧化鋅對於徑向拉伸強度沒有顯著影響，但是X光繞射分析發現氧化鋅使得磷灰石的(002)結晶面強度變寬。滲覆幾丁聚醣並不影響複合材料的強度。在體外浸泡實驗發現，塊材拉伸強度急速降低，在 3 天後損失達 40%，之後維持穩定值。

關鍵詞：溶膠凝膠法、鈣磷酸鹽、幾丁聚醣、氧化鋅、仿生複合材

Abstract

Hydroxyapatite (HA) ceramics are currently used as bone graft substitutes because of its osteoconductivity. Zinc promotes proliferation and differentiation of osteoblast cells and acts as a stimulator on bone growth. Naturally polymeric chitosan has many good biological properties like biodegradation that make chitosan a suitable compound for biomedical applications such as micropheres, membranes, and scaffolds. We prepared Zn-containing apatite/chitosan composite by sol-gel route and freeze-drying method. In the sol-gel method, the pH value of precursor solution is adjusted. The chitosan solution will introduce the inorganic materials. The properties and in vitro behavior of hybrid materials are evaluated. The XRD patterns show that apatite phases can be obtained at 500°C for control condition, but for pH 9 at lower temperature of 300°C. The lower pH value used for preparation of the sol-gel derived powder, the higher crystallinity was. The tensile strength of monolithic apatite derived from the sol-gel processing at pH 9 (5.3 MPa) is significantly higher than that of samples without pH adjustment (3.8 MPa). There was no significant effect of ZnO addition on strength value, however, enhancing an oriented growth of the HA phase on the (002) plane. Incorporation of chitosan into bioceramic bulks did not affect strength value. When immersion in Hank's solution, the tensile strength decreased rapidly after immersed in Hank's solution for 3 days with a reduction of about 40%, followed by a steady value.

Keywords: Sol-gel, calcium phosphate, chitosan, ZnO, biomimetic composites.

二、緣由與目的

Hydroxyapatite (HA) is the main component of bone and tooth tissue. Because of its osteoconduction, HA has been used in medical and dental fields for bone substitutes [1]. Sol-gel method, a wet chemistry synthesis route at

low temperature and molecular-level mixing, has recently attracted a great of attention [2,3]. Kim and Kumta prepared nanocrystalline powders of HA from $Ca(NO_3)_2{\cdot}4H_2O$ and P_2O_5 using a simple sol-gel approach. The presence of amorphous hydroxyapatite in the as-dried gel precursor can transform single phase (50-150 nm) of HA after heat treatment at 900°C for 12 h in air [3]. As is known, zinc is an essential trace element having stimulatory effects on bone formation *in vitro* and *in vivo*. Ito *et al.* found that ZnTCP/HA ceramic with a zinc content of 1.2 wt% significantly increased osteoblastic cell proliferation of rat stroma cells *in vitro*. In particular, zinc could increase bone formation in rabbit compared to the control without zinc [4]. Chitosan is a very abundant naturally occurring polysaccharide obtained by deacetylation of natural chitin. Chitosan and some of its complexes have been studied for use in a number of biomedical applications. These include wound dressings, drug delivery systems and space filling implants [5].

The brittle and rigid nature of HA severely limits its biomedical applications. Thus, the use of a hybrid composite comprised chitosan and HA, resembling the morphology and properties of natural bone, may be one way to solve the problem of CaP's brittleness without reducing mechanical properties [6]. Natural bone is actually an inorganic/organic composite mainly made up of nano-structured hydroxyapatite and collagen fibers. Of most importance to synthesize biomimetic composites having good biocompatibility, high bioactivity and great bonding properties should be performed. Yin *et al.* studied a biodegradable composite scaffold for bone tissue engineering consisting of chitosan, gelatin and β-tricalcium phosphate, which resulted in an improved compressive strength and especially compressive modulus [7].

In the preliminary project, we synthesize HA via sol-gel route, followed by adding zinc oxide and by sintering the inorganic materials. After that, the incorporation of chitosan into zinc-containing apatite will form an organic/inorganic hybrid composite material. The degradation behavior of the hybrid bodies was evaluated by monitoring changes in tensile strength, when immersed in simulated body fluid, in addition to characterization.

三、實驗方法

3.1. Preparation of hybrid composites

Apatite powder were prepared by the sol-gel method using calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$), triethyl phosphate ($(C_2H_5O)_3PO$, TEP) as raw materials. Briefly, the TEP was hydrolyzed in 2N HNO_3 for 1 h under stirring. $Ca(NO_3)_2{\cdot}4H_2O$ was dissolved in ethanol for 1 h, followed by adding TEP solution for 3 hour, achieving Ca/P ratio of 1.67. The pH value of the mixed solution containing $Ca(NO_3)_2{\cdot}4H_2O$ and TEP was further adjusted up to pH 9. The sample without pH adjustment is referred to as the control. Afterwards, aging and drying were performed before calcination at 300°C. 1wt% ZnO was added to the above-mentioned material and pressed by biaxial pressing at 200 MPa, and sequentially sintered at 900°C, which is the optimized parameter. The 0.5 wt% chitosan solution dissolved in acid solution infiltrated the zinc-containing apatite composite bulk through vacuum suction, followed by freeze drying procedure to prepare the apatite/Zn/Chitosan hybrid composite. Biodegradation behaviors of all samples were obtained by soaking in Hank's solution, which ionic composition is similar to that of human blood plasma, for predetermined periods of time at 37°C.

3.2. Characterization of hybrid composites

For evaluation of pH effect, the dry gel of the samples without ZnO and chitosan was examined by thermal gravimetric analysis at a heating rate of 10°C/min from ambient temperature to 1000°C. The phase composition and microstructure were analysis by X-ray diffraction and scanning electron microscopy. Diameter tensile strength (DTS) of various samples was measured using Shimadzu EZ-Test. One-way ANOVA statistical analysis was used to evaluate the significance ($p < 0.05$).

Their degradation behavior was focused on DTS change.

四、結果與討論

4.1. Characterization of as-prepared bodies

4.1.1 Morphology

Concerning pH effect, in both TGA curves of sol-gel derived apatite without and with pH adjustment (Fig. 1), there is evaporation of volatile liquids such as ethanol and adsorbed water before 200°C [2,8]. The rapid weight loss occurred at the region of 200~300°C relates to the evaporation of crystalline water in $Ca(NO_3)_2 \cdot 4H_2O$ in the precursor [3]. Furthermore, the sample weight appreciably decreased at the temperature between 300°C and 550°C. We suspect that this is caused by the removal of NO_3 groups, in agreement with the results of other studies [2,3,8]. There is no significant weight loss however, observed above 550°C, indicating that the precursor generates a stable phase after heat-treatment at temperatures of more than 550°C. The weight loss of the sample with pH 9 condition reached 70%, but the control was 60%. The higher pH value used in the preparation of apatite, the denser apatite crystals was. It leads to the apatite bulk made from pH 9 to be higher tensile strength that from the other condition, as described later.

To further clarify pH effect on phase composition of sol-gel derived material, the samples were heat-treated at different temperatures. It is found that apatite phase can be obtained at 500°C for control sample, but for pH 9-sample at lower temperature of 300°C. It can therefore be inferred that the pH in the precursor solution may be an effective factor for achieving HA phase. It can be also seen that the appearance of extra CaO phase occurred at temperature more than 500°C, possibly due to the decomposition of calcium nitrate precursor that remained either partially reacted or unreacted in the gel to calcium oxide [2,3]. It is reasonable that the crystallinity of apatite was increased with increasing temperature for the two samples. In Fig. 2 XRD patterns of ZnO-containing samples indicates that the addition of ZnO promote the (002) preferred orientation peak compared to the other apatite peaks. As for chitosan incorporation into the ceramic samples, there is no evident change in X-ay diffraction patterns due to the chitosan is amorphous [6].

Fig. 3 shows that SEM pictures of 900°C-sintered apatite alone bulks. It seems that pH value did not affect the microstructure of apatite, which consisted of small particles. It is without doubt that the sample infiltrated with 0.5 wt% chitosan shows a smooth morphology than that without chitosan.

4.1.2 Mechanical properties

Concerning diametral tensile strength, the value of 5.3 MPa for monolithic apatite derived from the sol-gel processing at pH = 9 was higher ($p < 0.05$) than corresponding control group of 3.8 MPa. This can be explained by the reason that higher pH value makes the structure of apatite phase denser, as mentioned above. However, there was no significant difference regarding effect of ZnO addition on DTS value ($p > 0.05$). Similarly, in the present study the small amount of chitosan incorporation did not influence the strength value. That is that the variations in the tensile strength of various samples containing ZnO and chitosan did not depend on each other, as shown in Fig. 4, indicating no significant difference. Nevertheless, all pH 9-related groups seem to have a higher value than their corresponding control-related groups. The tensile strength values were in the range of 4~5 MPa.

4.2. Characterization of immersed bodies

Fig. 4 also shows the changes in the tensile strength of various samples that immersed in Hank's solution for different periods of times. The results revealed that, when immersed in Hanks' solution, the six different types of samples gradually lost the strength with increasing initial immersion time. In the case of the control group, the strength was significantly reduced from the initial strength of 3.8 MPa down to 2.4 MPa after three-day immersion with a reduction of about 35%, showing biodegradation. As for pH 9-group, it

also showed the similar behavior but seriously. However, immersion up to 90 days, the strength values did not go on declination. On the other hand, no significant differences could be found for the 90-day-immersed samples derived from different pH values. When the samples contained ZnO and/or chitosan, the resulting hybrid bodies also lost their strength as same trend as those of matrix samples. This deterioration in the strength seems unavoidable for present samples immersed in physiological solution. The immersion-induced decline in mechanical strength was due to less stable zones (particle surfaces or interface regions of grains) of a hybrid body, where the degradation occurred more rapidly [9].

From Fig. 5, it can be seen that much more particle-like agglomerates were formed on the 0.5 wt% chitosan covered surface after immersion for 30 days, which was due to the formation of precipitated apatite, suggesting a good bioactivity. Zinc-containing apatite/chitosan composites may be a bioactive and biodegradable material and can be used in bone substitute, but they needs more *in vivo* experiment to exam the biological properties.

五、結　論

The pH value was an important factor controlling characteristics of sol-gel derived apatite. The samples made from the condition at pH 9 presented a stronger diametral tensile strength than that of control sample. A small amount of added ZnO did not affect the tensile strength and microstructure of ZnO-containing apatite composites with an exception of (002) preferred orientation. Chitosan incorporation did not also influence the strength value. When immersion in Hanks' solution, the tensile strength of all samples decreased rapidly after immersed in Hank's solution for 3 days with a reduction of about 40%, showing biodegradation. After that, the strength maintained a steady value.

六、成果自評

The preliminary study of this project focuses on the investigation of properties of Zn-containing apatite/chitosan. The results will be submitted to SCI journal, in addition to one paper published in domestic journal (Chung Shan Medicine Journal). As for Si-containing hybrid composites, the results are being taken into account to apply USA patent.

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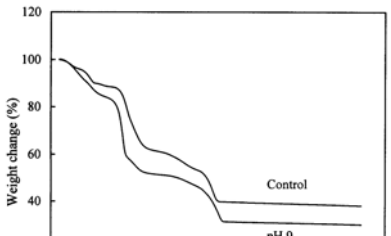

Fig. 1. Thermo-gravimetric analysis of the as-dried Ca-P precursor obtained from control and pH 9 groups.

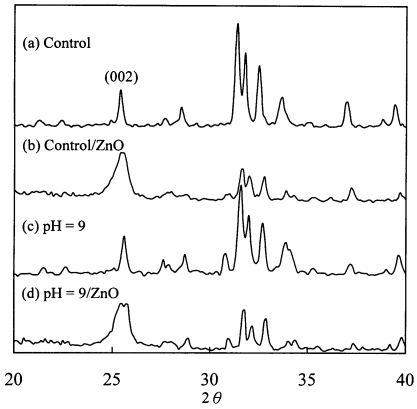

Fig. 2. XRD patterns of samples obtained without and with pH adjustment and containing ZnO after heat treatment at 900°C.

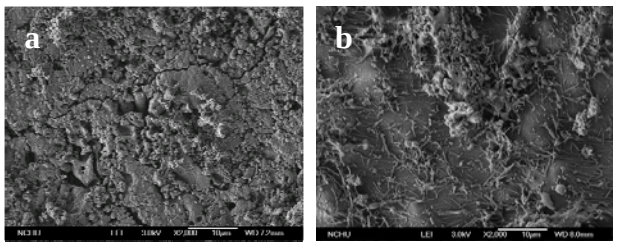

Fig. 3. SEM images of sol-gel derived apatite without (a) and with chitosan (b).

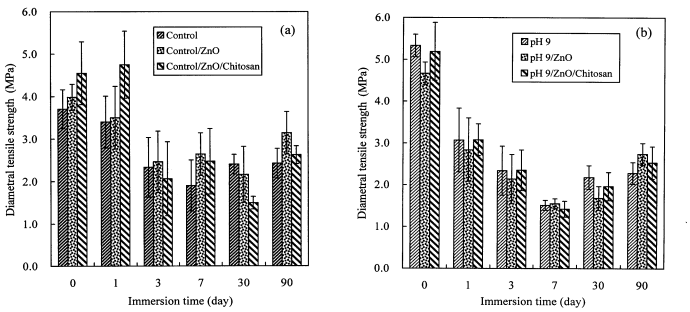

Fig. 4. The changes in diametral tensile strength of control groups (a) and pH 9-related groups (b) as a function of immersion time.

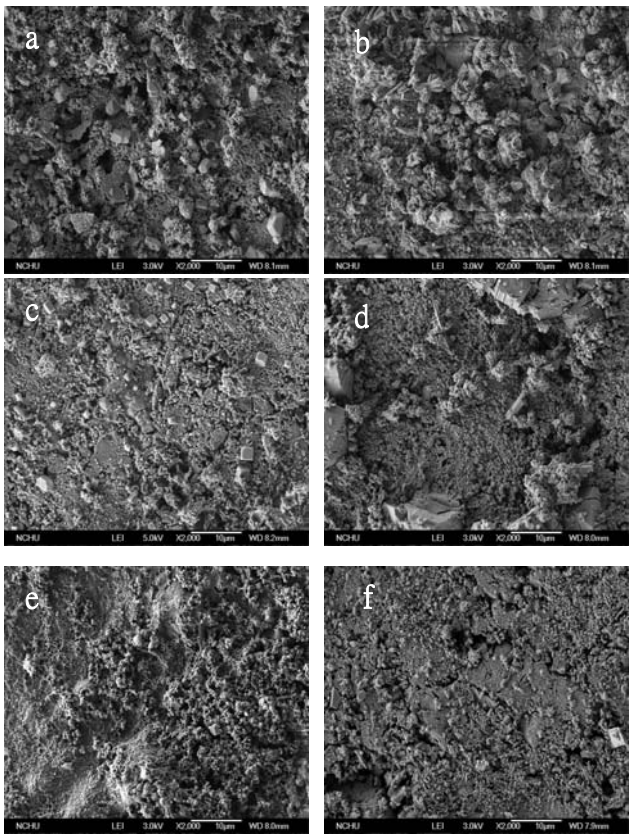

Fig. 5. SEM images of the samples after immersion in Hanks' solution for 30 days (a,c,e)and 90 days (b,d,f). (a,b) control sample containing only apatite, (c,d) pH 9- derived apatite bulk, (e,f) pH 9- derived apatite bulk containing ZnO and chitosan.