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磷酸鈣/幾丁聚醣仿生植體研發

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執行單位：中山醫學大學牙科材料研究所

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磷酸鈣/幾丁聚醣仿生植體研發

Development of calcium phosphate/chitosan biomimetic implants

計畫編號：NSC 92-2614-B-040-020

執行期限：92 年 8 月 1 日至 93 年 7 月 31 日

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一、中文摘要

組織工程區分為骨架、細胞及生長因子等三大要素。骨架扮演整個組織主要支撐的角色，搭載著細胞與生長因子，其性質之優劣決定受損組織之再生、修復及重建成效。因此骨架材料的特性如孔隙率、孔洞大小、機械性質需加以評估以求適合組織成長。本計劃以燒結製程製備三維多孔體，前期先以磷酸鈣及多孔劑為原料，後續再製造磷酸鈣/幾丁聚醣複合體。特性評估乃將各材料浸泡於 Hanks 模擬體液中，研究其疲勞行為、機械性質、重量損失，及微結構形態。結果發現多孔體結構由巨孔與微孔構成，多孔陶瓷體其強度與添加劑(PVA)息息相關。經疲勞測試後強度隨浸泡時間增加而降低，拉伸強度、彈性係數、重量損失亦如此。加入幾丁聚醣於多孔磷酸鈣鹽材料後所形成的仿生植體並不影響多孔體的拉伸強度。

關鍵詞：組織工程、多孔性骨架、機械性質、鈣磷酸鹽、幾丁聚醣

Abstract

The framework of tissue engineering consists essentially of scaffold, cell, and grown factors. The scaffold is mainly supporting structure and plays a role of cell and growth factor carrier. The properties of the scaffold affect the performance of regeneration, repair and reconstruction of damaged tissue. Thus, characterization such as porosity, porous size, and mechanical property of scaffold materials used are needed to evaluate for the growth of the tissue. In this program, a highly three-dimensional porous structure is designed by sinter processing, which calcium phosphates and a porogen were used as raw materials. After which, the preparation of calcium phosphate/chitosan biocomposite was carried out. Besides mechanical property, morphology, and weight change in Hanks solution, the cyclic fatigue behaviors of the samples were assessed. Experimental results showed the pore microstructure of the as-sintered bodies to be made up of the macropores and micropores. The initial strength of the porous ceramic bodies was dependent on the contents of the PVA additive. Porous bodies subjected to cycling fatigue in Hanks' solution remarkably decreased in the strength. With increasing immersion time in solution, the tensile strength and elastic modulus of various porous bodies decreased. The weight loss data confirmed the degradation behavior of the porous bodies in Hank's solution. The introduction of chitosan did not adversely affect the tensile strength of the porous calcium phosphate/ chitosan biomimetic composites.

Keywords: tissue engineering, porous scaffold, mechanical property, calcium phosphate, chitosan.

二、緣由與目的

Tissue engineering is regarded as one of the bioscience for guiding the body to regenerate or repair tissues, when organ and tissue loose or fail [1-3]. The scaffold is the temporary supporting structure of tissue-engineered constructions [1-5]. Aside from suitable surface chemistry, the scaffold architectures provide sufficient space and suitable mechanical properties for tissue regeneration [1-2]. Ideally, the resorption rate of an implant should not exceed the rate of bone formation and the resorption-induced reduction in implant strength should closely match the increase in strength of the healing tissue [2].

Porous biodegradable synthetic materials, such as calcium phosphates, poly(lactic acid) (PLA), and poly(glycolic acid) (PGA), are currently tested as implants for the regeneration of damaged and diseased tissues [2-5]. These synthetic biomaterials were chosen on the basis of their biocompability and mechanical properties. Concerning the fabrication of porous ceramic bodies, several techniques, such as the use of polymeric sponge [6] and organic additives [7], and molding processes [8], have been used. For example, Prado da Silva et al. used three different organic additives, potato starch, almond crust, and wax spheres, to produce porous structures of a CaO-P_2O_5 glass reinforced hydroxyapatite (HA) [7]. The available synthetic biodegradable calcium phosphate ceramics for bone tissue regeneration include HA, β-tricalcium phosphate (β-TCP), and calcium polyphosphate [3-4,6].

Natural bone is actually an inorganic/organic composite mainly comprised of nano-structure hydroxyapatite and collagen fibers. Of most importance to synthesize biomimetic composites having good biocompatibility, high bioactivity and great bonding properties should be performed. Chitosan is a very abundant naturally occurring polysaccharide. Due to its biocompatibility, biodegradability, and nontoxicity, it has attracted much research in biomedical and drug-delivery applications [9].

Biodegradation of tissue-engineered materials is characterized by changes in the mechanical and physicochemical properties (disintegration and dissolution) of the material after implantation or after immersion in physiological solution. Little information on the variations in mechanical properties of porous calcium phosphates, when immersed in simulated body fluid, has been reported. In this project, porous calcium phosphates were prepared by sintering mixtures of monocalcium phosphate monohydrate (MCPM) and polyvinyl alcohol (PVA). The degradation behavior of the porous bodies was characterized by monitoring changes in tensile strength and modulus as well as weight loss. The fatigue test in wet condition was also performed. Additionally, we infiltrated chitosan solution into the porous ceramic body to produce a biomimetic composite.

三、實驗方法

Commercially pure MCPM and PVA powders were used. The as-received MCPM powder was directly mixed with PVA in a vacuum mixer. Four different types of the porous ceramic bodies were prepared from mixtures of MCPM and PVA powders with different ratios (4:1, 3:1, 2:1, and 3:2 by weight). The mixture was uniaxially pressed at 10 MPa, and then sintered at $900^{o}C$. The morphology was observed under a SEM. The measurement of the porosity was carried out using a conventional Archimedes immersion technique.

The extracellular solution with an ionic composition similar to that of human blood plasma, Hank's balanced salt solution, was used as supporting solution and for the immersion test. For fatigue test, the sintered samples were fatigued under diametral compression test in Hanks' solution at room temperature. For immersion test, the specimens were immersed in solution for the predetermined periods of time at $37^{o}C$. Their degradation behavior was also monitored through sample weight change. The diametral tensile testing was conducted on an EZ-Test machine.

On the composite part, the as-sintered calcium phosphate bodies were incubated in different concentrations of chitosan solutions to make up the composite, which solidified by freeze drying. The mechanical properties and microstructure of the calcium phosphate/chitosan were also evaluated.

四、結果與討論

4.1. Characterization of as-sintered bodies

4.1.1. Morphology

The SEM micrographs showed the pore structure of calcium phosphate bodies essentially to be as an assembly of macropores and micropores. Macropore sizes as large as hundreds of micrometers were generated and micropores were less than 10 μm. PVA served as a pore-forming agent because of the evolution of water and carbon dioxide during sinter processing, leading to the development of the highly porous microstructure. When sintered at 900°C for 3 hr in air, porous bodies of around 80% porosity were achieved. C4P1 sample had the higher porosity possibly due to the double role of PVA both as binder and pore-former.

4.1.2 Mechanical properties

The variations in the tensile strength of as-sintered bodies were found to depend on the PVA contents. The addition of PVA up to 25 wt% achieved increased tensile strength. Indeed PVA is a very commonly used binder that can improve the strength, in addition to the role as pore-former [10]. However, higher PVA contents adversely affected the mechanical properties with a reduction of up to 67% of the highest value. The porous bodies consisting of smaller macropores exhibited higher strength in comparison with those with larger macropore.

4.2. Characterization of immersed bodies

4.2.1. In vitro fatigue test

Fig. 1 shows that the stability of the porous bodies was apparently affected by the cyclic loading, with a remarkable decrease in the strength as the number of cycles increased. For example, sample C3P1 fatigued in Hanks' solution for 9×10^5 cycles had a significant degradation down to 40% of the original strength.

4.2.2. Weight loss

The resulting weight loss indicated that sample C4P1 had the largest weight loss with degradation time, reaching up to 11% after 90 days; however, the weight loss still remained about 6% over 90 days of immersion for all other samples (Fig. 2). The decrease in sample weight might be explained by degradation of such porous materials.

4.2.3. Mechanical properties

The variation in the tensile strength with immersion time of the series of porous bodies is presented in Fig. 3. The results revealed that, when immersed in Hanks' solution, the four different types of porous samples gradually lost the strength with increasing immersion time. The strength of sample C3P1 was significantly reduced from the initial strength of 1.2 MPa down to 1.0 MPa after one-day immersion. When immersed for 90 days, its strength decreased to 0.4 MPa. The tensile strength of immersed calcium phosphates mixed with 20 wt% PVA in green sample (C4P1) significantly declined by about 60% after immersion for 90 days. When the green bodies comprised 33 and 40 wt% PVA, the resulting porous bodies lost 58% and 64% of the as-sintered strengths after 90-day immersion, respectively. This deterioration in the strength seems unavoidable for biodegradable porous ceramics immersed in physiological solution and has also been observed in other studies [11]. The immersion-induced decline in mechanical strength was due to less stable zones (particle surfaces or interface regions of grains) of porous ceramic body, where the degradation occurred more rapidly [11].

Concerning the changes in the elastic modulus against immersion time for porous bodies, there was also a pronounced decrease in the elastic modulus for all porous bodies after immersion in solution over 90 days, similarly to the tensile strength.

4.3. Characterization of porous composites

Fig. 4 shows the microstructure of calcium phosphate/chitosan composites, reflecting the retention of the porous structure. On the other hand, the introduction of chitosan did not adversely affect the tensile strength of the porous calcium phosphate/chitosan biomimetic composites, as shown in Fig. 5.

五、結　論

Results showed the amounts of PVA added as a pore-former and binder to calcium phosphate ceramics to have an important effect on mechanical properties. The fatigue resistance of all porous bodies was found to greatly decline and their texture delaminated when immersed in Hanks' solution.

六、成果自評

The preliminary study of this project focuses on the investigation of biodegradation properties of porous calcium phosphate in physiological simulated solution. The results have been accepted by SCI journal, Ceramics International in 2004, in addition to one paper published in domestic journal (J Med Biol Eng 2003;23:159-164). The in vitro fatigue and degradation properties of calcium phosphate/chitosan composites are being evaluated because the samples will be immersed up to six months in solution.

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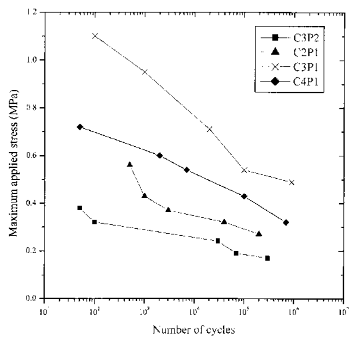

Fig. 1. Maximum stress applied in diametral tensile stress cyclic fatigue versus the number of cycles.

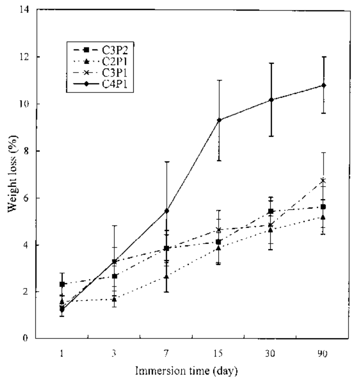

Fig. 2. Weight loss of various porous bodies after immersion in Hanks' solution.

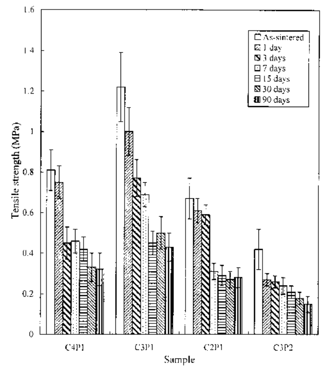

Fig. 3. The variations of tensile strength against immersion time for different porous bodies.

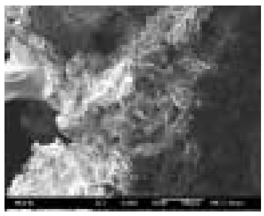

Fig. 4. SEM micrographs of C4P1 bodies at 900^{0}C after treated with 2% chitosan.

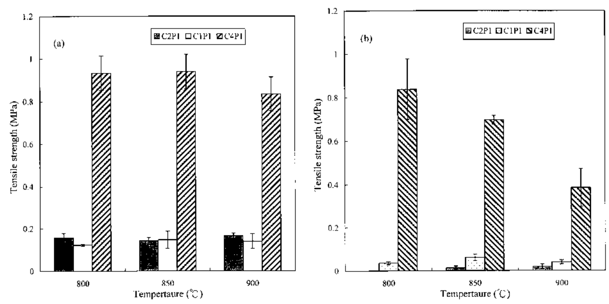

Fig. 5. Tensile strength of various composite bodies with different weight ratio of MCPM and PVA after different sintering temperature, and then adding 2% (a) and 0.5% (b) chitosan.