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行政院國家科學委員會專題研究計畫成果報告  
應用於骨組織工程 Alginate/HAP 多孔海綿體之製備及活體外  
與活體內相關性質之研究

Porous Alginate/HAP Sponges for Tissue Engineering: Preparation,  
In Vitro, and In Vivo Studies

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一、中文摘要

本文探討由 Alginate 製備三維多孔性海綿體及其特性，並將 HAP 加入 Alginate 溶液中以強化其機械強度及生物相容性。此海綿體是以三步驟製得：首先加入二價陽離子（如  $\text{Ca}^{2+}$ 、 $\text{Sr}^{2+}$ 、 $\text{Ba}^{2+}$ ）至 Alginate 溶液當中而形成水膠，其次冷凍，最後再經由冷凍乾燥製備三維多孔性海綿體。結果顯示多孔性海綿體不僅具有良好的內部連結孔洞結構，並且也具備很好的機械強度。為了探討其生物相容性，本文並將細胞株植入海綿體上。使用 Alginate 海綿體做為組織工程之模板，應用於骨的組織再生，極具潛力。

**關鍵詞：**組織工程、生物降解性高分子、支架、海藻酸鹽、氫氧基磷灰石、多孔海綿體、骨組織再生

**ABSTRACT**

This paper describes the preparation and characterization of a three-dimensional, porous sponges made from alginate. The alginate solution was mixed with hydroxylapatite(HAP) to increase its mechanical properties and biocompatibility. The sponge was prepared by a three-step procedure: first gelation of the alginate solution to form a hydrogel with bivalent cations (such as  $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$  and  $\text{Ba}^{2+}$ ), then freezing, and finally drying by

lyophilization to produce a three-dimensional, porous sponge. The results demonstrate that the porous sponges not only have well interconnected pore structure, but also have well mechanical properties. In order to examine its biocompatibility, rat osteogenic sarcoma cell line (OGS) was seeded into the scaffolds. Using alginate sponges that serve as a template for tissue engineering has a potential use in the regeneration of bone tissue.

**Keywords:** Tissue engineering, Biodegradable polymers, Scaffolds, Alginate, Hydroxylapatite, Porous sponges, Bone tissue regeneration

**INTRODUCTION**

Tissue engineering uses a three-dimensional porous, biodegradable polymer scaffold that seeds the cells and serves as template for tissue regeneration [1]. It is a potential alternative to tissue or organ transplantation. An important part in tissue-engineering research is to find how to prepare a biodegradable, biocompatible, and highly porous scaffold. In this study, we developed a method to fabricate alginate/HAP scaffold. Alginate is a nature polymer that extracted from algae. It is highly hydrophilic, biocompatible and relatively economical. Alginate forms stable gels in the presence of certain bivalent cations. However, the scaffold

made from Alginate is very soft and weak. This may limit its further application as a template for tissue regeneration. We proposed the use of hydroxyapatite as a reinforcing material to make novel, porous alginate/HAP composites. The incorporation of HAP may enhance the mechanical properties of the composite materials. The effects of freezing temperature on the properties of scaffolds including pore size, density, porosity, morphology, and mechanical properties were studied.

In addition, since HAP is an osteoconductive material, it may also enhance the bone regeneration rate *in vivo*. We seeded osteo-correlated cell, osteogenic sarcoma into the sponges, to examine the biocompatibility.

## MATERIALS AND METHODS

The technology for sponge preparation is based on three steps. The first step was to make the composite solution by mixing alginate, HAP powder, and cross-linker in double-distilled water. Then, the composite solution was frozen to the desired temperature. Finally, the frozen composite was dried by lyophilization to develop the pore structure. Table 1 lists the preparation condition for Alginate/HAP sponges. The properties of sponge were then characterized by mechanical testings and morphology observation.

For cell culture, the sponges were sterilized by immersed in 75 % alcohol solution for 30 min. The samples were transferred to a 24-well polystyrene plate and immersed in DMEM-FBS for 1 day. Each sponge was seeded with  $10^6$  osteogenic sarcoma cell line (OGS) in a 5 %  $\text{CO}_2$  incubator at  $37^\circ\text{C}$  with 99% humidity. After 5 days of culture, OGS cell line seeded within alginate sponges were fixed for examination by scanning electron microscopy (SEM) observation.

## RESULTS AND DISCUSSION

The morphology of sponge displayed a highly porous, well- interconnected pore structure. Figure 1 shows the typical

morphology of the alginate/HAP (100/0) sponge. The well-interconnected pore structure of scaffold is developed through phase-separation. In phase-separation, it contains two phases: the polymer-rich and polymer-poor phases. The polymer-poor phase is mostly solvent, such as water in this case. Freeze-drying technique was used to remove the ice cube and the pores were left behind. The polymer-rich phase contains most of polymer solution and would form the cell walls around the pores. The thermal property of various scaffolds shown in Figure 2 demonstrated that Alginate solution was not changed during the scaffold fabrication process.

We examined the effect of the quenching temperature on the sponge microstructure and found that pore size decreased with decreasing the quenching temperature as indicated in Table 2. It is suggested the cooling rate is more rapid at lower quenching temperature, thus smaller ice crystals in alginate/HAP solution were obtained. Accordingly, the pore structure with smaller pore size was developed. It is also noted that the pore sizes in top, bottom surfaces, and cross- section area were different. For the scaffold prepared at the same freezing temperature, the pore size in the bottom surface was the smallest among the others. This is because the bottom surface of the scaffold was exposed directly to the freezer and the cooling rate was very fast, thus smaller pore size was formed.

Since the scaffold made from neat Alginate was very soft and weak, we used hydroxyapatite as a reinforcing material to fabricate alginate/HAP composite scaffold. In general, the compressive strength and modulus of scaffolds increased with increasing HAP content as shown in Figures 3 and 4. The quenching temperature may not play a significant role in affecting the mechanical properties of the scaffolds. The Alginate/HAP (50/50) scaffold prepared at  $-40^\circ\text{C}$  shows the best mechanical properties among the other scaffolds.

Table 3 lists the porosity of the prepared scaffolds. The porosity of neat Alginate scaffold was above 83% at each freezing temperature. However, the porosity of the composite scaffold was decreased as the

amount of HAP increased. The low porosity of the scaffold may limit the efficiency of the nutrition supply during the cell culture.

Because the scaffold fabricated in this article has a uniformly distributed and highly interconnected pore structure, cells were attached and distributed evenly on the pore walls of scaffold, as shown in Figures 5 and 6.

## CONCLUSIONS

Alginate/HAP scaffolds not only have well interconnected, highly porous structure but also have great mechanical properties. OGS cell lines seeded into the alginate/HAP scaffolds demonstrate good biocompatibility. These natural polymeric sponges may be applied to tissue engineering applications.

## REFERENCES

1. Vacanti CA, Langer R, Schloo B, Vacanti JP. Synthetic polymers seeded with chondrocytes provides a template for new cartilage formation. *Plast Reconstr Surg* 1991; 88:753-759.

Table 1. Preparation conditions of alginate/HAP scaffolds  
(Cross-linker: 0.03M CaCl<sub>2</sub>)

freezing temp.	Alginate/HAP ratio
-10 °C	100/0, 75/25, 50/50
-40 °C	100/0, 75/25, 50/50
-80 °C	100/0, 75/25, 50/50
liquid N <sub>2</sub>	100/0, 75/25, 50/50

Table 2 Pore size of alginate scaffolds  
(Alginate/HAP ratio=75/25)

temp.	top	bottom	cross-section
-10°C	193(±7)	195(±5)	196(±22)
-40°C	162(±10)	129(±5)	158(±9)
-80°C	98(±6)	42(±2)	109(±5)
liquid N <sub>2</sub>	Pore size<10um		Ladder structure

Table 3. Porosity of alginate/HAP scaffolds  
(Cross-linker: 0.03M CaCl<sub>2</sub>) (N=50)

temp.	Alginate	Alginate/HA=75/25	Alginate/HA=50/50
-10°C	87.2%	79.6%	68.8%
-40°C	86.5%	80.8%	69.7%
-80°C	87.3%	79.4%	73.3%
liquid N <sub>2</sub>	84.0%	78.5%	66.4%

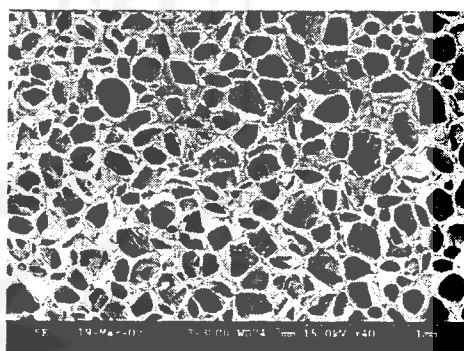
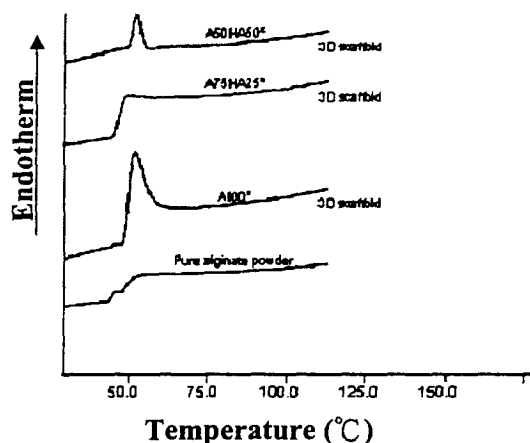


Figure 1. Scanning electron micrograph of alginate/HAP (100/0) sponge (40X).



\*A:alginate; HA:Hydroxyapatite

Figure 2. The glass transition temperatures ( $T_g$ ) of alginate powder and the prepared scaffold with different alginate/HAP ratio .

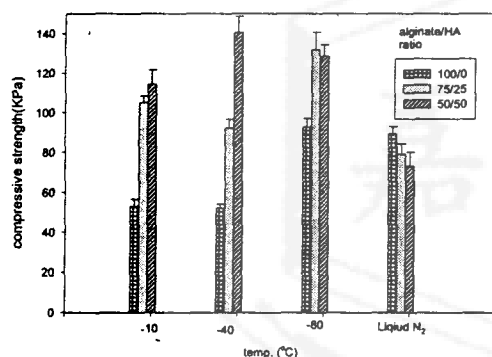


Figure 3. Compressive strength of scaffolds made from different weight ratio of alginate/HAP in different quenching regime.

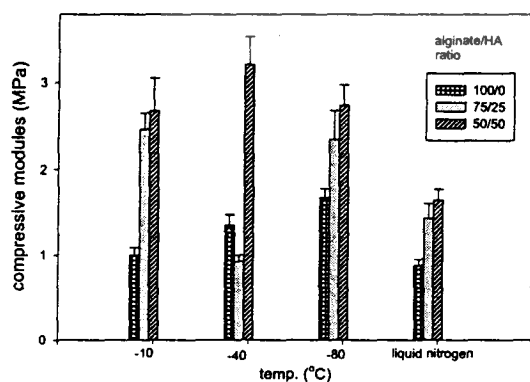


Figure 4. Compressive modulus of scaffolds made from different weight ratio of alginate/HAP in different quenching regime.

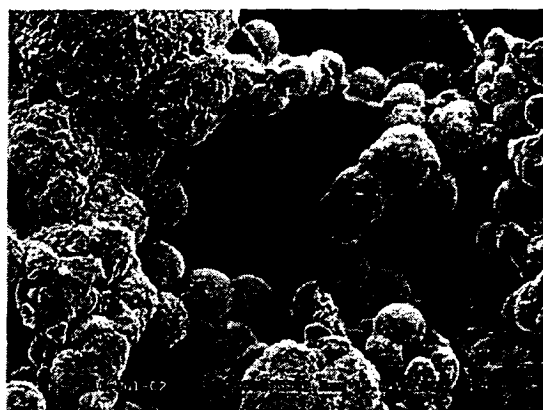


Figure 5. Scanning electron micrographs of OGS cell line-seeded alginate/HAP (75/25) sponge after 5 days in culture. (500X)

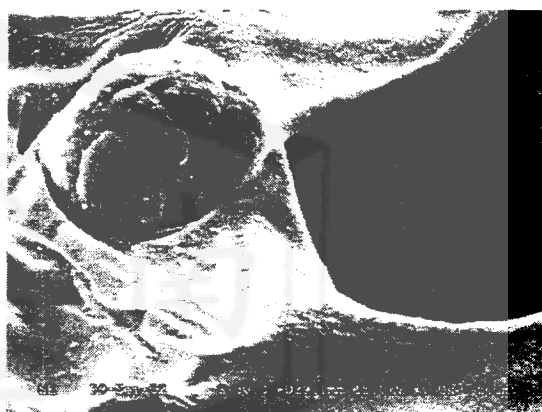


Figure 6. Scanning electron micrographs of OGS cell line-seeded alginate/HAP sponge (100/0) after 5 days in culture. (1000X)