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단신

# 승화-압착법에 의한 수산화아파타이트/키토산 필름 제조

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# Hydroxyapatite/Chitosan Film by Freeze-Drying Assisted Compressing Method

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**Keywords:** Hydroxyapatite, Chitosan, Sublimation-assisted compression (SAC), Mechanical strength, Flexible

## INTRODUCTION

Currently, the functional treatment of fracture non-unions and bone loss is a significant challenge in the field of orthopedic surgery. HAp has long been used as a biocompatible and osteoconductive substitute in the field of orthopedic surgery, immediate tooth replacements, pulp-capping material and repair of bone defects, *etc*.[1-3] However, it is difficult to shape HAp in the specific forms required for bone repair and implantation on account of its hardness and lack of flexibility. Furthermore, HAp powders used in the treatment of bone defects have intrinsic problems in that they migrate easily from the implanted sites. In addition, they do not disperse well and tend to agglomerate, which limits their applications in the area of clinic medicine.[4] Therefore, novel composites of HAp and organic polymers have attracted considerable attention to compensate for the weak mechanical and conformational properties of inorganic biomaterials.[5-6] Chitosan, a naturally occurring biopolymer, has attracted considerable attention in wound dressings,[7] drug delivery systems,[8-9] space-filling implants,[10] and tissue engineering.[11] Chitosan is an *N*-deacetylation product of chitin, and consists of glucosamine and *N*-acetylglucosamine units linked through 1/4 glycosidic bonds.[12] In addition, chitosan has high heat resistance due to the intramolecular hydrogen bonds formed between the hydroxyl and amino groups.[13-14] Nanocomposites of HAp/chitosan have been prepared with increased osteoconductivity and biodegradation for orthopedic use.[15] On this basis, this study developed a novel route for preparing HAp/chitosan films using a freeze-drying assisted compressing method, known as a sublimation-assisted compre-

ssion (SAC) method, to increase the osteoconductivity and biodegradation as well as provide ideal mechanical strength for orthopedic use. Homogenous HAp/chitosan composites were prepared using the SAC method. The improvement in mechanical and morphological properties of the composite was examined as a function of the amounts of HAp added to the chitosan solution. The mechanical properties, composition and microstructure were characterized using a materials testing machine as well as by thermal gravimetric analysis (TGA) and scanning electron microscopy (SEM).

## EXPERIMENTAL SECTION

A chitosan-dispersed aqueous solution was prepared by dissolving chitosan powder into acetic acid. The mixture was then stirred to obtain a homogeneous polymer solution. HAp powder was added to the prepared solution with vigorous stirring to make a polymer/HAp mixture. The HAp was dissolved in the solution, and the surface potential of the mixture was measured using a zeta-sizer (ZS-nano, Malvern, United Kingdom) before an ice-molding process to determine the dispersity of the colloid in solution. A composite foam of HAp/chitosan was prepared using a freeze-drying method. In this procedure, the foam was achieved by solid-liquid separation of the mixture with the subsequent removal of the solvent by sublimation. The composite was finally compressed to form a flexible thin film.

*Table* 1 shows the compositions of the films. The composition of the HAp/chitosan film was determined by TGA (SCINCO model # 1000, Korea) in a $N_2$ environment (flow rate, 30 cc/min) at temperatures ranging from 25 $^{o}$C to 800 $^{o}$C at a heating rate of 15 $^{o}$C/min. FT-IR spectrophotometry (FT-IR 6300, JASCO, Japan) was used to determine the chemical composition of the HAp/chitosan film. The morphology of the HAp/chitosan film observed using a Hitachi S4700 SEM. The tensile strengths were measured using a materials testing machine (LLOYD, AMETEK, United Kingdom) at a crosshead speed of 5 mm/min and a span of 10 mm. Five rectangular pieces of each film with the same size were measured.

*Table* 1. Composition of the HAp/chitosan films

| HAp/chitosan composite films | HAp (g) | Chitosan (g) | Ratio |
|---|---|---|---|
| HAp A | 0 | 0.5000 | 1/5 |
| HAp B | 0.1250 | 0.5000 | 1/4 |
| HAp C | 0.2500 | 0.5000 | 1/2 |
| HAp D | 0.5000 | 0.5000 | 1/1 |
| HAp E | 0.7500 | 0.5000 | 3/2 |
| HAp F | 1.0000 | 0.5000 | 2/1 |

## RESULTS AND DISCUSSION

SEM images of HAp/chitosan film (2/1 weight ratio) are shown in *Fig*. 1A and B. *Fig*. 1A is the surface SEM micrographs of HAp F film. As shown in the SEM micrographs, HAp particles can be easily identified on the polymer matrix surface, more often assembled into aggregates, The dispersion was found to be more homogeneous for high HAp contents (*Fig*. 1B), as seen in *Fig*. 1B, a qualitative good adhesion was found between the chitosan matrix and HAp particles. *Fig*. 1C shows the tensile strength of the HAp/chitosan films in various proportions. From the figure, we can see the films prepared from a 1:1 (weight ratio) HAp solution had a higher tensile strength than the other films. In addition, this film (weight ratio, 1/1) showed a more homogenous and uniform morphology. The tensile strength of the prepared films was characterized using a mechanical strength machine to determine their mechanical behavior. Six specimens were tested at weight ratios of 0/5, 1/4, 1/2, 1/1, 3/2 and 2/1, and their tensile strengths were compared. As shown in *Fig*. 1C, heating and compressing caused a significant increase in the mechanical strength of the HAp/chitosan film. The strength of the film prepared with compressing was 10 times higher than that of the film prepared without compressing.

*Fig*. 1D shows the TGA curves of the HAp/chitosan films prepared at various concentrations (1/4,

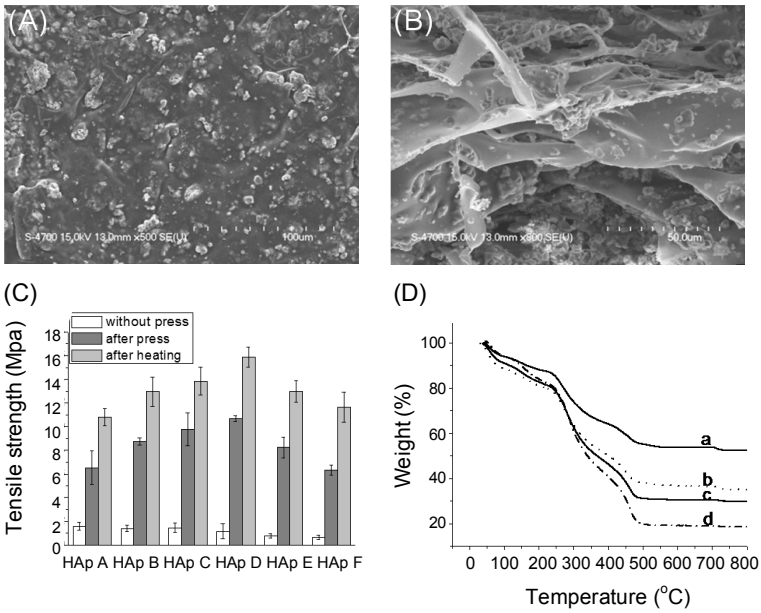


*Fig.* 1. (A) and (B) SEM images of HAp/chitosan film with weight ratio of 2/1. (C) Tensile strength of the HAp/chitosan films. (D) TGA curves of HAp/chitosan films of (a) 1/4, (b) 1/2, (c) 1/1, (d) 3/2, respectively.

1/2, 1/1, 3/2). From the TGA curves, the sample weight decreased rapidly with increasing temperature, particularly in the ranges, 40° ~ 130 °C and 250° ~ 600 °C.[4] The broad endothermic peak near 100 °C was assigned to the loss of water. The peak at approximately 500 °C was assigned to the thermal decomposition of chitosan. The decomposition temperature decreased with increasing HAp contents. This means that the thermo stability of the film increases with increasing HAp concentration. The film was homogeneous with HAp powder distributed uniformly in the film showing the highest tensile strength.

## CONCLUSIONS

A novel freeze-drying method, called the SAC method, was used to produce high tensile strength and flexible HAp/chitosan films. The optimum weight ratio of the HAp/chitosan films to produce the highest tensile strength was 1:1. This film showed homogeneity, uniformity and enhanced mechanical properties. The maximum limit of HAp incorporated in chitosan for satisfactorily homogeneous HAp/chitosan composites was found to be 2/1, as indicated by SEM. This prepared film makes it suitable for use as a patch-type controlled delivery system for bone substances in orthopedics as well as in the osteoconductive treatment of multi-fractured bone.

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## REFERENCES

1. Liu, Y. L.; Schoenaers, J.; De Groot, K.; De Wijn, J. R.; Schepers, E. *J. Mater. Sci. Mater. Med.* **2000**, *11*, 71.
2. Kitsugi, T.; Yamamuro, T.; Nakamura, T.; Kotani, S.; Kokubo, T.; Takeuchi, H. *Biomaterials*, **1993**, *14*, 216.
3. Kriakose, T. A.; Narayana, S. K.; Palanichamy, M.; Arivuoli, D.; Dierks, K.; Bocelli, G.; Betzel, C. *J. Cryst. Growth.* **2004**, *263*, 517.
4. Yamaguchi, I.; Tokuchi, K.; Fukuzaki, H. et al *J. Biomed. Mater. Res*. **2000**, *55*, 20.
5. Taguchi, T.; Kishida, A.; Akashi, M. *J. Biomater. Sci. Polym. Edn.* **1999**, *10*, 331.
6. Furukawa, T.; Matsusue, Y.; Yasunaga, T.; Shikinami, Y.; Okuno, M.; Nakamura, T. *Biomaterials*, **2000**, *21*,

889.
7. Hirano, S.; Itakura, C.; Seino, H.; Akiyama, Y.; Nonaka, I.; Kanbara, N.; Kawakami, T. *J. Agric. Food Chem*. **1990**, *38*, 1214.
8. Aiedeh, K.; Gianasi, E.; Orienti, I.; Zecchi, V. *J. Microencapsul*. **1997**, *14*, 567.
9. Miyazaki, S.; Yamaguchi, H.; Takada, M.; Hou, W. M.; Takeichi, Y.; Yasubuchi, *H. Acta. Pharm. Nord.* **1990**, *2*, 401.
10. Muzzarelli, R.; Baldassarre, V.; Conti, F.; Ferrara, P.; Biagini, G.; Gazzanelli, G.; Vasi, V. *Biomaterials*, **1988**, *9*, 247.
11. Minuth, W. W.; Sittinger, M.; Kloth, S. *Cell Tissue Res.* **1998**, *291*, 1.
12. Yamaguchi, I.; Lizuka, S.; Osaka, A. et al *Elsevier Science* **2003**, *214*, 111.
13. Ogawa, K.; Hirano, S.; Miyanishi, T.; Yui, T.; Watanabe, T. *Macromolecules*, **1984**, *17*, 937.
14. Lee, Y. L.; Khor, E.; Ling, C. E. *J. Biomed. Mate. Res.* **1999**, *48*, 111.
15. Sreedhar, B.; Aparna, Y.; Sairam, M.; Hebalkar, N. *J. Appl. Polym. Sci*. **2007**, *105*, 928.
16. Yamaguchi, I.; Tokuchi, K.; Fukuzaki, H. et al *J. Biomed. Mater. Res.* **2001**, *55*, 20.