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Biodegradable Inorganic-Organic Composite Artificial Bone Substitute
-Part 3. *In vitro* properties of the composite substitute-

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=Abstract=

To develop an artificial bone substitute that is gradually degraded and replaced by the regenerated natural bone, the authors designed and produced a composite that is consisted of calcium phosphate and collagen. Human umbilical cord origin pepsin treated type I atelocollagen was used as the structural matrix, by which sintered or non-sintered carbonate apatite was encapsulated to form an inorganic-organic composite.

With cross linking atelocollagen by UV ray irradiation, the resistance to both compressive and tensile strength was increased. Collagen degradation by the collagenase induced collagenolysis was also decreased.

Key words : Bone substitute, Collagen, Cross link, Strength, Degradation

INTRODUCTION

An artificial bone substitute was designed as an inorganic-organic composite that would be gradually degraded and replaced by natural bone after implantation. The composite consists of a calcium phosphate, especially a carbonate apatite that comprises about 80% of the bone apatites, and a type I collagen according to the main compositions of the natural bone. A carbonate apatite with similar crystallinity to the natural bone could be obtained[1], and human umbilical cord origin type I collagen was extracted. Telopeptides were removed from the collagen by pepsin to decrease immunogenecity to use as the composite matrix[2]. Type I collagen comprises about 23~25% in cortical bone and 30% in cancellos bone in volume as protein[3].

To develop a biodegradable artificial bone substitute, an inorganic-organic composite that consists of carbonateapatite and type I atelocollagen extracted from human umbilical cord, was prepared. Physical properties of the composite substitute were investigated in vitro.

MATERIALS AND METHODS

1. Preparation of the composite substitute

Carbonate apatite powder and type I atelocollagen were prepared according to the previously reported methods[1, 2] Carbonate apatite powder synthesized at 58℃ and sintered at 980℃ was mixed with human umbilical cord origin type I atelocollagen solution and controlled to pH 7.4 at room temperature. The collagen concentrations in the mixed paste were controlled to 10, 12, 15, 20 v/v%. The pastes were dried and stored at 4℃ in an UV ray chamber to enhance

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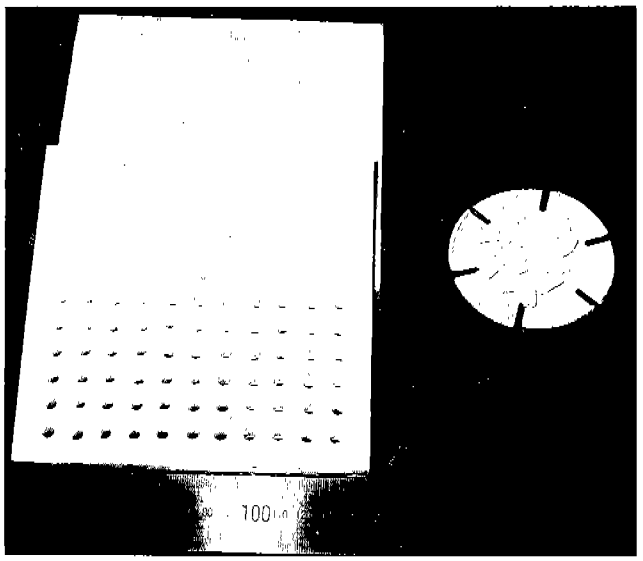

Fig. 1. Teflon mold and fabricated carbonate apatite-atelocollagen rods

collagen cross links and to sterilize.

2. Compressive strengths of the composite

Wet carbonate apatite-type I atelocollagen paste was placed in a teflon mold to produce rods. The rods (6 mm of diameter, 10 mm of height) were irradiated by UV ray with wave length of 254 nm for 4 hours at 4℃ (Fig. 1).

After UV ray irradiation to the composite paste, the shrinkage rate of the specimen was measeured.

The compressive strengths of the rods, which were irradiated by UV ray for 30 minutes, 1, 2, 3, 4 and 8 hours, were investigated by a standardized measuring device (INSTRON model 8511,Instron Co, MA, U.S.A.).

Each group consists of 10 specimens, and the data were treated by one-way ANOVA.

3. Collagenolysis of the composite

To investigate the collagenolysis rate of the cross linked carbonate apatite-collagen composite, the composite rods (3mm of diameter, 5mm of height) irradiated by UV ray were placed in 10 ml of 0.9% NaCl solution, and incubated at 37℃ for 48 hours. 2 ml of the suspension was used as the solution specimen for the Neuman and Logan's method of hydroxyproline assay. The solution specimens were placed into a 10 ml of 0.05N $H_2NC(CH_2OH)_3$ [tris (hydroxymethyl) aminomethane] containing 2 ml of collagenase (C-0130, Sigma Chemical Co., St. Louis, MO, U.S.A.) and controlled to 10, 20, 30, 40, 50 unit/ml in an experimental glass tube. After incubation at 37℃ for 5, 10, 15, 30, 60 min, 1 ml of ethanol was added to stop the collagenase digestion activity. 1 ml of the supernatant was obtained as a specimen, and mixed with 0.2 ml of 6N HCl and hydrolyzed in a dry constant temperature oven at 110℃ for 24 hours. The incubated liquid specimen containing derived hydroxyproline from the collagenase digested carbonate apatite-collagen rod was analyzed by a tungsten spectrophotometer (Spectronic 20, G-02650-24, Cole-Palmer Instrment Co., Ill, U.S.A) at 560m , and selected a 20g/ml hydroxyproline as a standard reagent. Each experimental group consists of 4 specimens, and the data were analyzed by two way ANOVA.

After incubation of the composite rods for the collagenolysis, the calcium concentration in the solution was also analyzed to measure the released carbonate apatite quantity. The emulsions were dissolved completely in 0.1N HCl, and the quantity of the calcium ions in the solution was measured by an atomic absorption analyzer (AA-60, Shimadzu Co., Kyoto, Japan).

RESULTS AND DISCUSSION

An inorganic and organic composite which would be degraded and replaced by regenerative bone after implantation was designed by authors. A carbonate apatite with similar crystallinity was synthesized, and mixed with type I atelocollagen which was purified from human umbilical cord. Though the carbonate apatite is weak in the compressive strength, the composite demonstrated higher compressive strength than the apatite alone (Fig. 2). This would be concerned to the elasticity of the collagen. The mixed collagen matrix should absorb and distribute the compressive load. The comressive strength of the collagen is related to the cross links between the collagen fibrils. In this study, UV ray irradiation was performed to the preformed carbonate apatite and atelocollagen composite to enhance the collagen cross links within the composite. The size of the

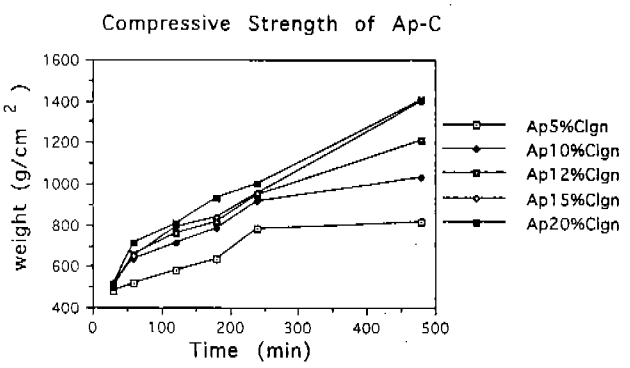

Fig. 2. Compressive strength of the inorganic and organic composite.

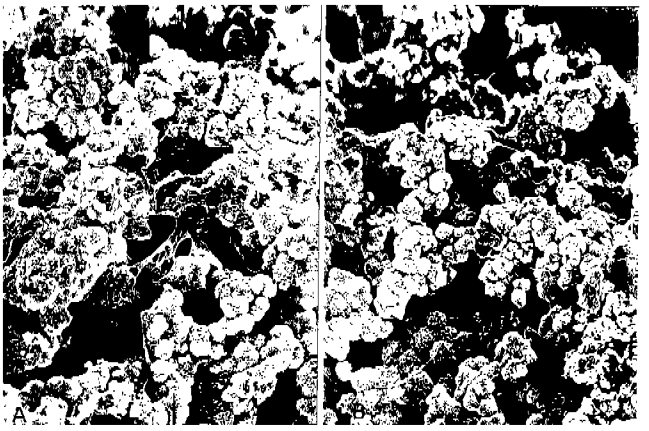

Fig. 4. Composite before **(A)** and after **(B)** UV ray irradiation (by SEM)

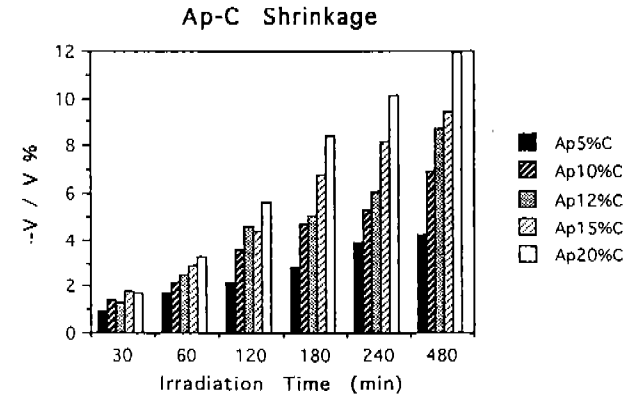

Fig. 3. Shrinkage rate of the composite by UV ray irradiation

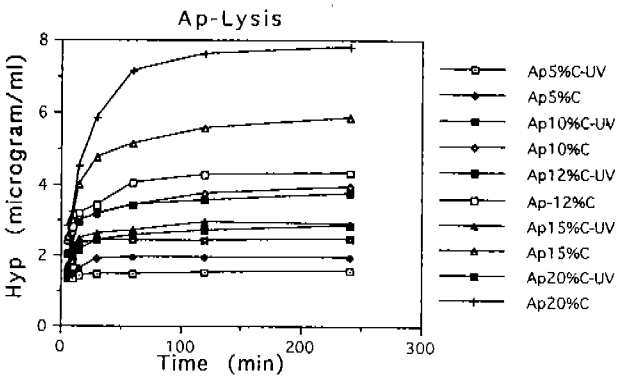

Fig. 5. Released hydroxyprolines from the composite by collagenlysis

composite rods shrinked after UV ray irraidation, and this shrinkage would be related to the collagen cross linking rate (Fig. 3). UV ray has been known as a recommendable tool to promote collagen intermolecular bindings and concentrates the collagen molecules[4]. The UV rays on 2~260 wave length activate the amines radicals of the peptide, and lead to form intramolecular aldol condensations and intermolecular schiff base bindings. The resulred shrinkage improves not only the resistance to the compressive strength but also the inorganic grain encapsulation by the collagen matrix (Fig. 4).

To investigate degradation of the collagen matrix by collagenase, the composite was incubated in normal saline solution, and the released hydroxyroline amount was chased. Hydroxyproline is a typical amino acid that exists in the collagen molecules, and the released hydroxyproline's amount during collagenolysis by collagenase can be measured by Neuman and Logan's assay. The released hydroxyproline amount from the lower-cross inked composite rod was more than from the higher-cross linked one (Fig. 5). This means that the highly cross linked collagen matrix in the composite is less degradable. Collagenolysis of the composite matrix results in inorganic particle release. The amount of the released calcium ions from carbonate apatite due to the collagenlysis increased as time increases (Fig. 6). Therefore, the released inorganic ingredient of the composite would be a supplementary additive to the regenerated bony ground substance. If the amount of the de-

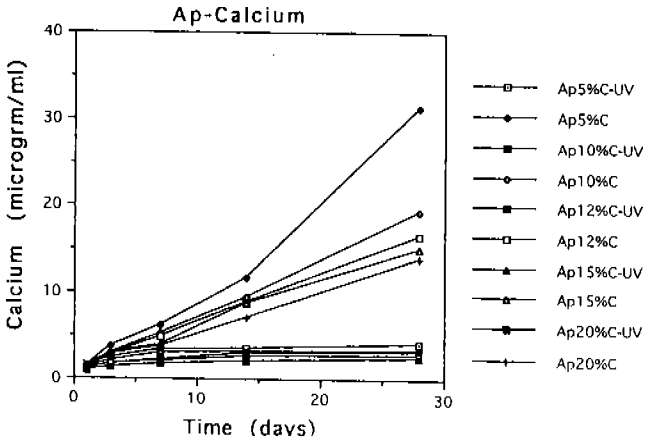

Fig. 6. Released calcium from the composite by collagenolysis

graded collagen matrix is same to the regenerated bony collagen quantity, the composite would be replaced by the natural bony tissue[5, 6].

CONCLUSION

The mechanical and biological properties of the biodegradable inorganic and organic composite were investigated in vitro. The followings are conclusions.

1. Comressive strength of the composite is increased by UV irradiation.
2. Collagenolysis rate of the composite decreases as the UV irradiation time increases.
3. Encapsulated inorganic substances are released following to the collagenolysis.
4. Degradation rate of the composite would be controllable by UV irradation time.

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REFERENCES

1. Lee CK, Suh H: *Biodegradable Inorganic-Organic Composite Artificial Bone Substitute.* Part 1. Synthesis and Crystallinities of Inorganic Substances. J KOSOMBE, 15:195-199, 1994.
2. Suh H, Lee CK: *Biodegradable Inorganic-Organic Composite Artificial Bone Substitute.* Part 2. Collagen purification and its physical and biological properties. J KOSOMBE, 15:341-346, 1994.
3. Fox SI: *Human Physiology,* 4th ed., pp 35-39, Wm. C. Brown Publishers, Iowa, 1993.
4. Nimni ME: *Collagen.* CRC Press, Florida, 1988.
5. Quteish D, Singh G, Dolby AE: *Development and testing of a human collagen graft material.* J Biomed Mater Res, 24: 749-760, 1990.
6. Nimni ME, Cheung D, Strates B, Kodama M, Sheikh K: *Chemically modified collagen: biomaterial for tissue replacement.* J Biomed Mater Res, 21:741-771, 1987.