

Evaluation of Calcium Polyphosphate as an Implant Material : an Animal Study

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I. Introduction

Various types of bone grafts have been used in the treatment of periodontal intraosseous defects¹⁻³. Autogenous bone grafts appear to possess a good osteogenic potential. But they have lack of availability in clinical use, and the harvesting of graft tissue may cause patient morbidity.

In contrast, allografts get much wider availability and little patient morbidity associated with graft procurement. However, problems with unreliable graft incorporation, immune response, and possible disease transmission represent clear drawbacks to their use. While several synthetic graft materials have also been introduced, most alloplastic materials function primarily as a biocompatible defect filler⁴. Several attempts have been made to resolve these problems use of biodegradable polymers and combination of ceramics with bioactive polymers such as collagen

and polylactides⁵⁻⁷.

Chitosan is a biodegradable cationic polysaccharide composed of N-acetylglucosamine residues which is known to accelerate wound healing and bone formation⁸. Our previous reports corroborated bone defects healing⁹. It is hypothesized that the major path for chitosan breakage down in vivo is through lysozyme, which acts slowly to depolymerize the polysaccharide¹⁰. The biodegradation rate of the polymer is determined by the amount of residual acetyl content, a parameter that can easily be varied⁸.

Porous alloplastic implants have been studied extensively for their use in oral and maxillofacial applications¹¹. The use of these materials allows for recovery of the cosmetics and continuity of the surrounding bony structures without the concerns associated with the use of autogenic implants. They have several advantages in periodontal and craniofacial applications.

Ceramics, porous block hydroxyapatite(HA), which is one such alloplastic material, has been shown to be an effective implant material in short- and long-term application¹²⁾. With advances in ceramics technology, the application of calcium phosphate materials has received considerable attention as bone substitutes for several decades. Calcium phosphate as bone substitutes are believed to be biocompatible and osteoconductive when implanted in bone defects¹³⁻¹⁶⁾. Numerous animal studies provide histologic evidence of the long-term biocompatibility of porous HA and of its favorable interaction with soft tissue and bone^{17,18)}. In addition, these studies indicate the lack of an inflammatory response to HA implants^{19,20)}.

Calcium phosphates are generally considered materials of choice as bone substitutes. While calcium phosphate ceramics meet some of the needs for bone replacement, they are limited by their inherent stiffness, brittleness and low fatigue properties relative to bone²¹⁾ and are generally not resorbed during bone remodeling²²⁾. Among the many candidates for bioabsorbable or transient implants, the use of bioabsorbable Calcium Polyphosphate (CPP) has twofold advantages over that of the other bioabsorbable polymeric materials. That is, CPP is chemistry similar to natural bone and has higher stiffness. The CPP are controlled the rates of biodegradability by method of preparation and type of CPP²³⁻²⁵⁾. Regardless of its additive components, CPP granules showed osteoconductivity and biocompatibility, but they exhibited very slow degradation rate⁸⁾.

The purpose of this study was to evaluate Calcium Polyphosphate(CPP) as a bone graft material and to compare the bone formation and resorption of CPP between short term and long term data.

II. Materials and Methods

1. Manufacturing Calcium Polyphosphate (CPP)

Interconnected porous calcium polyphosphate (CPP) blocks were prepared by condensation of anhydrous $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (Duksan Chemical Co., Inc.) to form non-crystalline $\text{Ca}(\text{PO}_3)_2$. From the latter, an homogenous melt was created by thermal treatment, quenched in distilled water, and the block was then milled to produce CPP powder. CPP granules were prepared according to the pore size 45ppi. Pore size of CPP(45ppi) is approximately 450-550 μm .

The CPP granules with chitosan were prepared by use of CPP powders. The each powder of CPP, CaSO_4 , and chitosan were mixed into 5:1:1 in 5% chitosan solution as binder by weight ratio. The mixed compound was penetrated into mesh which pore size is 800 μm and then dried in the fan oven. The size of CPP granules with chitosan were 300-500 μm .

2. Animal experiments

The 3 year-old male adult beagle dogs were bred exclusively for biomedical studies and bucco-gingival health was checked before experimentation, and all teeth of the dog were preliminarily scaled and polished under general anesthesia. Antibiotic treatment with spiramycin and metronidazole was given for 5days.

Surgical procedures. All surgical procedures were performed under general anesthesia with intramuscular rompun[®](1.5mL/kg) followed by anesthesia with ketar[®] (5mg/kg). During surgery, the animals received lactated Ringer's solution and 1g of cephalosporin antibiotic perfused intravenously. The extractions were performed on right and left second, third and fourth mandibular premolars and on right and left second and third max-

illary premolars.

Thus, 10 teeth were extracted per animal. As premolar teeth are biradicular, 20 alveolar extraction sites were available for bone filling. All alveolar sites were checked after extraction and thoroughly debrided with a dental curet to remove the periodontal ligament. Extraction sites were grafted with biomaterial or left unfilled; i.e, the mesial socket of a tooth was left unfilled and the distal socket filled with the composite biomaterial.

Antibiotic treatment by intramuscular injection of cephalosporin(15mg/kg, b.i.d)was continued for 48hours after surgery. The animals were checked daily and fed with a soft consistency diet. Sutures were removed under short general anesthesia 2 weeks after implantation (day 14) and a normal diet was then given.

The animals were sacrificed 3 month and 1 year after implantation by intravenous injection of over-dosed sodium pentobarbital.

Sample preparation. Mandibular and maxillary osseous segments were immediately dissected from the animals and fixed in paraformaldehyde solution. They were fixed with thin metal pins before radiographs were performed to localize each socket.

The sockets were individually separated by cutting into the intra and interdental septa with a diamond saw, according to the pins' position. Each socket was dehydrated in graded ethanol and embedded in a glycolmethylmethacrylate resin.

3. Histological evaluation and Bone ingrowth measurements

Both treated and control mandibular and maxillary sites were evaluated with light microscope (LM). For each socket, 30 μ m thick sections were cut with a hard tissue microtome along the long axis of the root implantation site and then multiple-stained for light microscope observations.

The surface of the resin block obtained from the central area of the socket was prepared for LM observations. Bone ingrowth was investigated and compared in filled and control mandibular and maxillary sites and quantitatively evaluated using a Image Access (TDI scope eye, Korea). Results are given as the percentages of newly formed bone in mandibular and maxillary extraction sites.

4. Statistical analysis

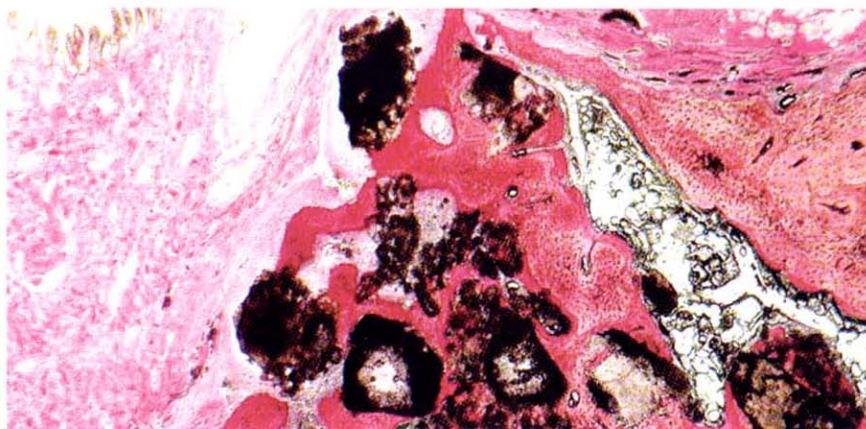


Figure1. Newly bone formation without fibrous tissue encapsulation (multiple stain, original magnification $\times 20$)

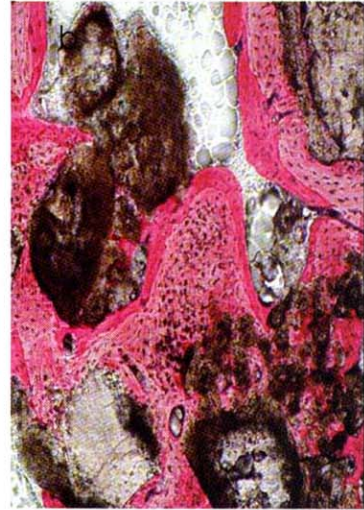
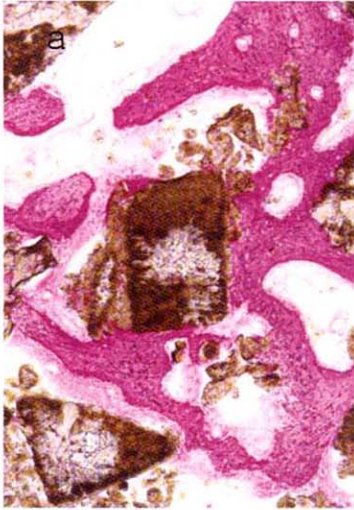


Figure2. There is smooth and irregular contact between newly formed bone and CPP granules, (a) 3month (b) 12month (multiple stain, original magnification $\times 100$)

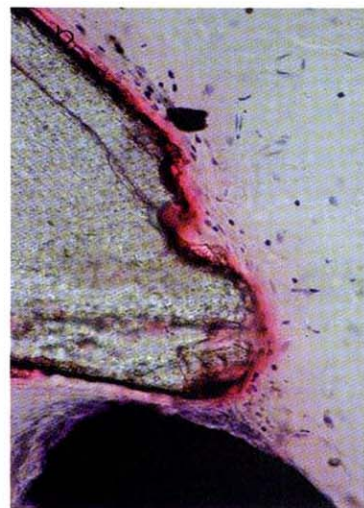
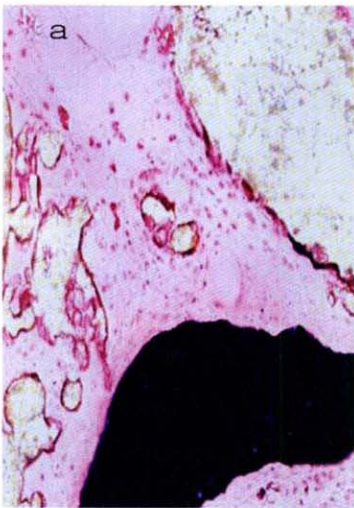


Figure3. Tissue response of CPP granules. A few inflammatory cell infiltrated in 12month, (a) 3month (b) 12 month (H&E stain, original magnification $\times 400$)

Differences in new bone formation between CPP granules with chitosan and CPP granules with Na_2O and demineralized freeze-dried bone(DFDB) sites were studied for statistical purposes with one-way

ANOVA test. The independent samples t-test used to compare the results between 3month and 12 month, P values $< 0,05$ were considered statistically significant.

Table1. % area of New Bone Formation in experimental sites

Group	Mean % of regenerated area	
	3 month	12 month
1	44.04±4.7*	58.87±2.0*
2	62.64±6.1*	69.21±3.0*
3	66.48±1.0*	70.16±2.8*
4	80.46±5.7*	82.07±7.5*

* : Mean ± S,D

: There were significant differences % area of new bone formation between group 1 and the other groups(p < 0.05).

Group1 : control

Group2 : demineralized freeze-dried bone

Group3 : CPP chitosan granule

Group 4: CPP granule with Na₂O

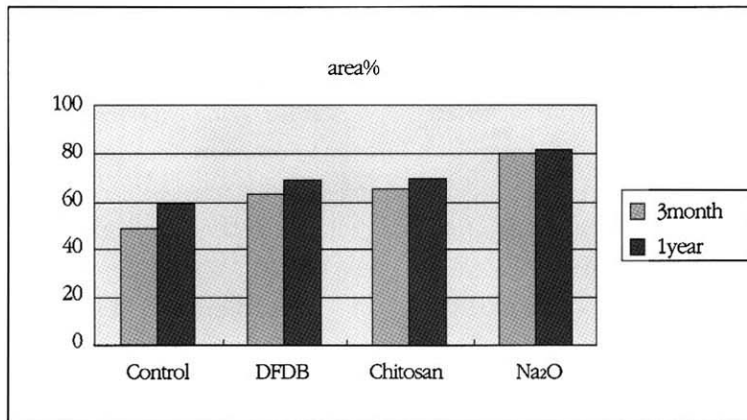


Figure 4. Area % of new bone formation

III. Results

1. Histologic findings

All control and experimental sites healed uneventfully with no clinical evidence of inflammatory response. Histologically, notable lack of fibrous encapsulation and presence of newly formed bone (figure 1). Like 3 month results, irregular and smooth surfaces were present between CPP granules and newly formed bone (figure 2). The bone showed large marrow spaces with no evidence of active bone formation. Unlike 3 month results, there was

little osteoclastic activity evidenced by few multinucleated giant cells, but osteoblast-like cells were lining newly formed bone as like 3 month (figure 3).

2. Measurement of newly formed bone

The one-way ANOVA showed that all the treatments produced statistically significant higher gain in new bone formation than did the control group (p < 0.05). There was no significant difference between experimental sites with implant material compared between 3 month and 12 month (Table 1). There was a slight increase of newly formed bone at

12 month but no significant difference (figure 4).

IV. Discussion

The trials carried out to test bone graft substitutes in the repair of periodontal lesions have employed a range of experimental protocols. This study to compare the new synthetic bone substitutes in fresh extraction socket⁹⁾. The overall results of this study generally parallel an earlier report based on 3-month findings. The healing time was extended to 12 month in anticipation that the control sites might respond with substantially better osseous repair. Such was not the case, if any, improvement in the control sites occurred. Similarly, CPP granules grafted sites showed little overall change in mean % with longer evaluation period. CPP granules in experimental sites showed very slow degradation pattern evidenced by little multinucleated giant cell compared 3 month finding. That may pose a problem for the replacement of these devices with new bone and may alter the mechanical properties of the newly formed bone.

In present study, chitosan matrix were employed for enhancement of osteoconductivity and degradation rate of CPP granules. But contrary to our expectation, no significant differences between CPP granules with Na₂O and CPP granules with chitosan were found in matrix degradation and the amount of induced new bone by the two. These findings indicate that the addition of chitosan to CPP granules probably does not evoke any effects to degradation of the matrix or healing of defect, and also does not serve any additional osteoconductive effect.

The knowledge of the degradation mechanism is far from complete, but it is generally known that the hydrolytic degradation of CPP is due to the disruption of hydrolyzable -P-O-P bonds in CPP by

water molecules²⁶⁾. Bunker reported on the mechanism and kinetics of dissolution for polyphosphate ceramics and that the dissolution of polyphosphate ceramics is congruent independent of ceramic composition, the pH, or the sample surface/solution volume ratio (S/V)²⁷⁾. It is known that protons enhance the dissolution rates of polyphosphate ceramics, but the hydrated polyphosphate released in solution limits the dissolution²⁸⁾. In this study, addition of Na₂O may increase the biodegradation rate initially, but during the degradation, that retards the biodegradation. So optimal percentage of Na₂O and other additive component will be investigate in future study.

In this study, regardless of its additive components, CPP granules showed osteoconductivity and biocompatibility, but they exhibited very slow degradation rate. As bone substituting materials, CPP is available in various forms and its degradation rate is controllable, so in future study, we investigate the optimal forms of CPP and additive components to increase the degradation rate.

V. Conclusion

1. CPP granules showed very slow degradation rate.
2. There was significant difference between CPP granules with chitosan, CPP granules with Na₂O and control group ($p < 0.05$).
3. There was no significant difference between CPP granules with chitosan and CPP granules with Na₂O ($p > 0.05$).

VI. References

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꿀이식재로서의 Calcium Polyphosphate 평가: 동물 연구

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치주조직재생을 도모하기 위한 전통적인 시술방법으로는 여러 가지 꿀이식재를 이용한 꿀이식술이 오래 전부터 이용되고 있다. 이번 실험의 목적은 장,단기간의 신생골의 형성과 CPP의 흡수를 비교하여 꿀이식재로서 Calcium Polyphosphate(CPP)를 평가하는 것이다. 이번 실험에 사용된 CPP는 무수 $\text{Ca}(\text{H}_2\text{PO}_4)_2$ 를 condensation 하여 무결정의 $\text{Ca}(\text{PO}_3)_2$ 를 얻고 이를 용융하고 냉각시킨후 분쇄하여 얻은 것으로 키토산이나 Na_2O 를 첨가한 후 3세된 비글견에 이식하여 관찰하였다. 양성 대조군으로 동결탈회건조골을 이용하였다. 조직학적으로 3개월 소견과 같이 섬유조직의 게재없이 신생골의 형성이 관찰되었다. 12개월 후의 신생골의 형성은 3개월 결과에 비해서 동결탈회건조골이나 키토산, Na_2O 를 넣은 CPP 과립에서 더 많은 비율로 나타났다. 음성 대조군과 이식재를 넣은 군간에는 유의성이 있는 것으로 나타났고($p < 0.05$), 또한 키토산을 첨가한 CPP 과립과 Na_2O 를 첨가한 CPP 과립 사이에는 신생골의 형성에 유의성이 없었다. 이식한 CPP 과립의 경우 흡수소견이 3개월 결과에 비해 크게 증가하지 않았다. 이번 실험에서는 첨가물에 상관없이 CPP 과립은 골유도성과 생체적합성을 보였다. 그러나 흡수속도가 매우 느려 신생골로 대체되는 여부를 알 수 없었다. 향후 연구에서는 흡수속도를 증가시킬 수 있는 적당한 CPP 형태와 첨가물을 밝혀내어야 할 것이다.

주요어: Calcium Polyphosphate, 골유도성, 생체 적합성, 키토산, Na_2O , 신생골형성, 흡수성