# THE EFFECT OF INCINERATED TOOTH PARTICLES ON OSTEOGENESIS ACCORDING TO PARTICLE SIZE

Lee, Kyu-Chil., D. D. S. Yoon, Chang-Keun., D. D. S., M. S. D., Ph. D.

Department of Prosthodontics, College of Dentistry, Chosun University.

#### I. INTRODUCTION

It is important to choose a material which is not only biocompatible with, but also resembles the tissue for which it is substituted. Hydroxylapatite (HA) belongs to this category. Since Hency<sup>5)</sup> showed that it was possible to generate tight bonds between bone and hydroxylapatite, HA has been the focus of recent studies. In the past 3 years, HA has come into widespread use as alveolar ridge augmentation, tooth root substitutes, repair of periodontal lesions, and other emerging dental applications.

On the other hand, an investigation by Cho et al.<sup>3)</sup> demonstrated that incinerated tooth ash was mainly composed of hydroxylapatite and a small amount of white—lockite. Their conclusion was that the tooth ash powder had high compatibility and bioadhesiveness in vitro and in vivo. Hong et al.<sup>3)</sup> also reported potential use of the tooth ash root as a root substitute for preservation of the alveolar ridge. Concerning the form, there were a few controversies. Shapoff et al.<sup>13)</sup> and Jarcho et al.<sup>7)</sup> suggested that small particles enhance osteogenesis. However many researchers reported good clinical results of plug type implantation.<sup>4,10,15)</sup>

The objectives of the present study were to observe the osteogenesis according to particle size, when 40 mesh and 200 mesh particle tooth ash and Calcitite were implanted in the fresh

extracting sockets in beagle dogs.

# II. MATERIALS AND METHODS

Calcitite\* (40 mesh) and tooth ash powder were used in this study. The tooth ash powder was made by the following method. Teeth with small cavities or caries-free were obtained from the department of oral surgery in the Dental School Infirmary, Chosun University. Carious material and calculus were removed from the teeth and cleaned with an ultrasonic cleaner. The teeth were incinerated in a furnace at 900°C and ground in a motar and pestle for amalgam so that the powder would pass through a 200 mesh sieve. A cylindrical type of tooth ash 1 cm high and 1 cm in diameter was made under 1000 Kg/cm<sup>2</sup> of pressure using these 200 mesh tooth ash particles. The cylindrical tooth ash was still grayish in color and sintered again in a porcelain furnace at 1100°C until it was pure white. Each sintered mass was pulverized again an divided into particles of 200 mesh and 40 mesh by sieves.

Eight young adult beagle dogs (6 to 8 months old and weighting 12.0-17.7kg) were divided into four groups according to sacrificing schedule. 1.5cc of Rampun\*\* was injected intramuscularly and then each dog was anesthetized with an appropriate amount of intraveneous Ketamin HCL (40 ml/kg).\*\*\* Two premolars of the right and

left mandibles and maxillae were removed by means of odontectomy. The upper and lower right sockets were left to heal without implant and it served as control site for the implanted sites. Calcitite particles were implanted in the fresh extracting sockets on the left mandibles and maxillae. 40 mesh tooth ash were implanted on the left mandibles and maxillae, and 200 mesh tooth ash on the right mandibles and maxillae. The powders mixed with saline solution were plugged in the sockets by amalgam carrier and plugger, the extracting wound edges were sutured with 4-0 silk after implantation was done. A antibiotic, Acopex\*\*\*\* 250mg was administered intramuscularly to prevent infection. A fluid diet containing rice and milk was supplied for 3 days after surgery. Healing processes were carefully observed clinically and the dogs were sacrificed at 1st, 2nd, 3rd, and 4th weeks after implantation. The extracted and implanted sites were resected in block and Softex radiographs were taken. For the histological observation the tissue specimens were fixed with 10% formalin. The fixed specimens were decalcified in Plank Rychol solution and invested with paraffine. The histologic slices were made at 4-6  $\mu$ m in thickness and stained with hematoxylin-eosin.

#### III. RESULTS

#### 1. Clinical findings

Clinical inspection of both jaws after implantation showed redened spots around the suture silk but normal healing occurred in the extracting sockets at both implanted and control sites within 3 days after implantation.

The alveolar ridge after four weeks was thorough-

ly covered with normal gingival epithelium and no inflammation was seen during the whole experimental period.

# 2. Roentgenographic findings

The particulate materials in the sockets were easily interpreted on roentgenographs immediately after implantation. The particles filled the sockets from crest to bottom. Sometimes particles in a few sockets were lost at or below the crestal margin within the first 2 weeks of implantation. The materials were no longer lost after 2 weeks and the increased density of extracted socket was seen and the upper border of the extracting sockets became smooth with continuation and contour. The Calcitite and 40 mesh particles of tooth ash were loosely arranged in the sockets but 200 mesh was well condensed. (Fig. 1,2,3 and 4)

#### 3. Histologic findings

An overview of histologic specimen (under X 10) showed that implanted sites were covered with oral epithelium form two or three weeks depending on specimens. The alveoli of the control group were normally filled with matured bony trabeculation at four weeks (Fig. 5a and Fig. 6a). Otherwise there was no bone in the center of the alveolus socket but the opening of the alveolus was covered with newly formed bone in the specimen of 200 mesh tooth ash. (Fig. 5b and Fig. 6b). The specimens of 40 mesh tooth ash and calcitite disclosed that there was no bony closure of the openings showing scattered particles above crestal margin. (Fig. 5c, d and 6c, d) However the implanted sites showed delay of bony growth into the center compared with the control site. The implanted particles were surrounded with connective tissue in 40 mesh tooth ash and Calcitite was occurred in the alveolus under the crestal margin. On the other hand the particles of 40 mesh tooth ash and Calcitite over the crestal

<sup>\*</sup> Calciteck Inc.

<sup>\*\*</sup> Bayer, Korea

<sup>\*\*\*</sup> Yuhan, Korea

<sup>\*\*\*\*</sup> Yuhan, Korea

<sup>\*\*\*\*\*</sup> CM BM-2, Jira, Japan

margin were interfaced with dense connective tissue until four weeks. The osteoid increased progressively fusing with each other and varying stages of maturity (lamellae, lacunae containing osteocytes, and Haversian systems) were observed. No signs of inflammation or untoward reaction were noted. The space occupied by Calcitite appear white, which means complete decalcification of 40 mesh tooth ash were not clear and the indistinctive fine mesh like tissue was observed.

#### IV. DISCUSSION

It is well known that hydroxylapatite, allograft material elicits no foreign body reaction and that it has a close chemical and crystal resemblance to bone mineral. In terms of the molecular level ashed tooth powder most closely resembled to tooth and bone. The biocompatibility of ashed tooth power was proved by Cho et al.<sup>3)</sup> and Hong et al.<sup>6)</sup> In the present study two kinds of tooth ash particles were used, one is 40 mesh and the other is 200 mesh.

It was suggested by Shapoff et al. 13) that small particles enhanced osteogenesis. Jarco et al. 7) also noted that more bone deposition was disclosed on particulate implantes than on plug implants. Bell 1) stated that the implantation of particles was not only clinically an expedient procedure but had also none of the postoperative problems, such as loss of implantation, migration of the implant, submucosal prominence, dehiscence and surgical maintenance or resubmergences encountered with cone implantation. These facts were the rationale to choose a particulate type in the study.

Shapoff et al<sup>13)</sup> attempted to determine if particle size was a factor to be considered in the osteogenesis of freeze—dried bone. The conclusion of their study showed that small particles (100–200  $\mu$ m) enhanced osteogenesis compared to large particles (1000–200  $\mu$ m). In an earlier study,

Mowlem<sup>9)</sup> suggested that small particles of bone might enhance the osteogenic effect of an autogenous cancellous bone graft. Robinson<sup>12)</sup> hypothesised that small particles of bone were more rapidly resorbed and enhanced osteogenesis. Rivault et al.<sup>11)</sup> and Jonck<sup>8)</sup> also reported that fine bone shavings  $(12-25 \ \mu m)$  could induced osteogenesis.

The results of this study showed that 40 mesh particles of tooth ash and Calcitite enhanced osteogenesis when compared to fine particles (200 mesh) of tooth ash. These findings were not coincidence with the studies mentioned previously.

On contrary Urist 14) demonstrated that freeze-dried bone of 250-420 µm inhibited cartilage formation and bone induction and better results were obtained when larger particles of 100 to 200 µm were used. There has been very little agreement about particle size. This might be due to different materials used in each experiments. It was stated by Jarcho et al. That this better result of particulate implant sites was related to mechanical stabilization. But they did not state definite particle size. In general, Calciteck, Inc. 15) supplied 20-40 mesh for alveolar ridge augmentations and finer 40-60 mesh for filling of periodontal lesions. Jarcho et al. suggested small particles were more rapidly stabilized by invading fibroblasts and osteoblasts than the more bulky one. This conclusion was based on that mechanical stabilization was an important prerequisite for osseous formation. In the present study 40 mesh tooth ash and Calcitite enhanced osteogenesis in the same rate but no calcification occurred in the 200 mesh sites. The data from this study suggested that large 40 mesh particles were more compatible with osteogenic activity than fine 200 mesh.

Because fine 200 mesh tooth ash formed mass like bulky root type in extracting sockets, vascularization and invading fibroblasts and osteoblast were inhibited. Conclusively this study proved us potential use of 40 mesh tooth ash as a substitute for pure hydroxylapatite.

The long term observation, however, should be conducted and the fate of tooth ash particles and the effect on preservation of alveolar ridge should then be determined.

#### V. CONCLUSION

A short—term study was conducted to evaluated the effect of particle size on osteogenic activity of ashed tooth powder allografts and to compare them with dense synthetic hydroxylapatite, Calcitite, Experimental implantations were performed using 200 and 40 mesh ashed tooth particles, and 40 mesh Calcitite particles in fresh extraction sockets of beagle dogs.

The following conclusions were drawn by clinical, radiographic and histologic evaluation.

- 1. No untoward tissue reaction was observed in any of the cases.
- 2. The osteogenic activity of 40 mesh tooth ash appeared makedly higher than that of 200 mesh tooth ash.
- 3. 40 mesh tooth ash particles and Calcitite enhanced the osteogenesis, but delayed calcification compared with the control group.
- 4. The osteogenic activity between 40 mesh tooth ash particles and Calcitite was the same.

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### 국문요지

## 치아회분의 입자 크기에 따른 골형성에 대한 영향

조선대학교 치과대학 보철학교실

# 이규칠・유창근

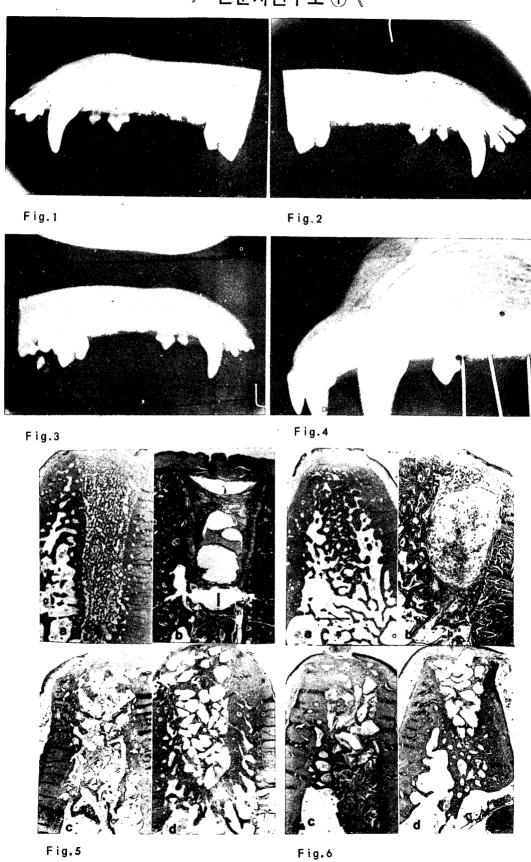
본 실험의 목적은 치아 회분이 입자 크기에 따라 골형성에 어떠한 영향을 주며 순수 합성수산화 apatite인 calcitite 와는 어떠한 차이가 있는가를 평가하기 위하여 수행하였다. 이를 위하여 200 mesh와 40 mesh의 치아 회분말과 40 mesh calcitite를 잡종견의 신선한 발치와에 매식후 1주, 2주, 3주, 4주에 각각 희생하여 임상, X-선상 및 현미경적으로 관찰한 바 다음과 같은 결론을 얻었다.

- 1. 모든 경우에 조직의 거부반응이 없었다.
- 2. 40 mesh치아 회분의 경우가 200mesh의 치아회분의 경우 보다 골 형성 능력이 현 저히 높았다.
- 3. 40 mesh 치아회분과 calcitite 모두 비교군에 비하여 골 형성이 지연되었다.
- 4. 40 mesh치아 회분과 calcitite의 골 형성 능력은 유사하였다.

#### Legend of Photographs

- Fig. 1. Radiograph of Calcitite implantation site at 1 week.
- Fig. 2. Radiograph of 40 mesh tooth ash implantation site at 1 week.
- Fig. 3. Radiograph of 200 mesh tooth ash implantation site at 1 weeks.
- Fig. 4. Radiograph of 40 mesh tooth ash implantation site at 4 weeks.
- Fig. 5. Photomicrograph of cross section at 3 weeks, a: control, b: 200 mesh tooth ash, c: 40 mesh tooth ash, d: Calcitite. (hematoxylin and eosin stain, magnification 10X).
- Fig. 6. Photomicrograph of cross section at 4 weeks, a: control, b: 200 mesh tooth ash, c: 40 mesh tooth ahs, d: Calcitite, (hematoxylin and eosin stain, magnification 10X).
- Fig. 7. Photomicrograph of control site at 1 week, showing osteoid foci growing from the base and around bone of extracting socket (hematoxylin and eosin stain, magnification 40X).
- Fig. 8. Photomicrograph of 200 mesh tooth ash at 1 week, showing same features as control but delaying osteogenesis. (Hematoxylin and eosin stain; magnification 40X).
- Fig. 9. and 10. Photomicrographs of 40 mesh tooth ash and Calcitite at 1 week, showing that osteogenesis was highly active from around bone of extracting socket.
- Fig. 11. and 12. Photomicrographs of 40 mesh tooth ash and Calcitite at 3 weeks; showing that 40 mesh tooth ash and Calcitite enhanced osteogenesis and in particular direct bony fusion was seen in tooth ash site. (homatoxylin and eosin stain, magnification 40X).
- Fig. 13. and 14. Photomicrograph of 40 mesh tooth ash and Calcitite at 4 weeks, highly matured bone and direct fusion were seen in both sites.

# 》 논문사진부도 ①《



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# 》논문사진부도 ②《

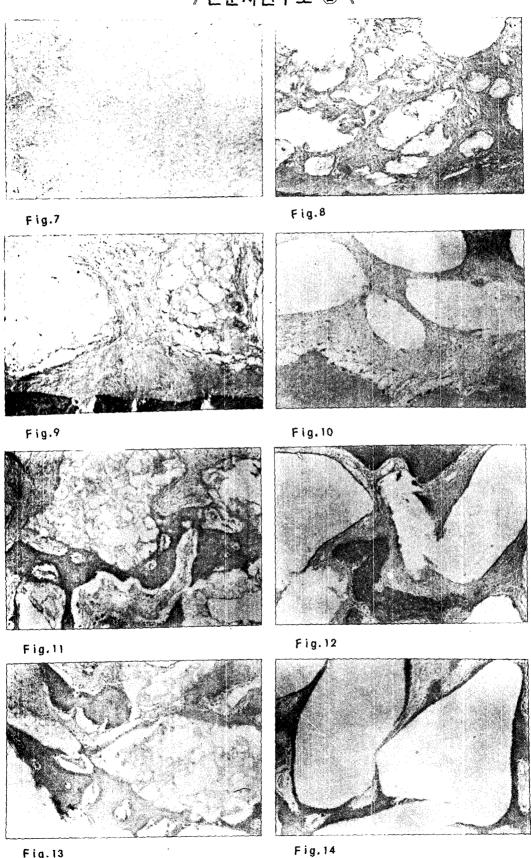


Fig. 13