

Bone Tissue Formation in Extraction Sockets from Sites with Advanced Periodontal Disease: A Histomorphometric Study in Humans

Jae-Jin Ahn, DDS, MS, PhD¹/Hong-In Shin, DDS, PhD²

Purpose: To investigate postextraction bone formation over time in both diseased and healthy sockets.

Materials and Methods: Core specimens of healing tissues following tooth extraction were obtained at the time of implant placement in patients treated between October 2005 and December 2007. A disease group and a control group were classified according to socket examination at the time of extraction. The biopsy specimens were analyzed histomorphometrically to measure the dimensional changes among 3 tissue types: epithelial layer, connective tissue area, and new bone tissue area. **Results:** Fifty-five specimens from sites of previously advanced periodontal disease from 45 patients were included in the disease group. Another 12 specimens of previously healthy extraction sockets were collected from 12 different patients as a control. The postextraction period of the disease group varied from 2 to 42 weeks. In the disease group, connective tissue occupied most of the socket during the first 4 weeks. New bone area progressively replaced the connective tissue area after the first 4 weeks. The area proportion of new bone tissue exceeded that of connective tissue by 14 weeks. After 20 weeks, most extraction sockets in the disease group demonstrated continuous new bone formation. The control group exhibited almost complete socket healing after 10 weeks, with no more new bone formation after 20 weeks. **Conclusions:** Osseous regeneration in the diseased sockets developed more slowly than in the disease-free sockets. After 16 weeks, new bone area exceeded 50% of the total newly regenerated tissue in the sockets with severe periodontal destruction. In the control group, after 8 weeks, new bone area exceeded 50% of the total tissue. INT J ORAL MAXILLOFAC IMPLANTS 2008;23:1133-1138

Key words: advanced periodontal disease, bone formation, coronal corticalization, extraction socket healing, timing of implantation

Studies concerning healing of human extraction sockets have found that sockets are filled with new bone, by as much as two thirds, in 40 days and completely filled with new bone in 10 weeks.¹⁻⁵ Those studies focused mainly on the microscopic tissue changes of healing sockets rather than quantitative analysis of different tissue types.

Recently, Cardaropoli et al⁶ reported a long-term animal experiment on extraction socket healing in dogs where the amount of new bone was measured

for the first time. New mineralized bone occupied 48% of the extraction socket on day 14, and 88% of the extraction socket was filled with bone on day 30.⁶

Following tooth extraction, the periodontal ligament (PDL) loses its functionality and disappears. However, the remnants of PDL cells differentiate into a variety of cell types, including fibroblasts, osteoblasts, and osteoclasts.^{7,8} There are a few studies that suggest that PDL fibroblasts have osteoblastlike properties.^{9,10} Lin et al¹¹ found that PDL fibroblasts actively proliferate after tooth extraction, migrate into the coagulum, form dense connective tissue, and differentiate into the osteoblasts that form new bone during socket healing. Therefore the state of the PDL and the remaining socket wall would be the main influential factors for the osseous regeneration.

Information about healthy socket healing cannot be directly applicable to the actual surgical situation of teeth missing as a result of severe periodontal destruction.¹²⁻¹⁹ Some authors compared the healing

¹Affiliated Professor, School of Dentistry, Kyungpook National University, Korea; Private practice, Daegu, South Korea.

²Professor, Chief, Institute for Hard Tissue and Bio-tooth Regeneration, Department of Oral Pathology, School of Dentistry, Kyungpook National University, Daegu, South Korea.

Correspondence to: Dr Jae-Jin Ahn, 43-13, Daebong-dong, Jung-gu, Daegu, 700-809, South Korea. Fax: +82-53-427-2285. E-mail: ahn@dr-ahn.co.kr

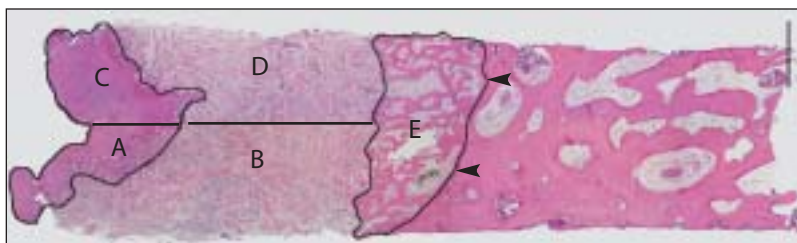


Fig 1 Measurement example. A = thickness of epithelium (1.01 mm here); B = thickness of connective tissue (2.83 mm); C = epithelial area (3.2 mm,² 17.4%); D = connective tissue area (11.1 mm,² 59.6%); E = new bone area (4.3 mm,² 23%). Area of total newly regenerated tissue = 18.6 mm² (100%). Arrowheads indicate demarcation between new bone and old basal bone in this 6-week specimen.

pattern of healthy sockets with that of sockets modified with bone substitute and/or membrane.^{20–24} However, the internal histomorphologic changes of the human socket recovering from severe periodontal destruction have not been studied as they apply to implant dentistry.

The healing socket consists of 3 new tissue components: epithelium, connective tissue, and bone tissue. Interactive dynamic changes take place between these 3 components during the healing period. The aim of this study was to investigate the amount of new bone tissue that develops during the postextraction period and to compare the healing patterns in disease-free extraction sockets with that in sockets with severe periodontal destruction.

MATERIALS AND METHODS

The study protocol was approved by the Kyungpook National University Hospital ethics review committee, and written informed consent was obtained from all subjects. Cores were removed from healing extraction sites at the time of implant placement to evaluate the healing pattern histologically between October 2005 and December 2007. Those were obtained from both periodontally involved sockets and from essentially healthy sockets. A disease group and a control group were classified and recorded according to socket examination at the time of extraction.

Harvesting of Specimens

The category of advanced periodontal disease comprised severe tooth mobility with deep pocket, chronic periodontal abscess, large periapical abscess or granuloma, persistent sinus tract, radiograph showing no lamina dura and destruction in the interseptal bone of molars, etc. At the time of extraction, a simple flapless technique was used, and curettage was not done. The remaining bony walls and the depth of defect were not measured, but it was confirmed clinically that all surrounding walls were severely destroyed. One or 2 extraction site biopsy specimens were taken from each subject. The biopsy core was taken from the center of the healing socket using a trephine bur (2.8 × 22 mm). The biopsy site was then

prepared for implant placement. It was intended that each core specimen should have 4 tissue components in a single piece: new epithelium, new connective tissue, new bone tissue, and old basal bone (Fig 1). During retrieval, some portions of the specimens were damaged, and these data were not included.

For the control group, core specimens were obtained from disease-free extraction sockets in the same way as in the disease group. The causes of extractions were tooth fracture, endodontic failure, or caries. The surrounding alveolar walls were intact.

Histologic Examination

All the biopsy specimens were fixed in 10% buffered formalin and demineralized in 10% ethylenediaminetetraacetic acid solution, except for the control mucosa. A 7- μ m-thick longitudinal section, representing the central part of each core specimen, was stained with hematoxylin and eosin (H&E) for histologic examination. The histologic section was examined using a light microscope (Olympus BX51, Japan), and photographs were taken with the equipped camera.

Histomorphometric measurement of the tissue image was performed using the software Image & Microscope Technology (iMT Technology, Daejeon, Korea) (Fig 1). Areas of measurement were as follows.

- Thickness and area of epithelial layer
- Thickness and area of connective tissue: collagen fiber–dominant soft tissue existing between the epithelium and the new bone area
- Area of new bone tissue: woven bone, osteoid, and adjoining vascular fibrous connective tissue
- Total area of newly regenerated tissue: all tissue above the old basal bone within the obtained core (Fig 1).

Statistical Analysis of Data

Means and standard deviations were calculated for each item measured (ie, each type of tissue). A 2-way analysis of variance was used to evaluate the statistical significance. For those measurements with $P < .05$, a paired t test was used to evaluate the difference between each postextraction period within the disease group, while an unpaired t test was used to evaluate the differences between the 2 groups.

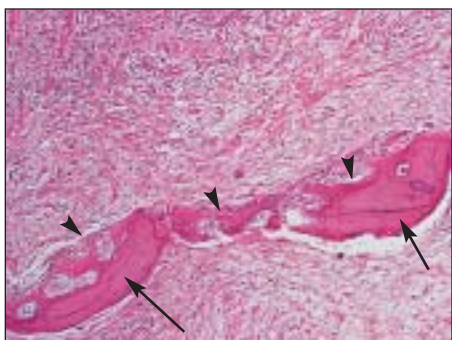


Fig 2 In this 2-week specimen (disease group), 2 bone fragments (arrows) existed separately from the host bone. In these fragments some lacunae were vacant. New woven bone and osteoid were lined by active osteoblasts (arrowheads) (H&E; $\times 100$).

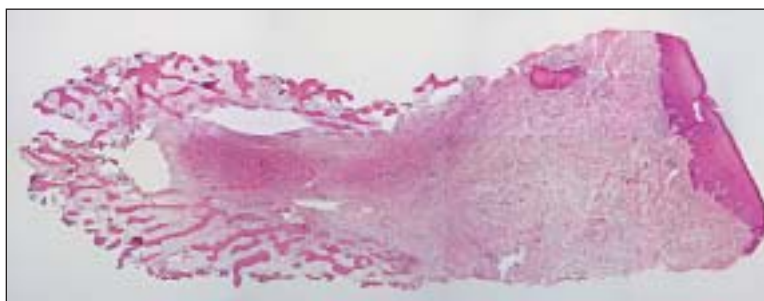


Fig 3 In this 8-week specimen (disease group), new woven bone growing vertically toward the center forms a well-arranged meshwork. New bone area increases from the base and sides of the socket (H&E; $\times 40$).

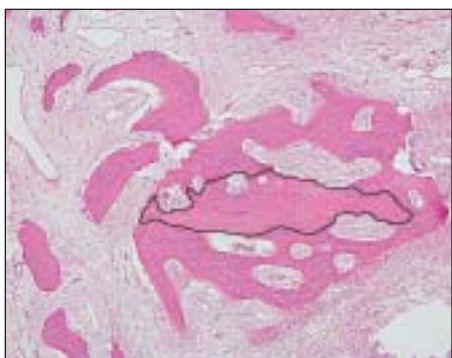


Fig 4 In this 9-week specimen (disease group), the nonvital bone fragment (inside line) is completely intermingled with the new woven bone (H&E; $\times 100$).

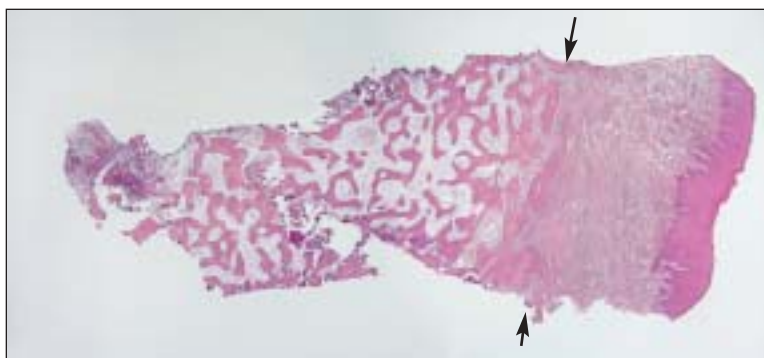


Fig 5 In this mature healing socket (16 weeks, disease group) the trabeculae are interconnected in a meshwork pattern. The horizontal trabeculae form a hard tissue bridge (arrows), namely coronal corticalization, which appears at the crestal margin of the socket (H&E; $\times 40$).

RESULTS

Disease Group

A total of 55 specimens from 45 patients (25 men and 20 women, aged 30 to 85 years [mean 55.9 years]) was included in this group. Teeth had been extracted 2 to 42 weeks prior to specimen retrieval. Biopsy samples were obtained from 27 maxillary molars, 7 maxillary premolars, 16 mandibular molars, 4 mandibular premolars, and 1 maxillary incisor.

Control Group

Twelve core specimens of previously healthy extraction sockets were collected from 12 patients (7 men and 5 women, aged 46 to 75 years [mean 59.1 years]). Teeth had been extracted 2 to 46 weeks prior to specimen retrieval. Biopsy samples were obtained from 1 maxillary molar, 5 maxillary premolars, 4 mandibular molars, and 2 mandibular premolars.

Microscopic Observations

Disease Group. 2 to 4 Weeks. New epithelial cells developed quickly from the adjacent normal epithelium, which covered most of the extraction surface. Most of the extraction site was filled with a mixture of granulation tissue and fibrous connective tissue. The characteristic feature in this healing period was the presence of vital or nonvital bone fragments in the healing tissue. Such fragments were observed in 6 of the 9 specimens (Fig 2).

5 to 10 Weeks. Immature trabeculae from the base and the socket walls were growing vertically toward the center of the socket, forming an interconnected meshwork (Fig 3). Vital or nonvital bone fragments were completely intermingled with new woven bone (Fig 4).

11 to 20 Weeks. In some mature healing sockets, a newly formed hard tissue bridge, namely woven bone representing coronal corticalization, was seen closing the coronal aspect of the socket (Fig 5).

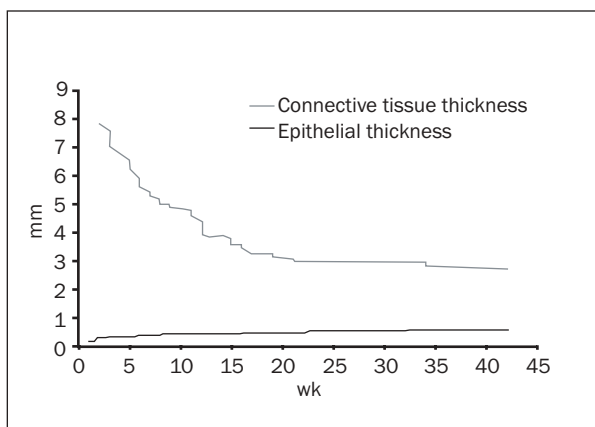


Fig 6 Thickness of epithelium and connective tissue in the disease group (n = 55).

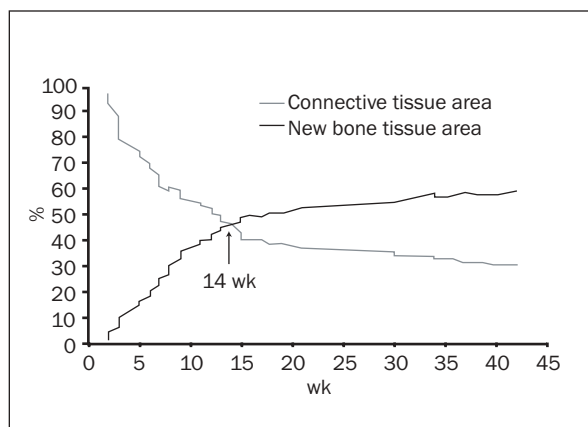


Fig 7 Area proportion of new bone tissue and connective tissue in the disease group (n = 48).

Table 1 Area Proportion (%) of New Bone and Connective Tissue in the Total Newly Regenerated Tissue Area in the Sockets at Different Time Points

Group/tissue	2-4 wk	5-10 wk	11-20 wk	≥ 21 wk
Disease group				
New bone tissue	1.5 ± 1.7* (n = 9)	31.5 ± 10.7* (n = 14)	52.1 ± 10.1* (n = 17)	56.1 ± 8.0 (n = 8)
Connective tissue	93.4 ± 4.6 (n = 9)	61.7 ± 11.4 (n = 14)	39.8 ± 8.8 (n = 17)	37.3 ± 8.2 (n = 8)
Control group				
New bone tissue	15.6 ± 7.5** (n = 3)	52.3 ± 9.0** (n = 3)	69.6 ± 6.2** (n = 3)	65.9 ± 4.0 (n = 3)
Connective tissue	75.6 ± 10.9 (n = 3)	40.3 ± 7.5 (n = 3)	21.2 ± 8.4 (n = 3)	27.5 ± 4.0 (n = 3)

P* < .05 between the different periods within the disease group; *P* < .05 between the disease and the control group of the same period.

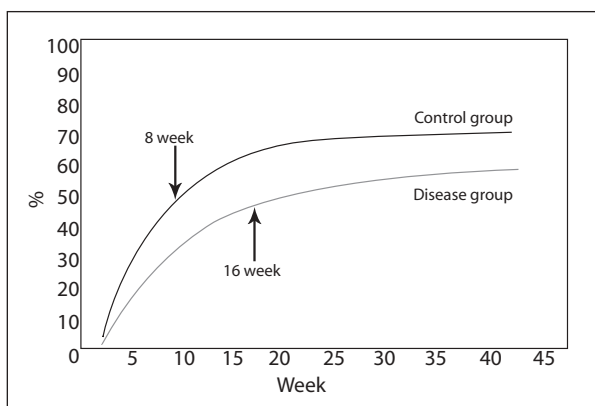


Fig 8 Comparison of new bone tissue area between the disease group (n=48) and the control group (n=12). Arrows indicate the points when new bone tissue areas exceed 50% of the space.

21 to 42 Weeks. A lamellar structure was not visible in new bone, but hematopoietic bone marrow was identified in a few cases.

Coronal corticalization was demonstrated when osseous regeneration of an extraction socket was completed. Coronal corticalization was observed in 8 of the 39 specimens that had healed for at least 10 weeks.

Control Group. Coronal corticalization was identified in all 8 specimens that had healed for more than 10 weeks.

Histomorphometric Findings

Disease Group. Linear Measurement. The epithelial layer changed least among the 3 tissue types, and the thickness of connective tissue decreased gradually over time (Fig 6). The mean thickness of epithelium was 0.45 ± 0.23 mm (n = 55).

Area Measurement. Connective tissue occupied most of the socket during the first 4 weeks after extraction. New bone tissue was observed in a very small proportion at 2 weeks. The new bone area had progressively replaced the connective tissue area after 4 weeks. Meanwhile, the connective tissue area decreased in an inverse proportion to the new bone area (Table 1). The proportion of new bone area exceeded that of the connective tissue area at 14 weeks, and the new bone area increased up to 42 weeks to occupy 60% of the space at its maximum (Figs 7 and 8).

Control Group. The mean percentage of new bone area of the control group (n = 12) was much greater than that of the disease group. The new bone area reached a maximum of 70% of the space by about 20 weeks (Table 1, Fig 8).

DISCUSSION

The healing of an extraction socket can be influenced by many factors, including general health, age, local disease, tooth location, and time since extraction.^{25–27} If a subject is healthy and an extraction socket has no local disease, all sockets seem to undergo a similar course of healing, which was identified in earlier histologic studies.^{1–6}

The findings of the present investigation demonstrated that the healing pattern of the diseased socket was more complicated and unpredictable than that of the healthy socket. It was thought that the defect of the socket walls and absence of the PDL would result in decreased bone formation. However, new bone formation of the diseased extraction socket occurred, albeit much more slowly than in the disease-free socket ($P < .05$, Table 1).

The origin of osteoblasts was studied using a cell-labeling technique in the rat extraction socket by Lin et al.¹¹ The result indicated that PDL fibroblasts are the major contributors to the osteoblast population involved in new bone formation, and the endosteal and paravascular fibroblasts play only a minor role in the early healing. Further research is necessary regarding the level of remaining bone walls as well as the presence of the PDL and their influence on the healing process.

Of interest is the presence of vital or nonvital bone fragments, which appeared separately from the host bone. Such fragments were observed in 14 of the 55 diseased specimens, but none were found in the healthy sockets. These might be regarded as fragments that had detached from the host bone as a consequence of chronic irritation from bacterial toxins. New woven bone and active osteoid were forming on these isolated bone fragments (Fig 2). Such bone fragments were found more frequently in earlier stages of healing, but at later points it was difficult to identify those fragments because of intermingling with new bone (Fig 4). Vital or nonvital bone fragments might play a role like a nucleus of ossification, or they might serve as autogenous bone graft in the healing process. Accordingly, it is cautiously suggested that unnecessary instrumentation in the extraction site might result in the removal of these advantageous vital or nonvital bone fragments and could diminish the healing potential of the surrounding walls.

Coronal corticalization was first observed by Ohnishi et al²⁸ in an experimental rat wound with guided bone regeneration, and Cardaropoli et al⁶ found a hard tissue bridge consistently covering the marginal portion of extraction sites in dog specimens obtained after 60 days of healing. In the present study, coronal corticalization was identified in only 8

of 39 disease group specimens with at least 10 weeks of healing. In contrast, in the control group, coronal corticalization was identified in all 8 specimens older than 10 weeks. The presence of coronal corticalization is regarded as a strong indicator for completion of bone formation in the extraction site because it means that the defect has regained the external cortical architecture of the bone. This information about coronal corticalization and new bone formation following extractions could be useful in determining the optimal timing of implant placement.

CONCLUSIONS

The present human study demonstrated that the healing pattern of diseased sockets is more complicated and unpredictable than that of healthy sockets. New bone formation in extraction sockets with previously advanced periodontal disease developed more slowly than in disease-free sockets. The percentage of new bone area exceeded that of the connective tissue area by 14 weeks. After 16 weeks, the new bone area exceeded 50% of the total newly regenerated tissue in sockets with severe periodontal destruction. In healthy sockets, new bone area exceeded 50% of the total tissue after 8 weeks.

ACKNOWLEDGMENTS

This work was supported by a Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (MOST) (No. M10646020001-06N4602-00110).

REFERENCES

1. Amler MH, Johnson PL, Salman I. Histological and histochemical investigation of human alveolar socket healing in undisturbed extraction wound. *J Am Dent Assoc* 1960;61:32–44.
2. Amler MH. The time sequence of tissue regeneration in human extraction wounds. *Oral Surg Oral Med Oral Pathol* 1969;27:309–318.
3. Boyne PJ. Osseous repair of the postextraction alveolus in man. *Oral Surg Oral Med Oral Pathol* 1966;21:805–813.
4. Evian CI, Rosenberg ES, Coslet JG, Corn H. The osteogenic activity of bone removed from healing extraction sockets in humans. *J Periodontol* 1982;53:81–85.
5. Avery JK. *Oral Development and Histology*. Philadelphia: BC Decker, 1988:282–290.
6. Cardaropoli G, Araújo M, Lindhe J. Dynamics of bone tissue formation in tooth extraction sites. *J Clin Periodontol* 2003;30:809–818.
7. Somerman MJ, Archer SY, Imm GR, Foster RA. A comparative study of human periodontal ligament cells and gingival fibroblasts in vitro. *J Dent Res* 1988;67:66–70.

8. Yamashita Y, Sato M, Noguchi T. Alkaline phosphatase in the periodontal ligament of the rabbit and macaque monkey. *Arch Oral Biol* 1987;32:677–678.
9. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. *J Periodontol Res* 1991;26:144–154.
10. McCulloch CA. Origins and functions of cells essential for periodontal repair: The role of fibroblasts in tissue homeostasis. *Oral Dis* 1995;1:271–278.
11. Lin WL, McCulloch CA, Cho MI. Differentiation of periodontal ligament fibroblasts into osteoblasts during socket healing after tooth extraction in the rat. *Anat Rec* 1994;240:492–506.
12. Botticelli D, Berglundh T, Lindhe J. Hard-tissue alterations following immediate implant placement in extraction sites. *J Clin Periodontol* 2004;31:820–828.
13. Cardaropoli G, Araújo M, Hayacibara R, Sukekava F, Lindhe J. Healing of extraction sockets and surgically produced—augmented and non-augmented—defects in the alveolar ridge. An experimental study in the dog. *J Clin Periodontol* 2005;32:435–440.
14. Chen ST, Wilson TG Jr, Hämmerle CHF. Immediate or early placement of implants following tooth extraction: Review of biologic basis, clinical procedures, and outcomes. *Int J Oral Maxillofac Implants* 2004;19(suppl):12–25.
15. Covani U, Cornelini R, Barone A. Bucco-lingual bone remodeling around implants placed into immediate extraction sockets: A case series. *J Periodontol* 2003;74:268–273.
16. Covani U, Bortolaia C, Barone A, Sbordone L. Bucco-lingual crestal bone changes after immediate and delayed implant placement. *J Periodontol* 2004;75:1605–1612.
17. Paolantonio M, Dolci M, Scarano A, et al. Immediate implantation in fresh extraction sockets: A controlled clinical and histological study in man. *J Periodontol* 2001;72: 1560–1571.
18. Quirynen M, Assche NV, Botticelli D, Berglundh T. How does the timing of implant placement to extraction affect outcome? *Int J Oral Maxillofac Implants* 2007;22:203–223.
19. Rimondini L, Bruschi GB, Scipioni A, et al. Tissue healing in implants immediately placed into postextraction sockets: A pilot study in a mini-pig model. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100(3):e43–50.
20. Araújo MG, Lindhe J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *J Clin Periodontol* 2005;32:212–218.
21. Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. *Clin Oral Implants Res* 2003;14:137–143.
22. Chen ST, Darby IB, Adams GG, Reynolds EC. A prospective clinical study of bone augmentation techniques at immediate implants. *Clin Oral Implants Res* 2005;16:176–184.
23. Iasella JM, Greenwell H, Miller RL, et al. Ridge preservation with freeze-dried bone allograft and a collagen membrane compared to extraction alone for implant site development: A clinical and histologic study in humans. *J Periodontol* 2003;74:990–999.
24. Wilson TG Jr, Carnio J, Schenk R, Cochran D. Immediate implants covered with connective tissue membranes: Human biopsies. *J Periodontol* 2003;74:402–409.
25. Amler MH. Age factor in human alveolar bone repair. *J Oral Implantol* 1993;19:138–142.
26. Amler MH. Disturbed healing of extraction wounds. *J Oral Implantol* 1999;25:179–184.
27. Novaes AB Jr, Marcaccini AM, Souza SLS, Taba M Jr, Grisi MFM. Immediate placement of implants into periodontally infected sites in dogs: A histomorphometric study of bone-implant contact. *Int J Oral Maxillofac Implants* 2003;18:391–398.
28. Ohnishi H, Fujii N, Futami T, Taguchi N, Kusakari H, Maeda T. A histochemical investigation of the bone formation process by guided bone regeneration in rat jaws. Effect of PTFE membrane application periods on newly formed bone. *J Periodontol* 2000;71:341–352.