A Light and Scanning Electron Microscopy Study of Bone Healing Following Inferior Alveolar Nerve Lateralization: An Experimental Study in Rabbits

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Purpose: *The purpose of this study was to evaluate the bone healing kinetics around commercially pure titanium implants following inferior alveolar nerve (IAN) lateralization in a rabbit model.* ***Materials and Methods:*** *Inferior alveolar nerve lateralization was performed in 16 adult female rabbits (*Oryctolagus cuniculus*). During the nerve lateralization procedure, 1 implant was placed through the mandibular canal, and the IAN was replaced in direct contact with the implant. During the 8-week healing period, various bone labels were administered for fluorescent microscopy analysis. The animals were euthanized by anesthesia overdose, and the mandibular blocks were exposed by sharp dissection. Nondecalcified samples were prepared for optical light and scanning electron microscopy (SEM) evaluation.* ***Results:*** *SEM evaluation showed bone modeling/remodeling between the IAN and implant surface. Fluorochrome area fraction labeling at different times during the healing period showed that bone apposition mainly occurred during the first 2 weeks after implantation.* ***Conclusions:*** *The results obtained showed that bone healing/deposition occurred between the alveolar nerves in contact with a commercially pure titanium implant. No interaction between the nerve and the implant was detected after the 8-week healing period. Appositional bone healing occurred around the nerve bundle structure, restoring the mandibular canal integrity and morphology.* Int J Oral Maxillofac Implants 2008;23:457–462

Key words: *bone healing, dental implants, inferior alveolar nerve lateralization, light microscopy, scanning electron microscopy, titanium*

The success of implant treatment has often been reported to be above 90% in regions where appropriate bone quantity and quality are present. However, clinical situations where low bone quantity and quality are present generally require therapy prior to implantation, which lengthens therapy time and increases treatment morbidity.

Several surgical techniques specific to the posterior mandible have been employed to allow implant placement in regions of inadequate bone height and width.[1-5] These surgical procedures include bone grafting, distraction osteogenesis,[6,7] implant placement toward the lingual aspect of the bone,[8] placement of implants of smaller diameter, and inferior alveolar nerve (IAN) lateralization or transposition.[9–13]

Jensen and Nock[14] described a technique for the placement of endosseous implants in conjunction with the IAN transposition. Several other investigators have described modifications[8–20,22,23] of IAN lateralization or transposition techniques. Although these techniques present high implant survival rates, this technique and its variations have fallen from favor in clinical practice because of the negative impact of trans-surgical nerve manipulation (temporary or permanent postsurgical neurologic disturbances).

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Table 1 Sequence of the Polyfluorochrome Sequential Labeling and Days of Application

Days postsurgery	Substance	Injection (mg/kg)
14	Alizarin	3 g/100 mL + 2 g $NaHCO_3$
21	Alizarin	3 g/100 mL + 2 g $NaHCO_3$
28	Calcein	3 g/100 mL + 2 g $NaHCO_3$
35	Calcein	3 g/100 mL + 2 g $NaHCO_3$
42	Tetracycline	3 g/100 mL + 2 g $NaHCO_3$
49	Tetracycline	3 g/100 mL + 2 g $NaHCO_3$
56	Sacrifice	

Several investigators have described clinical neurologic disturbances after the IAN procedure related to various techniques.[8-20] A previous study[24] demonstrated that bone healing occurred around the nerve in the first weeks after surgery, restoring the morphology of the mandibular canal. However, no study has been conducted to demonstrate the influence of implants placed during IAN lateralization on healing around the IAN of tissue in direct contact with the implant surface.

The objective of this study was to evaluate the bone healing kinetics around commercially pure titanium implants following IAN lateralization in a rabbit model.

MATERIALS AND METHODS

Following approval from the Ethics Committee For Animal Research (University of São Paulo protocol 065/2001), 16 adult female New Zealand white rabbits weighing ~3 kg (*Oryctolagus cuniculus*) in good systemic health were acquired and kept under a soft diet and water ad libitum. All animals remained healthy throughout the study.

Surgical Procedures

The surgical procedure comprised the surgical placement of 1 commercially pure titanium implant in the posterior aspect of the mental foramen. All surgical procedures were conducted under general anesthesia (intramuscular ketamine injection of 20 mg/kg). A blade was utilized to remove the hair at the surgical region. This was followed by skin decontamination with an antiseptic iodine-based solution. Local anesthesia was induced with lidocaine containing 1:100,000 epinephrine.

For the nerve lateralization procedure, a midline incision from the mandibular symphysis to the hyoid bone was performed for subperiosteal exposure of the basal cortical bone of the mandible. An intraoral procedure was not possible because of the incompatibility between the implant size utilized and physical dimensions of the animals.

The cortical bone surrounding and posterior (to 2 mm) to the mental foramen was removed, and a window 3 mm in height was carefully opened for the IAN exposure. The osteotomy dimensions were recorded, and nerve lateralization was performed. Following IAN lateralization, a commercially pure titanium implant 3.25 mm in diameter and 8.5 mm in length was placed bicortically through the base of the mandible following the manufacturer's instructions. After verification of implant stability, the IAN was carefully repositioned in contact with the implant surface, and standard layered suture techniques were utilized to close the wound.

For the mineral apposition assessment during the 8-week healing period, sequential polyfluorochrome labeling[25] used (Table 1).

Specimen Retrieval and Histologic Evaluation

Eight weeks after the surgery, the animals were euthanized by anesthesia overdose. Following the euthanization, a longitudinal incision was made to expose the animal's heart for perfusion through the ventriculus sinister cordis. Sodium chloride (NaCl) solution was utilized to eliminate the circulating blood, and a modified Karnovsky solution (2.5% glutaraldehyde, 2% paraformaldehyde, 0.1 mol/L sodium phosphate buffered solution with a pH of 7.3) was used for fixation. After perfusion, mandible block sections containing the implants and adjacent structures were removed for dissection.

For 8 blocks, the nerve was carefully exposed and fixed in the same Karnovsky solution utilized for perfusion at 4°C for 24 hours. The samples were then washed in sodium phosphate buffered solution for en bloc scanning electron microscopy (SEM) evaluation. A few specimens were subjected to a decalcification process by means of a 20% chloridric acid solution for 48 hours to preserve structures around the implants.

In the remaining 8 animals, after specimen retrieval, the blocks were fixed in 10% neutral buffered formalin solution. The samples were then washed in running water for 12 hours and dehydrated in a graded series of ethanols from 70% to 99%, remaining for 24 hours in each solution. After dehydration, the samples were immersed in 2 xylene baths for 24 hours and 48 hours, respectively. The blocks were then embedded in a methacrylate solution (Tecnovit VCL, Sigma). After polymerization, the nondecalcified sections were processed according to established cutting and grinding techniques.[26,27]

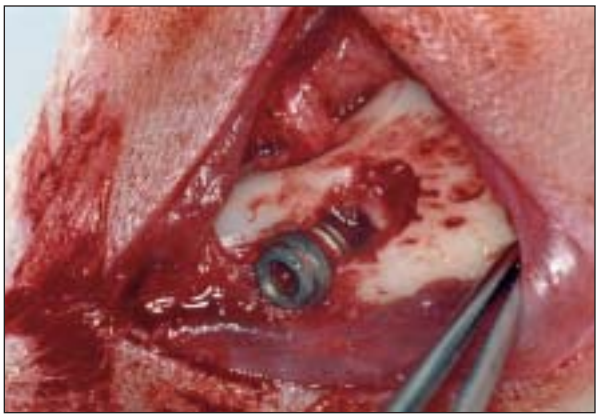

Fig 1 An extraoral incision was performed for nerve lateralization and implant placement. Note the intimate contact between implant and nerve.

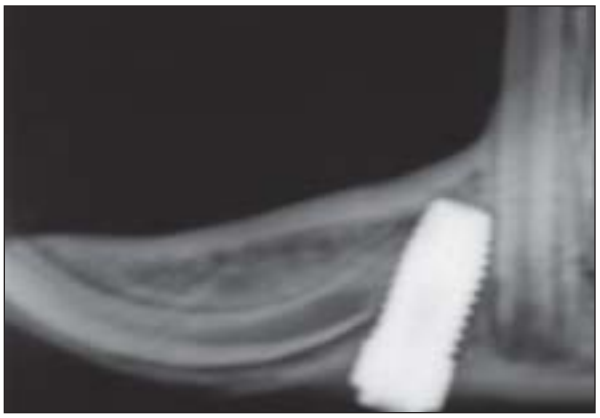

Fig 2 Postsurgical radiograph showing implant location in the rabbit's mandible. Note the bicortical engagement achieved prior to nerve repositioning at the implant surface.

The nondecalcified sections (~ 50 µm thickness) were analyzed under a fluorescent light microscope with filters (Nikkon Eclipse E 1000 Proc. FAPESP 99/10320-3). The amount of fluorescent area fraction labeled at the region between nerve and implant was determined with computer software (Software Image Pro-Plus V. 4.1). The area fraction for the fluorochrome markers administered at different times in vivo was evaluated by 1-way analysis of variance (ANOVA) at the 95% level of significance.

Following area fraction label quantification, the nondecalcified samples were stained by Masson trichrome stain for qualitative evaluation of bone ingrowth between the implant and IAN. Gold sputter-coating of the nondecalcified samples was carried out for SEM analysis (qualitative analysis) of the interaction between bone, IAN, and the implant.

RESULTS

Nerve lateralization and implant surgical placement was performed without complications through the extraoral incision (Fig 1). Figure 2 shows implant bicortical engagement in close proximity to the mental foramen in the mandible.

Postsurgical review and follow-ups showed uneventful healing of the surgical sites for all animals, and no clinical signs of infection, inflammation, or implant exposure were observed. Following euthanization and bone block retrieval, clinical evaluation showed that all implants were stable in the bone blocks.

Light microscopy and SEM analysis of all the specimens showed bone ingrowth between the implants and the neurovascular bundle (Figs 3 and 4). No direct contact between the nerve bundle and implant surface was observed after the 8-week healing period (Fig 3a).

Table 2 Area Fraction for the Tissue Labels Administered in µm²

	Area Fraction Labeled	
Fluorescent marker	Mean	SD
Alizarin	160388	34119[a]
Calcein	60186	33443[b]
Tetracycline	21992	15334[b]

$P < .01$.

Fluorescent microscopy (Fig 3b) revealed large areas of alizarin red labeling in close proximity to the implant surface. Calcein and tetracycline labels were observed in regions between implant surface and nerve and in proximity to the nerve. Histomorphometric analysis showed that the bone around the neurovascular bundle was primarily labeled by alizarin, followed by calcein, and tetracycline in decreasing amounts of percent area fraction labeled (Table 2). The 1-way ANOVA statistics summary for area fraction labeled is presented in Table 2. The area fraction labeled was significantly higher for the alizarin, and was followed by calcein and tetracycline.

Scanning electron microscopy analysis of bone sections with a thickness of ~ 50 µm showed that the nerve structure was well preserved and surrounded by newly formed bone, forming a new canal (Fig 4a). Higher-magnification (Fig 4b) electron micrographs further supported the finding of no contact between the IAN and implant surface due to the presence of new mineralized tissue between them (Fig 4b).

Bone block decalcification enabled the removal of the mandibular basal cortical plate and exposure of the IAN and implant region of interest (Figs 5a and 5b). Removal of the IAN from the bone blocks showed a newly formed mandibular canal bone wall (Fig 5b).

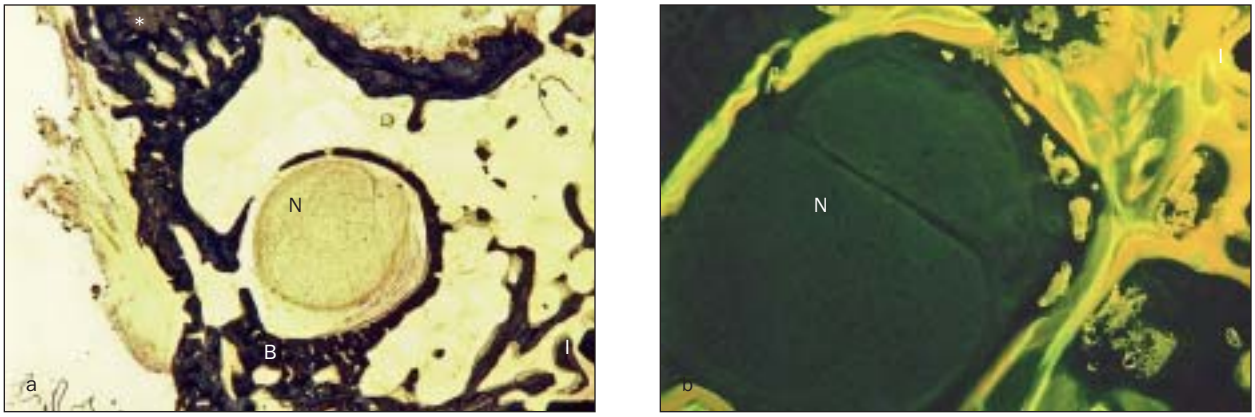

Fig 3 *(a)* Light microscopy showed bone formation (B) between the implant (I) and the nerve (N) (original magnification ×10). Notice bone ingrowth at the area of surgical access to the nerve bundle (*). (Masson trichrome). *(b)* Fluorescent microscopy imaging showed that greater label interdistance occurred between alizarin (red), followed by calcein (green) and tetracycline (orange) (original magnification ×30). This presence of higher MAR labeling between alizarin labels reveal that bone ingrowth occurred primarily over the first weeks of healing (Table 2).

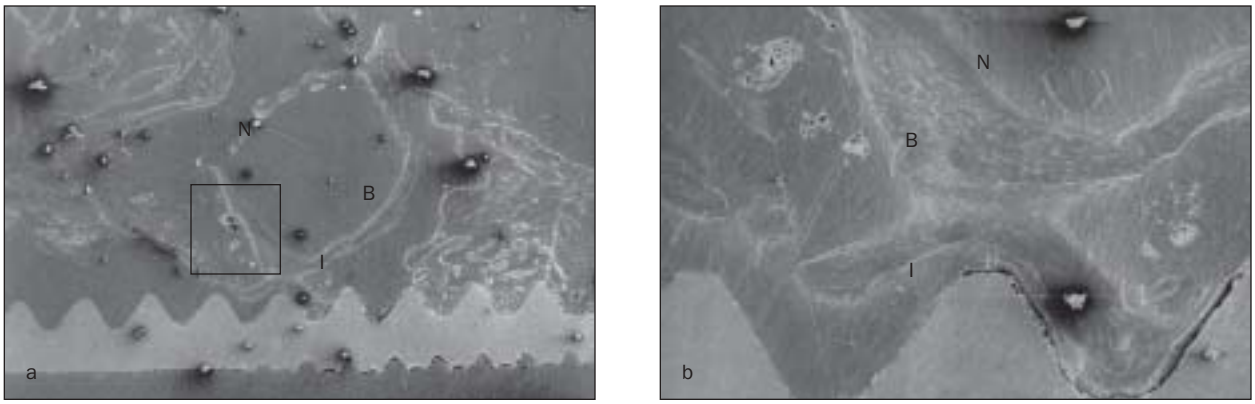

Fig 4 Scanning electron micrographs showing the relationship between the IAN and the implant surface. *(a)* Bone (B) ingrowth between the IAN (N) and implant (I) and *(b)* detailed micrograph showing the IAN (N) surrounded by new bone (B).

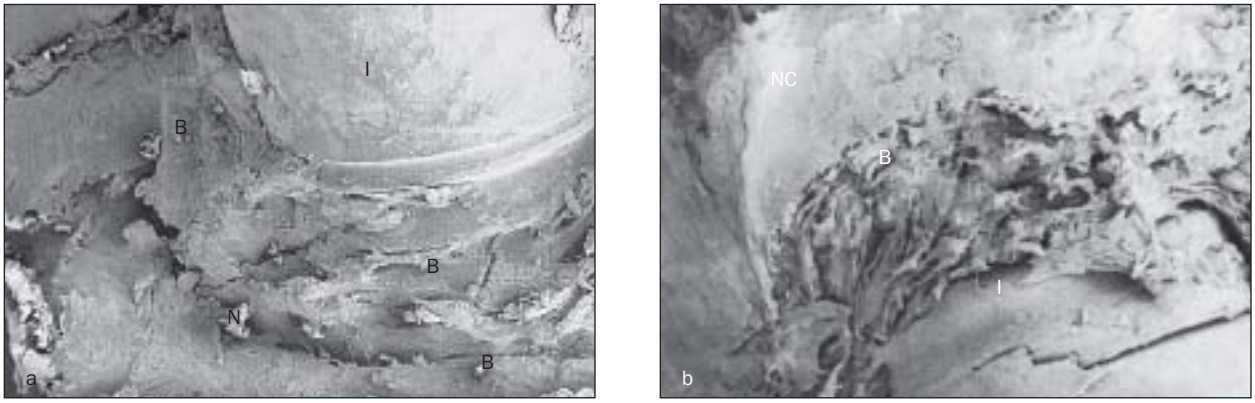

Fig 5 Scanning electron micrographs obtained following cortical selective removal by means of 20% chloridric acid solution. *(a)* The implant (I) and the lateral bone walls (B) of the IAN (N). *(b)* Removal of the IAN revealed the presence of a newly formed mandibular canal bone wall (NC).

DISCUSSION

Lateralization of the IAN was routinely practiced in clinical dentistry prior to implant placement. This surgical technique provides better biomechanical support for single and multiunit prosthetic treatment modalities in the posterior mandible by allowing the placement of longer implants. According to the literature,[9,13] implant survival rates at nerve lateralization sites vary from 93.8% to 100%. However, postsurgical neurologic disturbances have led the technique to fall from favor in clinical practice.

Although clinical research shows that in the majority of the cases nerve impairment is transient,[8,28] the interplay between surgical trauma extension, nerve injury nature and site, and subsequent neuroma formation,[29] added to a lack of knowledge regarding the repair mechanisms of the nerve and bone, has resulted in inconsistent transurgical and postsurgical procedures to avoid or minimize postsurgical nerve damage. Variations in surgical techniques have been addressed in the literature[29–32] with variable success rates concerning oral rehabilitation and sensory nerve re-establishment. The present study aimed to provide information concerning the wound healing of nerve lateralization procedures where the nerve was in direct contact with the implant surface.

The results of the present study showed that modeling/remodeling of bone in close proximity to the direct contact between the IAN and implant surface (Figs 1 and 2) resulted in total isolation of the nerve bundle (Figs 3 to 5). This shows that the wound healing process includes processes to help restore the original presurgical anatomy, avoiding long-term nerve structure contact with the implant surface.

The bone tissue activity evaluated through a sequence of fluorescent labels showed that bone modeling between the IAN bundle and implant surface takes place as early as 14 days postoperatively at high bone mineral apposition rates (Fig 3b). Observation of the label position suggests that the healing occurred first around the implant surface and that bone apposition closer to the IAN followed later in vivo (calcein and tetracycline labels, Fig 3b).

The wound healing pattern observed through fluorescent labels suggests rapid bone formation at the implant surface soon after implantation, which is a desirable feature from several perspectives. First, mechanical stability is achieved at the mandibular posterior region shortly after implant placement (as per observation of the healing at regions away from the IAN-implant surface contact region, where qualitatively comparable results were observed). Second, rapid isolation of the IAN from the implant surface may effectively decrease any potential nerve sensitivity due to contact to the implant surface (ie, thermal sensitivity).

The remodeling observed at regions in proximity to the IAN at later implantation times (calcein and tetracycline) showed that following initial repair and modeling of the surgical site, remodeling leading to the new formation of a mandibular canal wall (Figs 4 and 5) may further support decreases in nerve sensitivity.

CONCLUSION

According to the results obtained with this animal model, it can be concluded that wound healing of bone following nerve lateralization where no barriers were utilized to avoid IAN contact with the implant surface resulted in early isolation of the IAN from the implant surface, which was followed by the re-establishment of the mandibular canal.

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REFERENCES

1. Bartling R, Freeman K, Kraut RA. The incidence of altered sensation of the mental nerve after mandibular implant placement. J Oral Maxillofac Surg 1999;57:1408–1412.
2. Bavitz JB, Harn SD, Hansen CA, Lang M. An anatomical study of mental neurovascular bundle–implant relationships. Int J Oral Maxillofac Implants 1993;8:563–567.
3. Deporter D, Pilliar RM, Todescan R, Watson P, Pharoah M. Managing the posterior mandible of partially edentulous patients with short, porous-surfaced dental implants: Early data from clinical trial. Int J Oral Maxillofac Implants 2001;16:653–658.
4. Ellies LG, Hawker PB. The prevalence of altered sensation associated with implant surgery. Int J Oral Maxillofac Implants 1993;8:674–679.
5. Ellies FG, Smiler DG, Quadland MW, Babbush CA, Krogh PHJ. Inferior alveolar nerve repositioning: Is there cause for concern? Dent Implantol Update 1995;6:30–32.
6. Sawaki Y, Ohkubo H, Yamamoto H, Ueda M. Mandibular lengthening by intraoral distraction using osseointegrated implants. Int J Oral Maxillofac Implants 1996;11:186–193.
7. Hu J, Tang Z, Wang D, Buckley M. Changes in the inferior alveolar nerve after mandibular lengthening with different rates of distraction. J Oral Maxillofac Surg 2001;59:1041–1045.

8. Rosenquist BO. Fixture placement posterior to the mental foramen with transpositioning of the inferior alveolar nerve. Int J Oral Maxillofac Implants 1992;7:45–50.
9. Onstad MS. Repositioning of inferior alveolar nerve for dental implants. Dent Implantol Update 1998;9:53–56.
10. Kahnberg KE, Henry PJ, Tan AE, Johansson CB, Albrektsson T. Tissue regeneration adjacent to titanium implants placed with simultaneous transposition of the inferior dental nerve: A study in dogs. Int J Oral Maxillofac Implants 2000;15:119–124.
11. Hirsch JM, Brånemark P-I. Fixture stability and nerve function after transposition and laterization of the inferior alveolar nerve and fixture installation. Br J Oral Maxillofac Surg 1995; 33:276–281.
12. Rosenquist BO. Nerve transpositioning to facilitate implant placement. Dental Economics 1995;85(10):92–93.
13. Friberg B, Ivanoff CJ, Lekholm U. Inferior alveolar nerve transposition in combination with Brånemark implant treatment. Int J Periodontics Restorative Dent 1992;12:441–449.
14. Jensen O, Nock D. Inferior alveolar nerve repositioning in conjunction with placement of osseointegrated implants: A case report. Oral Surg Oral Med Oral Pathol 1987;63:263–268.
15. Kan JY, Lozada JL, Boyne PJ, Goodacre CJ, Davis WH, Hanisch O. Endosseous implant placement in conjunction with inferior alveolar nerve transposition: An evaluation of neurosensory disturbance. Int J Oral Maxillofac Implants 1997;12:463–471.
16. Yoshimoto M, Allegrini S Jr, Oshiro M, Teixeira V. Lateralização do nervo alveolar inferior em Implantodontia: Relato clínico. Jornal Brasileiro de Clínica Estética em Odontologica 1999;3(17):53–57.
17. Sethi A. Inferior nerve repositioning in implant dentistry: A preliminary report. Int J Periodontics Restorative Dent 1995; 15:474–481.
18. Wagner JR. Alternative methods for repositioning of the inferior alveolar neurovascular bundle. Dent Implantol Update 1994;5:69–72.
19. Sethi A. Repositioning the contents of the inferior alveolar canal to accommodate the root form implants. Dent Implantol Update 1994;5:21–22.
20. Smiler DG. Repositioning the inferior alveolar nerve for placement of endosseous implants: Technical note. Int J Oral Maxillofac Implants 1993;8:145–150.
21. Sethi A. Inferior nerve repositioning in implant dentistry: Clinical report. Implant Dent 1993;2:195–197.
22. Peleg M, Mazor Z, Chaushu G, Garg AK. Lateralization of the inferior alveolar nerve with simultaneous implant placement: A modified technique. 2002;17:101–106.
23. Ferrigno N, Laureti M, Fanali S. Inferior alveolar nerve transposition in conjunction with implant placement. Int J Oral Maxillofac Implants 2005;20:610–620.
24. Yoshimoto M, Konig B Jr, Allegrini S Jr. Bone healing after the inferior alveolar nerve lateralization: A histologic study in rabbits (Oryctolagus cuniculus). J Oral Maxillofac Surg 2004;62(9 suppl 2):131–135.
25. Rahn BA. Die polychrome sequenzmarkierung. Freiburg, Germany: Habilitations-Schrift, 1976.
26. König B Jr, Lopes CC. Bone remodeling analysis after placement of dental implants using polyfluorochrome sequential labeling. Ann Anat 2002;184:241–244.
27. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. J Oral Pathol 1982;11:318–326.
28. Higuchi KW, Folmer T, Kultje C. Implant survival rates in partially edentulous patients: A 3-year prospective multistudy center. J Oral Maxillofac Surg 1995;53:264–268.
29. Miloro M, Halkias LE, Mallery S, Travers S, Rashid RG. Low-level laser effect on neural regeneration in Gore-tex tubes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:27–34.
30. Pitta MC, Wolford LM, Mehra P, Hopkin J. Use of Gore-tex tubing as a conduit for inferioralveolar and lingual nerve repair: Experience with 6 cases. J Oral Maxillofac Surg 2001;59:493–496.
31. Pogrel MA, Maghen A. The use of autogenous vein grafts for inferior alveolar and lingual nerve reconstruction. J Oral Maxillofac Surg 2001;59:985–988.
32. Khullar SM, Brodin P, Barkvoll P, Haanaes HR. Preliminary study of low-level laser for treatment of long-standing sensory aberrations in the inferior alveolar nerve. J Oral Maxillofac Surg 1996;54:2–7.