

**A new bone surgical laser technique: technical aspects and applications in dentistry**

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**1. ABSTRACT**

Ten patients requiring the extraction of a severely-deteriorated molar or premolar before placement of a dental implant for prosthodontic rehabilitation were selected (6 women; 4 men). The sockets were curetted and decontaminated with an 810 nm wavelength diode laser using a 400 micron fiber at close distance (1 mm) from the target area, power setting 2.5 W, pulsed mode (10 msec t-on, 10 msec t-off for five seconds, three repetitions for each bone wall, 30 seconds pause between each irradiation). The socket filled with  $\beta$ -TCP plus Tissucol and primary closure was attempted. In addition all patients were treated with a 810 nm GaAlAs laser, in continuous wave mode, defocused hand-piece, 50 J/cm<sup>2</sup> ( 1W for 50 seconds) after surgery and on days 3, 5, 7 postoperatively. At 18 months after prosthodontic treatment and loading, the implant was stable. Laser therapy, combined with a graft of biomaterial composed of  $\beta$ -TCP and tissucol, prevented alveolar crest resorption following tooth extraction. Formation of new bone of acceptable quality and quantity permitted placement of osseointegrated dental implants.

**2. INTRODUCTION**

Tooth extraction is a very well documented procedure, but healing events within post-extraction sockets may lead to progressive bone resorption (1-2). Because ridge dimensions are so critical, preservation of the alveolar crest after tooth extraction is essential to maintain the vertical and horizontal dimensions of the alveolar ridge (3). A reduction of about 50% in both horizontal and vertical directions has been observed over 12 months, with two-thirds of the reduction occurring in the first 3 months (3-4). The rate and pattern of bone resorption may be altered if pathologic and traumatic processes have damaged one or more of the bone walls of the socket. (4-5). These morphological changes may affect the successful placement and osseointegration of dental implants.

Augmentation of the existing alveolar bone is therefore often necessary to obtain excellent functional and aesthetic restorations using implants. Several studies have evaluated soft and hard tissue healing within extraction sockets from the clinical and histological standpoints,

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**Figure 1.** Periapical radiograph of deteriorated right mandibular first molar before extraction

following tooth removal in humans and animals (6-8) and several biocompatible materials and / or autogenous bone have been used to treat bone atrophy of the alveolar ridge, thus allowing ideal implant placement (3,9-12). Although autogenous bone is still considered the gold standard for grafting procedures, limitations including donor site morbidity from bone graft harvesting techniques (13) have prompted the search for suitable synthetic grafting materials. A variety of bone substitutes are available; these differ in origin, consistency, particle size, porosity and resorption. (14)

Beta-tricalcium phosphate ( $\beta$ -TCP), a synthetic alloplastic material, has been used for bone regeneration in a multiplicity of surgical procedures, with satisfactory clinical and histological results in both animal models (15-16) and human trials (17-18).  $\beta$ -TCP may be a suitable bone substitute that will biodegrade and be replaced by newly-mineralizing bone tissue without fibrous tissue proliferation. (17). Likewise, with the goal of improving healing of large bone defects, the application of various techniques including laser therapy have been studied (19) primarily evaluating the bactericidal effect of various laser systems on periodontopathic bacteria, as diverse *in vitro* studies have been shown (20-23). Clinical investigations have provided proof that the diode laser is an effective tool to treat periodontitis and peri-implantitis. Moritz *et al.* (24) demonstrated that the 805-nm semiconductor laser has a bactericidal effect and helps to reduce inflammation in periodontal pockets. Application of the 809-nm GaAlAs laser is reported to have a lethal effect on anaerobic gram-negative rods found around deteriorating implants (25) and on the bacterial biofilm (26)

Several *in vivo* and *in vitro* studies have also investigated the use of laser therapy in the biomodulation of bone repair, through accepted photochemical and photobiologic effects (27, 19) with the aim of providing patients with more comfortable postoperative recovery and faster healing.

In a recent article, Pinheiro (28) demonstrated that laser photobiomodulation (LPBM) might have a positive effect upon early healing of bone defects treated

with a combination of hydroxyapatite and guided bone regeneration.

The purpose of this study is to describe a new bone surgical laser technique and to show preliminary clinical and histological results in bone reconstruction in dentistry.

### 3. MATERIALS AND METHODS

Ten patients requiring the extraction of a severely deteriorated molar or premolar due to a localized periodontal disease before placement of a dental implant for prosthodontic rehabilitation were selected ( 6 women, 4 men). All patients were non-smokers in good systemic conditions ( no osteoporosis, no diabetes ) and with good oral hygiene. In each case replacement would be through an implant – supported full-veneer crown (Figure 1). The alveolar preservation protocol was approved by the Ethical Review Board of the University of Genoa, and written informed consent was obtained from patients after the risks and benefits had been explained. Data recorded for each patient included age, anatomical location of tooth scheduled for extraction, ridge dimensions before and after regenerative procedures (Table 1).

#### 3.1. Procedure

In all cases, treatment was carried out under local anesthesia (articain 2% with epinephrine 1:100.000) by a single dental surgeon. After administration of local anesthesia, the tooth was extracted using an ultrasonic device with a specific tip to preserve the residual bone (surgybone-silfradent S.r.l.- S.Sofia (FC) - Italy) After tooth extraction an intra-sulcular incision was made with a vertical buccal releasing incision. A full split-thickness flap was elevated to expose the marginal bone to allow visualization and measurement of the alveolar bone level, the socket was thoroughly curetted to remove all inflammatory tissue. A template, fabricated on the study model including one tooth anterior and posterior to the compromised tooth, was used for the vertical measurement to serve as a fixed reference guide. The horizontal ridge width was measured with a standardized periodontal probe at the midpoint of the alveolar crest.

Laser decontamination of the socket was performed with an 810 nm wavelength diode laser (enjoy-Sweden and Martina-Due Carrare, Padova, Italy) using a 400 micron quartz optic fiber. The freshly-cleaved fiber was inserted in the socket and gently moved around the post-extraction site at a slight distance ( 1 mm) from the bone walls ( power setting 2.5 W, pulsed mode 10 msec t-on, 10 msec t-off for five seconds, three repetitions for each bone wall, with 30 seconds pause between each irradiation).

In all cases,  $\beta$ -TCP (Angipore- Sweden and Martina-Due Carrare-Padova) with Tissucol (Baxter S.p.a- Rome-Italy) was placed in the alveolar socket, completely occupying the space from the crest to the apex of the socket. Tissucol was diluted 1:10 and mixed with  $\beta$ -TCP to obtain a sandy consistency. The resultant coagulum was transferred to the socket in small amounts with a sterile biomaterial

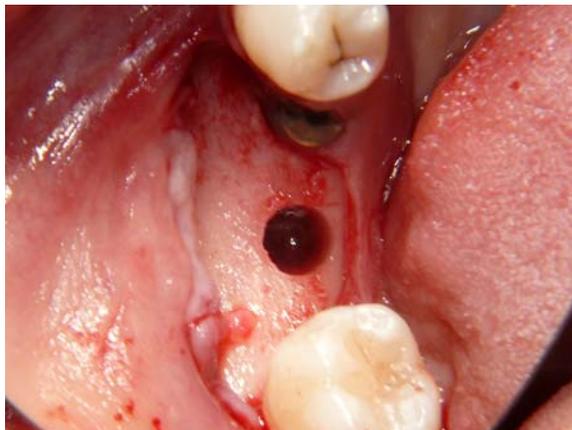
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**Table 1.** Data recorded for each patient included age, anatomical location of tooth scheduled forextraction, ridge dimensions before and after regenerative procedures

N° of patients	Age (years)	Locations	Ridge dimensions, mm, (mean ± sd?)			
			Mesio-buccal		Vertical	
			Before treatment	After treatment	Before treatment	After treatment
10	28 to 63 (43 mean)	4 premolars 6 molars	10.5 +/- 1	8 +/-1.5	0.6 +/-1.4	0.4 +/- 1.5



**Figure 2.** Bone sample collected from the treated extraction socket, during dental implant placement.



**Figure 3.** The bone was found to be smooth and solid when prepared for implant insertion and clinically, no particles of material were visible.

syringe and condensed by manual pressure with a sterile condenser. After one - two minutes the mixture became hard and stable, allowing the flap to be repositioned coronally, in order to achieve tension-free adaptation at closure; it was sutured with a single interrupted resorbable suture on the releasing incision and horizontal mattress sutures on the occlusal part (ethibond excel 4.0- Ethicon- Partica di Mare-Pomezia Roma, Italy).

### 3.2. Postoperative follow-up

Low-level laser irradiation was applied for 60 seconds at the time of surgery and on days 3, 5, 7 postoperatively. The tissues were irradiated with 810 nm GaAlAs laser, continuous wave (CW) 1 W and fluence 50 J/cm<sup>2</sup> using a specific non-contact flat-wave hand-piece held

at 1 cm from the target area, i.e. the soft tissue covering the bone defect. Spot size was 1 cm and exposure time was 50 seconds. The patient was prescribed a course of antibiotics for five days (amoxicillin and clavulanic acid 1 gr. twice daily) and pain medication (paracetamol 1gr. daily) plus mouthwash twice daily (0.12% chlorexidine) with postoperative instructions to continue use for 7 days.

**Follow-up:** Patients were examined at 3, 5 and 7 days, then at 1, 2 and 4 months postoperatively; radiographs were taken at 1 week and 4 months postoperatively. After 4 months, during dental implant placement the custom acrylic template was again positioned to check the distance from the alveolar crest, and the horizontal ridge was again measured by means of a periodontal probe at the midpoint of the alveolar crest. Then a trephine drill, 3 mm inner diameter, 8 mm length, was used to collect a bone sample from the treated extraction socket. (Figure 2).

**Histopathology.** The bone biopsy specimen was prepared for decalcified histological analysis. The surgical specimen was decalcified and subsequently embedded in paraffin. The tissue blocks were sliced into 3-µm-thick sections for routine histological examinations with Hematoxylin & Eosin. The parameters evaluated included the percentage of new bone formation, the percentage of residual graft and the percentage of fibrous tissue.

## 4.RESULTS

### 4.1. Clinical data

Healing was uneventful. By the seventh day the socket was completely covered with gingiva. During the 4 months of observation, no loss of material, no signs of infection, exudation or fistula formation at the area of the extraction were observed; ridge preservation and wound healing were noted. Measurement of the alveolar ridge on the day of implant placement revealed slight horizontal bone resorption, but no significant changes in the vertical dimension of the alveolar ridge. The buccopalatal dimension of the alveolar socket was 10.5 mm +/- 1.0 mm before β-TCP plus Tissucol placement and 8 mm +/-1.5 mm after 4 months. The vertical ridge changes at the buccal and lingual sites were 0.6 mm +/- 1.4 mm and 0.4 mm +/- 1.4 mm respectively. Clinically, no particles of material were visible and the bone was found to be smooth and solid when prepared for implant insertion (Figure 3). Radiographically, by the fourth month of follow-up, the alveolar socket appeared to be filled with radiodense bone tissue except for the most cervical portion of the socket. By 4 months post-extraction, the cervical radiolucency had disappeared and uniform radiodense bone was found throughout the healing extraction socket (Figure 4). At follow-up 18 months after prosthodontic treatment and loading, the implants were stable and surrounded with



**Figure 4.** Periapical radiograph taken 4 months following socket preservation showing complete bone fill of the socket (an implant was placed during surgery in the mesial site, while the distal site was filled with  $\beta$ -TCP mixed with Tissucol).



**Figure 5.** Photomicrograph of a biopsy core taken 4 months after placement of  $\beta$ -TCP mixed with Tissucol. Showing a great deal of active new bone formation associated with residual  $\beta$ -TCP particles.

healthy tissue. There were no complaints or complications during this period of observation.

#### 4.2. Histopathology

The section showed lamellar bone with  $\beta$ -TCP particles incorporated into newly formed bone creating a dense lamellar bone, and normal bone marrow. Histologically, a great deal of active new bone formation was noted (Figure 5). This was as large, mature, lamellar bone. In some areas, new bone deposition was associated with residual  $\beta$ -TCP particles. It appeared that resorbing  $\beta$ -TCP was present as dispersed particles. No residual tissucol was noted in proximity to the  $\beta$ -TCP, and no fibrous tissue or inflammatory cellular infiltration was observed.

### 5. DISCUSSION

Post-extraction vertical and horizontal bone resorption are known events; there are several reasons to consider preservation of the alveolar socket immediately following tooth extraction as a technique of interest.

Various methods have been described to maintain alveolar ridge dimensions after tooth extraction (3,5,6,8-17). Although autogenous bone is still considered the gold standard for grafting procedures, ridge preservation using the Guided Bone Regeneration technique can lead to improve ridge height and width dimensions when compared to tooth extraction alone (10). Other techniques such as grafting bone-substitute materials have also been used for ridge preservation (3,5,9,12) with promising results.

The peculiarity of the technique we are presenting in this article is related to the synergistic effect of different procedures that are commonly used in dentistry, but they have never been applied together i.e the use of a supra-ablative laser light fluence to reduce pathogen contamination of the socket, a graft of  $\beta$ -TCP with Tissucol fibrin glue to achieve a ridge preservation and the use of sub-ablative laser light fluence to induce a photo-biomodulation in the immediate tissue area that would enhance healing.

In our patients, the socket after extraction was decontaminated with an 810 nm wavelength diode laser by means of a 400 micron fiber. The fiber was moved along the post-extraction site at a slight distance (1 mm) from the bone walls and  $\beta$ -TCP with Tissucol fibrin glue was placed in the socket, occupying the space from the crest to the apex of the socket.

The antimicrobial efficacy of diode laser light has been evaluated in many *in vitro* studies. (23,24,25). Moritz *et al.* (24) demonstrated that the 805-nm semiconductor laser has a bactericidal effect and helps to reduce inflammation in periodontal pockets. In general all these studies conclude that laser therapy combined with conventional treatment promotes better results in the periodontal treatment.

Application of the 809-nm GaAlAs laser is reported to have a lethal effect on anaerobic gram-negative rods found around deteriorating implants (25) and on the bacterial biofilm (26), however laser irradiation can induce biological damage in tissues through photothermal and photomechanical interactions. The type and response depends on the optical and thermal properties of the tissue, laser wavelength, selected power density and the pulse duration. The absorption by the target tissue of laser energy dictates the extent of thermal damage (22).

Due to the possible contra-indications of near IR photonic energy on bone, in our clinical study the fiber was moved along the post-extraction site at a slight distance (1 mm) from the bone walls, thus allowing the blood within the socket in providing thermal relaxation, yet predisposing to light scattering and reduction in energy density and establishing a preferred laser tissue interaction with target chromophores (hemoglobin).

The clinical and histological results in all cases showed that the total volume of newly-formed bone was of good quality and quantity, including both mineralized bone and bone marrow.

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It's reported that  $\beta$ -TCP particles in the extraction socket are osteoconductive, and also that when particles of  $\beta$ -TCP are mixed with the blood clot and surrounded by the bone walls of the alveolar socket, osteogenic cells, including undifferentiated mesenchymal stem cells start migrating from the existing bone surface between and over the surface of the particles, stimulated mostly by an adhesive glycoprotein (fibronectine), a component of the forming blood clot. (17,18) However, significant resorption of the  $\beta$ -TCP particles may be expected 3–6 months after placement (15).

At 4 months after applying the alveolar socket preservation technique, the small amount of the  $\beta$ -TCP graft remaining did not compromise placement of the osseointegrated dental implant. Moreover, the  $\beta$ -TCP particles had become incorporated into the new-formed bone, creating dense lamellar bone. Biodegradation of  $\beta$ -TCP comes about by both osteoclastic activity and chemical dissolution by tissue fluids (29).

Histologically, the data obtained showed  $\beta$ -TCP to be a highly-porous material and that dissolved  $\beta$ -TCP particles can be incorporated into the newly mineralized lamellar bone.

Treatment with low-level laser (810 nm GaAlAs) in continuous wave irradiation, applied for 50 seconds after surgery and on days 3, 5, 7 postoperatively, probably also had a biomodulating effect on bone repair; this is achieved through its photochemical and photobiologic properties, as documented by Pinheiro (27) and AboElssad (19).

The mechanism that leads to a positive effect of laser light on different tissues remains not fully understood, as there are possibilities to be considered, such as stimulation by the laser light of porphyrins and cytochromes to increase cellular activity, increasing the concentrations of adenosine 5'-triphosphate (ATP) and alkaline phosphatase (ALP) and the release of calcium (Ca) (27).

This study suggests that the new laser technique described, combined with a graft of biomaterial composed of  $\beta$ -TCP plus Tissucol can prevent alveolar crest resorption following tooth extraction and permit formation of new bone with placement of osseointegrated dental implants. However, further studies to investigate this surgical and post-surgical protocol and to test the hypothesis in isolation (ex: no antibiotics and laser decontamination to prove if the uneventful healing is due to laser therapy or due to the use of antibiotics or non-augmented socket left to heal without primary flap closure vs augmentation and flap but without laser use vs augmentation with flap and laser use) will be needed.

## 6. REFERENCES

1.A. Tallgren: The continuing reduction of the residual alveolar ridges in complete denture wearers: a mixed-longitudinal study covering 25 years. 1972. *J Prosthet Dent* 89, 427–435 (2003)

2.M.G. Araújo, F. Sukekava, J.L.Wennström, J. Lindhe: Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *J Clin Periodontol* 32, 645–652 (2005)

3. A. Barone, N. Aldini, M.Fini, R. Giardino, J.L.Calvo Guirado, U. Covani: Xenograft versus extraction alone for ridge preservation after tooth removal: a clinical and histomorphometric study. *J Periodontol* 79,1370-1377 (2008)

4. S.T.Chen, T.G. Jr Wilson, C.H. Hämmerle: Immediate or early placement of

implants following tooth extraction: review of biologic basis, clinical procedures, and outcomes. *Int J Oral Maxillofac Implants* 19 (Suppl),12–25 (2004)

5. B.M.B. Brkovic, H.S. Prasad, G. Konandreas, R.Milan, D.Antunovic, G.K.B. Sandor, M.D. Rohrer: Simple preservation of a maxillary extraction socket using beta-tricalcium phosphate with type 1 collagen: preliminary clinical and histomorphometric observations. *J Can Dent Associat* 74, 523-528 (2008)

6. G. Cardaropoli, M. Araújo, R. Hayacibara, F. Sukekava, J. Lindhe: 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* 32,435–440 (2005)

7. M. H. Amler: The time sequence of tissue regeneration in human extraction wounds. *Oral Surg Oral Med Oral Pathol* 27, 309-318 (1969)

8. L. Schropp, A. Wenzel, L. Kostopoulous, T. Karring: Bone healing and soft tissue contour changes following single-tooth extraction: A clinical and radiographic 12 months prospective study. *Int J Periodontics Restorative Dent* 23, 313-323 (2003)

9. M. Simion, I. Rocchietta, C. Dellavia: Three-dimensional ridge augmentation with xenograft and recombinant human platelet-derived growth-BB in humans: reports of two cases. *Int J. Periodontics Restorative Dent* 27,109-115 (2007)

10. P. Proussaefs, J. Lozada: Use of titanium mesh for staged localized alveolar ridge augmentation: Clinical and histologic-histomorphometric evaluation. *J Oral Implantol* 32, 237-247 (2006)

11. P.N.R.Nair, J.Schug: Observation on healing of human tooth extraction sockets implanted with bioabsorbable polylactic-polyglycolic acids (PLGA) copolymer root replicas: a clinical, radiographic, and histologic follow-up report of 8 cases. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 97, 559–569 (2004)

12. D. Boix, P. Weiss, O. Gauthier, J. Guicheux, J.M. Bouler, P. Pilet, G. Daculsi, G. Grimandi: Injectable bone substitute to preserve alveolar ridge resorption after tooth

## Running title

extraction: a study in dog. *J Mater Sci Mater Med* 17,1145–1152 (2006)

13. J. Clavero, S. Lundgren: Ramus or chin grafts for maxillary sinus inlay and local onlay augmentation: comparison of donor site morbidity and complications. *Clin Implant Dent Relat Res* 5, 154–160 (2003)

14. A. Palti, T. Hoch: A concept for the treatment of various bone defects. *Implant dent* 11, 73-78 (2002)

15. Z. Artzi, M. Weinreb, N. Givol, M.D. Rohrer, C.E. Nemcovsky, H.S. Prasad, H. Tal: Biomaterial resorption rate and healing site morphology of inorganic bovine bone and beta-tricalcium phosphate in the canine: a 24-month longitudinal histologic study and morphometric analysis. *Int J Oral Maxillfac Implants* 19, 357–368 (2004)

16. A.J. Haddad, S.A. Peel, C.M. Clokie, G.K. Sándor: Closure of rabbit calvarial critical-sized defects using protective composite allogeneic and alloplastic bone substitutes. *J Craniofac Surg* 17, 926–934 (2006)

17. I.R. Zerbo, A.L. Bronckers, G.L. de Lange, G.J. van Beek, E.H. Burger: Histology of human alveolar bone regeneration with a porous tricalcium phosphate. A report of two cases. *Clin Oral Implants Res* 12, 379–384 (2001)

18. I.R. Zerbo, A.L. Bronckers, G. de Lange, E.H. Burger: Localisation of osteogenic cells and osteoplastic cells in porous beta-tricalcium phosphate particles used for human maxillary sinus floor elevation. *Biomaterials* 26,1445–1451 (2005)

19. N.S. AboElsaad, M. Soory, L.M. Gadalla, L.I. Ragab, S. Dunne, K.R. Zalata, C. Louca:

Effect of soft laser and bioactive glass on bone regeneration in the treatment of infra-bony defects (a clinical study). *Lasers Med Sci* 24,387-395 (2009)

20. Y. Ando, A. Aoki, H. Watanabe, & I. Ishikawa: Bactericidal effect of Erbium YAG Laser on periodontopathic bacteria. *Lasers in Surgery and Medicine* 19, 190–200 (1996)

21. C.A. Henry, B. Dyer, M. Wagner, M. Judy & J.L. Matthewa: Phototoxicity of argon laser irradiation on *Porphyromonas* and *Prevotella* species. *J Photochem Photobiol B Biol* 34, 123–128 (1996)

22. D.W. Coffelt, C.M. Cobb, S. MacNeill, J.W. Rapley, W.J. Killoy: Determination of energy density for laser ablation of bacteria. *Journal of Clinical Periodontology* 24, 1–7(1997)

23. M. Kreisler, W. Kohnen, C. Marinello, J. Schoof, E. Langenau, B. Jansen, B. d'Hoedt: Antimicrobial efficacy of semiconductor laser irradiation on implant surfaces. *The International Journal of Oral & Maxillofacial Implants* 18, 706–711 (2003)

24. A. Moritz, U. Schoop, K. Goharkhay, P. Schauer, O. Doertbudak, J. Wernish, W. Sperr: Treatment of periodontal pockets with a diode laser. *Lasers in Surgery and Medicine* 22, 302–311(1998)

25. G. Bach, C. Neckel, Ch. Mall, G. Krekeler: Conventional versus laser-assisted therapy of periimplantitis: a five-year comparative study. *Implant Dentistry* 9, 247–251 (2000)

26. S. Sennhenn-Kirchner, S. Klaue, N. Wolff, H. Mergeryan, M. Borg von Zepelin, H.G. Jacobs: Decontamination of rough titanium surfaces with diode lasers: microbiological findings on in vivo grown biofilms. *Clin. Oral Impl. Res* 18, 126–132 (2007)

27. A.L. Pinheiro, M.E. Gerbi: Photoengineering of bone repair processes. *Photomed Laser Surg* 24,169-178 (2006)

28. A.L. Pinheiro, M.E. Martinez Gerbi, F. Jr. de Assis Limeira, E.A. Carneiro Ponzi, A.M. Marques, C.M. Carvalho, R. de Carneiro Santos, P.C. Oliveira, L.M. Nôia M, Ramalho: Bone repair following bone grafting hydroxyapatite guided bone regeneration and infra-red laser photobiomodulation: a histological study in a rodent model. *Lasers Med Sci* 24, 234-240 (2009)

29. J.X. Lu, A. Gallur, B. Flautre, K. Anselme, M. Descamps, B. Thierry, P. Hardouin: Comparative study of tissue reactions to calcium phosphate ceramics among cancellous, cortical, and medullar bone sites in rabbits. *J Biomed Mater Res* 42,357–367 (1998)

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