

Original Article

Histometric analyses of cancellous and cortical interface in autogenous bone grafting

Henrique Duque Netto¹, Sergio Olate^{2,3}, Leandro Klüppel⁴, Antonio Marcio Resende do Carmo¹, Bélgica Vásquez⁵, Jose Albergaria-Barbosa⁶

¹Dental School, Federal University of Juiz de Fora, Juiz de Fora, Brazil; ²Dental School, Universidad de La Frontera, Temuco, Chile; ³Center for Biomedical Research, Universidad Autónoma de Chile, Chile; ⁴Dental School, Federal University of Ponta Grossa, Brazil; ⁵Univeersidad de Tarapacá, Chile; ⁶Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil

Received June 18, 2013; Accepted July 7, 2013; Epub July 15, 2013; Published August 1, 2013

Abstract: Surgical procedures involving the rehabilitation of the maxillofacial region frequently require bone grafts; the aim of this research was to evaluate the interface between recipient and graft with cortical or cancellous contact. 6 adult beagle dogs with 15 kg weight were included in the study. Under general anesthesia, an 8 mm diameter block was obtained from parietal bone of each animal and was put on the frontal bone with a 12 mm 1.5 screws. Was used the lag screw technique from better contact between the recipient and graft. 3-week and 6-week euthanized period were chosen for histometric evaluation. Hematoxylin-eosin was used in a histologic routine technique and histomorphometry was realized with IMAGEJ software. T test was used for data analyses with $p < 0.05$ for statistical significance. The result show some differences in descriptive histology but non statistical differences in the interface between cortical or cancellous bone at 3 or 6 week; as natural, after 6 week of surgery, bone integration was better and statistically superior to 3-week analyses. We conclude that integration of cortical or cancellous bone can be usefully without differences.

Keywords: Bone defects, bone grafting, animal study

Introduction

Surgical procedures involving the rehabilitation of the maxillofacial region frequently require bone grafts [1]. This graft incorporation happens dynamically through reabsorption and apposition of the new bone tissue [2], and it can be influenced by factors that are inherent to the patient or external factors [3-5].

One factor that can interfere with the incorporation process is the graft's structural characteristic [6] that can be cancellous, cortical or a mixture of them [7]. Medullary grafts are typically revascularized faster involving the entire region, whereas cortical grafts are slower and incomplete [7].

Thus, the bone incorporation is made harder because of the fewer blood vessels in the graft region. This reduces the number of precursor osteoblast cells [8]. As a result, the cortical

graft incorporation process is slower than the cancellous one, and at the end of the process there are still regions that did not undergo any bone tissue formation and they show remaining islands of grafted material [9]. Despite that, several studies show the efficacy for both grafts in bone reconstruction before implant insertion [10-12].

Since that cortical and cancellous bone has morphological and chemical differences [13], the object of this study is to assess autogenous graft block incorporation through a histometric analysis when grafts are of the cortical-medullary or cortical-cortical contact.

Material and method

A descriptive research in an animal model was designed for histological analyses. This research was approved by the Ethic Commission for Animal Experimentation of Campinas State University under protocol number 1343-1.

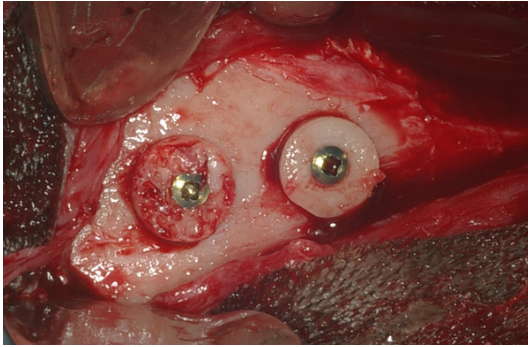


Figure 1. Intraoperative image of graft position being one with cortical-bone-contact recipient and another with cancellous-bone-contact recipient. All graft was fixed with one compression 12 mm screws.

Preoperative

Were selected 6 adult beagles dog (15 kg approximately) for the study. Thirty minutes before the procedure the animals received intramuscular benzathine benzylpenicillin (0.1 ml/kg) and dexamethasone (0.5 mg/kg). Before the surgical procedures, the animals were sedated with the anesthetic inductor ketamine chlorohydrate (0.15 mL/kg) and underwent general anesthesia receiving a 3% pento-barbital sodium (30 mg/kg) intravenous.

Surgical procedure

The bone graft was obtained from de parietal bone. With a trephine of 8 mm diameter was realized the bone cut until the dura mater which preserved with a carefully dissection. With a hand piece (10.000 rpm), two cortical-cancellous grafts (from each side of parietal bone) measuring 5 mm high (2 mm cortical and 3 mm cancellous bone) and 8 mm diameter were removed. Bone fragments that were removed were fixed on the frontal bone region without gap between the graft and recipient area, using 12 mm (1.5 mm system) metallic screws with a compression (lag screw) technique. Recipient site was not decorticalized (**Figure 1**).

Blocks fixtures were randomly; one of them was fixed with the cortical region to recipient contact (Group I) and another one was fixed with the cancellous region to the recipient contact (Group II). After bone graft's stabilization and fixation, periosteum and temporal muscles were approximated with sutures by using absorbable stitch (polyglactin 910). Superficial

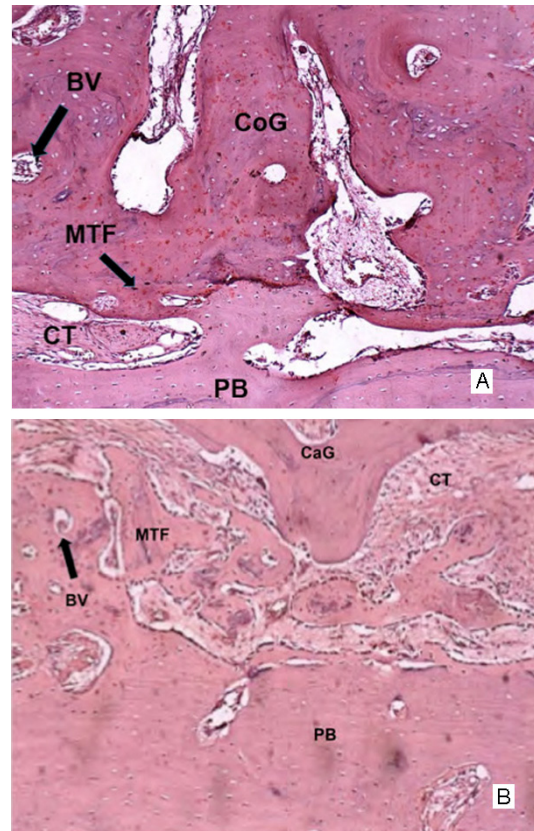


Figure 2. A, B: Histological analysis (10x) in a 3 week period. A: Group I - Cortical interface (CoG). B: Group II - Cancellous interface (CaG). Calcified material inside (MT), blood vessel (BV) and connective tissue (CT) are observed.

planes were sutured with monofilament 4-0 nylon stitch.

Euthanasia

Animals were randomly divided into two groups corresponding to the two euthanasia periods: the first group consisted of 3 animals which were euthanized three weeks after surgical procedure; the second group consisted of 3 animals which were euthanized six weeks after surgical procedure, which corresponds to 1 and 2 months in humans, i.e., (primary and secondary repair according to Turner *et al.* [14]). Euthanasia took place with a 19.1% potassium chloride intravenous overdose until cardiorespiratory arrest. Following this stage, access to the animal's skull was created and the grafted region exposed.

Bone blocks were obtained by cross and coronal sectioning of the bone with a 702 tapered

Autogenous bone graft interface

Table 1. Descriptive histological analyses for both groups in 3 and 6 week

Group	Connective tissue	Inflammatory process	Mineralized tissue formation	Blood vessels	Recipient area
I - 3 week	Present	Absent	Present	Present	Well delimited
II - 3 week	Present	Absent	Present	Present	Well delimited
I - 6 week	Present	Absent	Present	Present	Not delimited
II - 6 week	Present	Absent	Present	Present	Not delimited

Table 2. Cortical vs. cancellous bone interface analyses for both groups on 3 and 6 week period

Period	Interface	n (slides)	Mean (%)	SD (%)	Standard Error Mean (%)	P value
3 week	Cortical	12	11.57	29.80	8.60	0.961
	Cancellous	12	11.62	17.71	5.11	
6 week	Cortical	12	30.26	30.51	8.80	0.365
	Cancellous	12	28.72	49.04	14.15	

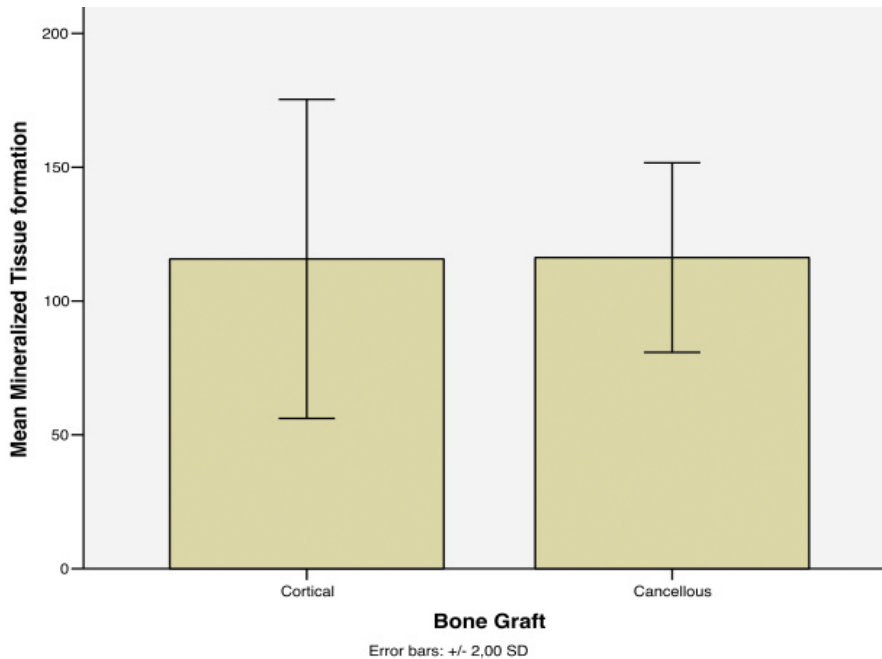


Figure 3. Cortical and cancellous interface standard error deviation values on 3 weeks period.

drill in high-speed turbine under constant saline solution irrigation with a 5 mm to 10 mm safety margin for the previously operated areas.

Histological analysis

Specimens were immersed in 4% buffered formalin. Posteriorly, the specimens were subsequently dehydrated in an ascending series of ethyl alcohols and infiltrated with methylmethacrylate. The hardened blocks were positioned in a microtome (Microslice 2, Ultra Tec, Santa

Ana, CA) and sectioned perpendicularly to in-interface recipient-graft to obtain sections of about 30 μm ; then were stained using hematoxylin-eosinophile for light microscopy analysis. Finally, were obtained 4 slices by each graft area.

Histological analysis was done using a light microscope (DMLB, Leica, Bensheim, Germany), and for histomorphometric analysis the images were acquired in X4 magnification using a camera (CCD IRIS;

Sony, Tokyo, Japan) coupled to a light microscope (BX50; Olympus, Tokyo, Japan). Each area was quantified using the public domain image-processing program IMAGEJ (National Institutes of Health, Bethesda, MD); the type of tissue was identified manually, marked and assigned to a color. In detail, the connective tissue, blood vessel and old bone were described and areas of new bone formation were calculated per total bone defect area and expressed as a percentage [15, 16].

Autogenous bone graft interface

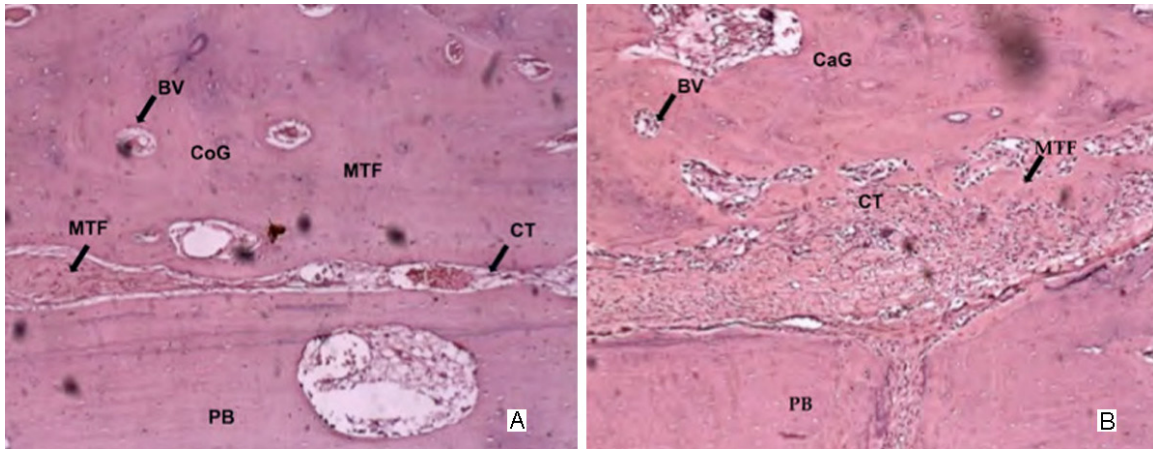


Figure 4. A, B: Histological analysis (10x) in a 6 week period. A: Group I - Cortical interface (CoG). B: Group II - Cancellous interface (CaG). Is observed more calcified material inside (MT) than 3 weeks period, blood vessel (BV) and connective tissue (CT).

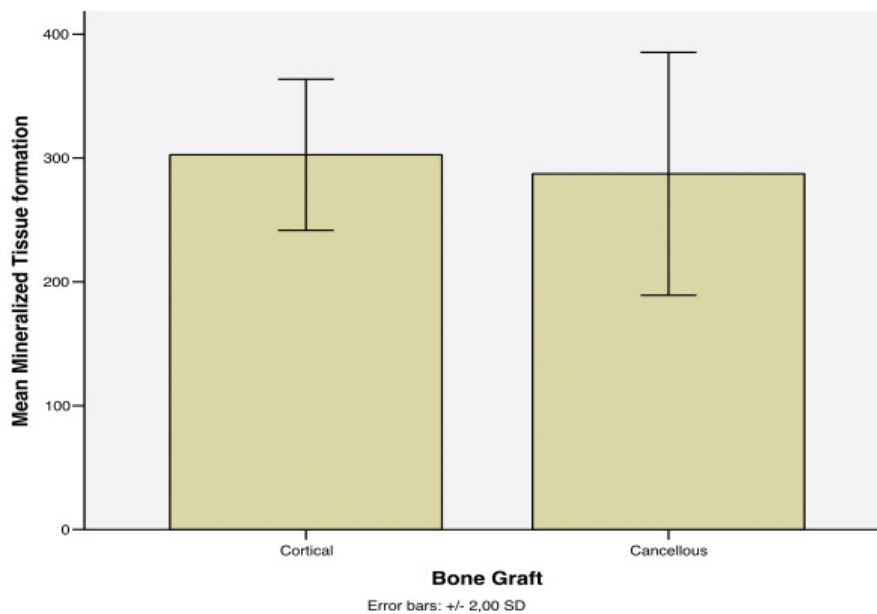


Figure 5. Cortical and cancellous interface standard error deviation values on 6 weeks period.

Statistical analysis

Descriptive statistics were used for histological analysis referring the presence or not of blood vessel, connective tissue and mineralized tissue formation. For 48 examined slices with histomorphometric analysis, t test for paired analysis was used (Biostat 5.0 software) with a 5% significance level.

Results

3 weeks

Descriptive analyses noted on the interface for both group the presence of connective tissue fulfilling the interface between the graft and recipient area, as well as the mineralized tissue formation and blood vessels. The recipient area and graft were well delimited as shows **Figure 2**. **Table 1** resumes the 3 and 6 weeks period histological descriptive analyses.

The mean values for mineralized tissue formation (MTF) on the interface graft and recipient area in the group I (cortical interface) was 11.57% ($\pm 8.60\%$ of standard error mean) and for group II (cancellous interface) was 11.62% ($\pm 5.11\%$ of standard error mean) as shows **Table 2**. It was not observed statistically significant difference ($p=0.961$). Mineralized tissue formation showed statistically the same results in each group (**Figure 3**).

Autogenous bone graft interface

Table 3. Comparison between 3 week vs. 6 week period for cortical and cancellous interface

Interface	Week Period	n (slides)	Mean (%)	SD (%)	Standard Error Mean (%)	P value
Cortical	3 week period	12	11.57	29.80	29.80	0.000
	6 week period	12	30.26	30.51	30.51	
Cancellous	3 week period	12	11.62	17.71	5.11	0.000
	6 week period	12	28.72	49.04	14.15	

6 weeks

Descriptive analyses noted on the interface in both groups the presence of blood vessels and less connective tissue fulfilling the interface between the graft and recipient area, being this region almost fulfilled by mineralized tissue formation. The recipient area and graft were not evidenced as shows **Figure 4**.

The mean values for MTF on the interface graft and recipient area for the group I was 30.26% ($\pm 8.80\%$ of standard error mean) and for the group II was 28.72% ($\pm 14.15\%$ of standard error mean) as shows **Table 2**. It was not observed significant difference ($p=0.365$). MTF showed statistically the same results in each group (**Figure 5**).

When analyzed the comparison between the 6 weeks and 3 weeks periods, both groups, cortical and cancellous interface, presented significant difference ($p=0.000$) for MTF as shows **Table 3**.

Discussion

The use of bone graft for treatment of congenital or acquired bone deficiencies has increased in line with dental implant therapy [17, 18]. This study used autogenous bone, since this is the gold standard because of its properties of osteoconduction, osteoinduction, and osteogenesis. Also, this material is the most used to treat these kinds of defects [19].

The lag screw technique reduces the space between the graft and recipient area [20]. This technique, used on the present research, reduced the gap on the interface graft-recipient area, improving the incorporation of the graft probably because it promotes primary reparation.

Amongst the parameters for assessing graft incorporation is the presence of conjunctive tis-

sue, blood vessels, and newly formed mineralized tissue [21]. This study observed in both groups the same kind of cells in both euthanasia periods. In both cases there was graft incorporation at the 6-week assessment, which corresponds to 2 months in human beings as described by Turner *et al.* [14]. Gerressen *et al.* [22], in a human sinus floor elevation surgery research used iliac bone with pure cancellous in one sinus and a mixture of cortical and cancellous in another sinus; the results demonstrate non differences between density or quality in both maxillary sinus; also, don't show differences between 4 or 7 month of evaluation.

Besides some authors noted that decorticalization of receipt area have influence on bone graft healing, improving their integration [4, 5, 7], we observed that both of interface were integrated without any preparation of recipient area, suggesting that it does not influence on bone graft integration.

Patient factors can influence the graft incorporation process [5, 7]. External factors include the graft's structural characteristics [6], the cancellous-type showing a better repair process when compared to cortical grafts because of a greater angiogenesis. This supposedly facilitates osteoblast precursor cell migration to the graft region, thus speeding up the incorporation due to greater absorption and new mineralized connective tissue formation [7]. Despite that, this study could not observe any statistic difference between the two groups in mineralized tissue formation for both euthanasia periods. This way, it may be suggest that, when analyze the probability to relapse, both grafts are similar, in agreed with other authors suggests [6, 7].

Based in our result, it may be concluded that both the cancellous or cortical graft interface in autogenous block does not influence in bone graft incorporation.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Sergio Olate, Division of Oral and Maxillofacial Surgery, School of Medicine, University of La Frontera, Claro Solar N° 115, Temuco, Chile. Phone: 56-45-325000; E-mail: sergio.olate@ufrontera.cl

References

- [1] Jensen J, Sindet-Pedersen S. Autogenous mandibular bone grafts and osseointegrated implants for reconstruction of the severely atrophied maxilla: a preliminary report. *J Oral Maxillofac Surg* 1991; 49: 1277-87.
- [2] Schliephake H. Bone growth factors in maxillofacial skeletal reconstruction. *Int J Oral Maxillofac Surg* 2002; 31: 469-84.
- [3] Issa MJP, Tiozzi R, Pitol DL, Mello SAS. TGF- β and new bone formation. *Int J Morphol* 2006; 24: 399-405.
- [4] Jensen SS, Broggin N, Hjorting-Hansen E, Schenk R, Buser D. Bone healing and graft resorption of autograft, anorganic bovine bone β -tricalcium phosphate. A histologic and histomorphometric study in the mandible of minipigs. *Clin Oral Impl Res* 2006; 17: 237-43.
- [5] Wang YN, Feng CC, Liu JL, Li F, Fu L, Wang Z. Transforming Growth Factor beta Alleviates Acute Graft-versus-Host-Disease after Allogeneic Bone Marrow Transplantation in Murine Model. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2008; 16: 1135-9.
- [6] Tong L, Buchman SR. Facial bone grafts: contemporary science and thought. *J Craniomaxillofac Trauma* 2000; 6: 31-41.
- [7] Nunamaker DM. Experimental models of fracture repair. *Clin Orthop Relat Res* 1998; 355 Suppl: S56-65.
- [8] Bhatt KA, Chang EI, Warren SM, Lin SE, Bastidas N, Ghali S, Thibboneir A, Capla JM, McCarthy JG, Gurtner GC. Uniaxial Mechanical Strain: An In Vitro Correlate to Distraction Osteogenesis. *J Surg Res* 2007; 143: 329-36.
- [9] Kübler A, Neugebauer J, Oh J, Scheer M, Zöller JE. Growth and proliferation of human osteoblasts on different bone graft substitutes. An in vitro study. *Implant Dent* 2004; 13: 171-9.
- [10] Serra E Silva FM, Ricardo de Albergaria-Barbosa J, Mazzonetto R. Clinical evaluation of association of bovine organic osseous matrix and bovine bone morphogenetic protein versus autogenous bone graft in sinus floor augmentation. *J Oral Maxillofac Surg* 2006; 64: 931-5.
- [11] Silva FM, Cortez AL, Moreira RW, Mazzonetto R. Complications of intraoral donor site for bone grafting prior to implant placement. *Implant Dent* 2006; 15: 420-6.
- [12] Sverzut AT, Stabile GA, de Moraes M, Mazzonetto R, Moreira RW. The influence of tobacco on early dental implant failure. *J Oral Maxillofac Surg* 2008; 66: 1004-9.
- [13] Kuhn LT, Grynpsas MD, Rey CC, Wu Y, Ackerman JL, Glimcher MJ. A comparison of the physical and chemical differences between cancellous and cortical bovine bone mineral at two ages. *Calcif Tissue Int* 2008; 83: 146-154.
- [14] Turner AS. Animal models of osteoporosis – Necessity and limitations. *Eur Cells Mater* 2001; 1: 66-81.
- [15] Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res* 1987; 2: 595-610.
- [16] Recker RR. *Bone Histomorphometry: Techniques and Interpretation*. Boca Raton, FL: CRC; 1983.
- [17] Callan DP, Rohrer MD. Use of bovine derived hydroxiapatite in the treatment of edentulous ridge defects: a human clinical and histologic case report. *J Periodontol* 1993; 64: 575-82.
- [18] Pallesen L, Schou S, Aaboe M, Hjorting-Hansen E, Nattestad A, Melsen F. Influence of particle size of autogenous bone grafts on the early stages of bone regeneration: a histologic and stereologic study in rabbit calvarium. *Int J Oral Maxillofac Implants* 2002; 17: 498-506.
- [19] Chaves Netto HDM, Olate S, Chaves MMGA, Barbosa JR, Mazzonetto R. Análisis histológico del proceso de reparación en defectos óseos. Reconocimiento de defectos críticos. *Int J Morphol* 2009; 27: 1121-1127.
- [20] Ellis E 3rd. Is lag screw fixation superior to plate fixation to treat fractures of the mandibular Symphysis? *J Oral Maxillofac Surg* 2012; 70: 875-882.
- [21] Franceschi C. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. *Mech Ageing Dev* 2005; 126: 351-61.
- [22] Gerresen M, Hermanns-Sachweh B, Riediger D, Hilgers RD, Spiekermann H, Ghassemi A. Purely cancellous vs. corticocancellous bone in sinus floor augmentation with autogenous iliac crest: a prospective clinical trial. *Clin Oral Impl Res* 2009; 20: 109-115.