CLINICAL ORAL IMPLANTS RESEARCH

Oscar Salomó-Coll
José E. Maté-Sánchez de Val
María P. Ramírez-Fernandez
Federico Hernández-Alfaro
Jordi Gargallo-Albiol
José L. Calvo-Guirado

Topical applications of vitamin D on implant surface for bone-to-implant contact enhance: a pilot study in dogs part II

Authors' affiliations:
Oscar Salomó-Coll, Federico Hernández-Alfaro, Jordi Gargallo-Albiol, Department of Oral and Maxillofacial Surgery, International University of Catalonia, Barcelona, Spain
José E. Maté-Sánchez de Val, María P. Ramírez-Fernandez, José L. Calvo-Guirado, International Dentistry Research Cathedra, Universidad Católica San Antonio de Murcia (UCAM), Murcia, Spain

Corresponding author:
José L. Calvo-Guirado
International Dentistry Research Cathedra
Universidad Católica San Antonio de Murcia (UCAM) N 135 Guadalupe 30107 Murcia, Spain
Tel.: +34 968933773
Fax: +34 968268353
e-mail: joseluis.calvoguirado@gmail.com

Key words: bone remodeling, Bone-to-implant contact, immediate implants, osseointegration, vitamin D

Abstract

Objective: The aim of this study was to evaluate the effect of topical application of vitamin D over implant surface, placed immediately to the extraction, throughout histological and histomorphometric analysis of peri-implant tissue.

Material and methods: Six American foxhound dogs were used in the study. Mandibular premolar distal roots were extracted. Twenty-four immediate conical C1 implants (MIS, Barlev, Israel) were randomly assigned to the distal site on each site of the mandible in three groups: (Group CI) 12 titanium implants alone; (Test Group DI) 12 titanium implants supplemented with vitamin D. Prior to implanting, test implants (DI) were submerged in vitamin D 10% solution. No treatment was applied at control implants (CI). After 12 weeks, animals were sacrificed. Block sections were obtained and processed for mineralized ground sectioning. Bone-to-implant contact (Total BIC and BIC%), new bone formation (NBF), interthread bone (ITB), and histological linear measurements (HLM) were analyzed.

Results: At 12 weeks, all implants were clinically stable and histologically osseointegrated. BIC evaluation showed Total BIC mean and SD values for DI (48.96 $\pm$ 2.14), CI (44.56 $\pm$ 1.75) ($P < 0.05$), BIC% DI (43.59 $\pm$ 0.98), and CI (42.67 $\pm$ 9.26) ($P > 0.05$). For interthread bone formation, values were as follows: DI (15.21 $\pm$ 3.87), CI (14.79 $\pm$ 1.45) ($P > 0.05$), no statistically differences. Regarding peri-implant new bone formation, no statistically differences could be found between the two groups DI (31.87 $\pm$ 1.23), CI (27.18 $\pm$ 2.38) ($P > 0.05$). For linear measurements, test group (DI) showed statistically significant less buccal crestal bone loss (CBL) DI (0.37 $\pm$ 0.12)*, CI (1.26 $\pm$ 0.8) ($P < 0.05$), and vitamin D implants showed less lingual junctional epithelium DI (1.58 $\pm$ 0.43)*, CI (2.18 $\pm$ 0.48) ($P < 0.05$). No differences were observed in the buccal mucosa.

Conclusion: With the limitation of animal studies, topical application of vitamin D on dental implants could reduce crestal bone loss and increase 10% more bone-to-implant contact at 12-week follow-up period.

Immediate implants have demonstrated achieving survival outcomes similar to delay implant placement (Lazzara 1989; Werbitt & Goldberg 1992; Botticelli et al. 2008; Lang et al. 2012). After immediate implant placement bone loss and soft tissue, recessions may occur during the first year after placement (Kan et al. 2009, 2011) that can compromise functional as well as esthetic outcomes (Evans & Chen 2008; Cosyn et al. 2012; Degidi et al. 2012; Lang et al. 2012; Tan et al. 2012). Main reason for buccal bone loss and soft tissue recession is caused by higher percentage of bundle bone present in the buccal wall, which will inevitably disappear after extraction, causing resorption of the buccal wall and so a reduction in bone volume (Pietrokovski & Massler 1967; Schropp et al. 2003; Cardaropoli et al. 2003; Araújo & Lindhe 2005a; Buser et al. 2011). This anatomical characteristic must be taken into consideration if pronounced buccal bone resorption needs to be avoided following immediate implant placement (Covani et al. 2004; Araújo et al. 2005b, 2006a, 2006b; Barone et al. 2011).

During immediate implants, the space between the buccal wall and the implant is called *jumping distance* or *buccal gap* (Botticelli et al. 2003), several authors stress the

Date:
Accepted 31 August 2015

To cite this article:
Salomó-Coll O, Maté-Sánchez de Val JE, Ramírez-Fernandez MP, Hernández-Alfaro F, Gargallo-Albiol J, Calvo-Guirado JL. Topical applications of vitamin D on implant surface for bone-to-implant contact enhance: a pilot study in dogs part II. *Clin. Oral Impl. Res.* **00**, 2015, 1–8
doi: 10.1111/clr.12707

need for gap filling at the time of immediate implant placement, depending on the dimensions of the gap to minimize bone loss (Botticelli et al. 2004a, 2004b, 2004c; Ferrus et al. 2010; Bottini et al. 2012). This involves the introduction of a grafting material that aims to minimize bone resorption and improve implant osseointegration. But new bone formation will be influenced by the chemical and physical properties of the graft material and procedure (Schwarz et al. 2008). Many protocols, techniques and biomaterials have been proposed for improving bone healing after dental extraction, but given the heterogeneity of biomaterials and techniques, no single protocol has proved better than the others and none have achieved the full maintenance of initial bone volume (Barone et al. 2011; Vignoletti et al. 2012; Calvo-Guirado et al. 2013). No bone substitute is able to respond to physiological loads or biochemical stimuli in the way that living tissue does (Hench 2002).

In recent years, research has focussed on improving bone substitutes and implant surfaces to achieve faster and better osseointegration by morphologic or biochemical modification (Calvo-Guirado et al. 2010a; Delgado-Ruíz et al. 2011). These modifications can improve bone quality and quantity around dental implants, reducing economic costs and treatment times, improving osseointegration, and consequently survival time. Biochemical modifications consist of the application of biological mediators over the implant surface or into the biomaterial to induce specific cell and tissue responses, such as grow hormone (Gómez-Moreno et al. 2009; Calvo-Guirado et al. 2011; Muñoz et al. 2012), melatonin (Calvo-Guirado et al. 2010a, 2010b; Tresguerres et al. 2012;), or vitamin D (Cho et al. 2011).

In our previous study (part I) related to the evaluation the effects of topical applications of melatonin over implant surfaces placed immediately after extraction, we demonstrated that improved bone formation around immediate implants and reduced lingual bone and lingual peri-implant mucosa, after 12 weeks of osseointegration (Salomó-Coll et al. 2015).

Vitamin D was discovered in 1922 and compends a group of fat steroid hormones. These hormones can be found in several forms such as ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Vitamin D_3 is formed by the skin from cholesterol under the UV exposure, and the most active form is 1α, 25-dihydroxyvitamin D3 (1α,25-(OH)2D3), named as calcitriol. This biomolecule has a fundamental role in bone and calcium homeostasis (Christakos et al. 2013), acting directly in calcium absorption in the intestines and kidney (Cooper 2000; Van Driel et al. 2006), and it enhances bone reabsorption and reduces calcium and phosphate excretion. Vitamin D receptors are present in osteoblasts and have direct effect on cells by regulating gene expression (Van Driel et al. 2006; Van Leeuwen et al. 2001; Cho et al. 2011; Christodoulou et al. 2013), as well as other proteins involved in bone formation like osteocalcin (Ong et al. 1998). Vitamin D plays a major role on bone health (Cooper 2000).

The aim of this study was to determine whether the topical application of vitamin D over the implant surface is effective in improving osseointegration, using histomorphometric analysis and histological linear measurements at 12 weeks after immediate implant placement.

Material and methods

1. Study design

Six American foxhound dogs of approximately 1 year of age, each weighing approximately 13–15 kg, were used in the study. Three conical implants were inserted in each hemiarch and divided randomly into three different groups. This study includes results obtained from two of the groups ($n = 24$); results for the other group were published in Part 1.

The Ethics Committee for Animal Research at the University of Murcia, Spain, approved the study protocol which followed guidelines established by the European Union Council Directive of February 1st 2013/53/CEE). Animals were quarantined for application of antirabies vaccine, antihelmintics and vitamins, but vitamin D was applied topically. Pre- and postoperatively, the animals were kept in kennel cages; received appropriate veterinary care with free access to water and standard laboratory nutritional support throughout the trial period. All animals presented intact maxillas, without any oral viral or fungal lesions. Clinical examination determined that the dogs were in good general health.

2. Surgical procedures

First stage

The animals were pre-anaesthetized with 10% zolazepam at 0.10 ml/kg and acepromazine maleato (Calmo-Neosan; Pfizer, Madrid, Spain) 0.12% – 0.25 mg/kg and medetomidine 35 μg/kg (Medetor 1 mg, Virbac, CP-Pharma Handelsgesellschaft GmbH, Burgdorf, Germany). The mixture was injected intramuscularly in the femoral quadriceps. Animals were then taken to the operating theater where, at the earliest opportunity, an intravenous catheter was inserted (diameter 22 or 20 G) into the cephalic vein and propofol was infused at the rate of 0.4 mg/kg/min as a slow constant rate infusion. Anesthetic maintenance was obtained using volatile anesthetics, and the animals were submitted to tracheal intubation with a Magill probe for adaptation of the anesthetic device and for administration of oxygen-diluted volatile isoflurane (2%). Additionally, local anesthesia (Articaine 40 mg, 1% epinephrine, Normon, Madrid, Spain) was administered at the surgical sites. These procedures were carried out under the supervision of a veterinarian surgeon.

Bilateral mandibular premolar (P_2, P_3, P_4,) extractions were performed in each dog. Teeth were sectioned in a bucco-lingual direction at the bifurcation using a tungsten-carbide bur (Fig. 1a). Distal roots were individually extracted, using a periotome and forceps without damaging bony walls (Fig. 1b). Mesial roots were filled with MTA and composite to maintain a minimum function during implant healing and to prevent endodontic pathology.

A randomization scheme was generated using the Web site randomization.com (http://www.randomization.com). A scheme was created for the 24 implants (12 implants with vitamin D2 10% [DI], 12 implants alone [CI] (Salomó-Coll et al. 2015) randomized into six groups [six dogs]). Each dog received six conical C1 implants (MIS, Barlev, Israel), with sandblasted and acid etched surface, three per hemiarch, randomly assigned on the mandible. Before implant placement, test implants were submerged in vitamin D2 at 10% in acetone solution (ref 705489, Sigma-Aldrich, St. Louis, MO, USA) (Fig. 1c). No treatment was applied to control implants (CI). Minimal full-thickness mucoperiosteal flaps were elevated, and implants were placed. All specimens received six conical self-tapering implants from MIS® (MIS® Implant Technologies, Barlev, Israel) (Fig. 1d). Implant position was determined in relation to shape and volume of the alveolar process and the buccal wall position. Apical portion of the socket was prepared using manufacturers conventional drilling protocol. All implants were placed at the same buccal bone level, and implant shoulder was at the same level than buccal bone crest. Each mandible received six conical screwed implants. (3.75 × 10 mm). After implant

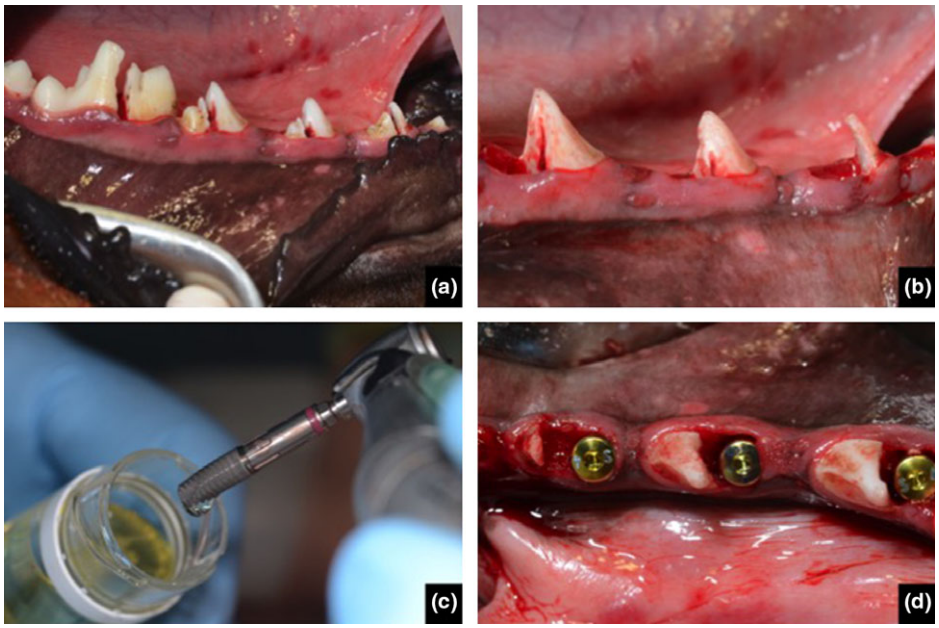

Fig. 1. (a) Mandibular premolars odontosections (P_2, P_3, P_4,); (b) Distal root extraction (c) C1 dental implant with 10%. Vitamin D dopping surface (d) Three implants randomly placed immediately in the distal socket with healing abutments in place.

placement, standard 4 mm height healing abutments (CS-HS475, MIS® Implant Technologies, Carmiel, Israel) were placed to allow a nonsubmerged healing protocol. After abutment placement, occlusion was checked to avoid interferences during biting. No grafting materials were used in the gaps remaining between bony plates and implants. The flaps were closed with simple interrupted nonresorbable sutures (Silk® 4-0, Lorca Marin, Lorca, Spain). After the surgical procedures, the animals received antibiotics twice daily (Amoxicillin 500 mg. Clamoxyl L.A.; Pfizer), and analgesics three times a day (Ibuprofen 600 mg, Rimadyl; Pfizer). The sutures were removed after 2 weeks. Animals were fed a soft diet for 7 days after surgery. The animals had free access to water and were fed with moistened balanced dog chow. The wounds were inspected daily for any clinical signs of complications and the healing screws cleaned.

Second stage

Digital radiographs were taken at 12 weeks (Kodak 6100, Eastman Kodak, Rochester, NY, USA) (Fig. 2). Afterward, the animals were sacrificed (12 weeks after implant placement by means of Pentothal Natrium (Abbot Laboratories, Madrid, Spain) perfused through the carotid arteries with a fixative containing a mixture of 5% glutaraldehyde and 4% formaldehyde. The mandibles were dissected, and block sections including the implant sites and surrounding soft and hard tissues were removed using a saw.

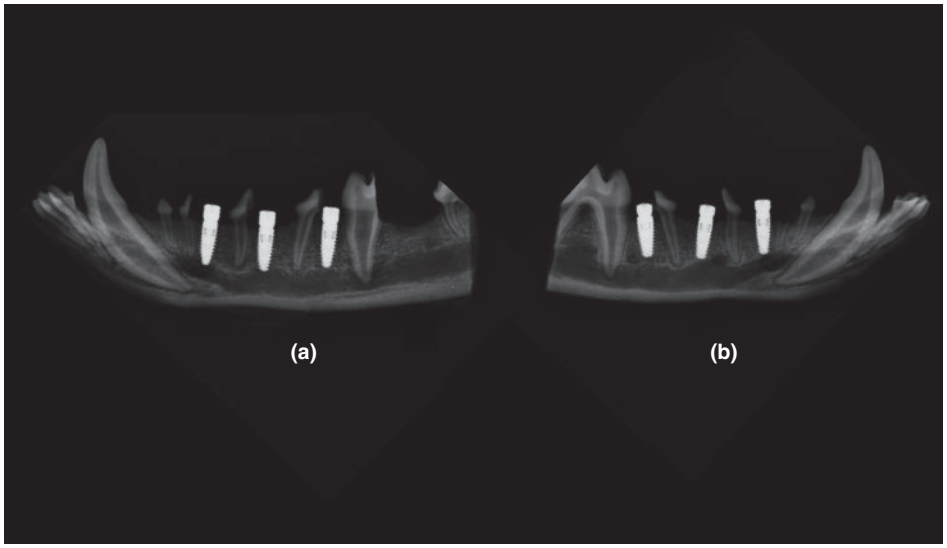

Fig. 2. (a) Digital radiographs of Vitamin D C1 dopped implants after 12 weeks of healing; (b) Digital radiographs of SLA C1 implants after 12 weeks of healing.

Histological and histomorphometric analysis

Biopsies were processed for ground sectioning according to the methods described by Donath and Breuner. Samples were dehydrated in increasing grades of ethanol up to 100%, infiltrated with methacrylate, polymerized, and sectioned at the buccal–lingual plane using a diamond saw (Exakt Apparatebau, Norderstedt, Hamburg, Germany). Two sections were cut from each biopsy specimen. The first was cut from the center of the implant and the second from the surrounding bone. Each block was sectioned with a high-precision diamond disk to about 100 mm thickness and ground to approximately 40 mm final thickness with an Exakt 400s CS grinding device (Exakt Apparatebau).

Sections were stained with toluidine blue, and a semi-quantitative evaluation of BIC was performed. To obtain a single digitally processable overview image of each implant, four images of the same implant were taken with a 10X lens and assembled into a single image (Image-Pro Plus 4.5; Media Cybernetics Inc., Immagini & Computer Snc., Milan, Italy). A 1-mm-wide zone around the implant surface, reaching up to the original implantation level, was defined as the region of interest (ROI). Within the ROI, hard tissue was defined digitally as old bone or newly formed bone. To improve the differentiation between native and newly formed bone, light and dark blue chromaticity were digitally enhanced. Finally, the contact length between bone and implant surface (BIC) was determined.

Bone-to-implant contact (BIC) in each histological section was calculated by measuring the length of the implant surface in contact with bone tissue, in comparison with the total length of the implant surface, expressed as a percentage (Total BIC). The percentage of mineralized bone in direct contact with the titanium surface was determined by counting inside the threaded zone (Interthread Bone). New bone formation was calculated as the interthread bone and the bone in direct contact with the implant perimeter (New Bone Formation). BIC percentages were calculated around the entire implant body, from the first point of bone-to-implant contact, at the most coronal point, evaluating mineralized bone in

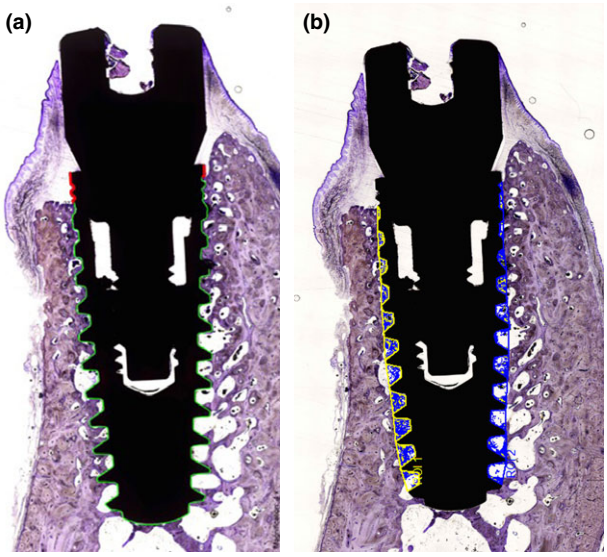

Fig. 3. (a) BIC scheme: Green lines shows Total BIC and red line show crestal bone loss. (b) BIC scheme Yellow line delimitates interthread space and blue line shows New Bone Formation.

contact with the implant surface linearly (BIC %) (Fig. 3a,b). Histomorphometric analysis was performed using a video camera (Sony 3CCD, Berlin, Germany) with 10X magnification. Interthread images were digitalized (Axiophot-System, Zeiss, Oberkochen, Germany) and stored, and reference points were plotted.

The following buccal and lingual measurements were made at X10 magnification (as illustrated in Fig. 4):

D1: distance from the top of the peri-implant mucosa and the apical portion of the junctional epithelium (P-aJE).

D2: distance from the apical portion of the junctional epithelium to the first point of bone-to-implant contact (aJE-fBIC).

D3: distance from the implant shoulder to the first point of bone-to-implant contact (IS-fBIC) (Crestal Bone Loss: CBL).

D4: distance from the top of the peri-implant mucosa to the first bone-to-implant contact (P-fBIC) (Peri-implant Mucosa: PIM).

D5: distance from the implant shoulder to the bone crest (IS-BC).

D6: distance from the first point of bone-to-implant contact to the bone crest (fBIC-BC).

Measurements were performed with a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a high-resolution video camera (3CCD, JVC KY-F55B, JVC®, Yokohama, Japan) and interfaced to a monitor and PC (Intel Pentium III 1200 MMX, Intel®, Santa Clara, CA, USA). This optical system was connected to a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image capture capabilities (Image-Pro Plus 4.5; Media Cybernetics Inc., Immagini & Computer Snc., Milan, Italy).

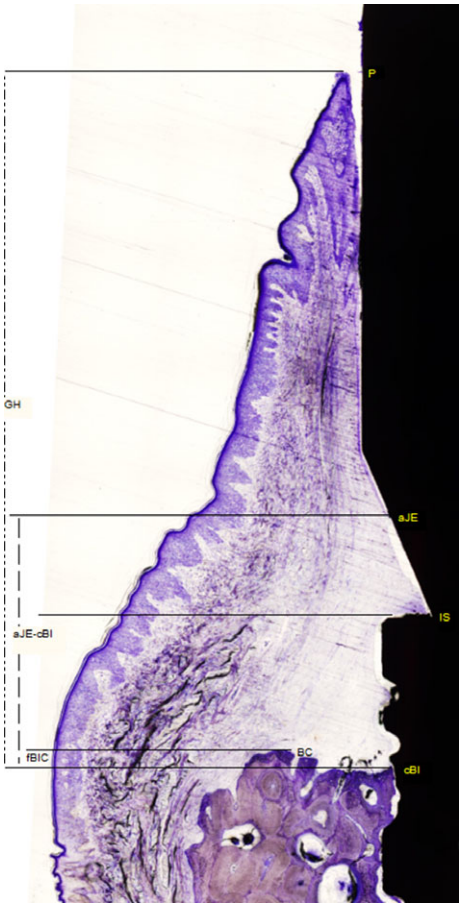

Fig. 4. Linear Measurements scheme: P,top of the peri-implant mucosa; aJe,apical portion of the junctional epithelium; fBIC: first point of bone-to-implant contact; IS, Implant shoulder; BC, buccal crest.

Radiographic evaluation

Digital radiographs were taken at implant placement and 12 weeks later to verify implant osseointegration and to assess changes to postsurgical crestal bone levels (Kodak 6100; Eastman Kodak, Rochester, NY, USA). Exposure parameters were standardized. No radiolucent images or signs of osseointegration disorders were observed. X-ray analysis revealed that all 24 implants showed uneventful osseointegration.

Statistical analysis

Firstly, factors such as individual difference and position of the implant could be excluded as not significant. Despite having several samples on the same specimen, implants were considered dependent clustered data related to the dogs which were considered independent variable. Dependent variables included the histomorphometric measurements previously described. Values were expressed as medians and means ± standard deviation. As the distribution of data was not normal, a nonparametric Wilcoxon signed rank test was applied. The level of statistical significance was established at $P < 0.05$. All histomorphometric parameters were analyzed using descriptive methods (SPSS 21.0 software, Chicago, IL, USA).

Results

Histomorphometric and histological results

The results of the different histomorphometric measurements are presented in Table 1. BIC% values at 12 weeks varied slightly high between vitamin D (43.59 ± 0.98), which was no statistically significant ($P < 0.05$) when comparing to bone surrounding control implants CI (42.67 ± 1.45). Analyzing Total BIC, statistically significant values were found between DI (48.96 ± 2.14) vs. CI (44.56 ± 1.75) ($P > 0.05$). Regarding to interthread bone, values for vitamin D were 19.56 ± 0.78 and it was found when comparing to control implants CI (16.23 ± 1.24) ($P < 0.05$).

For peri-implant new bone formation, vitamin D showed statistically significant differences ($P > 0.05$), comparing test group DI (31.87 ± 1.23) vs. CI (27.18 ± 2.38).

Figs 5 and 6 showed histological image of control [CI] buccal and lingual, respectively. Figs 7 and 8 show test implants [DI], buccal and lingual, respectively. All histological images revealed good bone healing without any signs of inflammation. Newly formed bone was similar for both groups, in direct contact with implant surface, mostrating

Table 1. **Medians, means and standard deviation for total BIC, BIC%, new bone formation and interthread bone after 12 weeks of healing. Nonparametric Wilcoxon signed rank test for related samples**

	Control group C1		Test group D1		
Variable	Mean ± SD %	Median	Mean ± SD %	Median	*P* value
New Bone Formation	27.18 ± 2.38	26.3	31.87 ± 1.23	30.6	0.021*
Interthread Bone (ITH)	16.23 ± 1.24	15.9	19.56 ± 0.78	18.6	0.345
Total BIC	44.56 ± 1.75	43.6	48.96 ± 2.14	47.9	0.035*
BIC%	42.67 ± 1.45	41.8	43.59 ± 0.98	42.7	0.167

*Differences between values achieving statistical significance ($P < 0.05$).

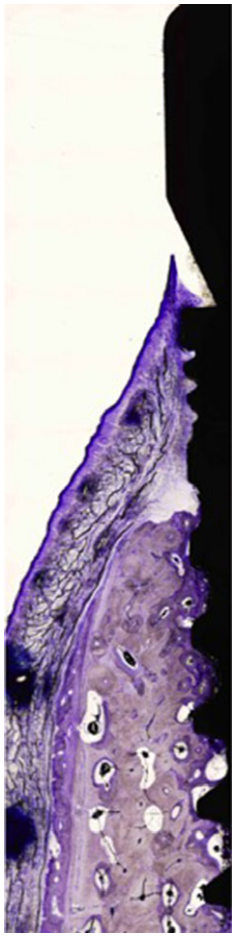

Fig. 5. Buccal ground section of an untreated implant [CI] after 12 weeks of healing. Hematoxilin eosin stain, original magnification ×10.

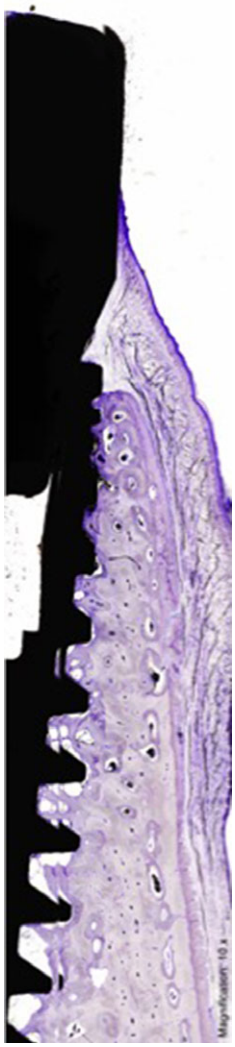

Fig. 6. Lingual ground section of an untreated implant [CI] after 12 weeks of healing. Hematoxilin eosin stain, original magnification ×10.

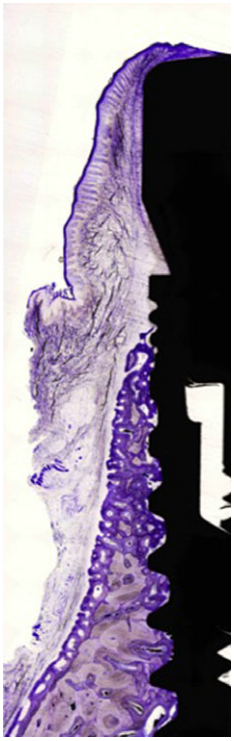

Fig. 7. Buccal ground section of a test implant [DI] after 12 weeks of healing. Hematoxilin eosin stain, original magnification ×10.

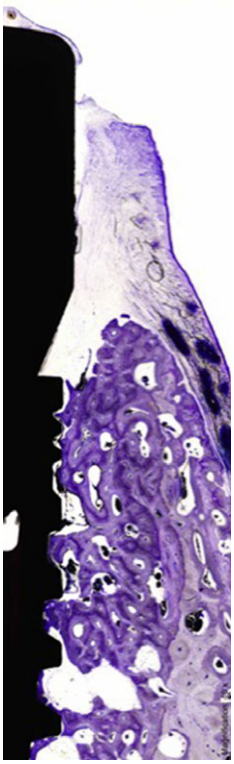

Fig. 8. Lingual ground section of a test implant [DI] after 12 weeks of healing. Hematoxilin eosin stain, original magnification ×10.

active remodelating activity with mixed pattern of mature, and immature bone was observed, densely organized. No presence of fibrous connective tissue was observed at the bone–implant interface.

Linear measurements

The results of the different linear measurements are presented in Table 2. Few statistically significant differences could be found among the variables. Vitamin D group showed statistically less buccal crestal bone loss ($P < 0.05$) DI (0.37 ± 0.12), CI (1.26 ± 0.81), and less lingual junctional epithelium DI (1.58 ± 0.43)*, when comparing to control implants CI (2.18 ± 0.48).

Radiographical analysis

No evidence of large loss of bone around the implant or the presence of radiolucency was found. Good contact between the implants and the host bone was observed. All 24 implants showed uneventful osseointegration at 12 weeks during X-ray analysis. No statistical values could be obtained because of difficulties on X-rays standardization.

Table 2. **Medians, means and standard deviation for linear measurements at 12 weeks of healing**

	Control group C1 (n = 12)		Test group D1 (n = 12)		
Buccal	Mean ± SD	Median	Mean ± SD	Median	P value
P-aJE buc	2.59 ± 0.71	2.11	2.78 ± 0.24	2.67	0.034*
aJE-cBI buc	0.91 ± 1.04	0.87	1.97 ± 0.98	1.71	0.154
IS-cBI buc (CBL)	1.26 ± 0.81	0.91	0.37 ± 0.12*	0.29	0.032*
P-cBI buc (PIM)	3.98 ± 0.32	3.11	4.68 ± 0.97	3.91	0.023*
IS-BC buc	1.73 ± 1.05	1.12	1.89 ± 0.95	1.79	0.214
fBIC buc	0.51 ± 0.71	0.47	0.45 ± 0.82	0.39	0.281
	ALONE (n = 12)		VITAMIN D (n = 12)		
Lingual	Mean ± SD	Median	Mean ± SD	Median	P value
P-aJE lin	2.18 ± 0.48	1.97	1.58 ± 0.43	1.1	0.028*
aJE-cBI lin	0.66 ± 0.93	0.60	0.79 ± 0.65	0.143	0.590
IS-cBI lin (CBL)	0.96 ± 0.62	0.89	0.98 ± 1.23	0.478	0.187
P-cBI lin (PIM)	3.67 ± 1.74	3.12	3.58 ± 0.53	0.443	0.378
IS-BC-lin	1.18 ± 0.43	0.92	1.22 ± 0.37	0.052	0.143
fBIC-lin	0.22 ± 1.11	0.18	0.44 ± 0.99	0.410	0.016*

*Differences between values achieving statistical significance ($P < 0.05$). Nonparametric Wilcoxon signed rank test for related samples.

Discussion

The use of biocompatible materials to improve bone formation around dental implants has been widely documented in the literature. A wide variety of substances have been studied to improve bone regeneration and peri-implant bone response: grow hormone, morphogenetic proteins, calcium coatings, fluorine coatings, magnesium, or vitamin D (Calvo-Guirado et al. 2010b, Young-Jin et al. 2011; Tresguerres et al. 2012; Cutando et al. 2008). Links between vitamin D and bone metabolism have been reported in the literature, and its effect on stimulating osteoblast proliferation and differentiation (Kato et al. 2015), but the effect of the topical application of osteoinductive elements like vitamin D over immediate implants is still subject of investigation.

The present experiment demonstrated that bone formation around immediate dental implants treated with topical application of vitamin D was not improved, when comparing with control implants. No statistically differences could be found between two groups regarding bone-to-implant contact, new bone formation, or interthread bone. Vitamin D implants showed less buccal crestal bone loss values and exhibit less lingual junctional epithelium. The absence of statistically differences could be caused by the inadequate way of administration of the vitamin D, because of the fact that specimens were at good general health, even though vitamin D has demonstrated to have its major effect on population with low levels of vitamin D. Another possibility is because vitamin D has also effect on bone reabsorption (Naito et al. 2014), or cause of the absence of sunlight, which should active vitamin D, while dogs were kept in kennels.

In a study, Kelly et al. 2009; performed in rats, apart from vitamin D intake and light exposure, found that, at 2 weeks, implants in tests groups showed statistically less amount of BIC when compared to control implants. In that study, authors said that vitamin D deficiency significantly impaired the titanium implants osseointegration. In agreement with these results, in another study performed by Dvorak et al. 2012, ovariectomized rats found that at 6 and 8 weeks vitamin D deficiency only statistically significant increased Cortical BIC, and the authors explain this fact that their specimens only had a moderate vitamin D deficiency in comparison with Kelly's specimens that have a severe deficiency. Authors conclude that the overall process of peri-implant bone formation is not substantially changed by vitamin D deficiency.

In agreement with our results, Akhavan et al. 2012, in a diabetic rat model compared at 3 and 6 weeks, demonstrated that vitamin D orally supplemented had no effect on BIC formation and it is not time dependent. Authors suggest that the absence of statistically significant results could be caused by an inadequate dose of vitamin D intake; moreover, authors suggest that age, body weight, sex environmental factors, or genetic variations of vitamin D receptors also could influence the effect of vitamin D.

In a study, Hong et al. 2012 performed in healthy beagle dogs and created a surgical defects and supplemented the dogs with high dose of vitamin D and calcium during 4 weeks. At 4 weeks, animals were sacrificed and authors found that VitD/Ca supplementation increased new bone formation and bone density, and reduced crestal bone loss when compared to nonsupplemented groups.

In an experimental study, Naito et al. 2014, in the rabbit tibia, observed BIC and new bone formation of 28 implants at 6 weeks. In this study, authors used machined implants with 3 degrees of vitamin D coating and compared them to a control group (untreated machined implants). After 6 weeks, no statistically differences could be found among the four groups, authors explain that fact, because the machined surface cannot sustain enough concentration of vitamin D or the damage of the protein caused by the coating process. The authors use machined implants to potentiate the effect of vitamin D; they suggested that as rough implants improve osseointegration, and it can cause an alteration of the potential effect of vitamin D. Moreover, they mentioned that vitamin D has also a bone reabsorbing effect.

Cho et al. (2011) inserted the rabbit's tibiae implants with vitamin D coating. At 4 and 12 weeks, osseointegration level was determined through BIC values. At 4 weeks, test implants showed higher Total BIC values (37.08 ± 10.18) compared to control implants (28.01 ± 8.70). At 12 weeks, statistically significant differences were found between the two groups, control group (29.53 ± 9.49) and test group (39.10 ± 7.68), respectively, similar to our results.

Contrary to all results, Fügl et al. 2014 in a study in 60 rats divided into three groups (vitamin D deficiency + ungraft; vitamin D deficiency+ local calcitriol application and control group), they created two millimeters circumferential diameter defects and samples were obtained at 1 and 3 weeks. At 1 week, results were refused because values obtained were close to 0. At 3 weeks, new mineralized bone area values were statistically significant higher for vitamin D deficiency with ungrafted defects (17.5%), when compared to vitamin D deficiency+ local calcitriol application (15.9%) and control group (2.1%).

The potential effect of oral or intravenous vitamin D supplementation around titanium implants remains unclear. Experimental model, dose, vitamin D deficiency, calcium supplementation, administration via, receptor polymorphisms, sun exposure, and observation period seem to influence studies outcomes. The heterogenicity of the studies difficults rising a conclusion. In this study, no differences could be observed between the two groups.

Conclusions

Within the limits of this study, topical application vitamin D during immediate implants treatment seems not having enhanced effect on dental implants osseointegration, although implants with topical application of vitamin D exhibited less crestal bone loss and 10% more bone-to-implant contact.

Acknowledgements: The authors would like to thank Dr. Naroa Lozano (Department of Oral and Maxillofacial surgery, International University of Catalonia) and Nuria Garcia Carrillo (Veterinary, University of Murcia) for the help in the development of the research and manuscript.

References

Akhavan, A., Noroozi, Z., Shafiei, A.A., Haghighat, A., Jahanshahi, G.R. & Mousavi, S.B. (2012) The effect of vitamin D supplementation on bone formation around titanium implants in diabetic rats. *Journal of Dental Research* **9**: 582–587.

Araújo, M.G. & Lindhe, J. (2005a) Dimensional ridge alterations following tooth extraction. An experimental study in the dog. *Journal of Clinical Periodontology* **32**: 212–218.

Araújo, M.G., Sukekava, F., Wennström, J.L. & Lindhe, J. (2005b) Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *Journal of Clinical Periodontology* **32**: 645–652.

Araújo, M.G., Sukekava, F., Wennström, J.L. & Lindhe, J. (2006a) Tissue modeling following implant placement in fresh extraction sockets. *Clinical Oral Implants Research* **17**: 615–624.

Araújo, M.G., Wennström, J.L. & Lindhe, J. (2006b) Modeling of the buccal and lingual bone walls of fresh extraction sites following implant installation. *Clinical Oral Implants Research* **17**: 606–614.

Barone, A., Ricci, M., Calvo-Guirado, J.L. & Covani, U. (2011) Bone remodelling after regenerative procedures around implants placed in fresh extraction sockets: an experimental study in Beagle dogs. *Clinical Oral Implants Research* **22**: 1131–1137.

Botticelli, D., Berglundh, T., Buser, D. & Lindhe, J. (2003) The jumping distance revisited: an experimental study in the dog. *Clinical Oral Implants Research* **14**: 35–42.

Botticelli, D., Berglundh, T. & Lindhe, J. (2004a) Resolution of bone defects of varying dimension and configuration in the marginal portion of the peri-implant bone. An experimental study in the dog. *Journal of Clinical Periodontology* **31**: 309–317.

Botticelli, D., Berglundh, T. & Lindhe, J. (2004b) The influence of a biomaterial on the closure of a marginal hard tissue defect adjacent to implants. An experimental study in the dog. *Clinical Oral Implants Research* **15**: 285–292.

Botticelli, D., Berglundh, T. & Lindhe, J. (2004c) Hard-tissue alterations following immediate implant placement in extraction sites. *Journal of Clinical Periodontology* **31**: 820–828.

Botticelli, D., Renzi, A., Lindhe, J. & Berglundh, T. (2008) Implants in fresh extraction sockets: a prospective 5-year follow-up clinical study. *Clinical Oral Implants Research* **19**: 1226–1232.

Bottini, L.P., Ricci, L., Piattelli, A., Perrotti, V. & Iezzi, G. (2012) Bucco-lingual crestal bone changes around implants immediately placed in fresh extraction sockets in association or not with porcine bone: a non-blinded randomized controlled trial in humans. *Journal of Periodontology* **29**: 1–8.

Buser, D., Wittneben, J., Bornstein, M.M., Grütter, L., Chappuis, V. & Belser, U.C. (2011) Stability of contour augmentation and esthetic outcomes of implant-supported single crowns in the esthetic zone: 3-year results of a prospective study with early implant placement postextraction. *Journal of Periodontoly* **82**: 342–349.

Calvo-Guirado, J.L., Gómez-Moreno, G., López-Marí, L., Guardia, J., Martínez-González, J.M., Barone, A., Tresguerres, I.F., Paredes, S.D. & Fuentes-Breto, L. (2010a) Actions of melatonin mixed with collagenized porcine bone versus porcine bone only on osseointegration of dental implants. *Journal of Pineal Research* **48**: 194–203.

Calvo-Guirado, J.L., Mate-Sanchez, J., Delgado-Ruiz, R., Ramírez-Fernández, M.P., Cutando-Soriano, A. & Peña, M. (2011) Effects of growth hormone on initial bone formation around dental implants: a dog study. *Clinical Oral Implants Research* **22**: 587–593.

Calvo-Guirado, J.L., Ramírez-Fernández, M.P., Gómez-Moreno, G., Maté-Sánchez, J.E., Delgado-Ruiz, R., Guardia, J., López-Marí, L., Barone, A., Ortiz-Ruiz, A.J., Martínez-González, J.M. & Bravo, L.A. (2010b) Melatonin stimulates the growth of new bone around implants in the tibia of rabbits. *Journal of Pineal Research* **49**: 356–363.

Calvo-Guirado, J.L., Ramírez-Fernández, M.P., Negri, B., Delgado- Ruiz, R.A., Maté-Sánchez de-Val, J.E. & Gómez-Moreno, G. (2013) Experimental model of bone response to collagenized xenografts of porcine origin (OsteoBiol® mp3): a radiological and histomorphometric study. *Clinical Implant Dentistry and Related Research* **15**: 143–151.

Cardaropoli, G., Araújo, M. & Lindhe, J. (2003) Dynamics of bone tissue formation in tooth extraction sites. An experimental study in dogs. *Journal of Clinical Periodontology* **30**: 809–818.

Cho, Y.J., Heo, S.J., Koak, J.Y., Kim, S.K., Lee, S.J. & Lee, J.H. (2011) Promotion of osseointegration of anodized titanium implants with a 1,25-dihydroxyvitamin D3 submicron particle coating. *International Journal of Oral & Maxillofacial Implants* **26**: 1225–1232.

Christakos, S., Hewison, M., Gardner, D.G., Wagner, C.L., Sergeev, I.N., Rutten, E., Pittas, A.G., Boland, R., Ferrucci, L. & Bikle, D.D. (2013) Vitamin D: beyond bone. *Annals of the New York Academy of Sciencies* **1287**: 45–48.

Christodoulou, S., Goula, T., Ververidis, A. & Drosos, G. (2013) Vitamin D and bone disease. *Biomed Research International* **2013**: 396–541.

Cooper, L.F. (2000) Systemic effectors of alveolar bone mass and implications in dental therapy. *Periodontology 2000* **23**: 103–109.

Cosyn, J., Hooghe, N. & De Bruyn, H. (2012) A systematic review on the frequency of advanced recession following single immediate implant treatment. *Journal of Clinical Periodontology* **39**: 582–589.

Covani, U., Bortolaia, C., Barone, A. & Sbordone, L. (2004) Bucco-lingual crestal bone changes after immediate and delayed implant placement. *Journal of Periodontology* **75**: 1605–1612.

Cutando, A., Gómez-Moreno, G., Arana, C., Muñoz, F., Lopez-Peña, M., Stephenson, J. & Reiter, R.J. (2008) Melatonin stimulates osteointegration of dental implants. *Journal of Pineal Research* **45**: 174–179.

Degidi, M., Nardi, D., Daprile, G. & Piattelli, A. (2012) Buccal bone plate in the immediately placed and restored maxillary single implant: a 7-year retrospective study using computed tomography. *Implant Dentistry* **21**: 62–66.

Delgado-Ruíz, R.A., Calvo-Guirado, J.L., Moreno, P., Guardia, J., Gomez-Moreno, G., Mate-Sánchez, J.E., Ramirez-Fernández, P. & Chiva, F. (2011) Femtosecond laser microstructuring of zirconia dental implants. *Journal Biomedical Materials Research B: Applied Biomaterials* **96**: 91–100.

Dvorak, G., Fügl, A., Watzek, G., Tangl, S., Pokorny, P. & Gruber, R. (2012) Impact of dietary vitamin D on osseointegration in the ovariectomized rat. *Clinical Oral Implants Research* **23**: 1308–1313.

Evans, C.D. & Chen, S.T. (2008) Esthetic outcomes of immediate implant placements. *Clinical Oral Implants Research* **19**: 73–80.

Ferrus, J., Cecchinato, D., Pjetursson, E.B., Lang, N.P., Sanz, M. & Lindhe, J. (2010) Factors influencing ridge alterations following immediate implant placement into extraction sockets. *Clinical Oral Implants Research* **21**: 22–29.

Gómez-Moreno, G., Cutando, A., Arana, C., Worf, C.V., Guardia, J., Muñoz, F., Lopez-Peña, M. & Stephenson, J. (2009) The effects of growth hormone on the initial bone formation around implants. *International Journal of Oral and Maxillofacial Implants* **24**: 1068–1073.

Hench, L.L. (2002) Third-generation biomedical materials. *Science* **295**: 1014–1017.

Hong, H., Chou, T., Yang, J. & Chang, C. (2012) The potential effects of cholecalciferol on bone regeneration in dogs. *Clinical Oral Implants Research* **23**: 1187–1192.

Kan, J.Y., Rungcharassaeng, K., Lozada, J.L. & Zimmerman, G. (2011) Facial gingival tissue stability following immediate placement and provisionalization of maxillary anterior single implants: a 2-

to 8-year follow-up. *International Journal of Oral and Maxillofacial Implants* **26**: 179–187.

Kan, J.Y., Rungcharassaeng, K., Morimoto, T. & Lozada, J.L. (2009) Facial gingival tissue stability after connective tissue graft with single immediate tooth replacement in the esthetic zone: consecutive case report. *Journal of Oral and Maxillofac Surgery* **67**: 40–48.

Kato, H., Ochiai-Shino, H., Onodera, S., Saito, A., Shibahara, T. & Azuma, T. (2015) Promoting effect of 1,25(OH)2 vitamin D3 in osteogenic differentiation from induced pluripotent stem cells to osteocyte-like cells. *Open Biology* **5**: 140201.

Kelly, J., Lin, A., Wang, C.J., Park, S. & Nishimura, I. (2009) Vitamin D and bone physiology: demonstration of vitamin D deficiency in an implant osseointegration rat model. *Journal of Prosthodontics* **18**: 473–478.

Lang, N.P., Pun, L., Lau, K.Y., Li, K.Y. & Wong, M.C. (2012) A systematic review on survival and success rates of implants placed immediately into fresh extraction sockets after at least 1 year. *Clinical Oral Implants Research* **23**: 39–66.

Lazzara, R.J. (1989) Immediate implant placement into extraction sites: surgical and restorative advantages. *International Journal of Periodontics and Restorative Dentistry* **9**: 332–343.

Muñoz, F., López-Peña, M., Miño, M., Gómez-Moreno, G., Guardia, J. & Cutando, A. (2012) Topical application of melatonin and growth hormone accelerates bone healing around dental implants in dogs. *Clinical Implant Dentistry and Related Research* **14**: 226–235.

Naito, Y., Jimbo, R., Bryington, M.S., Vandeweghe, S., Chrcanovic, B.R., Tovar, N., Ichikawa, T., Paulo, G.C. & Wennerberg, A. (2014) The influence of 1α. 25-dihydroxyvitamin d3 coating on implant osseointegration in the rabbit tibia. *Journal of Oral Maxillofacial Research* **1**: 5-e3.

Pietrokovski, J. & Massler, M. (1967) Alveolar ridge resorption following tooth extraction. *Journal of Prosthetic Dentistry* **17**: 21–27.

Ong, J.L., Hoppe, C.A., Cardenas, H.L., Cavin, R., Carnes, D.L., Sogal, A. & Raikar, G.N. (1998) Osteoblast precursor cell activity on HA surfaces of different treatments. *Journal of Biomedical Materials Research* **39**: 176–183.

Salomó-Coll, O., Maté-Sánchez de Val, J.E., Ramírez- Fernández, M.P., Satorres-Nieto, M., Gargallo-Albiol, J. & Calvo- Guirado, J.L. (2015) Osseoinductive elements for promoting osseointegration around immediate implants: a pilot study in the foxhound dog. *Clinical Oral Implants Research* doi: 10.1111/clr.12596.

Schropp, L., Wenzel, A., Kostopoulos, L. & Karring, T. (2003) Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *International Journal of Periodontics and Restorative Dentistry* **23**: 313–323.

Schwarz, F., Rothamel, D., Herten, M., Wüstefeld, M., Sager, M., Ferrari, D. & Becker, J. (2008) Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. *Clinical Oral Implants Research* **19**: 402–415.

Tan, W.L., Wong, T.L., Wong, M.C. & Lang, N.P. (2012) A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. *Clinical Oral Implants Research* **23**: 1–21.

Tresguerres, I.F., Clemente, C., Blanco, L., Khraisat, A., Tamimi, F. & Tresguerres, J.A. (2012) Effects of local melatonin application on implant osseointegration. *Clinical Implant Dentistry Related Research* **14**: 395–399.

Van Driel, M., Koedam, M. & Buurman, C.J. (2006) Evidence that both 1α,25-Dihydroxyvitamin D3 and 24-Hydroxylated D3 enhance human osteoblast differentiation and mineralization. *Journal of Cellular Biochemistry* **99**: 922–935.

Van Leeuwen, J.P., Van Driel, M., Van den Bemd, G.J. & Pols, H.A. (2001) Vitamin D control of osteoblast function and bone extracellular matrix mineralization. *Critical Review in Eukaryotic Gene Expression* **11**: 199–226.

Vignoletti, F., Matesanz, P., Rodrigo, D., Figuero, E., Martin, C. & Sanz, M. (2012) Surgical protocols for ridge preservation after tooth extraction. A systematic review. *Clinical Oral Implants Research* **23**: 22–38.

Werbitt, M.J. & Goldberg, P.V. (1992) The immediate implant: bone preservation and bone regeneration. *International Journal of Periodontics and Restorative Dentistry* **12**: 206–217.

Young-Jin, C., Seong-Joo, H., Jai-Young, K., Seong-Kyun, K., Shin-Jae, L. & Joo-Hee, L. (2011) Promotion of osseointegration of anodized titanium implants with a 1α,25-dihydroxyvitamin D3 submicron particle coating. *International Journal of Oral and Maxillofacial Implants* **26**: 1225–1232.