J Clin Periodontol 2010; 37: 937–942 doi: 10.1111/j.1600-051X.2010.01608.x

Journal of Clinical Periodontology

Flapless immediate implant placement with or without immediate loading: a histomorphometric study in beagle dog

Juan Blanco[1], Antonio Liñares[1], Gabriel Villaverde[1], Javier Pérez[2] and Fernando Muñoz[3]

[1]Department of Stomatology, University of Santiago de Compostela, Spain; [2]Dental Technician, Lugo, Spain; [3]School of Veterinary of Lugo, University of Santiago de Compostela, Spain

Blanco J, Liñares A, Villaverde G, Pérez J, Muñoz F: Flapless immediate implant placement with or without immediate loading. a histomorphometric study in beagle dog. J Clin Periodontol 2010; 37: 937–942. doi: 10.1111/j.1600-051X.2010.01608.x.

Abstract

Objective: To assess the osseointegration process after flapless immediate implant placement with or without immediate loading.

Material and Methods: This study was carried out on six beagle dogs. Four implants were placed in the lower jaw (two per side) in each dog immediately after tooth extraction (3rd and 4th premolars). Flapless immediate implant placement was performed in one hemimandible (control). The same procedure was carried out in the contra-lateral side and immediate prosthesis was connected with occlusal contacts (test). After 3 months of healing, the dogs were sacrificed for histomorphometric analysis.

Results: Twelve implants were placed in each group. None of the implants and prosthesis was lost. The percentage of bone-to-implant contact (BIC %) was similar in both groups: 82.72% (test) and 76.96% (control). Differences were found neither in the inter-thread bone area (test: 83.45%, control: 80.65%) nor in peri-implant bone area (test: 94.37%, control: 94.81%).

Conclusion: In this animal model where the implants were well within the confines of the extraction socket, osseointegration following flapless immediate implant placement and loading can be achieved in the same manner as immediate placement without loading.

Key words: bone healing; flapless surgery; immediate implants; immediate loading

Accepted for publication 29 June 2010

Since the first published work on immediate implants (Schultze et al. 1978), the interest for this technique has increased in the last years. The advantages claimed by various authors are the following: decrease of the number of surgeries and of the overall treatment time (Lazzara 1989, Parel & Triplett 1990), ideal implant orientation (Werbitt & Goldberg 1992, Schultz 1993), bone preservation in the extraction area (Shanaman 1992, Denissen et al. 1993, Watzek et al. 1995) and optimum aesthetic of the soft tissues (Werbitt & Goldberg 1992).

Recently, it has been suggested (Paolantonio et al. 2001) that the placement of immediate implants could maintain the anatomy and the original shape of the alveolar ridge. However, published experimental studies (Botticelli et al. 2004, Araújo et al. 2005) failed to prove this hypothesis. We must emphasize that in these studies, surgery (dental extraction, immediate implant placement) was always performed raising a vestibular and lingual flap.

In this context, we should highlight that the surgical trauma (raising the flap). which implies the detachment of the periosteum and its connective desinsertion to the underneath bone surface, will cause vascular damage and an acute inflammatory response, which will mean the resorption of the exposed bone surface (Wilderman 1963, Staffileno et al.

Conflict of interest and sources of funding statement

This study was supported by Straumann Company (Straumann, Basel, Switzerland).
The authors declare that there are no conflicts of interests in this study.

1966, Wood et al. 1972, Bragger et al. 1988). This could partially explain the dimensional alterations suffered by the alveolus after tooth extraction (Araújo & Lindhe 2005) and by the alveolus after extraction and immediate implant placement (Araújo et al. 2005). In this way, Blanco et al. (2008) showed in the beagle dog that the resorption of the vestibular bone after immediate implant placement is minor when performed without flap, although without statistical significance.

On the other hand, in accordance with the Wolff's Law (Wolff 1892, Frost 1990, 1994), the function and stimulation of the bone lead to the conservation of the integrity of the structure of that tissue. Some authors have suggested that certain loads may increase the amount of mineralized bone at the interface and in the peri-implant bone (Wehrbein et al. 1998, Gotfredsen et al. 2001, Berglundh et al. 2005). In addition, other authors stated that immediate implant loading may stimulate bone formation and thus may influence the early stages of osseointegration (Piattelli et al. 1998, Romanos et al. 2002, 2003). However, Nkenke et al. (2003) did not find any difference between the immediately loaded and unloaded groups.

A recent systematic review (Atieh et al. 2009) assessed the outcomes of immediate single implants with immediate restoration/loading. Ten studies with 629 implants were included. The results demonstrated that immediate single implant restoration/loading in extraction sockets in the aesthetic zone was associated with a significantly higher risk of implant failure (risk ratio of 3.62). However, the bimodal approach (immediate implant placement and loading) showed favourable marginal bone changes after 1 year. The review and meta-analysis supported the potential advantages offered by this bimodal approach, but indicated a higher risk when compared with immediate restoration/loading in healed ridges.

The objective of this study was to assess the influence of flapless immediate implant placement and loading on the osseointegration process in the beagle dog model.

Material and Methods

Once approval from the Ethics Committee of the University of Santiago had been granted, this research was carried out using six beagle dogs. They were provided by the School of Veterinary Studies at the University of Cordoba, and were installed in the Animal Experimentation Service facility at the Veterinary Teaching Hospital Rof Codina of Lugo. The animals were maintained in individual kennels in a 12:12 light/dark cycle (lights on at 07:00 h) and $22 \pm 2^{\circ}C$, with regular chow and tap water. All experiments were performed according to the Spanish Government Guide and the European Community Guide for animal care.

Surgical procedure

Six beagle dogs about 2 years old and 20 kg of weight, were enrolled in the study. During surgical procedures, the animals were premedicated with acepromacine (0.05 mg/kg intramuscularly) and morphine (0.2 mg/kg intravenously). Immediately after, they were induced general anaesthesia by injection of propofol (2 mg/kg intravenously). Isofluorane (1.5–2%) and O_2 (100%) were used as inhalated anaesthetics.

In total, 24 implants were placed in these six dogs. All implants were 8 mm long, 3.3 mm diameter with Standard neck height (2.8 mm), Straumann Dental Implant System (Institute Straumann, Basel, Switzerland). All the implants had a sand-blasted and acid-etched surface (SLA). The implants were placed into fresh extraction sockets, and bone augmentation procedures were not attempted.

The lower 3rd and 4th premolars were carefully removed, separating the roots by means of tooth hemisectioning with the use of a fissure bur, and extracting them individually with elevators and forceps. After the extraction, immediate implants were placed into the distal sockets of each tooth. Four implants were placed in each dog (two in each hemimandible) according to the manufacturer's protocol (Straumann® Dental Implant System, Basel, Switzerland). The implants were placed so that the marginal level of the (SLA-coated surface was flush with the buccal bone crest. In order to achieve this, the buccal soft tissue height was measured with a periodontal probe immediately before implant installation and having in mind that the smooth surface of the implant had a height of 2.8 mm. Primary stability [implant stability quotient (ISQ) $\geqslant 60$] was measured immediately after implant installation.

Following implant installation, the experimental groups were randomly selected. One hemimandible (test group) received an immediate loading restoration by means of provisional abutments for bridges (Straumann® Dental Implant System) that were splinted by an acrylic stent that remained with occlusal contacts with the antagonist teeth. The occlusion was checked again at sacrifice. The implants in the contra-

Fig. 1. Clinical photographs illustrating the experimental surgery. (a) Extraction of premolar 3 and 4. (b) Flapless immediate implant placement in distal sockets. (c) Immediate loading with a resin provisional screw-retained bridge in the test group. (d) Photograph illustrating the experimental groups.

lateral hemimandible (control group) received short healing caps (1.5 mm height) aiming a non-submerged healing approach without loading (Fig. 1).

During the first week after surgery, the animals received Amoxicillin (500 mg, twice daily) orally and Meloxicam (0.1 mg/kg, once a day) orally. Throughout the experiment, the animals were fed a pellet diet. They were placed on a plaque control regimen that included tooth and implant cleaning three times per week with the use of toothbrush and dentifrice.

After 3 months of healing, the animals were euthanized with an overdose of sodium pentobarbital through the cephalic veins.

Histological preparation

The mandibles were removed and block biopsies of each implant were dissected using an oscillating saw (Donath 1993). The samples were fixed in 10% formol for 1 week. Next, the samples were dehydrated in different graded ethanol series (70–100%), and infiltrated with four different graded mixtures of ethanol and infiltrating resine, glicometacrilate (Technovit 7200®, VLC – Heraus Kulzer GMBH, Werheim, Germany) with 1% of Benzoyle Peroxide (BPO®: Heraus Kulzer GMBH). The last infiltration was performed with pure infiltrating resin under vacuum. The samples were then polymerized, first under low-intensity UV light for 4 h, followed by a polymerization under high-intensity UV light for 12 h and finally by keeping the samples heated for 24 h to assure complete polymerization.

The samples were glued to a sample holder. Longitudinal sections in the bucco-lingual direction of 200 μm were cut with a band saw and mechanically polished (Exakt Apparatebau, Norderstedt, Germany) using 1200 and 4000-grit silicon carbide papers (Struers, Copenhagen, Denmark) until 70 μm thick samples were obtained. All sections were stained with Levai Laczko tincture (Laczkó & Levai 1975) for both histological examination and histomorphometric analysis.

The samples on the permanent ports were observed using the Olympus® SZX9 microscope (Olympus, Tokyo, Japan). By means of the Olympus® DP12 digital camera (Olympus), the images were captured and transferred to the computer. With the Microimage® program, the points of interest were identified from the digital histological images in order to subsequently measure the variables. The researcher carrying out the measurements was blind with respect to the group to which each sample belonged. The histological outcomes to analyse were:

- Bone-to-implant contact (%) (BIC %).
- Bone area (inter-thread and peri-implant bone area).

The percentage of BIC was measured within the SLA border surface. To carry out this measurement on the digital image, the entire implant was scanned from SLA border. This was calculated dividing the length of the implant surrounded by bone by the total length of the implant with SLA surface, with the resulting value multiplied by 100. This figure indicates the implant level of integration with the surrounding bone.

The inter-thread bone area was defined as the area of bone inside the threads/complete area inside the threads multiplied by one hundred, starting from the most coronal thread down to the most apical thread.

The peri-implant bone area was measured in the zone from the tip of the threads up to 300 μm horizontally. It was determined as bone area/tissue area multiplied by100, again starting from the most coronal thread down to the most apical thread (Fig. 2).

Statistical analysis

The statistical analysis was performed using the Sigma-Stat® statistics program.

Descriptive statistics were taken for each of the variables and groups (mean values and standard deviation).

To compare the two groups in each variable, the Student's *t*-test for paired observations was used.

The dog was used as the unit for analysis ($n = 6$), using average results across similarly treated implants in the same dog and then compared. *p*-Values < 0.05 were considered statistically significant.

Results

In total, 24 implants were immediately placed following tooth extraction, 12 of them were immediately loaded and the other 12 remained without loading in a non-submerged healing approach. At the end of the experimental period, none of the implants was lost, and all the restorations were still in service and the occlusal contacts remained in the provisional bridge; however, all restorations showed abrasion of the occlusal aspects.

At the time of surgery. all the immediate implants had an ISQ value over 60, with a mean of 69.41 (range 62–75) and 68.33 (range 60–73) for the immediate loaded and unloaded group, respectively. No statistical significant difference was detected. After 3 months of healing, the ISQ values increased in both groups (Table 1).

Histological observations

The histological study showed that the buccal and lingual mucosa in each implant of both groups were covered by a keratinized oral epithelium that continued from the peri-implant marginal mucosa with the barrier epithelium facing the implants. Apical to this epithelium was an area of fibre-rich connective tissue, which apparently maintained strong contact with the implant. The central and external parts of the buccal and lingual bone plates were made up of lamellar bone characterized by a large density of secondary osteons.

Histomorphometric results

BIC % (Table 2)

The BIC % was higher at the immediate loaded (mean 82.72%, SD 9.54, range 61.37–93.47%) than at the unloaded group (mean 76.96%, SD 10.63, range 59.67–92.51%). The difference was, however, not statistical significant.

Bone area (Table 3)

The inter-thread bone area did not differ for the loaded (mean 83.45%, range 51.39–96.82%) and unloaded group (mean 80.65%, range 48.95–97.02%).

The peri-implant bone area showed no relevant differences between groups (loaded, 94.37%; unloaded, 94.81%).

Discussion

Experimental animal trials of immediate loading implants in healed sites have shown a good level of osseointegration (Piattelli et al. 1998, Romanos et al. 2002, 2003, Nkenke et al. 2003). The

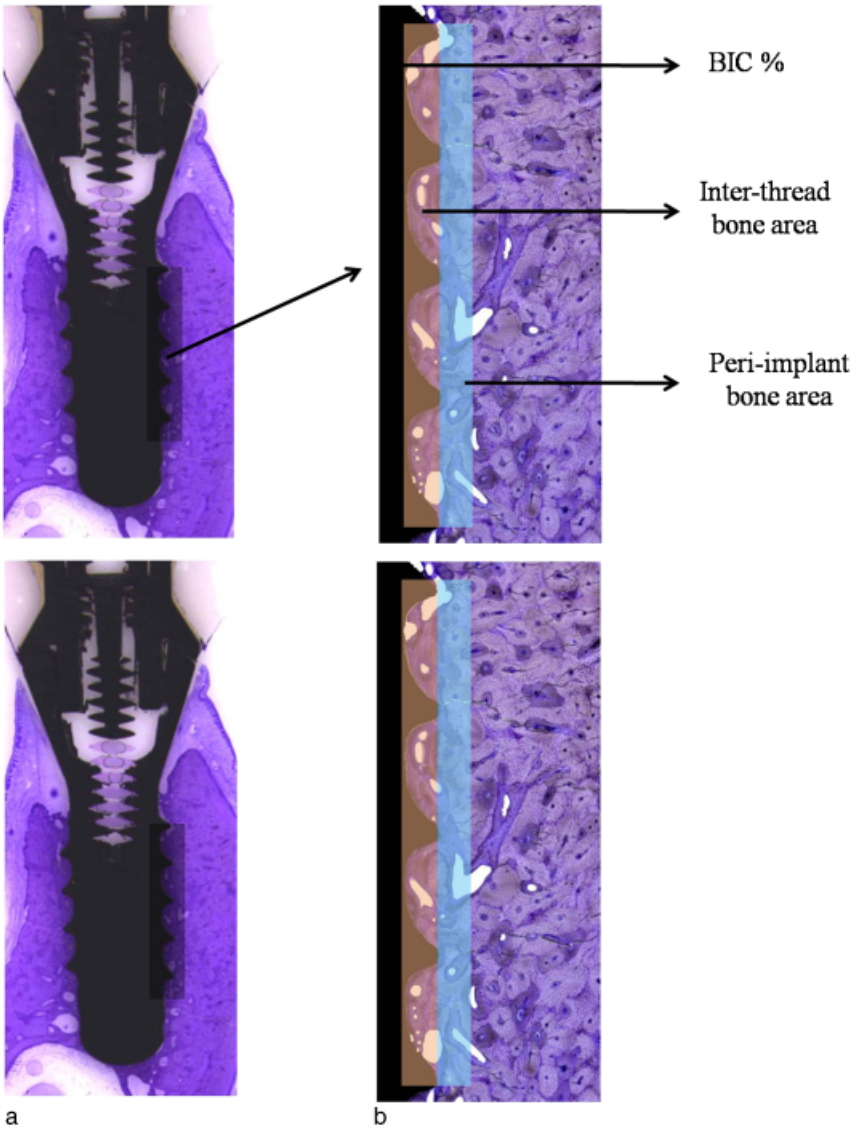

Fig. 2. Histomorphometric analysis. (a) Implant area included for the analysis of the inter-thread and peri-implant bone area. (b) Delimitation of the inter-thread area corresponding to the five threads, and delimitation of the peri-implant area from the picks of the threads up to 300 μm horizontally. Levai Laczko staining method. Original magnification $\times$ 8 and inset $\times$ 30.

Table 1. Implant Stability Quotient values (ISQ)

	Loading		No loading	
	baseline	3 months	baseline	3 months
Mean	69.41	73.08	68.33	74.25
SD	3.67	5.43	4.16	5.31
Minimum	62	60	60	65
Maximum	75	79	73	80
p-value	0.062		0.016	

Table 2. Bone-to-implant contact percentage (BIC %)

	BIC %	
	loading	no loading
Mean	82.72	76.96
SD	9.54	10.63
Minimum	61.37	59.67
Maximum	93.47	92.51

results of the present research are consistent with the above-mentioned studies. In addition, clinical trials have shown that immediate loading of dental implants can be achieved with good long-term success for removable and fixed prostheses (Chiapasco 2004, Gallucci et al. 2004, Schincaglia et al. 2007). In the present study, none of the implants and prosthesis was lost.

Dental implant primary stability has been demonstrated to be a key factor for implant survival rates. Sennerby & Meredith (1998) found the resonance frequency analysis technique to be helpful in deciding when to replace an immediately loaded temporary prosthesis with a permanent prosthesis after implant placement. Östman and colleagues (Östman et al. 2005, Östman 2008) reported low failure rates when using an ISQ of 60 as an inclusion criterion for immediate loaded implants in totally edentulous maxillae and in posterior mandibles. Schincaglia et al. (2007) carried out a randomized controlled trial, where patients received immediate loading of dental implants supporting fixed partial dentures in the posterior mandible, in which one side received machined-surface implants whereas the other received oxidized-surface implants. At implant placement, they recorded the maximum insertion torque and ISQ values. After 1 year of function, no implants were lost in the oxidized-surface group and two were lost in the machined-surface group. ISQ and insertion torque vales for both groups of implants were similar. The authors concluded that implants placed in the posterior mandible, inserted with $\geqslant$20 N cm and ISQ value $\geqslant$60, may be considered for being immediately loaded in partially dentate patients. Values above an ISQ of 60, as results obtained with the implants in this study, indicate a favourable response to immediate loading, while low ISQ values may be indicative of overload and ongoing failure. However, no clinical conclusions should be extrapolated from the present investigation, as the above-mentioned studies were performed in humans and with longer follow-up.

In the present study, it has been shown that osseointegration can be achieved following immediate implant

Table 3. Bone area percentage (inter-thread and peri-implant bone area)

	Inter-thread bone area (%)				Peri-implant bone area (%)			
	mean	SD	maximum	minimum	mean	SD	maximum	minimum
Loading	83.45	13.33	96.82	51.39	94.37	5.3	99.73	80.55
No loading	80.65	12.12	97.02	48.95	94.81	4.67	99.52	82.58
p-value	NS				NS			

NS, not significant.

placement with or without immediate loading. The amount of osseointegration measured as the percentage of BIC was similar in both groups and was not influenced by the loading protocol. The BIC % achieved in this study is in agreement with previously reported experimental models in dogs (Araújo et al. 2005, 2006, Blanco et al. 2008, de Sanctis et al. 2009) with similar type of implants. In a study by Mangano et al. (2009) in a non-human primate Papio ursinus model, with immediate implants and immediate loading *versus* no loading (submerged), they obtained a BIC % of 86.85% for the immediate loading group and 86.02% for the unloaded group, and these findings are in agreement with the results achieved in our study. In the same way is the study by Nkenke et al. (2003) in the mandible of minipigs. The results of their study reveal that the BIC, bone density and bone mineral apposition rate do not differ for loaded and unloaded implants during the observation period. However, Berglundh et al. (2005), in a study in the beagle dog analysing the bone tissue reactions to longstanding functional loading, showed a higher degree of BIC in the loaded group. One reason for these results could be the 10 months of follow up *versus* the 3 months in the present study. Therefore, it appears that immediate loading has no adverse effects on the new bone formation.

In summary, it can be concluded with the results of the present study that osseointegration can be achieved following flapless immediate implant placement with or without immediate loading. The bimodal approach (immediate placement and loading) may be a safe protocol if sufficient primary stability is obtained at implant installation.

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Address:
Juan Blanco
Department of Stomatology
School of Medicine and Odontology
University of Santiago de Compostela
C/Entreríos s/n.
15705. Santiago de Compostela
Spain
E-mail: jblanco@blancoramos.net

Clinical Relevance

Scientific rationale for the study: There is an increasing interest in immediate implant placement and immediate loading protocols, but from a biological point of view there are some aspects (osseointegration process, bone resorption, etc.) that are necessary to be clarified before recommending such a technique in daily practice. The results of an experimental study in the beagle dog are shown, where flapless immediate implant placement and immediate loading are compared with flapless immediate implant placement without loading.

Principal findings In this animal model study where the implants were well within the confines of the extraction socket, osseointegration following flapless immediate implant surgery and immediate loading can be achieved in the same manner as immediate placement without loading.

Practical implications: According to this study, osseointegration of immediate implant placement with immediate loading is achieved in the same way as immediate implant placement alone.