# ORIGINAL ARTICLE

# Peri-implant bone reactions around immediately loaded conical implants with different prosthetic suprastructures: histological and histomorphometrical study on minipigs

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Abstract The aim of this study was to evaluate peri-implant bone reactions around immediately loaded conical implants with metal and acrylic resin prosthetic restorations. Five splinted conical implants were inserted in each hemimandible of six minipigs at the alveolar crest level. Ten implants were inserted in each minipig. All the implants were immediately loaded. The implants were divided into a group with an acrylic resin prosthetic restoration and into another group with a metal prosthetic restoration. No postoperative complications or deaths of the minipigs occurred. All minipigs were killed after 3 months. No implant was lost. A total of 60 implants were retrieved and processed to obtain thin ground sections. Histology and histomorphometry showed the presence of compact, mature bone around all the implants. Bone was in close contact with the implant surface starting from the first or second implant threads. A high quantity of mineralized bone was present around immediately loaded conical, root form implants. No differences in the peri-implant bone response were found in the groups with different prosthetic reconstructions.

**Keywords** Animal study · Bone tissue · Conical implants · Immediate loading · Prosthetic superstructures

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## Introduction

Immediate loading of dental implants is currently one of the most interesting and studied topics in implant dentistry [1]. Although it has been shown to be clinically successful under long-term function [2, 3], limited knowledge exists regarding the healing and remodeling processes of the bone around these implants. Animal studies demonstrated osseointegration of immediately loaded definitive [4, 5] and provisional [6] implants. Histologic evaluations of immediately loaded implants showed a high degree of osseointegration under long-term function [7, 8]. Using screw implants with a microstructured surface and bone quality-adapted insertion procedures, osseointegration was achieved when implants were initially stable and splinted within the prosthetic suprastructures [9]. The importance of the implant geometry and surface characteristics, in an effort to achieve better bone anchorage, has been demonstrated [10] and various implant systems have been introduced over the past years in order to achieve a faster bone integration [11, 12]. Some authors focused their attention on the conical implant design. Nordin et al. [13] showed that a conical implant had a wider diameter in the cortical passage and that it resulted in bone resorption along the conical surface down to the first thread. Sakoh et al. [14], in an in vitro study, showed that conical implants had a higher primary stability than cylindrical implants. These authors found that the torque and push-out values of the conical implants were significantly higher, while the mean Periotest values of the conical implants were significantly lower. Quaresma et al. [15], in a finite element analysis, showed that a conical implant connected to a solid, internal, conical abutment produced lower stresses on the alveolar bone and prosthesis and greater stresses on the abutment compared to a cylinder implant connected to a screw-retained, internal hexagonal abutment. The use of miniature pigs in dental research increased significantly due to their similarity in gross anatomy and physiology to humans, as well as for other scientific, economic, and ethical reasons [16]. The roots of the teeth in the minipig are curved distally and the teeth have a higher number of roots than humans (e.g., the molars have four to six roots) [16]. The minipig is a valuable preclinical model that can be used in oral and craniofacial research [17-21] although the rate of bone regeneration in pigs is different from humans (pigs 1.2-1.5 mm/day; human 1.0-1.5 mm/day) [22]. The restorative materials used for implant-supported prostheses are another important point in implant dentistry due to the fact that they play a relevant role in the long-term success of osseointegrated implants [23]. However, in vivo studies histologically quantifying the reaction of the peri-implant hard tissues to different restorative superstructure materials are lacking [24]. The three most common groups of materials used for fixed prostheses are porcelain, acrylic, and metal. Impact loads are the lowest with acrylic, increase with metal, are greater with enamel, and further increase with porcelain. Porcelain and acrylic fractures can occur under excessive loads or even with a lesser load of longer duration, angulation, or frequency, and acrylic fractures more easily [25]. Few data are available regarding biomechanical reactions between immediately loaded implants rehabilitated with acrylic and metal suprastructures [26].

The aim of this study was to evaluate peri-implant bone reactions around immediately loaded conical implants with either metal or acrylic resin prosthetic restorations.

### Materials and methods

A total of six Göttingen minipigs, 14 to 16 months of age and with an average body weight of 35 kg, were used in this study. The study was approved by the Ethical Committee of the Veterinary Clinic of the University of Madrid, Spain. A total of 60 root form conical implants (RF, Bone System, Milan, Italy) were used. The animals were sedated with an intramuscular injection of ketamine (10 mg/kg), atropine (0.06 mL/kg), and stresnil (0.03 mL/kg). In the areas exposed to surgery, 4 mL of local anesthesia (2% lidocaine with 12.5  $\mu$ g/mL epinephrine; Xylocain/Adrenalin®, Astra, Wedel, Germany) was injected and three premolars and three molars were extracted in each hemimandible.

The tooth extractions were difficult in every case because the roots were divergent and usually curved distally. It was always necessary to separate the roots before extracting them.

After a healing period of 3 months, no migration of the neighboring teeth was observed and five splinted implants

10 mm in length and 4.1 mm in diameter were inserted in each hemimandible at the level of the alveolar crest (Fig. 1) under continuous external sterile saline irrigation. All the implants were immediately loaded (the same day of the insertion). The implants were divided into two groups; the implants in the first group, inserted in the left hemimandible, were rehabilitated with prosthetic restorations in acrylic resin (Fig. 2), while the implants in the second group, inserted in the right hemimandible, were rehabilitated with a prosthetic restoration in metal (Fig. 3). Both kinds of restorations were put in centric occlusion and checked with articulating paper [27]. The animals were inspected after the first few postoperative days for signs of wound dehiscence or infection and weekly thereafter to assess general health. No postoperative complications or death occurred. All minipigs were killed after 3 months. The animals were euthanized with an overdose of ketamine hydrochloride given intravenously. No implants were lost. A total of 60 implants were retrieved (Figs. 4 and 5). The implants and surrounding tissues were stored immediately in 10% buffered formalin and processed to obtain thin ground sections. The specimens were processed using the Precise 1 Automated System (Assing, Rome, Italy). The specimens were dehydrated in a graded series of ethanol rinses and embedded in a glycol methacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). After polymerization, the specimens were sectioned, along the longitudinal axis of the implants, with a high-precision diamond disk at about 150 µm and ground down to about 30 µm with a specially designed grinding machine. Three slides were obtained for each implant. These slides were stained with acid fuchsin and toluidine blue and examined with transmitted light under a Leitz Laborlux microscope (Leitz, Wetzlar, Germany).

Histomorphometry was carried out using a light microscope (Laborlux S, Leitz, Wetzlar, Germany) connected to a



Fig. 1 Five implants are placed in each hemimandible at the level of the alveolar crest



Fig. 2 Implants rehabilitated with acrylic resin restoration

high-resolution video camera (3CCD, JVC KY-F55B, JVC<sup>®</sup>, Yokohama, Japan) and interfaced to a monitor and personal computer (Intel Pentium III 1200 MMX, Intel<sup>®</sup>, Santa Clara, CA, USA). This optical system was associated with a digitizing pad (Matrix Vision GmbH, Oppenweiler, Germany) and a histometry software package with image-capturing capabilities (Image-Pro Plus 4.5, Media Cybernetics, Immagini & Computer Snc, Milano, Italy).

The histomorphometric measurements involved the mean percentage of bone to implant contact (BIC) in the three best consecutive threads in the cortical region, the bone area in all threads, as well as the bone area in the three best consecutive threads in the cortical region.

#### Results

The histological results in both groups were similar and will be presented together. Histological analysis of the bone-



Fig. 3 Implants rehabilitated with metal restoration



Fig. 4 Radiograph showing, before retrieval, the implants splinted and rehabilitated with acrylic resin restoration

implant interface revealed compact, mature bone found around all implants (Figs. 6 and 7). Many osteons were present. Only a few marrow spaces were present. Bone was in close contact with the implant surface (Fig. 8). Only a few remodeling areas were present. Only a few osteoblasts were present at higher magnification. No inflammatory cell infiltrate was present in the marrow spaces. A slight inflammatory cell infiltrate was observed in the periimplant soft tissues. No osteoclasts were observed. No gaps or fibrous, connective tissue was found at the boneimplant interface (Fig. 9). No epithelial downgrowth was present. First bone to implant contact was present at the level of the first or second implant thread. The quantitative histomorphometric analysis showed that:

- 1. bone to implant contact in all available threads around the implant =  $69.8\pm3.2$  (acrylic prosthetic restorations) vs  $68.1\pm2.1$  (metal prosthetic restorations; p=0.38);
- 2. bone to implant contact in the three best consecutive threads in the cortical region =  $84\pm2.7$  (acrylic



Fig. 5 Block sample retrieved after 3 months



Fig. 6 Low-power magnification of a histologic section of an implant rehabilitated with an acrylic resin restoration. Bone is present over a large portion of the implant surface. Acid fuchsin-toluidine blue,  $\times 8$ 

prosthetic restorations) vs  $83\pm3.6$  (metal prosthetic restorations; p=0.41);

bone area in all threads = 91±0.9 (acrylic prosthetic restorations) vs 89±1.1 (metal prosthetic restorations; p=0.32);



Fig. 7 Low-power view of a histologic slide of an implant rehabilitated with a metal restoration. Mature bone lines the implant perimeter. Acid fuchsin-toluidine blue,  $\times 8$ 



Fig. 8 Compact, mature bone with many osteons are found at the implant interface of an implant supporting an acrylic restoration. Acid fuchsin–toluidine blue,  $\times 40$ 

4. bone area in the three best consecutive threads in the cortical region =  $93\pm1.6$  (acrylic prosthetic restorations) vs  $91\pm1.8$  (metal prosthetic restorations; p=0.39).

# Discussion

Primary stability is related to mechanical interlocking, which is one of the most important factors for the development of osseointegration [28]. The implant geometry leads to a homogenous strain distribution in loaded peri-implant bone [29]. It has been demonstrated in animal experimental studies that immediate loading of dental implants could be performed without disturbing the early osseointegration process [30]. Several studies emphasized



Fig. 9 Peri-implant bone found around an implant restored with a metal prosthesis. No gaps or soft tissues are found at the bone–metal interface. Acid fuchsin–toluidine, blue  $\times 40$ 

that the osseointegration process depended on the implant design [10-20]. In the present study, a very high boneimplant contact percentage was present around immediately loaded conical, root form implants. The implant system used in this study was designed to allow implants to have a direct bone-implant contact over the whole implant surface directly after insertion. The use of a rough, sandblasted, and acid-etched surface was most probably helpful in obtaining a high bone-implant contact percentage in both groups. Clinical, radiographic, and histologic evidence supported the use of metal [24] and acrylic resin suprastructures as fixed prostheses for the restorations of endosseous dental implants [31]. However, when comparing porcelain and acrylic resin occlusal surfaces on osseointegrated implantsupported prostheses opposing natural teeth, no differences related to material could be detected in the load rates [32].

In the present animal study, no differences were found in the bone response in specimens retrieved after 3 months in the two groups (implants with metal superstructures and implants with acrylic resin superstructures). Acrylic resin had a low module of elasticity and could decrease the occlusal impact forces on the bone-implant area if compared to metallic suprastructures. Gracis et al. [33] evaluated the damping effect of five restorative materials rigidly connected to a Brånemark implant and subjected to an impact force. These materials included a gold alloy, a noble metal ceramic alloy, porcelain, a laboratory-processed light-activated microfilled resin, and a heat- and pressurepolymerized polymethyl methacrylate resin. The two resins were found to reduce the impact force by about 50% when compared to porcelain or the alloys. However, this potential protective role has never been fully demonstrated and, on the contrary, a significantly better distribution of bending moments was observed with the metal prostheses in comparison to the acrylic resin prostheses [34]. Sertgöz [35] used a three-dimensional field emission microscopy to study the effect of the superstructure material and occlusal surface material on the stress distribution in an implantsupported fixed prosthesis. The conclusion was that using a superstructure material with a lower modulus did not lead to substantial differences in the stresses in any of the parts of the model (e.g., prosthesis, screws, implants, surrounding bone), although the lower-modulus material did tend to concentrate stresses in the retaining screws.

The data of the present study were in agreement with other studies that observed no statistically significant differences of the force absorption quotient between the occlusal surfaces of gold, porcelain, and resin [36]. More recently, a study [37] used strain-gauged abutments to measure the force transferred to the implant after the application of a shock. This was measured in vitro and in vivo in five patients, and the different occlusal materials did not lead to different forces generated on the implants.

In the present histological specimens, a marginal bone resorption up to the first or second thread was observed. This could be related to the lack of oral hygiene or to an overloading of the implants. The data to assess the importance of inflammation of the peri-implant tissues and of occlusal overload are, however, still insufficient [38]. In a recent review, it has been stated that, even if in several clinical studies marginal bone loss around implants had been associated with high occlusal stress of the implants, a causative relationship with overload has not been established [39].

Even if there was a difference in resilience between acrylic resin and other materials, this difference was, probably, only measurable in vitro. Therefore, it seemed reasonable that prosthesis materials had no significant effects on the peri-implant bone stress, and the results of the present study showed that a protective role of resin for the bone–implant interface could not be demonstrated.

Additional studies are certainly necessary to evaluate the different types of implant-supported prostheses before final restorative recommendations can be made.

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**Conflicts of interest** The authors declare that they have no conflicts of interest.

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