CLINICAL ORAL IMPLANTS RESEARCH

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Study of the osseointegration of dental implants placed with an adapted surgical technique

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Date:
Accepted 21 July 2010

To cite this article:
Al-Marshood MM, Junker R, Al-Rasheed A, Al Farraj Aldosari A, Jansen JA, Anil S. Study of the osseointegration of dental implants placed with an adapted surgical technique
Clin. Oral Impl. Res. xx, 2010; 000–000.
doi: 10.1111/j.1600-0501.2010.02055.x

Key words: bone integration, dental implants, primary stability, surgical technique

Abstract

Objective: To study the osseointegration of dental implants placed with a modified surgical technique in Beagle dogs and to compare it with the conventional method.

Materials and methods: Dental implants were placed bilaterally in the mandible of Beagle dogs using the press-fit as well as undersized implant bed preparation technique. Micro computer tomography (micro-CT) and histometric methods were used to analyze the bone implant contact and bone volume (BV) around the implants.

Results: The bone-to-implant contact percentage (BIC: expressed as %), first BIC (1st BIC: expressed in mm), sulcus depth (SD: expressed in mm) and connective tissue thickness (CT: expressed in mm) were analyzed for both groups. The BIC percentage was significantly higher for the undersized installed implants ($P = 0.0118$). Also, a significant difference existed between the undersized and press-fit installed implants for the first screw thread showing bone contact ($P = 0.0145$). There were no significant differences in mucosal response (SD and CT) for both installation procedures. Also, no significant difference was found in the BV, as measured using micro-CT, between the implants placed with an undersized technique (59.3 ± 4.6) compared with the press-fit implants (56.6 ± 4.3).

Conclusion: From the observations of the study, it can be concluded that an undersized implant bed can enhance the implant–bone response.

Dental implants have become the first mode of treatment for lost teeth. This has revolutionized oral rehabilitation in partially and fully edentulous patients. Implant success is reported to depend on both the implant-to-bone response as well as the mechanical strength of the various implant components (Chuang et al. 2002; Abrahamsson et al. 2009) High implant success rates of the order of 78–100% have been reported over the past 15 years (Albrektsson et al. 1986). Despite these high rates of success, complications and failures still occur. Although a specific reason for the occurrence of these failures is difficult to indicate, it is known that the bone healing response is a very important parameter for success.

In view of the above mentioned, one of the main goals in dental implant treatment is to achieve optimal implant osseointegration. Of which, primary implant stability is considered to be a critical factor for obtaining successful osseointegration (Friberg et al. 1991). Studies have demonstrated that initial implant stability is determined by the density of the bone, the surgical technique used and the design of the implant (O'Sullivan et al. 2004; Abrahamsson et al. 2009). While different implant designs have shown similar results of higher stabilities in dense bone, initial stability can remarkably decrease in low-density bone, jeopardizing the osseointegration process and risking failure (Molly 2006). Low-density bone implant sites have been pointed out as the greatest potential risk factor for implant loss when working with standard surgical protocols (Garg 2002; Jacobs 2003). A clinical study with consecutively placed implants that were immediately loaded showed a higher failure rate in low-density bone, reinforcing that primary stability is a major factor in the success of immediately loaded implants (Glauser et al. 2001).

Because the bone quality and quantity are factors that cannot be controlled, the implant design and surgical technique may be adapted to the specific bone quality to improve the initial implant stability. Several strategies are available to optimize primary stability in bone of varying quality. Depending on the bone at the planned

implant position, an adaptive drilling procedure (e.g. by using a final drill size smaller than the recommended one) can be chosen to optimize implant stability (O'Sullivan et al. 2000; Beer et al. 2003; Shalabi et al. 2007a, 2007b; Tabassum et al. 2009). Other authors have proposed bone condensation using osteotomes (Summers 1994; Komarnyckyj & London 1998; Fanuscu et al. 2007), or even placing a submerged implant with its collar in a supra-crestal position to increase local compression of bone at the implant site (Davarpanah et al. 2000; Oh et al. 2003). In addition to the afore mentioned, other modifications to enhance the primary stability of oral implants are: the avoidance of pre-tapping, minimal or no countersinking, long/wide diameter implants, etc. (Glauser et al. 2001; Lekholm 2003; Sennerby & Gottlow 2008).

The primary stability, as obtained directly after implant installation is followed by a secondary stability phase, which is determined by the bone healing response. At the end of this secondary phase, the implant has to be completely osseointegrated, which has to be considered as a first guarantee for implant success. Even though few published clinical reports and animal studies have highlighted that under-sizing the implant bed results in a higher insertion torque during implant installation and a better primary implant stability (Beer et al. 2007; Shalabi et al. 2007a, 2007b; Tabassum et al. 2009), there is lack of histological data dealing with the processes as occurring during the secondary phase. For example, it cannot be excluded that the compressive forces as evoked on the surrounding bone after installation of an oral implant in an undersized implant bed, result in less osseointegration at the end of the bone healing phase. Hence, the current study was undertaken to evaluate the tissue response at the end of the secondary stability phase of dental implants placed according to two different surgical protocols in Beagle dogs.

The objective was to study the osseointegration of dental implants placed in the mandible of Beagle dogs using a conventional or undersized technique and left in place for 3 months. Bone as well as mucosal response were studied using micro computer tomography (micro-CT) and histological analysis.

Material and methods

Animals

Twelve adult Beagle dogs with an age of 9 and 16 months were used. The dogs weighed 10–15 kg. The design of the study was approved by the Ethics Committee on Animal Research, College of Dentistry Research Center (CDRC), King Saud University.

Extraction procedure

Teeth were extracted under general anesthesia under sterile conditions in an operating room. An intramuscular (IM) injection of ketamine hydrochloride (5 mg/kg) and diazepam (1 mg/kg) was used to sedate the animals before the procedure. The oral cavities of the animals were rinsed with a 1 : 1 mixture of povodine iodine 10% and chlorhexidine solution. The area around the lower premolars was locally anesthetized with an injection of lidocaine 2% with 1 : 100,000 epinephrine. Following complete anesthesia of tissues, the lower second and third premolars were extracted. The technique consisted of separating roots with a high-speed bur in the presence of an intense water spray and the roots were removed with forceps. The flaps were closed with interrupted absorbable sutures using 4-0 bbs Coated Vicryl and primary soft tissue closure was achieved without any additional procedure.

Implant surgical procedure

After a healing period of 3 months, the implant surgical procedures were performed under aseptic conditions by the same surgical team. Before surgery, the dogs were sedated and local anesthesia was injected in the field to reduce bleeding and to induce post-operative analgesia. Subsequently, an incision was made at the bone crest and a full thickness mucoperiosteal flap was raised both on the buccal and on the lingual side of the alveolar ridge. Using a low-speed drill, a graded series of burs and continuous saline irrigation, two implant sites per mandibular half were prepared. Thereafter, a self-tapping implant with a diameter of 4.1 and length of 8.5 mm (OSSTEM Implant System SS II, Seoul, Korea, http://www.osstem.com) was inserted in their designated positions.

For the installation of the implant fixtures, two different surgical approaches were used:

Group 1. Press-fit technique: Surgical preparation of the implant sites was accomplished with a consecutive series of drills to the final diameter of 3.8 mm. This included low-speed drilling using sterile saline as a coolant. The implants were placed in the created implant bed without any tapping.

Group 2. Undersized preparation procedure: The final drill used in the procedure had a diameter of 3.3 mm. A pilot study had already confirmed that indeed a 4.1 mm implant could be placed in such an undersized preparation.

All implants were inserted manually. The Group 1 implants were installed in one side of the mandible, whereas the contralateral side received the Group 2 implants. In the various animals, the implant bed preparations in the left and right mandibular side, according to the Group 1 and 2 specifications, were alternated. Further, the implants were placed with the lower margin of the ''smooth'' permucosal part in a flushed position with the crestal bone. After the placement of implants, the flaps were closed with interrupted absorbable sutures using 4-0 bbs Coated Vicryl and primary soft tissue closure was achieved without any additional procedure. A broad spectrum antibiotic (Gentamycin 4 mg/kg body weight) was administered intramuscularly for 7 days. The dogs were kept on a soft diet for 2 weeks after the surgical procedure. None of the implants in this study were loaded, but they did penetrate the gingiva with their permucosal part.

Three months after insertion of the implants, the dogs were sacrificed to harvest the jaw bone with the implants. After premedication with a combination of Haloperidol® and Fentanyl®, the dogs were anesthetized using 30 mg/kg Thiopental® after which 0.5 ml/kg Thromboliquine® was injected intravenously, followed by a lethal dose of Thiopental®. The vascular system was perfused with physiologic saline, followed by 4% neutral formaldehyde as a fixative. After perfusion, the mandibles were dissected out and immersed in 4% neutral formaldehyde.

Micro-computer tomography

After fixation in the formaldehyde solution and dehydration in ethanol 70%, three-dimensional micro-computer tomography (micro-CT) was used to analyze the bone volume (BV) of the implant surrounding bone mass. The specimens were wrapped in Parafilm M (Pechiney Plastic Packaging, Chicago, IL, USA) to prevent drying during scanning. Then, all samples were scanned at an energy of 101 kV and intensity of 96 μ.A with a resolution of 37.41 μ.m pixel using an aluminum filter (1 mm) (Skyscan-1072 X-ray microtomograph, TomoNT version 3N.5, Skyscan®, Kontich, Belgium). Cone-Beam reconstruction (version 2.15, Skyscan®) was performed. All scan and reconstruction parameters applied were identical for the specimens and calibration rods.

The data were analyzed by the CT Analyzer (version 1.9, Skyscan®). The region of interest (ROI) was specified as an annular area with a diameter of 1 mm surrounding the implants over a length of 3 mm. BV was determined in the area and expressed as percentage. BV (mm^3) was expressed as a percentage of the total ROI volume.

Histological procedures

After micro-CT imaging, the bone blocks with the implants were dehydrated in a graded series of alcohols for 9 days. Following dehydration, the specimens were infiltrated with methylmethacrylate. After polymerization in MMA, thin

(10 μm) non-decalcified sections were prepared in a mesio-distal direction parallel to the long axis of the implant using a modified diamond blade sawing microtome technique (Leica, SP1600, Nussboch, Germany) (van der Lubbe et al. 1988). All sections were stained with basic fuchsin and methylene blue and were examined with a light microscope (Zeiss – Axio Imager Z1 automated microscope with AxioCam MRc5 digital camera and AxioVision V6.3.2. acquisition software, Gottingen, Germany). Also, digital image analysis software (Leica Qwin Pro, Leica Microsystems Imaging Solutions, Cambridge, UK) was used for histomorphometrical measurements.

The following parameters were assessed (Fig. 1):

A. Percentage of bone-to-implant contact (%BIC):

The amount of interfacial bone contact was measured starting at the first bone contact till the last screw thread of the implant.

B. Marginal bone level:

The linear distance from the top of the implant to the to the first BIC (1st BIC) was measured (in mm) for each implant.

C. Soft-tissue attachment:

The following measurements (in mm) and calculations were performed for each implant:

(1) Sulcus depth (SD): linear distance between the top of the implant and the most coronal point of the junctional epithelium (cJE).
(2) Connective tissue thickness (CT): linear distance between aJE and the 1st BIC.

All quantitative measurements were performed on three randomly chosen sections of implant.

Statistics

All statistical analyses were performed with GraphPad® Instat 3.05 software (GraphPad Software Inc., San Diego, CA, USA). Percentage BIC, SD and CT data were analyzed using an unpaired t-test with Welch correction. Gaussian distribution of the data was tested using the method of Kolmogorov and Smirnov. First, BIC data were analyzed using a non-parametric Mann–Whitney test, as the data failed the normality test. Differences were always considered significant at P-values <0.05.

Results

All dogs showed an uneventful recovery of surgery and healing of the extraction as well as implantation sites. At the end of the study, six press-fit and four undersized implants were found to be lost. No clinical signs of inflammation or adverse tissue reaction were seen around the rest of the implants.

Micro-CT evaluation

The results of the BV measurements using micro-CT are depicted in Table 1. The undersized implants had a mean BV of 59.3 ± 4.6 compared with the press-fit implants (56.6 ± 4.3). Even though the BV in undersized implants was relatively higher than the press-fit, it was not statistically significant ($P = 0.1258$).

Descriptive histological evaluation

Light microscopic evaluation showed the presence of mature bone around all implants. The overall bone response to press-fit and undersized implants was very similar. The bone had either a very dense, compact structure or showed a more trabecular, spongy appearance (Figs 2–5). The bone was always found in intimate contact with the implant surface without any sign of intervening fibrous tissue layer. Occasionally, remodeling lacunae with osteoblasts were visible at the implant–bone interface. Only, at the marginal bone level, a difference in bone response was seen between the two surgical approaches. Around all undersized inserted implants, the first implant–bone contact occurred at or above the first implant screw-thread (Figs 2 and 3). In contrast, in about 50% of the press-fit inserted implants, the first screw-thread was not covered with bone tissue, but with fibrous tissue (Figs 3 and 4).

Mucosal response for both installation procedures was also similar. Around all implants, there was a limited down growth of the mucosal epithelium (Figs 4–6). The epithelium formed a stable junction with the implant surface. No gross inflammatory response was observed in the connective tissue.

Table 1. Bone volume data as measured by micro-CT

Bone volume	Mean ± SD	Sample size (*n*)
Undersized implants	59.3 ± 4.6	17
Press fit implants	56.6 ± 4.3	13

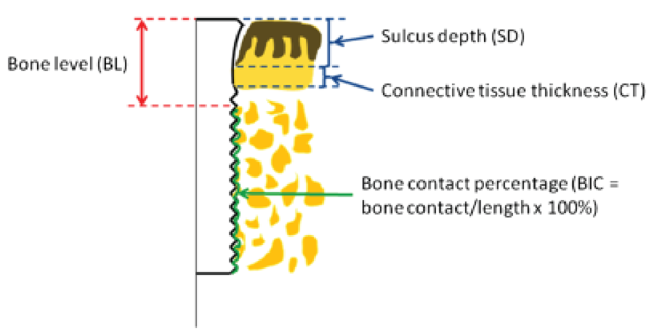

Fig. 1. Schematic drawing depicting the histomorphometrical parameters.

Histomorphometrical evaluation

The assessed parameters were BIC% (expressed as %), 1st BIC (expressed in mm), SD (expressed in mm) and CT (expressed in mm). The results are listed in Table 2 and 3.

Statistical testing of the BIC revealed a significant difference. The BIC% was significantly higher for the undersized installed implants ($P = 0.0118$). Also, a significant difference existed between the undersized and press-fit installed implants for the first screw thread showing bone contact ($P = 0.0145$). Further, no significant differences were seen in mucosal response (SD and CT) for both installation procedures (SD: $P = 0.1042$ and CT: $P = 0.2702$).

Discussion

Osseointegration refers to a direct bone-to-metal interface without the interposition of non-bone tissue. The concept of osseointegration has been defined at multiple levels such as clinically (Adell et al. 1981), anatomically (Branemark 1983), histologically and ultrastructurally (Linder et al. 1983). Primary implant stability is considered to play an important role in the successful osseointegration of dental implants (Albrektsson et al. 1981; Friberg et al. 1991). The primary implant stability depends on factors such as implant geometry, surgical procedure, site preparation and bone quality of the recipient site (Akca et al. 2006; da Cunha et al. 2004). Implant stability is often difficult to attain in bone of low density (Turkyilmaz & McGlumphy 2008). Several modifications of surgical technique have been described to increase the primary stability of implant in bone of low density. For example, Sennerby & Roos (1998) suggested that omission of tapping in low-density bone improves primary implant stability, while others have proposed bone condensation using osteotomes (Martinez et al. 2001), using a final drill size smaller than recommended (O'Sullivan et al. 2000), or even placing a submerged implant with its collar in a supra-crestal position (Davarpanah et al. 2000;

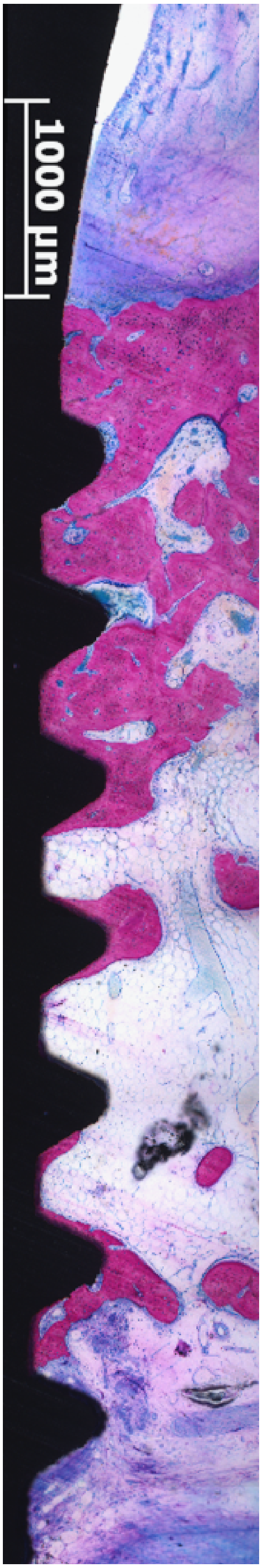

Fig. 2. Light micrograph of an undersized installed implant. No bone resorption is observed at the marginal level. The bone shows a trabecular appearance and is in close contact with the implant surface (Objective × 10).

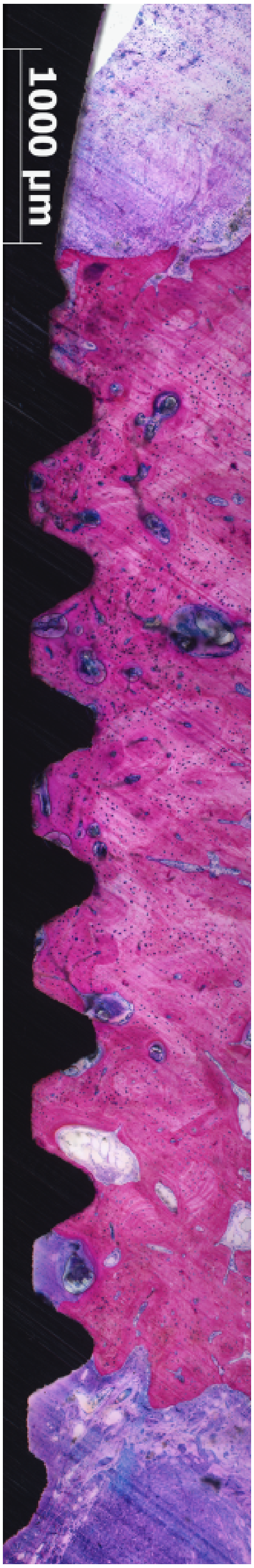

Fig. 3. Light micrograph of an undersized installed implant. The bone surrounding the implant is matured and is in close contact with the implant surface. Almost all screw threads are completely surrounded by bone (Objective × 10).

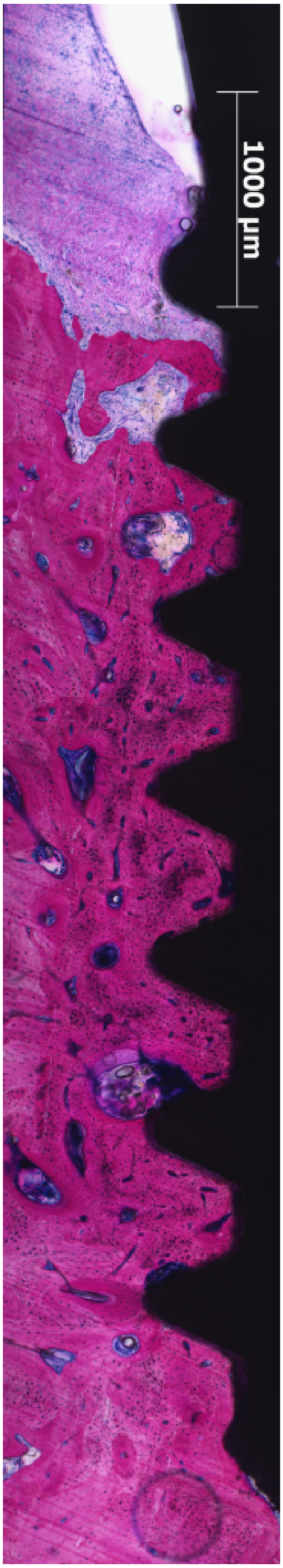

Fig. 4. Histological section of a press-fit installed implant. Dense bone is surrounding the implant. At the marginal level, the first screw-thread is exposed. The sulcus depth is limited. Junctional epithelium and connective can be discerned. No inflammatory response was observed in the connective tissue layer (Objective × 10).

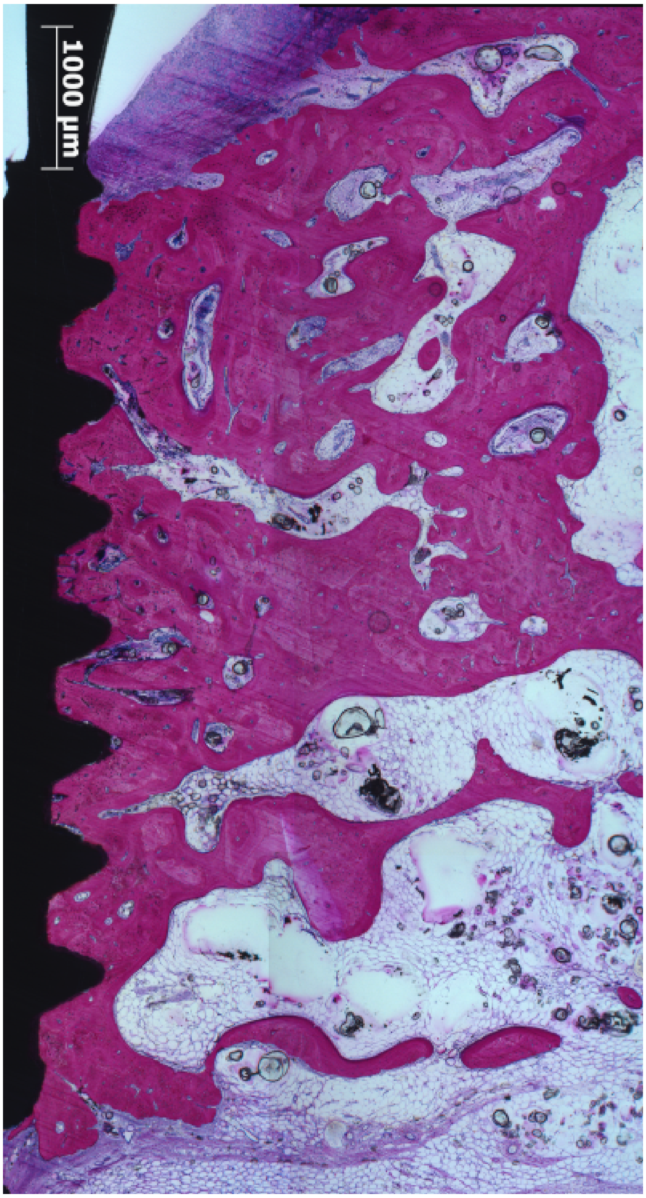

Fig. 5. Histological appearance of a press-fit inserted implant demonstrating trabecular bone around the implant. A lot of bone was present at the implant interface including the first screw-thread. The mucosal response was characterized by the formation of junctional epithelium with a thin layer of connective tissue (Objective × 5).

Hermann et al. 2001). Although these techniques are able to increase the initial implant stability, the final effect on the secondary stability of the implants is not clear. For example, it can be supposed that too much compression of the bone wall of the implant bed results in less osseointegration.

The present study compared the bone healing pattern and osseointegration of implants inserted with an undersized implant preparation and implants installed with the conventional procedure in a canine model. The results of the study showed that a significantly higher BIC was achieved in implants installed using the undersized technique. This observation is in agreement with the previous studies performed in *in vitro* models and in other animal models (Shalabi et al. 2006, 2007a, 2007b; Tabassum et al. 2009). Shalabi et al (2007a) found an enhanced BIC when implants were installed using an undersized preparation in trabecular bone of the femoral condyles of goats. This increased bone contact was suggested to be due to the occurrence of small bone fragments, which are created during the undersized installation of an implant in the alveolar bone bed (Shalabi et al. 2006). This bone debris was confirmed to possess osteogenic potential and can probably act as a kind of autograft (Dhore et al. 2008).

Relative motion of the implant during the early stages of bone healing can adversely affect the osseointegration (Cameron et al. 1973; Schatzker et al. 1975a, 1975b). The ideal implant design should mechanically interlock with the bone at the macro level to provide stabilization. Thread engagement, friction fit or a combination of both are the methods used for root form implants to achieve initial stabilization (Schatzker et al. 1975a, 1975b; Szmukler-Moncler et al. 1998).

The light microscopic examination of the samples from the undersized implants did not show any adverse tissue reaction in relation to the used technique. The healing as well as the bone adaptation were comparable to the conventional or press-fit technique. One of the main concerns to osseointegration is the heat generation during the preparation of the implant site with drills and condensation of the implant bed by the additional torque required for installing undersized implants (Bolz & Kalweit 1976; Albrektsson & Eriksson 1985; Tehemar 1999). However, in agreement with two earlier reports (Sharawy et al. 2002; Misir et al. 2009), the observations of the present study also showed no unfavorable effect on the adjacent bone. In contrast, around all undersized inserted implants, the first implant-bone contact occurred at a higher level as compared with the press-fit installed implants. This is in agreement with the observations of an earlier study on undersized implant bed preparation (Shalabi et al. 2007a).

The implantation site influences the osseointegration process through different levels of bone cellularity and vascularity (Spadaro et al. 1990). A healthy bone bed and minimal surgical trauma are important as it is the source of almost all cells, local regulatory factors, nutrients and vessels that contribute to the bone healing response. Bone healing around implants involves the activation of an osteogenetic, vascular and immunological cellular sequence at the implant surface (distance osteogenesis) or from the implant towards to the healing bone (Davies 1998). Vascularization is essential during osseointegration, as it influences tissue differentiation and ossification (Marco et al. 2005). Bone remodeling ultimately occurs in order to reshape or consolidate the bone at the implant site as well as to provide a mechanism for self repair and adaptation to stress.

Mechanical stimuli are described to accelerate the formation of bone (Frost 1983; Frost et al. 1986). The present study showed significantly improved BIC ratio with the undersized implants, which can be explained by the localized trauma caused by the condensation of the implant bed. The increased new bone formation of compressed bone grafts compared with native trabecular bone has been proven in previous animal experiments (Burri & Wolter 1977). Compression of the bone increases the bone density, while the blood supply is not disturbed severely. Because of its visco-

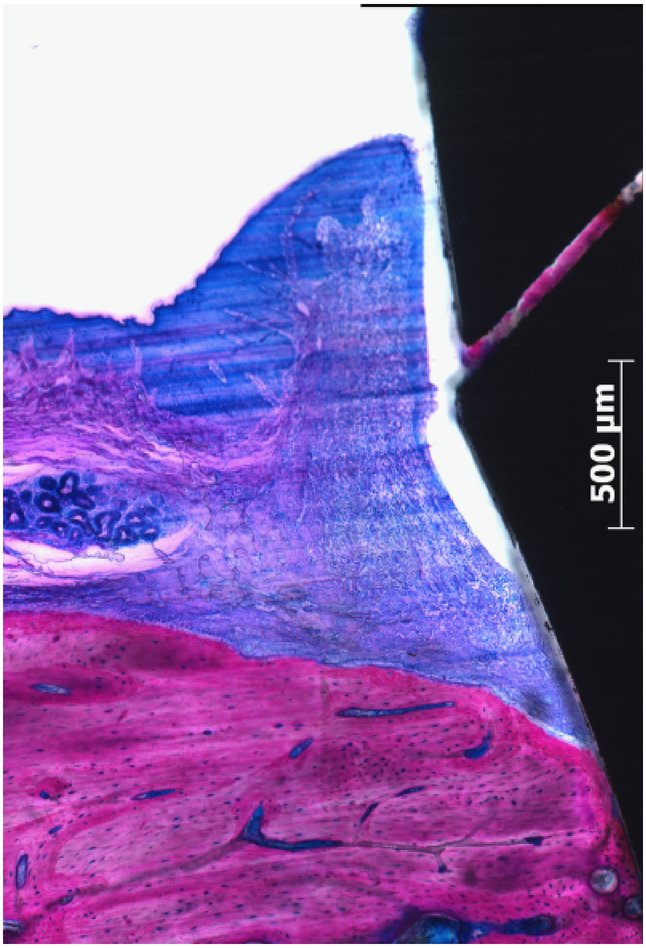

Fig. 6. Light micrograph of the mucosal response to an undersized installed implant (Objective × 10).

Table 2. Histomorphometrical data of the bone response

Histomorphometrical parameters	Undersized (Mean ± SD)	Press-fit (Mean ± SD)
BIC %	60.6 ± 14.2*	47.5 ± 11.7*
1st BIC (in mm)	3.7 ± 1.7**	5.8 ± 3.2**

*Significant difference ($P<0.05$).
**Significant difference ($P<0.05$).
BIC, bone-to-implant contact.

Table 3. Histomorphometrical data of the mucosal response

Histomorphometrical parameters	Undersized (Mean ± SD)	Press-fit (Mean ± SD)
Sulcus depth (in mm)	1.96 ± 0.61	2.35 ± 0.63
Connective tissue thickness	1.05 ± 0.26	1.45 ± 0.14

elasticity, bone maintains the ability to expand to twice its volume after a 20 MPa compression. Moreover, the osteocytes of the bone remain intact when the force of compression does not exceed 10–20 MPa (Nkenke et al. 2002).

From the observations in the present study, it can be concluded that an undersized implant bed preparation can enhance the implant–bone response. It can be suggested that this is due to osteogenic potential of the bone fragments as generated during the undersized implant installation. However, further studies are necessary to explain this phenomenon. The bone–implant interface is a very complex entity as well as the behavior of bone forming cells at this interface and the effect of stress and strain conditions (Brunski 1999).

Acknowledgements: The authors thank Hamdan Alghamdi for his help in the installation of the oral implants as well as Natasja van Dijk and Vincent Cuijpers for their valuable assistance in the histological preparation and evaluation. Maisa M. Al.Marshood was a recipient of a postgraduate research grant from CDRC, College of Dentistry, King Saud University.

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