Evaluation of the Surface Treatment on Bone Healing in a Transmucosal 1-mm Area of Implant Abutment: An Experimental Study in the Rabbit Tibia

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ABSTRACT

Purpose: The objective of the present study was to investigate the effect on bone tissue healing patterns in 1-mm area treated in the transmucosal surface of the abutment in the tibia of rabbits.

Materials and Methods: Forty-six abutments were divided into two groups: control group (CG) with 14 abutments with smooth surface and experimental group (EG) with 32 abutments presenting a 1-mm area of the transmucosal surface treated through sandblasting with microparticles of titanium oxide followed by acid etching. Five samples of each group were analyzed using an optical laser profilometer for surface roughness characterization. Thirty-six Morse taper implants (3.5 mm in diameter and 7 mm in length) were inserted 1.5 mm subcrestal into the tibiae of nine rabbits. The implants were removed after 8, 10, and 12 weeks for histological analysis. The histological slides were prepared and analyzed qualitatively in relation to the new bone at the interface bone-abutment and quantitatively, in relation to bone height from the base of the implant. These data were computed and statistically compared inside the groups using analysis of variance and the *U*-test between groups for same time.

Results: Both groups exhibited bone growth in the direction and over the surface of the abutments, with good healing. However, the EG group showed an increased height of bone formation in the crestal direction, and highly significant differences were observed ($p < .001$) between these measured values.

Conclusions: Under the limitations of the present study, histological follow-up at 8, 10, and 12 weeks showed that transmucosal 1-mm area of implant abutment with treatment of the surface facilitated the maintenance of bone height around the abutment compared with the same abutment with the totally smooth surface.

KEY WORDS: abutment, animal study, peri-implantar tissues, surface treatment

INTRODUCTION

The stability of the tissues around the transmucosal implant/abutment area is a physiological barrier to the apical migration of the junctional epithelium and prevents crestal bone resorption. Establishing the proper dimension and function of the soft tissue seal around dental implants is considered a prerequisite for achieving long-term stable peri-implant conditions.[1] Accordingly, extensive research has been performed to investigate the biological soft tissue seal at different types, materials, and roughness of dental implants.[1–4] The biocompatibility of the material used in the transmucosal part of the implant might, therefore, be an important factor for treatment success.

The preservation of stable relationships between overlying soft tissues and the underlying supporting crestal bone is critical for optimal form and function in implant-supported restorations. Morphological stability is particularly important in the anterior esthetic zone of the maxilla, where the anatomical integrity of

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esthetically critical marginal and papillary tissues is intimately dependent on stable crestal bone levels. Unfortunately, the loss of crestal bone, or "dieback," to the first coronal implant thread is commonly observed following abutment attachment, resulting in an average of 1.5- to 2.0 mm bone loss after the first year, often followed by an ongoing 0.1 mm loss each year thereafter.[5–8] Marginal soft tissue integration plays a fundamental role in establishing an effective seal between the oral environment and the endosseous part of a titanium implant. Generally, the peri-implant mucosa is recognized as scar tissue, exhibiting an impaired resistance to bacterial colonization.[9]

The relationship between the implant-abutment junction (IAJ) and implant-related crestal bone loss has received increasing attention and raised much concern.[5,8,10,11] Preclinical trials using a canine model have confirmed a 3-mm dimension of the peri-implant soft tissues.[5–8] The microgap created at the IAJ consistently resulted in an inflammatory infiltrate driving the healthy peri-implant connective tissue component apically, resulting in at least 1.5- to 2-mm crestal bone loss.[12] Preclinical and clinical studies have been conducted to reduce or minimize crestal dieback by examining the role of microchannels with defined three-dimensional shapes and depths in controlling fibroblastic and osteoblastic behaviors by limiting the apical migration of the junctional epithelium.[13,14] The results of these studies raise questions of whether similar results could be obtained after imposing roughness on the abutment surface. The altered surface, unlike traditional machined surface abutments, might provide improved opportunities for clot stabilization, thereby improving the quantity and quality of bone formation in this area.

In addition, direct bone tissue attachment to the abutment surface might potentially mitigate or altogether eliminate the negative sequelae secondary to microbial leakage from the IAJ microgap, thereby reducing the potential for peri-implant crestal bone loss. Previous studies have also demonstrated that the surface texture significantly influences fibroblast and epithelial cell attachment, suggesting that a certain surface roughness is needed for optimal soft tissue sealing.[15]

The purpose of the present pilot study was to use histological analyses to determine whether treatment of the surface within a defined healing abutment region increases or reduces crestal bone growth compared with a machined abutment.

Figure 1 The characteristics of the healing abutment used in this study.

MATERIALS AND METHOD

Preparation of Healing Abutments and Implant Samples

Forty-six C.P. titanium (titanium grade 4) healing abutments with a 3.5-mm diameter and 2.5-mm transmucosal height were used (Figure 1); 14 abutments had a machined surface, control group (CG), and 32 abutments presented treatment at a 1-mm area of the surface, experimental group (EG) (Figure 2). The EG abutments were treated with the same treatment used

Figure 2 Healing abutments with a smooth surface and with a 1-mm transmucosal region with surface treatment, respectively.

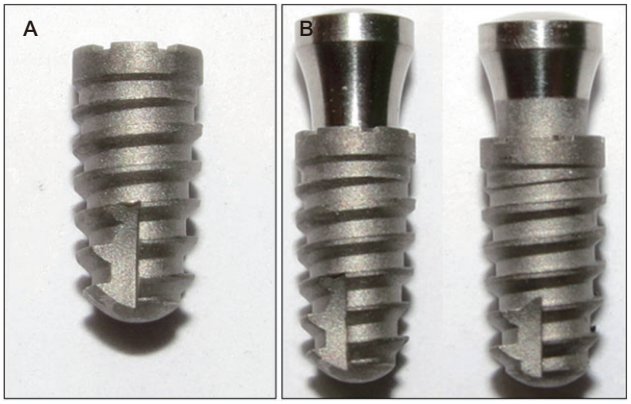

Figure 3 Image of the Morse-tapered implant and the implant with the abutment in position, respectively.

for the implant surface, that is, blasting with 50–100 μm TiO_2 particles, followed by ultrasonic cleaning with an alkaline solution Riozyme IV-E Neutro Gold (Indústria Farmacêutica Rioquímica Ltda, São José do Rio Preto, Brazil), washing with distilled water and pickling with maleic acid ($HO_2CCH_2CHOHCO_2H$). Each sample was prepared, packaged, and sterilized using the same requirements and care for implant packing. Five samples from each group were used for the roughness surface test.

Thirty-six conical implants, packaged and ready for commercialization, with Morse taper connections and surface treatment as described above, were used. The implant size was 3.5 mm in diameter and 7 mm in length (Figure 3A). The abutment/implant set is represented in the Figure 3B. All pieces (implants and healing abutments) were manufactured at Implacil DeBortoli (São Paulo/SP, Brazil).

Abutments Surface Analysis

Five samples of each group were analyzed using an optical laser Profilometer (Mahr GmbH, Brauweg 38 Gottingen, Germany) was used to measure the surface topography, measuring the absolute values of all profile points (Ra), the root-mean-square of the values of all points (Rq), and the value of the absolute heights of the five highest peaks and the depths of the five deepest valleys (Rz).

Animals and Surgical Procedure

Nine New Zealand white mature rabbits weighing approximately 4 kg were used in this study. This study was approved through the ethics committee of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. The rabbit represents a test system commonly used in orthopedics.[16] This animal model provides ideal conditions for the investigation of bone regeneration and implant osseointegration.[17,18] The rabbits were anesthetized through the intramuscular injection of ketamine (35 mg/kg; Agener Pharmaceutical, São Paulo, Brazil). Subsequently, a muscle relaxant (Rompum 5 mg/kg, Bayer, São Paulo, Brazil) and a tranquilizer (Acepran 0.75 mg/kg, Univet, Ribeirão Preto, Brazil) were intramuscularly injected. Additionally, 1 ml of local anesthetic (3% Prilocaine-felypressin, Astra Zeneca, São Paulo, Brazil) was subcutaneously injected at the site of surgery to improve analgesia and control bleeding. A skin incision with a periosteal flap was used to expose the bone of both proximal tibias. The bone site was prepared with burs under copious saline irrigation. Three implants from the EG group and one implant from the CG group were inserted into each animal, totaling two implants per tibia, with the position distributed by drawing lots prior to surgery. The implants were positioned at a 1.5-mm intrabone level with respect to the marginal border (Figure 4) and fixed in the inferior cortical. The tibia was selected as the implant site because of the simplicity of the surgical access.[19] The insertion torque of the implants was manually controlled by an experienced surgeon (SG), and subsequently, the abutment was positioned. The periosteum and fascia were sutured with catgut sutures, and the skin was sutured with silk sutures. Postoperatively, a single dose of 600,000 IU (Benzetacil, Virbac, São Paulo, Brazil) was used. After surgery, the animals were placed in individual cages with 12-hour cycles of light, a controlled temperature (21°C), and the ad libitum diet

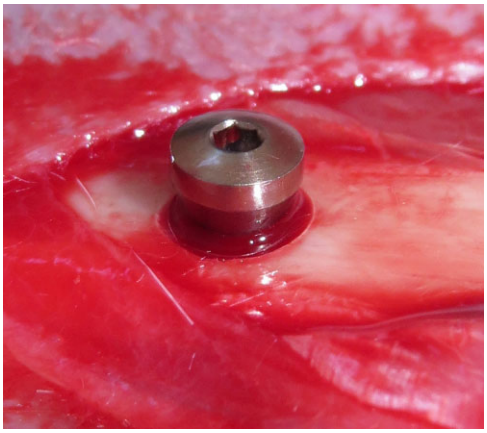

Figure 4 Image of the level position of the implant/abutment set in the bone.

typically used in the laboratory. No complications or deaths occurred during the postoperative period. All animals were sacrificed through an intravenous overdose of ketamine (2 ml) and xylazine (1 ml); three animals were sacrificed for each time point: 8 (*t*1), 10 (*t*2), and 12 weeks (*t*3) after implantation. Both tibias were removed, placed in 10% formalin solution and immediately transported to the laboratory (Biotecnos, Santa Maria, Brazil) for analysis.

Histomorphological Analysis

Bone blocks of the tibiae, with inserted implants and healing abutments, were removed from each animal, fixed in 10% of formaldehyde solution for 7 days, and dehydrated in increasing ethanol solutions (60%, 70%, 80%, and 99%) for 24–56 hours, as previously described.[20] Subsequently, the samples were embedded in Technovit 7200 VLC resin (Kultzer & Co., Wehrhein, Germany) and, after curing, the samples were sectioned using a metallographical cutter (Isomet 1000; Buehler, Germany), as previously described.[18] The disk samples were polished using an abrasive paper sequence (Metaserv 3000; Buehler, Germany) to a ~ 30-μm thickness and analyzed using light microscopy (Nikon E200, Nikon Corporation, Tokyo, Japan). The bone growth was measured with respect to the implant platform at the bone contact with the healing abutment, according to the scheme shown in Figure 5 using *Image Tool* software, version *5.02* for *Microsoft Windows*™. The measurements were made by both authors at different times, and a unique average of these values was computed. When the measured values were very different, measures were repeated by both examiners.

Statistical Analyses

The outcomes were longitudinally analyzed within the same group using the one-way analysis of variance (ANOVA) test for repeated measures. The comparison between the two groups in the same time was performed using the Mann-Whitney *U* test. These statistical analyses were performed using the software *SigmaStat 3.5*. (Systat Software, Inc., Point Richmond, CA, USA). The level of significance was set at $\alpha = 0.05$.

RESULTS

All test and control implants were successfully osseointegrated at the three times proposed for sacrifice. No evidence of localized infection was detected during the healing period at any of the implant sites.

Histologic Observations

In all samples from both groups, the bone tissue presented with complete healing, independent of time. The bone growth around the abutments was similar for both groups, with no qualitative differences observed in the samples studied, independent of time (Figure 6).

Abutments Surface Analysis

The EG group with surface roughness presented values for the mean and standard deviation of the absolute

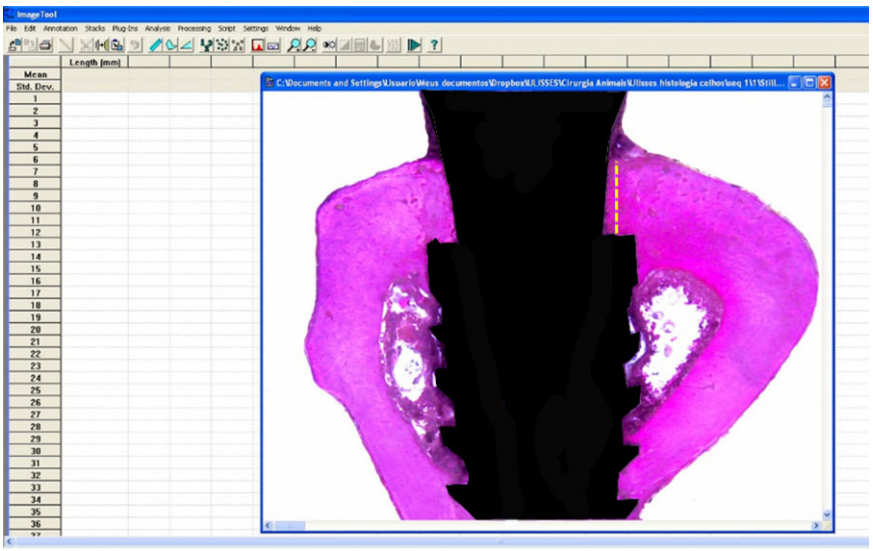

Figure 5 Image of the program used to measure the distance between the platform of the implant at the crestal bone.

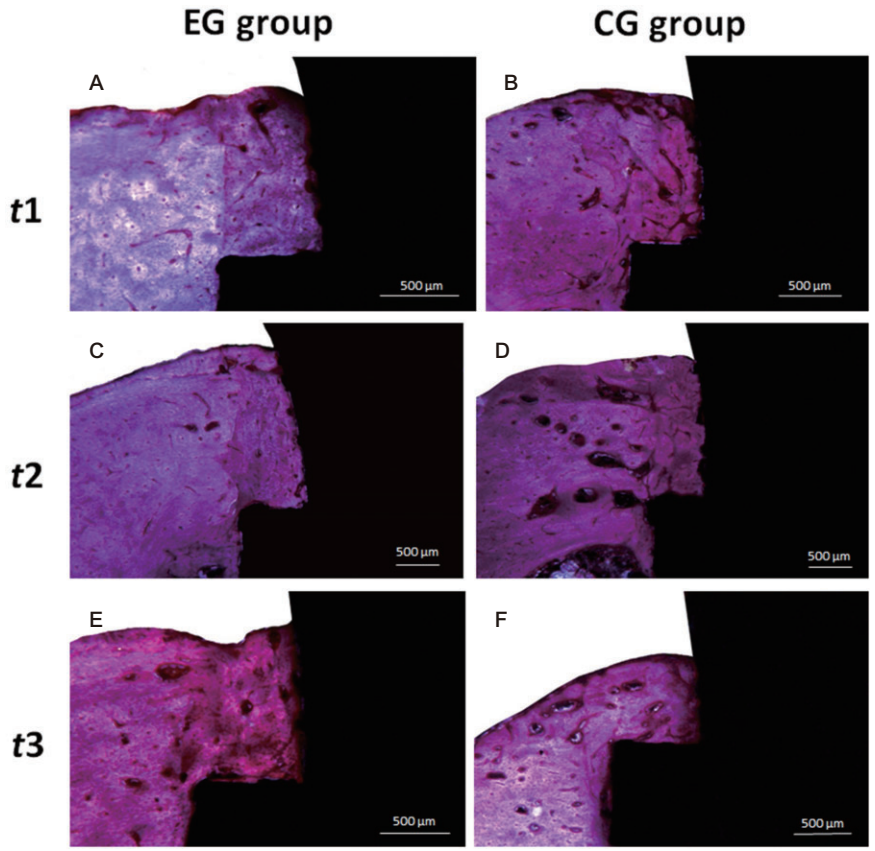

Figure 6 Images demonstrating progress in bone healing at 8 (A and B), 10 (C and D), and 12 weeks (E and F).

values of all profile points (Ra), the root-mean-square of the values of all points (Rq) and the average value of the absolute heights of the five highest peaks and the depths of the five deepest valleys (Rz) of 0.77 ± 0.12, 1.10 ± 0.16 and 5.09 ± 0.69 µm, respectively. The CG group presented a Ra of 0.14 ± 0.08 µm, Rq of 0.33 ± 0.13 µm, and Rz of 3.11 ± 0.77 µm.

Histomorphometry

The mean of the bones measured in each group (Figure 5) and the mean differences with respect to baseline values for the time in the EG group were 1.54 ± 0.14 µm (range: 1.33–1.85 µm; length variation (ΔL) = 0.52 µm) for time *t*1; 1.56 ± 0.07 µm (range: 1.48–1.69 µm; $\Delta L = 0.21$ µm) for time *t*2; and 1.59 ± 0.13 µm (range: 1.46–1.84 µm; $\Delta L = 0.38$ µm) for time *t*3. The values in the CG group were 0.91 ± 0.09 µm (range: 0.79–1.04 µm; $\Delta L = 0.25$ µm) for time *t*1; 0.88 ± 0.06 µm (range: 0.79–0.97 µm; $\Delta L = 0.18$ µm) for time *t*2; and 0.88 ± 0.15 µm (range: 0.68–1.02 µm; $\Delta L = 0.34$ µm) for time *t*3. These data are showed to visual observation of the difference among the groups in the bar graph of the Figure 7.

When the results of the measurements were compared between the two groups at each time using the proposed *U*-test, a highly significant difference was observed ($p < .001$). Moreover, making a single average for both groups between the times (1.56 µm for EG group and 0.89 µm for CG group), the value of the EG group is 75.3% bigger than the CG group.

Inside of the groups, these were observed using one way repeated measures analysis of variance, being that the differences in the mean values among the different times are not great enough to exclude the possibility that the difference is due to random sampling variability. Then, within each group at *t*1, *t*2, and *t*3, no significant difference was detected among the values, with $p = .372$ for the EG group and $p = .843$ for the CG group.

DISCUSSION

The aim of the present pilot study was to use histological analyses to determine whether treatment of the surface within a defined abutment region increases or reduces crestal bone growth compared with a machined abutment in a rabbit tibia model. The increase in the amount of peri-implant bone tissue in the crestal portion of the

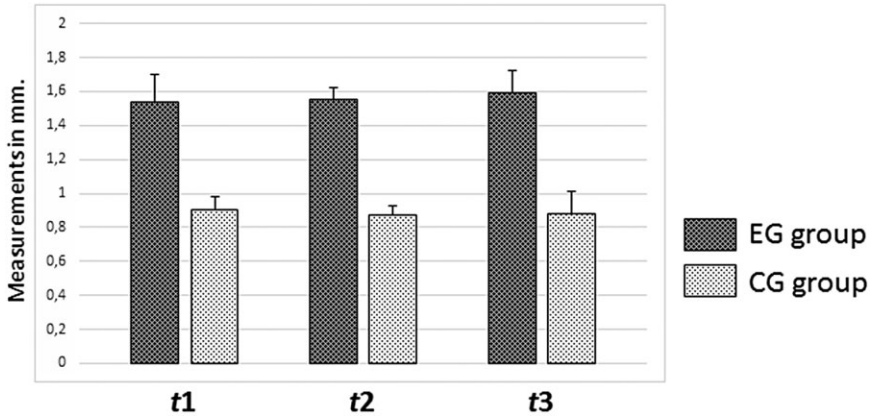

Figure 7 Bar graph and standard deviation of the distance from the implant platform at the crestal bone measured in the three groups.

implants could prevent inflammatory phenomena and/or esthetic problems. Healing abutments and not prosthetics abutments were used in the present study because it would not load applied on these. However, the idea in a normal clinical situation would be to use a definitive abutment with this treatment, which would not be substituted for drawing up the final prosthesis.

It has been suggested that the following factors primarily influence early implant bone loss in the crestal portion: microgap, when placed at or below the bone crest; implant crest module; occlusal overload; and the reformation of the biological width around dental implants.[11,21] The crest module is the region of the implant that receives the crestal stress to the implant after loading, and the implant design influences the intensity of stress on this area.[22] After the implant is loaded, bone loss has been observed down to the first thread in many submerged implant systems with different distances from the implant platform to the first thread.[23] It has been suggested that bone loss might be reduced at the first thread due to changes in the shear force of the crest module to a component of compressive force caused by the thread itself.[22,23] The implant neck design has been developed to improve the integrity of the soft tissue integration, and microtextured and macrotextured surfaces have also been explored. These designs primarily enhance the stability of the interface for both soft and hard tissue and minimize the marginal bone reduction in the first year of implantation. Moreover, the osseointegration at implants placed in sites with marginal defects is influenced by the surface characteristics of the implant.[24] Thus, in the present study, we presented an alternative to increase the amount of bone tissue in the peri-implantar area through the surface treatment of the transmucosal portion of the abutments, where was observed, using an overall average, an increase in 75.3% in relation of the conventional surface (without treatment).

Several types of chemical and physical surface treatments have been developed and marketed by dental implant manufacturers.[25] However, there is still no consensus on what the optimal condition for peri-implant bone growth should be. It is known that the bone response can be influenced by the implant surface topography at the micrometer level, and some indication exists that a nanometric surface can also have an effect. However, the mechanisms behind an optimal bone response in relation to a given type of surface still remain largely unknown. Some biological processes involved in the activation of the early stages of osseointegration, such as protein adsorption, cell–surface interaction, progenitor cell recruitment and differentiation, tissue formation at the interface between the body and the biomaterial, can be affected by the implant surface microroughness as well as by its physical-chemical surface properties.[26–28] Currently, several brands of implants use surfaces known as sandblasted and acid-etched (SLA) types, which are produced by sandblasting with titanium particles followed by a strong acid-etching bath with a mixture of HCl/H_2SO_4 at elevated temperature for several minutes are widely utilized and have been well documented in the literature.[29,30] These are moderately rough surfaces that usually present fine 2–4 μm micropits superimposed on the rough-blasted surface. Though well documented, the presence of residuals of alumina embedding on its

surface due to the fabrication process has been regarded as a potential risk for long-term osseointegration.[31–34] Alternatively, surfaces have been blasted with other biocompatible media such as calcium-phosphate bioactive ceramics[35] and titanium oxide.[36,37] The first comprises a resorbable medium that is actually bioactive, whereas the second method consists of particles that are made of the same biocompatible material as the implant[38] that demonstrated an excellent biologic response.[39]

During implant placement, obtaining high torque values of initial stability has been associated with successful osseointegration.[40,41] However, high clamping torque values are typically obtained at the cortical bone portion where the stress response is theoretically less. Thus, after preparation of the surgical site and intraosseous installation of the implant, most of the crestal bone is in contact with the clot and compression free (Figure 4), and the initial implant stability was obtained in the latero-lateral cortical portion of the tibia, not affecting the osseointegration of implants. Studies have shown that a marginal defect of approximately 1 mm between the bone wall and the metal surface after implant installation can heal with a high degree of bone fill and osseointegration.[42]

Implants installed in regular-sized alveolar ridges showed higher horizontal, but lower vertical, buccal bony crest resorption compared with implants installed in reduced alveolar ridges. Narrow abutments in reduced ridges and wide abutments in regular-sized ridges yielded less soft tissue recession compared with their counterparts.[43] Therefore, we used Morse taper connection type implants in the present study. This system comprises abutments with a considerable reduction in diameter compared with the diameter of the implant.

The marginal defects around titanium implants regenerate in 20–30 days through distance osteogenesis but, the bone fill of the defects is incomplete after 1 month.[44] Thus, we proposed sample collection times of 8, 10, and 12 weeks, when the bone healing is completed. However, the results showed no significant variation within groups at the proposed collection times, thereby strengthening the validity of the data collected, and because the implants did not receive loads at any point, the values could not be changed. On the other hand, when the data were compared between the groups at each time, significantly larger difference was observed for the EG group. The test (Mann-Whitney *U* test) used for the statistical analyses among the groups is justified because of the difference in the number of observations in each time (18 for EG group and six for CG group).

Further studies are needed to demonstrate the effects after loading these implants, including the behavior of the bone near and/or in contact with the abutment. Moreover, it would be important to assess whether the bone growth around the abutment increases the peripheral seal.

CONCLUSIONS

Within the limitations of this study, the histological follow-up at 8, 10, and 12 weeks indicated that the treatment of a 1-mm area of the surface increases the height of bone growth around the abutment compared with the same abutment with a totally smooth surface.

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