Histomorphometric analysis of bone healing at implants with turned or rough surfaces: An experimental study in the dog

Abstract

Objective

The objective of this study was to evaluate the influence of machined or moderately rough surfaces on osseointegration of implants.

Materials and methods

Three months after extraction of all mandibular premolars and first molars in six Labrador dogs, two implants, one with a novel turned surface (Combed) and a second with a moderately rough surface (ZirTi), were placed in each side of the mandible in the premolar region of each dog. The 3-D parameters to express roughness and density of peaks were $S_a = 1.399 \, \mu m$ and $S_{ds} = 0.065 \, \mu m^2$ for the ZirTi surface and $S_a = 0.600 \, \mu m$ and $S_{ds} = 0.314 \, \mu m^2$ for the Combed surface, respectively.

Abutments were attached and the flaps were sutured to allow non-submerged healing. The animals were sacrificed after four months of healing, and ground sections were obtained for histomorphometric assessments of the hard-tissue integration.

Results

All of the implants were osseointegrated. Mineralized bone-to-implant contact was $50.6 \pm 18.3\%$ and $56.3 \pm 18.6\%$, while bone density was $54.6 \pm 9.6\%$ and $43.0 \pm 9.0\%$ at the Combed and ZirTi surfaces, respectively. The difference between the two surfaces was statistically significant ($p = 0.046$) for both parameters evaluated.

Conclusion

Implant surface characteristics influence the degree of osseointegration of implants placed in the alveolar process.

Keywords

Animal study, bone healing, dental implants, osseointegration, bone levels, histometry, morphometry.
Introduction

Osseointegration processes are influenced by many variables, and osseointegration has been found to proceed faster in animals compared with humans and in the spongiosa compared with the cortical bone. Moderately rough surfaces have shown faster bone apposition compared with turned surfaces. Recently, various implant surfaces have been discussed with regard to osseointegration. Mainly those of four brands represented frequently in the international market were addressed. It was shown that the different surface treatments of the implants led to different values of the 3-D average roughness over a surface (Sa value), as well as of the density of peaks (Sds) and of the developed surface area ratio (Sdr). Different values of Sa and Sdr among the various surfaces were reported, including between 0.3 μm and 1.78 μm for Sa and between 24% and 143% for Sdr. It is interesting to note that the original Brånemark nontreated turned surface presented values of Sa of 0.9 μm and Sdr of 34%.

A comparison of the sequential healing between turned and rough (sandblasted, large-grit, acid-etched; SLA) surfaces was performed in an experimental study in dogs. Troughs were created in the space between threads so that, after implant placement, a chamber was obtained, and only the tips of the threads were in contact with the pristine bone. It was demonstrated that osseointegration within the chambers proceeded faster and reached higher levels at the SLA compared with the turned surfaces.

The study of the healing of the hard tissue at untreated turned surfaces and at surfaces blasted with zirconia particles and subsequently acid etched still needs clarification. Hence, the aim of the present experiment was to compare osseointegration at turned and moderately rough surfaces after four months of healing.

Materials and methods

The research protocol was submitted to and approved by the local ethics committee for animal research at the University of the State of São Paulo, Araçatuba, Brazil.

Clinical procedures

Clinical procedures, histologic preparation and data regarding marginal soft- and hard-tissue healing have been reported on previously. Briefly, six Labrador dogs (each approximately 23 kg and at a mean age of about three years) were used. At any of the surgical sessions, the animals were pre-anesthetized with Acepran (0.05 mg/kg; Univet-vetnil, São Paulo, Brazil) and sedated with Zoletil (10 mg/kg; Virbac, Fort Worth, Texas, U.S.) and Xilazina (1 mg/kg; Cristália Produtos Químicos Farmacêuticos, São Paulo, Brazil), complemented with ketamine (2.5 mg/kg; Cristália Produtos Químicos Farmacêuticos). Local anesthesia was also provided.

All premolars and first molars were extracted at both sides of the mandible. After three months of healing, an incision in the center of the alveolar crest was performed and full-thickness mucoperiosteal flaps were elevated. Two osteotomies were prepared in each side of the mandible in the premolar region and two 10 mm long and 3.8 mm wide titanium Premium (Fig. 1a) or Platform Premium (Fig. 1b) implants (Sweden & Martina, Due Carrare, Italy) were placed in the right and left sides of the mandible, respectively (Fig. 1c). The anterior implants had a turned surface (Combed, Sweden & Martina), while the posterior implants had a moderately rough surface (ZirTi, Sweden & Martina).

The ZirTi surface was first sandblasted using particles of zirconia and subsequently acid etched, while the Combed surface was obtained by a particular tooling process, developed and controlled to achieve a more homogeneous and rough surface in comparison with standard machined surfaces. The 3-D parameters to express roughness and density of peaks were Sa = 1.399 μm and Sds = 0.065 μm² for the ZirTi surface and Sa = 0.600 μm and Sds = 0.314 μm² for the Combed surface, respectively.

Abutments were attached at the top in the implants (Fig. 1d) and the flaps were adapted around the abutment–implant units to allow non-submerged healing (Fig. 1e). After the surgical procedures, the animals received ketoflex 1% (0.02 mL/kg; Cetoprofeno, Biofarm Química e Farmacêutica, Jabaquara, Brazil) and Pentabiotic (Fort Dodge Animal Health, Campinas, Brazil). The animals were kept in kennels and on concrete runs at the university’s field laboratory with free access to water and fed with moistened balanced dog food. The wounds were inspected daily for clinical signs of complications, and the abutment cleaned. Sutures were removed after two weeks. The animals were euthanatized four months after the surgery, applying an overdose
Healing of smooth vs. rough surface implants

Histological preparation

The implants and surrounding tissue were dehydrated in a series of graded ethanol solutions and subsequently embedded in resin (LR White, hard grade, London Resin Company, Berkshire, U.K.) and polymerized. The cuts were performed along the buccolingual plane following the long axis of the implants using a diamond band saw fitted in a precision slicing machine (Microslice 2, Ultratec, Santa Ana, Calif., U.S.) and then thinned. The histological slides were stained with Stevenel's blue and alizarin red and examined under a standard light microscope for histometric analysis.

Histometric evaluation

Under an Eclipse Ci microscope (Nikon, Tokyo, Japan), equipped with a DS-Fi2 (Nikon) digital video camera connected to a computer, the percentage of mineralized bone-to-implant contact (MBIC%) between the most coronal bone-to-implant contact (B) and the apex of the implant (A) was evaluated at 100× magnification. Moreover, the percentages of mineralized bone (MB%) and soft tissue contained in a region included between B and A and between the body of the implant to a distance of about 0.6 mm from it were determined. For this aim, a point-counting procedure was applied and a lattice with squares of 50 μm was superposed over the tissue at 200× magnification.

Data analysis

Mean values between the two implants included in each group were obtained for both MBIC% and MB% in each dog. An n = 6 was obtained. Differences between the two implant surfaces were analyzed using IBM SPSS Statistics (Version No 19.0; IBM Corp., Armonk, N.Y., U.S.) and applying the Wilcoxon signed-rank test for dependent variables. The level of significance was set at p = 0.05. A correlation between MBIC% and MB% was also calculated for all 24 implants, as well as for the 12 implants of each group.

Results

After four months of healing, no complications were observed and no implants had been lost. All of the implants were available for histological analysis. Data illustrating the outcomes at the marginal soft and hard tissue around the implants were previously described. Table 1 reports the mean values and standard deviations, as well as medians and 25th and 75th percentiles. In the text, only mean values ± standard deviations are reported.
The implants were well integrated into the mature bone, represented by mature lamellar bone and bone marrow surrounding the implant surface (Figs. 2 & 3). MBIC% was 50.6 ± 18.3% and 56.3 ± 18.6% at the Combed and ZirTi surfaces, respectively. The differences between the two surfaces were statistically significant (p = 0.046).

Bone density that was evaluated to a distance of about 0.6 mm from the implant surface and between the most coronal level of osseointegration (B) and the apex of the implant (A) was 54.6 ± 9.6% and 43.0 ± 9.0% at the Combed and ZirTi surfaces, respectively. The difference between the two surfaces was statistically significant (p = 0.046).

An outlier for MBIC% was identified (one dog), the values being below the first quartile. Data excluding the outlier are reported in Table 2. A statistically significant difference was no longer identified.

The correlation between MBIC% and MB% when all of the implants were taken into account yielded r = 0.3. However, if the groups of implants with different surfaces were considered separately, the values for the Combed and ZirTi surfaces were r = 0.80 and r = 0.02, respectively.

Discussion

Moderately rough surfaces have been shown to yield superior osseointegration potential compared with that of smooth surfaces. In an experiment in dogs, troughs were prepared around standard implants so that, after placement, chambers resulted between the implant body and the recipient bone. Moderately rough (SLA) and turned surface implants were used. A more rapid new bone apposition at the SLA compared with the smooth surface implants was observed.
After 12 weeks of healing, bone-to-implant contact was slightly below 60% at the SLA (percentage deduced from the graph in the article) and 36.8% at the turned surfaces. It has to be noted that the $S_a$ of these surfaces was $2.29 \pm 0.59 \mu m$ for the SLA and $0.35 \pm 0.17 \mu m$ for the turned surfaces, respectively.\(^4\)

In the present experiment, the roughness of the two surfaces used was different from that previously described. In fact, $S_a$ was about 0.6 $\mu m$ for the Combed and about 1.4 $\mu m$ for the ZirTi surfaces. This different roughness yielded different osseointegration, namely $50.6 \pm 18.3\%$ and $56.3 \pm 18.6\%$, in which the high standard deviation was mainly related to the presence of an outlier.

However, in the present study, the difference in MBIC$\%$ between the two surfaces was smaller than 6%, while in the previously discussed study,\(^4\) the difference was about 20%. This may be partly related to the differences in roughness of the two surfaces, but also to the different models used. In that study, a chamber was prepared around the implant body so that only the pitches of the threads were in contact with the pristine bony beds. This yielded a primary bone-to-implant contact to the parent bone of about 6.3–6.5%, as measured on the day of implant placement. Bone apposition had to cover a distance of 0.4 mm to reach the inner side of the chamber. It was shown that this bone apposition on to the implant surface proceeded faster at the rough compared with the turned surfaces. In the present study, no modifications were applied to the implant configuration so that a higher primary contact of the implant surface to the bone bed was expected. In fact, in another dog study, similar implants were used and, after five days of healing, an MBIC$\%$ of about 32% was observed.\(^7\) This higher bone-to-implant contact area may have resulted in greater osseointegration at the turned implants used in the present study com-

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Table 2

<table>
<thead>
<tr>
<th>MBIC$%$</th>
<th>Turned</th>
<th>Rough</th>
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<tbody>
<tr>
<td>Mean (SD)</td>
<td>57.1 (10.5)</td>
<td>63.3 (5.5)</td>
</tr>
<tr>
<td>Median (25$^{\text{th}}$; 75$^{\text{th}}$)</td>
<td>59.6 (56.8; 60.6)</td>
<td>64.5 (61.0; 66.5)</td>
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\(^*\) $p < 0.05$. 

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Figs. 3a–d
Photomicrographs of ground sections illustrating the healing after four months at the ZirTi surface. Stevenel’s blue and alizarin red stain. 100× magnification.
pared with that used in the above-mentioned study.

It is important to consider that MBIC% repre-
sents a percentage and not an absolute value of
mineralized bone in contact with the implant sur-
face. Even though the MBIC percentages may be
similar among surfaces with different 3-D para-
eters, the absolute values may be dissimilar
owing to the different roughness of the surfaces.
In the present study, bone density around the
implant surfaces was found to be higher at the
turned (54.6 ± 9.6%) compared with the mod-
erate rough (43.0 ± 9.0%) surfaces. It was
found that the correlation between MBIC% and
MB% was low (r = 0.3) when all of the implants
were taken into account. However, when the two
groups of implants, Combed and ZirTi sur-
faces, were considered separately, r = 0.8 and
r = 0.02 were observed, respectively. This gave
rise to the speculation that the surface config-
uration influenced not only the osseointegra-
tion, but also the response of the tissue in close
vicinity to the implant surface.

This outcome of the present study is in
agreement with that of a previously discussed study.4 In that study, after six, eight and twelve
weeks of healing, a higher amount of miner-
ialized tissue within the chamber at the turned
compared with the rough surfaces was found.
Moreover, during the same periods of healing,
it was observed that the percentage of miner-
alyzed tissue was increasing within the chamber
of the turned surface, while it remained quite
stable within the chamber of the rough surface.

It has to be observed that the present study
reports data on healing after four months and
without loading. Longer periods of healing may
yield different results. Moreover, the load may
influence the healing as well.8

A further limitation of the present study is the
lack of standardization of the sites, the implant
with the Combed surface being placed consis-
tently about 10 mm more mesially in the premo-
lar region of the alveolar process compared with
the ZirTi surface.

In conclusion and within the above-men-
tioned limitations of this study, it has been
shown that the surface characteristics of im-
plants affected the degree of osseointegration.
Both the turned and the moderately rough sur-
faces were osseointegrated to a similar degree
after four months of healing, with the moder-
ately rough surface providing only modestly
better osseointegration. However, the osseoin-
tegration process before four months at the
turned surfaces still requires greater elucidation.

Competing interests

The authors declare that they have no conflict of
interest regarding the materials used in the pres-
ent study.

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References

1. Botticelli D, Lang NP. Dynamics of
osseointegration in various human and animal
models—a comparative analysis.
→ Clin Oral Implants Res.
2016 May 23. doi:10.1111/clr.12872. [Epub
ahead of print].
2. Wennerberg A, Albrektsson T. On implant
surfaces: a review of current knowledge and
opinions.
3. Berglundh T, Abrahamsson I, Lang NP, Lindhe
J. De novo alveolar bone formation adjacent
to endosseous implants.
→ Clin Oral Implants Res.
4. Abrahamsson I, Berglundh T, Lindner E, Lang
NP, Lindhe J. Early bone formation adjacent to
rough and turned endosseous implant
surfaces: An experimental study in the dog.
→ Clin Oral Implants Res.
5. Baffone GM, Botticelli D, Pantani F, Cardoso
LC, Schweikert MT, Lang NP. Influence of
various implant platform configurations on
peri-implant tissue dimensions: an
experimental study in dog.
→ Clin Oral Implants Res.
2011 Apr;22(4):438–44.
6. Schroeder HE, Müenzel-Pedrazzoli S.
Correlated morphometric and biochemical
analysis of gingival tissue. Morphometric
model, tissue sampling and test of stereologic
procedures.
→ J Microsc.
7. Rossi F, Lang NP, De Santis E, Morelli F,
Favero G, Botticelli D. Bone-healing pattern at
the surface of titanium implants: an
experimental study in the dog.
→ Clin Oral Implants Res.
GS, Lang NP. Influence of immediate loading
on healing of implants installed with different
insertion torques—an experimental study in dogs.
→ Clin Oral Implants Res.