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The effect of different surgical drilling procedures on full laser-etched microgrooves surface-treated implants: an experimental study in sheep

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Abstract

Objectives: To evaluate the influence of instrumentation technique on the early osseointegration histomorphometrics and biomechanical fixation of fully laser-etched microgrooves implant surfaces in a sheep model.

Material and Methods: Six sheep were subjected to bilateral hip surgeries 3 and 6 weeks before euthanasia. A total of 48 implants (∅4.5 mm, 8 mm in length) were distributed among four sites (8 per animal) and placed in bone sites drilled to 4.6 mm (reamer), 4.1 mm (loose), 3.7 mm (medium) and 3.2 mm (tight) in diameter. After healing, the animals were euthanized and half of the implants were biomechanically tested, while the remainder was subjected to non-decalcified histologic processing. The histomorphometric parameters assessed were bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO). Statistical analysis was performed using a mixed-model analysis of variance with significance level set at $P < 0.05$.

Results: A general increasing trend is present from 3 to 6 weeks for most of the variables. The groups prepared to be press fit seemed to present higher values, which were maintained throughout the observation period. The reamer group presented the lowest BIC probably due to the drilling technique; however qualitatively, more new bone seemed to be in contact to the implant surface, at 3 weeks, whereas the implants placed in press-fit situations were mainly supported by cortical bone.

Conclusion: The laser-etched microgrooved implant presented osteoconductive and biocompatible properties for all surgical procedures tested. However, procedures providing increasingly higher press-fit scenarios presented the strongest histomorphometric and biomechanical responses at 3 and 6 weeks.

Surface topography is one of the decisive factors for osseointegration (Le Guehennec et al. 2007; Wennerberg & Albrektsson 2010). It has been reported in a number of *in vivo* studies that moderately roughened implant surfaces present higher bone responses compared with turned surfaces (Gotfredsen et al. 1995; Novaes et al. 2002; Piattelli et al. 2002), and the degree of surface roughness significantly influences osteoblast gene expression (Schneider et al. 2003). The roughened surface provides adequate base for firm cell attachment and cell spreading, due to the increased surface area (Mustafa et al. 2001), which positively influences proliferation and differentiation (Sammons et al. 2005; Zhao et al. 2006).

Techniques such as sandblasting and acid etching or anodic oxidation have been utilized to roughen the surface (Ravanetti et al. 2010), and implants treated with these methods have increased the affinity to bone resulting in enhanced osseointegration (Wennerberg & Albrektsson 2009). Moreover, a combination of topographical and chemical modification has been proven to promote osseointegration (Fini et al. 1999; Sul et al. 2005; Franco Rde et al. 2008). Hydrophilic surfaces have also been introduced to optimize cell–material interactions due to the high surface energy created by the sandblasting or oxidation (Buser et al. 2004; Schwarz et al. 2007; Jimbo et al. 2011).

One unique method to increase the roughness of the implant surface is the use of laser treatment (Yue et al. 2002; Tian et al. 2005). Not only does the laser ablation technology remelt and modify the outermost surface by

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rapid cooling, but it also increases the material corrosion resistance, hardness and biocompatibility (Gaggl et al. 2000; Hallgren et al. 2003; Faeda et al. 2009). It has been reported that laser-treated commercially pure titanium implants possessing a porous structure presented significantly higher removal torque values compared with the non-treated surfaces (Cho & Jung 2003; Hallgren et al. 2003).

Computer-controlled laser ablation technique has the capability of designing desired surface topographies (Vaidya et al. 1998; Nayak et al. 2007). An interesting topography that can be applied with this approach is the application of nano- or microgrooves with complete regularity. It has been reported that osteoblasts cultured on microgrooves expressed osteogenic gene expression as compared to the surfaces without grooves (Lamers et al. 2010). It is thought to be beneficial for cell responses, because certain cell types such as osteoblasts or fibroblasts elongate themselves following the grooved topography and promote cell migration (Meyle et al. 1995; Walboomers & Jansen 2001; Ricci et al. 2008). The so-called contact guidance has been tested and discussed in many studies, and it is suggested that this specific surface topography is one of the factors involved in the cell adhesion due to the enhancement of linear focal contact (Ohara & Buck 1979; Clark et al. 1990; Meyle et al. 1995; Teixeira et al. 2003). It has been reported *in vivo* that when laser-induced circumferential microgrooves were applied in the neck segments of dental implants, bone regeneration occurred more rapidly than surfaces without the grooves (Ricci & Alexander 2001; Weiner et al. 2008). Furthermore, the unique structure seemed to induce both hard and soft tissue attachment, which in some studies suggest the facilitation of the biologic width (Weiner et al. 2008; Pecora et al. 2009).

An important aspect to consider when placing an implant with a specific modification is the drilling protocol. It has been suggested that the combination of different implant geometries and drilling protocols results in different interactions between the implant and the bone (Coelho et al. 2010) and has a great influence on the primary stability ultimately determining the following biologic stability (osseointegration) (Berglundh et al. 2003; Tabassum et al. 2010; Halldin et al. 2011). Thus, evaluating the biologic outcomes of the modified implants placed in sites prepared with different instrumental techniques would provide valuable information. The objective of this study was to biomechanically and histomorphometrically evaluate the influence of instrumentation technique of fully laser-etched microgrooved implants in a sheep model.

Material and methods

Implants and surgical procedures

This study utilized implants possessing a laser-treated microgroove in the coronal portion of the implant (Laser-Lok®; BioHorizons, Birmingham, AL, USA, 4.5 mm in diameter by 8 mm length). Four instrumentation techniques were performed by one experienced surgeon, with the final drilling diameters as described: reamer (R) 4.6 mm (final instrumentation hand reamer), loose (L) 4.1 mm, medium (M) 3.7 mm and tight (T) 3.2 mm. All drilling procedures followed the manufacturers' recommended drilling speeds (1200 rpm) under abundant irrigation with sterile saline.

The *in vivo* study comprised of six Finnish Dorset crossbred sheep (each weighing approximately 150 pounds) approximately 1.5 years old. The study was approved by the Ethics Committee for Animal Research at the École Nationale Vétérinaire d'Alfort (Maisons-Alfort, Val-de-Marne, France). The sheep remained in the facility for 2 weeks prior to the surgical procedures.

A total of 96 implants were distributed among four experimental groups ($n = 12$ per group and time-point, two per animal, one utilized for torque and the other for histologic measurements). The pelvis access was performed by an antero-posterior incision of 15 cm in length, which was followed by blunt dissection of the fat and muscle layers. The implants were placed sequentially from proximal to distal at distances of 2 cm from each implant's center at the central region of the bone.

On each sheep, eight implants were first placed in one side of the hip, and after 3 weeks, the other side of the hip underwent the same procedure (16 sites per animal). Three weeks later, the animals were sacrificed, and thus, each animal provided samples that remained *in vivo* for 3 and 6 weeks. The drilling technique procedures were sequentially changed as a function of implantation site per animal. Two rows of implants were placed in the pelvis, one medially and the other laterally.

Prior to the experimental procedures, the surgical area was shaved and aseptic procedures were carried out. The animals were then continuously monitored for heart rate, oxygen saturation, respiratory rate, temperature and tissue coloration prior to complete shaving of the intended surgical site and the adjacent areas and application of a povidone solution. After monitoring, the animals were transferred over to an automated system and aseptically draped.

Animals underwent tracheal intubation following an intravenous bolus anesthesia with short-acting barbiturates (sodium thiopental). The intubated animals were connected to an anesthesia workstation (Julian, Draeger) and patient monitor (Datex A/S3), which insured automatic volume-controlled ventilation and delivery of isoflurane in the gas mixture (carrier gars being air, closed system).

Halogenate anesthetic agent vapor concentration in fresh gas, oxygen concentration in fresh gas, ventilation rate and volumes were adjusted following the monitored values (oxygen saturation, end tidal halogenate agent concentration and end tidal carbonic gas pressure). In addition to isoflurane, animals received ketamine (1 mg/kg IV) and morphine (0.1–0.2 mg/kg/h).

Animals were treated with meloxicam NSAIDs (0.4 mg/kg IM the day of surgery and for the following 3 days). They received penicillin and streptomycin for 6 days starting the surgery day and were also treated with fentanyl percutaneous patches delivering 3 μg/h/kg.

Animals were housed in group pens in a barrier facility under a 12D-12N lightning cycle. They were provided with vegetal bedding and received each day hay and pellets providing the recommended ration for this species in energy, proteins and vegetal fibers. They had permanent access to fresh water. Daily examinations tracked any signs of adverse events such as pain, lameness and open wounds.

Removal torque and histomorphometry

The animals were euthanized after 6 weeks counting from the first implant surgery via anesthesia overdose, and the 3- and 6-week samples were retrieved by sharp dissection. Half of the implants were biomechanically tested using the removal torque testing. In brief, the removed bone–implant blocks were fixed in an electronic torque machine equipped with a 200 Ncm torque load cell (Test Resources, Minneapolis, MN, USA). The implant driver provided by the manufacturer was connected to the head of the torque machine, and the implants were carefully connected for the torque testing. The implants were torqued counterclockwise at a rate of 0.19618 rad/s, and the maximum torque for interface failure was recorded for each specimen.

The remaining implants were subjected to non-decalcified histology processing following

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the standard methodology (Donath & Breuner 1982). In brief, the retrieved samples were dehydrated in a graded series of ethanol and thereafter were infiltrated and embedded in methylmethacrylate. The embedded resin blocks were then subjected to non-decalcified histologic sectioning where the samples were first perpendicularly sectioned into slices with a slow-speed precision diamond saw (Isomet 2000; Buehler Ltd, Lake Bluff, IL, USA). After gluing the sections to a plexiglass with a light curing acrylate-based adhesive (Technovit 7210 VLC adhesive, Heraeus Kulzer GMBH; Wehrheim, Germany), the samples were grinded and polished under abundant water irrigation using a series of silicon carbide (SiC) papers (400, 600, 800, 1200) (Buehler Ltd) until a final thickness of 30 μm. Thereafter, the sections were then stained in 1% toluidine blue.

The bone-to-implant contact (BIC) along the implant was determined at ×50–200 magnification (Leica DM2500M; Leica Microsystems GmbH, Wetzlar, Germany) by means of specialized computer software (Leica Application Suite; Leica Microsystems GmbH). The area of visibly distinguishable bone (tissue stained in purple) within the implant thread chamber at ×100 magnification was calculated as bone area fraction occupancy (BAFO) using the same microscope and software.

All evaluations concerning removal torque testing, histologic preparation and histomorphometry were performed in a blinded manner.

Statistics

Preliminary statistical analyses showed no effect of implant site (i.e., there were no consistent effects of different positions along the bone) on all measurements. Therefore, site was collapsed in further analysis. Preliminary analysis also tested the normality assumption for the torque, BIC and BAFO data. Kolmogrov–Smirnov tests of both measures failed to show significant deviations from normality (P's ranged from 0.21 to 0.36, depending on the group and measure). Final statistical evaluation of the effects of time and drilling technique on the torque, BIC and BAFO measures then used a mixed-model analysis of variance with one grouping and one repeated factor, each with two levels. Statistical significance was set at 5% ($\alpha = 0.05$).

Results

Animals

The surgical procedures and follow-up showed no complications regarding the procedural conditions or other immediate clinical concerns. No postoperative complications were detected, and no implant was excluded from the study due to clinical instability immediately after euthanasia.

Removal torque

The ANOVA summary for the effects of time *in vivo* and drilling technique on torque is presented in Table 1. The mean removal torque values presented that there was a general increase in the torque values from 3 to 6 weeks except for group T (Table 2). The general trend seemed to be that the differences seen between the different groups at 3 weeks seemed to have evened out after 6 weeks. In detail, at 3 weeks, groups L and R presented significantly lower mean torque values (Ncm) than groups T and M. However, at 6 weeks, no significant differences were detected between all groups. When considering time and instrumentation, significant increase in torque values was observed for groups L and R between 3 and 6 weeks (Table 2).

Histologic and histomorphometric (BIC and BAFO) evaluation

Histologic observation presented bone formation in close contact to the implant for all groups at both time-points (Fig. 1). However, the content of the bone seemed to differ depending on the preparation with the press-fit groups (T and M) at 3 weeks presenting more cortical bone contact, whereas the groups L and R presented more new bone formation, which aligned the thread morphology. At 6 weeks, the bone in the chamber and in contact to the implant for all groups seemed to be comparable and the volume seemed to have increased compared with 3 weeks.

The ANOVA summary for the effects of time *in vivo* and drilling technique on BIC is presented in Table 3. The bone-to-implant contact (BIC) results, presented in Table 4, showed that at 3 weeks, group R had significantly lower values than groups T, M and L ($P < 0.05$, Table 4). No differences were observed between groups T, M and L. At 6 weeks, groups T and M were significantly higher than groups L and R. When considering time and instrumentation, a significant increase was observed for groups T and M between 3 and 6 weeks.

The ANOVA summary for the effects of time *in vivo*, drilling technique and their interaction on BAFO is presented in Table 5. The mean BAFO percentage results, presented in Table 6, showed that at 3 weeks, no significant differences were observed between groups. At 6 weeks, groups T and M were significantly higher ($P < 0.05$) than groups L and R. When considering time and instrumentation, only group T presented a significant increase between 3 and 6 weeks ($P < 0.05$).

Table 1. **ANOVA summary for torque**

Source	Numerator df	F	Sig.
Intercept	1	492.987	0.000
Time *in vivo*	1	23.542	0.000
Drilling technique	3	9.793	0.000

Table 2. **Estimated means and standard error for torque. The letters depict statistically homogeneous groups at each time *in vivo***

Time *in vivo*	Group	Estimated mean	SE
3 weeks	L	71.17	8.30
	M	91.67	8.30
	R	56.83	8.30
	T	107.83	8.30
6 weeks	L	92.33	8.30
	M	98.67	8.30
	R	92.67	8.30
	T	106.83	8.30

Discussion

This study investigated the effect of different drilling protocols for an implant possessing laser-etched micro grooves in the coronal portion of the implant. The sheep hip model was used because this region consists of a thin cortical layer and a marrow tissue underneath, which is similar to that of the human bone. This similarity is one of the reasons why this region is often used as a site for large bone augmentation in the oral cavity. This location has been used in studies and has shown consistent biologic outcomes (Stübinger et al. 2013; #103; Stübinger et al. 2010; #104; Ferguson et al. 2008; #105; Langhoff et al. 2008; #106). Due to the advancements in surface modification using different techniques, the quality and rate of osseointegration have considerably improved compared with the initially introduced turned or excessively rough implant surfaces (Junker et al. 2009; Suzuki et al. 2010). It has been confirmed that commercially available implants possess distinct surface topography both in the nano- and microlevels (Svanborg et al. 2010), which distinguishes one surface from another; in addition, the variations in the implant macrogeometry make each implant unique. In this regard, the laser-etched surface utilized in the current study is a unique

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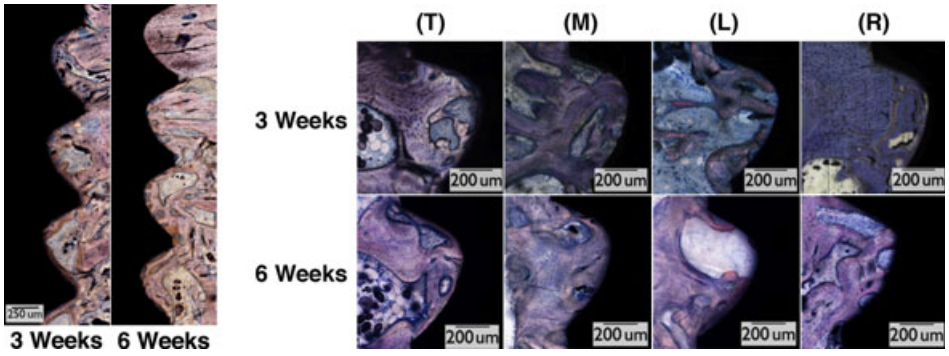

Fig. 1. An overview of the histologic sections observed in the study for both 3 and 6 weeks (bar: 250 μm), and magnified histologic image (bars: 200 μm) of the effect of four different instrumentation procedures for both 3 and 6 weeks.

Table 3. **ANOVA summary for BIC**

Source	Numerator df	F	Sig.
Intercept	1	760.400	0.000
Time *in vivo*	1	20.717	0.000
Drilling technique	3	13.600	0.000

Table 4. **Estimated means and standard error for BIC. The letters depict statistically homogeneous groups at each time *in vivo***

Time *in vivo*	Group	Estimated mean	SE
3 weeks	L	46.01	4.33
	M	50.53	4.33
	R	34.50	4.33
	T	51.03	4.33
6 weeks	L	42.99	4.33
	M	60.99	4.33
	R	39.02	4.33
	T	64.56	4.33

Table 5. **ANOVA summary for bone area fraction occupancy (BAFO)**

Source	Numerator df	F	Sig.
Intercept	1	618.420	0.000
Time *in vivo*	1	10.090	0.002
Drilling technique	3	8.600	0.000

Table 6. **Estimated means and standard error for bone area fraction occupancy (BAFO). The letters depict statistically homogeneous groups at each time *in vivo***

Time *in vivo*	Group	Estimated mean	SE
3 weeks	L	40.61	4.81
	M	49.00	4.81
	R	37.97	4.81
	T	45.19	4.81
6 weeks	L	41.46	4.81
	M	58.09	4.81
	R	44.33	4.81
	T	56.25	4.81

surface because the surface topography alteration does not undergo the traditional acid etching or sandblasting, and the computer-controlled procedure provides an intended structure, which has been suggested to be an important factor for enhanced bone apposition (Yoshinari et al. 2003; #110; Wennerberg et al. 2012; #111). Moreover, it has been suggested by Berglundh et al. that the healing sequence of different implant geometries using different osteotomy techniques has shown different bone-healing properties (Berglundh et al. 2003). In this study, we have used the same implant design for all groups, which meant that the objective was to purely observe the effect of the prepared osteotomy size.

Bone regeneration around the implant can be divided into two different types when focusing on the surgical procedure and the implant geometry (Marin et al. 2010). The former being the press-fit situation where, normally, the osteotomy diameter is smaller than the diameter of the implant and, as a result, presents high primary stability (Natali et al. 2009). The latter where a closed chamber is created in between the implant thread and the surrounding bone allows blood to be sustained within the chamber and results in bone formation (Leonard et al. 2009; Coelho et al. 2010). It has been reported that the press-fit situation is influenced greatly by the biologic remodeling process, where the contacting bone is resorbed by the osteoclast activity and thereafter bone apposition due to osteogenesis (Dhert et al. 1998; Slaets et al. 2006). The overall stability of the implant has been suggested to decrease the most during the stability transition stage from primary (mechanical) to secondary (biologic) stability, and this is one of the critical stages where the implant could disintegrate (Jimbo et al. 2007). On the other hand, the latter with a less press-fit situation naturally has lower primary stability; however, because majority of the implant surface is contacting the blood, bone remodeling will not undergo resorption, but induce contact osteogenesis (Davies 1998). This is probably why the reamer group (group R) presented the lowest values for all evaluations at 3 weeks and increased after 6 weeks. These results suggest that the bone formed at 3 weeks for group R is still immature and eventually mineralizes, so that it withstands higher removal torque values at 6 weeks and this biologic phenomenon is clearly different to that of the press-fit groups, which accords to the above raised past studies.

The biomechanical removal torque testing showed that the press-fit groups (medium and tight) presented significantly higher removal torque values than the other groups at 3 weeks, which was surprisingly higher than expected, because it has been known that the bone has a relaxing effect due to its viscoelastic properties and the stability levels would decrease within 1 week (Halldin et al. 2012). Furthermore at 6 weeks, the same group continuously maintained high removal torque values (no significant difference compared with 3 weeks), which seemed to have been supported by the significant increase in BIC. This is a clear indication that the implant stability was maintained at a high level; however, during the healing process, the secondary (biologic) stability successfully altered the primary (mechanical) stability. The stability alteration without the so-called stability dip has been suggested to be an essential factor for earlier bone healing around the implant (Cochran 2006 #107), and it has been suggested that the surgical protocols along with the implant surface modification is one of the major contributing factors for the upregulated stability threshold (Barewal et al. 2003; #108; Cochran et al. 1998; #109).

The general observation of the histologic sections displayed intimate bone contact with all groups and, at 3 and 6 weeks, the encircling line of osteoblasts indicating ongoing bone formation in close contact with the surface, which suggests the positive effect of the laser-etched microgrooves in the cortical (implant neck) region. It can be speculated that the contact guidance effect of the microgrooves attracted osteogenic cells and aligned them in the direction of the grooves, which may have triggered the differentiation and mineralization

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of them. Although with the undecalcified ground section stained with toluidine blue, it is difficult to quantify or identify the existence of osteoclasts or macrophages, it can be said that all groups represented progressive bone regeneration without any signs of inflammation or bone resorption represented by large bone lacunae. The results of the current study confirmed the previous *in vitro* reports, where the alkaline phosphatase activity significantly increased due to the presence of the microgrooves (Kenar et al. 2006; Im et al. 2012; Jiang et al. 2012).

Conclusion

Based on the results obtained, it can be concluded that the laser-etched microgrooved implant surface presented biocompatibility and osteoconduction. Furthermore, a drilling protocol generating a press-fit situation between the implant and the bone seemed to present high primary (mechanical) stability and was successfully altered by secondary (biologic) stability, suggesting that for this specific design implants, the press-fit setup seemed to be most ideal in clinical situations.

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