Original article

Biomechanical and histomorphometric properties of four different mini-implant surfaces

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Summary

Objectives: To investigate the effects of surface roughness on the removal torque and bone-to-implant contact of four different orthodontic mini-implants.

Materials and methods: Mini-implants and circular discs were made from alloy Ti6Al4V grade 5. On the basis of surface treatment, the study was divided into four groups—group 1: machined (n = 32), no surface treatment; group 2: acid etched (n = 32), with hydrochloric acid; group 3 (n = 32), grit blasted with alumina; and group 4, grit blasted + acid etched (n = 32). Mean surface roughness (Ra) and quadratic average roughness (Rq) from each group were measured two dimensionally in non-contact mode by the optical profilometer. Contact angle measurement of discs from each group was done with a contact angle goniometer. Contact angle of liquids with different hydrophobicity and hydrophilicity was measured: 1. highly hydrophilic liquid sodium chloride (NaCl), 2. lightly hydrophobic liquid dimethylsulfoxide, 3. distilled water, and 4. human blood. One hundred and twenty-eight miniscrews, differing in surface treatment, were placed into the tibias and femurs of adult male New Zealand white rabbits. Rabbits were euthanized after 8 weeks and removal torque and bone-to-implant contact were measured.

Results: Surface roughness of group 3 was significantly greater than other groups (P < 0.05). Group 4 had significantly lower contact angle measurements, both for blood and sodium chloride (NaCl; 40.26 degrees, 27.20 degrees) when compared to other three groups (P ≤ 0.01). Group 4 had significantly higher torque and bone-to-implant contact than group 3 (P = 0.007), group 2 (P = 0.003), and group 1 (P = 0.0002).

Conclusion: Surface roughness and wettability of mini-implants influence their biological response.

Introduction

Mini-implants have been used in contemporary orthodontics as an absolute source of skeletal anchorage (1, 2). Attaining maximum or absolute anchorage has always been an arduous goal for the practicing orthodontist (3, 4). Traditionally, numerous appliances and techniques have been devised for preserving anchorage, such as Nance holding arch, transpalatal bars, extra-oral traction, use of multiple teeth as the anchorage segment, and applying differential moments (1, 2). However, all these methods have inherent disadvantages: complicated designs, need for exceptional patient cooperation, and elaborate wire bending.

Mini-implants offer many advantages, including a simpler, shorter, less-invasive, more-economic procedure and can be placed in different areas of alveolar bone, including between the roots of the teeth and does not require patient compliance. Moreover, it can be used for borderline cases requiring absolute anchorage, such as
bidental alveolar protrusion, distalization of the entire dentition, and complex skeletal open bite.

The success rate of mini-implants as reported in the literature varies from 60 to 91 per cent and depends both on the host-related and mini-implants related factors (5–7). Host-related factors include bone quantity and quality, whereas screw-related factor includes length, diameter, shape of threads, and surface topography of the mini-implants (5, 7). Surface treatment of mini-implants is intended to provide better integration potential with the bone, thus can aid in resisting the dynamic forces (orthodontic and orthopaedics) and moments applied during the orthodontic treatment. Studies have shown that surface-treated implants have more primary and secondary stability (8–12).

The removal torque reflects the characteristics of the mini-implant–bone interface and is used to evaluate the anchorage capability of mini-implant because removal torque is the resistance force required to remove a mini-implant. Furthermore, it has been correlated with the amount of bone in contact with implant, leading to changes in biomechanical characteristic of the bone–implant interface. Implant surface properties are one of the major factors affecting integration with bone, but the mechanism involved in this process has not been clearly elucidated (11, 13).

Therefore, the purpose of this in vitro and in vivo study was to determine the correlation between the surface properties, removal torque, and bone-to-implant contact (BIC) of four different surface-treated mini-implants. The success rate of mini-implants has improved from the past, but failures still do occur, which led us to change the surface topography of mini-implants in this research.

Our chief aim was to determine if rough surface mini-implants (surface-treated) integrate better than machined (smooth surface) mini-implants. Bone to mini-implant integration was measured both by histomorphometry (bone to mini-implant contact) and mechanical analysis (removal torque). The null hypothesis was that different surface-treated mini-implants have no significant influence on the removal torque and bone-to-mini-implant contact.

Materials and methods

All mini-implants (Dentaurum Co., Ispringen, Germany) and circular discs (3 mm diameter and 3 mm thick; Dentaurum Co.) were made from titanium alloy (Ti6Al4V) grade 5. The 6-mm-long mini-implants were self-drilling and the outer thread diameter of mini-implant was 1.6 mm; inner core diameter of shank was 1.3 mm (threads are 0.15 mm deep on each side). The shank and threads are cylindrical for the top two-thirds of the mini-implant, and the lower one-third is tapered (Figure 1 and Supplementary Figure 1).

Machined ‘mini-surface’ surfaces were modified by subtractive method, acid etching with hydrochloric acid (HCl), blasting with non-resorbable blasting material (alumina) and a combination of blasting first with alumina particles and then acid etching with HCl.

On the basis of surface treatment, the implants and the circular discs (Figure 2 and Supplementary Figure 2) were categorized into four types—Group 1: machined—smooth surface mini-implants (no surface treatment). Group 2: acid etched—mini-implants were acid etched with 0.11 mol/l HCl at 65°C for 20 minutes. After etching, implants were dried in an oven for 24 hours. Group 3: grit blasted—mini-implants were blasted with alumina particles of grain size 25–50 µm. Group 4: grit blasted with acid etching—‘mini-implants’ were blasted first with alumina particles of grain size 25–50 µm and then etched with 0.11 mol/l HCl at 65°C for 20 minutes. After etching implants were dried in an oven for 24 hours.

In vitro experiments

The in vitro experiments were done on circular discs. The discs (Dentaurum Co.) received the same surface treatment as the ‘mini-implants’ (Dentaurum Co.) and were sterilized according to manufacturer’s instructions. Circular discs were used to measure the surface roughness and contact angle. Circular discs were used instead of mini-implants for in vitro experiments, as flat surface is required to measure the surface roughness and contact angle accurately.

Surface roughness

The implant surface roughness was measured using an Optical Profilometer (Proscan 2000, Scantron, London). Surface roughness parameters (mean surface roughness: Ra and quadratic average roughness: Rq) of six discs (3 mm in diameter, 3 mm in height) from each group were measured (2 mm² area) two dimensionally in noncontact mode using an optical profilometer (Figure 2). Each disc was measured five times. Additionally, the surface morphology of two mini-implants and two discs from each group was observed with a low vacuum scanning electron microscope (JEOL, JSM 5310LV, Japan).

Wettability/contact angle measurement

The contact angle measurement of five discs from each group was done with a contact angle goniometer (BP Medical Supplies, Brooklyn, New York, USA). Contact angle of distilled water was
used as a reference measurement, and the results were compared with the contact angle of liquids with different hydrophobicity and hydrophilicity: 1. highly hydrophilic liquid sodium chloride NaCl (0.150 M NaCl), 2. lightly hydrophobic dimethylsulfoxide (DMSO), and 3. human blood. Institutional review board clearance (NS1004-08) was obtained for the human blood. Single reading was measured from each disc at room temperature using a droplet of liquid. Height (b) and diameter (d) of the droplet was measured to calculate the contact angle ($\theta = 2 \tan^{-1}(2b/d)$) (Supplementary Figure 3) (14–16).

In vivo experiments

Methodology

Animal care committee of Indiana University-Purdue University, Indianapolis, approved the research and the approval number was DS0000883R. A total of 128 mini-implants, differing in surface treatment, were placed into the tibias and femurs of eight (4–5 months of age) male New Zealand white rabbits. On the basis of surface treatment, the mini-implants were divided into the four types listed above—group 1: machined ($n = 32$ mini-implants), group 2: acid etched ($n = 32$ mini-implants), group 3: grit blasted ($n = 32$ mini-implants), and group 4: grit blasted with acid etching ($n = 32$ mini-implants).

Each rabbit received a total of 16 implants, four each in the mid-diaphysis regions of the tibia and the femur of each hind leg. The placement of implants was randomized. The distance between adjacent implants was 20 mm (Figure 3A–D).

Randomization and mini-implant placement

Implants placement was randomized (statistician generated randomization using SPSS software and the authors were blinded about the randomization) according to the site of placement and type of mini-implant.

Anaesthetic/analgesic procedure

The rabbits were induced with an Acepromazine/Torbugesic (50/50 mixture at 0.15 ml/kg given i.m., not to exceed 0.45 ml total) to tranquilize as a pre-anaesthetic. The tranquilizer was administered 30–60 minutes prior to administering profound surgical anaesthesia with isoflurane and xylazine 25 mg/kg, xylazine 5 mg/kg mixture, and then the administration of B-euthanasia 1 ml/4.5 kg i.v. After death was confirmed, the femur and tibia of the rabbits were disected free and each specimen was assigned an identifying number to which the principal investigator was blinded.

Surgery

All procedures were performed under sterile conditions. The rabbit’s legs were shaved using an electric razor, the remaining hairs were removed using Nair® lotion (Church & Dwight Co., Princeton, New Jersey, USA) and the legs were surgically prepared and draped. An incision approximately 5 cm in length was made along the medial surface of the femur and the bone surface was surgically exposed by blunt dissection. In the tibia, because of decreased muscle mass and soft tissue thickness, a tissue punch supplied by the manufacturer of the implants, was used to expose the skin and the periosteum. All mini-implants preparations (holes) were drilled 20 mm apart with an internally irrigated, twist drill of 0.3 mm in length and 1 mm in diameter (Figure 3A–D). The implants were then screwed into prepared holes.

Euthanization

Rabbits were euthanized 8 weeks after the surgical procedure by first anaesthetizing with a ketamine 25 mg/kg, xylazine 5 mg/kg mixture, and then the administration of B-euthanasia 1 ml/4.5 kg i.v. After death was confirmed, the femur and tibia of the rabbits were disected free and each specimen was assigned an identifying number to which the principal investigator was blinded.

Tissue preparation for histomorphometric analysis

The mini-implants within the bone were 2D x-rayed using the Skyscan® MicroCT (model: 1072 Skyscan®, Aartselaar, Belgium) to determine implant orientation within the bone block. The specimen were then mounted onto a plastic slide for further grinding with different grinding papers (K320, K500, K800, K1000, and K1200) on an Exakat® grinding system (Exakt Medical Instruments, Oklahoma City, Oklahoma) until one side of the implant was exposed completely. The exposed side was then polished on the Exakt® grinding machine with a polishing paper. It was then mounted to a second slide using the Exakt light cure resin. The first slide was popped off and then the block was ground on the Exakt until the second side was exposed completely. Once the section reached the desired thickness (40–50 μm), the sections were polished as described above and readied for fluorescent and polarized light microscopy analysis.

Torque testing

Torque testing was completed immediately after euthanization. Removal torque measurement (Gauge Tohnichi® model 6BGT, 0–150 N cm range, Tohnichi Mfg. Co., Tokyo, Japan) was done immediately after the bone was harvested. The gauge was positioned on the hexagonal implant head and an increasing torque was applied and removed at the first ‘give’. The peak torque registered by the instrument was recorded.

Bone-to-implant contact

BIC was measured on grinded section after staining it with toluidine blue. This parameter tells about the actual bone contact with the implant surface.
Statistical analysis
Non-parametric tests were used to compare the outcome variables between different treatment groups since the data were not normally distributed and to account for the possibility of heteroscedasticity arising from the variances within groups not being equal. A one-sample Kolmogorov–Smirnov test was used to examine normality of distribution of the data. Kruskal–Wallis tests were used to compare the differences in distribution of the outcome across the four levels (treatment groups) of the independent variable. This was followed by multiple pairwise comparisons using the Mann–Whitney U-test to examine the differences in distribution of the outcome variable between different treatment groups. Since multiple pairwise comparisons introduce the possibility of type 1 errors, Bonferroni corrections were used to account for the same. For each of the analyses, there were four treatment groups and hence six pairwise comparisons were made with Mann–Whitney U-tests. To account for type 1 errors using the method of Bonferroni, a $P$-value of 0.008 was deemed to be statistically significant when the Mann–Whitney U-tests were used. All statistical tests were two sided. All statistical analyses were performed using SPSS Version 20.0 software.

Results
Characterization of mini implant surfaces
Machined: Figure 4A shows the surface morphology of machined implant surfaces. The tool marks were created during the manufacturing of the mini-implants. The unidirectional surface irregularities indicate the direction of the turning process. Acid etched: Figure 4B shows the surface morphology of the acid etched implant surface. A fine roughened isotropic surface was noted with regular elevations and depressions, but without any pits. Grit blasted: Figure 4C of grit blasted implant shows highly irregular surface with elevations, depressions, and irregular shaped cavities (pits). Grit blasted with acid etching: Figure 4D shows the surface morphology of grit blasted, followed by acid etching, implant surfaces. A much more uniform surface roughness was observed, when compared to grit blasted surface, with numerous elevations, depressions, and micro pits. Compared to grit blasted implants, the pits were more uniform and smaller in size.

Profilometer
The mean surface roughness (Ra) and quadratic average roughness (Rq) of different implant groups are listed in Supplementary Figure 4A–D and Figure 5. The acid etched (1.78 and 3.27) and machined group (1.13 and 2.56) had significantly lower ($P = 0.001$) mean value of surface roughness and quadratic average roughness than grit blasted (4.88 and 7.06) and grit blasted and acid etched (3.69 and 4.97, Figure 7). Grit blasted with acid etching had significantly ($P = 0.001$) lower surface roughness (Ra and Rq) than grit blasted (Figure 5). Comparison of acid etched to machined showed no statistically significant ($P = 0.35$) differences in mean and quadratic surface roughness values (Figure 5).

Contact goniometer
Mini-implant surface treatment directly affects its wettability. The interaction between group and liquid was significant ($P = 0.0002$).

Removal torque
There was no significant ($P = 0.67$) interaction between bone type and implant surface. Grit blasted with acid etching had significantly higher torque than grit blasted (tibia: 13.67 > 9.07 N cm; femur: 18.21 > 14.12 N cm; $P = 0.0075$), acid etched (tibia: 13.67 > 9.78 N cm; femur: 18.21 > 12.87 N cm; $P = 0.0035$), and machined (tibia: 13.67 > 4.08 N cm; femur: 18.21 > 6.49 N cm; $P = 0.0001$; Figure 9). Grit blasted ($P = 0.0009$) and acid etched ($P = 0.0007$) had significantly higher torque than machined but were not significantly different from each other ($P = 0.82$; Figure 7).

Bone implant contact
There was no significant interaction between bone type (femur or tibia) and mini-implant surface ($P = 0.70$). The femur and tibia did not have significantly different BIC% ($P = 0.87$). Grit blasted with acid etching had significantly higher BIC than grit blasted (tibia: 66.34 > 53.07 per cent; femur: 68.94 > 49.10 per cent; $P = 0.0003$), acid etched (tibia: 66.34 > 50.64 per cent; femur: 68.94 > 48.30 per cent; $P = 0.0001$), and machined (tibia: 66.34 > 39.30 per cent; femur: 68.94 > 45.28 per cent; $P = 0.0001$) (Figures 8 and 9 A–D). Grit blasted, acid etched, and machined were not significantly different from each other ($P = 0.08$ for grit blasted versus machined, $P = 0.16$ for acid etched versus machined, $P = 0.74$ for grit blasted versus acid).

Discussion
The null hypothesis of this study that surface treatment of mini-implants has no influence on the removal torque and bone to mini-implant contact was rejected. Surface roughness of implants has been considered an important parameter for bone integration as it influences cell (osteoblast and fibroblast) adhesion, adsorption, and differentiation (10, 17, 18). Mean surface roughness (Ra) is the arithmetic average of the roughness profile, whereas quadratic surface roughness (Rq) is the root mean square deviation of the roughness profile.

Suzuki et al. (19) showed that machined implants have a surface roughness of 0.5–1.2 μm. In our research, machined implants had a Ra of 1.13 μm, whereas the Ra of acid etched mini-implants was
1.78 µm. The Ra acid etched implants usually depend on three factors: concentration of acid, type of acid and etching time, whereas the Ra of grit blasted mini-implants is a function of particle type, particle size, and the blasting pressure. In our study, the Ra of grit blasted mini-implants was 4.88 µm, whereas Ra of grit blasted + acid etched mini-implant was Ra = 3.69.

Altering the surface properties (roughness) of mini-implants affects the quality of bone integrated to mini-implants. Ra values above 1.2–1.5µm are considered favourable for integration with bone. However, severe roughening of mini-implants may lead to peri-implantitis and risk of ionic leakage, thus hindering bone integration (20, 21). Rq along with Ra gives an important estimate regarding the surface roughness of the implant surfaces. Rq value can be 20–150 per cent more than Ra value. In our research, the Ra and Rq values of grit blasted mini-implants were significantly higher than machined, acid etched, and grit blasted + acid etched mini-implants.

Surface energy and wettability are usually quantified by the contact angle of liquid with surface (14, 15). The numerical values of contact angles indicate whether an implant surface is hydrophilic or hydrophobic. The present research shows that grit blasted with acid etching (group 4) is the most hydrophilic surface for all the liquids tested (Figure 6A and B). The grit blasted (group 3) and acid etched (group 2) group were equally hydrophilic for all liquids tested (statistically insignificant). Furthermore, the machined implant (group 1) was the least hydrophilic (contact angle values highest for the liquids tested) group among all the groups. In vitro and in vivo studies have shown that hydrophilicity is necessary for protein adsorption and cell adhesion (22, 23). Furthermore, Shibata et al. (24) and Buser et al. (25) showed that increased hydrophilicity of the mini-implant surfaces can enhance the adsorption proteins containing an arginine–glycine–aspartagine (RGD) sequences on their surfaces. They further stated that increased adsorption of RGD proteins contributes to cell adhesion and differentiation of osteoblasts, thus increasing the bone mini-implant integration (24, 25).

Removal torque test have been used consistently over the time, to evaluate integration potential (primary and secondary stability) of mini-implants. Theoretically, rough implant surfaces (Ra > 1.5 µm) are capable of establishing stronger biomechanical interactions with the peri-implant bone tissue than machined implant surface. The removal torque values in our study are consistent with the results from previous studies, which have shown a significant increase in bone retention of implants with increasing Ra and Rq values, except that in our study, both in tibia and femur, grit blasted with acid etching (group 4; Ra = 3.69 µm) implants had higher removal torques when compared to those of grit blasted (group 3) mini-implants (Ra = 4.88 µm; Figure 7) (26–28). Surprisingly, despite statistically different Ra and Rq values, in our study, we were unable to statistically differentiate between the removal torques of acid etched (group 2) implants (Ra: 1.78) and grit blasted (group 3) implants (Ra: 4.88 µm). Possibly, the blasting material used for developing grit blasted implants, alumina (Al₂O₃), often remains embedded in the implant material, even after the ultrasonic cleaning of the implants, and these alumina particles are released into the surrounding bone and interfere with bone mini-implant integration.

This problem is usually overcome by passivating the implant surfaces using different acids, and probably this was the reason of getting higher removal torque values in the grit blasted + acid etched mini-implants. Another plausible reason could be enhanced osteo-conduction (migration and differentiation of osteoblasts precursor) through the attachment of fibrin and osteogenic cells, resulting in bone apposition on the surface of the grit blasted + acid etched mini-implants. 

![Figure 4](https://academic.oup.com/ejo/article-abstract/37/6/627/2599941/631) (A) Machined, (B) acid etched, (C) grit blasted, and (D) grit blasted and acid etched scanning electron microscope images of titanium discs at 2000 magnification.

![Figure 5](https://academic.oup.com/ejo/article-abstract/37/6/627/2599941/631) Mean and quadratic surface roughness of four differently treated titanium discs. *Ra and Rq of grit blasted implant is significantly greater than machined, acid etched and grit blasted + acid etched mini-implant.
mini-implant, when compared to grit blasted implant having significantly higher Ra and Rq values.

Research studies have showed that the bone integration is more for the rough surface implants, when compared to machined implant and thus results in more stable bone–implant interface (10, 11, 26). Our research has similar findings, i.e. all the rough surface implants (Ra > 1.5) were having significantly higher removal torque than the machined surface implants.

Studies have even speculated that higher biomechanical fixation of rougher surface implants, compared to machined surfaces, was primarily due to mechanical interlocking between the implant surface and the surrounding bone (10, 11, 26).

However, it is very difficult to compare studies, particularly because the techniques used for altering the surface topography (different types of acid used, particle size of alumina, different types of particles used for blasting, blasting pressure) of machined implant vary considerably, and even more, the techniques used for surface topographical characterization (2D (Ra) versus 3D (Sa), laser profilometer versus optical profilometer) vary considerably; hence, a surface that is termed rough in one study may be termed smooth in another. In reality, even a machined surface may vary considerably in roughness, as is the case for grit blasted, acid etched, and a combination of grit blasted and acid etched. Even more, the animals used in studies are different (changes the healing process, bone remodelling

Figure 6. (A) Contact angle of dimethylsulfoxide (DMSO) and water of four differently treated titanium discs. *Grit blasted + acid etched has a significantly lower contact angle for water and DMSO than machined, acid etched and grit blasted mini-implant. (B) Contact angle of NaCl and blood of four differently treated titanium discs. *Grit blasted + acid etched has a significantly lower contact angle for NaCl and Blood than machined, acid etched and grit blasted mini-implant.

Figure 7. Removal torque of different surface-treated implants.

Figure 8. Bone-to-implant contact of different surface-treated implants in femur and tibia.
activity, cortical to trabecular bone ratio) and the surgical techniques of placing the implants vary from study to study.

A prerequisite for a success of mini-implant is the establishment of a direct BIC without the interposition of fibrous tissue. Research has shown that the specific surface properties of implants may have an impact on the adsorption of proteins and subsequently the initial regulation of cell adhesion (29–32). Additionally, it has also been shown that the surface properties of implants control the type of tissue, which develops at the bone–implant interface (33–35). Buser et al. (36) suggested a tendency for an increased BIC with increasing roughness or changing the micro-topography of the implant surface. In contrast, London et al. (37) and Novaes et al. (38) did not find any significant change in BIC with different surface-treated implants, but treatments that added roughness to the implant surface were having superior BIC, than found for the machined surface. Furthermore, other studies did not report any significant increase in bone-to-implant contact between rough surfaced and machined implants (27, 39–42).

This research showed significantly higher BIC with grit blasted with acid etching mini-implant when compared to the other three mini-implant surfaces. It is important to observe that increased BIC on grit blasted with acid etching implants compared to the other three implants was due to direct bone apposition along the implant surfaces, as evident by toluidine blue staining.

There were no statistical differences among the other three groups of implant, but numerically both in tibia and femur machined group had least BIC. It has been suggested that vascularization and initial stabilization of implants play essential roles in the early stages of peri-implant wound healing (43, 44).

However, whether the increased stability of rough surfaced implants is due to mechanical interlocking, increased contact, or modified bonding, or a combination of these, is still controversial and unknown. Removal torque is a dynamic test of the three-dimensional (3D) relationship between implant and bone, but BIC measurement is a two-dimensional static parameter. Thus, more research is needed to exactly determine the parameters evaluating the 3D bone structure relationship to adjacent implant.

Clinical extrapolation: Rough surfaces mini-implants can be used for dentofacial orthopaedics and situations requiring more and dynamic forces. Grit blasted implants should not be used in poor

Figure 9. (A) Machined, (B) acid etched, (C) grit blasted, and (D) grit blasted and acid etched bone-to-implant contact.
bone quality patients, as it can hinder the process of integration with the bone.

Conclusion
Orthodontic miniscrews surface properties were investigated both through in vitro and in vivo analysis and the study concluded that

1. The present study indicates that surface roughness parameters (Ra and Rq) were significantly more for grit blasted implants > grit basted and acid etched > acid etched > machined.
2. Contact angles for liquids tested: machined > grit blasted = acid etched > grit blasted with acid etching.
3. The removal torque of the mini-implants both in tibia and femur were in following order: grit blasted and acid etched > grit blasted = acid etched > machined.
4. Our histomorphometric results showed a significantly higher percentage of bone-implant contact with the rough surface implant than the machined. It must also be stressed that higher bone-implant contact percentage found around rough surface mini-implants could be especially useful in exacting clinical conditions like poor quality bone and early or immediate loading.

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Supplementary material
Supplementary material is available at European Journal of Orthodontics online.

References


