Histomorphological and torque removal comparison of 6 mm orthodontic miniscrews with and without surface treatment in New Zealand rabbits


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Summary

Aim: The purpose of this study was to assess the difference of removal torque values (RTV) and the bone-to-implant contact (BIC) between the sand-blasted, large grit, and acid-etched (SLA) surface-treated and the machined surface (MA) miniscrews.

Material and methods: Miniscrews used in this study were 6 mm long with a diameter of 1.5 mm. A total of 23 SLA miniscrews and 24 MA miniscrews were placed into the distal femoral condyle of 24 New Zealand rabbits. Removal torque test and the BIC was histologically evaluated at 0 and 8 weeks.

Results: There was no statistical difference between the RTV in the MA group versus the SLA group at both 0 and 8 weeks. Comparing 0–8 weeks, there was no significant difference in RTV of the SLA group (P = 0.48), however the change in the MA group was statistically significant (P = 0.006). Histological observation showed a significant decrease in BIC comparing 0 and 8 weeks for the MA group. The BIC ratio at 8 weeks was statistically significantly higher in the SLA group compared to the MA group.

Conclusion: SLA surface preparation does not increase the RTV of miniscrews. Further investigations under loading and a large sample size are required.

Introduction

In orthodontics, anchorage control is essential in most cases to achieve a successful outcome. When maximum anchorage is required, additional appliances are often needed to support the anchoring teeth. While intraoral aids may be limited in their anchorage value, the extraoral options, such as headgear, requires strict patient cooperation (1).
the other skeletal anchors (9). The overall success rates of miniscrews are variable and has been reported to range from 59.4 to 100%, whereas the dental implants have less than a 7–9% risk of failure over 10–15 years (10–12). Compared to conventional implants which are generally loaded after osseointegration and are intended to be permanent, orthodontic miniscrews are usually loaded right after insertion and are intended to be removed at the end of orthodontic treatment (13).

Various surface treatments have been studied for the past several years in order to achieve faster bone integration (14). Among those, the sand-blasted, large grit, and acid-etched (SLA) surface was found to have higher removal torque value (RTV) and bone-to-implant contact (BIC) compared to other surface treatments (15, 16). Oh et al. (17) evaluated the differences between SLA-treated miniscrews and smooth surface orthodontic miniscrews with length of 9.5 mm and an outer diameter of 1.8 mm under loading. The results showed that 6 weeks after placement, the SLA group presented a higher mean RTV (8.29 Ncm) than the smooth group (3.34 Ncm) and histological analysis revealed a higher new bone formation along the screw in the SLA group. They concluded that the SLA-treated miniscrews may endure higher orthodontic forces without loosening. The same finding was confirmed by the results from the study by Jeon et al. (18). A study by Vande Vannet et al. (2007) showed osseointegration with or without load application using semi-self-tapping miniscrews and concluded that the resistance to unscrewing is dependent on the amount of compact bone surrounding a titanium implant (19).

The results from previous studies also suggested that the wider the miniscrews, the better the success rate (9, 20). However, increased diameter and length will increase the risk of root damage during placement (21). The smaller miniscrews have been developed so they can be placed at various areas of the dental arch. The smaller devices widen the clinical applications in orthodontics and decrease surgical trauma.

The purpose of this study was to assess the difference of RTV and the BIC between the 6 mm SLA surface-treated and the conventional machined surface (MA) orthodontic miniscrews.

**Material and methods**

**Miniscrews**

The miniscrews used in this study were self-drilling, 6 mm in length with a diameter of 1.5 mm. They were constructed from Ti-6Al-4V titanium alloy by Russell Symes & Co Pty Ltd, New South Wales, Australia, replicating the Aarhus anchorage® system (American Orthodontics, Medicom eG, Tuttlingen, Germany) to allow for direct comparisons with a commonly used anchorage system. A total of 47 miniscrews were divided into two groups; the SLA group (n = 23, surface treatment by Biomaterials Korea Inc.) and the standard MA group (n = 23) to act as controls (Figure 1). All miniscrews were sterilized as per manufacturing recommendations prior to use.

**Animals and anaesthesia**

Twenty-four adult male New Zealand rabbits weighing 3.5–4 kg were used in this study. All rabbits were given a week acclimatization period prior to the surgical procedure. The experimental protocols and the methods were approved by Western Sydney Local Health District Animal Ethics Committee (Ethics approval No. 5081-04-11). The rabbits were sacrificed in two time points, at 0 and 8 weeks after 8 weeks of healing.

The animals were anaesthetized with a combination of Ketamine 35 mg/kg of body weight and Xylazine 5 mg/kg of body weight intramuscularly followed by continuous delivery of 4% isofluorane with 35% O₂ gas via mask. Before surgery, the animals also received intramuscular injection of opioid, Buprenorphine (Temgesic 0.01 mg/kg), and subcutaneous injection of antibiotic, Enrofloxacin (Baytril 10 mg/kg), and non-steroidal anti-inflammatory drug (NSAID), Meloxicam (Metacam 0.2 mg/kg).

**Surgical procedure**

After the animals were anaesthetized, both legs of each rabbit were shaved, washed, and decontaminated with a mixture of povidone–iodine and 70% ethanol.

Incision on the anteromedial surface of the lower femur, down to bone was made under aseptic conditions. The fascia and periosteum were dissected layer by layer. The anteromedial aspect of the femur and the medial femoral condyle were exposed so that the landmark of the medial epicondyle could be seen.

Miniscrews were placed using a hand-held screwdriver by the same operator (KA). One of each miniscrew type (MA or SLA) was placed approximately 3 mm superior to the medial epicondyle. None of the screws were loaded.

In the 8 week animals (n = 12), the incisions were sutured in separate layers (muscle, subcutaneous, and skin) using a combination of 4-0 and 3-0 Vicryl™ sutures (Ethicon Inc., New Jersey, USA). The rabbits recovered without complications and were kept in separate cages. After the surgery, they received subcutaneous injection of antibiotic, Enrofloxacin (Baytril 10 mg/kg), and NSAID, Meloxicam (Metacam 0.2 mg/kg), for another 6 days.

**Removal torque analysis**

A digital torque gauge (HTG2-500Nc, Imada Corporation, Northbrook, Illinois, USA) with a resolution of 0.1 Ncm was used...
to measure the resistance to reverse torque rotation. The gauge was securely fastened engaging the head of the miniscrew while the leg of the animal was stabilized as the miniscrew was removed. For the 0 week animals, RTV were measured immediately after miniscrew placement.

For the 8 week animals, the areas were re-accessed using the same surgical procedures to perform the RTV tests.

After the completion of RTV measurements, the animals were sacrificed using an overdose of pentobarbitone sodium by intravenous injection (200 mg/kg).

**Histomorphometric analysis**

After the animals were sacrificed, the femur-miniscrew blocks were sectioned and fixed in 10% formaldehyde solution for 48h followed by 70% ethanol. The blocks were then dehydrated, embedded in methacrylate resin, and sectioned with IsoMet® 5000 linear precision diamond saw (Beuhler Ltd, Illinois) at a thickness of approximately 300 µm. The best sections were subsequently stained with 2% toluidine blue. The histologic analysis was carried out by bright-field polarized light transmission microscopy. The bone-implant contact ratio (%BIC) of miniscrews was calculated using Image J Launcher software (Java version 1.1.4. for Windows, Microsoft, Redmond, Washington).

**Statistical analysis**

The data were analysed using IBM SPSS Statistics Version 21 (Copyright © IBM Corporation and other(s) 1989, 2012). A Generalized Estimating Equations was used to model the maximum RTV and %BIC separately by weeks and by screw type. A Bonferroni correction of α = 0.05/4 = 0.0125 was used to take into account multiple comparisons. Therefore P values under 0.0125 are considered statistically significant.

**Results**

All the animals recovered from anaesthesia without complications and had good wound healing.

**Removal torque values**

The RTV at 0 and 8 weeks are displayed in Figure 2 and Table 1.

At 0 week, the SLA surface group showed higher mean RTV (7.21 Ncm) than the MA miniscrews (5.38 Ncm) (P = 0.05). The mean RTV of machined and SLA surface groups at 8 weeks were 8.00 and 6.59 Ncm, respectively. The difference between the two miniscrews at 8 weeks was not statistically significant (P = 0.89).

Comparing 0 week to 8 weeks, there was no significant difference in RTV of SLA group (P = 0.48), however the change in the MA miniscrews was statistically significant (P = 0.006, Table 1).

**BIC ratio (%)**

The mean BIC ratios are shown in Table 2. The decrease in BIC ratios comparing 0 and 8 weeks for the MA group was statistically significant (P = 0.003) but not for the SLA group (P = 0.28). On average, BIC ratio was higher for (Figure 3A–3D) the SLA group at 8 weeks (P = 0.001). These results need to be interpreted cautiously since there were a small number of observations in each group.

**Discussion**

Most of the dental implants and miniscrew studies use tibia of the New Zealand rabbits as an implant placement site (17, 18, 22, 23). However, after several trial experiments on rabbit cadavers as well as cone beam CT and cross sections of the bone were analysed, we have seen that there was insufficient trabecular bone in the tibial diaphysis, and therefore in our study, the miniscrews were placed on the distal femoral condyle. In another study by Sennerby et al. (24), the implants were placed in the tibia of the rabbits and the authors found that the rabbit’s tibia consisted of cortical layer and marrow tissue with no cancellous bone. The implants were held by first one or two threads in the cortical bone while the major subcortical portion of the implants protruded into the marrow cavity. Since the aim of our study is to investigate and compare the anchorage potential and success rates, through osseointegration, of the SLA surface-treated and MA miniscrews, cancellous bone was necessary. Thus, femur was chosen to be the miniscrew placement site in our study. We have administered Meloxicam and Temgesic in our study for pain relief. Goodman et al. (25) showed that COX-2 inhibitors, when applied for a short period of time, have minimal effect on bone. However, in a critical view, it was concluded that there was a lack of basic science.
research defining the exact mechanism with which NSAIDs could interfere with bone cells and also the conduction of well-randomized prospective clinical trials are warranted (26).

There are several techniques for measuring implant stability and osseointegration such as Periotest, resonance frequency analysis, and removal torque test. Among these, the RTV has been widely used to evaluate the relationship between the implant surface and bone (27–30). The force needed for removal of implant increases with the torsion resistance of the bone–implant interface (31) which indicates the anchorage capability of the implant (32, 33). RTV studies are undertaken in humans as well as animals (34, 35). Kim et al. (34) showed that the RTV in a 37 patient sample with early loaded mini screws ranged between 3.94 and 35.41 Ncm. The previous animal studies of dental implants and miniscrews comparing MA to surface-treated (SLA) dental implant/miniscrews showed that after the healing period, SLA implants had higher mean RTV than MA implants (14, 15, 17, 18, 34) and the RTV increased with time (22). This is in contrast to the findings of the present study which showed that the RTV of the SLA miniscrews were not significantly different compared to the MA miniscrews at both 0 and 8 week periods. At

Figure 3. The lower power micrographs of SLA and MA miniscrews at 0 and 8 weeks. (A) SLA miniscrew at 0 week; (B) SLA miniscrew at 8 weeks; (C) MA miniscrew at 0 week; (D) MA miniscrew at 8 weeks.
0 week, the SLA surface group showed slightly higher mean RTV (7.21 Ncm) than the MA group (5.38 Ncm); however, this was not statistically significant ($P = 0.05$). For the MA group, the increase in RTV at 8 weeks (5.38 versus 8.00 Ncm) was significant while in the SLA group, there was a slight insignificant decrease (7.21 versus 6.59 Ncm). The increase in RTV of MA miniscrews from 0 to 8 weeks is expected, however the reduction in SLA is unexpected. However, this reduction was statistically insignificant and could be attributed to the variations in the bone density and thickness in this region, which we tried to eliminate using the femoral condyle rather than tibia. Kim et al.'s (36) study also showed that the removal torque of SLA miniscrews were not statistically different compared to MA miniscrews in a beagle sample after loading. Comparison of our results with other studies is difficult as other studies have loaded the miniscrews and utilized different animal models and surgical site. Chang et al. (22) have used loaded and unloaded MA and SLA miniscrews and found no differences between the loaded and unloaded conditions in most groups. In the loaded condition, the RTV of the SLA surface increased earlier, at 4 weeks.

The direct measurement from histomorphologic sections is the most precise method to measure BIC ratios (22). Previously, when comparing MA, SLA, and sandblasted and alkaline-etched (SL/NaOH) surface miniscrews in New Zealand rabbits, it was shown that the BIC ratio increased with time in all groups after 4 weeks and the BIC ratio of the SLA surface miniscrews was significantly higher than the MA surface miniscrew at 4, 8, and 12 weeks (22). The result from the present study does not support the finding from the study by Chang et al. (22) that the BIC ratio increases with time. In the present study, the decrease in BIC ratio from 0 to 8 weeks in the MA group was statistically significant ($P = 0.003$) but not in the SLA group ($P = 0.28$). The SLA group showed more BIC ratio than MA group at 8 weeks.

Our study had some limitations apart from the small study sample, which might explain the differences of our results with others. In the present study, the histological sections showed the variation in bone quality of the miniscrew sites. The sectioned site for the histomorphometric analysis does not always provide overall information about the specimen due to the limitation of the sections that are obtained via ground-sectioning method. However, the thickness of bone and the bone type in which the implant is inserted was found to be an important factor associated with removal torque (1, 37, 38). The characteristics of the treated surface of the implants also are important. Different surface treatment methods can affect the roughness of the surface (21). The optimization of the rough surface does not depend only on the size but also on the cross section. Study by Hansson and Norton (39) showed that half-spherical micropits had the highest retentive capacity and 1–5 µm was the optimal diameter with the highest pit affactivity factor. Moreover, Sul et al. (23) found that the oxide properties of titanium implants include oxide thickness, micropore configurations, and crystal structures and they also significantly influence the bone tissue response in the evaluation of RTV.

Even though the BIC ratio remained higher in the SLA group compared to the MA group after 8 weeks, the hypothesis that SLA miniscrews might offer advantages in stability and resistance to removal torque which could be used to withstand heavy or dynamic forces was not justified with the results of this study as there was no significant difference in RTV after 8 weeks. However, the RTV required to remove the osseointegrated miniscrew in the present study was clinically acceptable and comparable to the findings from previous studies (17, 18).

Conclusion

This animal experiment showed that SLA surface preparation does not increase the RTV of miniscrews. Further investigations are required to test these results in a larger sample and under loading.

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