Cortical bone microdamage produced by micro-osteoperforation screws versus orthodontic miniscrews: an in vitro study

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Background/objective: The alternative use of Orthodontic Miniscrew Implants (OMIs), traditionally used for skeletal anchorage, to facilitate micro-osteoperforations (MOPs) for accelerating orthodontic tooth movement has been reported in previous studies. The objective of the present in vitro study was to compare the microdamage generated by OMIs and MOP-purposed screws of similar dimensions in porcine cortical bone.

Materials and methods: Forty rectangular porcine cortical bone specimens of 1.5 mm thickness were produced and divided into two equal groups. According to group allocation, either a single MOP screw or OMI was inserted and later removed. A sequential staining protocol was carried out to distinguish true microdamage created upon screw insertion and removal from iatrogenic damage. The bone specimens were imaged by a confocal laser scanning microscope, and five histomorphometric measurements described and quantified the generated microdamage.

Results: On the entry (outer) bone surface, the OMI screws produced greater microdamage which reached statistical significance across all of the histomorphometric parameters. In contrast, a statistically significant increase in microdamage was created following MOP screw insertion on the exit (inner) bone surface, but only in three assessment parameters, recorded as total damage area, as well as diffuse damage area and radius.

Conclusions: Overall, the present study showed that 1.5 mm OMIs produced slightly greater microcrack-type and diffuse damage-type microdamage than the 1.6 mm diameter MOP screws. However, these differences were small and considered clinically insignificant.


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Introduction

The average active duration of orthodontic treatment ranges between 18 months and 3 years. The rate-limiting factor in controlling tooth movement is considered to be bone resorption at the bone and periodontal ligament interface. Surgical and non-surgical adjuncts marketed to reduce orthodontic treatment duration aim to accelerate tooth movement by increasing the number and function of osteoclasts through a variety of mechanisms. Surgical adjuncts rely upon the induction of a regional acceleratory phenomenon (RAP) in which a localised intensified remodelling process occurs as a response to a noxious stimulus. The RAP response initiates increased activity of osteoclasts and osteoblasts, as well as increased levels of inflammatory markers.

The use of corticotomies for the purpose of accelerating tooth movement dates back to the 1950s, when it was first introduced by Köle, who created surgical incisions limited to cortical bone. It was initially believed that corticotomies removed the mechanical resistance to orthodontic tooth movement. Since then, various procedures have evolved and in 2001, Wilcko et al. described the Accelerated Osteogenic Orthodontic
(AOO) technique, which involved raising a full thickness soft tissue flap, followed by corticotomy incisions and intra-marrow penetrations as well as placing a bone graft. More recently, a minimally invasive technique of applying corticotomies using micro-osteoperforations (MOPs) have been reported, in which small, shallow transmucosal holes are created in alveolar bone adjacent to teeth intended to be moved. Teixeira et al. first performed MOPs using a handpiece and round bur in an animal study. An increased number of osteoclasts and bone remodelling activity was reported as a result. It was believed that the localised trauma triggered the release of pro-inflammatory chemokines and cytokines, inducing osteoclastic upregulation and activity. A randomised controlled clinical trial by Alikhani et al. used a screw tip specifically for the purpose of facilitating MOPs, and found that MOPs increased the rate of canine retraction 2.3-fold, compared with control specimens without MOPs. However, the overall evidence of the effectiveness of MOPs is contentious. A recent systematic review concluded that, based on limited evidence, a single MOP does not accelerate orthodontic tooth movement, while a contrary review reported that the rate of tooth movement increased after MOP application.

The use of orthodontic miniscrew implants (OMIs) as an alternative means of performing MOPs was later explored by Cheung et al. in a rat study when it was reported that the rate of molar protraction increased, by 1.86 fold, compared with a control group. In addition, the study found decreased bone volume and bone density, as well as 44% more osteoclasts around the first molars on the side where MOPs were performed. Conversely, a split-mouth clinical trial on OMI-facilitated MOPs by Alkebsi et al. concluded that the experimental protocol of three MOPs performed distal to maxillary canines requiring retraction made no difference in the rate of tooth movement when compared with a control side.

The microdamage of cortical bone created by OMIs has been previously investigated as an indication of the levels of targeted remodelling activity and associated impact on OMI stability. Multiple parameters known to affect peri-implant microdamage and OMI stability have been investigated and include insertion torque, screw design and cortical bone thickness. Several studies have investigated the impact of the method of insertion on the stability of OMIs with conflicting outcomes.

Current knowledge suggests that there have been no prior studies on the comparison of microdamage created by MOP-specific screws and OMIs. Therefore, the aim of the present study was to determine if there was a difference in the microdamage generated by MOP screws and OMIs of similar dimensions. The null hypothesis is that there is no difference in the microdamage generated by the two types of screws.

Materials and methods
In keeping with Institutional Ethics Committee guidelines, eight fresh pig tibia bones were obtained from a local abattoir. The ends of the tibia bone were discarded, and a band saw was used to split the remaining portion into three equal parts, following which, the soft tissue and periosteum were stripped off using a scalpel. Following the methodology of Nguyen et al. which included a power and sample size calculation, forty flat cortical bone pieces were prepared from the bone segments using a slow speed sectioning machine (Leica SP1600, Leica Germany). The dimension of the prepared bone specimens was approximately 15 mm by 20 mm, and of a thickness of 1.5 mm to represent the average thickness of the human maxilla and mandibular cortical bone. A polishing protocol was then carried out to remove the majority of the iatrogenic preparation cracks. The top and bottom surfaces of the bone blocks were polished ten times with increasingly finer grades of silicon carbide micromesh while submerged in distilled water. Using digital callipers (CD-6”CX, Absolute Digimatic, Mitutoyo, Japan), the thicknesses of the bone sections were checked before being wrapped in gauze soaked in 10% phosphate buffered solution. Between the time of bone preparation and experimentation, the bone pieces were kept at -23°C to prevent desiccation and to prevent changes in their physical properties.

The bone blocks were divided into two groups of twenty comprising group 1 (MOP group) and group 2 (OMI group). Group 1 had a single 1.6 mm × 7 mm Excellerator PT MOP screw (PROPEL Orthodontics, Ossing, NY) placed into each bone specimen, while group 2 had a 1.5 mm × 6 mm self-drilling Aarhus Anchor OMI screws (MEDICON eG, Tuttlingen, DE) placed in each bone specimen. Half of the OMI
and MOP screw groups (subgroups 1A and 2A) had screws inserted with a torque-limiting hand driver (G00234, Rocky Mountain Orthodontics, Denver, CO, USA), while the other half (subgroups 1B and 2B) were inserted using a motor-powered handpiece (Elcomed, W&H Dentalwerk Bürmoos GmbH, AT).

The staining method applied in the present study was also adapted from Nguyen et al.'s protocol in which a series of calcium-binding fluorochromes of increasing affinity were used in order to differentiate between true microdamage caused by screw insertion and iatrogenic damage. For ease of identification, the edge of each bone specimen was labelled with at least one scratch on the entry surface using a scalpel prior to the first stain. The specimens were then soaked in xylenol orange \(5 \times 10^{-4}\) M solution for 30 min, to identify damage created during the bone preparation process. The specimens were rinsed under distilled water for 8 min to remove excess stain.

The bone specimens were subsequently supported and secured between two acrylic plates which formed part of a customised holding jig to facilitate the screw insertions. Loading compression levels upon insertion were also measured by an Omega miniature compression load cell (R4-F6-76535, N2Surplus Inc., Roanoke, VA, US), which was connected to a laptop computer running LabVIEW software (National Instruments Australia, Macquarie Park, NSW, AU). To ensure complete insertion, subgroups 1A and 2A had screws inserted with a torque-limiting hand driver with insertion torque limited to 40 N cm. Subgroups 1B and 2B had insertion carried out via a torque controlled surgical handpiece, with insertion torque and speed controlled at 40 N cm and 25 rpm, respectively. After miniscrew insertion was completed, the bone blocks were removed from the holding jig and soaked in calcein solution \(1 \times 10^{-3}\) M for 30 min to identify microdamage created by screw insertion, and rinsed again for 8 min. The minscrews were removed and excess bony debris protruding from the entry and exit surfaces was carefully removed with a scalpel to allow the specimens to be laid flat. The bone specimens were finally immersed in calcein blue solution \(1 \times 10^{-4}\) M for 30 min, followed by another 8-min rinse, to identify damage caused by screw removal. All miniscrew insertions and removals were conducted by a single operator to eliminate introducing inter-operator differences in technique.

The bone specimens were imaged with an Olympus FV3000 confocal laser scanning microscope (Olympus, Japan). A 561DPSS laser (569-700 nm red spectrum), a 488 Argon laser (496-593 nm green spectrum) and 405 Diode laser (413–480 nm blue spectrum) were used to excite the xylenol orange, calcein and calcein blue, respectively. Scans of the entry and exit surfaces of each bone block were carried out separately at 1.25 magnification to a maximum depth of 600 µm at 10 µm intervals. The images of each surface were then digitally superimposed to produce a two-dimensional representation of the total damage. ImageJ software was used to mark and quantify the detected microdamage. Measurements of the five histomorphometric parameters of microdamage were recorded, namely total microdamage, total diffuse damage, maximum crack length, maximum crack radius, and maximum diffuse damage radius (Figure 1). Diffuse damage was identified as patches of more intensely stained mineralised matrix that had been disrupted by locally intense deformations, while microcracks were linear and had sharp borders, extending to a range of 100µm, and usually stopping at osteonal cement lines.

Burr and Stafford’s criteria were applied for the identification of a microcrack, which were: (1) Intermediate in size, larger than a canaliculi but smaller than vascular channels, (2) sharp borders with a halo of basic fuchsin staining, (3) stained through the depth of the section, and (4) more deeply stained than the intervening space when the depth of focus was changed. Only minor differences in generated microdamage were found between the insertion methods within the OMI and MOP groups. These were considered unlikely to be of clinical significance, and the data of microdamage generated by the OMI and MOP subgroups were combined within their respective groups for the comparison of generated microdamage between OMI and MOP screws.

**Statistical analysis**

Descriptive statistics of all miniscrew variables categorised by mode of insertion and screw type were performed. Linear regression models were applied to investigate the association between bone damage measures and type of screws (MOP and OMI). All entry and exit data were analysed separately. \(P\)-values...
less than 0.05 were considered statistically significant. The software used to carry out statistical analysis was SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

**Results**

The prepared bone specimens did not vary greatly from the targeted 1.5 mm thicknesses in the MOP groups and OMI groups, with mean measurements of 1.51 mm (SD 0.08) and 1.52 mm (SD 0.07), respectively. Compression data showed relatively consistent readings with the mean loading compression values ranging from 25.06 N to 26.13 N between the groups. Of the MOP and OMI screws inserted by a motor handpiece \((n = 20)\), the mean insertion torque of the MOP group (7.95 N cm) was lower than the OMI group (9.95 N cm) by 2.00 N cm, which was not statistically significant.

**Difference in bone microdamage generated by MOP and OMI screw tips**

On the entry surface, the OMI screws generated greater microdamage and reached statistical significance across all histomorphometric parameters; total damage area, diffuse damage area, maximum crack length, maximum damage radius and maximum diffuse damage radius, with mean differences of 3.49 mm\(^2\), 2.83 mm\(^2\), 0.49 mm, 0.86 mm and 0.57 mm\(^2\), respectively \((P < 0.05)\) (Table I). On the exit surface, there was a statistically significant difference in diffuse damage of both histomorphometric parameters, as well as in total damage area, where greater damage was displayed following the use of MOP screws. The difference was 1.28 mm\(^2\), 0.71 mm\(^2\) and 0.20 mm\(^2\) with regards to increased total damage area, diffuse damage area and maximum diffuse damage radius, respectively \((P < 0.05)\) (Table II).

**Discussion**

Changes to bone properties and differences in initiated bone remodelling have been demonstrated to occur as a result of different types of microdamage. Diffuse damage, comprising submicroscopic cracks, has been shown to produce a bone toughening effect.\(^{27}\) Once past a compression threshold, linear microcracks appear and the property of fracture toughness decreases.\(^{27}\) In vivo studies have demonstrated an association between microcracks and resorptive bone remodelling.\(^{28,29}\) Mori and Burr demonstrated increased intra-cortical resorption and the appearance of resorptive cavities localised to the vicinity of fatigue-induced microcracks.\(^{30,31}\) In an in vivo study on rat ulnae subjected to fatigue-induced microdamage, Herman et al. provided a distinction between the types of microdamage and its effects on the induction of targeted bone remodelling.\(^{31}\) While
Table I. Comparison of histomorphometric variables between OMI and MOP groups on the entry surface.

<table>
<thead>
<tr>
<th></th>
<th>Total damage area [mm$^2$]</th>
<th>Diffuse damage area [mm$^2$]</th>
<th>Maximum crack length [mm]</th>
<th>Maximum damage radius [mm]</th>
<th>Maximum diffuse damage radius [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Micro-osteoperforation screws</td>
<td>6.05</td>
<td>1.72</td>
<td>3.12</td>
<td>1.05</td>
<td>1.64</td>
</tr>
<tr>
<td>Orthodontic Miniscrew Implants</td>
<td>9.53</td>
<td>1.96</td>
<td>5.95</td>
<td>1.17</td>
<td>2.12</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>-3.49</td>
<td>-4.55, -2.42</td>
<td>-2.83</td>
<td>-3.36, -2.29</td>
<td>-0.49</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>0.01*</td>
<td>&lt;0.05*</td>
<td>0.00*</td>
</tr>
</tbody>
</table>

Table II. Comparison of histomorphometric variables between OMI and MOP groups on the exit surface.

<table>
<thead>
<tr>
<th></th>
<th>Total damage area [mm$^2$]</th>
<th>Diffuse damage area [mm$^2$]</th>
<th>Maximum crack length [mm]</th>
<th>Maximum damage radius [mm]</th>
<th>Maximum diffuse damage radius [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Micro-osteoperforation screws</td>
<td>7.07</td>
<td>1.48</td>
<td>3.46</td>
<td>0.70</td>
<td>2.04</td>
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<tr>
<td>Orthodontic Miniscrew Implants</td>
<td>5.79</td>
<td>1.07</td>
<td>2.75</td>
<td>0.41</td>
<td>1.84</td>
</tr>
<tr>
<td>Difference (95% CI)</td>
<td>1.28</td>
<td>0.52, 2.04</td>
<td>0.71</td>
<td>0.36, 1.06</td>
<td>0.20</td>
</tr>
<tr>
<td>P-value</td>
<td>0.00*</td>
<td>&lt;0.05*</td>
<td>0.17</td>
<td>0.53</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>
the number of resorption cavities was positively correlated with the number of microcracks, neither the cumulative loading history nor diffuse damage foci were associated with bone remodelling activity. Furthermore, it was observed that osteocyte viability was only affected by microcrack-type damage, which was related to bone resorption induced by apoptotic osteocytes. Therefore, histomorphometric parameters differentiating between both types of microdamage were selected for the present study, and have also been used in previous studies investigating OMI-related microdamage associated with pilot holes and on varying diameters of OMIs. Using an animal model, Lee and Baek studied the difference in microcrack generation between varying OMI diameters of the self-drilling variety, using a surgical handpiece with insertion torque controlled at 40 Ncm. The 2 mm OMI diameter groups had significant increases in microcracks related to maximum crack length, the number of cracks and accumulated crack length, with the result suggesting a 1.5 mm OMI diameter as the preferred choice over a 2 mm OMI to minimise microcracks. Liu et al. however, found varying OMI diameters from 1.4 mm to 2.0 mm had no differential impact on microcrack formation, provided that pilot holes were predrilled prior to insertion. Where cortical bone thicknesses were 2 mm or greater, Shank et al. found that microcrack formation was reduced when a pilot hole was predrilled prior to OMI insertion.

It is possible that the methodology of these studies may have been flawed as the use of basic fuchsin stain has been reported to be fraught with the risk of introducing artefacts caused by dehydration of the bone specimens when stained by an ethanol-based dye for prolonged periods. The present study adopted the staining protocol and imaging technique piloted by Nguyen et al. for microcrack detection created by OMI insertion, in which a series of calcium-binding fluorochromes of increasing affinity were used to label areas of bone damage at different stages of the experiment to distinguish iatrogenic damage, damage caused by OMI insertion, and damage caused by OMI removal. The selected chelating fluorochromes emit fluorescence at different wavelengths which enabled ease of distinction under laser scanning confocal microscopy. Laser confocal microscopy has been demonstrated to provide images of high spatial resolution, with an ability to detect minute microcracks of 10 µm in great visual detail.

**Microdamage created by OMIs and MOP screw tips**

Recent animal studies have explored the use of OMIs to facilitate MOPs for the purpose of accelerating orthodontic tooth movement. The purpose of the present study was to investigate whether perforations produced by OMIs provide an alternative option to MOP screw tips. It was determined that the amount of microdamage generated by both MOP screw tips and OMIs was comparable, but generated small differences in statistical significance. The clinical significance of the differences was, however, questionable. This suggested that OMIs are a suitable alternative to MOP screws for performing MOPs, and supports the additional function of OMIs to include performing MOPs. Furthermore, the OMI group produced increased values of the parameters relating to microcrack type damage overall, which was associated with bone resorptive remodelling necessary for orthodontic tooth movement. These observations suggest a slight advantage in the use of OMI screws over MOP screw tips for the purpose of performing osseous perforations.

The present results are reflective of a single use application. Although MOP screws are disposable items, they are designed for the creation of multiple MOPs within a single clinical setting. OMIs, however, are designed as a single-use item for the purpose of providing skeletal anchorage. Despite the manufacturer’s recommended guidelines, several authors have reported the successful clinical reinsertion of OMIs without screw fractures. Chung et al. evaluated the mechanical properties of used OMIs and reported significant tip deformation requiring greater insertion force for complete insertion into artificial bone blocks. Similarly, an unpublished ex vivo study by Luong et al. revealed a reduction in volume of OMI tips after a one-time use, but no significant difference in microdamage created upon a single reuse. Therefore, it is suggested that OMIs may be considered as an alternative to MOP screws for facilitating MOPs for at least two insertions. Further research would be necessary to investigate if there is a difference in microdamage generated by used MOP and OMI screws. Furthermore, establishing the maximum amount of safe reuse of MOP and OMI screws for the purpose of MOP creation would have clinical value and generate further research.
The diameter of OMIs should also be taken into account if they are considered for reuse as MOPs, as smaller diameter OMIs have a higher risk of screw fracture and therefore, make removal difficult. Wilmes et al. compared fracture resistance of OMIs of different diameters ranging from 1.3 mm to 2.0 mm and found reduced fracture torque values with the smaller diameter OMIs. Based on previous studies, OMI diameters of 1.5 mm to 2.0 mm were able to be successfully reused at least once with little risk of screw fracture. The difference in microdamage created by the MOP and OMI groups may also be attributed to differences in screw material and design. The OMI screws used in the present study were 1.5 mm in diameter and made of titanium alloy, while the MOP screws are only manufactured with a diameter of 1.6 mm and of surgical grade stainless steel. High grade stainless steel has a higher modulus of elasticity and fracture resistance compared with titanium alloys. Titanium alloy OMIs are most commonly used for their highly biocompatible properties, but the successful use of stainless steel OMIs has been reported. Due to their properties, previous authors have advocated the use of stainless steel OMIs when placement sites are planned in areas of denser bone such as the mandibular bucal shelf and infra-zygomatic crest. Few studies have been carried out to determine the influence of OMI material on stability. A histological study by Brown et al. found no difference in microdamage between immediately loaded titanium alloy and stainless steel OMIs six weeks after placement in rabbit tibias. Although this provided an insight into the difference in secondary stability as some healing and targeted remodelling would have already occurred, further studies are required in order to establish if screw material influences the created microdamage upon insertion. Therefore, the difference in microdamage produced by OMIs of different materials and MOP screw tips would be a relevant area for further investigation.

Other design characteristic differences may explain the difference in microdamage found in the present study. For the purpose of achieving OMI stability for successful skeletal anchorage, the thread pitch and diameters of the shaft and thread are other important determining design characteristics. Within limits, a greater pitch advances the OMI farther into the cortical bone per turn, and produces greater axial stress and torque. It has been shown that 0.8 mm is the optimum thread ascent, while the difference between the shaft diameter and thread diameter should be approximately 0.4 to 0.6 mm. Fluting is an additional design parameter which allows bone chips to be carried away from the cutting edge as the screw advances. Although it has been shown that there is no difference in insertion torque between fluted and non-fluted OMIs, an animal study by Truong et al. reported a 37% increase in removal torque and a reduced amount of peri-implant bone when the fluted screws were removed 2 weeks after placement. The results of the present study only reflect the difference in microdamage creation between the MOP screw and OMIs of a particular design. Future studies should be carried out to investigate whether a significant difference exists in the microdamage created between MOP screws and OMIs of different designs.

Conclusion
Based on results of the present study, upon insertion, 1.5 mm OMIs produced slightly greater microcracks than the 1.6 mm diameter MOP-purposed screw tips. This finding not only supports the additional function of OMIs to include facilitating MOPs, but also implies the potential for a slight advantage in performing MOPs using 1.5 mm diameter OMIs to produce a greater RAP effect for the purpose of accelerating orthodontic tooth movement. In addition, using OMIs as an alternative to MOP screws for performing MOPs could potentially aid in minimising the inventory of an orthodontic practice in which OMIs are already being used for augmenting orthodontic anchorage.

Conflict of Interest
The authors declare that they have no conflict of interest.

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