Original

Histological Study of Porous TiNi Alloy Miniscrew Immediate Load with Different Force Levels in a Beagle Dog

Yi Liu and Ying Zheng

Department of Orthodontics, School of Stomatology, China Medical University, Shenyang, China

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Abstract: The purpose of this study was to compare the stability of porous TiNi alloy miniscrew implants with different force levels (0 g, 200 g, 400 g and 600 g) loaded immediately after placement. Using a randomized split-mouth design, we placed 16 miniscrews into 3 skeletally mature male beagle dogs. All the miniscrews were equally divided into 4 groups. The result showed that there was more fibrous tissue repair between the miniscrew-bone interfaces with the increase of the force levels. The 600 g load caused serious bone trabecular damage on the neck of miniscrew, whereas no significant effect on tissue around miniscrews has been apical. Not only was the miniscrew-bone interface completely mechanically integrated, but it also formed osteoid-like tissue. There was more fibrous healing, with the increasing of the force levels. Bone trabecular around the neck of the miniscrew destroyed, when the case of 600 g loaded.

Keywords: Anchorage, Miniscrew, Immediate loading, Miniscrew-bone interface, Implant

Introduction

Stable anchorage is the key to the success of orthodontic treatment, which also plays an essential role in the treatment design. Recently, more and more orthodontists pay close attention to miniscrew. Many factors affect the stability of miniscrew, such as length, diameter, material, thread shape, surface treatment, location of the miniscrew, the way of loading force, timing of load, and so on. In addition, it should also be concerned about the condition of the patient, such as age, sex and oral hygiene. Porous structure could change the surface of the miniscrew, imitate the natural morphology of the cancellous bone and form a mechanical lock between miniscrew-bone interfaces, increase contact area, benefit to the ingrowth of the bone. The study was about the stability of immediately loaded Porous TiNi alloy miniscrew with different force levels.

Material and Methods

Animals

In this study, 3 skeletally mature male beagle dogs between 1 and 2 years of age and weighing 14.0 ± 1.0kg were used. The housing, care, and experimental procedures were approved of by the Animal Care and Use Committee of China Medical University. Because of the periodontal ligament and alveolar bone similar to those in humans, beagle is a widely used animal model. The beagles were fed with semiliquid diet and were monitored for normal behavior.

Porous TiNi alloy miniscrew implant

The Porous TiNi alloy miniscrew implants used for this experiment were 1.6mm in diameter by 8mm in length (supplied by Eastnorth University ceramic institution). Its preparation process was added TiH2 into the Ti and TiNi alloy as pore-forming agent. And based on added 40 wt% TiH2 pore-forming agent, we also added into NH4HCO3 as a second pore forming agent into 50 wt% Ni sample. The pores consisted of 100 -500 μm large pore and 1-50 μm small pore as well as the micropore of under 1 μm, which formed a perfect microrough surface; the distribution of this porosity was suitable for the biomedical requirements of miniscrew.

Animal model of immediately loading

Surgical procedures were performed aseptically under intraperitoneal 3% pentobarbital (1ml/Kg) anesthesia. Four locations were chosen for the placement of miniscrews: on the mesial and distal buccal alveolar bone of the maxillary canine, and the root bifurcation attached gingival of the third and premolars (Fig 1). The miniscrew supporting device buccal alveolar bone was 90° (pilot drill 1.3 mm in diameter, speed 800 rpm, physiological saline cooling).

The miniscrews was immediately loaded at the different force
Figure 1. Porous TiNi alloy miniscrew implant

Figure 2. Positions of the miniscrews in the maxillae of the dogs

Figure 3. A1: Methylene blue-basic fuchsin staining, multiple fibrous tissue around the neck of the miniscrew and osteoid-like tissue on the edge of the mature bone; A2: Methylene blue - basic fuchsin staining, apical aspects of miniscrew, miniscrew lost, multiple fibrous tissues adhered and little born contacted around the apical aspects of the miniscrew; A3: Toluidine blue staining, much fibrous tissue around the neck of the miniscrew and the osteoid tissue at bone contact area; A4: Toluidine blue staining, apical aspects of miniscrew, miniscrew lost, a thin layer of fibrous tissue was visible by the miniscrew -side of shedding light blue osteoid; A5: Toluidine blue staining, apical aspects of miniscrew, miniscrew lost, a thin layer of soft tissue surrounding the interspace, shedding light blue osteoid on soft tissue periphery.

Figure 4. B1: Methylene blue - basic fuchsin staining, neck of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; B2: Methylene blue - basic fuchsin staining, apical aspects of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; B3: Toluidine blue staining, neck of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue obviously visible; B4: Toluidine blue staining, apical aspects of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue obviously visible; B5: Methylene blue - basic fuchsin staining, neck of miniscrew, miniscrew with surrounding bone tissue closely integrated; B6: Methylene blue - basic fuchsin staining, neck of miniscrew, miniscrew with surrounding bone tissue closely integrated; B7: Toluidine blue staining, apical aspects of miniscrew, miniscrew with surrounding bone tissue closely integrated.
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Figure 5. C1: Methylene blue - basic fuchsin staining, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; C2: Methylene blue - basic fuchsin staining, apical aspects of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; C3: Toluidine blue staining, neck of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; C4: Toluidine blue staining, apical aspects of miniscrew, miniscrew with surrounding bone tissue closely integrated, osteoid tissue visible; C5: Toluidine blue staining, apical aspects of miniscrew with surrounding bone tissue closely integrated.

(used closed coil nickel-titanium spring), and they were divided into 4 groups according to force magnitude: 0 g, 200 g, 400 g, 600 g (0g as control group).

All the animals received prophylactic antibiotics penicillin (800 All units/day) postoperatively, and were fed with semiliquid diet to avoid damage the appliances.

The appliances were actived every two weeks to maintain the force. We found one miniscrew in 600 g lost at the 4th week; and another miniscrew in 400g group was observed inflammation. In this study, the miniscrews were loaded interactively, so a total of four miniscrews were removed.

After 8 weeks, all the animals were sacrificed with overdose of anaesthetic. Arranged to the miniscrew as center, each sample was cut mesiodistally into 1.5-2.0mm bone tissue block. Perfused transcardially with 10% Formaldehyde solution overnight at 4 ℃, radiographs were taken and measured to determine the direction of miniscrew. The specimens were dehydrated and embedded in paraffin. Three sections were cut from each sample. The sample was cut from neck apical aspects into 200-300 μm and prepared for histology observation.

Sections were stained with toluidine blue, and methylene blue - basic fuchsin stain. To observe the combination of miniscrew-bone interface and formation the new bone, optical microscope was used.

Results

The result showed that the shedding light blue osteoid, dark blue mature calcified bone tissue in toluidine blue staining; and red mature bone tissue, blue soft tissue, purple calcification edge in methylene blue-basic fuchsin staining, though the cortical bone...
Table 1. Differences among 4 Groups in Clinical and Optical Microscope Observation

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical Observation</th>
<th>Optical Microscope Observation</th>
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<tbody>
<tr>
<td>0g</td>
<td>4 miniscrews were stable.</td>
<td>There were fibrous tissue around the neck and apical aspects of the miniscrew and osteoid-like tissue on the edge of the mature bone.</td>
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<tr>
<td>200g</td>
<td>4 miniscrews were stable.</td>
<td>Miniscrew and surrounding bone tissue were closely integrated, and a few gaps, soft tissue and osteoid tissue were visible.</td>
</tr>
<tr>
<td>400g</td>
<td>2 miniscrews were stable. 2 miniscrews had been removed due to inflammation.</td>
<td>Miniscrew and surrounding bone tissue were integrated more closely; osteoid tissue was visible. removed due to inflammation.</td>
</tr>
<tr>
<td>600g</td>
<td>4 miniscrews were stable.</td>
<td>Miniscrew lost on the neck of miniscrew in the 600 g group, fibrous tissue were around the neck of the miniscrew, bone trabecular destroyed, free bone trabecular appeared by miniscrew-side. While the apical aspects slices showed that miniscrew with surrounding bone tissue were closely integrated, obviously osteoid-like tissue was closed to the miniscrew edge.</td>
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under an optical microscope. In this study, two staining results were consistent.

The 0 g group
In the 0 g group, there were fibrous tissue around the neck and apical aspects of the miniscrew and osteoid-like tissue on the edge of the mature bone (Figure 3. A1-5).

The 200 g group
In the 200 g group, miniscrew and surrounding bone tissue were closely integrated, and a few gaps, soft tissue and osteoid tissue were visible (Figure 4. B1-7).

The 400 g group
In the 400 g group, miniscrew and surrounding bone tissue were integrated more closely; osteoid tissue was visible (Figure 5. C1-5).

The 600 g group
Miniscrew lost on the neck of miniscrew in the 600 g group, fibrous tissue were around the neck of the miniscrew, bone trabecular destroyed, free bone trabecular appeared by miniscrew-side. While the apical slices showed that miniscrew with surrounding bone tissue were closely integrated, obviously osteoid-like tissue was closed to the miniscrew edge (Figure 6.

Discussion
The miniscrew has gained wide acceptance as direct and indirect orthodontic anchorage\(^3,4\). Animal experiments and clinical studies\(^5,6\) showed that miniscrews could be loaded immediately with forces typically used in clinical orthodontics and some orthopedic situations.

But there are several limitations. In recent years, miniscrew gained good effects in some difficult cases, but the varying degrees of movement or even clinical failures happened occasionally. The study focused on stability of immediately loaded Porous TiNi alloy miniscrew. Park\(^7\) stressed that mobility of the screw might be a crucial factor of failure (inpreparation). The absence of mobility indicates that the miniscrew-bone interface forms osseointegration.

When achieving osseointegration, the orthodontic force can be loaded increased without negative effect on the stability of the miniscrew. Scholars believed that\(^8-14\): the implant loaded before osseointegration has the same anchorage effect as the implant loaded after osseointegration. The possibility of immediate loading is attributed to the successful mechanical interdigitation on the miniscrew-bone interface. And also they pointed that immediate loading can help improve the stability of mechanical interdigitation.

Morphology observation in this study under the light microscope showed that there was little bone healing but much fibrous healing on the neck and apical aspects of the miniscrews in the 0 g group. Remodeling at the periosteal bone was slightly
more active in the loaded specimens than in the controls. The reasons might be there was no stimulation of the force, the bone tissue recovered more slowly after damage. This observation was similar to the study of Yan Chen: immediate loading does not inhibit osseointegration of miniscrews but stimulates bone adaptation 9).

The study indicated that immediate loading of the miniscrew could get better results. Miniscrew-bone interfaces were closely conjunction on the neck and apical aspects of the miniscrews in the 200 g, 400 g and 600 g groups. And osteoid tissue appeared in 600 g group. No connective tissues interpose or new bone formed between the miniscrew-bone interface means osseointegration, which is Hickham's theory10). According to the theory, two months after immediate loading, all stable miniscrews had a certain quantity of osseointegration.

Some scholars16-22) believed that partial overpressure formed fissure, which led to osteoclasia and resorption. Research observed that there was a large number of fibrous tissue surrounding the neck of miniscrew under optical microscope in 600 g group. Miniscrew lost was reported in both immediately and delayed loaded20, 21). The failure depended on the amount of force applied; the increased force appeared to be a contributing factor 22). Observation suggested that 600 g orthodontic force caused the bone tissue significantly destruction and not repaired within the experimental time. Though miniscrews did not fall off, miniscrews loaded with 600 g showed significantly more movement than those loaded with 200 g and 400 g. The possible reason was that miniscrew with surrounding bone tissue were closely integrated, obviously osteoid-like tissue was closed to the miniscrew edge in apical slices. But whether 600 g force could maintain osseointegration for a long time or bone destruction on neck could repair over time and form new bone, still need a further study.

Inflammation was one of the main reasons that caused the failure of miniscrew. Zarb23) stressed that failure of miniscrew might have been due to traumatic surgery and early loading, since 1-stage implants could not eliminate the bacterial and epithelial intrusions. Therefore, good oral hygiene is important to reduce infection. In 6 week, miniscrew loaded 400 g was observed inflammation around the miniscrew, 2 miniscrews had been removed to avoid result in affection.

Porous TiNi alloy could be considered successfully for clinical use. The clinical success of anchorage with Porous TiNi alloy miniscrew was loaded immediately.

In conclusion, 1) Miniscrew-bone interface was not only completely mechanical integrated, but also formed osteoid-like tissue; 2) Porous TiNi alloy could use as orthodontic anchorage, There was more fibrous healing, with the increasing of the force levels; and 3) Bone trabecular around the neck of the miniscrew destroyed, when the case of 600 g loaded.

References

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