Osseointegration and biomechanical properties of the onplant system

Xiang Chen, a Guoxin Chen, b Hong He, c Cong Peng, a Ting Zhang, d and Peter Ngan e
Wuhan, China, and Morgantown, WV

Introduction: Onplants can be used as temporary anchorage devices for orthodontic tooth movement and orthopedic protraction of the maxilla. The device requires 3 to 4 months of osseointegration and can be removed after the orthodontic treatment. The purpose of this study was to investigate the degree of osseointegration and the biomechanical properties of onplants during various healing periods in an animal model.

Methods: Sixteen rabbits were used in the study, and 3 onplants were placed on the calvaria of each rabbit (n = 48). The rabbits were divided into 4 healing-period groups with 12 onplants in each group: 2, 4, 8, and 12 weeks. At the end of the healing periods, the animals were killed, and bone blocks, each containing an onplant, were prepared for either histologic examination or biomechanical characterization.

Results: The histologic and histomorphometric results showed significant increases in bone formation or bone contact ratio at the bone-onplant interface in the 8-week and 12-week groups when compared with the 2-week and 4-week groups (P < .05). Under a light microscope, smaller and fewer osteoblasts—a sign of maturity of the osteoblast—were observed in the 8-week and 12-week groups when compared with the 4-week group. However, evaluation of the biomechanical properties of the onplants showed that the shear force increased with the length of healing period (7.56 ± 2.92, 75.30 ± 9.64, 155.56 ± 12.15, and 305.71 ± 12.74 N for the 4 healing periods, respectively), and significant differences were found between the 8-week and the 12-week healing periods (P < .05).

Conclusions: These results suggest that osseointegration occurred mainly after the 4-week healing period. The shear force of onplants increased with healing time, suggesting that shear force is not necessarily determined by the area of newly formed bone, but to a certain degree depends also on the density of the newly formed bone. The notion of loading onplants for orthodontic tooth movement as early as possible needs further clinical study for verification. (Am J Orthod Dentofacial Orthop 2007;132:278.e1-278.e6)
region. An incision is made in the palatal mucosa from the premolar area toward the midline. The tissue is tunneled under, in full-thickness fashion, past the midline to the eventual implantation site. The onplant is then slipped under the soft tissue and brought into position, and the incision is sutured. A vacuum-formed stent is worn by the patient for 10 days for the initial stabilization.

Preclinical studies in monkeys and dogs showed that onplants can be used as stable anchorage to move teeth and as abutments for distraction osteogenesis of the mandible. In these experiments, onplants provided 3.06 N of continuous force and 711 N of shear force. Clinically, onplants have been used successfully to close space orthodontically, help move molars distally, erupt teeth occlusally, and provide anchorage for forward protrusion of the maxilla. The success of an onplant as orthodontic anchorage clinically depends on the amount of force it can withstand. Studies on the strength of the union between the bone and the onplant device are lacking in the literature. Studies on the biomechanical properties of oral implants suggested that dual-etched implants have more rapid rates of pull-out strength than implants with machined surfaces and remained significantly stronger throughout the study periods. Research on onplant healing after osseointegration can provide useful information for clinicians on the timing for loading onplants. A common research method to evaluate implant or onplant healing is to measure the extent of bone contact along the implant surface that can be assessed at the light microscopic level or with histomorphometry. Onplants can be removed from experimental animals with the surrounding bone. Thin sections can be viewed under the microscope, and the percentage of the bone contact ratio can be calculated. This can be compared with in-vitro shear strength between the bone-onplant interface to determine whether there is a relationship between the degree of osseointegration and the strength of the union between bone and onplants. We hypothesized that the in-vitro shear force of onplants increased significantly with the length of healing and that the ability of onplants to withstand shear force is related to the degree of osseointegration or the percentage of bone contact.

The objectives of this study were to evaluate the degree of osseointegration at the onplant-bone interface qualitatively and quantitatively by histomorphometric examination and vital staining, and to assess the biomechanical properties of the onplant attachment to bone by measuring the shear force during various healing periods.

MATERIAL AND METHODS

Sixteen male rabbits were used in this study. Three onplants were placed in each rabbit, 1 on each side of the midline suture, and another on the anterior region of the calvaria. The onplants (n = 48) were then randomly divided into 4 healing periods with 12 onplants in each group: 2 weeks, 4 weeks, 8 weeks, and 12 weeks. In each group, 1 onplant with 3 wafers was used for histomorphometric analysis, and the other 2 were used for shear force measurement.

The onplant device was a thin titanium disk; its fitting surface was smooth. On the nonfitting surface, there was a threaded hole into which a male portion could be attached. The onplants were 7.7 mm in diameter and 2.8 mm in thickness with rounded edges to avoid soft-tissue dehiscence (Fig 1).

The fitting surface of the onplant was polished with sand paper from 240# to 2000# (Hubei Tianma, Wuhan, China) and etched with hydrochloric acid/sulphuric acid. Figure 2 is a scanning electron microscope photograph of the onplant fitting surface indicating the etched pattern and nondirectional surface topography.

Mature, healthy male rabbits weighing 2.0 to 3.0 kg were used. All procedures were performed in sterile conditions. The animals were anesthetized with ketamine (44 mg per kilogram intramuscularly) and xylazine (5 mg per kilogram intramuscularly). The rabbits’ scalps were shaved, surgically prepared, and draped. To place the 3 onplants on the calvaria, a skin incision was made, and 2 skin flaps were reflected laterally to expose the 2 sides of the cranium. Three periosteal incisions, 10 mm in length, were made, and 3 subperiosteal tunnels were created (1 on each side of the midline, and the third on the anterior region of the cranium), with a small periosteal elevator. The onplants were placed in the created tunnels. The tunnels were closed by suturing the free edges into the adjacent periosteum. The skin flaps were closed and sutured tightly by using subcutaneous and simple interrupted sutures. Each rabbit received an immediate postoperative dose of...
penicillin (Hubei Pharmaceutical, Wuhan, China). The rabbits were killed at 2, 4, 8, or 12 weeks, respectively, after the surgery according to their groups. Each cranium was harvested and cut into blocks (each block contained 1 onplant with at least 3 mm of bone around the onplant). The onplants on the anterior region of the cranium were fixed in 4% paraformaldehyde, embedded in Spurr’s resin, and sectioned into wafers about 100 μm in thickness, perpendicular to the onplant-bone interface, by using a large-scale heavy-duty sectioning system with a diamond-wafering blade (Leica Company, Wetzlar, Germany). Three sections were acquired from each sample. The undecalcified ground sections were stained with methylene blue or the compound of hematoxylin and eosin and methylene blue (Wuhan Bell Chemical Reagent, Hubei, China). The other samples were prepared for the force evaluation experiment.

The sections were viewed under an inverted microscope (Leica) and photographed. The photographs were analyzed by using the Image J program (a computer-based image analysis system, Toronto Western Research Institute, Toronto, Canada). If no new bone is formed between the onplant and the calvarium surface, there will be space between the 2 surfaces. The percentage of newly formed bone at the onplant-bone interface was calculated by dividing the length of the newly formed bone by the total bone-implant interface length seen on each section at high magnification.12 For each experimental group, 12 sections were analyzed, and the average was used to calculate the bone-contact ratio.

Excised of soft tissue immediately, the 30 bone blocks (each containing 1 onplant) were stored in normal saline solution at 4°C and prepared for the shear force test. Bone blocks were embedded in a self-cured orthodontic acrylic resin material in a custom-made mold and left to cure in normal saline solution at 4 for 1 hour. Then, they were attached to the lower part of the testing machine. The shear force test was performed at a speed of 1 mm per minute for each onplant until failure. The shear force at the point of failure was recorded in newtons.

The data for the histomorphometric and shear force experiments were analyzed by using ANOVA and the Kruskal-Wallis and Dunn unpaired tests. Significant differences among the 4 experimental groups were set at the level of $P < .05$.

**RESULTS**

Health conditions were normal in all rabbits, with no infection of the wounds noted. Complete periosteum was found on 46 of the 48 onplants with successful osseointegration. No infection was observed in the surrounding tissue and the new bone formed at the onplant-bone interface. Two onplants in the 4-week group were not integrated and were removed from the sample. In these samples, incomplete and thin periosteum was found at the onplant-bone interface with soft-tissue dehiscence. In the successful onplants, new bone was formed along the sides of the onplants.

Table I shows the mean percentage of new bone contact area at the onplant-bone interface for the 4 healing periods. Significant differences were found between the 4-week (36.2%), 8-week (60.1%), and 12-week groups (67.2%) compared with the 2-week group ($<0.1\%$). Significant differences were also found between the 8-week and the 12-week groups compared with the 4-week group. No differences were found between the 8-week and 12-week groups.

**Table I. Bone contact ratio for healing periods**

<table>
<thead>
<tr>
<th>Group</th>
<th>Samples (n)</th>
<th>Bone contact ratio (%)</th>
<th>SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 week</td>
<td>4</td>
<td>&lt;0.1</td>
<td>0.04</td>
<td>27.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>4 week</td>
<td>4</td>
<td>36.2$a,b$</td>
<td>4.14</td>
<td>9.23</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>8 week</td>
<td>4</td>
<td>60.1$a,b$</td>
<td>7.07</td>
<td>1.99</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>12 week</td>
<td>4</td>
<td>67.2$a,a$</td>
<td>8.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from each onplant were averaged and stood for 1 sample. Same letter indicates no difference between groups.

**Fig 2.** Scanning electron microscope image of polished and acid-etched onplant fitting surface showing etched pattern and nondirectional surface topography (original magnification $\times$ 1000).
Figures 3 to 6 show the photomicrographs of the histologic sections of the 4 groups. In the 2-week group, there was space between the onplants and the bone surfaces. Cells were found to attach only at the margin of the onplants (Fig 3). In the 4-week group, there were a few regions of bone osteogenesis, with the osteoblasts abnormally arranged (Fig 4). In the 8-week and 12-week groups, new bone was consistently present along the onplant surfaces (Figs 5 and 6).

There were fewer osteoblasts in the 8-week and 12-week groups than in the 4-week group under the microscope. The osteoblasts were also smaller; this is a sign of the maturity of the osteoblasts. In the 12-week group, new bone was found connecting the cranial bone to the onplant in some regions.

Table II shows the shear debond force for the 4 healing groups. ANOVA showed significant differences among the groups. Onplants in the 2-week group withstood a shear force of 7.56 ± 2.92 N. Onplants in the 4-week group withstood a shear force of 75.30 ± 9.64 N.
In the 8-week and 12-week groups, the shear forces were $155.56 \pm 12.15$ N and $305.71 \pm 12.74$ N, respectively. Because the healing periods were not long, the fractures mainly occurred at the onplant-bone interface.

**DISCUSSION**

In this study, calvaria bone was chosen to study the biomechanical property and degree of osseointegration at the onplant-bone interface because it is more accessible than the oral cavity. In addition, calvaria bones develop similarly to the maxillary and mandibular bones: they all develop via intramembranous bone formation, and the precursor cells are neural crest derived cells.13 The onplants were made of pure titanium, which has good biocompatibility with bone.14 Undecalcified thick sections were used because titanium in general is hard to cut, and the bone-onplant interface could be easily broken or disturbed.

Various studies have examined the effect of implant surface topographies on bone healing.15-17 In general, a greater percentage of bone-to-implant contact was found adjacent to micro-rough titanium surfaces when compared with smooth surfaces. The onplants used by Hoffman and Block1 incorporated a mesh on the fitted surface and a layer of hydroxyapatite to enhance osseointegration to bone. In our study, the onplant surface was polished and acid-etched with 98% (w/w) sulphuric acid and 37% (w/w) hydrochloric acid at 60°C for 30 minutes. The result was found to be satisfactory. The shear force between the onplant and the bone surface increased significantly with healing time for the entire study period. We could not determine when shear force will level off from our 4 healing periods. In 1995, Block and Hoffmann1 studied onplants with dogs and monkeys, and concluded that after a healing period of 5 months, the onplant was sufficiently anchored by the hydroxyapatite-coated biointegrated interface to resist up to 711 N of shear debond force; this was greater than that of our study. The length of the healing period and the hydroxyapatite coating might be the reasons for the enhanced shear force. Hydroxyapatite coating has been shown to induce the differentiation of osteoblasts.18 In this study, histomorphometric analysis showed that the mean bone contact ratio at the interface was significantly higher in the 8-week and the 12-week groups when compared with the 2-week and 4-week groups. No significant difference was observed between the 8-week and 12-week groups. However, the shear force at the onplant-bone interface increased throughout the 12-week study period, and significant differences were found between the 8-week and 12-week groups. These results suggest that the biomechanical properties of onplants cannot be determined only by the areas of the newly formed bone but might also be affected by bone density to some degree.

In addition, new bone was formed along the sides of the onplants; this might be an important factor for the stabilization of the onplants. Higher circumferential tissue mineralization might occur with increased healing time, thereby affecting the stabilization and shear force of the onplants to some degree.

In the study, smaller and fewer osteoblasts were observed in the 8-week and 12-week groups when compared with the 4-week group with the microscope. The presence of mature osteoblasts suggests that osseointegration of the onplants occurred mainly between the 4-week and 8-week healing periods after placement of the onplants. This observation is somewhat qualita-
tive and should be substantiated by measuring the levels of alkaline phosphate and osteocalcin.

Infection is a common risk factor for the failure of osseointegration. In this study, 2 onplants failed to osseointegrate because of infection. Incomplete and thin periosteum was found on their surfaces. Complete periosteum is important for success of integration because onplants can tightly contact the bone surface under the pressure of the periosteum. At the same time, complete periosteum can separate the onplant from the outside environment to prevent infection. Mucoperiosteum is thick in the human palate, making the use of onplants possible.

A direct comparison between the healing under onplants and the healing around implants is not appropriate because onplants are placed on relatively inactive bone surfaces, whereas implants are placed in freshly prepared sockets in bone. When onplants are placed in subperiosteal tunnels over the cortical layer of cranial bone, the only source of osteoblasts and other cells with osteogenic potential is the periosteum. Implants, on the other hand, have additional sources of cells from endosteum and bone marrow spaces. Therefore, a slower healing rate generally is expected under onplants when compared with implants. In addition, for successful osseointegration, cells should be induced to migrate from the periphery of the onplants toward the interface. Growth factors (TGF-β BMP-2 RGD) have been shown to expedite cell differentiation toward the interface. Growth factors (TGF-

CONCLUSIONS

Osseointegration of the onplant occurred mainly after the 4-week healing period. However, the shear force between the bone-onplant interface increased significantly with the length of healing throughout the 12-week period. These results suggest that the shear force of onplants is not determined solely by the area of newly formed bone but to a certain extent by the density of the newly formed bone and the circumferential new bone formed along the onplants.

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REFERENCES