Effect of Biologic Aging of Implants on Osseointegration in the Dog

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The objective of this preclinical study was to investigate the effect of biologic aging of implants on osseointegration in the canine. According to multiple comparisons, there was a significant difference in bone-to-implant contact (BIC) between control implants placed 6 months after manufacture and 2-week-old implants (P = .016), and between control and newly prepared implants with acid-etching (P = .019). However, there was no significant difference in BIC between newly prepared implants with acid-etching and 2-week-old implants. In all groups, BIC at 12 weeks was significantly higher than at week 4 (P < .05). There were no significant differences in bone volume (BV) regardless of area and time. Biologic aging of implants might affect osseointegration in the bone marrow zone at 4 weeks of healing. Although implant aging did not greatly affect BIC and BV at 12 weeks of healing in this study, further research is required to determine an appropriate period of biologic aging of implants that yields significant clinical effects.


The titanium implant has been indispensable in dental treatment since Brånemark discovered osseointegration in 1952.1 A long healing time was required for the successful function of early implants, whose surfaces were smoothed by machine milling. Clinicians at that time were interested only in replacing missing teeth with implants. Now they are working to reduce healing times to enable immediate loading of dental implants.

An essential factor for immediate loading is implant stability, also known as total stability, which is the sum of primary stability during implant placement and secondary stability during healing.2–4 It is commonly known that total stability of an implant reaches its lowest point at 4 to 6 weeks after placement. This phenomenon, called a stability dip,2–4 influences the success of immediate loading. Therefore, increased primary stability and reduced stability dip are essential for immediate loading. To achieve these, researchers have developed implant designs and surface treatments to enhance early function and reduce healing time of implants.

Meanwhile, surface bioactivity of dental implants after manufacture has been found to degrade over time.5–7 This deterioration of bioactivity occurs due to implant surface absorption of organic materials.
such as hydrocarbons from the atmosphere, cleansing solution, and water during manufacture and storage.\textsuperscript{8,10} X-ray photoelectron spectroscopy spectra have shown increases in the atomic percentage of carbon on the implant surface of 16% to 62% over time.\textsuperscript{5}

Hydrocarbon contamination changes the electrical properties of the implant surface, which is naturally negatively charged. A divalent cation such as Ca\textsuperscript{2+} is attracted to the negatively charged implant surface, followed by negatively charged proteins before cells adhere to the implant surface. However, osseointegration is interrupted as proteins and extracellular matrix cannot combine with the oxide layer of an implant surface contaminated by hydrocarbons,\textsuperscript{11} rendering the titanium surface bioinert.

Protein absorption, attachment and proliferation of osteogenic cells, and mineralization on the implant surface are essential to successful osseointegration. It has been reported that an aged implant surface shows inferior performance compared to a newly prepared acid-etched implant surface with respect to all these factors.\textsuperscript{5}

An in vivo experiment using a rat model revealed that the biomechanical strength of bone–titanium integration for 4-week-old acid-etched implants was less than half that for newly prepared implants. It was also found that the percentage of bone-to-implant contact (BIC) was < 60% for 4-week-old acid-etched implants, compared to > 90% for newly prepared acid-etched implants.\textsuperscript{5}

Although many studies have been published on implant aging and its resolution,\textsuperscript{5,7,11–15} most were cellular experiments and few have been carried out with small animals such as rats. The present authors are not aware of any study that shows the effect of implant aging in animals larger than the rat. Even though the application of results from dog experiments to human has limitations, data from tests using larger animals such as the dog might be more useful compared to cellular experiments or tests with small animals. The objective of this study is to investigate the effect of implant aging on osseointegration in the dog via histomorphometry.

**Materials and Methods**

**Implant Surface Characterization**

A total of 36 implants (3.5 mm in diameter and 8.5 mm in length, Magic Grip Straight Fixture, Oneplant), all with sandblasted/acid-etched surface, were used in the experiment. All implants were made at the same time (October 21, 2013) and placed in a sealed container (traditional method). For surface rejuvenation, 24 implants were treated with 67% sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) at 120°C for 75 seconds and cleaned ultrasonically in distilled water twice for 10 minutes each time.\textsuperscript{5} Of the 24 acid-etched implants, 12 were placed in a sealed container and stored in a dark room (23°C and 60% humidity) for 2 weeks.\textsuperscript{5}

**Experimental Design**

In group A, the control group, implants were placed 6 months after manufacture. In group B, implants with fresh surfaces as prepared following the protocol mentioned above were placed. In group C, implants with 2-week-old surfaces as prepared following the protocol mentioned above were placed. Half of each group was obtained from the animals 4 weeks after implant installation, and the other half 12 weeks after.

**Surgical Procedure**

Six young adult male mongrel dogs weighing approximately 30 kg each were used in this study. The animals had intact maxillae and mandibles, no periodontitis, and normal dentition. The animals were in good general health. Animal care and treatment protocols were approved by the Animal Care and Use Committees, Yonsei Medical Center, Seoul, Korea (approval no. 2013-0109).

All surgeries were performed by the same operator under general anesthesia in a sterile operating room. The animals received a subcutaneous injection of atropine (0.06 mg/kg) and an intravenous injection of xylazine (Rompun, Bayer) (0.2 mg/kg) and tiletamine/zolazepam (Zoletil, Virbac) (5 mg/kg). Inhalation anesthesia was performed using 2% isoflurane. During the surgeries, a heating pad was applied to the animals. The P1, P2, P3, and P4 mandibular premolars on both sides were extracted. At 2 months after the extraction, a full-thickness...
mucoperiosteal flap with midcrestal incision was elevated under the same general anesthesia condition as teeth extraction. Six implants were placed in the extraction areas of each mandible with a torque of 30 Ncm according to the manufacturer’s recommendation. All implants were connected with healing abutments. The same postoperative management was performed as for the extraction of teeth. All sutures were removed after 7 days. The animals were fed a liquid diet and sacrificed by anesthesia drug overdose 4 weeks and 12 weeks after placement of implants (Fig 1).

**Histologic Preparation**

Specimens were fixed in 10% buffered formaldehyde solution (pH 7) and dehydrated in ascending concentrations of alcohol (up to 100%), then embedded in methacrylate. Embedded specimens were sectioned buccolingually and ground to a thickness of < 35 μm. Sectioned specimens were stained with hematoxylin-eosin and observed via light microscopy.

**Histomorphometry**

Each implant section was analyzed using light microscopy (BX50, Olympus) coupled to a videocamera capture system. Magnification was ×100 and ×200. Measurements were made with computer-based histomorphometric measurements (IMT iSolution Lite ver 8.1, IMT i-Solution). The peri-implant tissue was divided into an upper zone (blue line) and a lower zone (red line) of the implant (Fig 2); both zones were within a 500-μm vicinity. BIC of bone tissue located
within 50 μm of the implant surface without intervention of soft tissue was calculated as follows:

\[
BIC(\%) = \left( \frac{\text{sum of the length of bone-to-implant contact}}{\text{circumference of the implant}} \right) \times 100
\]

\[
BV(\%) = \left( \frac{\text{bone area in the area of interest}}{\text{area of interest}} \right) \times 100
\]

Results

Of 36 implants, 34 were successful, and there were no complications. Two implants were not included in the analysis because there was severe marginal bone loss. There were statistically significant differences in BIC between the groups in the lower zone of the implant at 4 weeks of healing (\(P < .05\)) (Table 1 and Fig 3). Based on multiple comparisons, there was a significant difference in BIC between groups A and C (\(P < .017\)), and between groups A and B (\(P = .017\)). There was no significant difference in BIC between groups B and C. In all groups, BIC at 12 weeks of healing was significantly higher than at 4 weeks (\(P < .05\)).

The results showed that there were no significant differences between the groups in BIC in the upper zone of the implant at 4 and 12 weeks of healing. At 12 weeks, there were no significant differences in BIC between groups in the lower zone of the implant (Fig 3).

In the upper zone of the implant, BV at 4 weeks was significantly higher than at 12 weeks (\(P < .05\)) (Table 2). However, there was no significant difference in BV in the lower zone of the implant regardless of the healing time. Table 2 shows that there were no significant differences in BV between groups at 4 and 12 weeks of healing regardless of area.

Discussion

In this study, the difference in BIC between rejuvenated and nonrejuvenated surfaces was only seen in

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**Table 1 Comparison of Bone-to-Implant Contact (BIC) Between Groups**

<table>
<thead>
<tr>
<th>Area</th>
<th>Healing time</th>
<th>Group A Mean (%) ± SD</th>
<th>Group B Mean (%) ± SD</th>
<th>Group C Mean (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper zone of implant</td>
<td>4 wk</td>
<td>80.0 ± 15.8</td>
<td>83.6 ± 7.5</td>
<td>84.2 ± 9.1</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>93.3 ± 2.3</td>
<td>92.0 ± 4.8</td>
<td>87.5 ± 6.3</td>
</tr>
<tr>
<td>Lower zone of implant</td>
<td>4 wk</td>
<td>63.5 ± 6.5</td>
<td>77.4 ± 5.2*</td>
<td>79.4 ± 12.7*</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>79.2 ± 7.1</td>
<td>83.3 ± 14.7</td>
<td>76.3 ± 9.8</td>
</tr>
</tbody>
</table>

*Statistically significant difference compared to group A (\(P < .05\)).

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**Statistical Analyses**

Statistical analyses were performed using SPSS 12.0 for Windows (SPSS). Kruskal-Wallis test was used to assess differences in BIC and BV; \(P < .05\) was considered significant. To avoid accumulation of errors from multiple comparisons, Mann-Whitney test with Bonferroni correction was performed.
the lower zone at 4 weeks. Surface rejuvenation with acid etching may help increase the success rate of immediate loading in patients by improving osseointegration between cancellous bone and implant. A previous study reported that implants treated for surface rejuvenation before placement showed no stability dip, regardless of the degree of primary stability.16

The BIC of group B was not generally higher than that of group C, suggesting that even if the period of implant aging is shorter; BIC and BV can be lower depending on factors such as implant thread design, surface treatment, and condition of host. Dental implant thread geometry was reported to affect BIC in an in vivo study using the tibiae of rabbits.17 At the cellular level, 2 weeks of implant aging might suffice to influence osteoblast cell density, alkaline phosphatase activity, and calcium deposition, whereas this time frame might not have a profound impact on BIC and BV in large animals such as the dog.

All groups showed a high percentage of BIC in the upper zone of the implant due to the good quality of cortical bone in the dog mandibles. The reason for the reduction in BIC on the lower zone of the implant may be that the canine mandible is composed of very large marrow space (Figs 3 and 4). Albrektsson and Johansson18 hypothesized that approximately 50% BIC is necessary for a successful prosthetic result. All groups in this study satisfied this requirement, suggesting that host bone quality (bone density and amount of cortical bone) plays an important role in limiting the effect of implant aging.

Group A did not show any significant differences from groups B and C except in healing in the lower zone of the implant at 4 weeks. This indicates that commercially used 6-month-old implants have no clinical problems, although the implant surface undergoes changes such as loss of hydrophilicity due to implant aging. Despite the lack of explicit evidence, it is widely assumed that most implants on the market in South Korea have a 5-year shelf life. Few studies have found that the standardized period reduces osseointegration due to biologic aging. Further research on the shelf life of implants is required. As a recent advance, implants are embedded in liquid such as calcium solution and stored in sterilized containers. The liquid storage seems to prevent hydrocarbon contamination and surface deterioration, eventually promoting osteogenesis.

Surface rejuvenation with acid-etching was effective in slightly increasing BIC. However, it seems less effective compared to methods used for surface rejuvenation in previous in vivo studies (surface rejuvenation using UV).15,16,19 This might be ascribed to mechanisms such as hydrocarbon removal, protein absorption, proliferation of osteogenic cells, and osteoblast differentiation. The exact mechanism of surface rejuvenation has not been elucidated and merits further investigation.

### Conclusions

This study, although based on a limited number of samples, indicates that surface rejuvenation with acid etching offsets the biologic aging of implant-enhanced BIC in the lower zone of the implant at 4 weeks. This result suggests that a newly prepared implant might be more effective in successful loading before the stability dip than a biologically aged implant due to a slight improvement in osseointegration in the bone marrow zone and a period of stability dip. In the case of an adequate healing period (> 12 weeks), implant aging did not affect BIC and BV in large animals such as the dog. However, further study is required to determine the standard period of biologic aging of the implant in terms of desirable clinical effects. The mechanisms of biologic aging of implant and of surface rejuvenation also await elucidation.

### Table 2 Comparison of Bone Volume (BV) Between Groups

<table>
<thead>
<tr>
<th>Area</th>
<th>Healing time</th>
<th>Group A Mean (%) ± SD</th>
<th>Group B Mean (%) ± SD</th>
<th>Group C Mean (%) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper zone of</td>
<td>4 wk</td>
<td>80.5 ± 8.3</td>
<td>72.1 ± 17.2</td>
<td>81.5 ± 14.7</td>
</tr>
<tr>
<td>implant</td>
<td>12 wk</td>
<td>66.5 ± 12.6</td>
<td>72.1 ± 15.9</td>
<td>69.8 ± 8.4</td>
</tr>
<tr>
<td>Lower zone of</td>
<td>4 wk</td>
<td>32.2 ± 20.9</td>
<td>33.0 ± 22.1</td>
<td>53.2 ± 23.5</td>
</tr>
<tr>
<td>implant</td>
<td>12 wk</td>
<td>43.4 ± 14.7</td>
<td>42.0 ± 21.4</td>
<td>37.5 ± 20.6</td>
</tr>
</tbody>
</table>

*Statistically significant difference compared to group A (P < .05).
Acknowledgments

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References