Chapter 5

Peri-implantitis induced by stainless steel ligature in beagle dog

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International journal of periodontics and restorative dentistry.

2017, 37(3): e170-e179
ABSTRACT

The purpose of this study is to develop a new method for a peri-implantitis model in beagle dogs in which a stainless steel ligature (SSL) was used independently. Thirty six Staumann dental implants were placed in 6 beagle dogs 1 month after all mandibular premolars were extracted. Three month later, SSLs were placed in a submarginal position of implants to induce peri-implantitis and were not replaced during the 12-week tissue breakdown period. Inducing peri-implantitis in the Beagles with an SSL is a rapid, effective and simple method.

KEY WORDS: dental implant, dog, peri-implantitis, stainless steel ligature
INTRODUCTION

Peri-implantitis has been defined as “an inflammatory process around an implant, characterized by inflammation of the soft tissue and a loss of the supporting bone”. Peri-implantitis was found in 28% to 56% of subjects and in 12% to 43% of the implants. It remains one of the major causes of a loose or lost dental implant and it accounts for 5% to 11% of dental implant failure.

Animal models have been used extensively to study the pathology and treatment of peri-implantitis. One of the animal models most commonly used is the Beagle dog. In the past 20 years, various ligature materials, such as cotton, silk, plastics and nylon, have been used to cause peri-implantitis in dogs. A cotton ligature is the material most commonly used to induce bone loss and peri-implantitis in dogs. The cotton ligature is placed submarginally around the implant which results in a progressive inflammation and breakdown of the soft and hard tissues of the peri-implant. Although it succeeded in inducing bone loss around the implants in 6 to 48 weeks, the use of a cotton ligature has some drawbacks: 1) the cotton ligature is soft and so it is difficult to place it precisely at the bone-implant contact; 2) it needs replacement to maintain the stimulation, which means more frequent general anesthesia, an operation with accompanying risks including the death of the animal; 3) the ligation period needed for inducing peri-implantitis varied greatly from 6 to 48 weeks. Therefore, the efficiency of a cotton ligature is hard to predict.

A stainless steel ligature (SSL) has been used in orthodontic treatment for a long time due to its good flexibility and a certain degree of hardness. It is easy to handle and it can be placed precisely at bone-implant contact. This creates a space where bacteria for the local infections can accumulate. An SSL twisted with a silk ligature was used to induce periodontitis in beagle dogs which did not need replacement. A surgical steel ligature wrapped with cotton thread to induce periodontitis in mongrel dogs has also been reported. Although SSL has been used together with gauze to induce peri-implantitis in mongrel dogs, no bone loss data was provided and the model is not discussed.
The aim of the study was to develop a peri-implantitis beagle dog model by using an SSL without the need for replacement. To adequately demonstrate the characters of this new method, we measured peri-implant probing depth, the width, depth and area of bone defect on X-ray films 3 weeks and 12 weeks after ligation. EDX analysis was also used to exclude the possible influence from released metal ions during the peri-implant tissue breakdown period.

MATERIALS AND METHODS

Surgical procedures

The outline of the experiment was presented in Fig.1. Six two year old male beagle dogs weighing between 13 and 14 Kg were used in this experiment. The animal experiment was approved by the Ethics Committee of Zhejiang Chinese Medical University, China. The dogs were housed individually and maintained on a commercial diet and water ad libitum. All surgery was performed under general anaesthesia using intravenous pentobarbital sodium (25mg/kg) with the addition of Penicillium (5×10^4 U/kg) and atropine (0.03mg/kg) 30 minutes before surgery. Local anaesthesia (1% lidocaine with 1: 100000 adrenaline) and skin disinfection (0.5% iodophor solution) were used at the implantation sites. All mandibular premolars were extracted to establish recipient sites for implants.

After healing for 4 weeks a full thickness flap was elevated and three Straumann® implants (SØ 3.3mm RN, SLA® 8mm, Straumann AG, Basel, Switzerland) were inserted into the edentulous area on each side. Healing abutments were placed on all implants and surrounding gingiva tissue was secured and sutured to allow the implant sites to heal and for osseointegration to occur (Figure 2).
Figure 1. The outline of the experiment. SSLs were placed around the neck of implants at week 0 (+Lig) and removed at week 12 (-Lig).

Figure 2. Clinical photographs for implant installation. Small-diameter holes (pilot hole) were drilled at extraction sites in order to guide the titanium screw (A). The pilot hole was slowly widened to allow placement of the implant screw (B). Once the implant is in place, a protective healing abutment was placed on top (C). Surrounding gingiva tissue was secured and sutured to allow the site to heal and osseointegration to occur (D).
Stainless steel ligature induced peri-implantitis

A plaque control program was initiated in which the teeth and implants were cleaned twice a week with a toothbrush. Twelve weeks after implantation, a clinical and radiological examination indicated that all the implants were successfully osteointegrated. Stainless Steel Ligatures (SSL, 0.010 inch, 270-0010, Ormco corporation, US) were placed and forced as deeply as possible into the apical position of the margin of the peri-implant mucosa. We wrapped the SSL 6 turns around each implant (Figure 3A). The excess part of each ligature was snipped and the remaining end was pressed as close as possible to the surface of the implant to avoid irritating the gingiva directly (Figure 3B). After the ligatures were secured, the dogs were fed a soft diet. SSLs were not replaced during the breakdown period. At week 12 when about half the initial bone around the implant was lost, the SSLs were removed. The treatment of peri-implantitis at week 16 will be reported separately.

Clinical measurements

The peri-implant probing depth was recorded immediately before placing the SSL (Baseline), immediately after removing the SSL at week 12 and four weeks after the removal of SSL at week 16 (Figure 1), which is the end of the period for inducing the peri-implantitis. The periodontal examination and scaling was carried out by an experienced dentist using a periodontal probe (PCP 8, Hu-Friedy Co., Chicago, Illinois, USA), with a constant probing force of 0.2N. The data were recorded at mesial, distal, buccal and lingual aspects of each implant at baseline, week 12 and 16 (Table 1).
Figure 3. Clinical photographs for the procedure of SSL-induced peri-implantitis. At baseline, SSL were placed in a submarginal position around the neck of the implants to allow sub-marginal plaque formation (A).

The excess SSL were snipped and the remaining part was well adjusted to avoid irritating gingiva directly (B). Obvious plaque accumulation, suppuration from the open pockets, gingival recession and bleeding on probing could be found at week 12 (immediately before ligature removal) (C). Clinical view of the crater-like peri-implantitis bone defect after flap elevation at week 16 (D).

Radiological measurements

Standardized periapical radiographs taken with a digital image system (Kodak 2100, Intraoral X-ray System, Carestream Health, Inc., Croissy-Beaubourg, France) were used to measure the bone loss around the implants at week 3 and week 12 (Figure 1). A film holder system was connected with a radiographic tube to standardize the position of the sensor, the implant and the x-ray source. The bone defect depth was calculated by subtracting the distance of the bone to implant contact from 8 mm,
which is the length that the implants were inserted into the bone in our study (Figure 4). The magnification of the radio image on length was corrected by dividing 8mm into the length of implant on the image. The mesial and distal values were averaged to give a mean for the analysis. The width of the bone defect on the radiographs was calculated by subtracting 3.3mm, the diameter of the implant, from the linear distance between the two peaks of saucer shaped bone defect (Figure 4). The magnification of the radio image was also corrected by dividing 3.3mm into the width of the implant on the image. The bone defect area was calculated by the point counting method described by Aydin et al. All measurements were repeated three times and the mean values were used for analysis. Furthermore, the average bone defect depth per week (ABDD) was calculated and was compared with the data that was previously reported in several studies using the conventional cotton ligature to induce peri-implantitis in beagle dogs.

Table 1. Mean ± SD of probing depth (mm) at four sites of each implant at three time points.

<table>
<thead>
<tr>
<th>Time</th>
<th>Mesial Mean (SD)</th>
<th>Distal Mean (SD)</th>
<th>Buccal Mean (SD)</th>
<th>Lingual Mean (SD)</th>
<th>Average of four sides Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (+Lig)</td>
<td>1.6 (0.7)*</td>
<td>1.9 (0.3)</td>
<td>2.0 (0.6)</td>
<td>1.8 (0.5)</td>
<td>1.8 (0.4)+</td>
</tr>
<tr>
<td>Week 12 (-Lig)</td>
<td>4.8 (1.2)♀</td>
<td>5.1 (1.5)</td>
<td>3.3 (1.0)♀</td>
<td>5.5 (1.4)</td>
<td>4.7 (1.1)</td>
</tr>
<tr>
<td>Week 16</td>
<td>3.9 (1.1)</td>
<td>4.4 (1.0)</td>
<td>3.7 (1.0)♀</td>
<td>4.0 (1.2)♀</td>
<td>4.0 (0.9)∗</td>
</tr>
</tbody>
</table>

* Significant lower (p<0.01) than Distal and Buccal sides. ♛ Significant lower (p<0.05) than Distal side. ♛ Significant lower (p<0.05) than Lingual sides. ♛ Significant lower (p<0.05) than Lingual side. ♛ Significant lower (p<0.01) than Mesial, Distal and Buccal sides. ¥ Significant lower (p<0.01) than Distal side. ¥ Significant lower (p<0.05) than Lingual side. ¥ Significant lower (p<0.05) than Distal side. ¥ Significant lower (p<0.01) than Week 12 and Week 16.
Figure 4. Schematic of evaluation of bone defect depth, width and area in standardized radiographs at week 3 and week 12. The value of bone defect depth was calculated by subtracting the distance of the bone to implant contact from 8 mm and the mesial and distal values were averaged to give a mean for the analysis. The width of the bone defect on the radiographs was calculated by subtracting 3.3 mm, the diameter of the implant, from the linear distance between the two peaks of saucer shaped bone defect. The bone defect area was calculated by the point counting method.

Energy Dispersive X-Ray (EDX) spectroscopy analysis for the possible metal elements released from SSL

In order to check for the possible erosion of an SSL, the bone tissue surrounding the implants was collected to measure the possible metal element released from the SSL by Electron Microscopy Energy Dispersive X-ray spectrometry (SEM-EDAX) (model XL20, FEI Company, Netherlands). Three samples of the bone to implant
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junction were randomly selected, in each of which, 10 points were randomly selected for the measurement.

Statistical analysis

The data of the peri-implant probing depth and bone defect were statistically evaluated with SPSS software (version 18 for windows, SPSS Inc., Chicago, IL, USA). All data are presented as means and standard deviations (SD). Repeated measures ANOVA were used for comparison of the probing depth at four sites of the implants with the means of overall probing depth at three times, namely baseline, week 12 and week 16. The paired t-test was used to compare the mean values of the morphometric measurements in x-ray films obtained at week 3 and week 12. All the significance levels were set at p<0.05.

RESULTS

All implants were successfully osteointegrated 3 months after insertion. At week 3, most of the implants showed obvious chronic inflammation of the soft and hard tissue and no implants were loose or lost. At the end of the SSL induced tissue breakdown period at week 12 one implant in Dog 1 was loose and two additional implants in Dogs 1 and 6 were exfoliated because of extensive tissue destruction and bone loss around implant. These three implants were extracted when they were found to be invalid for our study.

Clinical measurements

Large amounts of plaque were found in the sites of implantation at week 3. Marked signs of chronic inflammation of soft tissue, e.g., bleeding on probing, suppuration from open pockets and obvious gingival swelling or recession was observed. The means of the probing depth of mesial, distal, buccal and lingual side of each implant at three times are presented in table 1. Repeated measures ANOVA were used to compare the mean probing depth of the 4 sides, at each time. The results showed
that for the baseline, there were significant differences between the 4 sides, F (1,721, 48.198)=11.577, p<0.01 because the mesial side had a lower probing depth than that of the other 3 sites. At week 12, significant differences were found between the 4 sides, F (3.84)=42.047, p<0.01, because the buccal side had a lower probing depth than the other 3 sites. At week 16, there were significant differences between the mesial, distal and buccal side, F (3,69)=7.469, p<0.01, because the buccal side had a lower probing depth than the other 2 sites. A significant difference in the overall probing depth was found between the three times, F (2,46)=75.044, p<0.01, because the probing depth at the baseline was lower than at week 12 and week 16 (Table 1). The data showed a non-normal distribution and so the comparison of the probing depth at four sites of each implant taken at the three times was repeated using the Friedman test and the Wilcoxon signed-rank test. The results were the same as those using repeated measures ANOVA.

**Radiographic measurements – bone loss around dental implants**

Considerable amounts of bone loss were found during the 12 weeks period of SSL ligation and the typical saucer shaped bone defect was observed at week 3 and week 12 (Figure 5). The paired t-test was used to compare the mean values of morphometric measurements in x-ray films obtained at week 3 and week 12. The results given in Table 2 and Fig.6 show that there was a significant difference for the depth, width and area of bone defect between the 2 times. The T values and degrees of freedom are given in Table 2.

**EDX analysis results**

EDX analysis showed peaks associated with calcium and phosphorus (Figure 7), which are the major inorganic constituents of bone tissue. No metal particles were found except sodium.
Figure 5. Radiographs obtained at week 3 (A) and week 12 (B). Saucer-shaped bone defect around the implant can be found at week 3. At week 12, more obvious bone loss was observed.

Table 2. Average of morphometric measurements in x-ray film at two time points (mean ± SD).

<table>
<thead>
<tr>
<th>Bone defect</th>
<th>BL-Week 3</th>
<th>Week 3-Week 12</th>
<th>BL-Week 12</th>
<th>Value</th>
<th>T (df) §</th>
<th>PCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (mm)</td>
<td>1.6 (0.6)</td>
<td>2.4 (1.2)</td>
<td>4.0 (0.8) *</td>
<td>40%</td>
<td>T (33)=10.334</td>
<td>100%</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>4.9 (1.2)</td>
<td>1.9 (1.3)</td>
<td>6.8 (0.8) *</td>
<td>72%</td>
<td>T (33)=7.908</td>
<td>100%</td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>6.5 (2.0)</td>
<td>9.3 (4.6)</td>
<td>15.8 (4.8) *</td>
<td>41%</td>
<td>T (33)=9.747</td>
<td>100%</td>
</tr>
</tbody>
</table>

BL: Baseline. PCT: Percentage of the values of morphometric measurement parameters at different breakdown period divided by the values at week 12.

§: T represents t value and df represents degrees of freedom in the pared t test.

*Statistically significant bigger (p<0.01) than week 3 group.
Figure 6. Average of morphometric measurements in x-ray film at two time points (mean ± SD). *Statistically significant difference between week 3 and week 12 (p<0.01).

Figure 7. Energy Dispersive X-Ray spectroscopy analysis of the chemical content in the samples. No metal particles released from SSL were found.
DISCUSSION

Materials used to induce peri-implantitis in the dog model

The beagle dog model is well known in the study of peri-implantitis and has been widely used for diagnosis, treatment and prognosis. Many materials, such as a cotton ligature, a silk ligature and rubber elastics have been used to induce inflammation\textsuperscript{19}. A cotton ligature is the one most extensively used. On the contrary, there hasn't been any report on peri-implantitis dog model induced by SSL independently. In order to make a comparison between SSL method and cotton ligature method, six articles (Table 3) were chosen by a literature review in the PubMed database from 1990 to 2014 by the following criteria:. (1) Search terms and key words included: “peri-implantitis” OR “periimplantitis” AND “dental implants” AND “animal study” OR “dogs” AND “ligature”; (2) Publication in the international peer reviewed literature; (3) English language. (4) Beagle dog model; (5) Cotton ligature induced peri-implantitis; (6) The bone defect depth on x-ray film was provided. Some of parameters related to the breakdown of the hard tissue, such as the ligation period, interval of ligature replacement (ILR), the times of ligature replacement (TLR) and the bone defect depth (BDD) were listed or could be deduced. The average bone defect depth per week (ABDD) was calculated for comparison. These parameters were compared with our study (Table 3). In studies associated with cotton ligature, the ILR ranged from 2 to 4 weeks and an average of 3 weeks. The TLR ranged from 1 to 4 with an average of 3. In contrast, the value of ILR and TLR was zero in our study because we did not replace the SSL. The BDD of 4mm induced by an SSL at week 12 in our study was significantly larger than in the articles reviewed. The value of ABDD of the SSL both at week 3 (0.54mm) and week 12 (0.33mm) is significantly larger than for a cotton ligature in the studies in the literature review except the one made by Lindhe (0.53mm). The T values and degrees of freedom are given in table 3. Significant variations were found in ligation time, ILR, TLR and ABDD during the tissue breakdown in the articles reviewed (Table 3). The largest value of ABDD of 0.53mm\textsuperscript{3} is three times greater than the smallest of 0.16mm\textsuperscript{8}, let alone the large variation of TLR from 1\textsuperscript{3} to 4\textsuperscript{17}. These data show that the repeatability and stability of the results of peri-implantitis in beagles.
induced with a cotton ligature should be treated with caution. After 12 weeks the SSL could induce a BDD of 4mm which is significantly larger than in all other studies given in Table 3. The ABDD of the SSL was larger except for the study done by Lindhe et al\(^3\). These results show that an SSL is more efficient in inducing peri-implantitis. Most importantly, replacing the SSL was unnecessary. This saves manpower and resources and reduces the risks from general anaesthesia.

**Table 3.** Results of radiograph measurements from selecting literatures and our study. Mean values (n=6) are represented together with the standard error of the mean. In the group comparisons (post-hoc, LSD-test in ANOVA), significant differences (p<0.05) are denoted with an asterisk.

<table>
<thead>
<tr>
<th>Material</th>
<th>References</th>
<th>Ligation period (week)</th>
<th>ILR (week)</th>
<th>TLR</th>
<th>BDD (mm)</th>
<th>ABDD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J.Lindhe (1992)(^4)</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>3.20*</td>
<td>0.53*</td>
</tr>
<tr>
<td></td>
<td>Persson (1999)(^5)</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>2.12*</td>
<td>0.17*</td>
</tr>
<tr>
<td></td>
<td>Persson (2001)(^5)</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>3.20*</td>
<td>0.27(^fi)</td>
</tr>
<tr>
<td></td>
<td>Sennerby (2005)(^6)</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>3.10*</td>
<td>0.26*</td>
</tr>
<tr>
<td></td>
<td>Schwarz (2006)(^4)</td>
<td>12</td>
<td>3</td>
<td>4</td>
<td>3.10*</td>
<td>0.26*</td>
</tr>
<tr>
<td></td>
<td>Berglundh (2007)(^7)</td>
<td>16</td>
<td>2</td>
<td>3</td>
<td>2.51*</td>
<td>0.16*</td>
</tr>
<tr>
<td>Mean</td>
<td>Present study</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.60</td>
<td>0.54</td>
</tr>
<tr>
<td>SSL</td>
<td></td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>4.00</td>
<td>0.33</td>
</tr>
</tbody>
</table>

ILR: Interval of ligature replacement;
TLR: times of ligature replacement;
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BDD: bone defect depth;
ABDD: average bone defect depth per week

(Rounding rules were applied to calculate the ligation period, ILR and TLR).

† T represents t value and df represents degrees of freedom in the one-sample t test.

‡ The value of BDD was measured at week 10, 4 weeks after ligature removed (Titanium fixtures, Branemark System, Nobel Biocare AB, Goteborg, Sweden).

§ The value of BDD was simply calculated from the available data in the article. The mean value of BDD was 1.77mm in the left side and 2.12mm in the right side 1 month after ligature removal (3 months for tissue breakdown and 1 month for spontaneous progression). We chose the larger value for comparison (Titanium fixtures, Branemark System, Nobel Biocare AB, Goteborg, Sweden).

# The value of BDD was directly provided in the article (ITI dental implants with SLA surface).

& The value of BDD was simply calculated from the available data in the article. There were 6 groups in the experiment, but no overall mean value for all the BDD. We chose the largest value of BDD from 6 groups (The type of implants used in the experiment was not mentioned).

* Significant difference (p<0.01) between the selecting articles and our study at week 12 in the BDD or ABDD.

¶ Significant difference (p<0.05) between the selecting articles and that of our study at week 12 in the ABDD.
We compared our data with that of other experiments instead of setting up a control group in which peri-implantitis was induced by a cotton ligature. The reason for that is that the size of cotton ligature and the precise protocol for placing it at the right position was not mentioned in the articles selected in table 3. We were not confident that we could repeat the method as good as it was. In fact, a large variation of ligation period, from 6 weeks to 16 weeks, TLR from1 to 4, and ABDD, from 0.17mm to 0.53mm, can be found in the 6 selected articles\textsuperscript{3, 8, 15-18} and an even bigger variation of these parameters can be found in other articles not included in our study\textsuperscript{9, 20}, which suggests caution in repeating the use of a cotton ligature. So we just compared our data of the SSL with that of cotton ligature directly.

\textbf{Characters of SSL-induced peri-implantitis}

The greatest probing depth occurred immediately before removing the ligature week 12(Table 1). It had decreased 4 weeks after the removal of ligature, that is at week 16. This finding is in agreement with the study of peri-implantitis induced by a ligature in Macaca mulatta monkeys done by Hanisch et al\textsuperscript{20}, in which the mean probing depths of implants in the maxilla, mandible and overall decreased significantly one month after removing the ligature. This is also similar to the peri-implantitis induced by a ligature in mongrel dogs\textsuperscript{21}. However, they did not suggest a reason for the result. In our view, it may have been because the swelling gingiva recovered partially after the SSL was removed and the height of the gingival papilla decreased. This would reduce the reading on the gauge of the periodontal probe.

The mean value of the depth, width and area of the bone defect in the peri-implantitis induced by an SSL increased significantly from week 3 to week 12. A considerable part of the bone destruction in depth (40\%) and area (41\%) was formed from baseline to week 3 (Table 2). An even bigger part of the bone defect width (72\%) was formed from baseline to week 3. These results show that the bone destruction rate is highest from baseline to week 3 and then decreases from week 3 to week 12. This is demonstrated by the different slopes of the lines in Fig.6.
The formation rate of the bone defect depth and width was steady from baseline to 60 days in a study of the bone defect depth and width in peri-implantitis mongrel dogs\textsuperscript{21}. A possible reason is that the bone destruction was more even because the cotton ligature was replaced every 20 days. But the mean width in their experiment after 20 days ligation was 2.18 mm and this is much less than the average bone defect width of 4.9 mm at week 3, 21 days after ligation, in our experiment.

Similarly, Hanisch et al found an average bone defect width of only 2.0 mm 12 months after the removal of cotton ligatures in monkeys\textsuperscript{20}. An explanation may be that more of an SSL can be placed round the neck of the implants than of a cotton ligature. This gives more mechanical stimulation and more space for bacterial accumulation, especially in the horizontal direction. More horizontal bone defect than vertical bone defect could therefore be created in the early stages.

From the slopes of the lines in Fig. 6, it is able to speculate that the depth of the bone defect could be about 2.4 mm after 6 weeks. This depth is comparable with some previous studies in Table 3\textsuperscript{7, 8, 15}. This suggests that if a critical bone defect depth in the peri-implantitis dog model is not necessary, six weeks’ ligation is enough using an SSL.

**Mechanism of ligature induced peri-implantitis**

A cotton ligature promotes a minor local trauma at the bone to implant contact point and allows the formation of a subgingival biofilm. This causes an acute inflammatory reaction and early tissue breakdown and bone loss. One potential problem is that thread type ligatures might become loose, suffer wear and even get lost during the process and the ligature may fail to induce bone loss around the implants\textsuperscript{3, 22}. Similarly, an increasing distance between the bottom of the bone defect and the ligature weakens the stimulus from the ligature. The bone loss cannot then continue until a new ligature is pushed into the bone to implant contact\textsuperscript{22} and this must be repeated regularly and requires frequent general anesthesia. Furthermore, the induction site may be affected by the additional manipulations introducing extra variables in the experiment possibly because the
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tensile strength of the cotton ligature around the neck of the implant decreases and then the relative security of the knot is also poor. In contrast, as SSL has a certain degree of hardness, they separate the gingiva from the protective cover screw, making it easier to place more SSL at the bone to implant contact. This makes it stable on the surface of the implant at the first ligation. This may give more mechanically injurious effects and more space for bacteria to accumulate. This would be highly conducive to prompting a bone defect around the implant. Due to the tensile strength and stability of the SSL, the tension of the ligation in our study was well maintained 12 weeks after ligature was placed, so there was no need at all to replace it.

Possible metal particles release from the SSL

Stainless steel has been frequently used in internal fixation and clinical orthodontic treatment because of its low cost, mechanical strength and its characteristics of easily bending and shaping. However, the use of stainless steel has some drawbacks such as surface corrosion and high rate of locally and systemically released corrosion products\(^23\). Some in vitro studies indicated that corrosion products of stainless steel had a toxic influence on osteoblasts and their proliferation and differentiation\(^24\). The release of elements from the alloy also results in some adverse biological effects such as toxicity, allergy, mutagenicity, and carcinogenicity\(^25\). We are concerned with the effect of metal ions released from the SSL in our experiment. An EDX analysis was performed in our experiment to detect any metal ions which had possibly been released from the SSL. Previous studies indicated that the oral environment is dynamic, where factors like the variation of pH, temperature, salivary conditions, mechanical load, microbiological and enzymatic activity all have effects on the rate of corrosion of metals\(^26-28\). In our study, SSL was placed in a pocket that formed between an implant and its surrounding bone defect walls. The ligation position of the SSL was relatively "quiet" as it was separated from open oral environment and it was not affected by any mechanical loading. The ligation time of the SSL is shorter in comparison with other experiments\(^26-29\). These factors probably account for the absence of metal ions released in our experiment.
We can cautiously exclude the possible effect of releasing metal ions on the breakdown of the soft and hard tissue in peri-implantitis in our experiment. The peri-implantitis should result from the bacterial accumulation on the SSL around the neck of the implant during the period of breakdown.

CONCLUSIONS

Within the limitation of the current study, we can conclude that SSL method is more rapid, more effective and less complicated than cotton ligature method for inducing peri-implantitis in the Beagles.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Arjen van Wijk for his assistance on statistic guide and Prof. Dr. Tony Hearn as a native English speaker for editing.
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