

## Original Article

# A modified method for inducing periodontitis in dogs using a silk-wire twisted ligature

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This study was designed to assess the effectiveness of a modified silk ligature twisted with wire for inducing advanced periodontitis. Periodontitis was induced in five premolars and one molar of 20 healthy dogs over a 60-day period. The dogs were divided into four groups according to the ligature-inducing materials used: soft moistened food only, wire ligature (WL), silk ligature (SL) and twisted ligature with silk and wire (SWL). Periodontal indices were recorded, and dental radiographs were taken before and after 60 days of ligation. The ligatures were checked daily and the day the ligature fell out was noted. The period during which the ligatures were maintained was significantly shorter for the SL group compared to the SWL group ( $p < 0.05$ ). Results of the clinical examination showed that almost all periodontal status parameters including the plaque index, gingival index, clinical attachment level, and bleeding on probing were significantly exacerbated in the SWL group compared to the other groups ( $p < 0.05$ ). Radiographic evaluation demonstrated that alveolar bone levels were significantly lower in the SWL group than the other groups on day 60 ( $p < 0.05$ ). These results suggested that experimental periodontitis induced by SWL could be an effective method for investigating periodontitis in canine models.

**Keywords:** dog, experimental model, periodontitis

## Introduction

Periodontal inflammatory disease is one of the most prevalent disorders affecting both humans and small animals [1,7]. Therefore, several studies have examined the occurrence and treatment of periodontal disease using models of experimentally induced periodontitis. This condition has been induced in rats using a range of methods including pathogen intake, endotoxin injection, high

carbohydrate feeding, or ligature placement around the tooth cervix [12,13,20]. Compared to rat models, ligature induction of periodontitis is used more frequently in dogs [3,17,21].

Ligation materials used to induce periodontitis including silk, cotton, and nylon are placed between the tooth and periodontium to mechanically widen the periodontal pocket and to facilitate plaque accumulation in the dento-gingival region [5]. Several studies have induced experimental periodontitis using a more invasive method in which a full-thickness gingival flap is made and the alveolar bone is destroyed prior to ligation [3,18]. These methods take several months to promote advanced periodontitis, and thread-type ligatures might be lost due to loosening or wearing during this period. In general, ligatures that are lost during the periodontitis induction phase are immediately replaced to maintain the same induction period and environment [3,13]. These reparative procedures require redundant general anesthesia. Furthermore, the induction site can be affected by additional manipulations that may act as variables in the experimental procedure. Therefore, the present study was designed to identify more effective methods for periodontitis induction using modified ligatures, which can promote cases of more advanced periodontitis using a less invasive approach and reduce unnecessary interventions compared to previously established techniques.

## Materials and Methods

The protocol used for this study was approved by the Institutional Animal Care and Use Committee (SNU-100208-3; Seoul National University, Korea). Twenty beagle dogs (13 females and 7 males) approximately 1.5 years old were used in this study. All experimental operations and examinations were performed with dogs

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under general anesthesia induced by a combination of medetomidine (0.01 mg/kg; Orion Pharma, Finland), tramadol (2 mg/kg; Samsung Pharm, Korea), and a commercial solution of zolazepam and tiletamine (2.5 mg/kg; Virbac, France) administered *via* intramuscular injection. To prepare the healthy gingiva, all teeth were scaled and polished using a piezo-type ultrasonic scaler (BonArt, USA), and tooth brushing was performed once daily without anesthesia for the following 2 weeks. During these periods of prophylactic care, the beagles were fed a hard pellet diet to reduce plaque formation [11].

### Experimentally induced periodontitis

Two weeks after scaling, experimental periodontitis was induced on the left upper second premolar (PM2), third premolar (PM3), and fourth premolar (PM4) as well as the left lower PM3, PM4, and first molar (M1). Prior to ligation, the gingival attachment was incised slightly by inserting a number 11 scalpel blade (Ailee, Korea), and the periodontal ligaments were undermined until a periodontal pocket depth reached up to 3 mm with a straight elevator (Osung MND, Korea). After undermining, shallow notches for ligature retention were made in the mesial and distal cervical region of each tooth with a round bur (#1; Komet, Germany). An ancillary notch was placed in the mesial surface of the upper PM4.

The dogs were randomly assigned to four groups. Periodontitis was induced in three groups (Fig. 1) by tying the ligatures around the cervical region of the tooth using dental ligature wire [wire ligature (WL),  $n = 5$ ; ClassOne Orthodontics, USA], 2-0 braided silk [silk ligature (SL),  $n = 5$ ; Ailee, Korea] or a twisted wire with 2-0 silk [silk-wire twisted ligature (SWL),  $n = 5$ ]. The remaining group did not undergo any surgical intervention but was fed only soft-moistened food as a control (SF,  $n = 5$ ). For pain control, tramadol (4 mg/kg, intramuscular injection) was

administered twice a day for 3 days after placing the ligatures. To promote plaque formation, soft-moistened food was given to all groups for the following 60 days. The ligatures were checked daily and the day the ligature fell out was recorded.

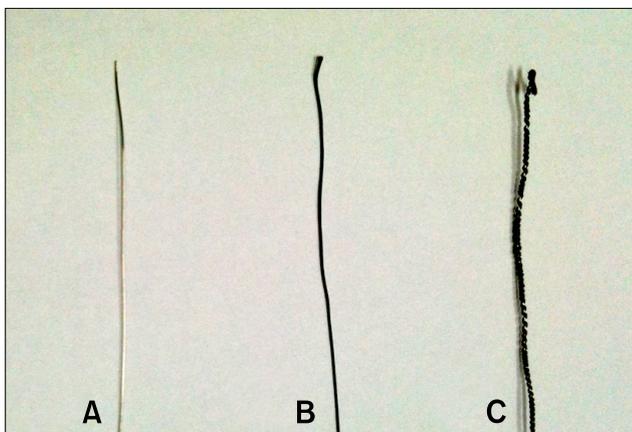
### Periodontal status evaluation

Before (day 0) and 60 days after (day 60) ligature placement, the clinical periodontal parameters were recorded and digital dental radiographs were taken to evaluate the periodontal status. Clinical parameters included the plaque index (PI), gingival index (GI), periodontal pocket depth (PPD), clinical attachment level (CAL), and bleeding on probing (BoP) [10,16,19]. All measurements were taken at three sites per tooth. If a ligature fell out, the parameters were measured on that day. All measurements were taken by one experienced clinician (S.E. Kim, Seoul National University, Korea) using a Williams periodontal probe (Osung MND, Korea).

Digital intraoral radiographs (AFP Imaging, USA) were obtained to evaluate the amount of alveolar bone loss after ligature application. An intraoral size 2 sensor (AFP Imaging, USA) was positioned to take dental radiographs using bisecting and parallel techniques for the maxillary and mandibular teeth, respectively, with the same exposure protocol. The alveolar bone level (ABL) was measured using Adobe Systems (USA) software. This measurement was performed at the mesial and distal margin of each tooth except for the upper PM4. ABL of upper PM4 was only measured at the medial margin of the tooth because the ABL at the distal margin was difficult to distinguish from other superimposed dental structures such as the upper M1. Distances from the alveolar bone margin and cemento-enamel junction to the root apex were measured and the ABL was calculated as a ratio of these two lengths.

### Statistical analysis

All data was analyzed using SPSS software (ver. 12.0; SPSS, USA). The maintenance period and clinical parameters for each group are expressed as the mean  $\pm$  SD. Differences in the maintenance periods between the SWL and WL or SL groups were assessed using Student's *t*-test. The clinical parameters measured on day 0 and day 60 in the same group were analyzed using a paired *t*-test to compare the progression of periodontitis in the same tooth within each group. A one-way analysis of variance was used to perform an intergroup comparison of the clinical periodontal parameters and ABL changes on days 0 and 60 with Tukey's method as a *post hoc* test. Data within the 95% confidence level were considered significant.



**Fig. 1.** Ligature materials. (A) 0.012-inch ligature wire, (B) 2-0 braided silk, (C) twisted ligature with silk and wire.

## Results

### Ligature maintenance periods

Table 1 lists the maintenance periods of each ligature and the maintaining ratio according to ligature material. The SF group was excluded from this analysis because the teeth in these animals were not tied with any material. Among the 30 teeth in each group, 26 and 25 ligatures were maintained for 60 days in the WL and SL groups, respectively. All ligatures were retained by the SWL group during the entire experimental period. Among the teeth that did not retain the ligatures, most were upper PM4 ( $n = 6$ , 66.6%). The mean ligature maintenance period was significantly shorter for the SL group than the SWL group ( $p = 0.046$ ). Although the ligature maintenance period for the WL group was also short compared to the SWL group, this difference was not significant ( $p = 0.051$ ).

**Table 1.** Ligature maintenance periods and the ratio of ligature loss in each group

Group	Ratio of ligature loss (n / N)	Ligature maintaining period	
		Day*	<i>p</i> -value <sup>†</sup>
WL	4 / 30	54.7 ± 14.6	0.051
SL	5 / 30	53.2 ± 16.2	0.046
SWL	0 / 30	60.0 ± 0.0	1.000

\*Data are expressed as the mean ± SD. <sup>†</sup>Statistical significance relative to SWL group. WL: wire ligature, SL: silk ligature, SWL: silk-wire twisted ligature, n: the number of ligatures that fell out for the group, N: the number of teeth with ligatures in the group.

### Periodontal status

The mean and standard deviation values for the clinical periodontal parameters on days 0 and 60 are listed in Table 2. Baselines for the clinical parameters of periodontal status and ABL among the study groups were not significantly different on day 0. After the 60-day period of periodontitis induction, significant increases in all periodontal parameters were observed for the WL, SL, and SWL groups compared to day 0. On the other hand, only the PI, GI, and PPD increased significantly in the SF group ( $p < 0.05$ ).

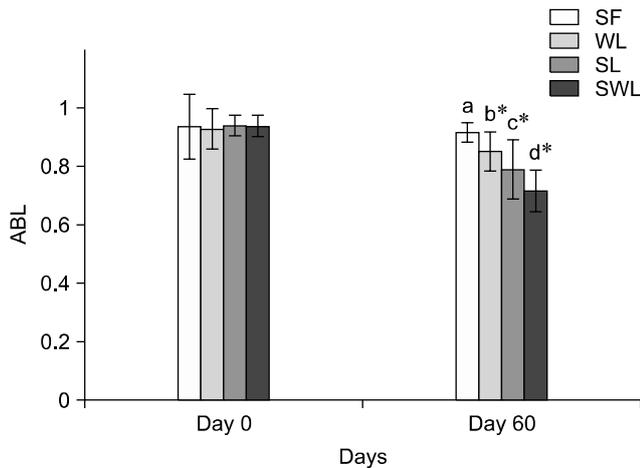
In the intergroup comparison of GI, CAL, and BoP, significant differences were observed among each experimental group, and these parameters were increased in a similar manner up to day 60 in each experimental group (Table 2). SWL animals showed the most dramatic changes followed by the SL and WL groups ( $p < 0.05$ ). The SF group had the best GI values compared to the other groups ( $p < 0.05$ ) with no significant increases in the CAL ( $p = 0.596$ ) or BoP ( $p = 0.203$ ) compared to baseline values. The mean PI scores for the SF and WL groups on day 60 were not significantly increased compared to that of the SL group, which was significantly lower than the SWL group score ( $p < 0.05$ ). In addition, the mean PPD on day 60 was significantly lower in the SF and WL groups than the SL and SWL groups ( $p < 0.05$ ).

Fig. 2 shows ABL changes observed in the experimental groups. The ABL of the SF group did not significantly change from days 0 to 60. On the other hand, the WL, SL, and SWL groups showed significantly lower ABLs on day 60 compared to day 0 ( $p < 0.05$ ). The ABLs of each group were significantly different on day 60; the SWL group had the lowest ABL followed by the SL, WL, and SF groups ( $p < 0.05$ ).

**Table 2.** Clinical parameters for each group

Day	Parameter	SF	WL	SL	SWL
Day 0	PI	0.3 ± 0.5	0.2 ± 0.5	0.2 ± 0.4	0.2 ± 0.4
	GI	0.4 ± 0.7	0.3 ± 0.7	0.2 ± 0.6	0.2 ± 0.6
	PPD	1.25 ± 0.41	1.32 ± 0.41	1.37 ± 0.45	1.33 ± 0.46
	CAL	0.08 ± 0.27	0.02 ± 0.15	0.06 ± 0.23	0.04 ± 0.21
	BoP	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3
Day 60	PI	1.9 ± 0.8 <sup>a,*</sup>	2.0 ± 0.7 <sup>a,*</sup>	2.3 ± 0.7 <sup>b,*</sup>	2.9 ± 0.4 <sup>c,*</sup>
	GI	0.9 ± 0.7 <sup>a,*</sup>	1.9 ± 0.6 <sup>b,*</sup>	2.1 ± 0.6 <sup>c,*</sup>	2.8 ± 0.4 <sup>d,*</sup>
	PPD	1.64 ± 0.52 <sup>a,*</sup>	1.83 ± 0.55 <sup>a,*</sup>	2.07 ± 0.85 <sup>b,*</sup>	2.13 ± 0.79 <sup>b,*</sup>
	CAL	0.10 ± 0.40 <sup>a</sup>	0.97 ± 0.57 <sup>b,*</sup>	1.91 ± 0.77 <sup>c,*</sup>	2.41 ± 0.67 <sup>d,*</sup>
	BoP	0.2 ± 0.4 <sup>a</sup>	0.6 ± 0.5 <sup>b,*</sup>	0.7 ± 0.5 <sup>c,*</sup>	0.9 ± 0.3 <sup>d,*</sup>

<sup>a,b,c,d</sup>Values with different superscript letters across each row are significantly different ( $p < 0.05$ ). \*Significantly increased values within a single group compared to the same parameters measured on day 0 ( $p < 0.05$ ). PI: plaque index, GI: gingival index, PPD: periodontal pocket depth, CAL: clinical attachment level, BoP: bleeding on probing, SF: negative control, WL: wire ligature, SL: silk ligature, SWL: silk-wire twisted ligature. Data are expressed as the mean ± SD.



**Fig. 2.** Alveolar bone levels before (day 0) and 60 days after (day 60) ligation in each group. <sup>a,b,c,d</sup>Values with different superscript letters were significantly different on the same day ( $p = 0.000$ ). \*Significantly decreased compared to day 0 in the same group ( $p < 0.05$ ). ABL: alveolar bone level, SF: negative control, WL: wire ligature, SL: silk ligature, SWL: silk-wire twisted ligature.

## Discussion

Periodontitis is a chronic inflammatory reaction that occurs as a consequence of interactions between the host immune system and oral pathogens [8,14]. Therefore, previous experimental studies investigating periodontal disease have generally used the animal models of periodontitis gradually induced by promoting plaque accumulation [3-5,9,11,15,18]. The model most similar to naturally occurring cases of periodontitis is one in which this condition is induced by a carbonate-enriched diet consisting of soft food. Nevertheless, the induction period for this method is greater than 5 to 7 month in dogs after clinical gingivitis has developed [9]. In our study, the control group (SF) showed no significant changes in CAL or BoP (factors that can represent destructive periodontal alterations) up to 60 days after the initial SF feeding.

Compared to the previous studies, we found that the use of hygroscopic and braided-type ligatures to promote periodontitis had a shorter induction period in dogs. In some studies, cotton floss with or without wire, which can provoke significant tissue reactions within the periodontal tissue, has been used to induce periodontitis [4,5,15,17,18]. In contrast to cotton, the degree of tissue reaction to a SL is quite low whereas wire has been shown to produce no reaction [2,6]. Although tissue reactions resulting from cotton do not directly cause an inflammatory response, we used silk with wire ligatures in the present study to reduce unnecessary environmental changes caused by the ligature.

For ligature-induced periodontitis, the ligature material not only forms a periodontal pocket subject to plaque accumulation but also serves as a constant irritant that can

harbor a multitude of oral pathogens [18]. Considering these characteristics, the loss of ligatures that enhance plaque formation might result in the failure to induce experimental periodontitis. For these reasons, other groups performed studies in which shallow notches were created on the mesial and distal cervix of the tooth using a round bur and act as retentive grooves for the ligature [3,13]. Another study employed a more invasive method in which a full-thickness gingival flap was created to secure the ligature to the alveolar bone crest [18]. In the present study, retentive grooves were made at two points of each tooth cervix to fix the ligature onto the initial location without loosening whereas a gingival flap was not made. Instead of using a relatively invasive method, we performed a closed undermining of the pocket up to a depth of 3 mm to expose the cement-enamel junction.

The period of ligature maintenance was significantly decreased in the SL group whereas all ligatures in the SWL group were maintained for 60 days. These results could be due in part by the fact that the tensile strength of silk sutures in tissue was reduced within 14 days and the relative security of the knot was also poor, whereas stainless steel ligatures were stable in the tissue [6]. The ligature maintenance period of the WL group also decreased compared to the SWL group, although this difference was not significant. The smooth surface of the wire might have cause the ligature to slip and fall out because the wire had been loosened by cervical connective tissue breakdown in the WL group.

Differences in clinical periodontal status varied significantly according to the ligature materials used. The SL and SWL groups, in which ligatures containing silk were used, showed significantly more advanced periodontal destructive changes than the other groups. Furthermore, most periodontal indices, including PI, GI, CAL, BoP, and ABL, were significantly worsened in the SWL group compared to the SL group. Considering the ability of ligatures to harbor oral pathogens while inducing periodontitis as mentioned above [18], these differences between the SL and SWL groups were attributed to the stability and surface area of silk. In the SWL group, the twisted ligature made with silk and wire could not only be firmly secured around the cervix of tooth due to the tension of the wire, but the coiling silk also provided a wide surface that housed a multitude of pathogenic micro-organisms.

Two cases of oral mucosal ulceration were encountered in both of the WL and SWL groups. The ligature used for both these animals included wires, and ulceration was attributed to a twisted knot of wire. Nevertheless, oral ulceration was not observed in all animals with ligatures containing wire. These results indicate that care should be taken when making the knot so as not to directly disturb the oral mucosa.

In summary, we found that ligatures composed of twisted

silk and wire were maintained for a significantly longer time than ones made of silk. Furthermore, the SWL induced more significantly advanced cases of experimental periodontitis compared to the other types of ligatures over the same period of time. According to these results, twisted silk and wire ligatures can be an effective method for studying periodontitis in dogs.

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