Dental Research Journal

Original Article

Inflammatory response of canine gingiva to a chemical retraction agent placed at different time intervals

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ABSTRACT

Background: Exposure of the gingival sulcus while controlling hemorrhage is prerequisites for maximizing treatment outcomes of cervical carious lesions and for obtaining quality impressions for the fabrication of indirect restorations with cervical finish lines. Gingival retraction cords saturated with different chemical agents are widely used for this purpose. The aim of this study was to investigate and compare the inflammatory potential of 15.5%ferric sulfate on connective tissue when placed at different times.

Materials and Methods: All procedures were performed on three dogs under general anesthesia. Retraction cords saturated with a 15.5% ferric sulfate solution were placed into the gingival sulcus and evaluated after 3 min and 10 min of exposure to the chemical agent. Excisional biopsies of the exposed gingival tissue were then obtained at intervals of 1 h, 24 h, and 7 days. For all specimens, histology evaluation was performed using light microscopy. Data collected from the microscopic images of all tissue specimens were analyzed by using the Wilcoxon Signed Rank and Kruskal-Wallis Tests. P value less than 0.05 was considered as significant.

Results: Histopathologic examination of the biopsied gingival tissue revealed that the ferric sulfate solution caused significant tissue changes at the beginning of both the 3-min and 10-min gingival exposure time ($P > 0.05$). However, the tissue returned to a normal histological appearance by the end of day 7 in all cases ($P > 0.05$).

Conclusion: The results of this study revealed that the biologic effects of 15.5% ferric sulfate solution are clinically acceptable and reliable when gingival exposure times of 3 min and 10 min are used for gingival retraction.

Key Words: Cord, ferric sulfate, retraction

Recieved: August 2012
Accepted: March 2013

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INTRODUCTION

The compatibility of prosthetic restorations with adjacent gingival tissue, masticatory function and appearance, is essential.[1] The finish line of crown restorations is generally considered to be very important in terms of esthetic demands. However, the compatibility of unacceptable finish lines with gingival tissue is often disregarded.[1]

Temporary displacement of the free gingival margin adjacent to cervical finish lines is required during fixed prosthodontic procedures to achieve an accurate impression of a prepared tooth using elastomeric materials for cast restorations.[2] The use of gingival retraction before taking impressions is the most favorable method of obtaining an accurate subgingival crown margin.[1]

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Gingival retraction techniques usually produce limited gingival recession and also protect sulcular tissues during tooth preparation.[3]

Several clinical methods are available for adequate tissue retraction.[2] Currently, gingival retraction can be achieved using mechanical, chemicomechanical, electrosurgical, surgical, and laser techniques.[4-6] However, mechanical and chemicomechanical applications are the most preferred and popular methods used in clinical practice.[1,7]

Apart from desirable hemostatic and astringent effects associated with gingival tissue retraction, chemical retraction agents are potentially harmful[1] to the gingiva as demonstrated by experimental animal and human studies.[8,9] Ideally, chemicals used as retraction solutions in chemicomechanical applications should not ellicit a negative systemic response nor cause local damage to the gingival tissue.[1]

There are several controversial reports of the histopathologic effects of retraction cord medicaments related to their duration of exposure to gingival connective tissue.[10-13] However, no universally accepted conclusion exists regarding this relationship. In addition, the long-term effects of retraction cord placement times in gingival tissues remains unclear. The aim of this study was to compare the inflammatory potential of gingival retraction cord saturated with 15.5% ferric sulfate solution on gingival connective tissue at different exposure times.

MATERIALS AND METHODS

The research protocol was approved by the Research and Ethics Committee at Jundi Shapour University prior to the onset of the study.

Inclusion criteria

Four healthy dogs weighing 20-25 kg were used as subjects and received a soft, nutritionally balanced diet during the study. Inclusion criteria included the following:

1. The presence of at least three teeth (molar and/or premolar teeth) in addition to a canine in every quadrant.
2. The absence of visible plaque and calculus.
3. The absence of caries or cervical lesions.
4. Normal appearance of the gingiva (color, texture, contour).
5. Probing depths of <3 mm and the absence of bleeding on probing.

Anesthesia

Anesthesia was induced with an intramuscular injection of 20 mg/mL of veterinary use grade xylazine hydrochloride (xylazine HCl, Injection, Teva Animal Health, Saint Joseph, MO, USA) and 5-13 mg/kg ketamine (Ketamine HCl, Putney, Portland, ME, USA).

Retraction cord management

Sixteen teeth and gingival retraction cords were used per dog (64 total) with 12 of the cords (Ultrapak Knitted Displacement Cord #00, Ultradent Products, South Jordan, UT, USA) being divided into two groups and saturated with ferric sulfate (Astringedent, 15.5% $Fe_2(SO_4)$ 3, Ultradent Products, South Jordan, UT, USA). The retraction cords in one group were placed for 3 min and for 10 min in the other group to establish different exposure times within each of the four dogs.

Before saturation, an examiner pulled the cords through a folded piece of clean filter paper held between the thumb and index finger to remove any air inclusions or bubbles trapped among the fibers that could substantially hinder thorough moistening of the cords. The bubble-free cords were then soaked for 20 min in the ferric sulfate solution. After the retraction cords are cut to the proper length, they were again soaked in the ferric sulfate solution for another 20 min before use.

Twelve of the prepared cords were placed into the buccal gingival sulci of maxillary right and left teeth for 10 min and mandibular right and left teeth for 3 min. Six cords were placed in each group for a total of 12 cords (saturated with distilled water per dog). The remaining 4 cords (two cords in each group) per dog were saturated with distilled water and placed into the buccal gingival sulci of four maxillary and mandibular canine teeth for 10 min and in four mandibular canines for 3 min [Figure 1].

These teeth were selected for the evaluation of the histopathologic appearance of the adjacent gingiva, but they were not included in the quantitative analysis. Table 1 summarizes the distribution of the 16 retraction cords for each dog.

Tissue biopsy

Following removal of the retraction cords, biopsy specimens were taken at 1 h, 1 day, and 7 days. The same dog was used at each time interval as a specimen donor [Figure 2].

The specimens were fixed, decalcified, and embedded in paraffin before the sections were cut in a series at 5 μm. The specimens were then stained with H and E to facilitate the evaluation of connective tissue, cells, and any alteration of the sulcular epithelium using light microscopy [Figures 3-8].

Table 2 shows the evaluation and scoring method used to evaluate the histopathologic results.

Statistical analysis

All data were analyzed using the Statistical Program of Social Science software (SPSS v.16, (SPSS Inc., Chicago, IL).

Two statistical methods were used to evaluate the results. The Kruskal-Wallis Test was used to show the statistical significance between times of specimen collection for each group. The Wilcoxon Signed Rank Test was used to evaluate the statistical significance between groups (3 min group and the 10 min group) at the end of each time period. P value less than 0.05 was considered as significant.

RESULTS

Following the removal of the cords and subsequent biopsies 1 h later, histopathologic changes in the connective tissue were evaluated in the acquired biopsy specimens. An increase in inflammatory cells (lymphocyte) was observed at hour 1 in both groups, but there was no significant difference between them ($P = 0.15$) [Figure 9].

However, statistical analysis revealed a significant difference between the two groups at the end of 24 h ($P = 0.034$) [Figure 10].

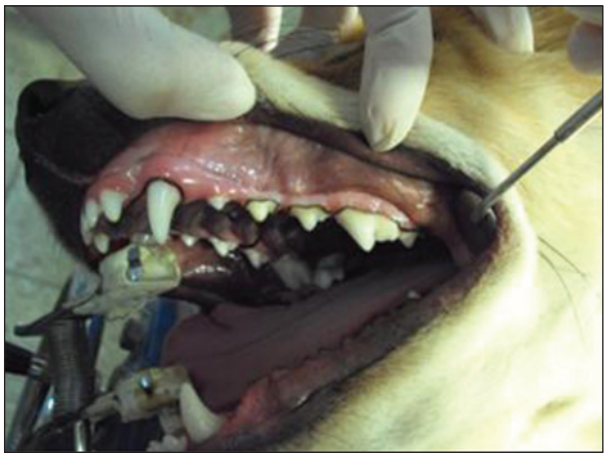

Figure 1: Retraction cord placement

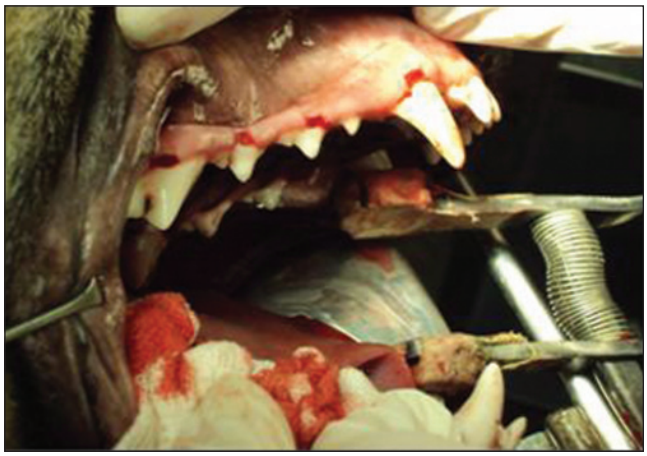

Figure 2: Performing the biopsy

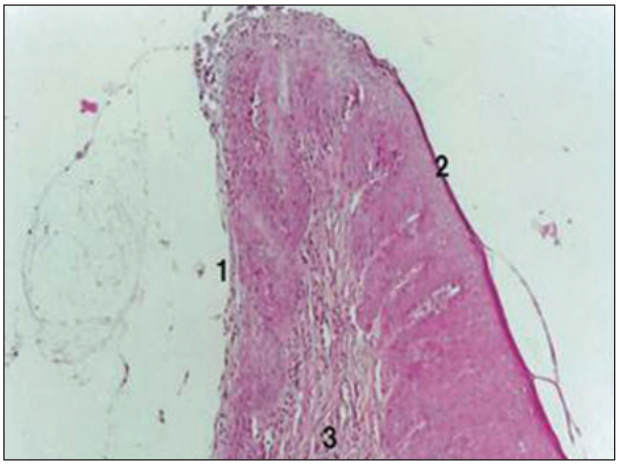

Figure 3: Histopathologic view of a 3-min placement times treated site at the end of day 7 with completely healed sulcular epithelium. (1) sulcular epithelium; (2) oral epithelium; (3) connective tissue (H and E, ×20)

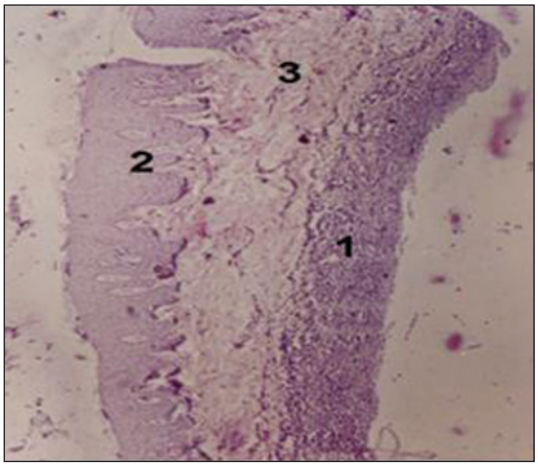

Figure 4: Histopathologic view of a 10-min placement time treated site at the end of hour 1: Severe inflammation in connective tissue and sulcular epithelium present. (1) sulcular epithelium; (2) oral epithelium; (3) connective tissue (H and E, ×20)

Table 1: Distribution of the 16 prepared retraction cords for each dog

No. of cords	Test site	Agent used	Exposure time
3	Right maxillary teeth	15.5% ferric sulfate solution	10 min
3	Left maxillary teeth	15.5% ferric sulfate solution	10 min
3	Right mandibular teeth	15.5% ferric sulfate solution	3 min
3	Left mandibular teeth	15.5% ferric sulfate solution	3 min
2	Maxillary canines	Distilled water	10 min
2	Mandibular canines	Distilled water	3 min

Table 2: The evaluation and scoring method used to evaluate histopathologic results of the biopsie

Score	Description of the biopsied tissue specimens
0	No inflammation
1	Slight inflammation (<25% inflammatory cells [lymphocyte] in the region)
2	Moderate inflammation (25-75% inflammatory cells [lymphocyte] in the region)
3	Severe inflammation (>75% inflammatory cells [lymphocyte] in the region)

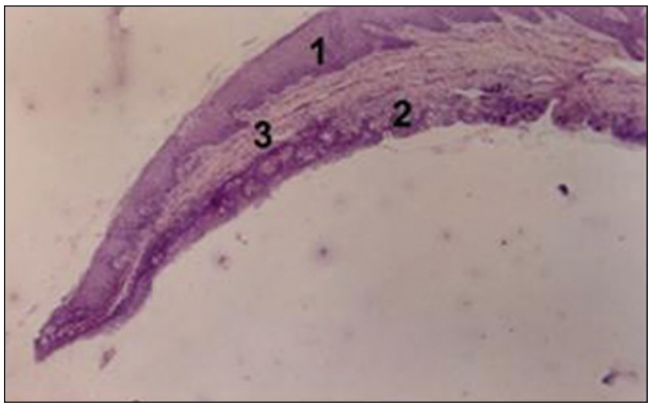

Figure 5: Histopathologic view of a 10-min placement timetreated site at the end of hour 24: Moderate inflammation in connective tissue and Sulcular epithelium present. (1) Sulcular epithelium; (2) oral epithelium; (3) connective tissue (H and E, ×20)

On the other hand, there was no significant difference between the two groups on day 7 and a decrease in the presence of inflammatory cells was observed at this interval ($P = 0.3$) [Figure 11].

DISCUSSION

Cosmetic dentistry has become an essential aspect of restorative dentistry.[2] Preparation of teeth for esthetic restorations requires the creation of a subgingival margin, or a finish line. To obtain a satisfactory impression of that finish line, tissue displacement or gingival retraction is necessary to facilitate the

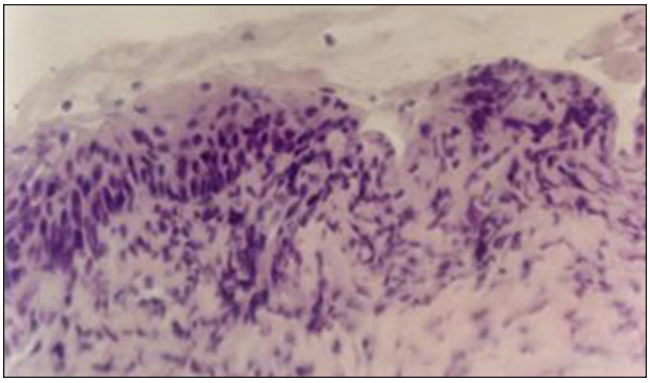

Figure 6: Histopathologic view of a 10-min placement times controlled site at the end of hour 1: Completely healed sulcular epithelium (H and E, ×0)

flow of impression material into the gingival sulcus. Numerous studies have evaluated gingival retraction materials and methods in recent years. As a result, five basic retraction techniques have been determined from these studies, which include: Chemicomechanical, mechanical, surgical, electrosurgical, and laser techniques.[3-10]

Although the chemicomechanical method has become the most preferred and popular method, controversy remains regarding the efficiency and reliability of chemical retraction solutions.

Previous studies have usually focused on the local effects of gingival retraction methods and the systemic effects of epinephrine.[8] Studies that evaluated the histopathologic effects of other medications are very limited. An insufficient number of studies that evaluated ferric sulfate as a chemical retraction have resulted in a lack of sufficient information to evaluate the histopathologic effects of this retraction agent. For this reason, a ferric sulfate solution was chosen for this study. Furthermore, the long-term effects of retraction cord placement times in gingival tissues remained unclear.

The present study compared the inflammatory potential of 15.5% ferric sulfate solution on gingival connective

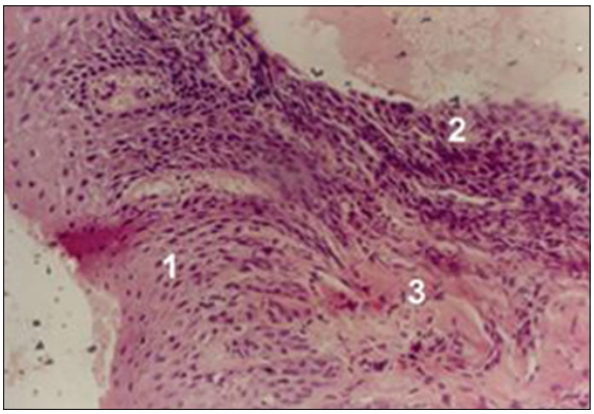

Figure 7: Histopathologic view of a 10-min placement time controlled site at the end of day 7: No inflammation in the connective tissue and sulcular epithelium. (1) oral epithelium; (2) sulcular epithelium; (3) connective tissue (H and E, ×10)

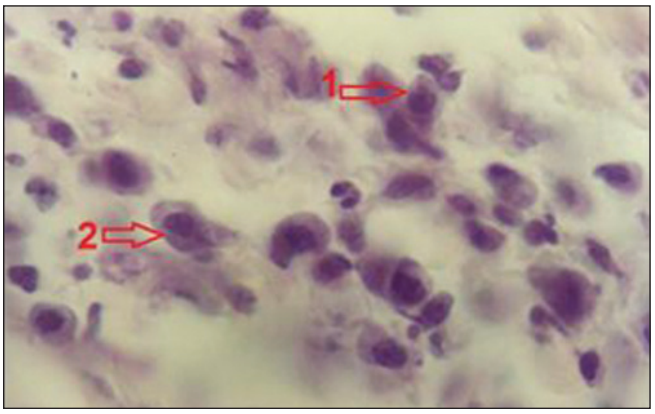

Figure 8: Histopathologic view of a 10-min placement time controlled site at the end of 1 h: An increase in the number of inflammatory cells in the connective tissue is present. (1) lymphocyte; (2) plasma cell (H and E, ×100)

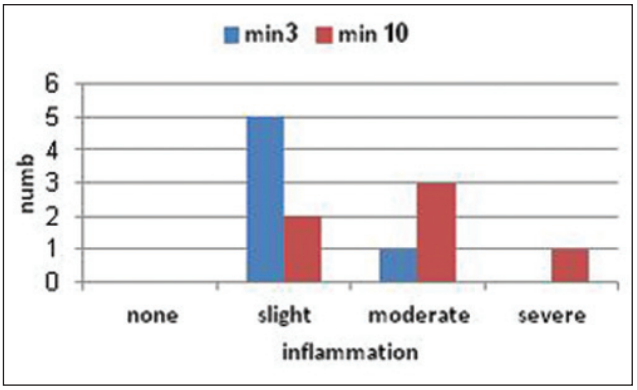

Figure 9: Evaluation of biopsied tissue at 1 h

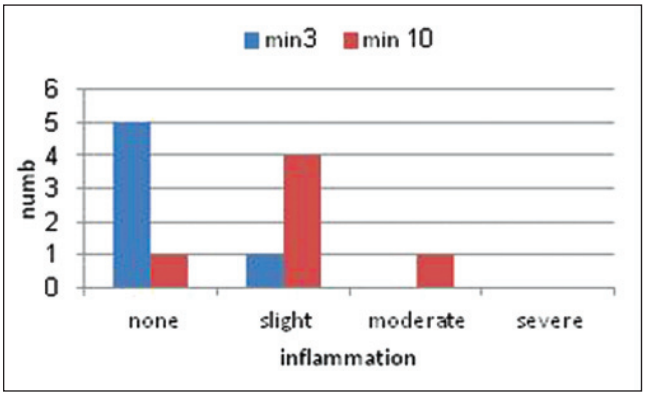

Figure 10: Evaluation of biopsied tissue at 24 h

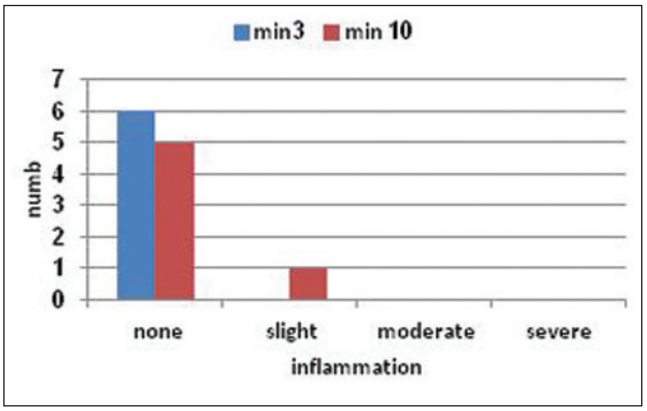

Figure 11: Evaluation of biopsied tissue at day 7

tissue with different exposure times to impregnated retraction cord. Since, it is preferable to perform gingival retraction prior to preparation of a tooth with a subgingival finish line; the aim of this procedure is to prevent damage to the sulcular epithelium. Failure to do so would result in the sulcular epithelium being more vulnerable to chemical trauma related to the degree of tissue damage resulting in gingival recession and undesirable exposure of the margin of the restoration. For these reasons, tooth preparations were not made prior to gingival retraction in this study.

Histopathologic examination of gingival tissue in the present study revealed that the ferric sulfate solution caused significant changes in gingival tissues at the beginning in both the 3-min and 10-min gingival exposure times to retraction cord impregnated with 15.5% ferric sulfate solution. However, the tissue returned to its normal histologic appearance at the end of day 7 after each exposure time.

Shaw *et al.*[13] have reported that the ferric sulfate medicament caused severe damage to connective tissue. In contrast, the results of the present study indicated that connective tissue was not damaged. The damage observed in the study by Shaw *et al.* could be due to tooth preparation being performed

before gingival retraction resulting in possible damage to the sulcular epithelium and connective tissue. Furthermore, the pressure applied during cord insertion could stimulate sulcular epithelium desquamation and connective tissue inflammation.

A study by Akca *et al.*[1] compared the effects of different retraction medicaments on gingival tissue. Statistical analysis revealed a significant difference between the study groups at the end of day 7. Among specimens obtained at 1 week following retraction cord removal, 15.5% ferric sulfate-treated tissue exhibited a slight inflammatory response. The pressure applied during retraction cord placement could influence sulcular epithelium desquamation and connective tissue inflammation. In addition, over saturation of the retraction cord may cause a longer interval of inflammation than in the present study. However, there was not a profound difference between the results of these two studies.

CONCLUSION

Based on the findings of this study and a review of the literature cited, materials used for gingival retraction should satisfy the following criteria if it is to be used in clinical procedures requiring gingival retraction:

1. The retraction materials must be effective in terms of its retraction effect.
2. Use of the materials should not cause significant irreversible tissue damage, and histologic healing should occur within 2 weeks following placement and removal.
3. Use of the material should not produce any potentially harmful systemic effects.

Considering the histopathologic results of this study, the biologic effects of 15.5% ferric sulfate solution are satisfactory. Both gingival exposure times (3-min and 10-min) used in this study are reliable and can be used effectively in clinical applications requiring gingival retraction.

ACKNOWLEDGMENTS

This report is part of a project financially supported and approved by the Research Deputy of Ahwaz Jundishapour University of Medical Sciences, Iran for which authors would like to thankfully acknowledge. The authors express their thanks and appreciation to Hossein Najafzadeh, and Mehdi Poor Mahdi, for their help.

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How to cite this article: Ahmadzadeh A, Majd NE, Chasteen J, Kaviani A, Kavoosi MA. Inflammatory response of canine gingiva to a chemical retraction agent placed at different time intervals. Dent Res J 2014;11:81-6.

Source of Support: Nil, **Conflict of Interest:** None declared.