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Title page

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Title

Periodontal tissue repair after sealing of the gap in vertical root fracture

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Abstract

The aim of the study was to determine whether sealing of fracture gap using adhesive resin through the root canal can prevent inflammation of periodontal tissue, and resealing the incompletely sealed fracture gap from outside, can resolve such inflammation in experimentally created vertical root fractures.

Vertical root fractures were created in incisor of beagles. In the experimental group, the fracture gap was sealed through the root canal with adhesive resin. After 5 weeks, sites with the clinical attachment level ≥4 mm were further divided randomly into the poor-replanting group and the poor-untreated group. In the poor-replanting group, the tooth was extracted and replanted after resealing the fracture gap with adhesive resin from the outer surface. Sites with clinical attachment level ≤3 mm after 5 weeks were considered as the satisfactory group. The poor-untreated group and the satisfactory group were subjected to no further treatment. The clinical attachment level was evaluated at baseline and after 2, 5 and 9 weeks. After 9 weeks, histological measurements were made to determine the length of the epithelial downgrowth and the area of alveolar bone resorption.

The clinical attachment level and the area of bone resorption were significantly smaller in the poor-replanting group and the satisfactory group than in the poor-untreated group ($p<0.05$). The results indicate the possibility that periodontal inflammation along the fracture line can be prevented and improved if the fracture gap is sealed.

Keywords:

Vertical root fracture, Attachment level, Periodontal inflammation, Adhesive resin, Gap sealing

Introduction

Vertical root fracture often causes a localized inflammation in the periodontal tissue surrounding the fracture line, a rapid increase in the probing depth, and bone resorption [1-4]. In cases of vertical root fracture, extraction is generally indicated for single-rooted teeth, while root resection and hemisection are indicated for multi-root teeth [1, 2, 5, 6]. Some authors attempted to conserve roots with vertical fracture by sealing the fracture gap with calcium hydroxide [7, 8] or glass ionomer cement [9-11] and there are also some reports of success achieved by bonding with resin. Masaka [12] reported cases of fractured roots that were preserved for 10 years by 4-methacryloxyethyl trimellitate anhydride/methyl methacrylate-tri-n-butyl borane (4-META/MMA-TBB) resin bonding. In addition, Sugaya et al. [13] carried out bonding using 4-META/MMA-TBB resin in 23 teeth with vertical root fracture, and of those, 18 teeth (78%) were conserved for an observation period of 6 to 74 months. Hayashi et al. [14] extracted 26 teeth with vertical root fracture, bonded the fractured roots, and replanted them. They reported results over an observation period of 4 to 74 months, and longevity was found as 69.2% at 36 months after replantation. These reports indicate the possibility that bonding of roots may be an option for the treatment of vertical root fracture. Moreover, in many of the cases where vertical root fractures were bonded with resin, improvement in the inflammation of periodontal tissue and a reduction in periodontal probing depth were reported even without debridement of the root surface.

Histopathologically, increased probing depth is indicative of two possible conditions. The first is the formation of a periodontal pocket by epithelial downgrowth and accumulation of bacteria in the periodontal pocket so that the condition resembles marginal periodontitis. The second is periodontal inflammation caused by bacteria in the root canal and the fracture gap, and while there is no epithelial downgrowth, the periodontal probe penetrates the inflamed connective tissue [15-17]. If the probe only penetrates inflamed connective tissue and the bacteria are localized in the root canal and the fracture gap, it is likely that elimination of the bacteria and sealing of the fracture gap will resolve the inflammation and repair the resorbed bone.

The aim of the present study was to examine histopathologically whether sealing of fracture gap using 4-META/MMA-TBB resin through the root canal can prevent inflammation of periodontal tissue, and whether resealing the incompletely sealed fracture gap from outside, can resolve such inflammation in experimentally created vertical root fractures.

Materials and methods

Experimental animals and sites

A total of 42 teeth in seven 12-month-old male beagles (weight 11.6 to 12.8 kg) were used for this study. The bilateral maxillary incisors I1, I2 and I3 served as the experimental sites. This experiment was carried out in accordance with the guidelines for the care and use of laboratory animals of the Graduate School of Medicine, Hokkaido University (approval no.01028).

Vertical fractures of the roots

For each tooth, pulpectomy was performed and vertical root fractures were created under general anesthesia using 0.1 ml/kg medetomidine hydrochloride (Domitor; Zenoaq, Fukushima, Japan) and 0.1 ml/kg ketamine hydrochloride (Ketalar; Daiichi Sankyo Propharma, Tokyo, Japan), combined with local anesthetic using 2% lidocaine hydrochloride containing 1/80,000 epinephrine (Xylocaine Cartridge, Dentsply-Sankin, Tokyo, Japan). The root canal was enlarged with a Peeso reamer #1 (Mani, Tochigi, Japan) and a K file (Mani, Tochigi, Japan). Then, the root was vertically fractured using a chisel and mallet (Fig. 1-A).

An impression was taken and the root canal was sealed with hydraulic temporary filling material (Caviton; GC, Tokyo, Japan). A cast post (Casting Silver Core; GC, Tokyo, Japan) was fabricated using the indirect technique.

Treatment for each group

In the experimental group, the root canal was thoroughly cleaned using an ultrasonic device (ST19A, U-file #30, ENAC 10WA; Osada, Tokyo, Japan) after 1 week. The root canal was treated with a solution of 10% citric acid and 3% ferric chloride (Green Activator; Sun Medical, Shiga, Japan) using a syringe (SS-01T2619S; Terumo, Tokyo, Japan) with a 30G irrigation needle (Clean wash needle; Nipro, Osaka, Japan). After 10 s, the root canal was irrigated with distilled water using a syringe and dried with mild air blow. 4-

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META/MMA-TBB resin (Super-Bond, Opaque Ivory; Sun Medical, Shiga, Japan) was mixed with monomer solution, a catalyst and polymer powder according to the manufacturer's instructions and loaded in a syringe (Terumo, Tokyo, Japan). The mixture was injected into the root canal and the fracture gap, after which a cast post was inserted and bonded (Fig. 1-B). In the control group, the root canal was not treated nor filled with resin, and the cast post was not bonded.

Fractured sites of the roots with the bonded post (Experimental group) were divided into subgroups based on the clinical attachment level after 5 weeks (Fig. 2). One group including sites with clinical attachment level ≤3 mm was considered as the satisfactory group and was subjected to no further treatment. Another group included sites with clinical attachment level ≥4 mm and was considered as the poor group which was further divided randomly into two groups: the poor-untreated group and the poor-replanting group. The poor-untreated group was subjected to no further treatment, while the poor-replanting group was treated as follows.

First, extraction was performed using forceps, with care taken to minimize damage to the periodontal ligament (Fig. 1-C). The fracture line was then prepared to a depth of approx. 1 mm using an ENAC 10WA with an SC-4 tip (Osada, Tokyo, Japan) without water irrigation (Fig. 1-D). After the fracture line had been prepared, the gap was thoroughly washed with saline and was treated with Green Activator for 10 seconds, washed with saline, and air-dried. The fracture gap was then sealed using Super-Bond. The tooth was left immersed in saline until the resin had hardened. After hardening was complete, excess resin was removed using a round bur (Dentsply Maillefer, Ballaigues, Switzerland) and a hand scaler (Gracey curette, Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA) (Fig. 1-E). Inflammatory connective tissue was curetted out of the extraction socket, and the root was replanted in the original position. The replanted root was splinted to the proximal teeth with Super-Bond.

Evaluation

The clinical attachment level was evaluated at the fracture site with a 15UNC Color-Coded Probe (Hu-Friedy) by measuring the distance from the cemento-enamel junction (CEJ) to the tip of the probe at baseline (immediately after root fracture), and after 2, 5 and 9 weeks.

The animals were sacrificed and tissue samples were prepared after 9 weeks. After fixation, the Super Bond was dissolved by immersion in acetone and the cast post was removed. Demineralization was

performed with formic acid sodium citrate. The sections were prepared perpendicular to the major axis of the root, and were stained with hematoxylin-eosin. Immunohistochemical staining of epithelial cells was carried out on some of the samples, using cytokeratin clone AE1/AE3 (Dako Japan, Tokyo, Japan) as the primary antibody. Histological measurements were made to determine the length of the epithelial downgrowth and the area of alveolar bone resorption.

To determine the length of the fracture line and epithelial downgrowth, the distance from the CEJ to the most apical fracture and epithelium was measured in a number of sections. The CEJ was distinguished by the morphology of the dentin, cementum and gingiva, because enamel was lost in the decalcified specimen. The area of bone resorption was measured on sections at 3.0, 3.5, and 4.0 mm from the CEJ toward the apex. The area of resorption was measured using image analysis software (Image J, Freeware, U. S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical Analysis

Differences in the clinical attachment level, the length of fracture line and epithelial downgrowth, and the area of bone resorption between the groups were analyzed using Kruskal-Wallis test and Mann-Whitney U test with Bonferroni correction. Differences in the clinical attachment levels between 5 weeks and 9 weeks were analyzed using Wilcoxon signed-rank test. All analyses were performed using SPSS Statistics Version 21 (IBM, Armonk, NY, USA).

Results

Final evaluation was was carried out in 28 teeth, because they were used as anchors after replantation, and those which fractured obliquely or fractured into three parts were excluded from the experiment. Where one tooth had two fracture lines, but clinical attachment levels after 5 weeks of ≤3 mm and ≥4 mm were sometimes both present in different sites on the same root, the ≤3 mm site was excluded from the evaluation in roots that were replanted. As a result, the numbers of sites that were evaluated were: control group -14 sites of 8 roots; satisfactory group -15 sites of 9 roots; poor-untreated group -7 sites of 5 roots; and poor-replanting group -7 sites of 6 roots.

There was no significant difference among the four groups in terms of clinical attachment level at baseline and 2 weeks ($p > 0.05$), but after 5 weeks, the clinical attachment level was significantly smaller in the satisfactory group than the other three groups ($p < 0.01$). After 9 weeks, there were no significant differences in clinical attachment level between the poor-untreated group and the control group ($p > 0.05$). However, the clinical attachment level was significantly smaller in the poor-replanting group than in the poor-untreated group ($p < 0.01$) (Table 1). Regarding the clinical attachment levels after 5 weeks and after 9 weeks, only the poor-replanting group showed significant improvement ($p < 0.05$).

The length of the fracture line was 6.2-11.9 mm and there was no significant difference among four groups ($p > 0.05$). The length of epithelial downgrowth was significantly smaller in the satisfactory group than in the poor-untreated or control groups ($p < 0.05$) (Table 2). In both the control group and the poor-untreated group, there was a considerable difference between clinical attachment level (6.4 ± 1.8mm and 6.4 ± 2.7mm, respectively) and length of epithelial downgrowth (1.94 ± 0.78mm and 2.00 ± 0.78mm, respectively) after 9 weeks.

The area of bone resorption was significantly smaller in the satisfactory group than in the poor-untreated or the control groups ($p < 0.05$), and significantly smaller in the poor-replanting group than in the poor-untreated group ($p < 0.05$) and the control group ($p < 0.01$) after 9 weeks (Table 2).

Histological observations at 9 weeks showed that in the control group, the alveolar bone around the fracture region had greatly resorbed and there was infiltration of inflammatory cells (Fig. 3A). The poor-untreated group showed the same findings as the control group (Fig. 3B). No pocket epithelium was seen in the deep part of the osseous defect in either of these groups. In the satisfactory group, the Super-Bond that sealed the fracture gap dissolved away during specimen preparation, but the pigment that was present in the Super-Bond was observed as black particles. Almost no inflammation was seen (Fig. 3C). In the poor-replanting group, immature, newly formed bone was observed, and there were very few inflammatory cells (Fig. 3D). In some of the samples, keratin-positive cells were seen on the surface of the resin (Fig. 3E). Cementum-like hard tissue was not seen on the resin in any of the samples.

Discussion

In this study, measurements were made on histological sections 3.0, 3.5, and 4.0 mm from the CEJ towards the apex. There are two reasons for that. Firstly, the mechanical damage at the time of extraction might

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affect the cervical region. Also, more roots might be excluded from the evaluation when histological measurements were made at the apical region because the fracture line was short. We assumed that there would surely be periodontal destruction at the regions 3.0, 3.5, and 4.0 mm from the CEJ towards the apex when clinical attachment level was 4 mm or more. Therefore, we included one group having sites with clinical attachment level ≥4 mm as the poor group. The other roots were classified as satisfactory group.

The length of fracture line might have an influence on the area of inflammation. The fracture line in the region 3.0-4.0 mm from the CEJ towards the apex was observed in all roots and the length of fracture line was not significantly different among four groups. Therefore, the difference in the length of fracture line of each group did not influence the results.

In a preliminary study, periodontal inflammation around the fracture line deteriorated over time up to 4 weeks after vertical fracture and remained stable thereafter. Based on this finding, fractured sites of the roots were divided into subgroups based on the clinical attachment level 4 weeks after sealing (5 weeks after baseline) in the experimental group. In this study, in the control and poor-untreated groups, the clinical attachment level was significantly greater after 5 weeks than after 2 weeks, and there were no significant differences between after 5 weeks and 9 weeks. In the Satisfactory group, the mean clinical attachment level was approximately 2 to 3 mm, and there was no clinical attachment loss after 9 weeks. From these findings, it appears that localized inflammation occurred in the periodontal tissue around the line of fracture not later than 5 weeks after vertical fracture and did not deteriorate from 5 to 9 weeks. Therefore, it would be appropriate that the experimental group was divided into subgroups based on the clinical attachment level after 5 weeks.

If there is a possibility of inflammation due to the presence of bacteria in the root canal and fracture gap, sealing of the canal and the fracture gap may prevent inflammation or resolve existing inflammation. Super-Bond was used in this study for sealing the fracture gap. This resin was chosen based on a number of clinical reports [13, 14, 18-21] describing good outcomes when Super-Bond was used to treat vertically fractured roots. Super-Bond comprises tri-n-butyl borane as a polymerization initiator. So when it comes in contact with moisture, radicals are generated and polymerization progresses [22]. Therefore, even if blood and periodontal tissue are present, it is still possible to obtain a high rate of polymerization and good biocompatibility.

In the present study, the control group had neither root canal filling nor fracture gap sealing and the poor-untreated group had root canal filling and fracture gap sealing through the root canal. But there was no

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significant difference in clinical attachment level or bone resorption area between these two groups which suggests that there was a failure in the adhesion and the seal through the root canal was incomplete. In the poor-replanting group, the tooth was extracted, and a fracture line was prepared from the outer surface and sealed, and then the root was replanted. As a result, the clinical attachment gain was enhanced and there was reduced bone resorption. From these findings, it appears that the causes of inflammation are bacteria in the root canal and the fracture gap. This is probably similar to reported cases in which teeth with unsuccessful root canal therapy were intentionally replanted after retrograde filling of the root apex, and subsequent healing was satisfactory [23-28]. Furthermore, the lack of downgrowth of pocket epithelium may be due to the repair of bone along with the removal of infection from the fracture line.

The present study compared the position of the tip of a periodontal probe and the most apical position of the pocket epithelium. As epithelial cells are hard to distinguish histologically when there is inflammation, they were identified by immuno-staining for keratin. In the control group, no epithelial cells were observed in the deep part of bone defect, even though the probe deeply penetrated the tissue and most of the bone defect was filled with inflammatory connective tissue. It has been reported in marginal periodontitis that even with constant probing pressure, as in cases of severe inflammation, the probe may penetrate beyond the apical termination of epithelium into the connective tissue [15-17]. So, it can be suggested that the greater probing depth in the control group was due to penetration of the periodontal probe into inflammatory connective tissue. Furthermore, as there was only slight downgrowth of pocket epithelium, it appears that inflammation of the periodontal tissue was mainly due to bacteria in the root canal and the fracture gap. However, the length of epithelial downgrowth was significantly greater in the poor-untreated group than the satisfactory group. This may suggest the possibility that root fracture which persists for a long time may cause down growth of pocket epithelium to form a periodontal pocket and thus marginal periodontitis occurs locally.

The results of the present study indicate the possibility that the inflammation of the periodontal tissue in cases of vertical root fracture can be prevented by sealing the root canal and the fracture gap through the root canal and in failure cases due to incomplete sealing, periodontal inflammation might be improved if the fracture gap is sealed from the outer surface of the root. However, the bonded roots were not subjected to occlusal load in this study, and the prevention of re-fracture, other than proper sealing of the fracture gap, is also a factor that may affect the prognosis of vertical root fracture. It is unclear whether the bonded roots

exhibit resistance to re-fracture when involved in a mechanical action such as occlusal force, and further studies are necessary to determine the clinical outcome .

Conflict of Interest

The authors declare that they have no conflict of interest.

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Figure captions

Fig. 1 Vertical fracture and sealing

(a): After vertical fracture

(b): After post bonding

(c): Extracted root

(d): After preparation of the fracture line with an ultrasonic file

(e): After sealing the fracture gap and removing excess resin

Fig. 2 Experimental design

Fig. 3 Histological sections at 9 weeks (3.5 mm toward the apex from the CEJ)

a: Control group. b: Poor-untreated group. c: Satisfactory group. d: Poor-replanting group. Bone ($\rightarrow$) has been repaired up to the region near the resin. e: Poor-replanting group (keratin stained, 1.5 mm toward the apex from the CEJ). Several layers of keratin-positive cells are in contact with the resin ($\leftarrow$).

Table 1. Clinical attachment levels in the experimental and control groups

Values represent mean $\pm$ S.D. (mm)

Significant differences between a and b (Mann-Whitney U test with Bonferroni correcction; $p < 0.05$, Wilcoxon signed-rank test; $p<0.05$)

Table 2. Length of epithelial downgrowth and area of bone resorption at 9 weeks

Values represent mean $\pm$ S.D.

Significant differences between different letters Mann-Whitney U test with Bonferroni correcction; $p < 0.05$)
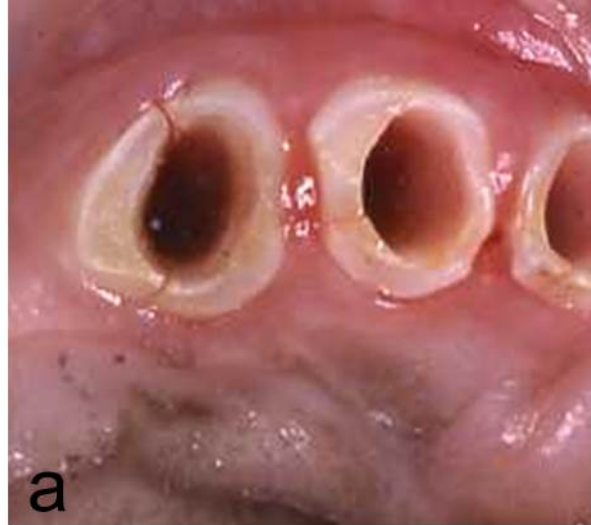
|				
Control				
Experimental				

Satisfactory group
  Clinical attachment level
  at 5W ≤3 mm				

Poor-untreated group
  Clinical attachment level
  at 5W ≥4 mm				

Poor-replanting group
  Clinical attachment level
  at 5W ≥4 mm				

Baseline   1W   5W   9W

- Pulpectomy
- Root fracture

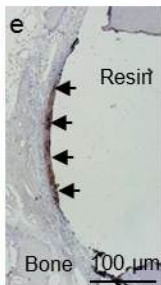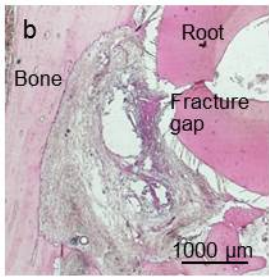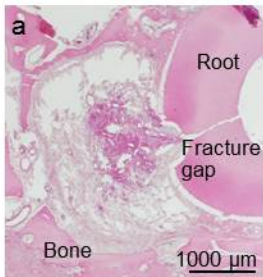- Sealing the fracture gap through the root canal
- Post bonding

- Extraction
- Sealing the fracture gap
- Replantation

- Specimen preparation
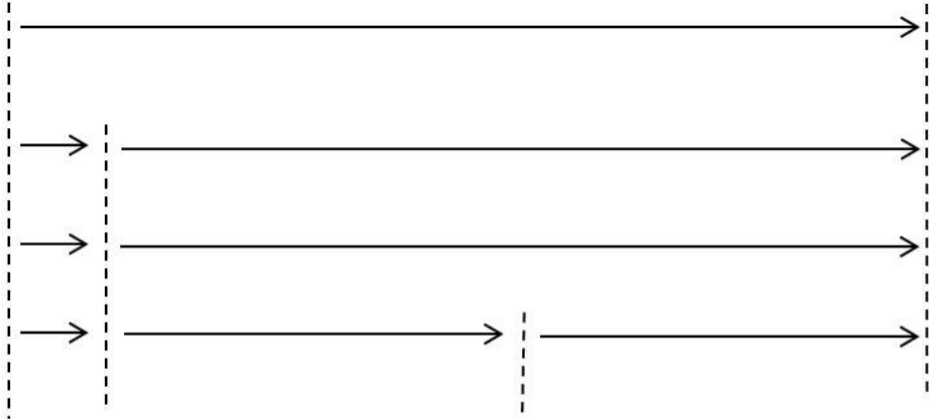
	Baseline	2 weeks	5 weeks	9 weeks
Control group	2.2 ± 0.7 [a]	2.8 ± 0.6 [a]	6.3 ± 1.8 [b]	6.4 ± 1.8 [b]
Satisfactory group	2.3 ± 0.5 [a]	2.4 ± 0.5 [a]	2.5 ± 0.5 [a]	3.0 ± 1.0 [a]
Poor-untreated group	2.1 ± 0.7 [a]	2.6 ± 1.0 [a]	6.3 ± 3.4 [b]	6.4 ± 2.7 [b]
Poor-replanting group	1.7 ± 0.8 [a]	2.8 ± 0.6 [a]	6.1 ± 1.8 [b]	3.0 ± 1.0 [a]

	Length of epithelial downgrowth (mm)	Area of bone resorption (mm^2)
Control group	1.94 ± 0.78 [a]	2.96 ± 1.43 [a]
Satisfactory group	0.91 ± 0.72 [b]	0.49 ± 0.48 [b]
Poor-untreated group	2.00 ± 0.78 [a]	3.05 ± 1.41 [a]
Poor-replanting group	1.46 ± 0.82	1.02 ± 0.72 [b]