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Author(s)	Sugaya, Tsutomu; Tomita, Mahito; Motoki, Youji et al.
Citation	Dental traumatology, 32(5), 397-401 https://doi.org/10.1111/edt.12270
Issue Date	2016-10
Doc URL	https://hdl.handle.net/2115/67216
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Type	journal article
File Information	DT32(5).pdf

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Title

Influence of enamel matrix derivative on healing of root surfaces after bonding treatment and intentional replantation of vertically fractured roots

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Running title

Healing of vertically fractured roots replanted with EMDOGAIN

Keywords

vertical root fracture, enamel matrix derivative, new cementum, root resorption, intentional replantation

Abstract

Background/Aim: The objectives of this study were to histopathologically evaluate cementum regeneration on root surfaces when enamel matrix derivative was used to bond a vertically fractured root, and to evaluate the effectiveness of enamel matrix derivative in inhibiting root resorption.

Material and Methods: A total of 40 roots from 24 maxillary premolars in beagles were used. The root was vertically fractured using a chisel and mallet. Super Bond was then used to bond the fractured root. In the experimental group, the root surface was treated with ethylenediaminetetraacetic acid and an enamel matrix derivative. The control group received no treatment. The root was then replanted in its original location. Histopathological observation and measurement using image analyzing software were carried out after eight weeks.

Results: In the experimental group, shallow resorption cavities developed on the root planed surfaces with new acellular cementum appearing over them. In the control group, however, no new cementum was seen on the planed surfaces, and there was connective tissue joining the roots. In some of the samples, resorption and multinucleated giant cells were seen. The experimental group showed a significantly larger volume of cementum formation ($p<0.001$), and the volume of root resorption was significantly smaller ($p=0.004$).

Conclusion: When bonding and replanting tooth roots after a vertical fracture, the application of enamel matrix derivative was effective in regenerating cementum on root surfaces from which periodontal ligament had been lost in the area around the fracture line, and in reducing the incidence of root resorption.

Keywords:
vertical root fracture, enamel matrix derivative, new cementum, root resorption, intentional replantation

Introduction

In vertical tooth root fractures, localized inflammation occurs in the periodontal tissue around the line of fracture, and the probing depth abruptly becomes deeper, with bone resorption occurring in many cases (1-4). The usual approach with a single-rooted tooth is to extract the tooth or, in the case of multiple roots, to perform root resection or hemisection (1, 2, 5, 6). A number of attempts to clinically preserve teeth have been reported, with successful outcomes of resin bonding, in particular (7-13). Sugaya et al (7) used 4-methacryloxyethyl trimellitate anhydride in methyl methacrylate tri-n-butyl borane (4-META/MMA-TBB) resin to bond 23 teeth with vertically fractured roots, and after six to 74 months of observation reported that 18 (78%) of the teeth could be preserved. Hayashi et al (9) extracted 26 teeth with vertically fractured roots, bonded the fractured roots and replanted them. After four to 74 months of observation, eight of the teeth ended up being extracted, and longevity was calculated as 88.5% at 12 months after replantation and 69.2% at 36 months. The outcomes described in these reports suggest that bonding treatment is effective for vertically fractured roots. However, these reports also indicated that in cases where there is significant destruction of periodontal tissue, deep pockets can develop postoperatively, and there can be residual bone defects, mostly leading to a poor prognosis. Consequently, in order to improve the success rate, regenerating cementum and periodontal tissue on the tooth surface, is thought to be important.

Because enamel matrix derivative (EMD) promotes the growth of periodontal ligament and facilitates differentiation into cementoblasts (14, 15), it is widely used to regenerate periodontal tissue that has been lost as a result of periodontitis (16-19). Moreover, it was found to be effective in inhibiting ankylosis and root resorption when used in replantation (20-26). No studies, however, have evaluated the effects on cementum regeneration or on

inhibition of root resorption along root surfaces from which periodontal ligament was lost when EMD was used in intentional replantation.

Given that, the objectives of the study described here were to histopathologically evaluate cementum regeneration and root resorption on root surfaces where periodontal ligament was lost in the area around the line of fracture when EMD was used in treatment of a vertically fractured root.

Material and methods

1) Experimental animals and sites

The experiment was performed in 40 roots of 24 teeth consisting of bilateral maxillary premolars P1, P2 and P3 from four 10-month-old female beagles. 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. 07037).

2) Vertical fractures of the roots

Under general anesthesia comprising 0.1 ml/kg of medetomidine hydrochloride (Domitor, Zenoaq, Fukushima, Japan) and 0.1 ml/kg of ketamine hydrochloride (Ketalar, Daiichi Sankyo Propharma, Tokyo, Japan), local anesthesia was administered with 2% lidocaine hydrochloride containing 1:80,000 epinephrine (Xylocaine Cartridge, Dentsply-Sankin, Tokyo, Japan). After removal of the crowns, the cervical third of root canal was prepared using a Peeso reamer #1 (Mani, Tochigi, Japan) and the apical part of the root canal was prepared using a K-file (Mani, Tochigi, Japan). After the root canal had been prepared, the root was vertically fractured using a chisel and mallet. The root canal was left open, without intracanal medication or temporary sealing.

2) Treatment method

Four weeks later, general and local anesthesia were administered, and, taking every effort not to damage the periodontal ligament, the tooth was extracted using only forceps. A #5 round bur (Mani, Tochigi, Japan) was used to clean the root canal wall and fractured surface of the root under irrigation with saline solution, and the infected tooth substance was removed (Figure 1-A). After thorough cleaning with saline solution, followed by air drying, the root canal walls and fracture surface were treated for 10 seconds with 10% citric acid with 3% ferric chloride (Green Activator, Sun Medical, Shiga, Japan), and were then washed with saline solution and air dried. We then used 4-META/MMA-TBB resin (Super-Bond, Sun Medical, Shiga, Japan) to bond the fractured root. After the resin had completely hardened in saline solution, excess resin was removed using a hand scaler. Additionally, the periodontal ligament was removed from the root surface around the line of fracture to a width of 1.4 mm by means of root planing (Figure 1-B). The teeth were then randomly classified into two groups based on the subsequent treatment approach.

Experimental group: The root planing site was treated for three minutes with 24% ethylenediaminetetraacetic acid (PrefGel®, Straumann, Basel, Switzerland), after which the area was washed with saline solution and an enamel matrix derivative (EMDOGAIN®, Straumann, Basel, Switzerland) was applied (Figure 1-C).

Control group: The root surface was not treated with PrefGel®, nor was EMDOGAIN® applied.

In both groups, granulation tissue was removed from the alveolar socket, after which the root was replanted in its original position and fixed to the proximal teeth with Super-Bond.

4) Evaluation method

After eight weeks, the dogs were sacrificed and histopathological observation and measurement were carried out. The fixed blocks were immersed in acetone to dissolve the Super-Bond, and were then demineralized and sliced at a thickness of 5 μm in the perpendicular direction along the longitudinal axis of the root, and stained with hematoxylin and eosin. Pathological measurements were done at a point 4 mm from the CEJ to the apical side. The morphology of healing on each planed root surface was then assigned to one of four classifications: (1) cementum, (2) connecting tissue (parallel fibers on the root surface, with no root resorption), (3) inflammatory root resorption (parallel fibers on the root surface, with root resorption), and (4) ankylosis. Measurements were done using image analyzing software (Image J, Freeware, USA).

Statistical analysis was performed using SPSS Statistics Version 21 (IBM, Armonk, NY, USA), and the Mann-Whitney *U* test was performed.

Results

Of the 24 teeth and 40 roots used in the experiment, roots that were not fractured or replanted because they were used as anchors after replantation, and those that fractured obliquely, or fractured into three parts, were excluded from the experiment. The remaining roots were classified into nine roots and 18 sites in the experimental group, and five roots and 10 sites in the control group, and these were used for measurement.

In the experimental group, shallow resorption cavity developed on the surfaces of roots that had been planed, with new cementum appearing over them. The new cementum was acellular (Figure 2). However, there was no formation of cementum on the Super-Bond that was used to seal the gap created by the fracture. In the control group, however, no new

cementum was seen and there was connective tissue on the surfaces of roots that had been planed. In some of the samples, the root surface had resorbed, and multinucleated giant cells thought to be odontoclasts were seen (Figure 3).

Histological measurements showed no significant differences ($p>0.05$) between the two groups in root planing length or ankylosis (Table 1). Compared to the control group, the experimental group had significantly longer cementum formation ($p<0.001$), and the length of root resorption was significantly smaller ($p=0.004$).

Discussion

The results of the study described here show that, when EMDOGAIN® was used in bonding and replantation of vertically fractured roots, cementum was regenerated on root surfaces from which the periodontal ligament had been removed by means of root planing. The effectiveness of EMDOGAIN® in regenerating cementum during flap surgery to treat periodontitis has been widely reported (16-19), and this study has shown that EMDOGAIN® is also effective in intentional replantation. In experiments in which dehiscence defects were created in monkeys and EMDOGAIN® was used, it was reported that 60 to 80% of the cementum was regenerated in a defect of 6 mm (27). In the present study, however, the cementum regeneration was smaller, at approximately 0.5 mm. This could be because, with intentional replantation, the periodontal ligament has actually been severed, making it necessary to repair the damaged periodontal ligament, and this possibly had a negative impact on regeneration. The newly generated cementum was acellular, and this finding is consistent with the cementum regenerated when EMDOGAIN® was used in periodontal surgery (27). The development of a resin cement capable of inducing cementum growth may further enhance the prognosis of this type of treatment.

In the control group, root resorption was seen on root surfaces from which the periodontal ligament had been removed by means of root planing, but in the experimental group, in which EMDOGAIN® was applied additionally, there was less root resorption. It has been reported that if surface root resorption of the cementum associated with replantation causes exposure of a contaminated dentinal canal, inflammatory root resorption will occur without the resorption cavity being repaired with newly generated cementum (28). In the present study, after the roots had been vertically fractured, the root canals were left open for four weeks to allow bacterial infection, so there was a possibility that bacteria infiltrated not only the root canal, but penetrated all the way to the dentinal tubules. This may be why inflammatory root resorption occurred in the area where the cementum was damaged or missing after replantation. In the experimental group, there was less inflammatory resorption than in the control group. This is thought to have been because EMDOGAIN® promoted the formation of cementum after surface resorption. Also, because it has been reported that EMDOGAIN® inhibits inflammation (29) and has an antibacterial effect (30-32), the inhibition of surface inflammation of the contaminated dentinal tubules may have played a role in reducing root resorption.

With respect to ankylosis of the damaged part of the periodontal ligament, there were no differences between the two groups. This could possibly be because the root canals were left open for four weeks following vertical fracture, causing bone resorption in the area around the line of fracture as a result of bacterial infection, and bone was not sufficiently regenerated after replantation. In the experiment, observation was carried out eight weeks after replantation, and if a longer period had been allowed, ankylosis might have occurred. Also, there have been reports in which ankylosis was diagnosed five years following replantation (33), and an observation period of eight weeks may be too short to evaluate

ankylosis. However, in research in which EMDOGAIN® was applied as part of intentional replantation, and the status of healing at the site where periodontal ligament was present was evaluated, the EMDOGAIN® was shown to reduce ankyloses (20,21,24,25), and the use of EMDOGAIN® can be expected to prove advantageous in healing at sites where there is residual periodontal ligament.

In many cases, if the root fractures vertically, the periodontal ligament in the area around the line of fracture is lost. If EMDOGAIN® is used and cementum is regenerated when performing intentional replantation, it is believed that periodontal pockets will become shallow following surgery, and there will be less root resorption, improving the prognosis for the fractured root. In the experiment described here, the root planing range was set at 1.4 mm, envisioning loss of the periodontal ligament in the area around the line of fracture. Because we were not able to regenerate all of the damaged cementum despite using this approach, this may be effective only when the volume of periodontal ligament lost after fracture is even smaller.

Acknowledgements

The authors thank Sun Medical for providing Super Bond used in this study.

The authors deny any conflicts of interest related to this study.

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Figure Legends

Figure 1: Preparation of the roots in the experimental group

A: Extracted root. B: After bonding. Root surface after root planing (↔). C: After application of EMDOGAIN®

Figure 2: Histological sections of experimental group

a: New cementum (arrowhead) was seen on the resorbed root surface.

Figure 3: Histological sections of control group

a: No new cementum was seen on surfaces where root planing was done (arrowhead).

b: Root resorption and multinucleated giant cells (arrow) can be seen.

Table 1. Root surface morphology after eight weeks

mean±S.D.(mm)

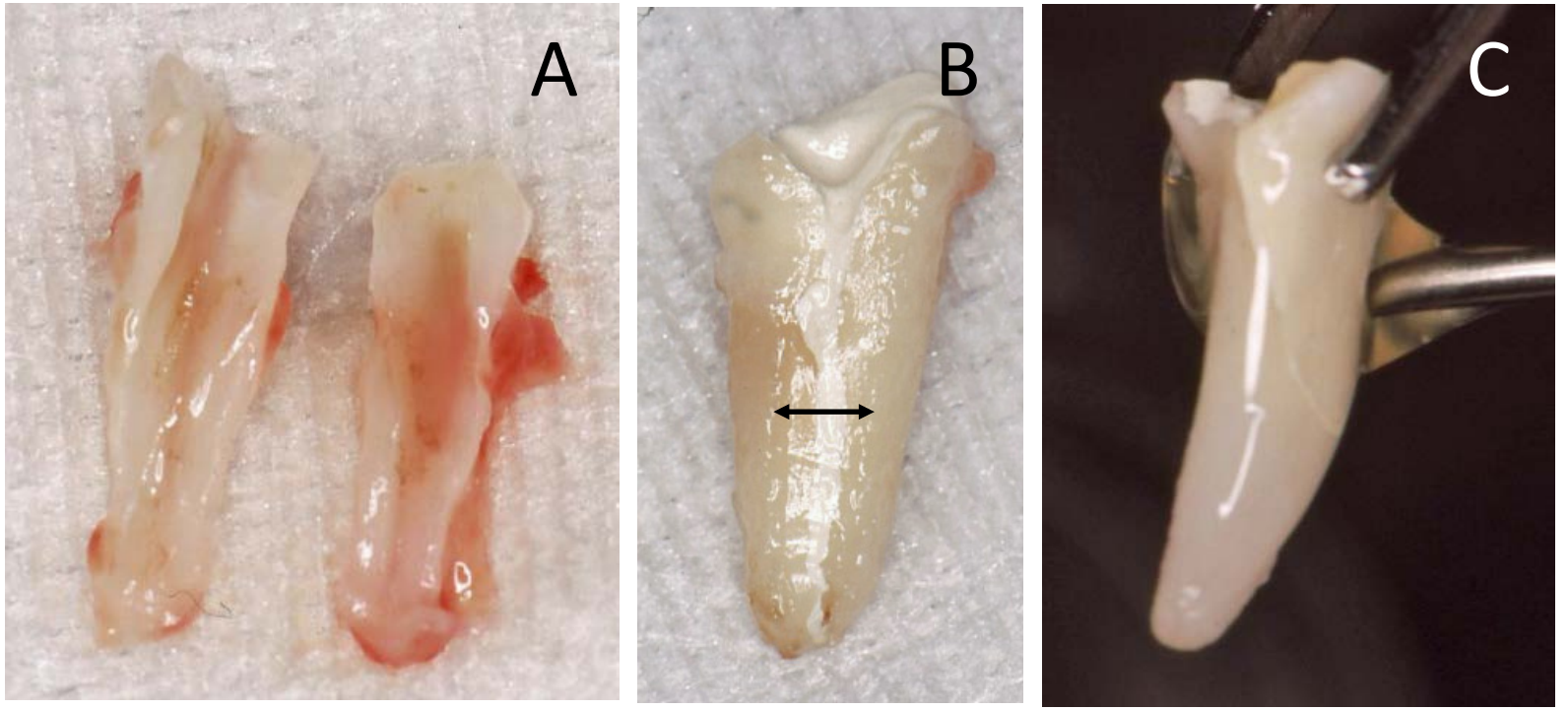
*: Significant difference between the two groups (Mann-Whitney U test, $p<0.01$)

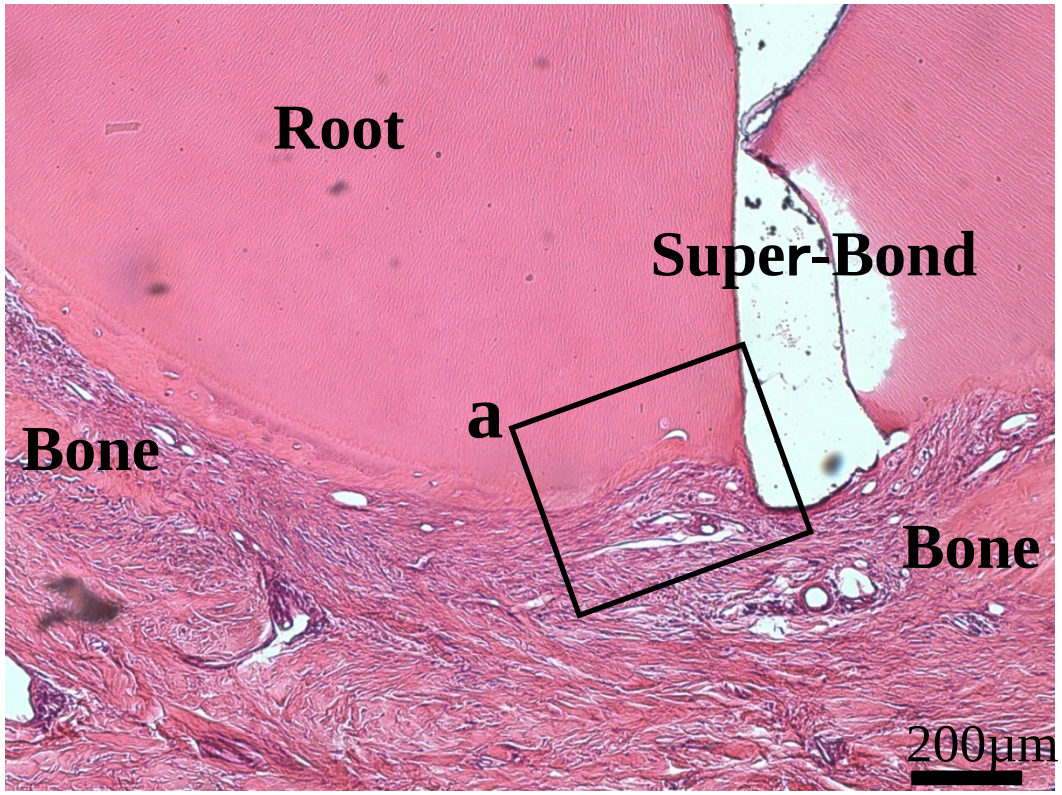
Table 1. Root surface morphology after eight weeks

	Root planed	New cementum *	Connective tissue	Inflammatory root resorption *	Ankylosis
Experimental group	1.39±0.31	0.49±0.34	0.63±0.37	0.03±0.13	0.22±0.34
Control group	1.27±0.23	0±0	0.58±0.46	0.53±0.57	0.15±0.34

mean±S.D.(mm)

*: Significant difference between the two groups (Mann-Whitney U test, $p<0.01$)


A
B
C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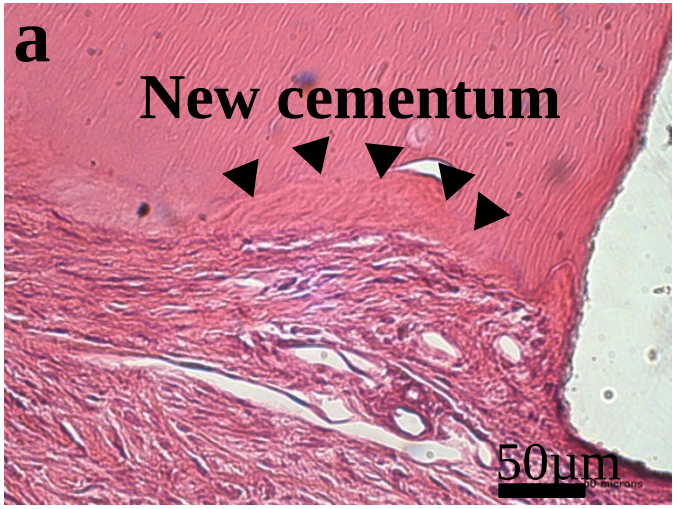
Bone
Bone
200μm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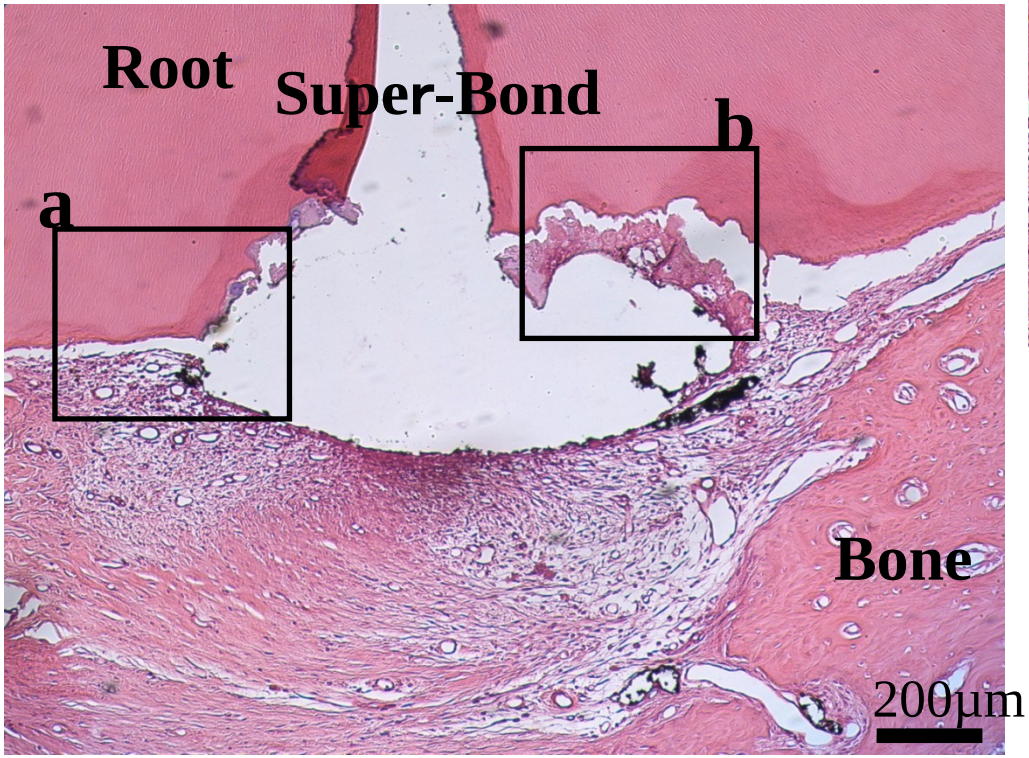
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a
b
Bone
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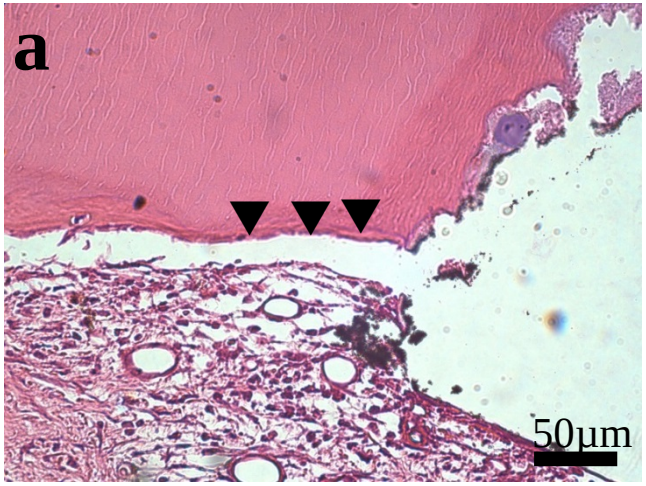
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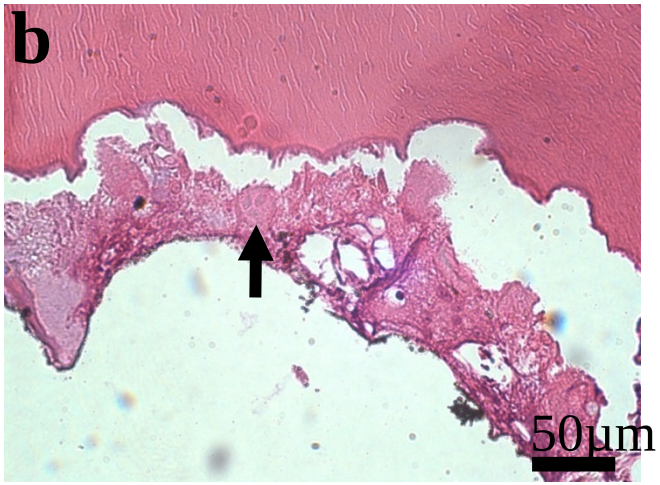
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