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Application of 4-META/MMA-TBB resin for fixation of membrane to tooth in guided tissue regeneration in dog

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The aim of this study was to evaluate 4-META/MMA-TBB resin as an alternative to sling sutures for fixation of membrane to tooth in GTR in terms of its effect on tissue regeneration. Dehiscence periodontal defects were created in 6 dogs which were divided at random into two groups, 3 dogs in each group: an experimental group, in which non-absorbable or absorbable membrane was fixed to the teeth with 4-META/MMA-TBB resin; and a control group, in which sling sutures were applied to fix the two types of membrane. Histologic and histometric evaluation was carried out at 8 weeks post-operatively to determine healing response in each group. Both methods of achieving membrane fixation to tooth were effective in inhibiting epithelial migration and encouraging formation of regenerated periodontal tissues around the root surfaces. These results suggest that 4-META/MMA-TBB resin is as effective as sling sutures in achieving membrane fixation in GTR and is, moreover, easier to apply.

Keywords: 4-META/MMA-TBB resin, Guided tissue regeneration (GTR), Membrane fixation

INTRODUCTION

Recently, two new approaches have been emphasized in the promotion of attachment of new tissues following periodontal therapy: one involves inhibition of epithelial tissue migration on the exposed root surface and promotion of cell attachment and differentiation to provide a more favorable environment for regeneration[1)]; the other focuses on proliferation of residual periodontal ligament (PDL)-derived cells on the exposed root surface based on the theory that PDL-derived undifferentiated mesenchymal cells are the only cells that differentiate into cementoblasts[2)]. Coronally-oriented cell growth requires growth inhibition of other tissues, particularly epithelial tissue, and this is the basis of the therapy known today as guided tissue regeneration (GTR)[2)]. However, evidence indicates that the most common form of surgical wound healing involves the proliferation of junctional epithelium[3,4)]. The apical migration of epithelial cells toward the root surface is inhibited during the early stages of periodontal wound healing, which induces re-growth of PDL cells toward the treated root surface and promotes attachment of new connective tissue[2,5-7)]. To achieve attachment, a barrier made of expanded polytetrafluorethylene (e-PTFE) membrane is inserted into the space between the gingival tissue on one side and the exposed root surface on the other. The role of this barrier is to prevent epithelial cell migration on the exposed root surface. Accordingly, in GTR therapy, the coronal barrier membrane is tightly fixed to the tooth by the sling suture procedure. However, Hardwick *et al*.[8)] have noted that inaccessibility of surgical site makes this procedure not only difficult, but also time-consuming. Furthermore, Murphy *et al*.[9)] have recently pointed out that the complexity of the method itself is one reason for subsequent failure of GTR. This suggests that a simplified and reliable method of membrane fixation would reduce GTR procedure-associated failure.

An extremely versatile material, 4-Methacryloxy ethyl trimellitate anhydride/methyl methacrylate-tri-n-butylborane (4-META/MMA-TBB) resin is used in restoration, direct pulp-capping[10)], treatment of fractured teeth[11-14)] and mucosal healing[15,16)]. When standard resin is used for periradicular treatment, bonding of materials may be hampered by contamination and difficulty in ensuring a dry surface. However, the bond strength of 4-META/MMA-TBB resin does not change, even on blood-stained dentin, and has been reported to be greatest on contaminated dentin[17)]. This ability to produce a durable bond under such adverse conditions offers advantages for periodontal surgery. Moreover, being easy to apply, using the brush-dip technique makes it ideal for use in periodontal surgery. While offering certain advantages in terms of applicability, however, the effect of 4-META/MMA-TBB resin on new tissue generation remains to be determined.

The aim of this study was to evaluate 4-META/MMA-TBB resin as an alternative to sling sutures for fixation of membrane to tooth in GTR in terms of its effect on tissue regeneration.

MATERIALS AND METHODS

Six healthy, male beagle dogs aged 30 months were used in this study. The animals were placed under

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general anesthesia with sodium pentobarbital (Somunopentyl®, Kyoritsuseiyaku, Tokyo, Japan) at a dose of 0.5 mg/kg. In order to reduce hemorrhage at surgical sites, local infiltration anesthesia (Xylestesin®, 1:80,000 epinephrine, Sumitomo 3M Ltd., Tokyo, Japan) was also applied. All experiments were performed according to the Guidelines for the Treatment of Experimental Animals at Tokyo Dental College.

Following intracrevicular incisions, full thickness mucoperiosteal flaps were elevated at both the vestibular and oral aspects of the jaw. Dehiscence periodontal defects were produced at a depth of approximately 7 mm as measured from the cemento-enamel junction (CEJ) using a slowly rotating cylindrical bur at the mesial buccal surfaces of both the 3rd and 4th mandibular premolars (Fig. 1a). To prevent spontaneous healing, a rubber base impression material was placed in each defect. In order to allow coronal displacement of the flaps, vertical and horizontal releasing incisions were performed. Following wound rinsing with sterile saline, the flaps were sutured with 4-0 silk. At 7 days after surgery, the sutures were removed. During the first 4 weeks after surgery, the rubber base impression material was removed from each defect and oral hygiene measures initiated. At 12 weeks after surgery, full thickness mucoperiosteal flaps were raised, the granulation tissue removed and root surfaces facing the defects scaled and planed. Using a small round bur, a reference notch indicating the bottom of the defect was prepared on the root surface. Following scaling and planing, in the experimental group, both the 3rd and 4th mandibular premolars were etched with a citrate agent (Green Activator, Sun Medical, Moriyama, Japan) and rinsed with distilled water, after which the following treatment was performed: non-absorbable (Gore-Tex® regenerative membrane W. L. Gore & Assoc., Flagstaff, AZ, USA) or absorbable (MEMBRANE®, GC Corporation, Tokyo, Japan) membrane was adjusted to cover the exposed root surface from the CEJ to a point 2–3 mm apical to the bone crest and fixed with 4-META/MMA-TBB resin (Super Bond C&B®, Sun Medical, Moriyama, Japan) (Figs. 1b and c). The resin sealed the membrane circumferentially to the teeth at the CEJ. In the control group, both membranes were tightly adapted to the teeth by sling sutures at the CEJ. Following placement of non-absorbable or absorbable membrane in both groups, the buccal and lingual flaps were positioned slightly coronally and sutured. These sutures were removed at 2 weeks after treatment.

The animals were euthanized with an intravenous overdose of sodium pentobarbital at 8 weeks after treatment. The jaw of each animal was removed, and specimens containing the experimental tissues placed in buffered formalin.

Histologic and histometric examination

For histologic and histometric examination, specimens were decalcified with 10% ethylenediamine tetraacetic acid (EDTA, Wako, Tokyo, Japan). The specimens were then dehydrated in ethanol, embedded in paraffin, serially sectioned to 5 μm in thickness in the buccolingual orientation and stained with hematoxylin-eosin. Six sections were used from each root for microscopic examination and histometric assessment at 40×magnification. The following distances were measured in mm: 1) from the notch (N) to the cemento-enamel junction (CEJ) to determine amount of defect; 2) from N to the coronal portion of new cementum (C) to determine amount of new cementum; 3) from N to the coronal portion of new bone (B) to determine amount of new bone; 4) and from the apical (AE) to the coronal (CE) portion of new epithelium to determine amount of new epithelium (Fig. 2). Statistical analysis was performed using the multiple comparison Scheffe test, with each animal serving as a statistical unit.

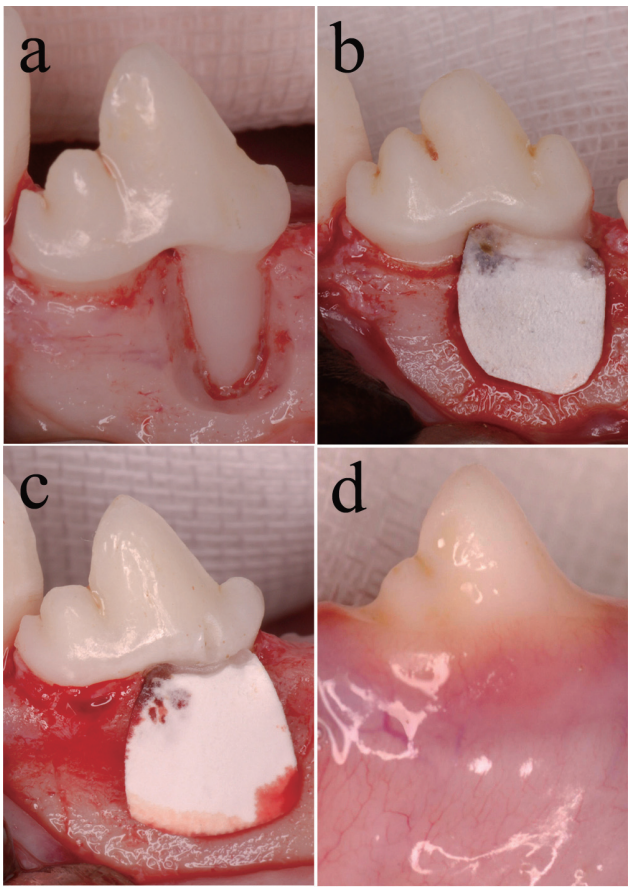

Fig. 1 Photographs of experimental group: dehiscence defects created (a); 4-META/MMA-TBB resin had fixed non-absorbable membrane (b) or absorbable membrane (c) to tooth; treated area at after 8 weeks of healing (d).

Scanning electron microscopy

The micromorphology of the membrane treated with 4-META/MMA-TBB resin was examined by scanning electron microscopy (SEM). The specimens were

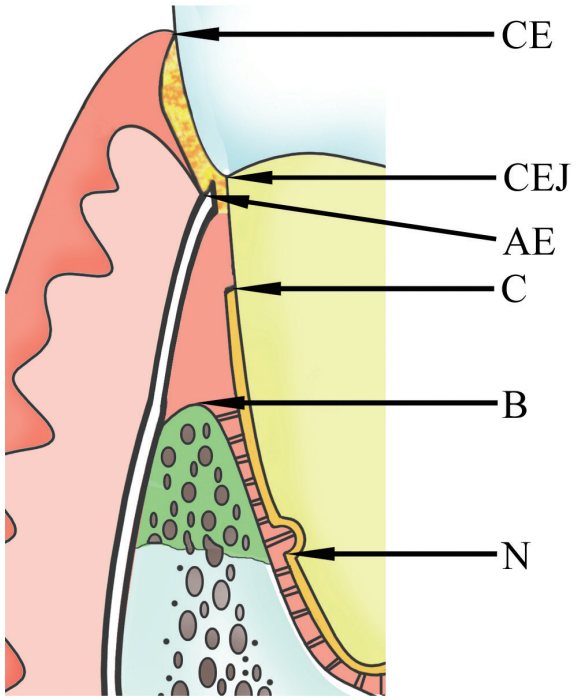

Fig. 2 Schema demonstrating reference points used to measure various linear distances in histological specimens. CE: coronal level of newly formed epithelium, CEJ: cemento-enamel junction, AE: apical level of newly formed epithelium, C: coronal level of newly formed cementum, B: coronal level of newly formed alveolar bone, N: apical border of notch.

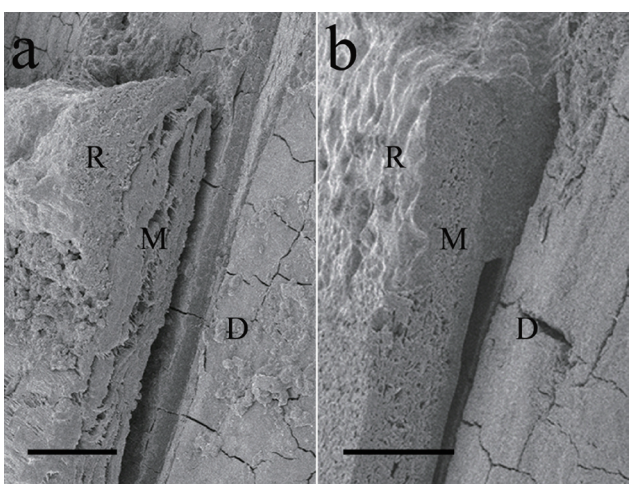

Fig. 3 SEM micrographs of interface between dentin and membrane.
a. (non-absorbable membrane); b. (absorbable membrane): Cross-sectional SEM view of specimen of 4-META/MMA-TBB resin (R). R had fixed non-absorbable membrane (M) or absorbable membrane (M) to dentin (D). Outer surface of membrane covered with R. Bars: a. 250 μm; b. 200 μm.

mounted separately on aluminum stubs and sputter-coated with Au-Pd alloy. The specimens were viewed and photographed using a scanning electron microscope (ERA-8900FE, ERIONIX, Tokyo, Japan).

RESULTS

Histologic observation

During the healing period, the gingiva adjacent to all experimental teeth exhibited only minute clinical signs of inflammation (Fig. 1d). No exposure of the membranes was detected. Observation by SEM revealed that the inner surface of the non-absorbable (Fig. 3a) or absorbable membrane (Fig. 3b) had been fixed to the teeth with the 4-META/MMA-TBB resin at the CEJ. The outer surface of the membrane was also covered with resin (Figs. 3a and b).

In the experimental group, histological analysis demonstrated that the 4-META/MMA-TBB resin (*) had fixed the coronal border of the membrane to the tooth (Figs. 4a and 5a). The teeth that received absorbable membrane showed a small amount of resorption at the coronal portion of the membrane (Fig. 5a). Migration of epithelium onto the surface of the 4-META/MMA-TBB resin (*) was observed in all test teeth (Figs. 4b and 5b). Apical extension of epithelial regeneration terminated at the coronal border of the membrane (Fig. 4b). A layer of epithelial cells lined the entire length of the 4-META/MMA-TBB resin surface. The apical end of the junctional epithelium was located just beneath the membrane (Fig. 5b). Very little inflammatory cell infiltration was observed in the subjacent connective tissue in either the non-absorbable (Fig. 4c) or absorbable membrane (Fig. 5c). In the areas adjacent to previously denuded root surface, GTR had induced an increase in cementogenesis and deposition of alveolar bone in all experimental teeth (Figs. 4a and 5a). New cementum with inserting collagen fibers had formed in the notch area, extending along the root surface coronally (Figs. 4d and 5d). The alveolar crest also showed an increase in height coronally to the notch in several areas (Fig. 5d).

In the control group without 4-META/MMA-TBB resin, the apical end of the junctional epithelium showed clear attachment to the CEJ. Regeneration of alveolar bone, cementum and PDL was discernible with both types of membrane (Fig. 6a). Insertion of periodontal ligament fibers into the newly formed cementum was clearly detected (Fig. 6b). No clear bone ankylosis or root resorption was observed at the root surface in any experimental teeth.

Histometric analysis

No difference was evident in length of defect between the experimental and control groups in either the absorbable or non-absorbable membrane groups. Length of new bone was greater in the experimental group than in the control group using absorbable membrane. With non-absorbable membrane, however, no statistically significant difference was evident in the

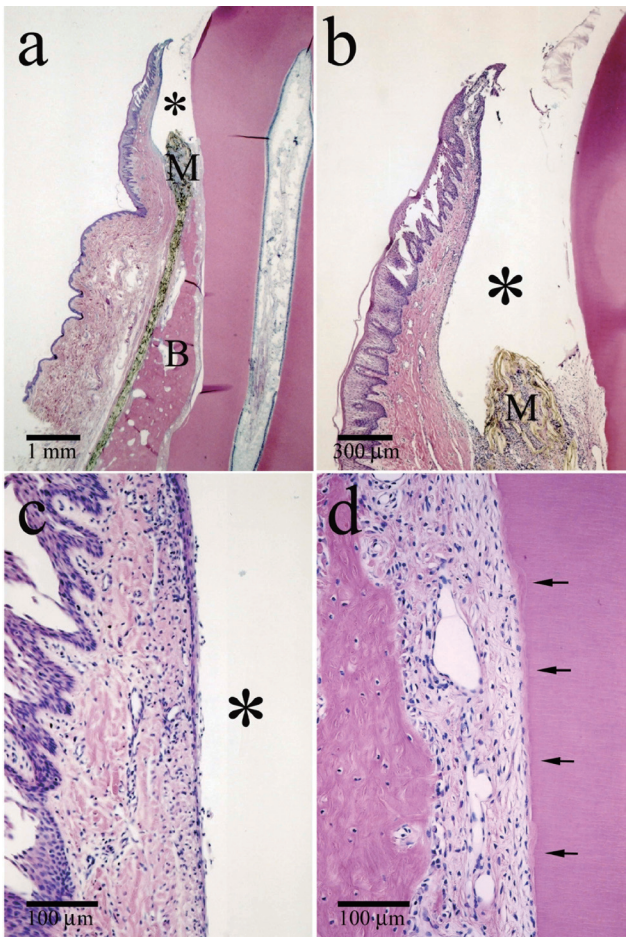

Fig. 4

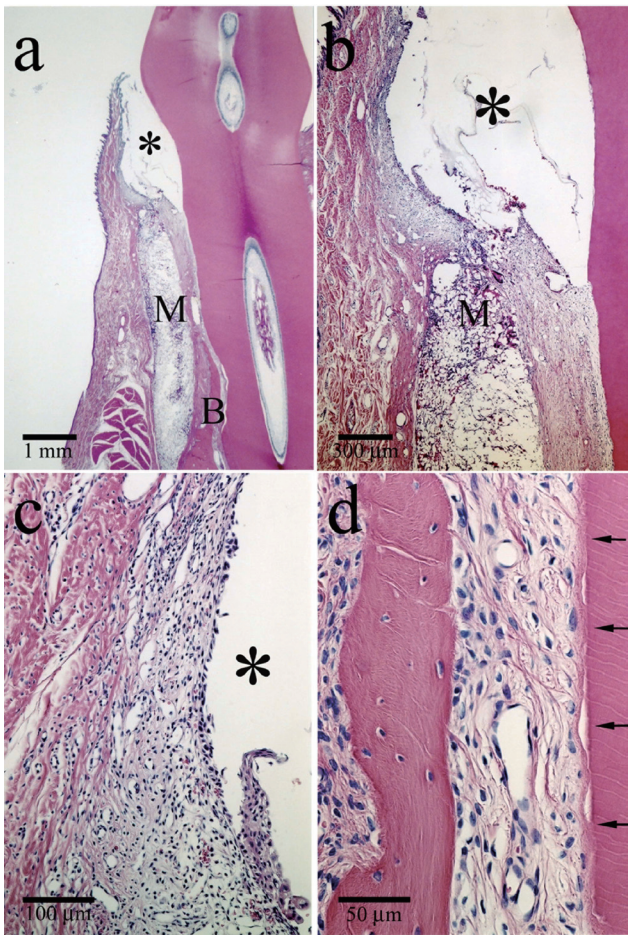

Fig. 5

Photographs of experimental specimens: 4-META/MMA-TBB resin (*) had fixed non-absorbable (Fig. 4) or absorbable (Fig. 5) membrane to tooth. Membrane covered root portion and newly formed bone observed in Fig. 4a and 5a. Higher magnification of gingival tissue seen in specimens in Fig. 4b and 5b. Regeneration of epithelium on surface of 4-META/MMA-TBB resin (*) observed. Higher magnification of connective tissue seen in specimen in Fig. 4c and 5c. No inflammatory cell infiltration observed in subjacent connective tissue. Higher magnification of periodontal tissue seen in specimen in Fig. 4d and 5d. Formation of new bone, cementum and periodontal ligament clearly evident.
M: membrane, B: new bone, arrow: new cementum, (*): 4-META/MMA-TBB resin (original magnification. a ×1.25, b ×5, c ×20, d: Fig. 4 ×20, Fig. 5 ×40. a, b, c and d: hematoxylin-eosin staining).

length of either new cementum or new bone. No difference was found in cementum formation between the experimental and control groups treated with absorbable membrane (Table 1). Migration of epithelium onto the resin material was seen with both non-absorbable and absorbable membrane. We measured the distances between the apical and the coronal portion of new epithelium. Although elongation of junctional epithelium was greater with both non-absorbable and absorbable membranes in the experimental group, it was still only minimal. A significant difference ($p<0.01$) in length of epithelium was observed between the two groups. Mean epithelium length was significantly longer in the experimental group (non-absorbable, 2.3 mm; absorbable, 1.8 mm) than in the control group (non-absorbable, 2.0 mm; absorbable, 1.0 mm: $p<0.01$) (Table 1).

DISCUSSION

The results showed that application of resin facilitated adequate periodontal tissue formation. This finding is consistent with that of Nyman *et al.*[18], supporting the effectiveness of 4-META/MMA-TBB resin in the promotion of coronal migration of PDL cells. The results of histologic and histometric observation in this study indicate that 4-META/MMA-TBB resin not only inhibited epithelial tissue migration toward the root surface, but also guided proliferation of PDL cells during wound healing. We measured the distances

Table 1 Results of histometric assessment (mm) of area surrounding periodontal tissue in 4-META/MMA-TBB resin (experimental group) and sling suture (control group)

Membrane	Observation Group	Defect X (SD)	P	New cementum X (SD)	P	New bone X (SD)	P	Epithelial regeneration X (SD)	P
Non -absorbable	Experimental	7.1 (0.1)	]NS	5.9 (0.1)	]NS	3.8 (0.1)	]NS	2.3 (0.1)	]*
	Control	6.9 (0.2)		5.9 (0.1)		3.8 (0.1)		2.0 (0.1)	
Absorbable	Experimental	7.3 (0.1)	]NS	6.8 (0.1)	]NS	2.9 (0.2)	]*	1.8 (0.1)	]*
	Control	7.2 (0.1)		6.8 (0.1)		0.6 (0.1)		1.0 (0.1)	

X=mean value; SD=standard deviation of the mean *p<0.01 (n=6)
NS=not significant

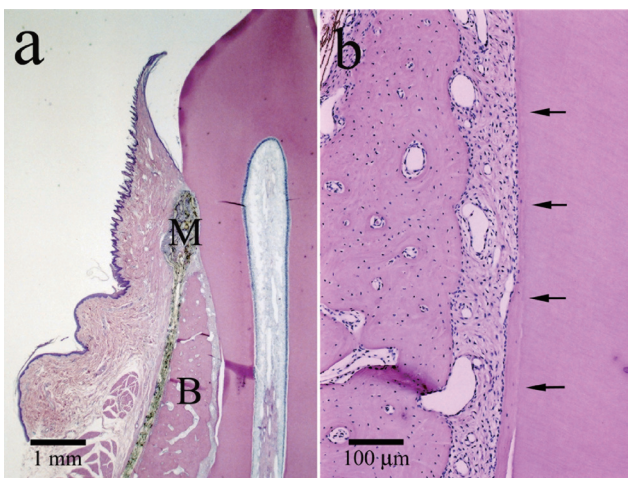

Fig. 6 Control specimen showing sling sutures fixed non-absorbable membrane to tooth. a: Portion of root covered in membranes showed new bone formation. b: Higher magnification of area surrounding newly formed periodontal tissue seen in Fig. 6a showing newly deposited cementum, new alveolar bone and new periodontal ligament. M: membrane, B: new bone, arrow: new cementum (original magnification. a ×1.25, b ×10. a and b: hematoxylin-eosin staining).

between the apical and the coronal portion of new epithelium. Although elongation of junctional epithelium with both non-absorbable and absorbable membranes was greater in the experimental group, it was still only minimal. This periodontal tissue formation was accompanied by regeneration of new alveolar bone, cementum and PDL tissue.

Formation of new periodontal tissues is probably accompanied by regeneration of new alveolar bone, cementum and PDL tissue. In particular, cells growing from residual PDL tissues to surgically exposed root surfaces may play an essential role in GTR. It has been suggested that GTR treatment promotes this mechanism by blocking contact between epithelial and gingival connective tissues and the exposed root surface[6,18]. Taken together with those of earlier studies[19], the results of the present study suggest the presence of progenitor cells from adjacent, undisturbed and healthy PDL or bone marrow.

The 4-META/MMA-TBB resin is widely employed as a dental adhesive. Nakabayashi has reported its adhesive properties and biocompatibility with enamel[20-22]. However, little is known about the application of 4-META/MMA-TBB resin to the fixation of membrane. In this study, the micromorphology of the membrane treated with this resin was examined by SEM. The structure of the e-PTFE membrane showed a characteristically porous structure. The resin had infiltrated the network of pores in the e-PTFE membrane, thus fixating it. On the other hand, with the poly-lactic-co-glycolic acid (PLGA) membrane, fixation was achieved through a chemical reaction with the resin.

The most important findings of the present study demonstrated that an epithelial cell layer hemmed the outer surface of the 4-META/MMA-TBB resin and reached the membrane surface. Generally, when applied to promote membrane fixation to tooth, 4-META/MMA-TBB resin is polymerized at the connective tissue interface before deep infiltration of monomers via blood or saliva, creating a space for subsequent epithelial tissue regeneration between the resin and connective tissue[23]. Our results showed no clear inflammatory reaction in the regenerated periodontal tissue. This lends support to the previously reported characteristics of 4-META/MMA-TBB resin as being a biocompatible material which adheres to metal, enamel and dentin that can be applied safely to the vital tooth[24]. It seems likely that 4-META/MMA-TBB resin induced less tissue irritation in the newly formed gingival tissue in this study, which is compatible with the results of Tsuchiya *et al.*[23] demonstrating that 4-META/MMA-TBB resin covered the wound by adherence with laminins and integrins in the initial process of epithelial regeneration.

Gingival recession and subsequent exposure of the coronal portion of the membrane has been described as a potential complication during healing following GTR-therapy[25,26]. Membrane exposure throughout the

healing process may influence new attachment formation[27]. No gingival recession or exposure of the membrane was observed in the present study. This was probably due to the biocompatibility of 4-META/MMA-TBB resin[28-30] allowing fixation of membrane to tooth while protecting against various stimuli such as bacterial inflammation. It should also be noted that the layer of regenerated epithelial cells which had adhered to the resin was sufficient to secure the subgingival region of the coronal membrane. It is possible that this, in combination with the regenerative ability of periodontal ligament, may have prevented apical displacement of the flap during healing and subsequent exposure of the membrane. The membrane is subsequently secured with resin over a period of 4–6 weeks, after which the resin should be removed by surgical means to prevent the built-up of bacterial deposits that often occurs otherwise.

The results showed that the sling suture procedure promoted sufficient healing . A tight fixation between membrane and tooth was achieved in both the control and 4-META/MMA-TBB resin groups. However, Hardwick *et al.*[8] noted that suturing a membrane to the defective region of a tooth was time-consuming and required a high level of physical skill. In fixing a membrane, it is important to achieve simplification of placement and post-operative management, regardless of location of surgical site. Individual variation will occur, and it is necessary to fix the membrane to the contours of the tooth.

In conclusion, the results of this study suggest application of 4-META/MMA-TBB resin as a useful alternative to sling sutures for membrane fixation in GTR, being easier to apply. Furthermore, application of 4-META/MMA-TBB resin for membrane fixation may eliminate failure in, and thus facilitate the clinical application of GTR.

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