SHORT COMMUNICATION

Vascular Regeneration after Tooth Replantation with Application of Enamel Matrix Derivative (EMD)

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Introduction

Application of enamel matrix derivative (EMD) for regeneration of periodontal tissue is a recent clinical topic¹⁾. The method is based on the idea that acellular cementum can be used to attach periodontal fiber bundles to the root surface. The enamel protein produced in the course of tooth genesis is thought to contribute to acellular cementum formation, and various studies have been conducted in this field²⁻⁴⁾. EMD has been observed by scanning electron microscopy (SEM) and autoradiography^{5,6)} to attach to the root before the formation of cementum. Actual periodontal treatment using an animal model has also been reported⁷⁾. Administration of EMD to artificial cavities, created in the incisor roots of monkeys, induced formation of acellular cementum attached to the dentin, while in the control group, only hard tissue with poor attachment was formed. Basic and clinical studies on periodontal tissue regeneration are underway based on the above results. In the present study, EMD was used in tooth replantation to observe changes in the regeneration of cementum and the vascular networks of periodontal ligaments.

Materials and methods

Two beagle dogs (12 and 15 kg) were used in this study. The experimental animals were put under general anesthesia (Nembutal[®], 25 mg intravenously ; Abbott, U. S. A) for performance of all experimental procedures.

1. Replantation procedures

The second, third and fourth premolars of the animals were all (upper, lower, right, and left) mesiodistally divided by a diamond bar with a dental turbine and were extracted. Endodontic treatment and root canal filling (Vitapex[®], NEO Dental Chemical Products, Japan) were performed on each distal root. Enamel matrix derivative (EMD ; Emdogain[®], BIORA AB, Sweden) was then applied to the root cementum of the right side teeth, while the left teeth served as controls, receiving no application. The distal roots were then replanted in the original alveolar sockets, and the mesial gingiva was sutured. An antibiotic (Penicillin G, 200,000 U) was administered postoperatively, and sutures were removed 7 days after the operation.

2. Morphological Analysis

The experimental animals were sacrificed at 30

days after tooth replantation. The common carotid arteries were cannulated and Ringer's solution containing 0.2% heparin was perfused until the jugular veins were cleared of blood. After perfusion, 2% glutaraldehyde phosphate buffer solution (pH 7.4) was injected into the carotid arteries to provide fixation. After fixation, histologic sections with HE staining were made from maxilla in the routine method and observed by light microscope (LM). To make vascular resin cast models (RCM), the coronoid processes were resected to expose the inferior alveolar arteries, which were then injected manually with synthetic resin (Mercor[®], Dai Nippon Ink, Japan). The soft tissue was digested by incubation at 40°C for approximately 2 weeks in a phosphate buffer solution (pH 8.4) containing 20% proteinase (Prozyme 6[®], Amano, Japan). All specimens were washed thoroughly with 40°C tap water and freeze-dried. The resin cast models were observed by stereoscopic microscope (Digital microscope[®], Keyence, Japan). The tooth root released by protease treatment was ion coated with platinum palladium, and specimens were examined by SEM (JSM 6301 F, JEOL, Japan).

Results

1. Control group

The cementum (C) in the control group was resorbed over a wide area, and convexoconcave shapes were observed on the root surface by light microscope (Fig. 1 a). In some areas (*), the cementum was resorbed to the extent that the dentin (D) was exposed. The exposed dentin (D) revealed sparse periodontal fibers (Fig. 1 b) at higher magnification. SEM observation of the root surface revealed resorption of cementum and assemblages of Howship's lacunae (*) (Fig. 1 c). Dentin tubules (arrows) appeared on the root surface where resorption reached the dentin. The original cementum surface (C) was seen as a higher plane. Regeneration of the vascular network of the periodontal ligament was assessed by stereoscopic microscope using the vascular resin cast model (Fig. 1 d). A convexoconcave shape (*) was found on the root surface. A vascular network of periodontal ligament (PDL) was regenerated in the crevices, and connected

to blood vessels in the alveolar bone (AB) via Folkmann's canals. On the area where it faced the root resorption (*), the vascular network was partially disconnected and formation was not complete (arrows).

2. Experimental (EMD) group

In the EMD group, the root surface was covered by cementum (C) and exposure of dentin (D) was not observed by light microscope (Fig. 2 a). Even in areas where resorption had been seen to reach the dentin (D) under higher magnification (Fig. 2 b), the surface showed a cover of acellular cementum (arrow). On the root surface, however, a calcific substance (*), which may be regarded as reparative cementum, had accumulated assemblages of Howship's lacunae, and the dentin tubule was not exposed (Fig. 2 C). A regenerated vascular network of periodontal ligament was observed in the vascular resin cast models (Fig. 2 d). The vascular network of periodontal ligament (PDL) had regenerated between the alveolar bone (AB) and the root, and the network was formed completely without any disconnection.

Discussion

The widths of periodontal ligaments change due to causes such as orthodontic tooth movements or occlusal trauma. The width, however, is known to recover to its original thickness. Microcirculation studies have revealed that bone resorption and formation take place along the areas where blood vessel configurations change, enabling the periodontal ligament to maintain a constant width⁸⁻¹⁰. It has also been suggested that a sensor in the ligament controls the width according to information received from the cementum¹¹. In addition to the fiber and blood vessels, the cementum is also thought to be involved in the regeneration of the ligament. After tooth replantation, ankylosis often occurs at the border of the exposed dentin and alveolar bone, which hinders the regeneration of the periodontal ligament¹² as well as the regeneration of blood vessels. In order to achieve successful tooth replantation, the periodontal ligament and cementum need to be preserved as much as possible to

Fig. 1 a (Control ; LM) : The cementum (C) was resorbed over a wide area, and convexoconcave shapes were observed on the root surface (*). Alveolar bone (AB), Dentin (D). Periodontal ligament (PDL).

b (Control ; LM) : In some areas, the cementum (C) was resorbed to the extent that the dentin (D) was exposed. The exposed dentin revealed sparse periodontal fiber.

c (Control ; SEM) : Root surface revealed resorption of cementum and assemblages of Howship's lacuna (*). Dentin tubules (arrows) appeared where resorption reached the dentin. The original cementum surface (C) is seen as a higher plane.

d (Control ; RCM) : RCM was observed by stereoscopic microscope. On the area where it faced the root resorption area (*), the vascular network was partially disconnected and formation was not complete (arrow). Alveolar bone (AB), Vascular network of periodontal ligament (PDL).

Fig. 2 a (EMD ; LM) : The root surface of the EMD group was covered by cementum (C). Alveolar bone (AB), Dentin (D), Periodontal ligament (PDL).

b (EMD ; LM) : Root resorption area was covered by acellular cementum (arrows) under higher magnification. Dentin (D), Cementum (C).

c (EMD ; SEM) : The assemblages of Howship's lacuna were found on the root surface. However a calcific substance (*), which may be regarded as reparative cementum, had accumulated and the dentin tubule was not exposed.

d (EMD ; RCM) : The vascular network of periodontal ligament (PDL) had regenerated between the alveolar bone (AB) and the root, and the vascular network was formed completely without any disconnection.

promote blood vessel regeneration. In an experiment with EMD, acellular cementum became attached to the root 8 weeks after replantation, but attached only poorly to intrinsic fibered tissue in controls¹³⁾. Circulation changes during periodontal ligament regeneration occurred at a rather early phase. Glomerular blood vessels proliferated into resorption fossa reaching the dentin, and blood vessel regeneration occurred 2 weeks after replantation¹⁴⁾. In regions where reparative cementum was not formed, ankylosis occurred in exposed dentin and the alveolar bone 4 weeks after replantation, and the periodontal fiber and vascular network were not regenerated. In this study, widely ranging cementum resorption was observed in the control group 4 weeks after replantation. In many cases, this reached the dentin and the dentin tubule was exposed. Attachment of the regenerated periodontal fiber rarely occurred in such areas, and some vascular resin casts of the network were found to be disconnected.

If replantation is successful, the resorption fossas become smaller at 3 weeks and the periodontal fiber and vascular network regenerate in the repaired cementum within 4 weeks¹⁵⁾. In the experimental (EMD) group of this study, the area with root resorption was smaller, and resorption fossa that reached the dentin was covered with repairing cementum. Regenerated fiber was found on the repairing cementum, and SEM images showed a covering of repairing cementum over the dentin tubules seen on the surface of resorption fossa. The periodontal ligament also formed a complete network as normal tissue. Taken together, the results suggest that circulation in the periodontal ligament regenerates if repairing cementum is formed 4 weeks after replantation, resulting in tooth adhesion.

This indicates that postoperative formation of repairing cementum is the key point in successful replantation. It is still necessary to observe in detail the effects of EMD and the regeneration of microcirculation by SEM. The present study suggests that effective use of EMD may accelerate the formation of repairing cementum and the regeneration of the periodontal ligament.

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