TITLE

Hybrid Layer Seals the Cementum / 4-META/MMA-TBB Resin Interface

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INITIALS

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Abstract

Although 4-META/MMA-TBB resin has adhesive properties to dentin, and has been clinically used for the bonding treatment of vertically fractured roots and apicoectomy, there has not been any investigation on the adhesion of 4-META/MMA-TBB resin to cementum. The purpose of this *in vitro study* was to evaluate the bonding and the sealing ability of 4-META/MMA-TBB resin to cementum. Bovine root cementum and dentin surfaces were treated with a citric acid and ferric chloride solution, and the 4-META/MMA-TBB resin was applied on the treated surfaces before testing. The micro-tensile bond strength and the leakage levels obtained for the cementum were almost equal to those for the dentin. In SEM and TEM observations, a hybrid layer approximately 2-3 μm in thickness, was observed at the interface between the resin and the cementum. It is concluded that 4-META/MMA-TBB resin adhered to cementum via a hybrid layer on cementum as previously reported for dentin.

Introduction

In recent years, dentin bonding systems have played a significant role for reducing marginal leakage and have been used for the bonding of vertically fractured roots [1-3]and apicoectomy [4-8].

Vertical root fractures usually lead to advanced periodontal breakdown with deep periodontal pockets and vertical bone defects. Many of these cases have hopeless prognoses resulting in extraction [9-11]. Recently, many procedures have been attempted for the treatment of vertically fractured roots and some of these approaches have used cyanoacrylate or glass-ionomer cement [12-14]. It has also been reported that the resin directly applied to the root-end surface resected by apicoectomy effectively prevented leakage from the root canals and the dentinal tubules into surrounding tissues[4-8]. However, the long-term prognosis of these procedures has not been reported.

The ideal material for the bonding treatment of vertical root fractures and root-end sealing should be well tolerated in a moist environment, have low cytotoxicity, be non-absorbable, bacteriostatic, dimensionally stable, biocompatible, easily manipulated, and allow connective tissue attachment.

4-META/MMA-TBB (4-methacryloxyethyl trimellitate anhydride/methyl methacrylate -tri-*n*-butylborane) resin has been used in orthopedics and prosthetic dentistry. This resin fulfils several criteria, such as adhesive properties to dentin *in vitro* and *in vivo* [15,16], little cytotoxicity, achieves a high level of polymerization under wet conditions, and is highly biocompatible after complete curing [17,18]. One difficulty often encountered during periradicular surgery is contamination and ensuring a dry field, which potentially interferes with the bonding of some materials. However it was reported that a 4-META/MMA-TBB resin showed little change in bond strength when dentin had blood applied and produced the highest bond strength to contaminated dentin [19]. The ability to produce a durable bond under these conditions would be advantageous for use in endodontic surgery. In addition, the ease of handling of this resin by the brush-dip technique (the liquid and powder are mixed together to form an adhesive ball) is useful for applications on the exposed surface without cavity preparation.

The application of 4-META/MMA-TBB resin for bonding treatment and as a root-end sealant has been previously reported; these clinical studies have suggested that 4-META/MMA-TBB resin seals vertically fractured roots and prevents refractures in the bonding treatment [1-3]. It was also reported that 4-META/MMA-TBB resin used as a root-end sealant following apicoectomy and intentional replantation indicated a good prognosis at six months after surgery [8]. Therefore it seemed clinically useful to use 4-META/MMA-TBB resin for bonding treatment and root-end sealing.

Cementum is a specialized tissue (20-200 μm thick) which is mineralized and covers the root surfaces, but may be porous in nature. While the bond to dentin (which makes up the majority of the root) is important, that to cementum is also important to prevent microleakage and bacteria gaining access to the underlying dentin. If the adhesion of the bonding material is weak, dead spaces may be generated between the cementum or the dentin, and the resin. These dead spaces may provide a niche for bacteria or a starting point for leakage of oral fluids to track along interfaces. Thus good bonding to not only dentin but also to cementum is required. However, there has not been any investigation on the adhesion of 4-META/MMA-TBB resin to cementum.

The purpose of this *in vitro* study, therefore, was to evaluate the bonding and sealing ability of 4-META/MMA-TBB resin to cementum, using the micro-tensile bond strength (MTBS) and dye leakage tests.

Materials and Methods

Adhesive and bonding procedures

Fifty extracted bovine incisors were used with the approval of local and institutional review committees. Twenty teeth were used for the micro-tensile bond strength test, twenty teeth for the dye leakage test and ten teeth for SEM and TEM observation. After soft tissue and debris were removed from the tooth surface with a curette scaler, the crowns were removed at the cement-enamel junction with a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The root pulp in each was then extirpated with a barbed broach.

Equal numbers of specimens were allocated to four groups. An aqueous solution of 10%citric acid and 3%ferric chloride, 10-3, (activator Green®, Sun Medical Co., Ltd., Kyoto, Japan) was applied to the surface of the cementum with a brush for 0 sec (group A), 5 sec (group B) and 30 sec (group C). The presence of cementum on the specimens was confirmed by observation under an optical light microscope before applying the solution. In the fourth group, the root surface was extensively root planed with a curette scaler, removing all cementum to yield a dentin surface to which 10-3 solution was applied for 5 sec (group D). In all specimens, 4-META/MMA-TBB resin (Super Bond C&B® clear type, Sun Medical Co., Ltd.) was applied on the treated surface according to the manufacturer's instructions using a brush-dip technique. The diameter of the cured resin was 3 mm (Fig. 1A). All specimens were stored overnight in distilled water at 37°C, and then prepared in the relevant way for the following tests/observations.

Micro-tensile bond strength (MTBS) test

After 24hours, a light–cured resin composite (Herculite®, Kerr, San Francisco, CA, USA) block was incrementally built up to a height of 5 mm on the surface of the 4-META/MMA-TBB resin, using a fresh layer of Super Bond C&B® as the adhesive (Fig.1B).

The specimens were sectioned perpendicular to the adhesive interface with a low-speed diamond saw under a water coolant. Three cementum - resin sections (1 mm thick) were obtained per tooth. The sectioned specimens were carefully trimmed into an hourglass shape using a high-speed super-fine diamond bur (SF 1104R, Shofu Inc., Kyoto, Japan) to result in a 1 mm bonded interface and an interface area of 1 mm^2 (Fig.1C). Each specimen was then attached to a testing apparatus with a cyanoacrylate adhesive (Model Repair Ⅱ Pink, Dentsply-Sankin Industry Co. Ltd., Ohtawara, Japan), and subjected to micro-tensile bond strength testing [20] in a tabletop material tester (EZ Test, Shimazu Corporation, Kyoto, Japan) with a crosshead speed of 1.0 mm/min.

The MTBS in MPa was derived from dividing the applied force (N) resulting in fracture by the bonded area (mm^2). If specimens failed before actual testing, the MTBS was determined only from the specimens that survived, with a note made of the number of pre-testing failures. The data were analyzed by One-way ANOVA and Scheffe's test at the 5% level of significance. All fractured surfaces were sputter-coated with Pd-Pt (Ion sputter E-1030, Hitachi Ltd., Tokyo, Japan) and observed by SEM (S-4000, Hitachi Ltd.).

Assessment of dye leakage

The specimens were prepared as described in the adhesive and bonding procedures and then immersed in a 0.5% basic fuchsine dye solution at room temperature for 24 hours. The specimens were rinsed with water, air-dried and sectioned through the center of the material perpendicular to the bonding interface. The extent of dye penetration along the interface from the periphery was measured with a measuring ocular in a light microscope (BX50, Olympus, Ltd., Tokyo, Japan). Only the greater length of dye penetration (from whichever periphery) was recorded for each specimen (Fig. 1D), and the leakage value calculated using: (dye penetration length / interface length) X100.

The data were analyzed by the Kruskal-Wallis test at the 5% level of significance.

SEM observation of cementum surface and bonding interface

Micromorphology of the cementum surfaces treated with 10-3 solution and the interface in groups A, B, and C were examined under SEM.

The bonding procedures were performed as previously described. The resin-bonded cementum disks were cross-sectioned perpendicularly to the bonding interface using a diamond saw. Each specimen was etched with a 6 N aqueous solution of phosphoric acid for 10 seconds and 10% NaOCl for 30 seconds to facilitate observation at the cementum-resin interface[21].

The specimens were subsequently fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) overnight at 4℃, then rinsed in a 0.1M sodium cacodylate buffer 3 times for 1 min per rinse, followed by dehydration in graded series of ethanol for 10min. Each specimen was desiccator-dried and then sputter-coated for SEM observation.

TEM observation of cementum-resin interface

Further specimens produced as described previously were sectioned into pieces of approximately 1 mm thickness and width and decalcified with 10% EDTA for 3 days at room temperature. They were then fixed in the same way as mentioned before, subsequently dehydrated in a graded series of ethanol, and then embedded in epoxy resin. Specimens were sectioned perpendicular to the cementum surface both with glass and diamond knives on an ultramicrotome (MT-2, Sorvall, Norwalk, CT, USA). The bonding interfaces for investigation were serially sectioned by alternating semi-thin and ultra-thin sections throughout the width of 80 nm. The ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (H-8000, Hitachi Ltd.) at an operating voltage of 100 kV.

Results

Micro-tensile bond strength

The mean MTBS of all groups are shown in Figure 2A. The mean MTBS of groups B, C, and D were significantly higher than that of group A, although a statistically significant difference was not observed among groups B, C and D.

Specimens of groups B, C and D revealed complete or partially cohesive failure within the resin, which was classified as cohesive failure or mixed failure. All specimens of group A showed adhesive failure between the cementum and the resin (Fig.2B).

Leakage value

The leakage values of groups B, C and D were significantly lower than the value of group A, and a statistically significant difference was not observed among groups B, C and D. One specimen of group A failed during sample preparation when sectioning. Two of the twenty specimens in group A showed dye penetration throughout the interface length of resin (Fig.3).

SEM and TEM analysis

In SEM observation, group A (non-treated cementum) specimens exhibited a rough surface covered with smear layer (Fig.4A), while the treated cementum of group C exhibited collagen fibrils (Fig.4B).

An acid resistant layer, the hybrid layer, approximately 2-3 μm in thickness, was observed at the interface of group C (Figs.5A, 5B). In group B, the hybrid layer was not clearly observed at the interface.

TEM examination of specimens of group C demonstrated the presence of an approximately 3- μm thick transitional zone, that appeared as a clear layer between the resin and the cementum. Collagen fibrils were recognized in the hybrid layer and the cementum (Figs. 6A, 6B). Specimens of group B could not clearly demonstrate the presence of a transitional zone.

Discussion

In this study we used bovine dentin. Although there may be some differences between bovine cementum and human cementum, it has been reported that the tensile bond strength of resin composite to bovine dentin was similar to that of human dentin under the same conditions[22]. Hence the authors of the present study are of the opinion that information about 4-META/MMA-TBB resin adhesion to cementum using bovine cementum is valid to make inferences for human study.

The results of the present study indicated good sealing of groups B, C, and D, and a statistically significant difference was not observed among them. Group A exhibited extensive dye penetration at the interface and significantly lower MTBS. The MTBS and leakage levels obtained for the cementum were almost equal to those of the dentin. In fractographical observation after micro-tensile bond strength testing, most specimens of groups B, C and D showed cohesive failure within the resin (group B, 78.6%; group C, 78.6%; group D, 90%). All specimens of group A showed adhesive failure at the interface of cementum and 4-META/MMA-TBB resin. The difference between group A and the others may be due to the smear layer-covered cementum surface.

It was previously reported that Super Bond C&B uses a 10-3 solution to remove the smear layer from the prepared dentin and hydroxyapatite from the surface dentin[23]. At the same time, the ferric chloride minimizes degradation of collagen in the zone of decalcification [24]. The high bond strength to dentin is due to the formation of a dense "resin infiltrated dentin layer" known as a hybrid layer [25] at the dentin / resin interface.

Despite morphological differences between cementum, dentin, and bone, they are composed mainly of collagen and hydroxyapatite. The sealing ability of 4-META/MMA-TBB resin to cementum could be due to the same mechanism of hybridization in dentin and bone. The 10-3 solution probably decalcifies hydroxyapatite crystals in the cementum and facilitates the resin monomers to infiltrate between the exposed collagen fibrils, thus, creating the cementum "hybrid layer". In this study, treated cementum surface exhibited collagen fibrils. This allows the cementum to remain highly receptive to the 4-META/MMA-TBB adhesive resin.

SEM and TEM micrographs of dentin are widely available in the literature[16,21, 23-25]. However, there has been no investigation on the adhesion of 4-META/MMA-TBB resin to cementum. We have demonstrated the existence of a hybrid layer between 4-META/MMA-TBB resin and cementum. SEM examination of group C established that there was a greater resistance to HCl dissolution of mineral components at the cementum/resin interface. TEM examination of specimens of group C demonstrated the presence of an approximately 3 μm -thick transitional zone, that appeared as a clear layer between the cementum and the resin. This hybrid layer at the cementum/resin interface demonstrated adhesive properties, thereby preventing dead space formation. This appeared to be similar to TEM examinations of a hybrid layer of human dentinal specimens[16] and "hybrid bone" [26].

However, SEM and TEM examinations of specimens of group B could not clearly demonstrate the presence of a hybrid layer. The difference may be due to the lack of depth of de-mineralized cementum in group B.

Regardless of treatment time, whether 5 sec or 30 sec, the test results imply that 4-META/MMA-TBB resin can adhere to the cementum effectively when the surface is treated with a 10-3 solution. The resin, therefore, potentially provides a good seal to cementum for root-end sealing and the bonding of vertically fractured roots. It could possibly be used for the external sealing of root perforations and fractures. When defects are treated with 10-3 solution and covered with 4-META/M.MA-TBB resin, they are likely to be sealed and restored, thus inhibiting bacterial penetration and colonization.

The results of this study demonstrate that 4-META/MMA-TBB resin adheres to cementum by inducing the formation of hybridized cementum in the short term. However, some studies demonstrated that a resin-free demineralized dentin zone exists at the base of the hybrid layer, as a result of incomplete resin infiltration [20, 25]. Moreover, the collagen fibrils that are not enveloped by resin may become susceptible to water degradation in the long-term[27, 28].

Further investigations on the durability of bonding are necessary.

Conclusion

4-META/MMA-TBB resin adhered to the cementum as well as dentin via the hybrid layer when the surface is treated with a 10-3 solution.

Acknowledgement

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Figures legends

Figure 1. Specimen preparation for MTBS and dye leakage test

(A) Application of 4-META/MMA-TBB resin on the treated surface

(B) Outline of sectioned specimen

(C) Sectioned specimen after trimming to produce an hourglass shape (adhesive area 1.0 mm^2)

(D) Assessment of dye leakage

Figure 2. Micro-tensile bond strength result

(A) Mean MTBS to cementum and dentin.

The number of specimens that survived the preparation process per total number of specimens are given in parentheses. Same letter revealed no significant difference ($p < 0.05$). The MTBS of groups B, C, and D was significantly higher than that of group A, although a statistically significant difference was not observed among groups B, C and D.

(B) Percentages of fracture modes of de-bonded surfaces.

Specimens of groups B, C and D revealed complete or partially cohesive failure within the resin, which was classified as cohesive failure or mixed failure. All specimens of group A showed adhesive failure between cementum and the resin.

Figure 3. Leakage value result

One specimen of group A was excluded because of failure in sectioning. Two of twenty specimens of group A showed dye penetration throughout the bonding interface. Same letter revealed no significant difference ($p < 0.05$). The leakage value of groups B, C and D was significantly lower than the value of group A, and a statistically significant difference was not observed among groups B, C and D.

(N)=number of specimens.

Figure 4. SEM micrographs of cementum surface morphology

(A) Group A: A typical specimen from this group showing the smear layer covered surface.

(B) Group C: A typical specimen from this group showing removal of the smear layer by using 10-3 solution for 30sec. Collagen fibrils could be observed.

Figure 5. SEM micrographs of the interface between cementum and 4-META/MMA-TBB resin

(A) Cross-sectional SEM view of a specimen of 4-META/MMA-TBB resin bonded to bovine cementum that had been treated with 10-3 solution for 30sec. The hybrid layer at the resin-cementum interface could be observed.

(B) Higher magnification of the area marked on Fig.4A; The width of the acid resistant 'hybrid layer' is about 3 μm.

Figure 6. TEM micrographs of the interface between cementum and 4-META/MMA-TBB resin

(A) Ultrathin cross-sectional TEM view of a specimen of 4-META/MMA-TBB resin bonded to bovine cementum that had been treated with 10-3 solution for 30sec. The presence of an approximately 3-thick transitional zone, that appeared as a clear layer between the resin and the cementum, was observed. Collagen fibrils were recognized in the hybrid layer and the cementum

(B) Higher magnification of the area marked on Fig.4C. The collagen fibrils (arrowed) are apparent in the hybrid layer.

(R, resin; H, hybrid layer; C, cementum)

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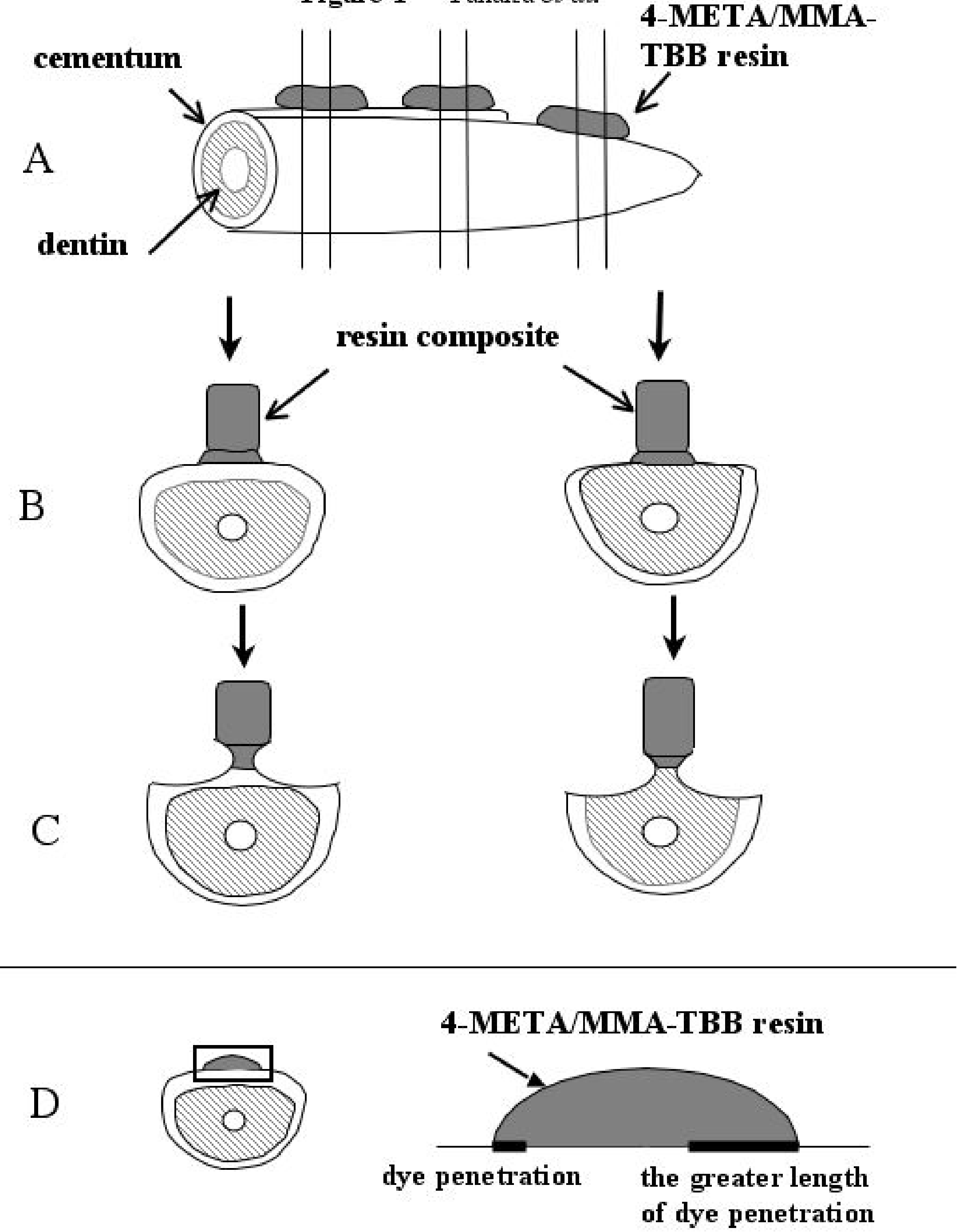

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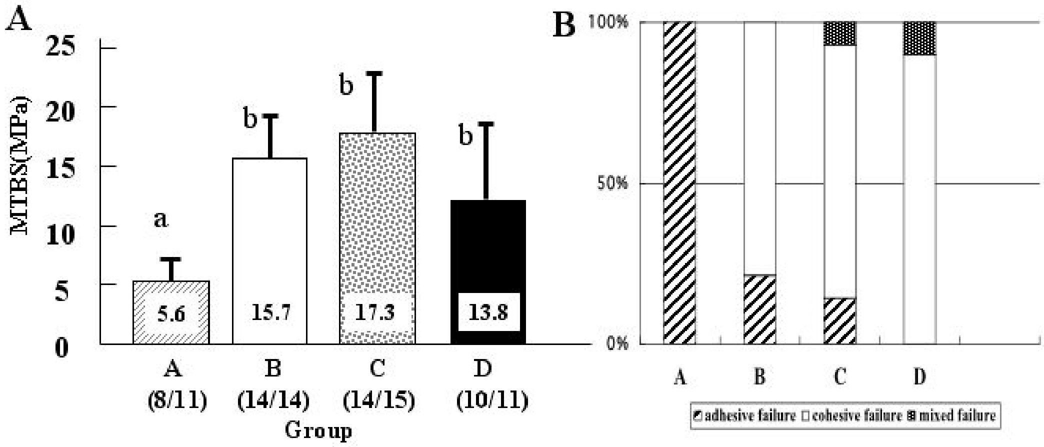

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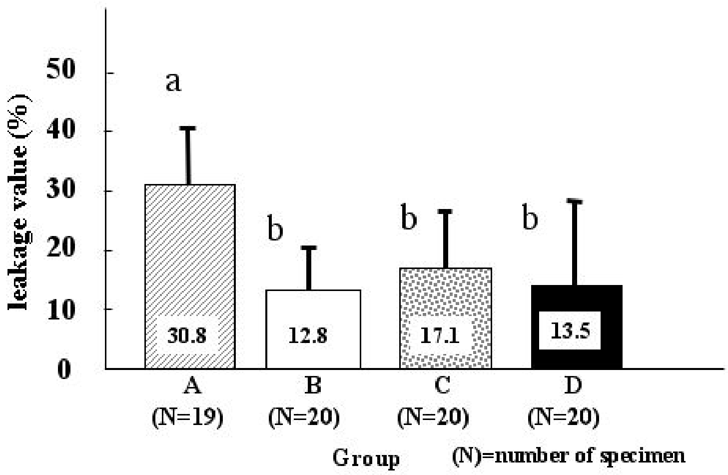

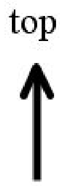

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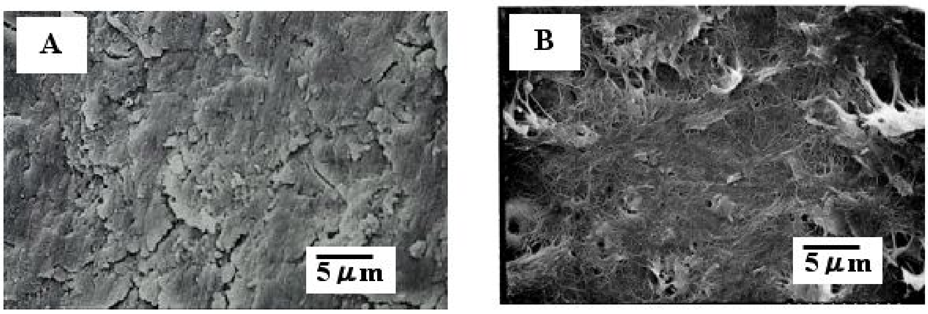

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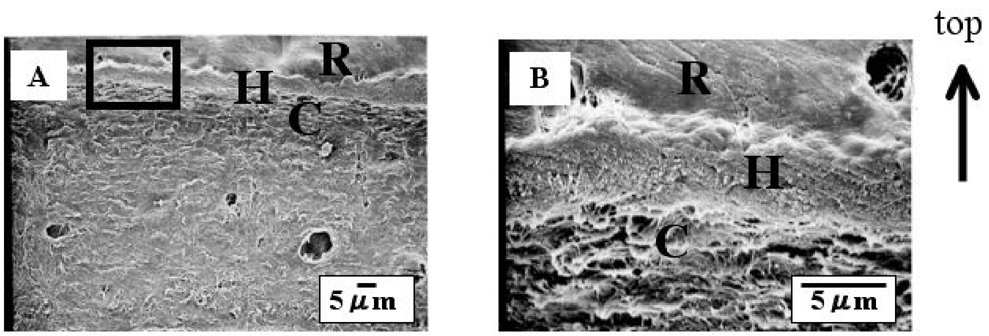

Figure 6 Tanaka *et al.*

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