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## **Influence of ascorbic acid on bonding of peroxide-affected dentin and 4-META/MMA-TBB resin**

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**Abstract** The purpose of this study was to evaluate the tensile bond strength to peroxide-exposed dentin. Furthermore, the effect of ascorbic acid on the bond strength of peroxide-exposed dentin was investigated. Extracted bovine dentin was exposed to 10% carbamide peroxide, 30% hydrogen peroxide or distilled water for 30 min, then treated with 10% ascorbic acid (0, 30, 90, 180 min), and conditioned with 10% citric acid/3% ferric chloride. The PMMA rod was bonded to the treated bovine dentin with 4META/MMA-TBB resin. A mini-dumbbell-shaped bonded specimen was prepared from these bonded assemblies and the tensile bond strength was tested. The fractured surfaces were also observed with SEM. Exposure to peroxide before bonding significantly reduced bond strength. The application of ascorbic acid to the peroxide-exposed dentin increased bond strength. On the other hand, an adverse effect of ascorbic acid was found in distilled water-affected dentin. Extended resin fibers were partially seen in the peroxide-exposed dentin. In conclusion, peroxide reduced the bond strength, and the stronger the oxidation, the weaker the obtained bond. Anti-oxidation with ascorbic acid recovered the bond strength, and the effect increased the longer the ascorbic acid was applied.

**Keyword** dentin bonding · anti-oxidant · polymerization · ascorbic acid · tooth bleaching · peroxide · 4-META/MMA-TBB resin

## Introduction

Tooth bleaching, e.g. at-home bleaching with carbamide peroxide (CP) and traditional bleaching with hydrogen peroxide (HP), has been used for discolored teeth and to satisfy esthetic needs. While tooth bleaching involves colorative esthetics, it does not involve morphological esthetics. Thus, an adhesive material, such as a microfilled resin composite or bonded laminate veneer restoration is sometimes used after tooth bleaching when both colorative and morphological restoration is needed [18].

The bond strength of current adhesive systems has been reported to be reduced significantly when bonding is performed immediately after bleaching for both enamel and dentin [2, 15-17, 23, 24, 26, 27]. It has been reported that allowing a one-week period between bleaching and restorative treatment reversed the enamel bond strength [2]. However, this method was not effective for bleached dentin [23, 27]. Thus, the bond strength to oxidant-exposed dentin such as peroxide and sodium hypochlorite (NaOCl) has generally been considered to be reduced [3, 14].

It has been well known that pretreatment with a combination of NaOCl and phosphoric acid increases the bond strengths with Panavia luting cements (Kuraray Medical) [9, 30]. On the other hand, an inverse effect of the pretreatment with NaOCl and phosphoric acid was obtained when using 4-META/MMA-TBB resin [3, 14]. As shown above, depending on the specific composition of the dentin adhesive, the application of NaOCl upon etching can increase or decrease bond strength [20, 29]. Differing from the many current adhesive systems, 4-META/MMA-TBB resin has some unique characteristics. Therefore, any difference to the current adhesive system may be found at the influence of peroxide on bonding of 4-META/MMA-TBB resin.

The reversal effect of anti-oxidants, e.g. ascorbic acid, sodium ascorbate, butyl hydroxianizole and sodium thiosulfate on the dentin bond strengths to the oxidant-exposed dentin has recently been some reported [14-16, 22, 32]. For

ascorbic acid, an adequate concentration has been reported to be 10% [22]. However, an adequate period of anti-oxidant application has not been discussed.

The purposes of this study were to clarify the influence of impairment in the resin bonding to dentin exposed to 10% CP or 30% HP and to investigate the reversal effect of ascorbic acid on bond strength with both peroxide-exposed and non-exposed dentin. The null hypotheses of this study were that 1) bond strength variation is not dependent on the type of peroxide, 2) ascorbic acid application time does not affect bond strength and 3) ascorbic acid has no effect for increasing the bond strength to dentin untreated with peroxide.

## **Materials and methods**

### *Specimen preparation and tensile bond testing*

Ninety-six extracted bovine teeth, frozen to maintain freshness, were defrosted and cut at the cervix to separate the crown and root. The coronal sides of the cut surfaces were sequentially abraded under a stream of water with SiC paper (#180- up to #600-grit) to prepare the flat dentin surfaces. To standardize the bonding areas, a 3.0 x 7.0 x 3.0 mm-acrylic frame was affixed to the abraded surface with double-sided tape (Nichiban, Tokyo, Japan).

For the CP groups, the exposed dentin areas of 32 teeth were subjected to a 10% CP gel (Opalescence<sup>®</sup> Regular, Ultradent Products Inc., South Jordan, Utah, USA) for 30 min, rinsed with distilled water for 60 sec, and dried. For the HP groups, the exposed areas of 32 teeth were subjected to a 30% HP solution (Wako Pure Chemical, Osaka, Japan) for 30 min, rinsed and dried in a similar way. For the DW groups, the exposed areas of 32 teeth were subjected to distilled water for 30 min and dried.

Each dentin specimen was then subjected to 10% ascorbic acid (Wako Pure Chemical) for 30, 90 or 180 min (30AA, 90AA and 180AA, respectively; each

sub-group had eight specimens) and the remaining eight specimens in each group were not treated with ascorbic acid (OAA). Each specimen was conditioned with 40  $\mu$ l of a 10<sup>-3</sup> solution (10% citric acid/3% ferric chloride) for 15 sec, rinsed with distilled water for 30 sec, and dried. Immediately after the acid conditioning, the surfaces were bonded to PMMA square rods (8.0 x 8.0 x 8.0 mm) using 4-META/MMA-TBB resin (Super-Bond<sup>®</sup> C&B; Sun Medical Co., Moriyama, Japan; also known as C&B Metabond<sup>™</sup> in North America, Parkell, Farmingdale, NY, USA) with a brush-dip technique. The bonded specimens were allowed to stand at room temperature for 60 min and then stored in 37 °C water for 24 h. They were then serially sectioned vertically to make 2.0-mm-thick bonded dentin slabs, using a low-speed diamond saw (Isomet<sup>™</sup>; Buehler, Lake Bluff, IL, USA). Each bonded slab was trimmed to a mini-dumbbell-shaped test specimen with a 3.0 x 2.0 mm cross-section at the adhesive interface [1, 11-13] using a diamond point (FG #211 regular; Shofu, Kyoto, Japan) in a high-speed air turbine handpiece with a copious air-water spray. One bovine tooth yielded only one bonded dentin slab. The prepared specimens were affixed to a disposable PMMA jig, and stressed in a tension using a universal testing machine (Tensilon RTC-1150-TSD; Orientec Co., Tokyo, Japan) at a cross-head speed of 0.5 mm/min. The cross-sectional dimensions of the fractured specimens were confirmed with a digital micrometer (Mitutoyo, Tokyo, Japan) after the tensile bond testing and tensile bond strength (TBS) was derived at the bonding area (mm<sup>2</sup>). When a specimen failed during processing (pre-testing failure), the TBS was set at 0 MPa [4, 28].

### *Statistical analysis*

The data were recorded and subjected to one-way and two-way ANOVAs, following Fisher's protected least significant difference (PLSD) test at the 5% level, to determine statistical significance using a commercially available statistical package (StatView<sup>®</sup> 5.0J, SAS Institute, Cary, NC, USA).

### *Scanning electron microscopy (SEM) observations*

After the tensile bond testing, each fractured specimen was desiccated in the box with silica gel for more than 24 h, placed on an aluminum stub, and sputter-coated with about 15 nm of Au-Pd using a Cool Sputter Coater (SC500A; VG Microtech, East Sussex, UK). The coated specimens were examined under a SEM (JSM-6340F; JEOL, Tokyo, Japan) at 15kV to determine the exact locus of the fracture.

### **Results**

TBSs of each group are shown in Table 1.

There is significantly difference among three groups (DW-0AA, CP-0AA and HP-0AA) when ascorbic acid was not applied (one-way ANOVA,  $p < 0.0001$ ). For DW-0AA (control), the highest TBS ( $12.5 \pm 5.1$  MPa) was obtained among all experimental groups. For CP-0AA, six specimens broke before TBS testing and two had low bond strength. For HP-0AA, all eight specimens broke before testing. Significant differences were found between CP-0AA and DW-0AA, and between HP-0AA and DW-0AA, respectively ( $p < 0.0001$ ).

One-way ANOVA revealed that AA-application significantly increased the TBS of CP groups ( $p = 0.0008$ ). AA-application group did not found the pre-testing failure (PTF). More than 90 min of ascorbic acid-application significantly affected the tensile bond strength (0 min vs 30 min:  $p = 0.2974$ , 0 min vs 90 min:  $p = 0.0096$ , 0 min vs 180 min:  $p < 0.0001$ ). Especially, 180 min of AA-application increased the TBS as no significantly difference was found to control DW-0AA ( $p = 0.1992$ ). One-way ANOVA also revealed that AA-application significantly increased the TBS of HP groups ( $p = 0.0494$ ). While AA-application decreased the number of PTF, the effect of AA on HP was smaller than that of CP. Three PTF was found even if AA was applied for 180

min, and remained five specimens obtained very low TBS. One-way ANOVA revealed that AA-application significantly decreased the TBS of DW groups ( $p = 0.0007$ ).

SEM photographs of fractured surfaces after the TBS testing are shown in Figs. 1 and 2. DW-0AA (control group) showed a mixture of fractures with mainly cohesive in the cured resin and partially at the bottom/within the hybrid layer (Fig. 1a). A mixed failure in the resin and adhesive interface was observed in the peroxide-exposed/ascorbic acid non-treated groups (CP-0AA and HP-0AA) (Fig. 1b and 1c). Furthermore, extended resin fibers were partially observed at the fractured surface (Fig. 1c).

A mixed failure in the cured resin and the top of the hybrid layer was observed in CP-180AA (Fig. 2a). A mixture of adhesive failure and cohesive failure in the dentin was observed in DW-180AA (Fig. 2b and 2c).

## **Discussion**

One of the purposes of this study was to determine the influence of peroxide on bond strength between dentin and 4-META/MMA-TBB resin. The results revealed that the exposure of dentin to peroxide decreased the bond strength, as reported in several previous studies [15, 16, 23, 27] (Table 1).

However, the some differences were found between CP and HP. For CP-0, two specimens could be tested for their TBS, and the remaining six were debonded while immersed in water, in spite of the specimen could be prepared. On the other hand, the all of the eight bonded specimens were fractured when sliced with the Isomet diamond saw. Even if after the treatment of ascorbic acid, CP specimens showed higher bond strength than that of HP specimens. The concentration of peroxide in the 30% HP is approximately nine-fold that in the 10% CP, because CP decomposes the two parts of urea and one part of HP. The differences may be due to the concentration of peroxide. In the above results, the first hypothesis was rejected.

In this study, the influence of peroxides on bond strength was larger than the previous reports, which were bonded with the current adhesive systems. Spyrides and colleagues [23] reported that the shear bond strength of bovine dentin exposed to 35% HP for 30 min to Single Bond/Z100 (3M ESPE) was reduced to 24% of the non-exposed control ( $4.2 \pm 2.5$  MPa vs.  $17.4 \pm 5.6$  MPa). Kaya and Turkun [15] also reported that the shear bond strength of dentin exposed to 35% HP for 10 min was a 53% reduction compared to the control ( $15.3 \pm 2.7$  MPa vs.  $28.4 \pm 1.7$  MPa) when Clearfil SE Bond/Clearfil AP-X (kuraray Medical). In contrast, the all eight specimens failed before testing at 35% HP for 30 min in this study. The differences were probably due to the unique characters of 4-META/MMA-TBB resin, as described below.

The pre-conditioner of adhesive used in this study includes 3% ferric chloride. The ferric ions ( $\text{Fe}^{3+}$ ) penetrate into the dentin and are not removed, even if the treated surface was sufficiently rinsed [25]. It has been well known that ferric chloride contributes to prevent the shrinkage of demineralized collagen and to make the water-soluble protein insoluble [8].

However, in the presence of ferric ion, especially ferric-citrate complexes, peroxides usually generate hydroxyl radical ( $\cdot\text{OH}$ ) directly via the Fenton reaction [5, 31]. The presence of excess radicals may interfere with the polymerization, because the resin fibers were extended in the SEM (Fig. 1). Thus, it was found that the combination of  $\text{Fe}^{3+}$  and peroxide induced the influence on bond strength.

For CP, the longer the ascorbic acid was applied, the higher the bond strength was obtained. For CP, the reversal effect of ascorbic acid was significantly found, because there was no significantly difference in TBS between DW-0 and CP-180 ( $p = 0.1992$ ). These results suggested that excess radical-generation from peroxide was interfered with by ascorbic acid [6, 21]. In contrast, the reversal bonding with ascorbic acid was not realized in the HP group. For HP, the large amount of radicals may be related to changes in the redox cycle [7, 31]. Oxygen free radicals are generally generated from

HP by decomposition. The oxygen radicals generated from HP are more destructive than the liberated oxygen [7]. Based on these results, the second hypothesis, that the application period of ascorbic acid is not affected, was rejected.

The application of ascorbic acid markedly decreased the bond strength in the DW group. These results required the rejection of the last null-hypothesis. We can consider two reasons for the low bond strength; oxidation and excessive demineralization. In the presence of both ferric ions and ascorbate,  $Fe^{3+}$  is changed to  $Fe^{2+}$ , thereby creating ascorbate free radicals, even if a peroxide is not applied. This reaction may inhibit the polymerization of the resin, monomer penetration or degradation of dentin substrates. In our study, however, the double-etching effect with 10-3 and ascorbic acid might also be correlated with the reduction in bond strength rather than the oxidation, because a fracture of the dentin structure was observed at the failed rod-side surface (Fig. 2c).

This study indicated that peroxide-exposure to dentin interfered the bonding of 4-META/MMA-TBB resin. Furthermore, it may suggest the deterioration of the bond reduction due to the interaction of ferric chloride and peroxide, and inadequate effect of the additional application of ascorbic acid. However, we could not prove their suggestion in this study design, and more investigations are needed to clarify these phenomenon.

## **Conclusion**

We found that exposure to peroxide and additional treatment with ascorbic acid on the bonding of 4-META/MMA-TBB resin to dentin as follows;

1. Peroxide reduced bond strength, and the stronger the oxidation, the weaker the bond.
2. The application of ascorbic acid recovered bond strength for peroxide-exposed dentin, and this tendency increased the longer the

ascorbic acid was applied.

3. Ascorbic acid had an adverse effect on non-peroxide-exposed dentin.

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## References

1. Arao T (1997) Effect of miniaturized dumbbell-shaped specimen to identify bonding of bovine dentin, J Jpn Dent Mater 16: 175-181.
2. Barghi N, Godwin JM (1994) Reducing the adverse effect of bleaching on composite-enamel bond. J Esthet Dent 6: 157-161.
3. Belli S, Zhang Y, Pereira PNR, Ozer F, Pashler DH (2001) Resin bond strengths of adhesive resins to pulp-chamber dentin. J Endod 27: 527-532.
4. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K, Lambrechts P (2004) Four-year water degradation of resin-modified glass-ionomer adhesive bonded to dentin. Eur J Oral Sci 112: 73-83.
5. Gutteridge JMC (1991) Hydroxyl radical formation from the auto-reduction of a ferric citrate complex. Free Radic Biol Med 11: 401-406.
6. Gutteridge JMC (1994) Biological origin of free radicals, and mechanisms of antioxidant protection. Chem Biol Interact 91: 133-140.
7. Halliwell B, Gutteridge JMC (1996) Chemistry of free radicals and related 'reactive species'. In: Free radicals in Biology and Medicine, 3rd ed. (Halliwell B, Gutteridge JMC, eds.), pp.53-67, Oxford Univ. Press, London.
8. Hanazaki Y, Watanabe A, Nakabayashi N (1999) The effect of water-soluble noncollagenous proteins on resin bonding to dentin. J Jpn Dent Mater 18: 375-379.
9. Inoue S, Murata Y, Sano H, Kashiwada T (2002) Effect of NaOCl treatment on bond strength between indirect resin core-buildup and dentin. Dent Mater J 21: 343-354.
10. Ishizuka T, Kataoka H, Yoshioka T, Suda H, Iwasaki N, Takahashi H, Nishimura F (2001) Effect of NaClO treatment on bonding to root canal dentin using a new evaluation method. Dent Mater J 20: 24-33.
11. Iwasaki Y, Toida T, Nakabayashi N (2004) Improved wet bonding of methyl methacrylate-tri-*n*-butylborane resin to dentin etched with ten percent phosphoric acid in the presence of ferric ions. J Biomed Mater Res 68A:

566-572.

12. Kameyama A, Kawada E, Takizawa M, Oda Y, Hirai Y (2000) Influence of different acid conditioners on the tensile bond strength of 4-META/MMA-TBB resin to Er:YAG laser-irradiated bovine dentin. *J Adhes Dent* 2: 297-304.
13. Kameyama A, Ihara S, Amagai T, Miake Y, Kawada E, Oda Y, Yanagisawa T, Hirai Y (2003) Adhesion of 4-META/MMA-TBB resin to heated dentin: Effects of pre-treatments with FeCl<sub>3</sub> and/or HEMA. *Dent Mater J* 22: 1-9.
14. Kataoka H, Yoshioka T, Suda H, Imai Y (1999) Effect of sodium hypochlorite on adhesion of 4-META/MMA-TBB resin to dentin. *Jpn J Conserv Dent* 42; 241-247.
15. Kaya AD, Turkun M (2003) Reversal of dentin bonding to bleached teeth. *Oper Dent* 28: 825-829.
16. Lai SCN, Mak YF, Cheung GSP, Osorio R, Toledano M, Carbalho RM, Tay FR, Pashley DH (2001) Reversal of compromised bonding to oxidized etched dentin. *J Dent Res* 80: 1919-1924.
17. Lai SCN, Tay FR, Cheung GSP, Mak YF, Carbalho RM, Wei SHY, Toledano M, Osorio R, Pashley DH (2002) Reversal of compromised bonding in bleached enamel. *J Dent Res* 81: 477-481.
18. Magne P, Belser U (2002) Bonded porcelain restorations in the anterior dentition: A biomimetic approach. pp 102-104, 1<sup>st</sup> ed, Quintessence Publishing Co, Chicago, USA.
19. Okamoto Y, Takahata K, Saeki K (1998) Studies on the behavior of partially oxidized tributylborane as a radical initiator for methyl methacrylate (MMA) polymerization. *Chem Letters* 27: 1247-1248.
20. Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F (2000) Effect of a sodium hypochlorite gel on dentin bonding. *Dent Mater* 16: 311-323.
21. Rose RC, Bode AM (1993) Biology of free radical scavengers: an evaluation of ascorbate. *FASEB J* 7: 1135-1142.
22. Soeno K, Taira Y, Matsumura H, Atsuta M, Suzuki S (2004) Adhesion of

- 4-META/MMA-TBB resin to collagen-depleted dentin: effect of conditioner with ascorbic acid/ferric chloride. *Dent Mater J* 23: 100-105.
23. Spyrides GM, Perdigao J, Pagami C, Araujo MAM, Spyrides SMM (2000) Effect of whitening agents on dentin bonding. *J Esthet Dent* 12: 264-270.
  24. Stokes AN, Hood JA, Dhariwal D, Patel K (1992) Effect of peroxide bleaches on resin-enamel bonds. *Quintessence Int* 23: 769-771.
  25. Takarada K, Nikaido T, Kojima M, Nakabayashi N (1990) Novel method to observe the interface between adhesive resin and dentin by staining Fe<sup>3+</sup> with tannic acid. *J Jpn Dent Mater* 9: 363-367.
  26. Titley KC, Torneck CD, Ruse ND (1992) The effect of carbamide-peroxide gel on the shear bond strength of a microfill resin to bovine enamel. *J Dent Res* 71: 20-24.
  27. Torneck CD, Titley KC, Smith DC, Adibfar A (1990) Adhesion of light-cured composite resin to bleached and unbleached bovine dentin. *Endod Dent Traumatol* 6: 97-103.
  28. Wakabayashi Y, Kondou Y, Suzuki K, Yatani H, Yamashita A (1994) Effect of dissolution of collagen on adhesion to dentin. *Int J Prosthodont* 7: 302-306.
  29. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P, Van Meerbeek B. (2005) Monomer-solvent phase separation in one step self-etch adhesives. *J Dent Res* 84: 183-188.
  30. Vargas MA, Cobb DS, Armstrong SR (1997) Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. *Oper Dent* 22: 159-166.
  31. Yazaki K, Kawada E, Oda Y (2003) An evaluation of the penetration of peroxide from tooth-whitener. *Biomed Res* 24: 325-330.
  32. Yiu CKY, Garcia-Godoy F, Tay FR, Pashley DH, Imazato S, King NM, Lai SCN (2002) A nanoleakage perspective on bonding to oxidized dentin. *J Dent Res* 81: 628-632.

## Figure Legends

Fig. 1: SEM micrograph of the fractured dentin-side surfaces of bonded specimens (0AA groups). a: DW-0AA (21.5 MPa; 1000x). Failure in the cured resin (R) in almost all parts, at the bottom of the hybrid layer (BHL) and partially within the hybrid layer (WHL) was observed. b and c: CP-0AA (pre-test failed; 400x and 1000x, respectively). Almost all parts show the adhesive failure (A) but failure in the resin (R) is only partially seen. Extended resin fibers branch out from the failure in the resin (pointer). Similar fibers were also partially observed at seven of eight specimens.

Fig. 2: SEM micrographs of the fractured surface of bonded specimens (180AA groups; Original magnification 1000x). a: dentin side surface of CP-180AA (11.8 MPa; 1000x). Fractured surface pattern is similar to that of DW-0AA (Fig. 1a). Failure both in the cured resin (R) and at the top of the hybrid layer (THL) is seen. However, the other site partially shows the fracture of dentin (data not shown). Arrow: Scratches of SiC paper. b: dentin side surface of DW-180AA (2.4MPa). A mixture of failed adhesive (A), dentin (D) and hybrid layer (HL) is observed. c: Rod-side surface of the same specimen to Fig. 2b. The failure in dentin was evidentially observed (D).

Table 1 Tensile bond strengths of each group (MPa, N=8)

Ascorbic acid application	DW			CP			HP		
	Mean ± S.D.	PTF	Statistics	Mean ± S.D.	PTF	Statistics	Mean ± S.D.	PTF	Statistics
0 min	12.5 ± 5.1	0	a	0.3 ± 0.7	6	f	0.0 ± 0.0	8	f
30 min	7.4 ± 4.9	0	b, c	2.7 ± 3.4	0	e, f	0.1 ± 0.1	5	f
90 min	5.4 ± 5.8	0	c, d, e	6.5 ± 7.3	0	b, c, d	0.3 ± 0.3	4	f
180 min	2.8 ± 2.2	0	d, e, f	10.1 ± 3.6	0	a, b	1.7 ± 2.6	3	e, f

Mean values designated with the same letter are not significantly different ( $p>0.05$ ).

PTF: The number of pre-testing failures.

Pre-test fractured specimens were regarded as 0 MPa.

Fig.1a

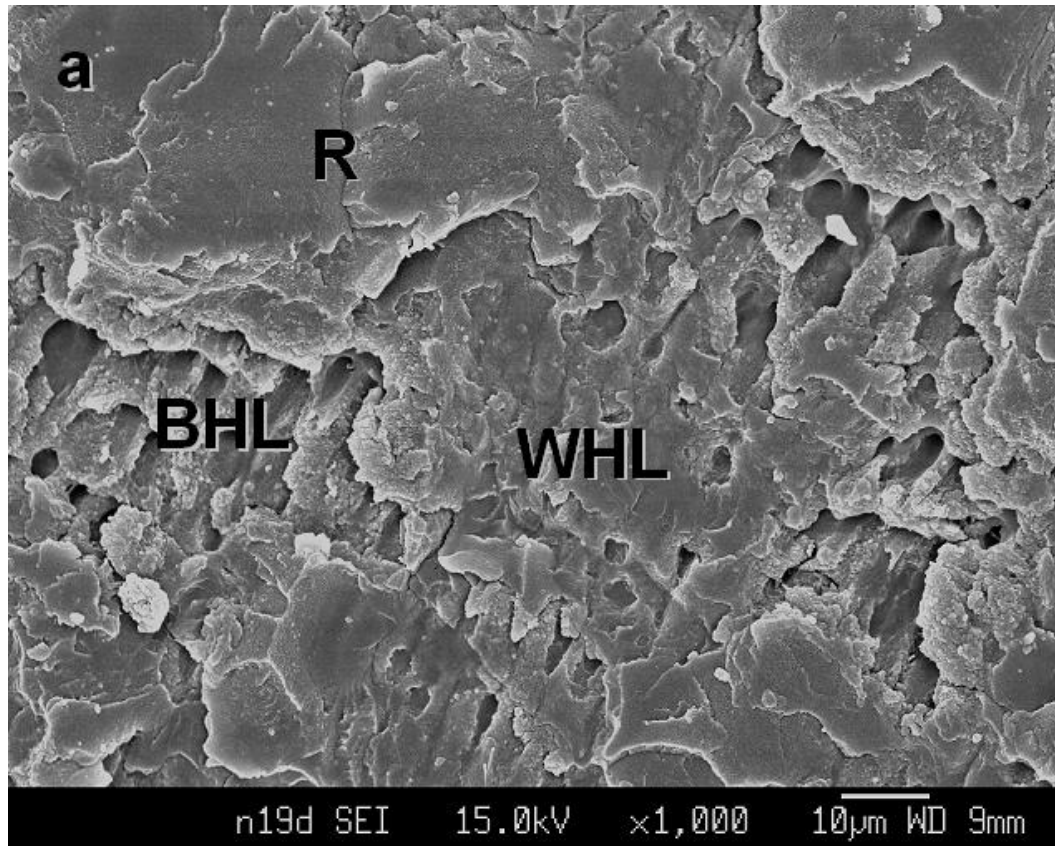
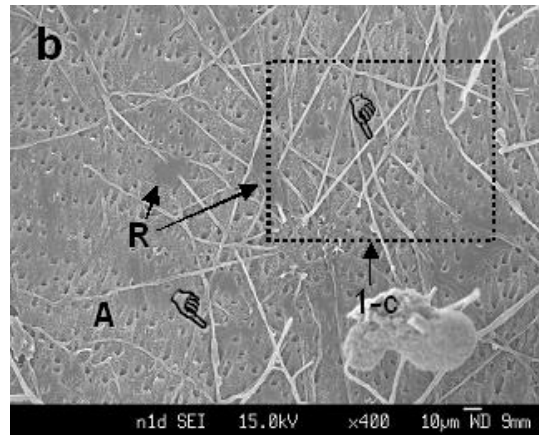


Fig.1b



**Fig.1c**

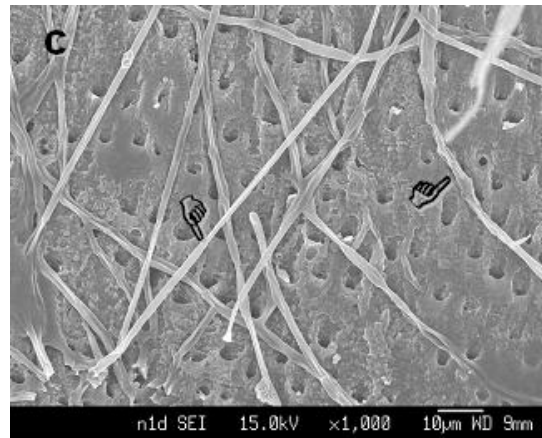
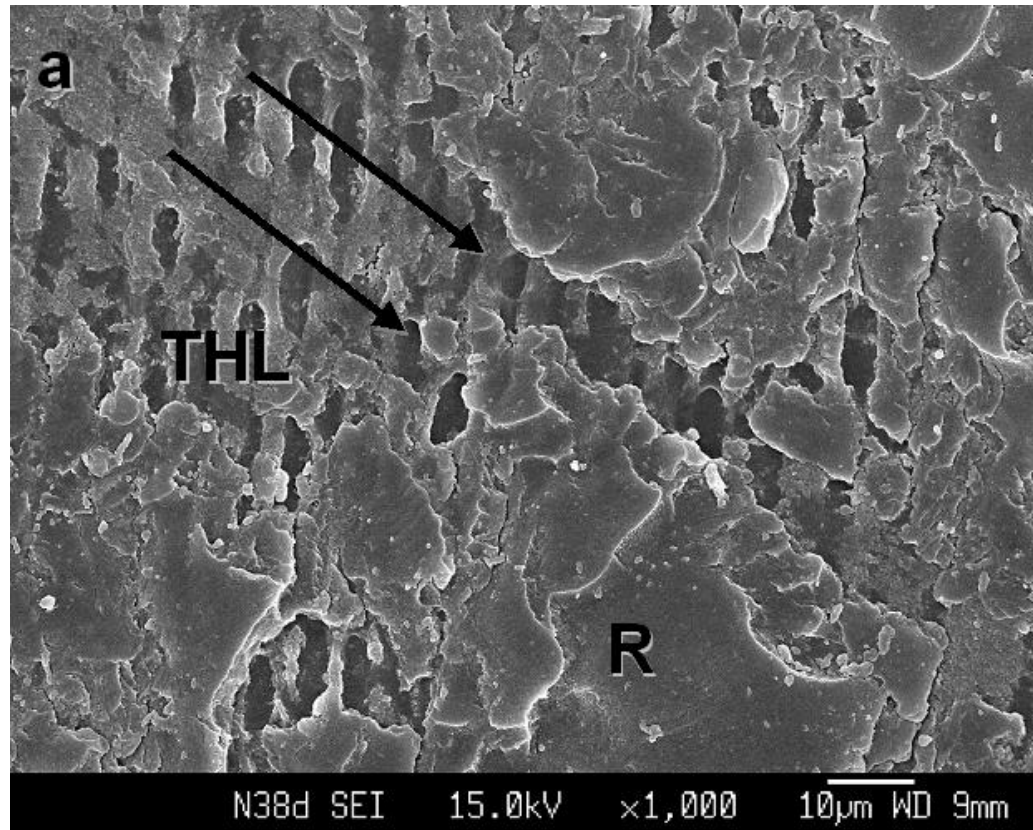
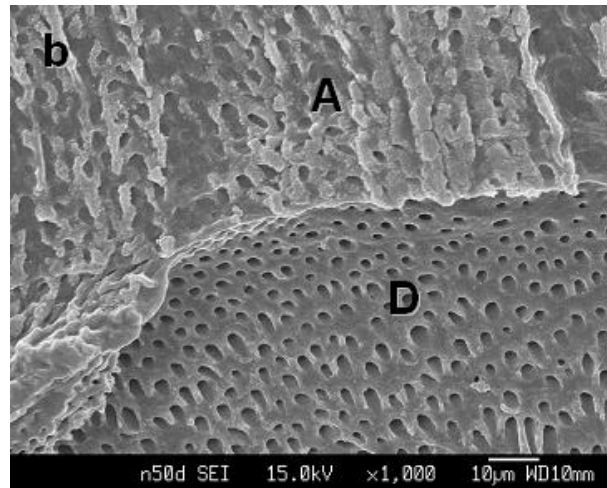


Fig.2a



**Fig.2b**



**Fig.2c**

