

Biological Bases of Dentin Hybridization

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ABSTRACT

The aims of this study were threefold: (1) to characterize and quantify the number, diameter and surface area of exposed dentinal tubules on the cross section of the human coronal dentin; (2) to determine if any such differences in these properties arise in relation to the distance from the dentinoenamel junction; and (3) to evaluate whether such differences can influence dentin hybridization. To accomplish these aims, scanning electron microscopy comparative observation was carried out on 60 prepared human premolars, which were divided into three groups of 20 samples each. The three sample groups were cut as follows: (1) in the central fissure region, one millimeter from the enamel-dentine junction; (2) halfway between the enamel-dentine junction and the pulp; and (3) one millimeter from the roof of the pulp chamber. Using one-way analysis of variance (one-way ANOVA) and a regression linear model, the data enumerated below were obtained. First, the mean number of the tubule openings was 19600/mm² on the first level, 32400/mm² on the second and 42300/mm² on the third. The mean tubule diameter on the first level was 0.67 µm, 1.52 µm on the second and 2.58 µm on the third. Finally, exposed tubules on the first level occupied 2.79% of total dentinal surface area, 23.90% on the second, and 87.78% on the third level. Therefore, significant statistical differences ($p < 0.01$) between all three groups of the specimens for all three properties were observed, as well as positive correlation between the dentin depth and each of these properties. This indicates that the dentin structural variety, which ultimately determines adhesion to dentine, involves a complex interaction between biological material (dentin) and the particular adhesion system applied.

Key words: dentin, dentinal tubules, dentin adhesives, dentin hybridization, hybrid layer, scanning electron microscopy

Introduction

During the past century, numerous histological investigations of human dentine structure have been performed^{1,2}. Dentine structure represents the biological basis of modern adhesive restorative treatment. Thus, interest in dentine structure is significantly correlated to contemporary understanding of the pulp-dentin complex and adhesion concepts. New and improved research techniques have enabled investigators to develop our understanding of the dentine structure, particularly those involving light microscopy and scanning electron microscopy (SEM). Although results of light microscopy have been limited, rapid development of dentine structure investigation began with use of SEM.

Using such techniques as SEM, detailed knowledge of hard dental tissue structure has become the basis for the development of concepts in modern adhesive restorative treatment. Specifically, while adhesion to enamel has neither been experimentally nor clinically problematic, adhesion to dentine has posed clinical issues given its heterogeneous structure. Three additional factors are important regarding the adhesion to dentine:

1. biological bases of the substrate;
2. condition and response of the pulp/dentine complex to the adhesive restorative treatment; and
3. materials selection³.

Dentinal tubules are long, narrow, conical shaped canals that radiate from the pulp throughout the entire thickness of dentin, making dentin a highly permeable tissue^{4,5}. Each tubule is surrounded by a collar of hypermineralized peritubular dentin. Intertubular dentin is less mineralized and contains more organic collagen fibrils. Peritubular dentin is more acid-sensitive. The diameter of the tubuli decreases from 2–3 μm at the pulp side to 0.5–0.9 μm at the dentinoenamel junction^{6,7}. These dentinal tubules contain the odontoblastic processes as a direct connection to the vital pulp. Dentinal fluid in the tubules is under a slight, but constant, outward pressure from the pulp. The intrapulpal fluid pressure is estimated to be 20 – 28 mmHg^{8,9}. The number of tubules increases from 7000–15000/mm² near the dentinoenamel junction to 45000–65000/mm² near the pulp¹⁰.

Because of the fan-shaped radiation of dentin tubuli, 96% of a superficial dentinal surface near the dentinoenamel junction is composed of intertubular dentin; only 1% is occupied by dentinal tubules, and 3% by peritubular dentin. Near the pulp, peritubular dentin represents 66% and intertubular dentin only 12% of the surface area, while 22% of the surface area is occupied by dentinal tubules^{11,12}.

Dentine structure determines the properties specific to dentine, i.e., permeability, humidity, and physical properties, such as hardness, strength, and elasticity. However, and importantly, since dentine structure is heterogeneous and since, consequently, dentine physiology varies, adhesion to dentine must, therefore, result from a complex interaction between biologic material (dentine) and the particular adhesion system¹³.

Dentin hybridization is procedure of modern restorative dentistry that is particularly suited to resolve the complications noted above. Hybridized dentin, otherwise known as hybrid layer, begins under the dentin surface after surface and subsurface demineralization and adhesive monomer infiltration into exposed collagen network¹⁴.

Three specific ultra-morphologic features have been described as resulting from the hybridization process. The first characteristic is a »shag-carpet« appearance that materializes. This reflects the loose organization of collagen fibrils directed towards the adhesive resin and often unrevealed into their micro-fibrils. The second characteristic is known as »tubule-wall hybridization« and represents the extension of the hybrid layer into the tubule wall area. Thus, so-called resin-tag formation in the opened dentin tubulus is circular and surrounded by a hybridized tubule orifice wall. In particular, the resin-tag neck, which, at the top takes up 5–10 μm of the tubule orifice, is thought to contribute most to retention and sealing effectiveness. The third characteristic is called »lateral tubule hybridization« and has been described as the formation of a tiny hybrid layer into the walls of lateral tubule branches which surrounds a central core of resin called micro-resin-tag¹⁵.

To restate the major aims of the present paper in the context of both dentin variety and the impact of it on the biological bases of the hybridization process, we must

first determine whether there are any differences in the number and diameter of exposed dentinal tubules and in the surface area that is occupied by dentinal tubules on the cross section of coronal dentin in relation to the distance from dentinoenamel junction to the pulp. Second, the data obtained from this effort will help us address the question of how such differences can influence dentin hybridization.

Materials and Methods

Sixty intact premolar human teeth, extracted for orthodontic reasons in patients ranging in age from 14–21 years, were collected for the investigation. Samples were selected with the intention of avoiding variability of dentin that is attend to be in dentin by age and pathologic processes as caries etc. After extractions, periodontal tissue was removed from the teeth, and the teeth were stored in 37% formaldehyde solution. Each tooth was cut in a mesiodistal direction in three levels by carbon disc and water cooling.

A SEM comparative observation was carried out on 60 specimens of human coronal dentine, which were divided into three groups, in relation to the distance from the enamel-dentine junction and the pulp. Coronal dentine in the region of the central fissure was observed on these three levels:

1. cross section of the coronal dentine, one millimeter from enamel-dentine junction (Figure 1);
2. cross section of the coronal dentine, halfway between enamel-dentine junction and the pulp (Figure 2); and
3. cross section of the coronal dentine, one millimeter from the roof of the pulp chamber (Figure 3).

To remove smear layer debris and inevitable peritubular dentin, all the specimens were treated with 37% orthophosphoric acid (Total-Etch, Ivoclar Vivadent) for 30

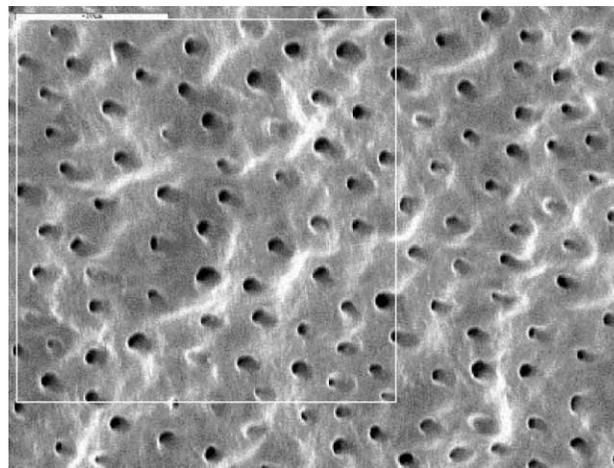


Fig. 1. Scanning electron microscopic image of cross section of coronal dentine one millimeter from dentinoenamel junction, enlargement 1200 \times , bar=20 μm , square 50 \times 50 μm .

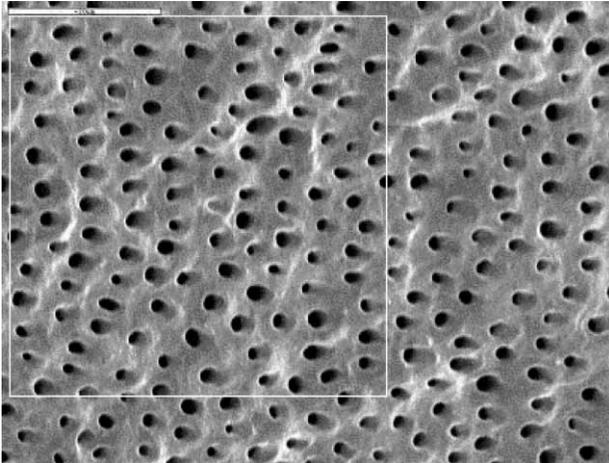


Fig. 2. Scanning electron microscopic image of cross section on half-distance between dentinoenamel junction and pulp, enlargement 1200 \times , bar=20 μm , square 50 \times 50 μm .

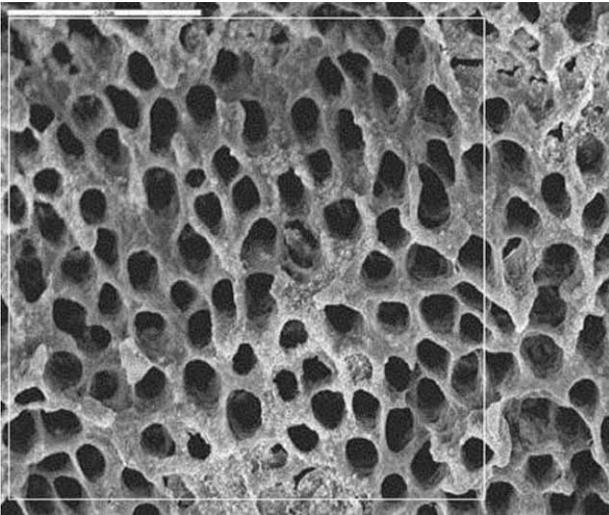


Fig. 3. Scanning electron microscopic image of cross section one millimeter from the roof of the pulp chamber, enlargement 1500 \times , bar=20 μm , square 50 \times 50 μm .

seconds, then washed and dried with compressed air for 5 seconds. All the specimens were steamed with layer of gold in a device S 150 Sputter Coater-Edwards. The specimens' surfaces were coated with a gold layer at a thickness of 10–15 nm in order to achieve improved electrical conduction. The specimens were examined in PLIVA d.d., Department for Quality Control, on SEM JSM-5800 (JEOL, Tokyo, Japan) 15 kV.

In SEM analysis, the reflected and secondary electrons are transformed into electric signals via a certain detector, which makes the technique very suitable for surface morphology and structure research. Because they are electrically charged, they can be diverted in a focus with an electromagnet, and they are brought over in a cathode pipe where we get an image. To avoid any potential atmospheric effects, these experiments are carried

out in a vacuum. The amount of reflected and secondary electrons depends on the tension we use, the detector's position and the specimen's surface. The final image of the specimen's surface, based on the electrons, is sharp with a very clear relief.

Openings of the exposed dentinal tubules were counted within a square dentinal surface area of 50 \times 50 μm . That number was divided by 2500 to arrive at the number of the openings of the dentinal tubules in a square micrometer ($\text{N}/\mu\text{m}^2$). This number was, in turn, multiplied by 10^6 to get the number of the openings of the dentinal tubules in a square millimeter (N/mm^2). The diameter of the exposed dentinal tubules was measured as the greatest diameter of irregularly shaped tubules. The percentage of surface area that is occupied by dentinal tubules was then calculated from number and diameter by the formula $\text{N} \times r^2\pi/2500 \mu\text{m}^2 \times 100\%$. Although dentinal tubules are irregularly shaped, for our calculation, they were idealised as regular circles, and the diameter includes peritubular dentin that was removed by acid etching.

Numerical data were represented by measurements of central tendency, including mean (X), median (C), mode (Mo) and standard deviation (SD). Measurements between all three groups were compared using one-way analysis of variance (one-way ANOVA) at $\alpha=0.01$ confidence and a regression model to identify differences among the groups. Statistical significance was considered as $p<0.01$.

Results

First, the mean number of the openings of the dentinal tubules on the first level was 19600/ mm^2 , 32400/ mm^2 on the second level, and 42300/ mm^2 on the third level (Table 1). Using the one-way analysis of variance at $\alpha=0.01$, we found a ratio of $F(\text{MS}_{\text{treatment}}/\text{MS}_{\text{error}})=305.22$ which was greater than $F_{0.99(2.57)} 4.98$. The results suggested that there is significant statistical difference ($p<0.01$) in the number of exposed dentinal tubules between all three groups of specimens. Furthermore, there is a positive correlation between the depth of dentin and the number of exposed tubules.

Second, the mean diameter of exposed dentinal tubules on the first level was 0.67 μm , 1.52 μm on the second level, and 2.58 μm on the third level (Table 2). Using the one-way analysis of variance at $\alpha=0.01$, we found a ratio of $F(\text{MS}_{\text{treatment}}/\text{MS}_{\text{error}})=261.57$ which was greater than $F_{0.99(2.57)} 4.98$. The results suggested that there is significant statistical difference ($p<0.01$) in the diameter of exposed dentinal tubules between all three groups of the specimens. Positive correlation between the depth of dentin and the diameter of exposed tubules exists.

Finally, the surface area that is occupied by exposed dentinal tubules on the first level was 2.79%, 23.90% on the second level, and 87.78% and on the third level of total dentinal surface (Table 3). Using the one-way analysis of variance at $\alpha=0.01$, we found a ratio of $F(\text{MS}_{\text{treatment}}/$

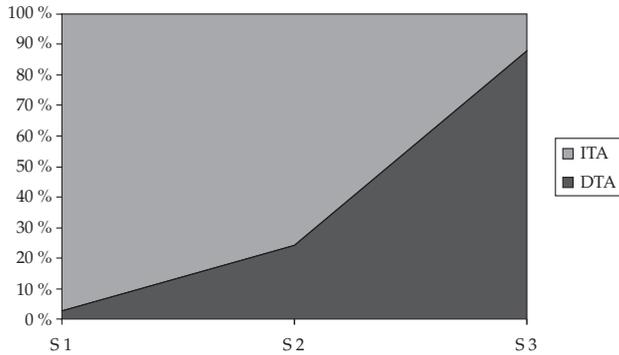


Fig. 4. Positive correlation between the surface area that is occupied by dental tubules (DTA) and the dentin depth. S1 – cross section of coronal dentin one millimeter from dentinoenamel junction, S2 – cross section on half-distance between dentinoenamel junction and pulp, S3 – cross section one millimeter from the roof of the pulp chamber, ITA – (intertubular area) area that is occupied by intertubular dentin, DTA – (dental tubules area) area that is occupied by dental tubules.

MS_{error}) = 470.16 which was also greater than F_{0.99 (2.57)} 4.98. These results also suggested that there is significant statistical difference (p < 0.01) in area that is occupied by exposed dental tubules between all three groups of the specimens. Once again, there is also positive correlation between the depth of dentin and the observed parameter (Figure 4).

Discussion

The results confirmed that there is significant statistical difference (p < 0.01) in surface area that is occupied by exposed dental tubules between all three groups of the specimens, as a specific consequence of differences resulting from variations in tubule parameters measured, such as number and diameter of exposed dental tubules. Number, diameter and also surface area of dental tubules on the cavity walls all vary with cavity depth, and these properties are also in positive correlation with dentin depth (Tables 1-3).

Our results correspond to the previously published investigations^{4,5,9,10}. Garberoglio et al. (1976) reported

TABLE 1
NUMBER OF EXPOSED DENTINAL TUBULES OF 60 HUMAN PREMOLARS WHICH ARE DIVIDED IN TO THREE GROUPS IN RELATION TO THE DISTANCE FROM THE ENAMEL-DENTINE JUNCTION AND THE PULP (N × 10³/mm²)

Specimens	X	C	Mo SD
S1	19.60	19.00	20.00 4.92
S2	32.40	29.40	29.60 6.74
S3	42.30	41.60	41.60 5.02

S1 – cross section of coronal dentine one millimeter from dentinoenamel junction, S2 – cross section on half-distance between dentinoenamel junction and pulp, S3 – cross section one millimeter from the roof of the pulp chamber.

TABLE 2
DIAMETER OF EXPOSED DENTINAL TUBULES OF 60 HUMAN PREMOLARS WHICH ARE DIVIDED IN TO THREE GROUPS IN RELATION TO THE DISTANCE FROM THE ENAMEL-DENTINE JUNCTION AND THE PULP (µm)

Specimens	X	C	Mo SD
S1	0.67	0.85	0.80 0.16
S2	1.52	1.40	1.60 0.23
S3	2.58	2.75	2.80 0.49

S1 – cross section of coronal dentine one millimeter from dentinoenamel junction, S2 – cross section on half-distance between dentinoenamel junction and pulp, S3 – cross section one millimeter from the roof of the pulp chamber.

that the number of dentinal tubules varies with the distance from the pulp¹⁰. The mean number of exposed dental tubules near the pulp was 59000/mm² and 10000/mm² near the dentinoenamel junction. The mean diameter of exposed dental tubules near the pulp was 3.2 µm and 0.5 µm¹⁰ near the dentinoenamel junction. Mjör et al. (1996) reported that the mean number of dental tubules near the pulp was 58000/mm², 29000/mm² halfway between dentinoenamel junction and pulp², and 10000/mm² near the dentinoenamel junction^{2,9}.

Number and diameter of exposed dental tubules are clinically important with respect to the concept of contemporary adhesive restorative treatment¹⁶. Openings of exposed dental tubules represent biological substrate for resin tag formation. The surface area that is occupied by dental tubules varies with the distance from the pulp¹⁷. Our investigation shows that the surface size that is occupied by dental tubules from overall substrate surface on the cross sections of coronal dentine measured at one millimeter from dentinoenamel junction, half-distance between dentinoenamel junction and pulp and one millimeter from the roof of the pulp chamber corresponds to the previously published results^{1,2,10,12}. Garberoglio et al. (1976) and Pashley (1989) reported that surface size that is occupied by dental tubules increases from 1% near the dentinoenamel junction to 88% near the pulp. They found that 96% of a superficial dental surface near the dentinoenamel junction is composed of

TABLE 3
SURFACE AREA THAT IS OCCUPIED BY EXPOSED DENTINAL TUBULES OF 60 HUMAN PREMOLARS WHICH ARE DIVIDED IN TO THREE GROUPS IN RELATION TO THE DISTANCE FROM THE ENAMEL-DENTINE JUNCTION AND THE PULP (%)

Specimens	X	C	Mo SD
S1	2.79	3.10	2.90 0.38
S2	23.90	20.25	23.80 2.56
S3	87.78	85.50	84.20 3.24

S1 – cross section of coronal dentine one millimeter from dentinoenamel junction, S2 – cross section on half-distance between dentinoenamel junction and pulp, S3 – cross section one millimeter from the roof of the pulp chamber.

intertubular dentin, while, near the pulp, intertubular dentin represents only 12% of the surface area^{10,12}. Our investigation is based on three presumptions: (1) that all peritubular dentin has been removed by acid etching, (2) that the surface area which is between exposed dentinal tubules belongs to intertubular dentin, and (3) that this surface area is in negative correlation with dentin depth (Figure 4). Importantly, it is this surface area of intertubular dentin that represents the biological substrate for hybrid layer formation¹⁸.

Pashley et al. examined the relative contribution of resin tags, hybrid layer formation and surface adhesion on the total bond strength to dentin as a function of dentine depth since the intrinsic structure of dentin varies with distance from the pulp chamber. They predicted that resin tags would contribute little to overall bond strength in superficial dentin where there are few tubules, but would contribute the majority of bond strength in deep dentine. According to the model, in superficial dentin, hybrid layer formation contributed most of the bond strength, while in deep dentine, it contributed little. The bond strength to sclerotic dentine is lower than to normal dentine due to the absence of resin tag formation¹⁹. Odontoblastic processes, that have greater diameter in a greater dentine tubules of deep dentin, can hamper resin tags formation and decrease bond strength of hybrid layer that is based on resin tags in deep dentin^{1,20}.

Gwinnett attempted to dissect dentin bonding into its component parts. When he bonded All Bond 2 dentin adhesive to smear layer-covered dentine, he obtained a shear bond strength of 10.2 MPa. He then removed the loose smear layer debris using an air-abrasive sodium bicarbonate powder spray which gave a shear bond strength of 20.4 MPa. The tubules remained occluded with grinding debris, but the surface appeared by SEM to be relatively free of smear layer. This surface was compared to that of fractured dentine, which gave a shear bond strength of 26.8 MPa. This increased strength in fractured dentine may be due to the formation of resin tags, although their diameter is less than that seen in acid-etched dentine. When Gwinnett acid-etched dentine, the shear bond strength of All Bond 2 rose to its maximum value, 32.7 MPa²⁰.

Most teeth that require adhesive restorative treatment are carious or have had caries lesion sometime previously. A number of tissue changes in the deep dentin and pulp take place as a result of caries (formation of tertiary dentine, sclerosis of the dentinal tubules, cellular changes in the pulp). With the exception of caries-affected dentine, sclerosis takes place in the exposed, abraded and cervical dentine. Both types of dentine sclerosis prevent resin tags formation. Bond strength values are

reduced in comparison to bond strength on the normal, physiologic dentine surface¹⁹.

Different dentine adhesive systems make different hybrid layers in terms of structural quality and quantity and in terms of the formation of the three mentioned ultra-morphologic features of hybrid layer without considering dentin depth. Conditioning of the root canal dentine with phosphoric acid and the use of one- and two-bottle-bonding systems gave a thicker and more uniform hybrid layer with considerably more resin tags than observed after the use of »self-etching« adhesives at the same dentin depth. This might provide a more durable bond of the post-to-root canal dentine²¹.

Morphological and structural variations in dentin may have influence on the bond strengths of the bonding systems in the coronal dentin to the floor of the pulp chamber^{22,23}.

Some adhesives do not bond well to deep dentin, especially »self-etching« adhesives with higher pH values, making them more susceptible to polymerization shrinkage stress that develops in cavities with high C-factors²⁴.

The differences in bond strength were thought to be related to the different bonding mechanisms of each material, as well as possible variations in the crown and root dentin substrates²⁵.

Modern concepts of adhesion to dentine are based on the hybrid layer and resin tag formation as a bond between dentine and resin, hard dental tissues and restorative materials respectively²⁶.

Conclusions

1. The number and diameter of dentinal tubules vary with respect to the distance from dentinoenamel junction and pulp chamber.
2. Surface area that is occupied by dentinal tubules varies with respect to the distance from dentinoenamel junction and pulp chamber.
3. Number, diameter and area that is occupied by dentinal tubules are in positive correlation with the dentin depth.
4. Dentin adhesion variety is a result of dentin structural variety.

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BIOLOŠKE ODNOVE HIBRIDIZACIJE DENTINA

SAŽETAK

Svrha ovog istraživanja je bila: (1) odrediti broj, promjer i površinu eksponiranih dentinskih tubulusa na koronarnim presjecima kroz humani dentin; (2) utvrditi da li se neki od nabrojanih parametara mijenja s udaljenosti od dentinsko-caklinskog spojišta i (3) posredno zaključiti kako moguće razlike mogu utjecati na postupak hibridizacije dentina. U tu svrhu je izvršena komparativna analiza scanning elektronskim mikroskopom 60 pripremljenih humanih premolara, koji su bili podijeljeni u tri skupine od po 20 uzoraka svaka. U svakoj od tri skupine učinjeni su različiti presjeci kroz koronarni dentin i to u skupini (1) na milimetar udaljenosti od centralne fisure i dentinsko-caklinskog spojišta; (2) na polovini udaljenosti od caklinsko-dentinskog spojišta do pulpe; i (3) na milimetar od krova pulpne komore. Dobiveni rezultati su obrađeni jednosmjernom analizom varijance (jednosmjerna ANOVA) i regresijskim linearnim modelom. Prosječan broj dentinskih tubulusa na prvoj razini presjeka je bio 19600/mm², na drugoj 32400/mm² i na trećoj 42300/mm². Srednja vrijednost promjera dentinskih tubulusa na prvoj razini presjeka je bila 0,67 μm, 1,52 μm na drugoj i 2,58 μm na trećoj. Ukupna površina pod dentinskim tubulusima na prvoj razini presjeka zauzimala je 2,79%, na drugoj 23,90% i na trećoj 87,78% površine od ukupne površine eksponiranog dentina. Postoji statistički značajna razlika (p<0,01) u sva tri promatrana svojstva između sve tri skupine uzoraka., kao i pozitivna korelacija između dubine dentina i tih svojstava. Za zaključiti je da je dentinska strukturalna raznolikost, koja ultimativno određuje adheziju na dentin, bitna za interakciju između biološkog materijala (dentina) i pojedinačnog adhezivnog sustava koji se koristi.