IN VITRO BEHAVIOUR OF NEW EXPERIMENTAL ADHESIVE SYSTEMS

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ABSTRACT. Bonding of current adhesives to dentin is usually through the formation of the interfacial hybrid layer between adhesives and dentin. Inter-tubular and intra-tubular resin infiltration leads to an increase of the quality regarding the dentinal adhesion. The aim of this study is in vitro testing of new experimental adhesives in comparison with commercial adhesive, regarding sorption and solubility as well as to investigate molecular chemical features of the adhesive/dentin interfaces. We used 4 experimental adhesives systems A1, A2, A3, A4 and IBond® (Heraeus) as reference material. Organic phase adhesive system consists of a mixture of monomers. As filler for these systems, besides hydroxyapatite, we used TiO_2 nanoparticles. FTIR spectroscopy was used to investigate molecular chemical features of the adhesive/dentin interfaces. The obtained data show the main advantages and disadvantages of the tested adhesive systems. The best values of water sorption and solubility both in water and artificial saliva present A2 and A3 adhesives.

Keywords: FTIR spectroscopy, TiO_2, dental adhesives, sorption, solubility

INTRODUCTION

The increase in the use of composite resins as a restorative material in dentistry is mainly due to their aesthetic features, favorable physical and mechanical properties, including high resistance to compression and wear,

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relatively low costs and simple application. Many studies have found failures of composite resins restoration due to the loss of integrity of the adhesive interface. A number of possible mechanical and chemical mechanisms have been proposed as reasons of dentin adhesion [1, 2]. The resin impregnation creates a transitional hybrid layer, that is neither resin nor tooth, but a hybrid of the two. The thin layer of resin reinforced dentin locks the two dissimilar substances together on a molecular level, sealing the surface against leakage. This layer that connects the adhesive to the subjacent dentin is believed to be both chemically and structurally heterogeneous, since its formation relies on many processes such as acid etching to remove mineral phase, adhesive penetrating into the demineralized collagen network in the presence of water and photo-polymerization of the adhesive [3, 4].

The characterization of the heterogeneity of the adhesive/dentin interfacial layer has thus been a topic of great interest. Fourier transform infrared (FTIR) spectroscopy has proven to be a good technique to investigate the physicochemical interactions at the dentin/adhesive interface [4]. Using FTIR spectroscopy, the degree of cure, relative chemical composition and homogeneity across the length and breadth of the adhesive/dentin interface can be determined [4].

Sorption and water solubility leads to a lot of chemical and physical effects, resulting negative influences upon the structure and functions of dental polymers including their capacity of dental adhesion retention. Ideally, the polymeric structure must be insoluble, with a high chemical and physical stability. However, most of the monomers used in the dental materials can absorb water and environment chemical substances, and can release components too.

The water contamination of adhesive materials during their preparation or application, significantly reduce their mechanical properties by 50%. A low solubility of the adhesive materials components is a fundamental condition to insure clinical success. Manufacturers have added hydrophilic monomers to hydrophobic dimethacrylates in an attempt to promote effective bonding between hydrated dentin and resin composites.

Many studies have found failures of composite resin restorations, due to the less of integrity of the interface [3]. This failure can lead to micro leakage and consequently post operatory sensitivity, marginal discoloration and secondary caries. Moreover, it has been shown that the movement of water from hydrated dentin may cause the formation of waterfilled channels within the polymer matrices of contemporary hydrophilic dentin adhesives [5,6]. Some findings suggest that the fluid sorption may lead to a plasticizing of the organic matrix and to its hygroscopic expansion witch can reduce the shrinkage stress [7].

The purpose of this study was to determine sorption, solubility and the physico-chemical interactions at the hard dental /adhesives interface.

RESULTS AND DISCUSSION

Water sorption and solubility mean values ($\mu g/mm^3$) are presented in figures 1, 2, 3 and 4. In the graphs are presented the weekly values of solubility, respectively of sorption. One way ANOVA showed that water sorption and solubility was different for tested materials ($p < 0.05$). The experimental adhesive A2 showed the lowest value, both in water and artificial saliva, followed by adhesive A3, with a small difference between them. That is due to the higher content of their filler (15% compared with 5% of A1 and A4). Adhesives A1 and A4 showed a higher water sorption and solubility, with a considerable difference between them and the first group adhesives. Regarding all tested adhesives, the values of sorption and solubility was stabilized after 21 days. In the same time, the solubility in water was higher than in artificial saliva in all tested adhesives. The negatives solubility values were recorded in this study. This can be explained by the presence of hydroxyl and carboxyl groups in monomers and their resultant polymers make them more hydrophilic and, supposedly, more prone to water sorption and increase the mas of the specimen.
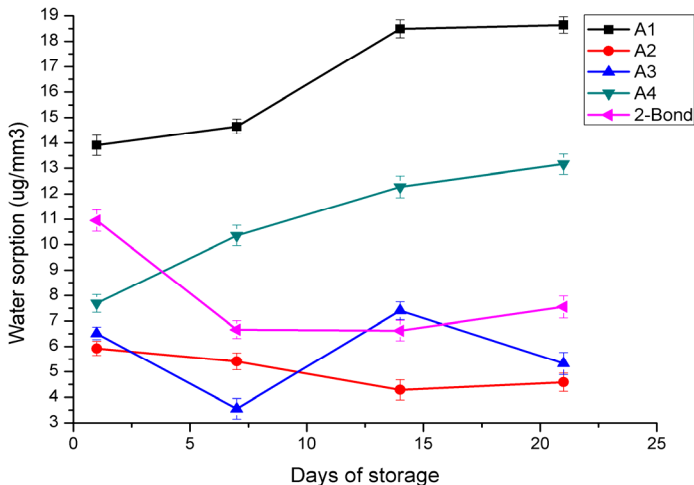

Figure1. Mean values and standard deviation for **water sorption** ($\mu g/mm^3$) of the adhesive materials tested after 1, 7, 14 and 21 days of **water storage**.
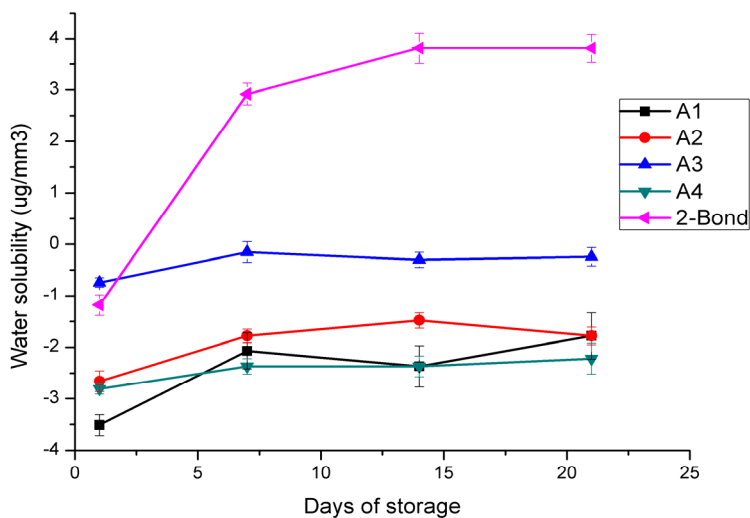
Figure 2. Mean values and standard deviation for **water solubility** (µg/mm^3) of the adhesive materials tested after 1, 7, 14 and 21 days of **water storage**.
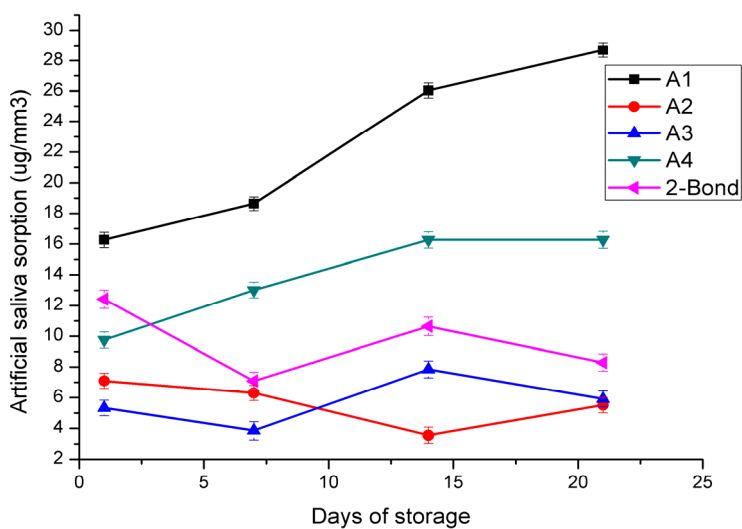

Figure 3. Mean values and standard deviation for (a) **artificial saliva sorption** (µg/mm^3) of the adhesive materials tested after 1, 7, 14 and 21 days of **artificial saliva storage**.
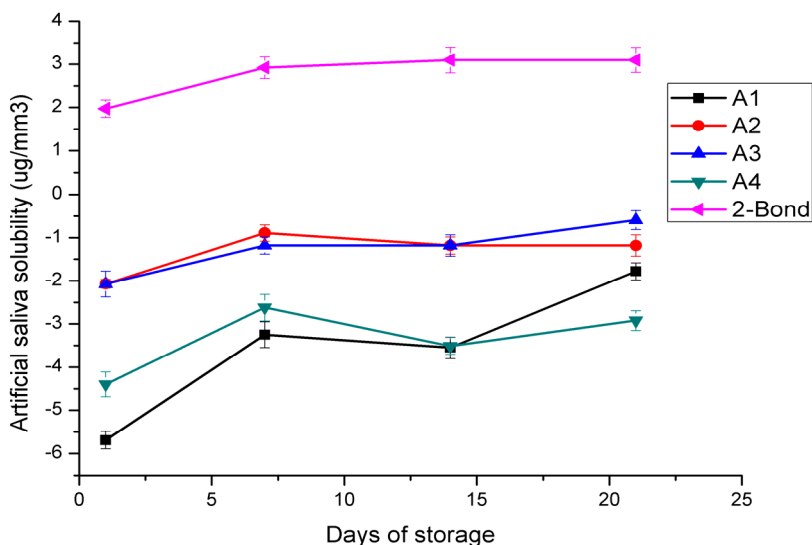
Figure 4. Mean values and standard deviation for **artificial saliva solubility** ($\mu g/mm^3$) of the adhesive materials tested after 1, 7, 14 and 21 days of **artificial saliva storage**.

The one-way ANOVA revealed highly significant differences between the materials for all the sorption and solubility values with some exceptions ($p < 0.05$). After storage in water, there was no significant difference between A2 and A3 at 1 day, between A2 and 2-Bond at 7 days as well as between A2 and 2-Bond at 14 days for the sorption (S). Additionally, there were no significant differences between all materials for the solubility (SL) and SL%, ($p = 0.196$ and $p = 0.245$, respectively). But there were significant differences between storage mediums. As shown in Fig. 1, and 3 the highest sorption (S) was obtained for A1 adhesive when it was immersed in artificial saliva followed by A4 and 2-Bond. A higher amount of TiO_2 in experimental adhesive composition, decrease the sorption and solubility. Moreover, no significant differences were noted between A2 and 2-Bond after 14 and 21 days sorption period. Water sorption and solubility of the experimental and commercial dental adhesives were all significantly dependent on material composition.

Thin sections of the adhesive/dentin interface specimens were analyzed using FTIR spectroscopy. In table 1 are presented the infrared spectroscopic v (cm^{-1}) wavenumbers of absorption maxima of dentin/ adhesive/ enamel interface of experimental dental TiO_2 adhesives and 2-Bond commercial adhesive.

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Table 1. Infrared spectroscopic v (cm^{-1}) wavenumbers of absorption maxima of dentin/adhesive/enamel interface of experimental dental TiO$_2$ adhesives and commercial adhesive

Band name of dental inter-face	Amide I	Amide II	Amide III	C=O	C=C	O-H	C-O	Collagen	PO$_4^{3-}$	Bis-GMA aromatic ring	TiO$_2$
Enamel							1405		1080 964 632		
Dentin	1660	1550	1240	1719	1637	1607	1039	1676 1458 1253	1076 965 588		
Adhe-sive					1639 1721		1182		1080 632	1610 1635	399
Dentin-adhe-sive			1240		1639 1721		1182	1458	1080 638	1635	400

It can be observed that the phosphate band (900–1200 cm^{-1}) of the dentin overlaps with the band of SiO$_2$ filler in the adhesive. In addition, the amide I region more or less overlaps the 1620–1680 cm^{-1} region of the adhesive.

The wavenumbers of absorption maxima from the dentin/adhesive interface spectra are specific from adhesive and dentine, in accordance with other studies [8-10].

Haller et al [11] reported an increase in the amount of interface with gaps over time of storage in water. He explained this occurrence as the effect of degradation of the polymeric structure, with hydrolytic degeneration of resin composite as the water diffuses along adhesive interface. In order to observe the gaps, some authors [6, 5-12] did not use a scanning electron microscope because it would require vacuum, causing loss of water from dentin and composite resin, which could alter the adhesive interface integrity. Instead of it, they used an optical microscope. The adhesive resin should create the hybrid layer (consisting of a collagen network exposed by etching and embedded in adhesive resin). This layer is an interface between dentin and adhesive and the quality of the dental restoration depends greatly on its properties. The quality and structure of the hybrid layer depends on a few factors. One of those is the adhesive type (Total etch/Self etch).

Eliguzeloglu et al [13] reported that hybrid layer thickness showed a significant difference between total etch and self-etch systems in non-carious sclerotic lesions without removal of the superficial layer. Hybrid layer thickness was increased when superficial dentin was removed.

Our study showed that all the tested adhesives are stabilized after 14 days when sorption and water solubility registered the best values. Adhesive A2 and A3 showed low solubility comparing with adhesive A1 and A4. This can be explained by the different composition of filler which is three times higher in adhesives A2 and A3. In the same time, the hydrophilicity of functional monomers can affect the chemical interaction and can influence water absorption and solubility in different adhesives.

Some studies have found that the water sorption causes expansion of the adhesive material until an equilibrium value [14-17]. Another study shows that most of the hygroscopic expansion occurs in the first two weeks, with the balance achieved in about eight weeks [18]. The process of water sorption depends on the composition of the adhesive (volume of filler, type of monomer of the resin matrix, volume of restoration and the cavity configuration [19, 20]. Contemporary adhesives contain increased concentration of hydrophilic resin monomers (HEMA, BPDM, MDP, Bis-GMA), to enhance their bonding to the wet dentine substrate [21-23]. The hydrophilic nature of copolymers facilitates water sorption from the oral environment when exposed to saliva and hydrated dentine. The absorbed moisture can alter the properties of adhesive resin [24]. The temperature at which the process is carried out is very important. In his study, Diamant at all [25] showed that the exposure to water at elevated temperature can produce irreversible effects in polymers that contain high concentration of very hydrophilic monomers. There is a correlation between de nature of monomers regarding their hydrophilicity, the high of moisture and temperature. Intra oral temperature changes can occur following eating and drinking. Under such conditions, formation of cracks as a result of swelling stress and chemical degradation trough hydrolytic reactions may compromise the adhesion [26]. Additions of TiO_2 nanoparticles increase the stability of adhesive, and the resistance to water sorption and solubility. Other studies revealed that TiO_2 present unique photocatalytic, antibacterial and UV-absorbing properties that recommend as beneficial additives in adhesives and resin composites [8, 27].

The strengths of bonding to dentin, especially bonding to the clinical relevant substrates, continue to drop as a function of time [28, 29]. Durability of this bond relies on the quality of the interface between adhesive and dentin. Understanding of the interfacial structure and chemistry is critical to reveal reasons for the low durable bond. The mechanical test techniques measure fracture resistance of adhesive/dentin specimens, and are not sensitive enough to identify interfacial defects that lead to crack initiation or

aqueous degradation [30, 31]. The differences in the distribution patterns of two adhesive regions within the specimens may be caused by different substrates during wet bonding processes. When bonding to dentin, the tubules perpendicular to the surface are filled with water after acid etching and water rinsing. The system of dental adhesive is considered to be the first choice in the treatment of dental restoratives. The results show that the introduction in the polymeric matrix of TiO_2, improves the physico-chemical properties of the adhesive and gives an antibacterial effect.

CONCLUSIONS

The reinforcement of TiO_2 in polymer matrices has shown interesting results in improving the water sorption and solubility properties. Based on the obtained results, we could consider that the TiO_2 (two-step self-etch systems – A2) could be a very promising filler for the dental adhesives.

Durability of the tooth restoration relies on the quality of the interface between adhesive and dentin. Understanding of the interfacial structure and chemistry is critical to reveal reasons for the low durable bond.

EXPERIMENTAL SECTION

Four new adhesives based on TiO_2 and a commercially available dental adhesive were tested: two "etch and rinse systems" adhesives (A2, A3), and two "two-step self-etch systems" adhesives (A1, A4) and the 2Bond® (Heraeus), etch and rinse adhesive system. The composition of the experimental adhesives is presented in table 2.

We used 20 extracted erupted human teeth, stored in artificial saliva. In each tooth were produced class I occlusal cavities following steps to prepare cavities by Black. All the cavities were etched with phosphoric acid (37%) for 15 seconds, followed by washing in running water for 20 seconds and drying. The adhesive solution was brushed onto the entire dentin surface, gently air-dried; the adhesive coated dentine surface was light-cured for 20 seconds using a conventional halogen light unit with an output intensity of $600 mW/cm^2$. A resin composite (Herculite XRV®, Kerr) was placed in three 1 mm thick increments over the adhesive and light-cured for 120 seconds. The prepared teeth were fixed on a methacrylate support and stored for 8 weeks in artificial saliva at 37ºC before being sectioned in 3 microns thick sections using a tungsten carbide knife fixed on a microtome (IsoMet 1000, Buehler). These sections were placed directly on the motorized stage for FTIR with a spectrometer (JASCO).

For the water sorption and solubility measurements, a teflon mold was used with 15 mm diameter x 1 mm thickness, according to ISO 4049. A glass cover slip was placed on top of the adhesive, which was light-cured for 40 s using a visible light (Woodpeker LED). After removing the specimen from the mould, photoactivation was repeated on its opposite surface for another 40 s. The obtained surfaces must be smooth and flat. Immediately after polymerization, the specimens were placed in a desiccator at 23ºC, repeatedly weighed (Analytical balance – Partner AS160/C/2, Partner Corporation, RO) after 24 h intervals until a constant mass (m_1) was obtained. The specimens were immersed in distillated water and maintained 7 days. The weight was daily determined by extracting from water and wiped with absorbing paper. After 10 minutes the specimens must be weighed thus registering the weight (m_2). The specimens were replaced in desiccator again, 4 hours long, until a constant weight had been achieved (m_3). This process will be repeated at 14 and 28 days. The test was done both in distilled water and artificial saliva ARTISIAL® (Joiveinal Laboratoires, France).

Table 2. The composition of experimental and commercial adhesive system

Adhesive system	Composition	Ratio
A1	(1) *Adhesive*- Bis-GMA, HEMA, TEGDMA, Polyacrylic acid,Ethanol and initiators, HAp-TiO2 (5%)	95/5
A2	(1) *Primer*- Bis-GMA, HEMA, Polyacrylic acid, Ethanol, Water and initiators; (2) *Adhesive*- Bis-GMA, HEMA, TEGDMA and initiators, TiO2 (15%)	85/15
A3	(1) *Primer*- Bis-GMA, HEMA, Polyacrylic acid, Ethanol, Water and initiators; (2) *Adhesive*- Bis-GMA, HEMA, TEGDMA and initiators, HAp-Ag (5%), HAp-ZnO (5%), TiO2 (5%)	85/15
A4	(1) *Adhesive*- Bis-GMA, HEMA, TEGDMA, Polyacrylic acid, Ethanol and initiators, TiO2 (5%)	95/5
2Bond, Heraeus Kulzer GmbH	(1) *Adhesive*- 4-META, Urethane dimethacrylate, Glutaraldehyde, acetone, water and initiators	100
Bis-GMA- 2,2-bis(3-(2'-hydroxy-3'methacryloyl-oxypropoxy)phenyl)propane [synthetised in ICCRR laboratory]; HEMA- 2-Hydroxyethyl methacrylate [Aldrich]; TEGDMA - triethyleneglycol- dimethacrylate [Aldrich]; 4-META- 4-methacrylo-yloxyethy trimellitate anhydride, Polyacrylic acid M2000 [Aldrich]		

Sorption and solubility were calculated for each specimen according to formulas:

$$Wsp = (m_2-m_1)/V \text{ and } SL = (m_1-m_3)/V \text{ (μg/mm}^3)$$

where: m_1 - sample weight before water immersion (μg);
 m_2 - sample weight after water immersion 24 hours (μg);
 m_3 - sample weight which was maintained in the desiccators until a constant weight had been achieved (μg);
 V – sample volume (mm^3).

ACKNOWLEDGMENTS

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