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MICROMORPHOLOGICAL CHARACTERIZATION OF THE ADHESIVE INTERFACE OF SELF-ADHESIVE RESIN CEMENTS

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1. Introduction

Adhesive dentistry is nowadays one of the most important fields of restorative dentistry. Scientific investigations focus on the physical and mechanical properties of adhesive materials and the evaluation of adhesion mechanisms as well as quality of adhesive bonding to dental tissues and different dental materials. Variability of adhesive materials is enormous. And especially in recent decades, since the constant demand for simplified adhesive materials and procedures persists, it is of utmost importance to examine these materials and prove their reliability and suitability in adhesive procedures.

Adhesion in dentistry represents the ability to bond different materials (metal, porcelain, resin composite etc.) to hard tooth tissues. Adhesive bonding techniques can be applied for the direct or the indirect restoration of lost tooth tissues. Resin composite fillings can be placed directly, whereas metal or ceramic crowns, inlays or onlays represent indirect restorations and are fixed to tooth tissues with resin containing luting agents. These resin luting agents are functioning similarly to resin composite filling materials: they consist of a resin matrix and fillers and require adhesive systems to mediate an adhesive bond between tooth and resin luting agent. In the classical adhesive procedure, hard tooth tissues are superficially demineralized and the smear layer (the debris which is left on the surface of tooth structures after bur application) is removed by a separate etching step. This creates a micro-retentive etched pattern which can be infiltrated by the compounds of the adhesive system. In this way, the micromechanical anchorage of resin material within the hard tooth tissues is established. The task of the adhesive system is to create a reliable bond between the hydrophilic tooth tissues and the hydrophobic resin composite luting agent.

Adhesive systems exist in huge varieties and differ from each other regarding the number of application steps and the mode of tooth tissue pretreatment. Mostly, they include two to three clinical steps which are quite time-consuming and technique sensitive. To overcome the technique sensitivity associated with a multi-step bonding procedure, simplified self-etching adhesive systems were introduced. In the most recent approach to simplification, the resin luting agent and the compounds of self-etching adhesive systems were combined to form self-adhesive luting agents, so called self-adhesive resin cements (referred to as SARC's in the present study). Through special functional monomers demineralization and infiltration of tooth tissue occur simultaneously, so no tooth tissue pretreatment is necessary anymore. The smear layer is not removed, but modified and included in the adhesive bond. Self-adhesive resin cements have an ability to bond to tooth tissue not only micromechanically as adhesive systems but also chemically as glass ionomer cements do. Radical and acid-base

polymerization reactions are claimed to enable fast and reliable bonding to tooth substrate. However, there is only scant research evidence about the efficacy of the bonding mechanism of self-adhesive resin cements.

The aim of this study was the depiction of different self-adhesive luting agents and analysis of the microstructure of the adhesive interface between dental tissues and SARC's in comparison to the adhesive interface of an established resin luting agent with separate self-etching adhesive system. The micromorphology was investigated employing different polymerization modes (light- or auto- polymerization) using low vacuum scanning electron microscopy (LV SEM). LV SEM is relatively new investigation method which allows for reduction of the specimen preparation procedure and at the same time examination of whole intact, demineralized or deproteinized specimen. It was the purpose of the present study to find out which morphological characteristics can be observed in specimens luted with SARCs and whether and how these characteristics resemble or differ from the morphological pattern of a luting agent with a separate adhesive system.

2. Literature review

2.1. Principles of adhesion in dentistry

The adhesion science in dentistry has developed rapidly since Buonocore in 1955 discovered selective enamel etching for the improvement of the adhesion of acrylic filling material to enamel (16). Restorative dentistry is no longer imaginable without adhesive procedures. Adhesion is a process whereby the “adherent” (or substrate) creates an intervening “interface” with an applied “adhesive”. In dentistry, there are different adherents like enamel, dentin, composite, ceramic, metal etc. The adhesives can involve single or multiple interfaces to mediate the bonding of, for instance, ceramic to metal or ceramic restorations to tooth tissues (51).

In restorative dentistry there can be several types of adhesion distinguished:

- Macro-mechanical adhesion is a bonding type that employs the macroscopic surface irregularities of macroretentive preparations. A typical example is the cementation of bridges and crowns with conventional phosphate or polycarboxylate cements.
- Similarly, microscopic irregularities are employed in the creation of micro-mechanical adhesion typical for composite resins and corresponding adhesive systems.
- Chemical adhesion is interfacial or true adhesion formed by chemical bonds between materials being joined. Glass ionomer cements possess true chemical adhesion potential.

The fundamental principle of micromechanical adhesion of composite resins to tooth tissues is based upon an exchange process, in which inorganic tooth material is substituted with synthetic resin (95). This involves two phases: calcium phosphates from tooth tissues are removed and microporosities are formed (25); the resulting calcium phosphate depleted collagen-network is infiltrated by the adhesive components (hybridization). The latter polymerize in situ and a hybrid layer is formed (25;98). Modern adhesive systems involve three approaches based on this two-phase process (98): etch-and-rinse approach, self-etching approach and glass ionomer approach each associated with different adhesive systems. Additionally, adhesive systems can be classified according to the number of clinical steps and the way they interact with the smear layer (65).

2.2. Etch-and-rinse adhesives

The technique employing separate conditioning of enamel and dentin is still considered to be the most effective one to achieve a stable bond to tooth tissues. Etch-and-rinse adhesives include a separate etching step. If the dentin and enamel are conditioned simultaneously, this technique is termed as “total-etch” technique. In the “selective-etch” technique enamel is selectively etched before the dentin pretreatment. The etchant is usually a 30-40% phosphoric acid, which is applied for 15-30 secs. to remove the smear layer and superficial hydroxyapatite and to expose the microporous collagen network. The etchant is then rinsed off. This is followed by a priming step and the application of an adhesive resin (25). If etching, priming and adhesive application are performed in separate clinical steps, adhesive system is designated as three step etch-and-rinse adhesive. There are simplified two-step etch-and-rinse adhesives where the primer is already combined with adhesive in one application step. The performance and handling of etch-and-rinse adhesives is dependent on the primer solvent (20;87). There are ethanol-based, acetone-based and water-based primers. The adhesives containing a water-based primer are supposed to be less technique-sensitive (25;64). Water moisturizes the etched dentin and preserves the collagen network from collapsing. When using acetone-based adhesives overdrying of dentin is not acceptable and so called “wet-bonding” (dentin should be moist) is important (25;98).

2.2.1. Adhesion to enamel

Enamel consists of 95-98% inorganic components, hydroxyapatite (HAp) crystals, which are arranged in prisms. Etching with 30-40% phosphoric acid removes about 10 µm of the top surface, exposing the prism cores (48). The applied adhesive system fills the resulting microscopic irregularities and forms taglike resin extensions (microtags and macrotags) after polymerization (48;98). The macrotags occupy the space around enamel prisms, but microtags are formed within etch-pits at the cores of enamel prisms. The microtags contribute most to the retention to enamel (98). When tested in different studies, the mean microtensile bond strength achieved with three-step and two-step etch-and-rinse adhesives was 39 and 40MPa, respectively (98). The wetting of a substrate depends on a surface energy. HAp has high surface energy, and the wetting of HAp by resin monomers of primer and adhesive in enamel is easier due to higher affinity than compared to dentin (82).

2.2.2. Adhesion to dentin

The complex structure of dentin makes adhesion to this tooth substance a challenge. Also, the reliability of adhesion to dentin is still not as high as that to enamel. Nevertheless, today’s

adhesives show superior results in the laboratory and have improved clinical effectiveness and performance of adhesion to dentin approaching that of enamel (98).

The inorganic part of dentin reaches app. 70 – 75%, whereas the water content is near to 10%. Dentin consists of intertubular and peritubular dentin, the latter is more mineralized and therefore harder. Due to the funnel structure and fan-like orientation of dentinal tubules, they occupy approximately 22% of the area in deep dentin close to the pulp and only 1% at the dentino-enamel junction (DEJ) and are connected with pulpal tissues (65;82). The content of water in dentin correlates with the depth of dentin and the volume of dentinal tubules. As the dentinal fluid can deteriorate the adhesive bonding to dentin, the bond strength to superficial dentin is higher than that to deep dentin (102).

The classical bonding mechanism of etch-and-rinse adhesive systems to dentin depends primarily on hybridization or infiltration of resin within the exposed collagen fibril scaffold (65;95;98). Phosphoric acid treatment on dentin removes the smear layer and enlarges the lumina of dentinal tubules by dissolution of peritubular dentin (37). It exposes a microporous network of collagen that is nearly totally depleted of HAp. In this case, a true chemical bond to collagen fibres is rather unlikely because for chemical bonding the functional groups of monomers need remaining HAp (98). If adhesive resin does not infiltrate the demineralized collagen network in its entire depth, “nanoleakage” is possible. Nanoleakage is a nanometer-sized space around naked collagen fibrils, where the resin has failed to infiltrate. These areas serve as a pathway for degradation of resin-dentin bonds over time (72). Nanoleakage is one of the greatest disadvantages associated with the use of etch-and-rinse adhesives.

Scanning electron microscope investigations revealed very specific features, characteristic for the morphology of the dentin-etch-and-rinse adhesive interface: about 3-4µm thick resin-dentin interdiffusion zone or hybrid layer (57), hybridization of the dentinal tubules and formation of resin tags and resin tags in lateral dentinal tubules.

2.3. Self-etching adhesives

The market-driven simplification of adhesive systems supported by the technique sensitivity of the wet bonding technique, led manufacturers to develop self-etching adhesives. Self-etching adhesives do not include a separate etching step; they consist of a complex mixture of functional monomers, solvents and water that are highly hydrophilic which renders the smear layer permeable to the adhesive resin. Therefore, the modified smear layer is incorporated within the resin-dentin interdiffusion zone. According to the number of resin application steps, self-etching adhesives are currently available as two-step or single-step systems, the latter also known as all-in-one adhesive systems, (40). Depending upon the pH

of the system, self-etching adhesives can be divided into strong, intermediate and mild ones (98). Functional monomers improve adhesion to tooth substrate also by chemical bonding to calcium in mild self-etching adhesives.

The pH of strong self-etching adhesives usually is 1 or below 1. This high acidity results in rather a deep demineralization effect (98). Strong self-etching adhesives exhibit a similar bonding mechanism and interfacial ultra-morphology as the etch-and-rinse adhesives do.

The pH of intermediary strong or moderate self-etching adhesives is about 1,5. Most typical is the two-fold build-up of the dentinal hybrid layer. Whereas the superficial layer of hybrid layer is completely demineralized, the base still contains undissolved HAp (98).

Mild self-etching systems have a pH of around 2 and are usually two-step systems. They demineralize dentin up to the depth of 1 μm. The presence of remaining HAp within the submicron hybrid layer may serve as a receptor for additional chemical bonding, especially for the functional monomers which have a potential to bond to calcium (104).

2.3.1. Adhesion to enamel

Due to milder etching properties of self-etching adhesives, their demineralization efficiency is lower as compared to that of phosphoric acid etching. The compromised potential to bond to enamel is considered to be one of the weakest properties of self-etching adhesives, this especially applies to all-in-one adhesives (98). Roughening of prismless enamel or a separate enamel etching step enhances the bonding capability of self-etching adhesives to enamel (64). In a transmission electron microscopical (TEM) study of self-etching adhesives, on the enamel a 1.5 – 3.2 μm thick, netlike resinous structure – “nanoretentive” interlocking - could be observed, indicating inter- and intracrystallite monomer infiltration (39). This accounts for the creation of a microretentive bond (39). The microtensile bond strength tests of self-etching adhesives showed lower values than those of etch-and-rinse adhesives (98). Nevertheless, two-step self-etching adhesives have an acceptable bond strength to ground enamel and dentin *in vitro* (64). The mean bond strength obtained from two-step self-etching adhesives was 30 MPa, whereas one-step self-etching adhesives produced only 16 MPa bond strength (98).

2.3.2. Adhesion to dentin

Discrepancies between the depth of demineralization and the depth of resin infiltration that can occur by separate acid etching and account for nanoleakage may be avoided by using self-etching adhesives (88). They remove minerals from the dentin surface while simultaneously replacing them with the resin monomers. This process leaves no voids, and

consequently, no nanoleakage can be expected at the interface. But on the other hand, Carvalho et al. reported that the self-etching adhesives cannot infiltrate the entire partially demineralized dentin (19). Incomplete infiltration is attributed to the residual water within the infiltration zone or different infiltration rates of adhesive co-monomers, and the generation of acidic but non-polymerizable, hydrolytic adhesive components. These components - even after polymerization - function as permeable membranes causing so called “water treeing” – water movement across the hybrid layer (19;21;25;40;64). “Water treeing” leads to reduced interfacial strength and premature hydrolysis.

Yuan et al. investigated the nanoleakage of etch-and-rinse, as well as two-step and one-step self-etching adhesives bonded to cervical cementum and superficial dentin by means of TEM examination. They observed that one-step self-etching adhesives showed less nanoleakage than two-step self-etching adhesives, whereas two-step self-etching adhesives showed better hybridization than etch-and-rinse adhesives (105). Self-etching adhesives hybridize dentin for up to 2 μm and have been reported to withstand stresses from polymerization shrinkage clinically (32). In general, the mild two-step self-etching adhesives form stronger bonds in comparison to all-in-one adhesives (25). This was also confirmed by Van Meerbeek et al. (98). They statistically compared and summarized numerous studies on microtensile bond strength of two-step self-etching and two-step etch-and-rinse adhesives and concluded that the effectiveness of them to bond to dentin was quite similar (98). Despite the detected increased porosity within the adhesive layer of two-step self-etching Clearfil Liner Bond II which Sano et al. detected in the SEM investigation, no significant decrease in μTBS occurred after 1 year *in vivo* function (73).

The thickness of the hybrid layer using strong self-etching adhesives approaches that of the hybrid layer of etch-and-rinse adhesive systems. Mild self-etching systems and glass ionomers form a submicron hybrid layer, which extends only up to 1μm (98). Some of the self-etching adhesives create a compact interdiffusion zone and can penetrate into dentinal tubules even forming tags, but some fail to produce even a satisfactory hybrid layer (32;79). In some tubule orifices the dissolved smear plugs appeared as voids within the resin-filled tubule (32).

2.4. The role of the smear layer in the adhesion process

When the adhesion process to tooth substances is described, the smear layer as an important part of adhesion has to be considered. Moreover, as the classification of current adhesives is based on an interaction mode with the smear layer, it is important to understand the changes occurring within the smear layer during the bonding procedure. In particular, the

smear layer and its properties influence the bonding ability of adhesive systems which modify the smear layer and include it in the adhesive bond.

After preparation of the dentin and enamel with burs or other instruments a 0.5 - 5µm thick smear layer covers the surface, filling the openings of the dentinal tubules. This debris consists of bacteria, saliva, blood cells and denaturated collagen (37;48;61). The smear layer fills the dentinal tubule orifices and forms smear plugs, but it is not always firmly attached to or continuous over the dentin substrate (37;82). The smear layer reduces the dentin permeability by up to 86% (65). The cohesive strength of the smear layer is ca. 5 – 10 MPa (60;65). The smear layer diminishes the water perfusion on the surface of bur-cut dentin, but the submicron porosity within it still allows the diffusion of dentinal fluid (65). The dentinal fluid can be detrimental for adhesion, disabling the ability of the hydrophobic components of adhesive to adhere to hydrophilic substrates. In this context, the polarity of functional monomers is very important for the wetting behavior (93). The smear layer of diamond bur-cut dentin tends to be more compact than that ground with silicon carbide sandpaper. The bond strength is also influenced by the thickness of a smear layer. For self-etching adhesives, a thin smear layer is more advantageous (65).

The early bonding systems preserved the smear layer because it was thought to protect the pulp, but it was an unstable bonding substrate. For achieving a good bond strength and good seal, dentin must be suitably conditioned to remove or modify the smear layer and to permit diffusion of monomers into the subjacent, partially demineralized collagen matrix (48).

2.5. Resin luting agents

The resin containing restorative materials with corresponding adhesive systems have found their application both for direct restorations and for indirect restorations. The direct restorations are usually performed with resin composite, whereas the indirect restorations use resin composite containing luting agents (= resin luting agents). The advantage of adhesive bonding of indirect restorations in comparison to direct resin composite restorations is the significant reduction in the polymerization shrinkage. Polymerization shrinkage causes deterioration of the bonding effectiveness to tooth tissues and therefore compromises the longevity of the adhesive bond. Resin luting agents with corresponding adhesive systems exhibit enhanced mechanical, physical and adhesive properties in comparison to luting agents such as phosphate cements, polycarboxylate cements, glass-ionomer cements and resin-modified glass-ionomer cements (100). Additionally, they are tooth-coloured and can be used for the cementation of dental materials such as ceramics to tooth hard tissues in aesthetically demanding regions. However, resin luting agents in combination with multi-step adhesives are quite technique sensitive due to complicated clinical procedures and therefore

susceptible to manipulation errors (100). Simplifications to reduce clinical steps were made and resulted in the development of so called self-adhesive resin luting agents (54). Adhesives and composite resin were combined into one material. These newly developed resin luting agents with incorporated adhesives are called self-adhesive resin cements (referred in the present study as SARC's). It is still unclear how the simplifications influenced the bonding effectiveness, what the nature of the adhesion is and how the simplified resin luting agents perform in the therms of clinical longevity. Especially, the morphology of the adhesive interface of new materials is little described. The question arises whether the features of the adhesive interface formed with etch-and-rinse or self-etching adhesives could also be found at the adhesive interface with SARC's.

2.6. Self-adhesive resin cements (SARC)

The first self-adhesive resin cement available on the market, RelyX Unicem, has been manufactured since 2002. Due to its clinical success RelyX Unicem is still a reference for *in vitro* and *in vivo* investigation data of other SARC's being developed. The new SARC's introduced recently to the market are listed in Table 2. However, detailed information on their composition and properties is limited and the information provided comes mainly from manufacturers.

2.6.1. Main composition

Similar to filling resin composites, SARC's consist of a resin and an inorganic filler part. These constituents can be delivered as paste-paste system (more frequently) or liquid-powder system (31).

The resin part of SARC's is mostly a mix of conventional monomers: mono-, di-, or multi-methacrylates such as Bis-GMA, UDMA, HEMA, TEGDMA etc. The particular acidic functional monomers are basically (meth)acrylate monomers with either carboxylic acid groups as 4-methacryloxyethyl trimellitic anhydride (4-META) and pyromellitic glycerol dimethacrylate (PMGDM), or phosphoric acid groups as 2-methacryloxyethyl phenyl hydrogen phosphate (phenyl-P) and 10-methacryloxydecyl dihydrogen phosphate (MDP), bis(2-methacryloxyethyl) acid phosphate (BMP) and dipentaerythritol pentaacrylate monophosphate (Penta-P) (31). These acidic monomers function as demineralizers of enamel and dentin via phosphate groups, and simultaneously as mediators for the chemical bond to calcium. The MDP monomer forms the most insoluble salts with calcium, whereas 4-META and phenyl-P have lower bonding potential to HAp and create less hydrolytically stable salts (43;93;104). The separation of acidic monomers from photoinitiators and ion-releasing glass fillers within the luting agent to avoid premature polymerization or acid-base

reaction is a challenge (31). In the freshly mixed SARC, depending upon the acidity and the concentration of functional monomers, pH is ca. 1.5 – 3 which corresponds to mild self-etching primer acidity. The pH rises rapidly in the first setting hour and approaches pH 7 by 24 – 48h (31;38).

The fillers of SARC's are combinations of barium fluoroaluminoborosilicate glass, strontium calcium aluminosilicate glass, quartz, colloidal silica, ytterbium fluoride etc. which are claimed to be fluoride-ion releasing. The filler content in SARC is somewhat lower than, for example, in compomers, and it is near to that of flowable resin composite, mostly reaching from 60 to 75% by weight (31).

2.6.2. Physical and mechanical properties

2.6.2.1. Bond strength to enamel and dentin

Numerous *in vitro* studies were conducted to investigate the microtensile (24;36;41;102), shear (3;50;70) and tensile (6) bond strength of SARC's. The bond strength of RelyX Unicem to enamel is about 14.5 MPa, which is significantly lower than the bond strength of resin luting agents ranging from 17 to 32 MPa but it is still significantly higher than of glass ionomer cements (3;24;71). The shear bond strength to untreated and etched enamel of Clearfil SA Cement was 9.8 and 17.6 MPa accordingly which exceeded the mean bond strength values of other SARC's: Maxcem, RelyX Unicem, Breeze, BisCem and seT (49). *In vitro*, bond strength of SARC to the enamel and dentin is generally lower than those of resin luting agents with separate adhesive system (3;24;50). Better bond strength can be reached by selective enamel acid-etching (24;27;41;49), but etching of dentin with phosphoric acid is reported to be detrimental for dentin bonding strength (24;41). Dentin pretreatment with polyacrilic acid gave controversial results: in some studies the bond strength of several SARC's to dentin was improved, whereas for other SARC's in the same studies significant differences in bond strength could not be detected (53;62;92). In the study of Mazzitelli et al. a pulpal pressure was used to determine its influence on microtensile bond strength of SARC's (RelyX Unicem, GCem, Multilink Sprint, BisCem) (54). RelyX Unicem and BisCem showed the highest bond strength values.

2.6.2.2. Polymerization characteristics

As SARC's are dual curing luting agents, there are several studies which investigate the influence of polymerization mode on material properties such as degree of conversion, shrinkage strain rates and shrinkage of SARC's (31;46;100). Auto-curing of Maxcem and Multilink Sprint caused higher shrinkage strain rates and shrinkage than that of RelyX Unicem (31;80). In the study of Kumbuloglu et al. (46) the degree of conversion in light- and auto-polymerization mode of SARC (RelyX Unicem) and resin luting agents with separate

adhesive systems (Panavia F, Variolink II and RelyX ARC) was compared. They found that RelyX Unicem had the lowest degree of conversion and reached 81% in light-polymerized mode and only 61% in auto-polymerized mode (46). Light curing of RelyX Unicem also resulted in higher shear bond strength to human dentin than auto-polymerization alone (70). Aguiar et al. examined the microtensile bond strength to dentin of Panavia F (resin luting agent with self-etching adhesive system) and SARC (RelyX Unicem, BisCem and GCem) depending upon polymerization mode (4). They found that the polymerization mode of RelyX Unicem and BisCem had no effect on the bond strength, whereas, the light-curing of Panavia F and GCem increased their bond strength to dentin. Cadenaro et al. found no significant difference in the microhardness between resin luting agent Panavia F and self-adhesive RelyX Unicem and Maxcem resin cements but they found that light-curing of resin luting agents generally resulted in higher hardness of materials than only auto-curing mode (17). Conversely, in the study of Pedreira et al. Panavia F exhibited higher initial microhardness than RelyX Unicem, Variolink and Duolink luting agents. Interestingly, three months storage in water significantly increased the microhardeness of RelyX Unicem (63). They concluded that the quality of curing seems to be unpredictable and highly material dependant. Self-adhesive GCem and RelyX Unicem cements showed the micro-mechanical properties as Vickers hardness, modulus of elasticity, creep and elastic or plastic deformation comparable to or even better than the resin luting agents with separate adhesive system (Dentin Build and Multilink Automix) (42). Light curing of RelyX Unicem resulted in almost two-fold increase of elastic modulus compared to self-curing mode (42). In the mentioned study eight commercially available SARC's including Clearfil SA Cement were tested. In conclusion, it can be said that light-activating improves the physical properties of SARC's as well as the effectiveness of their bond to tooth hard tissues.

2.6.2.3. Wear properties

SARC's (Bifix SE, Clearfil SA Cement, SpeedCem, RelyX Unicem, SmartCem 2, GCem and Maxcem Elite, iCem) showed good wear resistance in the toothbrushing wear test (1N applied force, 1.25 Hz brushing frequency, 20 000 cycles). However, ICem showed the lowest wear resistance to toothbrush abrasion (12). Most SARC's wore rapidly in comparison to resin luting agents with separate adhesive systems (AllCem and Variolink II Base) when higher loads of ACTA wear simulation machine (15N applied force, 400 000 cycles) were applied (12).

2.6.2.4. Marginal adaptation *in vitro*

There are several studies that have evaluated the marginal adaptation of SARCs and well-tried luting materials (10;11;33;56;75). Behr at al. luted Empress 2 inlays with SARC's (RelyX Unicem Clicker, Maxcem, Multilink Sprint) and resin luting agent Panavia F with self-etching

adhesive system and investigated the marginal integrity after storage in water for 90 days and additional mechanical and thermal loading in both dentin and enamel using dye penetration test and scanning electron microscopy (10). Panavia F showed the lowest dye penetration at the finishing lines followed by RelyX Unicem. Maxcem and Multilink showed considerable dye penetration up to 60% (10). Another dye penetration test and scanning electron microscopy investigation carried out by Behr et al. compared the marginal adaptation of a SARC (RelyX Unicem), with and without dentin pretreatment, with self-etching adhesive and established resin luting agents with corresponding adhesive systems (Variolink II and Dyract Cem Plus). It was found that the luting agents had comparable amounts of “perfect margin” even after simulation of five years oral stress, but the dye penetration was significantly lower with self-adhesive systems (11). In a similar study Mörmann et al. compared RelyX Unicem and Multilink with the resin luting agent Variolink and the glass ionomer cement Ketac Cem (56). It was observed that RelyX Unicem at cement-dentin interface had significantly higher marginal integrity than other luting agents. At crown-cement interface both SARC’s showed better results than Variolink and Ketac Cem. In the same study it was found that crowns luted with RelyX Unicem have higher fracture resistance (56). However, within enamel the marginal quality of luted IPS Empress inlays before and after thermo-mechanical load is still better using luting agents with etch-and-rinse adhesives (33).

The disadvantage of SARC’s is the enlarged number of pores and voids in the material as a result of mixing, particularly in RelyX Unicem after trituration (24;53). It was supposed that high viscosity of SARC hampers cement penetration, even if the surface is pretreated and the dentinal tubules are opened and smear plug free. Therefore it was suggested that to enhance the tixotropic properties of SARC and to reduce porosities at the interface, pressure should be applied during the seating and polymerization process (24;36).

2.6.2.5. *In vivo* studies

There are only few *in vivo* studies to assess the clinical performance of SARC’s. 1-year evaluation of 43 IPS Empress inlays luted with RelyX Unicem compared with a control group of 40 inlays luted with Variolink II showed clinically acceptable results (84). This is in line with the results reported by Peumans et al. who evaluated the two-year clinical performance of IPS Empress inlays luted with Relyx Unicem only or with selective enamel etching prior to the luting procedure with RelyX Unicem (69). Schenke et al. reported that selective enamel etching prior to luting seems to have no influence on marginal, partial ceramic crown or tooth integrity of the restored teeth after 1-year clinical performance (76). 2-year results of the same study showed slight tendency for better clinical results if selective enamel etching was performed (77).

2.6.3. Mechanism of adhesion and morphological characterization

2.6.3.1. Bonding of SARC's to enamel and dentin

Adhesive properties of SARC's are claimed to be based upon phosphoric-acid methacrylate monomers which demineralize and simultaneously infiltrate the tooth substrate, resulting in micromechanical retention (dual-cured red-ox polymerization reaction). Secondary reactions have been suggested to provide chemical adhesion to HAp (glass ionomer reaction). Gerth et al. (34) revealed in an X-ray photoelectron spectroscopy study, the chemical reaction of 86% of HAp calcium atoms with RelyX Unicem. In comparison the resin luting agent Bifix with corresponding etch-and-rinse adhesive system achieved only 65% (34). These findings confirmed the propriety of the concept suggested by Yoshida et al. This concept claims that functional monomers adhere easily to artificial HAp and create a very stable chemical bond with low water solubility (104). Generally, the materials containing MDP monomer (Clearfil SA Cement), 4-MET (GCem) and functional monomer of RelyX Unicem performed significantly better than other SARC's with regard to salt solubility (104).

2.6.3.2. Micromorphological characteristics of the adhesive interface

Immediately after mixing, RelyX Unicem is very hydrophilic and acidic, but these features change during the setting and it becomes hydrophobic and neutral (1). As the SARC's are one step luting agents, they principally interact with the smear layer. Although, the initial acidity of RelyX Unicem is quite high, almost no demineralization of the dentin surface was noted in a transmission electron microscopy (TEM) investigation reported by de Munck et al. (24). This was supposed to be due to the relatively high viscosity of this material and limited interaction/penetration time (in the study RelyX Unicem was light-cured directly after application) (24). A scanning electron microscopy (SEM) investigation of RelyX Unicem samples bonded to dentin revealed only superficial interaction with enamel and dentin. An irregular interdiffusion zone (hybrid layer) was revealed ranging from 0 - 2µm, probably corresponding to the rough (bur-cut) and irregular smear layer (24). The irregular interdiffusion zone formation could be explained by the similarity of RelyX Unicem adhesion mechanism to the glass ionomer cement one which also forms irregular interdiffusion zone (6). Although RelyX Unicem exhibited better marginal continuity than Maxcem Elite, the presence of an interdiffusion zone could not be confirmed in the SEM investigation of Goracci et al. (36). No resin tags could be detected in SEM investigations of Al-Assaf et al., De Munck et al., Goracci et al. and Yang et al. (6;24;36;102). The smear plugs remained undissolved in dentinal tubules. Where the smear plugs were absent (in the control samples of fractured dentin) RelyX Unicem infiltrated into the tubules and reacted with the tubule wall in a similar way as at the intertubular dentin surface (24).

2.7. Investigation methods of adhesive interfaces

During the last decades, diverse techniques have been used to investigate the adhesive interface between restorative materials and dental tissues (97). The most popular ones are different microscopy methods such as confocal laser scanning microscopy (CLSM) (8;12;45), transmission electron microscopy (TEM) (21;24;39;45;94;96;102;105), scanning electron microscopy (SEM) as high vacuum SEM (HV SEM) (6;8;22;26;45;57;79;96), field-emission SEM (FE SEM) (24;44;45;67;81) or environmental SEM (ESEM) (6;45). Numerous interface visualization techniques and specimen preparation procedures are used for HV SEM, FE SEM and TEM examinations. Basically, morphological studies were carried out on the demineralized/deproteinized specimens coated with conductive powder (SEM examinations) or on the ultrathin cut and stained samples (TEM investigations). SEM examinations mostly complemented the studies on mechanical properties (tensile, shear bond strength) of luting agents to determine the fracture mode (53). The major morphological structures of the resin-dentin interdiffusion zone observed in SEM could be confirmed and more detailed examination carried out using TEM (96).

2.7.1. Confocal laser scanning microscopy (CLSM)

CLSM makes optical tomograms or thin optical sections. The principle is based on the detection of the fluorescence emission from the focal plane or well-defined optical section. The detected light is converted into a video signal and appears as a two-dimensional image (101). CLSM does not require special specimen preparations and specimens can be viewed almost under normal environmental conditions. The non-destructive nature of CLSM is a great advantage (45). CLSM allows *in vivo* real-time evaluation and gives a subsurface image. It is useful to investigate the adhesive interface of materials sensitive to dehydration. Using labelling techniques with fluorescent markers, it is possible to assess the penetration depth of primers and adhesives (8), the micro- and nano-leakage around the restorations (97) or for measurements of wear loss (12). The disadvantage of CLSM is the limitation of resolution which does not allow submicron characterization of the tooth-resin interface. The other problem is the inability of fluorescent dyes to bind properly to resin solution and the possible dye elution from the resin makes the interpretation debatable (97).

2.7.2. Transmission electron microscopy (TEM)

TEM is one of the most powerful tools for the investigation of the resin-dentin or the enamel-resin interface offering the opportunity for high resolution (up to 1-2nm for most biological specimens) and good reliability with low incidence of artefact production. The specimen should be thin enough to permit transmission of at least 50% of the initial electrons. The

thickness of the sections determines the resolution obtainable with the TEM. The disadvantage of the TEM method is that the obtained image is two-dimensional and only a very small area observed which is not always representative for the whole specimen. TEM has also limited possibilities for complementary chemical analysis e.g. with energy-dispersive X-ray spectroscopy (EDX), the tool which possesses SEM (97). The preparation of ultrathin sections for TEM viewing is quite complex and requires experience. The preparation of a specimen for TEM examination may include demineralization, fixation, dehydration, embedding and staining to improve the image contrast. The applied specimen preparation technique depends upon what information of the ultrastructure is sought after. TEM allows analysis of the process of hybridization: the organization and quality of hybrid layer, orientation of collagen fibrils, their envelopment by resin, the depth of demineralization, resin interdiffusion, the effects of overdrying, etc (97).

2.7.3. Scanning electron microscopy (SEM)

2.7.3.1. High vacuum SEM (HV SEM)

SEM was one of the first and most widely used tools to investigate adhesive interfaces (97). For SEM examinations of dental specimens there are several preparation methods available: simple cross-fracturing or sectioning, total or partial demineralization of dentin substrate, deproteinization for removing dentin organic components or argon ion beam etching. Laboratory demineralization of dentin is simultaneously the test for the resistance of the hybrid layer to degradation. The resistance of the hybrid layer to demineralization can be evaluated by SEM and it correlates with high bond strengths in mechanical tests. Demineralization and infiltration with silver nitrate solution helps in revealing the nature and formation of the hybrid layer. However, with demineralization all information about dental structures to which the adhesive was bonded is completely lost (97).

To be able to observe the samples in a high vacuum environment, the sample should be adequately prepared. The main requirements are that the specimens should be resistant to high vacuum, have good conductivity and be absolutely dry. The standard laboratory protocols for the preparation of biological samples for HV SEM generally include fixation in glutaraldehyde or formaldehyde, dehydration in an ascending concentration of aqueous ethanol or acetone solutions, drying e.g. by the “critical point drying” method or the chemical HMDS (hexamethyldisilane) method and coating the specimen with electron-conductive material, mostly gold, gold-palladium or platinum (97). In HV SEM the specimen is observed under high vacuum conditions, the whole microscope column including the specimen chamber operates under high vacuum ($<10^{-5}$ Torr; 1Torr=133Pa) (13). HV SEM offers high resolution and large depth of field (13). The electron beam of primary electrons (PE) scans

the sample, where PE interact with surface electrons and are absorbed or scattered. The interaction of the sample surface with PE causes the emission of secondary electrons (SE) or backscattered electrons (BSE) as well as X-rays, which may then be captured with appropriate detectors and processed into a three-dimensional image. The changes in topography, composition and texture are determined by the number of emitted SE (97). BSE have high-energy and are useful to determine areas with different chemical composition (material contrast). Specimens containing substrates of higher atomic number generate brighter BSE images.

Basically, there are two types of electron sources possible which form an electron beam: the thermionic emission and the field-emission (13). In FE SEM, a field emission gun produces an electron beam which is smaller in diameter, more coherent and has a higher current density or brightness by up to three orders of magnitude compared with that achieved with conventional thermionic emitters of SEM. FE SEM in high vacuum mode allows the use of lower accelerating voltages in comparison to the thermionic HV SEM (15), allowing a better spatial resolution (up to 1,5nm) and significantly improved image quality (13). The FE SEM is complementary to TEM since the latter provides no information on the topography of the interface (15).

2.7.4. Environmental SEM (ESEM)

ESEM is the one of the techniques besides CLSM which allows for the investigation of specimens in a moist (up to 100% humidity) environment (23;45;58) irrespective of whether the specimen is wet, conductive or non-conductive. ESEM permits examination of unfixed biological samples in a low vacuum (up to 20 Torr) environment. However, a higher atmospheric pressure in the specimen chamber leads to poorer resolution.

There are two major aspects which differentiate ESEM from HV SEM: separation of the high vacuum electron column from the low vacuum specimen chamber by the means of special pressure limiting apertures (13) and the new type of detector which is adjusted to function in a gaseous environment (13;23). With a gaseous detection device (GDD) both SE and BSE images can be produced. GDD utilizes the ionization of the gas for detection of SE from the specimen surface (23).

2.7.5. Low vacuum SEM (LV SEM)

The low vacuum mode of SEM or LV SEM allows for the examination of surfaces of practically all specimens (wet or dry, insulating or conducting) within a highly reduced atmospheric pressure of 1 – 1.5 Torr. The introduction of the gaseous environment in the specimen chamber provides positive ion supply from the ionized gas and ensures the

suppression of a negative charge build-up on insulating specimens. The gas is also the detection medium. Different gases, separately or in mixture, can be introduced in the specimen chamber, for instance, nitrogen. One of the best imaging gases is water vapour due to its amplifying efficiency and useful thermodynamic properties; it allows the level of moisture around the specimens to be controlled (23;58). The water vapour functions as a cascade amplifier, amplifying the original (initial) SE signal from the sample.

The principle of signal generation in low vacuum mode is as follows:

- The PE beam (very energetic) penetrates the water vapour with little apparent scatter, scanning across the surface of the sample,
- SE are released from the surface of the sample,
- The water molecules are struck by these SE and produce SE themselves. They spread further in a cascade like reaction,
- At the same time positive charged ions of gas molecules drift towards the specimen surface and neutralise the negative charge on it (58).

The LV SEM operates in a highly reduced atmospheric pressure in the range of 1 – 1.5 Torr. By contrast, the column and the electron gun remain in the environment of standard pressure of 10^{-6} to 10^{-7} Torr. Diverse pumping systems and the use of pressure limiting aperture (PLA) – the electrode in the shape of truncated cone in diameter of 0.5mm - preserves the vacuum differences in specimen chamber and the electron gun chamber (23). The PLA constricts the field of view, because it is set on the pole-piece but without PLA the maximum pressure in the chamber could only be 1 Torr. The distance between PLA and the sample surface is kept small (less than 10mm) to reduce the number of molecules-atoms in the way of the PE beam (58).

Normally, for the maintenance of a specimen in wet conditions, the low pressure is advantageous, as well as the energy of primary beam should be low. Low pressure and low primary beam energy leads to poor signal amplification and image quality. To avoid that, a special separate Large Field Gaseous Secondary Electron Detector (LFD) was introduced. It allows the increased amplification of the charged particles which come from specimen and gas molecules.

With the FEI Quanta 400 FEG field emission scanning electron microscope (FEI Europe B. V., Eindhoven, The Netherlands) used in present study it is possible to operate either in HV SEM, ESEM and LV SEM modes and it allows observing of:

- electrically conductive or insulating samples

- delicate samples
- fully hydrated samples
- polymers
- mineralogical samples.

The preparation of specimens for HV SEM is time consuming and each of preparation phase separately causes stress and damage to the specimen (58). LV SEM cannot replace the HV SEM, but it is a very important additional method to visualize the microtopography and ultramorphology of native biological specimens, complementing the HV SEM imaging method.

2.7.5.1. Energy-dispersive X-ray spectroscopy (EDX)

The energy-dispersive X-ray spectroscopy (EDX) is an additional instrument to SEM that determines quantitatively and qualitatively the elements within a sample by irradiating the specimen with a high-energy electron beam and then analyzing from the specimen re-emitted characteristic X-rays (9). The X-rays are detected with a specific EDX Detector.

3. Objectives and hypothesis

Our focus was to characterize the micromorphology of the adhesive interface of self-adhesive resin cements with tooth hard tissue (enamel and dentin) in comparison to a well-established resin luting agent with separate self-etching adhesive system. The characterization was carried out using a new imaging method, low vacuum scanning electron microscopy, in order to evaluate the specimens in their native bonded state. This means that the tooth structures on the surface being observed were intact, just polished, and were not destroyed either by a demineralization/deproteinization procedure nor a coating with conductive layer. To validate the high informational value of minimally prepared native polished specimens, additional demineralized/ deproteinized and fractured specimens were processed.

The aim of the present study is to answer following questions:

1. Can characteristic morphological features of the adhesive interface described in the literature such as hybrid layer (interdiffusion zone), tags or other also be found with SARC's? Do SARC's infiltrate into the dentinal tubules and react with smear plugs? If tags appear, do they contain any fillers?
2. Could the structures like hybrid layer (interdiffusion zone) or resin tags observed in polished specimens be also confirmed by analyzing of demineralized/deproteinized and fractured specimens?
3. Does the polymerization mode (light- or auto-polymerization) influence the micromorphology of tooth tissue-luting agent adhesive interfaces?
4. Is the LV SEM method appropriate for the depiction and analysis of micromorphological characteristics of the tooth tissue-SARC's interface?

From what has been reported in the current literature, it is hypothesized that:

- The SARC's have only superficial interaction the with smear layer, therefore, the formation of luting agent extensions (tags) into dentinal tubules is not expected.
- Demineralization/deproteinization and fracturing of specimens help to reveal particularities of the adhesive interface of SARC's with tooth substances.
- The adhesive interface of self adhesive resin luting agents will show clear morphological differences depending on the polymerization mode. Auto-polymerization mode enhances the deeper luting agent diffusion and therefore a thicker hybrid layer (interdiffusion zone) can be detected.

4. Materials and methods

In order to observe the adhesive interface between tooth and luting agent in its native state in a low vacuum scanning electron microscope, a minimal specimen preparation was performed: specimens only underwent a polishing procedure. For validation of revealed micromorphological structures the polished specimens were exemplarily demineralized and deproteinized. Additionally, fractured and then polished specimens were prepared to correlate the micromorphological findings in the native fractured and native polished state.

4.1. Preparation of specimens

Forty-eight caries-free human third molars were chosen for the processing of specimens in order to observe the adhesive interface between tooth and luting agent in its native state. The teeth were stored in 0.5% chloramine solution for not longer than 1 month after extraction. After thorough depuration of teeth from soft tissues, they were stored in demineralized water at 4°C until further processing.

4.1.1. Sectioning procedure

The teeth were mounted with crowns down on microtome holders with methylmethacrylate resin (Paladur, Hereaus Kulzer, Germany). To avoid the influence of polymerization heat the mounted teeth were stored for 20 min at room-temperature in demineralized water. The holders were placed in the diamond saw microtome (1600 Leitz, Wetzlar, Germany) and the teeth were sectioned (adjustments on the feed-rate scale: 10 of 30) at the cemento-enamel junction under copious water cooling. To ensure that all discs were obtained from the mid-coronal region without enamel on the coronal side, 1.5 – 2 mm thick enamel-dentin discs were cut to have ~ 1mm sound dentin located directly above the pulp horns (Fig. 1). The discs were stored in demineralized water at 4°C not longer than one day before adhesive procedure. Before application of the adhesive procedures, the enamel-dentin discs were cross-sectioned under water cooling in bucco-lingual direction into two halves with a diamond separating disc (Trennscheibe, Komet, Germany) mounted in a hand piece (Intramatic 10C, KaVo, Germany) (Fig. 1).

4.1.2. Resin luting agents and experimental groups

As a control in the present investigation a well-established resin luting agent Panavia F (PAN) with self-etching adhesive system (one-step ED Primer II) was used. As representatives of self-adhesive resin cements Clearfil SA cement (CSA), RelyX Unicem Aplicap (RXU1) and RelyX Unicem 2 (RXU2) were selected. The chemical composition of

these resin luting agents is summarized in Table 3. The main feature of SARC's is their functional monomers which act as etchant, primer and adhesive simultaneously. Therefore, there is no need for a tooth tissue pretreatment or conditioning step. The bonding effectiveness is dependant on the activity of these functional monomers. The functional monomer of RXU1 and RXU2 has two phosphoric groups and two polymerizable groups, whereas CSA and PAN functional monomers contain only one acidic and one polymerizable group. Schematic illustration of these chemical formulas of functional monomers is given in Fig. 2.

After the cross-sectioning, the disc halves were randomly assigned to respective adhesive procedures with the four selected resin luting agents. With the different curing modes - auto-polymerization (AP) or light-polymerization (LP) - eight experimental groups were formed. For each experimental group 10 polished specimens (S01 – S10) were prepared (Fig. 3).

4.2. Adhesive procedure

4.2.1.1. Creation of smear layer

The smear layer was created just before the adhesive procedure. The coronal surface of every half-disc was wet-abraded for 60 secs on 600-grit silicon carbide sandpaper (Carbimet Paper Discs, Buehler, Germany) to create a uniform smear layer. Then the half-discs were briefly rinsed and gently air-dried with air/water spray. In Fig. 4 a smear layer created with silicon carbid sandpaper is shown using LV SEM mode.

4.2.1.2. Application of the luting agent

The half-disc was then positioned against the perpendicular wall of a plastic box, to preserve the cross-sectioned surface from embedding into the luting agent (Fig. 5). According to the manufacturers' instructions the luting agent was applied on each single half-disc, covered with a translucent strip (Universal strips, Frasaco, Germany) and the 2mm thick slice of the Vita Mark II Ceramic block (VITA Zahnfabrik, Bad Saeckingen, Germany) placed on top. On the ceramic slice a 420g weight was placed and held in position during the period of polymerization (LP or AP) as described below.

4.2.1.3. Polymerization

For light-polymerization, each luting agent under the applied weight was cured for 20 secs from 3 sides with the LED curing unit (Bluephase C8, Ivoclar Vivadent, Germany) with light intensity of 945 mW/cm^2 (measured with Cure Rite, Dentsply Caulk, USA). For auto-polymerization, the specimens were left under the applied weight for 10 mins in a dark chamber.

4.2.1.4. Coating of the luting agent and storage

After polymerization of the luting agent, the weight, ceramic slice and strip were removed. The luting agent was covered with a flowable resin composite (Tetric EvoFlow A2, Ivoclar Vivadent, Germany) and polymerized for 20s from three sides with the curing unit described above (Fig. 6). Then, each specimen was stored at 100% humidity in an incubator (U-10 Memmert, Schwabach, Germany) for 24 hours at 37°C.

4.3. Processing of specimens

4.3.1. Preparation of polished specimens

In the order to observe the adhesive interface in its native state between tooth and luting agent, 10 polished specimens (S01 – S10) per experimental group were prepared. After bonding (see 4.1.3), the interface was exposed, polished and then observed in the LV SEM (Fig. 3). Polishing of cross-sectioned surface (Fig. 5) was carried out using wet silicon carbide papers (Carbimet Paper Discs, Buehler, Germany) of decreasing abrasiveness from 600-grit to 1200-grit. Next, polishing was continued on a wet fabric tissue (8'' Mastertex PSA, Buehler, Germany) with an alumina suspension (Buehler, Germany) of decreasing roughness: 1.0µm, 0.3µm and 0.05µm grain size. Finally, the polished surface was cleaned using a wet fabric tissue and rinsed with water. The polishing was performed not more than 4 hours before examination of the specimens with LV SEM. Just before SEM examination, the specimens were repeatedly cleaned on a wet fabric tissue for 1min and thoroughly rinsed (Table 5).

4.3.2. Preparation of demineralized/deproteinized specimens

Those of the polished specimens which had exposed excellent adhesive interface quality and were suitable for further investigation underwent a demineralization/deproteinization procedure. From the polished specimens one specimen per experimental group was processed for documentation purposes.

4.3.2.1. Demineralization/deproteinization procedure

A demineralization/deproteinization procedure of the polished surface to remove the dentin and to further expose the interface was performed as described in Table 6. First, specimens were demineralized for 15 secs in 1N HCl solution (Merck, Darmstadt, Germany) and rinsed in twice distilled water. Following this, a deproteinization of specimens was carried out for 10 mins in 2% NaOCl solution (Speiko, Münster, Germany) and followed by rinsing in twice distilled water.

4.3.3. Preparation of fractured specimens

In addition to the polished specimens, one fractured specimen per experimental group was prepared. It was carried out in order to be able to relate the observed interdiffusion zone to the actual smear layer processed by the described method. Altogether eight specimens were processed this way. The enamel-dentin discs were obtained in the same way as for polished specimens (see 4.1.1). On the pulp side the enamel-dentin discs were notched across the middle to facilitate easy fracturing later (see Fig. 7). Then the smear layer was created on the coronal side of the disc, as described in 4.2.1.1 using wet 600-grit sandpaper for 1min. Afterwards, the luting agent was distributed in a thin layer on half the coronal side of the enamel-dentin disc, perpendicularly crossing the notch-line, so that later the fractured surface revealed a bonded and an unbonded area (see Fig. 7). The specimens were light-polymerized from 3 sides with the polymerization unit as described in 4.2.1.3. In the second set of discs prepared in that way, auto-polymerization was performed for 10 minutes under glycerine gel (Liquid strip, Ivoclar Vivadent, Schaan, Liechtenstein) for SARC's or Oxyguard II (Kurarey Medical Inc., Okayama, Japan) for Panavia F2.0, to avoid oxygen inhibition of the polymerization. After polymerization, the gel was rinsed off. No weight was applied either for light-polymerized specimens or for auto-polymerized ones. Next, that half of the disc with the bonded surface were covered with a thin flowable resin composite (Tetric EvoFlow A2, Ivoclar Vivadent, Schaan, Liechtenstein) layer and polymerized for 20 secs from three sides with the curing unit described above (see 4.2.1.3), to protect the luting agent layer from damage during further specimen processing. The specimens were then stored at 100% humidity for 24 hours at 37°C.

Just before SEM examination the specimens were fractured. After the documentation of the fractured surface at unbonded and bonded area, the side perpendicular to fractured surface was polished with an automatic grinder/polisher (Motopol 8, Buehler, England) using 600-grit and 1200-grit silicon carbide paper at 100 rpm for 30 secs. The polishing was continued on fabric tissue with decreasing (1.0µm, 0.3µm and 0.05µm) grain size of alumina powder at 50 rpm for 30 secs under copious water cooling, ending with wet fabric only. The polished bonded area was repeatedly investigated in LV SEM.

4.3.4. Methods of evaluation of specimens

4.3.4.1. LV SEM examination

For LV SEM examination the specimens were mounted on aluminium specimen stubs with self-adhesive carbon discs (Leit-C-Tab, Plano GmbH, Wetzlar, Germany) and plastic conductive carbon cement (Leit-C-Plast, Plano GmbH, Wetzlar, Germany). The middle of the polished specimen was marked on the flowable composite surface with a scalpel. For the

microscopic examination of specimens, the field-emission scanning electron microscope (FEI Quanta 400 FEG, FEI Company, FEI Europe, Eindhoven, The Netherlands) in low vacuum mode was used. Based on the literature, established LV SEM settings were used (30):

- Large Field Detector (LFD) for low vacuum environment
- Acceleration voltage of 4 kV
- Spot size 4.0
- Pressure limiting aperture (PLA, 500µm)
- Pressure in chamber of 1.5 Torr
- Working distance: ~6.5mm

4.3.4.2. Energy-dispersive X-ray spectroscopy (EDX) analysis

EDX analysis was performed once in this study. The fractured and consecutively polished specimen was first documented in LV SEM at original magnification. EDX mapping was then performed on the identical site and at the same original magnification (x3000) as the original SEM image for later comparison. A special pressure limiting aperture (PLA, 500µm) for EDX was used. The acceleration voltage was 6kV, spot size 5.5 and working distance was 10mm constantly. The site was scanned 512 times to obtain sufficient X-ray signal for elemental analysis. The element mapping of C, N, O, F, Ba, Mg, Na, Si, Al, P and Ca was performed.

4.4. SEM evaluation of interface morphology

4.4.1. Specimen documentation

4.4.1.1. Documentation of polished specimens

Eighty polished specimens (S01 – S10 per each experimental group) were systematically scanned, visualized and photo-documented at different areas of the tooth tissue-luting agent interface within enamel and dentin as shown in Fig. 6.

- **E** – enamel-luting agent adhesive interface at x800, x3000 and x6000 magnification
- **DEJ** – dentin-enamel junction overview image at x800 magnification
- **D1** – dentin-luting agent adhesive interface at x800, x3000 and x6000 magnification in the middle of the sample
- **D2** - dentin-luting agent adhesive interface at x800, x3000 and x6000 magnification laterally on the sample
- **D3** – additional, if adhesive interface at D2 was well visualized, dentin-luting agent adhesive interface on the opposite lateral side of the specimen at x800, x3000 and x6000 magnification.

A total of 10 (optionally 13) images per specimen were taken.

If a specimen had artefacts and it was impossible to gain a good quality of the adhesive interface, the documentation protocol was shortened to 5 images per specimen:

- **E** – enamel-luting agent adhesive interface at x800 and x3000 magnification
- **DEJ** – dentin-enamel joint overview image at x800 magnification
- **D1** – dentin-luting agent adhesive interface at x800 and x3000 magnification in the middle of the sample

4.4.1.2. Documentation of demineralized/deproteinized specimens

After the demineralization/deproteinization procedure the specimens were immediately examined in LV SEM. Identical SEM settings were used as for the polished specimens and the documentation of the identical sites at identical magnifications as for the polished specimens (see 4.3.4.1 and 4.4.1.1) was carried out.

4.4.1.3. Documentation of fractured specimens

Just before SEM examination the specimens were fractured as shown in Fig. 7 and the fractured surface documented at two sites within dentin area (1. LV SEM): unbonded with exposed smear layer at site (A) and bonded with the dentin-luting agent adhesive interface at site (B). After the first SEM examination (1.LV SEM) and documentation of the fractured surface at unbonded (A) and bonded (B) sites, the fractured surface was polished. The polished dentin-luting agent adhesive interface was repeatedly investigated in LV SEM (2.LV SEM). At the second time only the bonded and polished dentin-luting agent adhesive interface was imaged in the area shown by circle (C) (Fig. 7). The sites were documented at x3000 and x6000 original magnification using identical LV SEM settings as for the polished and demineralized/deproteinized specimens (see 4.3.4.1).

One fractured auto-polymerized Clearfil SA Cement (CSA, AP) specimen underwent EDX for surface elemental analysis in low vacuum environment. The EDX analysis on this particular CSA, AP specimen was carried out for exemplary chemical confirmation of the micromorphological findings revealed in the SEM observation of polished bonded interface.

4.4.2. Qualitative evaluation of adhesive interfaces of polished specimens

Ten specimens (S01 – S10) with the exposed polished surface from each experimental group were evaluated in LV SEM mode (Fig. 3). Representative SEM images of one specimen from each experimental group were selected for qualitative evaluation. They were demonstrated in overview figures showing the sites of documentation (E, D1, D2 and D3; see Fig. 6) at the original magnifications (x800, x3000 and x6000). Adhesive interfaces in SEM images were qualitatively analyzed for the presence of morphological structures described in

studies of Van Meerbeek et al. (98), de Munck et al. (24) and Goracci et al. (36). In enlarged SEM photomicrographs after each overview figure the integrity of adhesive interface, the presence of hybrid layer or interdiffusion zone and tags at the enamel-luting agent adhesive interface was evaluated. The hybrid layer or interdiffusion zone, tags, lateral tags, pores and smear plugs were analyzed at the dentin-luting agent adhesive interface. Additionally, the characteristics of luting agents and tooth hard tissues in these enlarged SEM images were described.

4.4.3. Qualitative evaluation of the adhesive interfaces of demineralized/deproteinized specimens

One polished specimen from each experimental group underwent demineralization/deproteinization procedure and was documented in LV SEM. The documented sites and magnifications were identical to those of polished specimens to allow later comparison. SEM images of each demineralized/deproteinized specimen were demonstrated in an overview figures: enamel-luting agent and dentin-luting agent adhesive interfaces depicted at x3000 and x6000 original magnifications. The qualitative evaluation was performed and the characteristics of the enamel-luting agent (integrity, hybrid layer or interdiffusion zone, tags) and dentin-luting agent adhesive interfaces (hybrid layer or interdiffusion zone, tags, lateral tags, pores and smear plugs), as well as the characteristics of luting agents and tooth hard tissues were described. After the overview of demineralized/deproteinized specimens, the SEM images were compared from identical sites in polished and subsequently demineralized/deproteinized specimens.

4.4.4. Qualitative evaluation of adhesive interfaces of fractured specimens

Morphological features of unbonded and bonded dentin-luting agent adhesive interfaces of fractured specimens were examined. SEM image sequences were made of fractured and of subsequently polished specimens as shown in the overview figures: fractured unbonded site (A), fractured bonded site (B), and polished bonded site (C) - dentin-luting agent adhesive interface at x6000 original magnification. Particularities dentin-luting agent adhesive interfaces (hybrid layer or interdiffusion zone, tags, lateral tags, pores and smear plugs), as well as the characteristics of luting agents and dentin were described.

4.4.5. Semi-quantitative evaluation of the interface micromorphology of polished specimens

Evaluation criteria

Adhesive interfaces of 10 polished specimens (S01 – S10) from each experimental group were semi-quantitatively analyzed for the presence of evaluation criteria described in

morphological studies of Van Meerbeek et al. (98), De Munck et al. (24) and Goracci et al. (36):

- at the enamel-luting agent adhesive interface - microgaps or integrity of the adhesive interface (present/not present) (36),
- at the dentin-luting agent adhesive interface - hybrid layer or interdiffusion zone, tags, pores and smear plugs as described in Table 7. Table 7-a shows the scores for criteria visualized with SEM images (24;98).

Semi-quantitative evaluation of the listed criteria was performed on the SEM images of polished specimens (S01 – S10) at x3000 original magnification. The enamel-luting agent adhesive interface was evaluated at (E) site (Fig. 6). From each experimental group the specimens with microgaps at the enamel-luting agent adhesive interface were counted.

The evaluation criteria of the dentin-luting agent adhesive interface as hybrid layer, tags, pores or smear plugs were scored at two sites per polished specimen: dentin middle (D1) and dentin lateral (D2 or D3 Fig. 6). The evaluation criteria were scored as detectable, questionable and not detectable (Table 7). The sites of dentin-luting agent adhesive interface which could not be evaluated for particular morphological criteria were referred as “failed specimens”.

Definition of micromorphological evaluation criteria

Adhesive interface: – the whole resin-tooth tissue complex, which include several layers of resin material or its constituents (hybrid layer, adhesive or primer layer, resin composite layer) and the corresponding interfaces between these layers as well as the material-tooth tissue interfaces. In these layers or between them demineralization, penetration and diffusion can be distinguished. *Synonym:* bonded interface.

Hybrid layer (HL) or resin-dentin interdiffusion zone (IZ): - the demineralized dentin surface impregnated with resin. The thickness of a hybrid layer for the etch-and-rinse adhesive systems can reach 4-5 µm, for self-etching adhesives the thickness of hybrid layer is mostly between 2-3 µm. For SARC’s, the presence of a hybrid layer is debatable, because its thickness does not exceed 1µm (98). But the interaction is not excluded, so the most used term for the hybrid layer of SARC’s is the nanohybrid layer or interdiffusion zone (98). In the SEM, this zone between the resin material and the dental tissues appears darker than the dentin due to the impregnation of the smear layer with the resinous phase of the resin luting agent and contains polymers rich in carbon atoms. Carbon atoms generate only a small amount of secondary electrons after irradiation with the primary electron beam, making these areas appear darker than surrounding tissue (Table 7-a). The back scattered electrons (BSE)

increase the material contrast allowing quite definite discrimination of the structures with different chemical compositions. Since heavy atoms with a high atomic number are stronger scatterers than light ones, SEM images with back-scattered electrons (BSE) contain compositional information.

Tags (T): - formed by the infiltration of the resin phase of adhesive systems or composite resin into dentinal tubules which may be either filler reinforced (FT) or without fillers being just the containing resinous phase (RT) (Table 7-a). Low viscosity resin is able to infiltrate even lateral dentinal tubules forming lateral tags (LT).

Pores: - in this study those are defined as luting agent irregularities appearing at the orifices of dentinal tubules. It is important to distinguish them from air bubbles and porosities within the bulk of material. Pores appear irregular whereas air bubbles are within the material and predominantly round and regular (Table 7-a).

Smear plugs (SP): - debris entrapped in the dentinal tubules during the creation of the smear layer or polishing procedure (Fig. 4). In SEM images they appear as loose masses filling dentinal tubules in variable depth, mainly 2 – 7 µm. If the smear plugs are enclosed by a resin sheath, they are forming tags and may have intimate connection to dentinal tubule walls and are therefore distinguishable from bare smear plugs (Table 7-a).

5. Results

5.1. Qualitative and semi-qualitative evaluation of the adhesive interface of polished specimens

For qualitative evaluation from each experimental group of polished specimens, SEM images of one characteristic specimen are depicted in overview Figures Fig. 8Fig. 15 showing the sites of documentation (E, D1, D2, and D3 as shown in Fig. 6) and the original magnifications (x800, x3000 and x6000). The reference number of the selected specimen (S01 – S10) is given in every overview figure. Morphological criteria of enamel-luting agent and dentin-luting agent adhesive interfaces as well as characteristics of tooth tissues and luting agents for each luting agent (PAN, RXU1, RXU2 and CSA) and polymerization mode (LP or AP) are described in the enlarged SEM photomicrographs after each overview figure. The same polished specimens (S01 – S10) were semi-quantitatively evaluated. Microgaps at enamel-luting agent adhesive interface were counted (Table 8). The morphological criteria (hybrid layer or interdiffusion zone, tags, pores and smear plugs) were scored as detectable, questionable and not detectable at dentin-luting agent adhesive interface (Table 9Table 12 and Fig. 16).

5.1.1. Enamel-luting agent adhesive interface of polished specimens

5.1.1.1. Qualitative evaluation of enamel-luting agent adhesive interfaces of polished specimens

The enamel-luting agent adhesive interface of luting agents in both polymerization modes is described in Fig. 8-a. No etching signs (surface irregularities) of enamel or deep infiltration (tag formation) could be observed at the adhesive interface of Panavia F2.0 (PAN) luting agent even at x6000 original magnification independent of polymerization mode (Fig. 8-a and Fig. 9-a). The enamel-SARC's adhesive interface appeared similarly to that of PAN (Figs.Fig. 10-a -Fig. 15-a). However, microgap formation in SARC's specimens was frequently observed which propagated predominantly adhesively between enamel and luting agent and were visible only at x3000 and x6000 magnifications (Figs. Fig. 10-a –Fig. 15-a). Even if separated from the enamel, a gap-free luting agent adaptation and absence of porosities along the adhesive interface was found in all specimens.

5.1.1.2. Semi-quantitative evaluation of enamel-luting agent adhesive interfaces of polished specimens

The frequency of specimens with evaluation criterion microgaps by experimental group is summarized in Table 8. Intimate adhesion without microgaps was present in all PAN specimens, in both polymerization modes. Almost all SARC's specimens (up to 10 per experimental group) revealed microgaps. Fewer microgaps appeared in the light-polymerized RelyX Unicem 2 experimental group (RXU2, LP; n=7). The highest amount of microgaps appeared in light-polymerized Clearfil SA Cement (CSA, LP; n=10) and auto-polymerized RelyX Unicem (RXU, AP; n=10) polished specimens. Influence of polymerization mode on the frequency of microgap formation could not be demonstrated.

5.1.2. Dentin-luting agent adhesive interface of polished specimens

5.1.2.1. Qualitative evaluation of dentin-luting agent adhesive interfaces of polished specimens

Morphological criterion: Hybrid layer or interdiffusion zone

The typical hybrid layer of PAN specimens is shown in the Fig. 8-c, whereas interdiffusion zone of SARC's is seen in Figs. 10, 12, 13 and 14, aspect b. Basically, the thickness of interdiffusion zone in SARC specimens did not exceed 1µm (RXU1, AP in Fig. 13-b or RXU2, LP in Fig. 14-b) and appeared irregularly even within one specimen (RXU1, LP in Fig. 12-b).

Morphological criterion: Tags

The resin tags within the dentinal tubules could be observed in PAN specimens (Fig. 8, aspect b and c, and Fig. 9, aspect b and c). Several tags contained fillers as seen in Figs. 8-b and 9-c. The tags could also be seen in some SARC specimens as in specimen of RXU2, LP (Fig. 14-b) and RXU2, AP (Fig. 15-b). Tags containing filler could not be detected in SARC specimens.

Morphological criterion: Pores

There were considerably less pores at the orifices of dentinal tubules in selected PAN specimens (Figs. 8 and 9, aspect b) than in SARC specimens (Figs. 10 and 12, aspect b). Pores appeared at the orifices of those dentinal tubules where no tags could be detected; these dentinal tubules were filled with smear plugs.

Morphological criterion: Smear plugs

Smear plugs which loosely fill the lumen of dentinal tubules could be detected in practically all specimens, irrespective whether the tags were or were not present in dentinal tubules. The typical appearance of smear plugs is shown in Fig. 8, aspect c and Figs. 9 -15, aspect b.

The characteristics of luting agents and dentin

Inclusions in dentinal tubules could be detected in the specimen of RXU1, AP (Fig. 13-b). Bubbles and voids (until 20 - 30µm in size) within the luting agent at x800 magnification could be detected in all experimental groups, but larger voids were observed in the specimens of RXU1 (Fig. 13, E and D1 at x800 magnification). Peritubular and intertubular dentin could be differentiated almost in every specimen (e.g. Fig. 8-c and Fig. 12-b).

5.1.2.2. Semi-quantitative evaluation of dentin-luting agent adhesive interfaces of polished specimens

Morphological criterion: Hybrid layer or interdiffusion zone

The detailed distribution of the scores for this criterion “Hybrid layer or interdiffusion zone” is outlined in Table 9. The scores of this criterion in polished specimens are summarized in Fig. 16(A). PAN specimens had the most distinct hybrid layer, especially in SEM images of the adhesive interface along lateral dentin; a hybrid layer could be detected in up to 7 of the 10 specimens. Within the SARC’s group, RXU2 showed an interdiffusion zone in 3 specimens, whereas an interdiffusion zone could only be detected in one specimen of CSA and RXU1. No clear differences between the luting agents could be detected with respect to polymerization mode.

Morphological criterion: Tags

The detailed distribution of the scores for criterion “Tags” is given in Table 10. The scores of this criterion in polished specimens are summarized in Fig. 16(B). The PAN specimens had both filler-reinforced and tags containing resin only (appeared in up to 7 specimens). Principally, within the SARC group, RXU2 and CSA luting agents revealed irregular resin tags in dentinal tubules (confirmed within 2-3 specimens per experimental group). The resin tags in SARC specimens were more frequently observed in the AP mode (2-3 confirming images of CSA and 1-2 SEM images of RXU2 luting agent) than in the LP mode (2 SEM images of RXU2 luting agent). There was only one RXU1 specimen where the tags could be detected. PAN luting agent had a similar incidence of tags in both curing modes. Tags were more frequently detected in SEM images of lateral dentin (near to DEJ; D2 or D3) than in the middle dentin (D1).

Morphological criterion: Pores

The detailed distribution of scores for the criterion "Pores” in polished specimens is given in Table 11. The scores are summarized in Fig. 16(C). Generally, PAN specimens exhibited slightly less pores at the orifices of dentinal tubules along the adhesive interface (1-2 specimens where pores could be detected) than SARC’s (2-3 specimens per experimental group). In the SEM images of auto-polymerized CSA and RXU2 specimens pores could not

be detected. The occurrence of pores increased in the CSA and RXU2 specimens cured in the light-polymerization mode. However, this does not apply to PAN and RXU1 specimens: they showed rather more porosity in auto-polymerization mode. The highest number of detectable pores was in the middle dentin (close to the pulp; D1).

Morphological criterion: Smear plugs

The distribution of scores for criterion “Smear plugs” is showed given in Table 12 and is visualized in Fig. 16(D). In all experimental groups smear plugs could be detected. Approximately 2-4 (on average 3) specimens per experimental group revealed the presence of smear plugs, unrelated to polymerization mode.

Failed specimens

The lowest number of failed specimens was revealed within the PAN group (Fig. 16). From all SARC’s, CSA and RXU2 specimens showed the highest number of failed specimens, followed by RXU1 specimens. Generally, slightly more SARC’s specimens failed in auto-polymerized mode more than in the light-polymerized mode. The observed fractures or gap sites were predominantly partial and propagated either along the dentin-enamel junction or adhesively in the middle dentin-luting agent interface. Therefore the adhesive interface on lateral part of dentin was more frequently intact.

5.1.3. Summary

Qualitative micromorphological characterization and semi-quantitative analysis of the enamel-luting agent and dentin-luting agent adhesive interface of polished specimens revealed several characteristic features. The PAN specimens of both polymerization modes showed gapless integrity of enamel-PAN adhesive interface as well as a distinct hybrid layer, resin tags and filler-reinforced resin tags at the dentin-PAN adhesive interface. Slightly lower number of specimens with microgaps at enamel-luting agent interface occurred in RXU2, LP in comparison to other SARC’s experimental groups. The interdiffusion zone at dentin-luting agent adhesive interface appeared quite thin and irregularly in SARC’s specimens. Up to 3 specimens revealed interdiffusion zone per experimental group. CSA and RXU2 specimens (2-3 confirming SEM images) were detected with resin tags in dentinal tubules. CSA showed the presence of tags only in auto-polymerized mode, whereas those were present in RXU2 specimens in both polymerization modes. Only one RXU1, AP specimen exhibited the presence of resin tags. Pores at the dentinal tubule orifices were revealed in the specimens of all experimental groups except for auto-polymerized CSA and RXU2 specimens. Smear plugs were detected in most specimens.

The influence of polymerization mode on the morphology of adhesive interface:

- The incidence of an interdiffusion zone within SARC specimens was not influenced by the polymerization mode.
- SARC formed resin tags more in self-curing mode, whereas PAN specimens were not influenced by polymerization mode in this aspect.
- The light-polymerized SARC specimens seem to have more pores in the orifices of dentinal tubules in comparison to auto-polymerized SARC, despite identical weight application on specimens.

5.2. Qualitative evaluation of adhesive interfaces of demineralized/deproteinized specimens

The demineralized and deproteinized polished specimens (one from each experimental group) were depicted as SEM image sequences of enamel-luting agent and dentin-luting agent adhesive interfaces at x3000 and x6000 original magnification in Fig. 17Fig. 24. The SEM images selected were taken at x3000 or x6000 original magnifications.

5.2.1. Enamel-luting agent adhesive interfaces of demineralized/deproteinized specimens

Neither in PAN, nor in SARC's specimens could a deep interaction with enamel be shown. Resinous microtags or signs of resin infiltration were not revealed by the demineralization/deproteinization procedure. Although polished specimens of PAN have shown excellent adhesion to enamel (no microgaps), no deeper resin infiltration into enamel could be determined in demineralized/deproteinized specimens (Figs. 17 and 18). A thin resinous layer representing an interdiffusion zone could be detected at the enamel-luting agent adhesive interface of SARC specimens (Figs. 19, 21, 23 and 24).

5.2.2. Dentin-luting agent adhesive interfaces of demineralized/deproteinized specimens

Morphological criterion: Hybrid layer or interdiffusion zone

The hybrid layer in PAN specimens was very well integrated with resin luting agent and in the demineralized/deproteinized state poorly distinguishable from the rest of the resin luting agent (Figs. 17 and 18). The interdiffusion zone of SARC specimens was sometimes separated from the bulk of the material by a thin resinous phase film which became visible under demineralized/deproteinized conditions as in light-polymerized CSA (Fig. 19) and light-polymerized RXU1 (Fig. 21) specimens. No signs of interdiffusion could be revealed in auto-polymerized CSA (Fig. 20) and auto-polymerized RXU1 (Fig. 22) specimens.

Morphological criterion: Tags

Filler reinforced tags were detectable in both auto-polymerized and light-polymerized PAN specimens. The resin tags in light-polymerized PAN specimen seemed to be longer and denser than in auto-polymerized PAN specimen. However, the fillers could be seen at the base of tags in both specimens (Figs. 17 and 18). The resinous phase of the adhesive also penetrated into lateral dentinal tubules – forming lateral resin tags (Fig. 17). With SARC luting agents resin tags could be detected in RXU2 and CSA specimens, irregularly sprouting out of the bulk of material (Figs. 20, 23 and 24). Several resin tags were porous (Fig. 20, CSA, AP) or hollow (Fig. 24, RXU2, AP). Filler particles could not be detected in the SARC resin tags. The tags which were found in polished PAN, CSA and RXU2 specimens could be confirmed in the demineralized/deproteinized specimens.

Morphological criteria: Pores and smear plugs

The pores at the adhesive interface after demineralization/deproteinization procedure remained on the interface of the resin luting agent as small indentations. Sometimes the smear plugs, if not rinsed off, remained on the surface of the luting material or dentin surface as in the specimen of RXU1, LP (Fig. 21).

5.2.3. Comparison of dentin-luting agent adhesive interface: polished vs. demineralized/deproteinized specimens

One corresponding site of dentin-luting agent adhesive interfaces on polished and then demineralized/deproteinized specimen from each experimental group is shown in the Figs. 25 - Fig. 28 for comparison of morphological characteristics. Principally, the micromorphological findings concerning the interdiffusion zone, tags and smear plugs revealed in the polished specimens could be also confirmed within identical demineralized and deproteinized specimens. Hidden by the dentinal tissue in the polished specimen of PAN, LP, lateral tags were found after the demineralization/deproteinization procedure (Fig. 25). The structures like *lamina limitans* could be revealed in the comparative sites of CSA, LP and RXU1, AP specimens (Fig. 26Fig. 27), but only after the demineralization/ deproteinization procedure. The interdiffusion zone was confirmed as a thin film covering the dentin surface after demineralization/ deproteinization of RXU2, AP specimen (Fig. 28).

5.2.4. Summary

Demineralization and deproteinization of specimens confirmed the presence of tags in the specimens of PAN, RXU2 and CSA. Moreover, the lateral tags became visible only in the demineralized/deproteinized state of PAN specimens. The interdiffusion zone of SARC's

after demineralization/deproteinization appeared predominantly as a thin resinous film and in several specimens it was separated from the bulk of material. Micromorphological feature such as *lamina limitans* became visible only in demineralized/deproteinized state of specimens. In polished specimens *lamina limitans* was not clearly discernible in the dentinal tubules. However, after the demineralization/ deproteinization procedure they appeared like the tags but remarkably longer or like an additional sheath within dentinal tubules.

5.3. Qualitative evaluation of adhesive interfaces of fractured specimens

The fractured specimens were prepared to correlate the thickness of standardized smear layer with the thickness of observed interdiffusion zone. Therefore, only dentin-luting agent adhesive interfaces of fractured and then polished specimens at x6000 original magnification were depicted as SEM image sequences (Fig. 29Fig. 36). The (A) SEM image is for unbonded fractured area, the (B) image for bonded fractured area and (C) is for bonded polished area on the specimen. Fig. 32-a shows the EDX analysis for carbon and silicon chemical elements carried out on the polished CSA, AP specimen (Fig. 32C).

5.3.1. Dentin-luting agent adhesive interfaces of fractured specimens

Morphological criterion: Hybrid layer or interdiffusion zone

The most distinct hybrid layer could be observed in the polished specimens of PAN, LP and PAN, AP (Fig. 29C and Fig. 30C). In the fractured light-polymerized PAN specimen (Fig. 29B) the ~2µm thick modified dentin interface could be observed. The hybrid layer in the PAN, AP polished specimen appeared more diffuse (Fig. 30C) and maybe therefore not visible in fractured state. The specimens of SARC showed no (Fig. 31, 32 and Fig. 35) or very thin (Fig. 33, Fig. 34 and Fig. 36) interdiffusion zone. Almost no dentin surface changes could be observed except for a fractured specimen of RXU1 (Fig. 33) where the luting agent has adhered to dentin indicating on interaction process.

Morphological criterion: Tags

Resin tags could be observed in fractured and polished state of PAN, LP and PAN, AP specimens (Fig. 29 and Fig. 30). The orifices of dentinal tubules appeared enlarged and modified (Fig. 29B) and resin of PAN infiltrated into dentinal tubules. In the specimens of CSA (LP), RXU1 (LP) and RXU1 (AP) the tags could be not found (Fig. 31, Fig. 33 andFig. 34). Smear plugs which were observed in RXU2, LP specimen seemed infiltrated with resin but not completely dissolved (Fig. 35B). The filler content in them as well as complete dissolution of smear plugs is questionable. Long resin tags (longer than 50µm) could be detected in the specimens of CSA, AP (Fig. 32B and C) and RXU2, AP (Fig. 36B and C).

The origin of these tags was determined on the CSA, AP specimen using energy dispersive X-ray analysis (EDX) which confirmed the high content of carbon chemical element in assumed tags (Fig. 32-a). Carbon atoms (monomer molecules) are the main components of the resin matrix of luting agents.

Morphological criterion: Pores

In the polished state of RXU1, LP specimen the typical pores in the orifices of dentinal tubules could be observed (Fig. 33C).

Morphological criterion: Smear layer and smear plugs

The created smear layer appeared as a barely noticeable thin film covering the surface of dentin and forming 2-7µm long smear plugs (Fig. 29A -Fig. 36A). The smear plugs occurred in nearly all dentinal tubules if not destroyed during the fracturing procedure. In PAN specimens smear plugs were dissolved in the dentinal tubules and replaced with resin tags. Except for CSA (AP), RXU2 (LP), RXU2 (AP) specimens (Fig. 32, Fig. 35Fig. 36) where the smear plugs appeared as infiltrated or substituted with resin, in other SARC specimens smear plugs remained in the dentinal tubules and no interaction with the resin of the luting agent could be observed (Fig. 31, Fig. 33Fig. 34).

The characteristics of luting agents and dentin

Some PAN specimens revealed inhomogeneous filler type with a hollow centre. These fillers appeared not only in polished specimen (Fig. 18), but also in fractured specimen (Fig. 30B).

5.3.2. Summary

The fractured and then polished specimens gave additional information about morphological characteristics of dentin-luting agent adhesive interfaces especially about the thickness of the smear layer. Superficial dentin surface modifications as well as resin tags were detected in fractured PAN specimens. The fractured specimens revealed the presence of extra long resin tags in the specimens of CSA and RXU2. The content of carbon chemical element representative for the resin in these tags was proved by EDX analysis on one CSA specimen. The smear plugs in RXU2 specimen were infiltrated with resin, but were not completely dissolved. The comparison between unbonded and bonded areas gave the opportunity to see what modifications, if any, occurred in smear layer and smear plugs. It was also possible to see whether the luting agent had reacted with them or not.

6. Discussion

6.1. Materials and methods

6.1.1. Tooth tissues

In the present study extracted caries-free upper and lower wisdom teeth with informed oral consent of patients were used. The teeth were delivered from the dental offices and stored in 0.5% chloramine solution from extraction until further processing but not longer than one month. Chloramine solution is often described as appropriate storage medium (10;27;29;36;54;66;67;96) and an alternative to distilled water (22;92), saline (59) and thymol solution (5;99). Obviously cracked and fractured teeth were excluded from the study.

There are controversial opinions about the influence of “age” of tooth substrate on the bonding effectiveness. The aged dentin contains less water and becomes more brittle, the dentin permeability decreases, it has higher hardness and therefore the etching effectiveness is lower (65). Tagami et al. (83) and Brackett et al. (14) could not find significant difference in the bond strengths to “old” and “young” dentin using diverse adhesive systems. By contrast, Tay et al. reported about 20% bond strength decrease of self-etching adhesive (Clearfil Liner Bond II) to sclerotic dentin of non-carious cervical lesions as compared to to normal dentin (90). Nevertheless, there are reports about excellent retention of the self-etching adhesive Clearfil SE Bond containing MDP monomer at 5 years period to sclerotic dentin *in vivo* (68).

Orientation of dentinal tubules has an effect on the formation of the hybrid layer, parallel oriented tubules form a thinner hybrid layer (65). In the literature data can be found that bonding to superficial dentin (closer to DEJ) results in higher strength; it shows 30-50% increase compared with deep dentin near to the pulp (65;102). Theoretically, deeper dentin (in the present study the middle of the specimens) in the SEM investigation should be more likely to show tags or other morphological features in the dentinal tubules due to the larger number of cut dentinal tubules. But practically these dentinal tubules are almost perpendicularly sectioned and therefore have very small cut area, thus it is difficult to detect morphological features within the dentinal tubules.

6.1.2. Smear layer

In the present morphological study a standardized smear layer was produced with 600-grit carbide paper, the most commonly used method for the creation of the smear layer (3;4;8;92;99;103). The standard smear layer should represent the smear layer created with a fine diamond bur in the clinical situation. It is important to have a thin smear layer to ensure

the complete diffusion of self-etching and self-adhesive agents with mild-etching properties into the smear layer and underlying dentin. If coarse diamond burs are used, an extra dentin pretreatment with conditioner may be necessary (98). Despite the standardized procedure, SEM imaging of fractured specimens revealed a more homogeneous and compact smear layer near to DEJ as compared to dentin near to the pulp. This can be explained by the larger area of intertubular dentin at the DEJ being involved in the creation of the smear layer.

There are many studies which try to determine the influence of the thickness of a smear layer on the bond strength of adhesive materials. In the most studies on bond strength of SARC's a smear layer was created by polishing with a 600-grit silicon carbide paper (3;4;99;103). Only a few used a 180-grit sandpaper (36;53). To create a clinically relevant smear layer, some studies used diamond burs (24;41) or carbide burs (50). Gisler et al. (35) tried to determine the shear bond strength of RelyX Unicem (SARC) and RelyX ARC (resin luting agent with etch-and-rinse adhesive system) to dentin in relation to surface roughness produced with 220- (the coarsest), 500-, 1000-, 2400- and 4000-grit (the finest) silicon carbide sandpaper and with a 15µm finishing diamond bur. They found that the roughness of the smear layer does not influence the shear bond strength of Relyx Unicem and which was even superior to that of RelyX ARC resin luting agent bond strength.

6.1.3. Luting agents

The one step self-etching primer system of the resin luting agent Panavia F 2.0, used in the present study as a control, has mild etchant properties. The functional monomers of mild self-etching adhesive systems have the ability to bond to hard tooth tissues through mirco-mechanical anchorage and real chemical bonding (74). ED Primer in combination with Panavia F2.0 luting agent has a good proven clinical performance and is often used to rank the other luting agents (10). Panavia primer is water based, contains amphiphilic monomers (HEMA, MDP and 5-NMSA) and plays an important role in the dual-cure effectiveness of resin composite (28). The amphiphilic monomers have a better ability to diffuse into the hybrid layer than hydrophobic monomers due to their low molecular weight (6). In the literature it is reported that MDP calcium salt exhibited the highest stability and reached the highest bond strength to hydroxyapatite in comparison to the other functional monomers (74;93). The investigation of adhesives containing functional monomers showed that MDP monomer was the most resistant to thermocycling (104). Clearfil SA Cement contains the amphiphilic functional monomer MDP as well. The identical functional monomers of both luting agents led us to select Clearfil SA Cement as one of the representatives of SARC's for this investigation. RelyX Unicem, as the first of the SARC's marketed, was the second selected self-adhesive resin cement for this morphological study. Other functional monomers

demineralized dentin much more, but MDP infiltrated partially demineralized dentin very well, keeping HAp around collagen, so that the collagen could be protected against hydrolysis (104). As the molecular formulas show (Fig. 2), RelyX Unicem and RelyX Unicem 2 functional monomer contains two phosphate groups and two polymerizable groups whereas MDP monomer contains one polymerizable and one phosphate groupTable 3. This suggests that the etching and polymerizing ability of RelyX monomer could be more active and occur in a shorter time than that of MDP monomer. So, it could be expected that RelyX Unicem monomers and thus the luting agent diffusion into the tooth tissues could be deeper than that of MDP monomer containing luting agents. However, observation of the interdiffusion zone in the SEM revealed no deeper interaction of RelyX Unicem and RelyX Unicem 2 luting agents with the dentinal tissues.

6.1.4. Specimen preparation

In preliminary studies the attempts were made to demonstrate the effectiveness of the adhesive interface of SARC on the clinically relevant specimens i.e. the indirect ceramic restorations (inlays or onlays) prepared with CEREC CAD/CAM System and luted on the whole prepared teeth. The results were unsatisfying due to the frequent presence of artefacts occurring during the microscopy: the dimensional changes in the vacuum environment of the different materials (dentin, enamel, luting agent, ceramic) with diverse physical properties (elasticity, hardness, water content etc.) led to entire or partial rupture of specimens along the adhesive interface. Therefore the specimen model was simplified in shape and reduced to the adhesive interface with the luting agent only. In the main trials, based on the study of Federlin et al. (30), the enamel-dentin half-discs were prepared and the luting agents were applied and polymerized according to the manufacturers' requirements and afterwards covered with flowable resin composite. In this way a non-constraining specimen model was created and the artefact formation in LV SEM was markedly reduced. The specimen preparation included separating the enamel-dentin discs into halves before the application of luting agents to eliminate the possibility of any other processing steps somehow mechanically affecting the adhesive interface, except for the polishing procedure.

6.1.5. Polymerization modes

In the present study light-polymerization of resin composite luting agents was carried out with an LED polymerization unit with intensity of 945mW/cm^2 for 20secs from three sides.

For auto-polymerization all later polished specimens were left under a weight for 10 minutes in a dark chamber. Vrochari et al. (100) evaluated different SARC's (RelyX Unicem, Maxcem, Biscem, Multilink Sprint) for the degree of cure in their self- and dual-curing mode and found

that degree of cure in auto-polymerization mode is very low (11 – 25%). The values in dual-curing mode were higher (26 – 42%). The Maxcem was found to have the lowest degree of cure. It should be added that in the present study after the auto-polymerization for 10 minutes in a dark chamber specimens were stored 24 hours to allow complete polymerization before further processing. The luting agents were polymerized under an applied weight. The weight was selected after the 10 measurements of finger pressure on the scales as though this was applied to the seating of an indirect restoration. As the finger pressure in a clinical situation would not always be identical, it was decided to apply a mean "finger force" of 420g onto all specimens in the present study. Goracci et al. have found out that interfacial strength and adaptation of luting agents are enhanced if a seating force is maintained during the initial curing period (36). In the study of Abo-Hamar et al. (2) was a load of 400g used during the cementation procedure.

The company instruction manual reports that RelyX Unicem is tixotropic material and that load during the polymerization is absolutely necessary to improve the flowability of RelyX Unicem and therefore better adaptation of luting agent (1).

6.1.6. Polishing, demineralization/deproteinization and fracturing procedure

The polishing of specimens was necessary to expose the adhesive interface on one flat surface. This is the simplest and the most conservative way to make the specimen observable with SEM. The LV SEM allowed the specimen to be examined in its native condition without covering it with a conductive layer. The polishing procedure was carried out on the silicon carbide sandpaper and on wet fabric tissue with decreasing grain size aluminium powder. To avoid the contamination and retention of aluminium powder particles to the adhesive interface as well as intrusion and retention of powder in cut dentinal tubules, extra attention was paid to rinsing in the polishing protocol. The duration of copious water spraying and rinsing of specimen was identical to the polishing duration. The polishing procedure can be considered as a mechanical test for the luting agents, which have to withstand the applied stress.

However, the majority of morphological studies were conducted by observation of specimens which were even more deteriorated - demineralized/deproteinized and sputtered (8;36;44;57). With the demineralization/ deproteinization procedure it is possible to liberate resin impregnated structures by superficial etching of inorganic and denaturation of organic tooth structures. The resistance of impregnated structures to demineralization/ deproteinization show the quality and efficiency of the resin interdiffusion process (97). However, in the present study the information about interdiffusion zone or hybrid layer was better seen in polished specimens. The demineralization and deproteinization of polished

specimens in the present study confirmed the presence of several morphological features seen as tags found in polished specimens. There were also characteristics which could not be detected in polished specimens but were revealed through demineralization/ deproteinization procedure, e.g. lateral tags. Demineralization/ deproteinization was especially advantageous for the determination of the real length of resin tags. However, the "real" length of tags is limited by the level of section of the polished specimens. Although, the demineralization/deproteinization procedure results in the important loss of informational value about the morphological structures, it can be concluded that demineralization and deproteinization complement the information gained from polished specimens.

In the present study the fracturing procedure was carried out to reveal the smear layer and correlate it with the thickness of hybrid layer or interdiffusion zone on the bonded side. With the exception of smear plugs, which are usually loosely arranged in dentinal tubules and so the loss of some during the fracturing was almost inevitable; the tooth and material structures remained unaltered. By fracturing the native specimen it is possible to investigate the content of the revealed dentinal tubules (without their modification) and to observe the changes to smear layer and smear plugs after their interaction with the luting agents. The disadvantage of fracturing was the possible breakage of the enamel-dentin disc and material in different planes and the additional stress application on the adhesive interface which could impede its quality.

6.1.7. Scanning electron microscopy

LV SEM as the method for visualization of luting agent adhesive interfaces

The visualization method selected for the present investigation was SEM in low vacuum mode. The study of Federlin demonstrated that this method is appropriate and advantageous in comparison to HV SEM for depiction of adhesive interfaces of diverse adhesive systems (30). The LV SEM was selected for the depiction of specimens because SEM images of good resolution and distinct material contrast can be obtained. LV SEM is also superior to the ESEM microscopy method. For ESEM characteristic increased water vapor concentration in the specimen chamber obscures the field of view. The hydration of a native specimen in LV SEM is reduced, and dehydration of the specimens is more likely to occur. Therefore, to investigate native and non-fixated specimens in LV SEM is a challenge. The persistent dehydration limits the investigation time, especially for such sensitive materials as SARC's are. Nevertheless, artefacts can arise both during the preparation procedures as well as during SEM examination.

The observation time should be reduced to a minimum to preserve specimens from desiccation. It was assumed that the artefacts occur due to water evaporation from the dentin

which leads to shrinkage. In the present study water vapour was used as the amplifying gas. Water is one of the best gaseous mediums and is appropriate for water-containing tissues providing humidity control around the specimen (23).

The chemical analysis of specimens in LV SEM is possible with energy-dispersive X-ray spectroscopy (EDX) method. The EDX analysis is useful for the qualitative and quantitative determination of chemical elements in a specimen surface. In the present study a single EDX analysis was carried out of the fractured/polished CSA, AP specimen to determine the chemical composition of detected tags and to confirm their origin was the luting agent. To permit the electron beam to penetrate deep enough into specimen surface, a large number of electron beam scans with increased acceleration voltage is irradiated onto the native specimen. It may cause damage to the surface of biological samples. Therefore, the suitability of EDX analysis for biological specimens is questionable. The authors of the study conducted to determine the morphology and chemical composition of different resin composites stated that EDX is non-destructive and can therefore be used with a variety of materials in solid, powder, liquid or wafer states (9). It must be added that in that study the materials were examined without the presence of biological material.

6.2. Results

6.2.1. Morphological considerations of enamel-luting agent adhesive interfaces

6.2.1.1. Enamel-luting agent adhesive interfaces in polished specimens

Microgaps

In the present study, adhesive microgaps could be detected at almost all enamel-SARC adhesive interfaces but not at the enamel-PAN with self-etching ED Primer adhesive system interfaces which were used as a control. The results show better adaptation and adhesion of the control resin luting agent. This is also supported by the available literature (24;27;36;41). Hikita et al. analyzed a failure mode of RelyX Unicem bonded to enamel (41). The authors found that in 78% of specimens adhesive failure occurred along the adhesive interface with RelyX Unicem whereas the luting agents with classical etch-and-rinse and self-etching adhesive systems exhibited mixed failures. Findings in the present morphological study support the data from mechanical microtensile bond strength tests because microgaps in SARC's specimens propagated predominantly along the adhesive interface. Overall, in the studies on mechanical properties of RelyX Unicem it was found that the microtensile bond strength or shear bond strength to enamel is lower than that of resin luting agents with multi-step adhesive systems. However, it is still higher than the bond strength of glass ionomer

cement to enamel (71). No deep interaction (presence of hybrid layer or interdiffusion zone) with enamel could be detected either at SARC's or at PAN adhesive interfaces. No enamel etching pattern could be demonstrated with LV SEM. Even if an interaction does exist, the so called "intercristallite nanoretention" of self-etching and self-adhesive materials, it can only be detected with TEM (39).

6.2.1.2. Enamel-luting agent adhesive interfaces in demineralized/deproteinized specimens

After demineralization/deproteinization procedure on several specimens the ultra thin interdiffusion zone could be detected as a thin film covered the luting agent. However, it is hard to determine whether this film is the reacted and infiltrated smear layer or only a superficial layer of luting agent adjacent to enamel surface. The morphological study of Di Hipolito et al. found a very thin nonuniform layer with short or poorly defined resin tags in demineralized/ deproteinized specimens of self-etching adhesive Clearfil SE Bond at the enamel adhesive interface (26). The interaction was tested on ground and intact enamel surfaces. Two other all-in-one adhesives, Adper and Prompt-L-Pop, were tested and revealed a more distinct hybridized layer with homogenous tags attributed to the higher aggressiveness and more hydrophilic resins present in all-in-one adhesives than in self-etching adhesive (26). The mild etching properties and relatively high viscosity of SARC's (in comparison to plain adhesive systems without filler content) give lower ability to penetrate deeper into the enamel surface. It could explain the absence of tags at the enamel-SARC's adhesive interface.

6.2.2. Morphological considerations of dentin-luting agent adhesive interface

It was the aim of the present study to determine which micromorphological characteristics described in literature could be found in the SARC specimens in comparison to the resin luting agent specimens with self-etching primer.

Hybrid layer or interdiffusion zone

In the present study it was hypothesized that the SARC's due to their viscosity, have only superficial interaction with smear layer. Therefore, micromorphological features such as a hybrid layer could be determined in the specimens, but deeper interaction such as the formation of tags as by resin luting agents with separate adhesive system cannot be expected. The analysis of PAN polished specimens confirmed a distinct interdiffusion zone. The fractured specimen of PAN, LP revealed a slightly modified surface of intertubular dentin and orifices of the dentinal tubules, indicating an interaction of ED Primer also with intertubular dentin. Goracci et al. (36) reported about a 1 - 2µm thick hybridized smear layer with short tags of PAN luting agent. The SEM investigation mentioned was carried out on the

positive epoxy resin replicas of demineralized/deproteinized specimens and the original magnification did not exceed x500.

Although the smear layer was standardized and always produced with 600-grit carbide paper, the irregular interdiffusion zone found in the polished specimens could point to the irregularity and variability of smear layer. In the literature it is suggested to create a smear layer with fine carbide burs if self-etching or all-in-one adhesives are used (78). The rough bur produces too thick smear layer which is problematic for the penetration of one-step adhesive materials. Especially, a thinner smear layer formation is suggested when mild self-etching adhesives are used (85). This would also be expected to be true for self-adhesive luting agents. The application of mild self-etching adhesives with agitation increases the depth of penetration (65). Theoretically, agitation could also enhance the demineralization and infiltration potency of SARC's.

The interdiffusion zone in SARC's in polished specimens in the present study could not always be detected – a thin interdiffusion zone (did not exceed 1 µm) could sometimes be revealed in the same specimens when demineralized/deproteinized. An irregular 0-2µm interdiffusion zone was detected in the TEM study of De Munck et al. (24). But in the study of Goracci (36) on positive replicas of demineralized and deproteinized specimens interdiffusion could not be detected even under applied seating pressure. The data found in the literature refer to RelyX Unicem, investigations on other SARC's used in the present study could not be found. In the present study the RXU2 specimens have more detectable interdiffusion zones than other SARC's, which could be explained by the lower viscosity of RXU2 as compared to RXU1. The smear layer of some SARC specimens seems to be impregnated with resin and separated from the bulk of material during the demineralization/ deproteinization procedure, whereas in the specimens of PAN the smear layer was integrated and not separately determinable. It could indirectly indicate that the smear layer in SARC specimens is not fully dissolved and is permeable for HCl and NaOCl while the ED Primer of PAN sealed or completely penetrated the smear layer.

Tags

In this study, it was not expected that SARC could form resin tags in dentinal tubules due to relatively high viscosity of SARC's. This is especially so because the morphological studies where RelyX Unicem, Maxcem, Multilink Sprint, GCem and BisCem were tested, do not show the luting agent infiltrating into dentinal tubules (36;55). CSA and RXU2 were not tested in the mentioned studies. By contrast, Panavia with self-etching ED primer showed the ability to infiltrate dentinal tubules (36). The PAN specimens in the present study showed the most resin tags. The ability of PAN to form the tags is probably due to the separate primer which modifies the smear layer and permits the deeper interdiffusion of Panavia luting agent.

In the present study, resin tags could be detected only in one polished RelyX Unicem specimen, supporting the opinion found in the literature that RelyX Unicem is not able to form tags. Only if the dentinal tubule was not already occluded with a smear plug, the resin of RelyX Unicem could intrude into dentinal tubule (24;36;102). But another two SARC's – Clearfil SA Cement and RelyX Unicem 2 polished specimens in the present study showed contrary results. There were several specimens where the presence of tags could be detected. The tags in polished specimens could be detected predominantly in lateral dentin (near to DEJ), where the angle of cut of the dentinal tubules was favourable. The cut of the dentinal tubules near to DEJ is oblique. Therefore, there is a greater possibility of exposing the tags than in the middle dentin where the dentinal tubules are cut perpendicularly. The morphology of detected CSA and RXU2 tags differ from the tags formed by PAN. The RXU2 and CSA resin tags were not filler reinforced and they were predominantly hollow and porous as revealed by demineralization/deproteinization of the polished specimens. The *lamina limitans*, the inner sheath of dentinal tubules, has also the hollow structure after demineralization/ deproteinization procedure (91). Therefore the differentiation of *lamina limitans* and resin tags in SEM is hindered. The ED Primer and PAN formed resin tags with filler intrusion at the base of tags. The depth of the filler infiltration could not be determined. The demineralized and deproteinized tags in PAN specimens were left connected to the luting agent whereas the SARC's formed tags which collapsed on the dentin surface separated at the junction with the rest of material. The tags were not only separated from the bulk of the luting agent, the interdiffusion zone after the demineralization/ deproteinization procedure separated from luting agent as well. The most critical aspect seems to be the connection of the CSA and RXU2 tags or interdiffusion zone to the bulk of material. Therefore the mechanical strength and the ability of these tags to create sufficient micromechanical anchorage in the dentinal tubules is questionable.

As shown by the imaging of demineralized/ deproteinized CSA and RXU2 specimens, the resinous tags in dentinal tubules approached a maximum of 100µm length. But the real length of resinous tags, even after the demineralization/deproteinization procedure, could not be precisely determined due to cross section of the specimens and the depth of demineralization. The formation of long resin tags could be explained by the absence of a counter pressure of dentinal fluid in the dentinal tubules from the pulpal side. Therefore the lower dental fluid pressure in the dentinal tubules could ease the infiltration of RXU2 and CSA resin. It would be interesting to investigate the influence of the pulpal pressure on the length of tags. The long resin tags of RXU2 and CSA may play important role in the decrease of post-operative sensitivity through occluding the dentinal tubules. On the other hand, if the

infiltration of resin occurs that deep in the dentinal tubules in a clinical setting it could cause an inflammatory reaction of the pulpal tissue.

Pores

The problem with porosity at the adhesive interface of SARC has already been described by De Munck et al. (2004) and Goracci et al. (2006). However, the study of Alster et al. claims that incorporation of small bubbles by stirring the luting agent contributes to stress reduction and to maintenance of a marginal integrity (7). The porosities are supposed to be associated with the relatively high viscosity of G-Cem and RelyX Unicem, therefore the appearance of this phenomenon could be minimized through the application of ultrasound as in the study of Cantoro et al. (18) or the application of weight as in the study of Goracci et al. (36). In our study, the polymerization of SARC was also carried out under an applied weight to minimize the porosities along the adhesive interface. Pores along the enamel-luting agent adhesive interface were not often detectable. By contrast, at the dentin-luting agent adhesive interface pores were regularly observed. Moreover, the pores along the dentin adhesive interface appeared mostly in particular places such as orifices of the dentinal tubules. The reason for that could be the residual moisture or water entrapped in dentinal tubules, which mixes with the hydrophilic resin content of the cement and dilutes the material. Excess water compromises the polymerization process of SARC's and results in incomplete polymerized regions or hydrogel which can be easily washed out during the polishing procedure. It is reported in the literature, that one-step self-etching adhesives due to their high hydrophilicity and permeability allow "water treeing" through the hybrid layer (21;25;86;88;89). The excess of water impedes the polymerization of acidic monomers. Incomplete neutralization of monomers can also inhibit the chemical curing of SARC components leading to reduced interfacial strength and premature hydrolysis (6). The difference between one-step and two-step self-etching adhesives is the absence of a hydrophobic resin layer between the hybrid layer and the composite resin which preserves the adhesive bond from further water infiltration (65). This layer is also absent at the adhesive interface of SARC's. To avoid excessive water in adhesive layer, Mathews et al. (52) tried to evaporate the water by heat drying and not by air-drying. The SEM evaluation showed no evidence of "water treeing" after application of the experimental method.

Smear plugs

The presence of smear plugs (resulting from creation of smear layer) is difficult to evaluate in polished specimens due to smear debris which may fill the dentinal tubules during the polishing procedure. Self-etching primer like ED Primer impregnate the smear plugs fixing them at the entrance of the dentinal tubule (15). So, even if the smear plugs in dentinal tubules have partially reacted with the luting agent, the non-reacted part during the polishing

procedure could be lost. Intact smear plugs and smear layer can be preserved if the specimens are fractured and without polishing. Therefore fractured specimens were additionally prepared to assess the real length of smear plugs and the thickness of the smear layer.

Characteristics of dentin

In the present study in some specimens the content of the dentinal tubules disappeared after the demineralization/deproteinization procedure disappeared and only brittle tubules covering sheaths were left. These thin brittle sheaths, which withstood the demineralization and deproteinization procedure, are considered to represent the *lamina limitans*. The difficulty in demineralized/deproteinized specimens is the differentiation of the tags from *lamina limitans* which as inner sheath of the peritubular matrix line the dentinal tubules, consisting of glucoseaminoglycans (GAG) and are resistant to demineralization and deproteinization. In sclerotized dentin the *lamina limitans* can also be mineralized (90). The function of *lamina limitans* presumably is the regulation of deposition of peritubular dentin, as GAG are known as inhibitors of mineralization (91).

6.2.3. The influence of polymerization mode on the morphology of adhesive interface

It was hypothesized that the micromorphology of the adhesive interface is affected by the polymerization mode. It was expected that in auto-polymerization mode the hybrid layer or interdiffusion zone of specimens would be more distinct due to the longer interaction time until complete setting of the luting agent. Unfortunately, there is no available data where the effect of the polymerization mode on the micromorphology of SARC's was investigated. In the majority of studies the luting agents were light-polymerized but there were no morphological studies included. Aguiar et al. determined the influence of the curing mode on the microtensile bond strength of PAN, RXU1, BisCem and GCem (4). They found that light-activation increased the bond strength of PAN and GCem, whereas the bond strength of RXU1and BisCem was not affected by the polymerization mode. In the present study, clear influence of polymerization mode on micromorphology could not be demonstrated. Although it was hypothesized that auto-curing of luting agents, especially of SARC's, would promote the deeper infiltration of resin and the detection of a thicker interdiffusion zone, no differences could be found in the thickness of the interdiffusion zone dependent upon the polymerization mode. No interaction with the dentin underlying the smear layer was shown in the study of Goracci et al. (36). Luting agents in that study were light-polymerized. Interestingly, tags in the present study were detected more frequently in the auto-polymerized SARC's specimens. PAN specimens had tags in both polymerization modes.

Pores along the adhesive interface were absent in the auto-polymerized specimens of CSA and RXU2, indicating that the longer interaction time could play a role in the adaptation of luting agents to the dentin surface. The better ability of hydrophilic resin to adapt to the dentinal surface during the longer auto-curing phase (mean duration until complete setting is 3 min) could explain the presence of tags and the reduced amount of pores at the adhesive interface of CSA and RXU2.

7. Summary

Resin luting agents are used to lute indirect restorations to hard tooth tissues. The luting procedure consists mostly of several tooth pretreatment steps as etching, priming and application of adhesive and only at the very end applying of a resin luting agent. Such a multi-step luting procedure with separate adhesive system is quite time-consuming and technique sensitive. Therefore, constant inquiry from the practitioners for the resin luting agents with simplified application procedure, have moved the manufacturers to develop self-adhesive resin cements which require no tooth tissue pretreatment anymore. Numerous *in vitro* and few *in vivo* studies of the first self adhesive resin cement RelyX Unicem showed adequate bond strengths to hard tooth tissues and acceptable short-term clinical performance.

The complex chemistry and relatively high viscosity of self-adhesive resin cements challenges the ability of them to interact with hard tooth tissues. Moreover, they interact directly with a smear layer. So, it was the question whether self-adhesive resin cements are able to create similar micromorphological adhesion pattern to resin luting agents with separate adhesive systems.

We tried to answer this question with the present SEM morphological investigation. It was the aim to analyze and compare the adhesive interfaces of a control resin luting agent with self-etching primer (Panavia F and ED Primer) and adhesive interfaces of self-adhesive resin cements (RelyX Unicem, RelyX Unicem 2 and Clearfil SA Cement). The task was to find out which morphological characteristics (tags, lateral tags, hybrid layer or interdiffusion zone, pores and other) could be found in the specimens of self-adhesive resin cements, compare them on native polished, demineralized/deproteinized and fractured specimens in low vacuum mode. It was the aim to find out whether the polymerization mode has the influence on the morphological findings. At least, to clear if the present LV SEM method can be considered as appropriate method for adhesive interface examinations.

It was hypothesized that the self-adhesive resin cements would have only superficial interaction with smear layer without ability to form resinous tags. This could not be confirmed because a few specimens of RelyX Unicem 2 and Clearfil SA Cement revealed the presence of tags in dentinal tubules. However, the interdiffusion zone was thin and appeared irregularly even within one SARC's specimen. This SEM morphological study confirmed quite a weak interaction of all three SARC's with hard tooth tissues. The weak adhesion to enamel led to microgap formation along the adhesive interface. There were more failed specimens in SARC's experimental group than in Panavia control group. In this aspect, RelyX Unicem 2

and Clearfil SA Cement performed worse than RelyX Unicem. The micromorphology of the adhesive interface of the control luting agent Panavia and its ED Primer appeared similar to that reported in the literature for resin luting agents with self-etching adhesive systems. A good material adhesion – low number of failed specimens, no microgap formation at enamel-Panavia adhesive interface, a distinct hybrid layer, tags and lateral tags were characteristic for a dentin-Panavia adhesive interface. The demineralization/deproteinization and fracturing of specimens revealed some characteristics extra like lateral tags and complemented the information obtained from polished specimens.

It was hypothesized that auto-polymerization would enhance the deeper diffusion of the luting agents and thicker interdiffusion zone could be detected. Specimen examinations showed insignificant differences of morphological findings. Although, tags were found mostly in auto-curing mode, the supposed thicker interdiffusion zone in auto-polymerized self-adhesive resin cement specimens was not present. Possibly the tags in Clearfil SA Cement and RelyX Unicem 2 specimens could be formed there, where the dentinal tubules were free of smear plugs. By contrast, ED Primer of Panavia has modified the smear plugs allowing resin to infiltrate into dentinal tubules in both curing modes, however, without influence on the thickness of the hybrid layer.

Using low vacuum SEM method it was possible to depict the characteristic adhesive interface of native, minimally prepared specimens. In particular, material contrast in LV SEM allowed features to be revealed such as the interdiffusion zone (hybrid layer) or tags.

Self-adhesive resin cements have shown less typical micromophological characteristics at adhesive interface when compared to a control resin luting agent in the present study. It can be concluded that self-adhesive resin cements have predominantly chemical interaction with smear layer. Although, the bond strength of self-adhesive resin cements *in vitro* studies is acceptable, for lasting clinical performance a combination of chemical and micromechanical adhesion with a formation of tags and sufficient interdiffusion zone would be more trustworthy.

8. Zusammenfassung

Befestigunskomposite werden benutzt, um die indirekten Restaurationen an Zahnsubstanz – Schmelz und Dentin adhäsiv zu befestigen. Die adhäsive Befestigung wird durch mehrere Vorbehandlungsschritte der Zahnhartsubstanz wie Ätzen, Primen und Bonden realisiert. Solches Vorgehen ist zeitaufwendig und Technik-sensitiv. Der Wunsch von Praktikern auf ein vereinfachtes Vorgehen führte zur Entwicklung der selbst-adhäsiven Befestigungskomposite. Bei der Anwendung der selbst-adhäsiven Befestigungskomposite die Vorbehandlung der Zahnhartsubstanz entfällt. Zahlreiche in vitro und in vivo Studien von ersten auf dem Markt verfügbaren selbst-adhäsiven Befesigungskomposit RelyX Unicem zeigten adäquate Haftwerte an Zahnhartsubstanz und akzeptable klinische Ergebnisse. Wegen der komplexen Chemie und relativ hohen Viskosität ist die Interaktion mit Zahnhartsubstanz für selbst-adhäsiven Befestigungskomposit eine Herausforderung. Besonderes, weil das Material direkt mit der Schmierschicht interagieren soll. Deshalb die Frage war, ob die Bindungszone der selbst-adhäsiven Befestigungskomposite die gleiche Mikromorphologie wie die von Befestigungskomposite mit separatem Adhäsiv-System hat.

Wir haben versucht, die Frage mit vorliegender niedrigvakuum-rasterelektronikroskopischen (LV REM) Untersuchung zu beantworten. Es war das Ziel, die Bindungszone von Befestigungskomposit mit selbstätzendem Primer (Panavia F und ED Primer) als Kontrolle und die Bindungszone von selbst-adhäsiven Befestigungskompositen (RelyX Unicem, RelyX Unicem und Clearfil SA Cement) zu vergleichen und analysieren. Die mikromorphologische Charakterisierung der Bindungszone von nativen polierten, demineralisierten und deproteinisierten und frakturierten Proben erfolgte nach Kriterien: Tags, laterale Tags, Hybridschicht oder Interdiffusionszone, Poren und anderen. Der Einfluss der Polymerisationsmodus auf die Mikromorphologie wurde zugleich untersucht. Es war das Ziel herauszufinden, ob LV REM eine geeignete Methode für die Analyse der Bindungszone ist.

Es wurde angenommen, dass die Bindungszone der selbst-adhäsive Befestigungskomposite nur eine oberflächige Interaktion mit Schmierschicht ohne Bildung der Tags aufweisen kann. Dies konnte nicht bestätigt werden, da mansche RelyX Unicem 2 und Clearfil SA Cement Proben die Anwesenheit der Tags zeigten. Die Interdiffusionszone war irregulär und dünn sogar innerhalb einer Probe. Die vorliegende REM Untersuchung wies eine schwache Interaktion der selbst-adhäsiven Befestigungskomposite mit Schmelz auf. Das führte zu Bildung der Mikrorisse entlang der Bindungszone. Es waren mehr Proben aus selbst-adhäsiven Befestigungskomposit Gruppen im Vergleich mit Panavia Kontollgruppe, die haben versagt. In diesem Aspekt, haben die RelyX Unicem 2 und Clearfil SA Cement Proben

schlechter als RelyX Unicem Proben funktioniert. Die Bindungszone in Panavia Proben mit separatem selbst-ätzenden ED Primer wies Ähnlichkeiten zu Micromorphologie der Befestigungskomposite mit separatem Mehr-Schritt Adhäsiv-System beschriebenen in der Literatur auf. Panavia Gruppe hatten niedrigen Anzahl der versagten Proben, keine Mikrorisse entlang Schmelz-Panavia Bindungszone, feststellbare obwohl auch dünne Hybridschicht, Tags, laterale Tags entlang Dentin-Panavia Bindungszone. Die Demineralisation und Deproteinisation als auch die Fraktur der Proben haben mansche zusätzliche Charakteristika wie laterale Tags offenbart und ergänzte die bekommene Information von polierten Proben.

Es wurde angenommen, dass in Auto-polymerisation des Befestigungskomposites eine tiefere Diffusion in Zahnhartsubstanz erlaubt und demzufolge eine dickere Interdiffusionszone feststellbar ist. Es wurde keine signifikante mikromorphologische Unterschiede zwischen beiden Polymerisationsmodi gefunden. Obwohl die Tags überwiegend in auto-polymerisierten Proben gefunden wurden, war die Interdiffusionszone nicht signifikant dicker. Wahrscheinlich, die Tags der RelyX Unicem 2 und Clearfil SA Cement Proben konnten sich dort bilden, wo Dentinkanälchen frei von Schmier-pfropfen waren. Im Gegensazt, der ED Primer von Panavia Befestigungskomposit modifizierte die Schmier-pfropfen. Dies erlaubte den Kunststoff in Dentintubuli unabhängig von Polymerisationsmodus zu diffundieren, auch wenn die Dicke der Hybridschicht nicht beeinflusst war.

Mit niedrigvakuum REM Methode war es möglich die charakteristische Bindungszone auf nativen, minimal präparierten Proben darzustellen. Besonderer Vorteil der LV REM ist guter Materialkontrast welcher erlaubt Darstellung solcher Besonderheiten wie Interdiffusionszone (Hybridschicht) und Tags.

In vorliegende Studie selbst-adhäsive Befestigungskomposite haben weniger typische mikromorphologische Charakteristika im Vergleich zu Kontrolle gezeigt. Es deutet darauf, dass selbst-adhäsive Befestigungskomposite nur eine oberflächige (chemische) Interaktion mit Schmierschicht haben mit unregelmäßiger Bildung echter Interdiffusionszone und Tags. Obwohl die Adhäsion und Haftwerte der selbst-adhäsiven Befestigungskomposite in in vitro Studien ausreichend sind, für einen dauerhaften klinischen Erfolg eine Kombination aus chemischen und mikromechanischen Verbund mit Bildung der Tags und ausreichender Interdiffusionszone wäre zuverlässiger.

9. Figures

9.1. Materials and methods

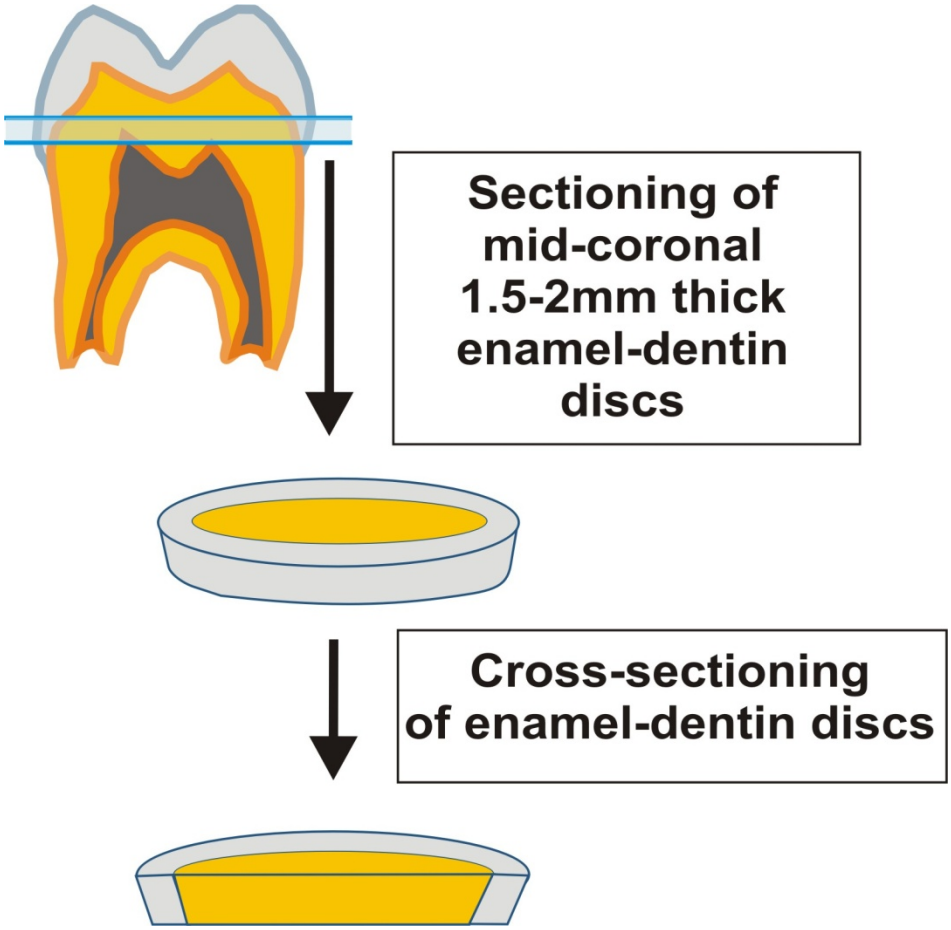


Fig. 1 Preparation of enamel-dentin half-discs.

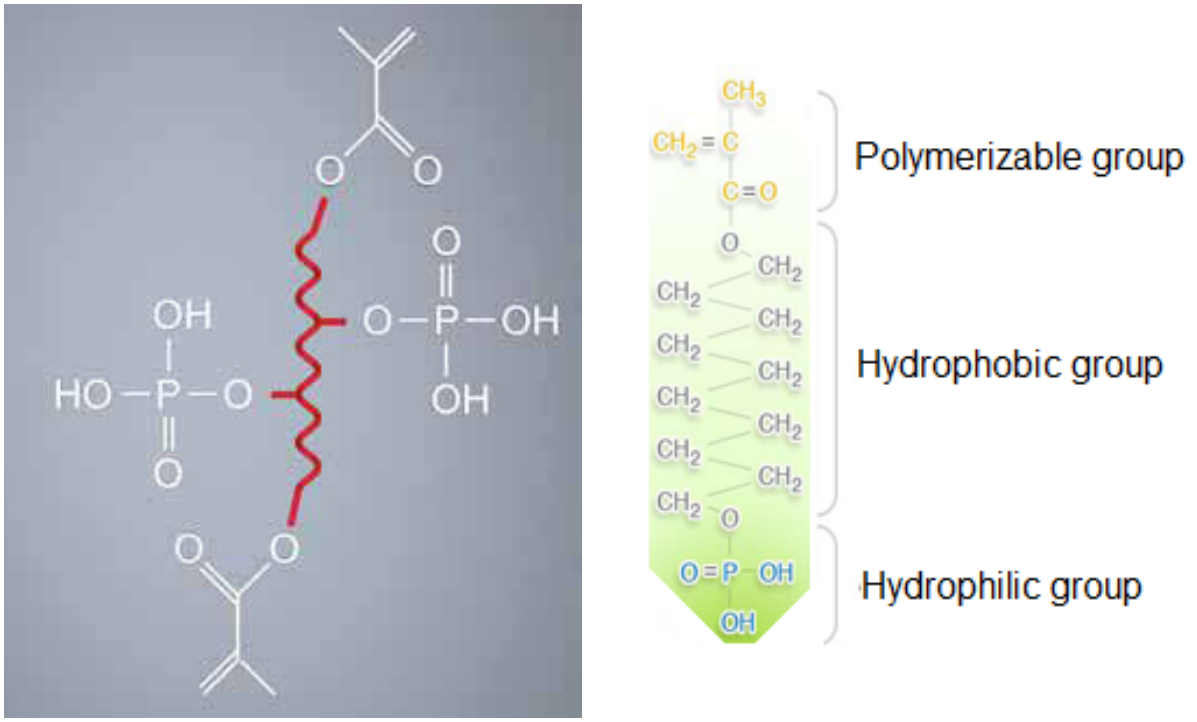


Fig. 2 Schematic illustration of functional monomers in selected SARC's: the functional monomer in RelyX Unicem and RelyX Unicem 2 (left) and MDP monomer in Clearfil SA Cement and Panavia F ED Primer (right) (1;47).

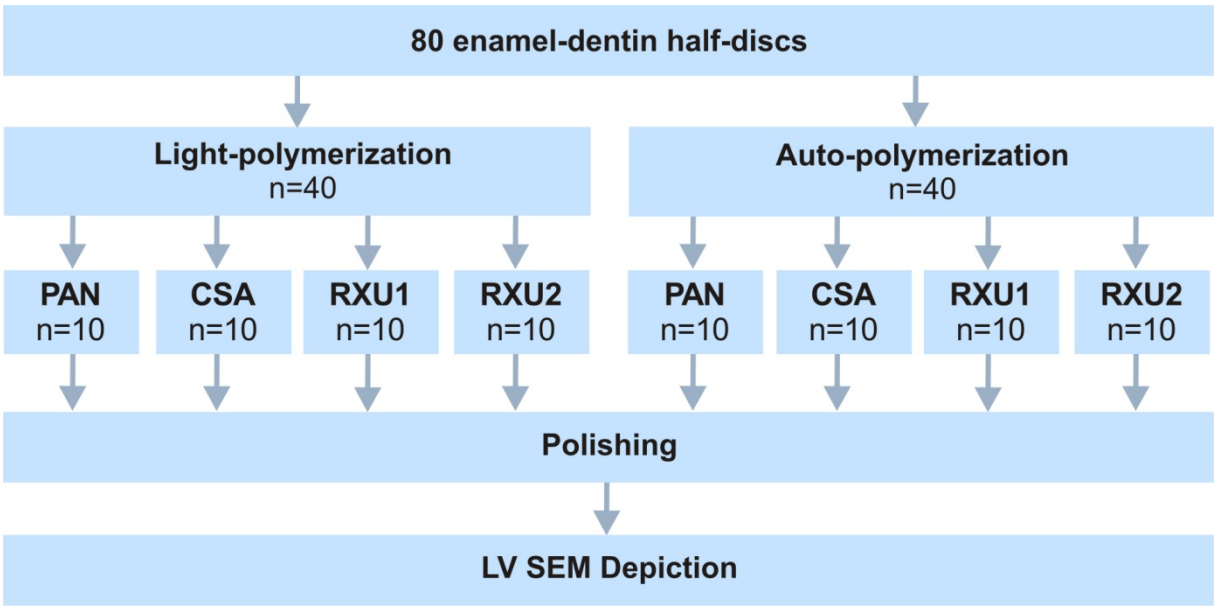


Fig. 3 Experimental groups and the number of polished samples per group (PAN = Panavia F+ED Primer, CSA=Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2).

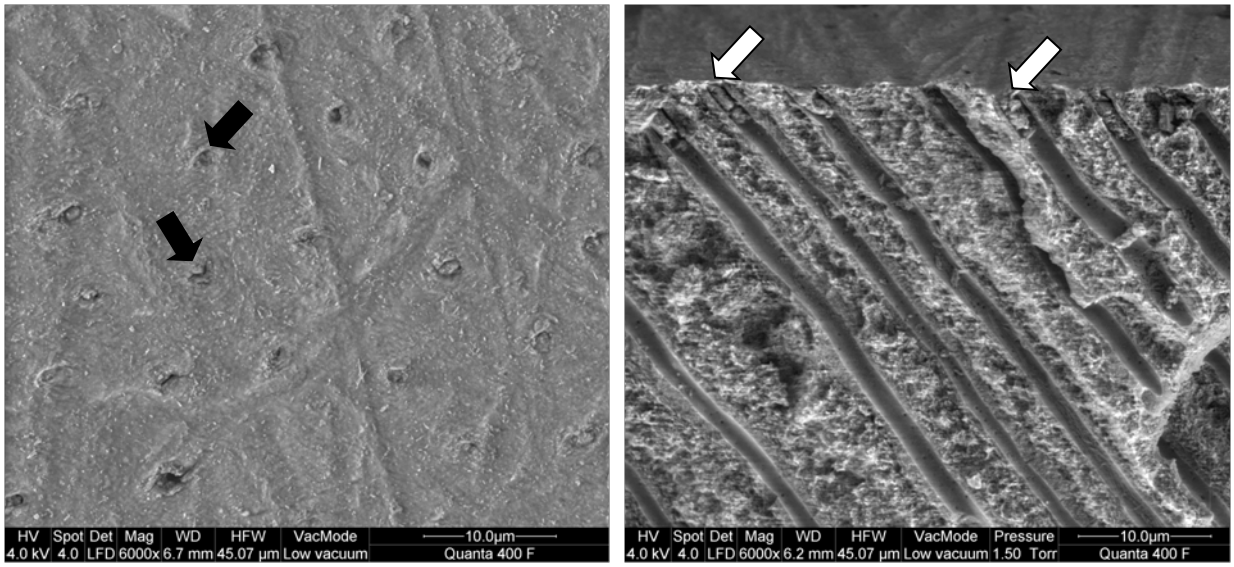


Fig. 4 The top view (left) and cross sectional view (right) of a fractured dentin disc with a smear layer created with 600-grit silicon carbide paper: smear layer occluded the orifices of dentinal tubules (black arrows) and smear plugs (white arrows) within dentinal tubules at x6000 original magnification.

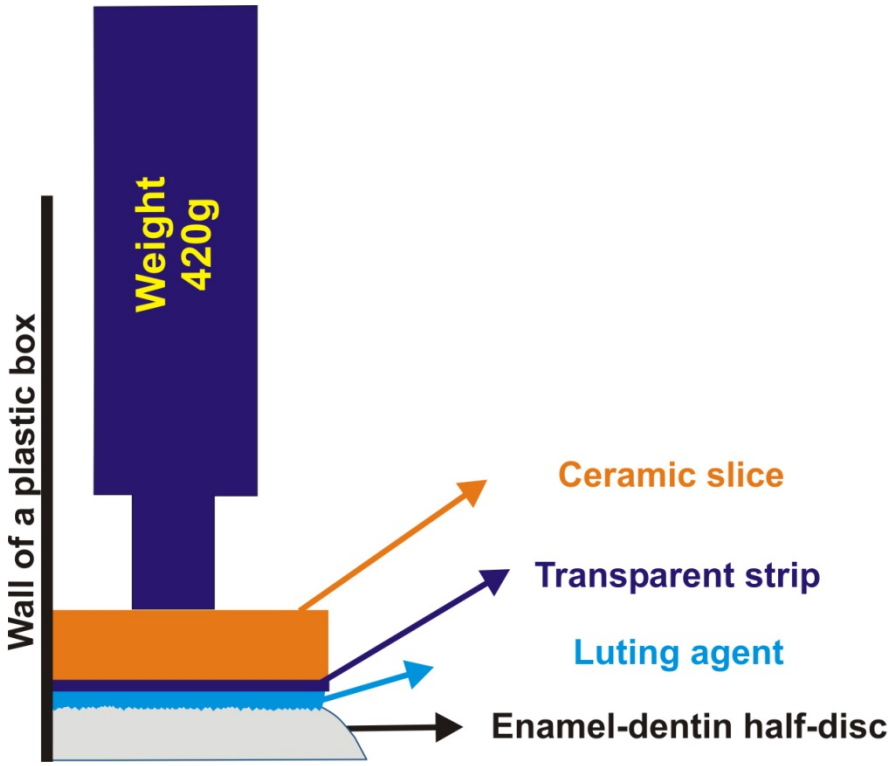


Fig. 5 A schematic picture of the adhesive procedure to show the interdiffusion zone between the luting agent and the tooth tissues.

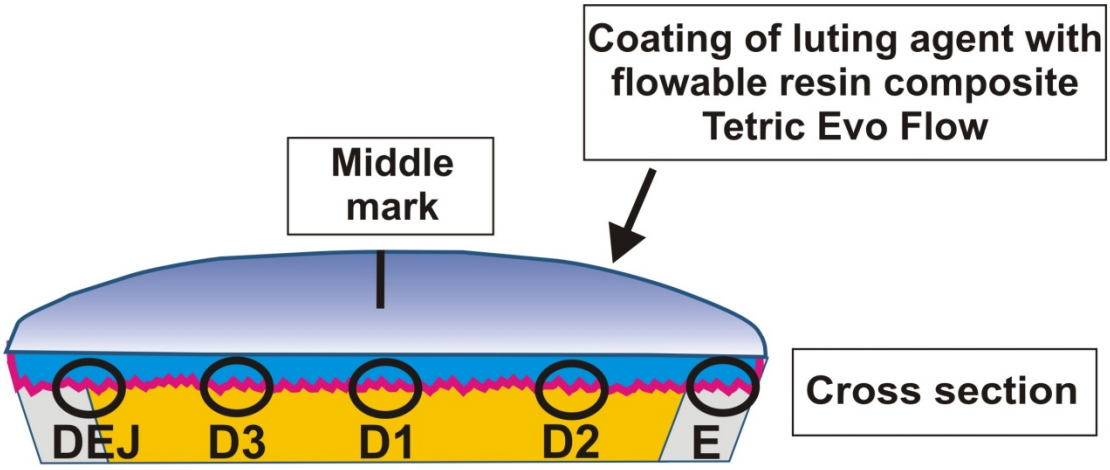


Fig. 6 Schematic illustration of SEM documentation protocol of polished cross-sectioned surface.

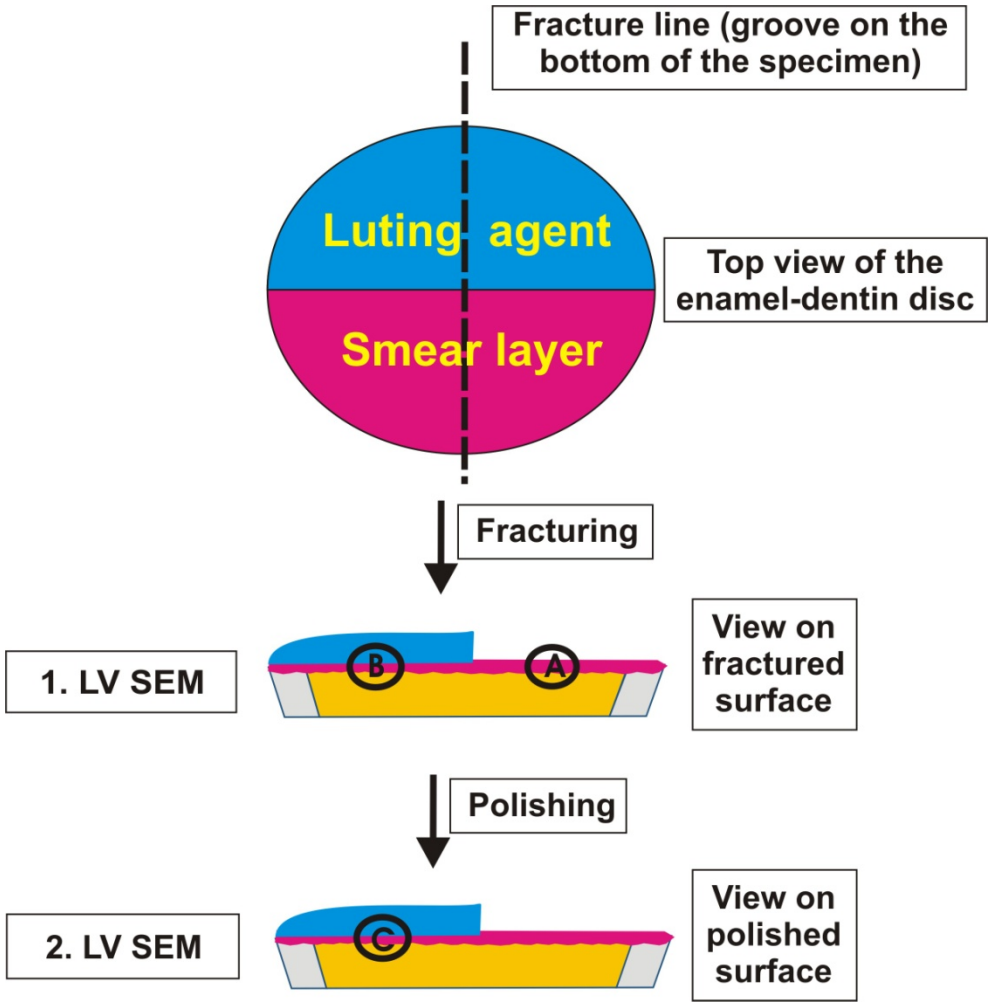


Fig. 7 Preparation of fractured and later polished specimen and examination of it in LV SEM. One half of the disc was coated with luting agent (blue) whereas the other half remained only with the smear layer created with a 600-grit silicon carbide paper (magenta). A, B and C in circles indicate the sites where the SEM images were taken.

9.2. Results

9.2.1. Qualitative evaluation of adhesive interfaces of polished specimens

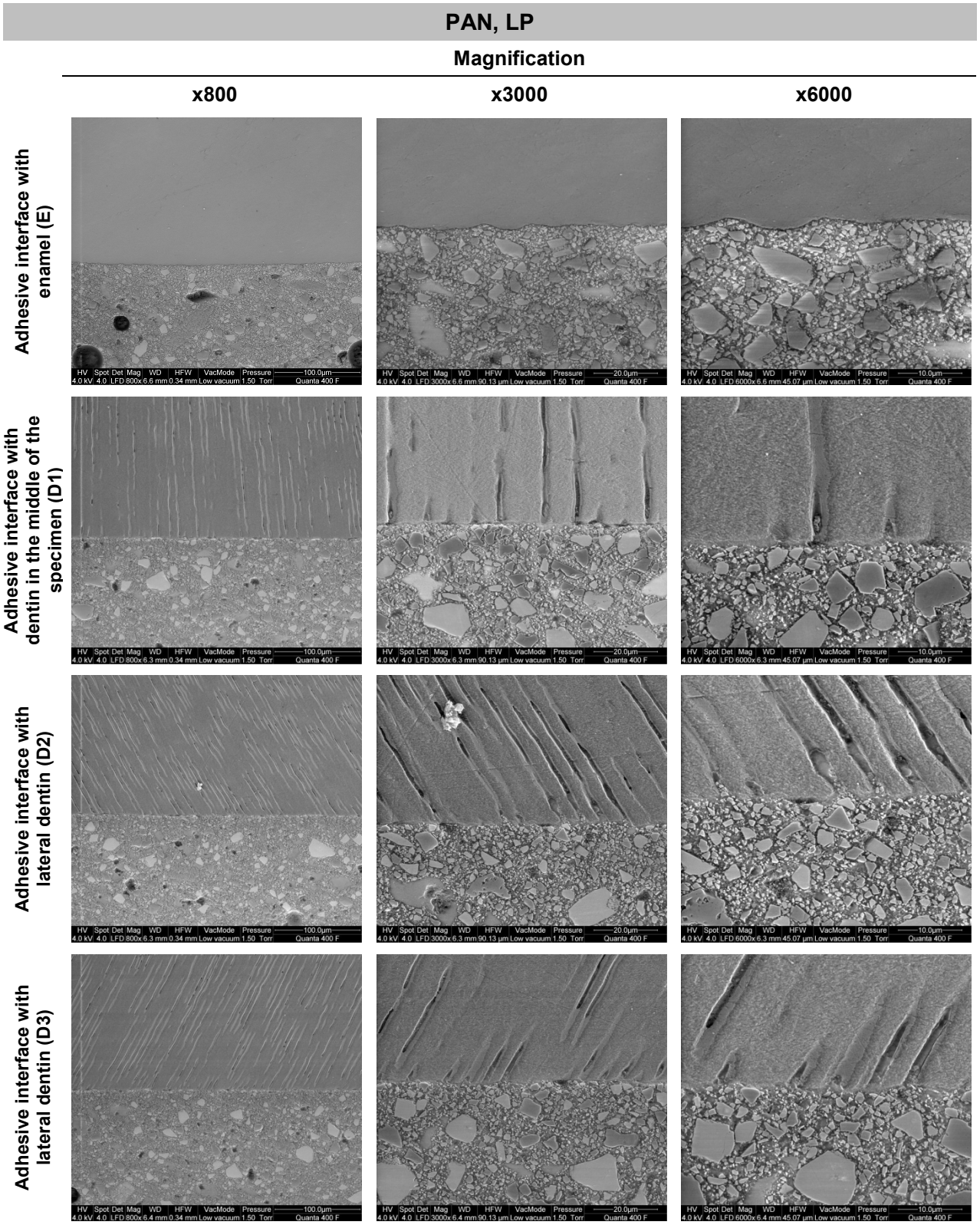


Fig. 8 Adhesive interface of the PAN, LP polished specimen No. S06. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

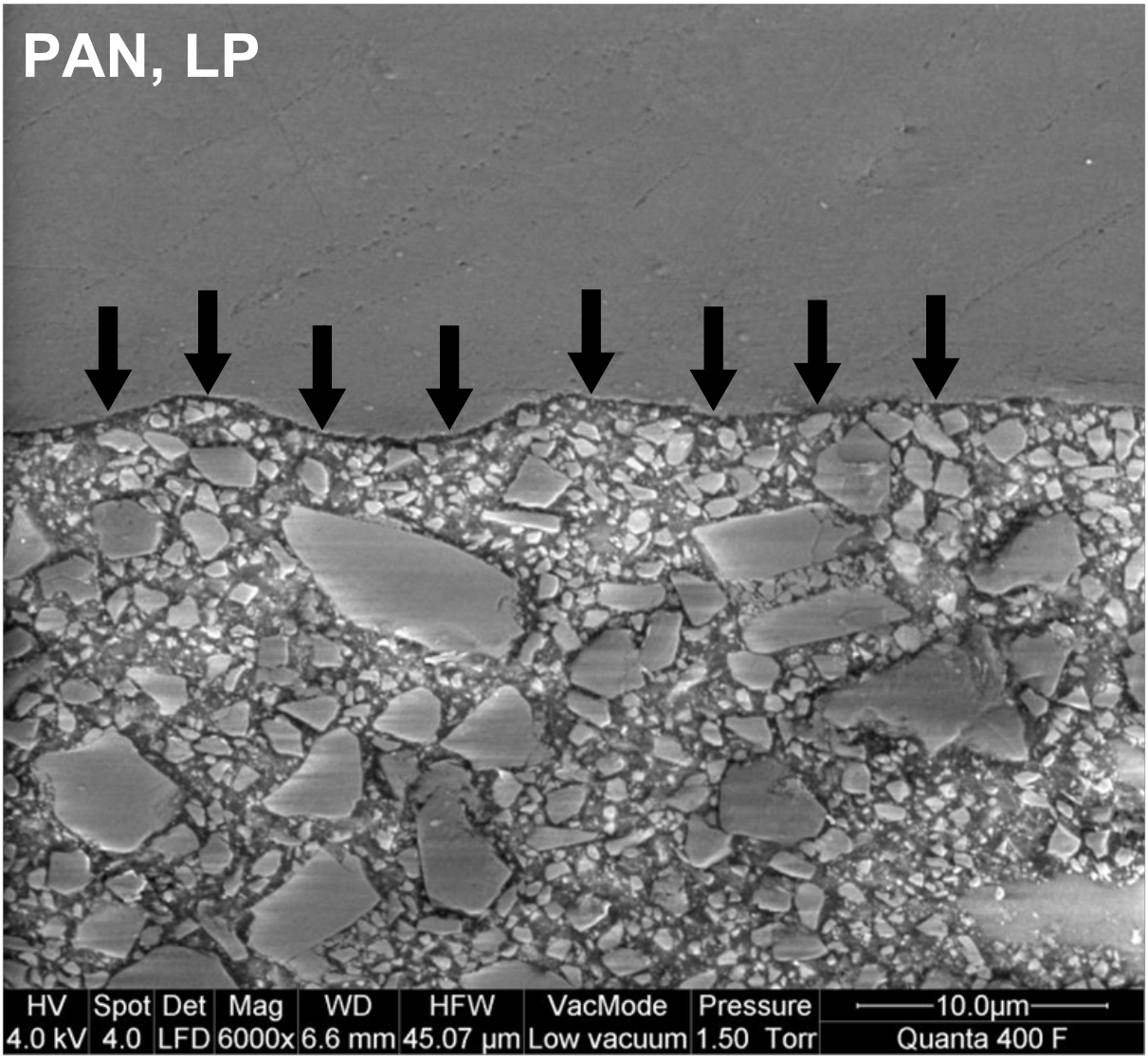


Fig. 8-a Characteristics of PAN, LP: enlarged depiction of the E image at x6000 magnification from Fig. 8. No microgaps could be observed in this nor in other PAN, LP specimens. The integrity of enamel-luting agent adhesive interface was perfect (black arrows) although no deeper interaction or etching pattern was revealed.

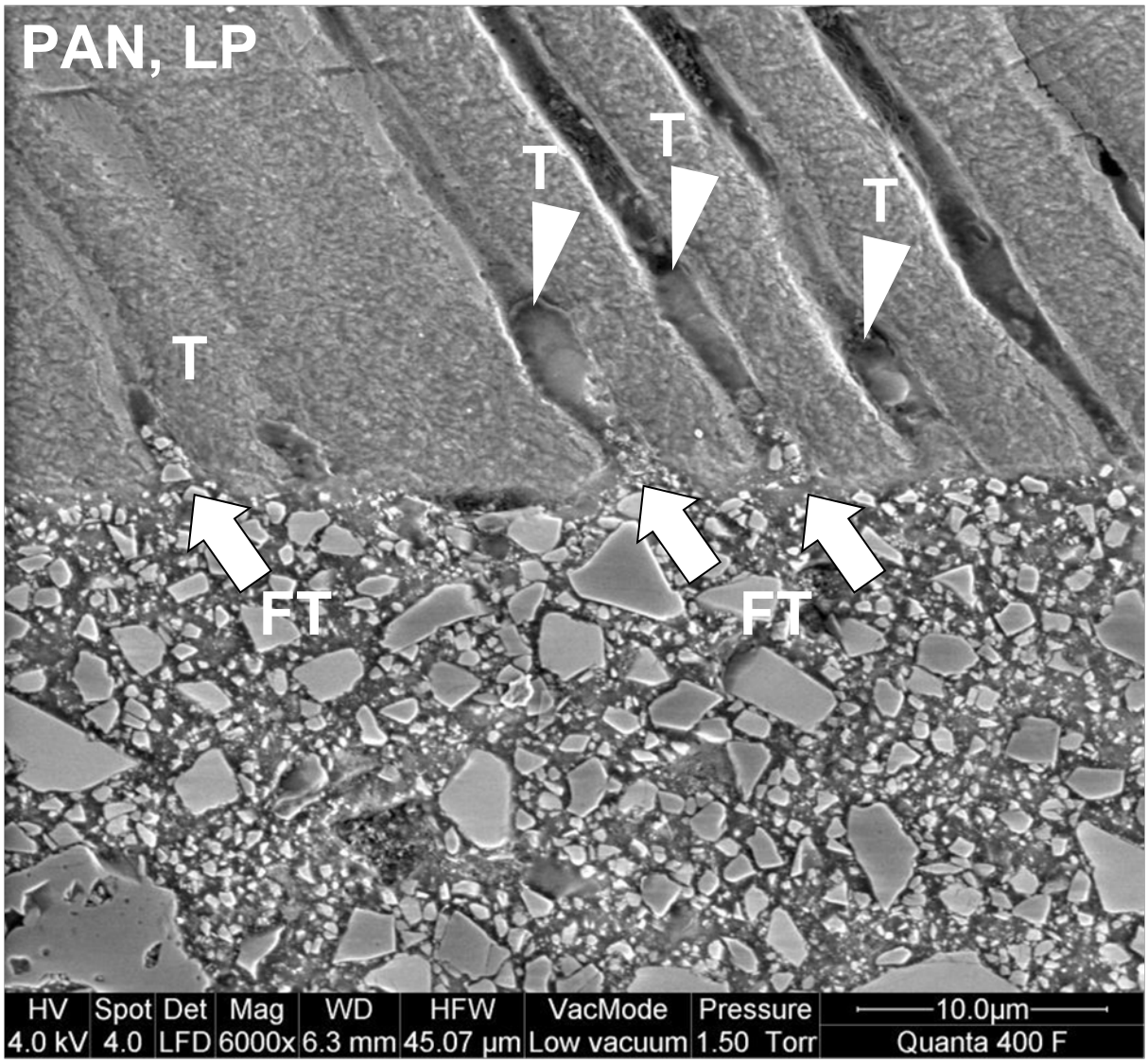


Fig. 8-b Characteristics of PAN, LP: enlarged depiction of the D2 image at x6000 magnification from Fig. 8. Resin tags (T; white arrow heads) and the filler reinforced tags (FT; white arrow) in dentinal tubules.

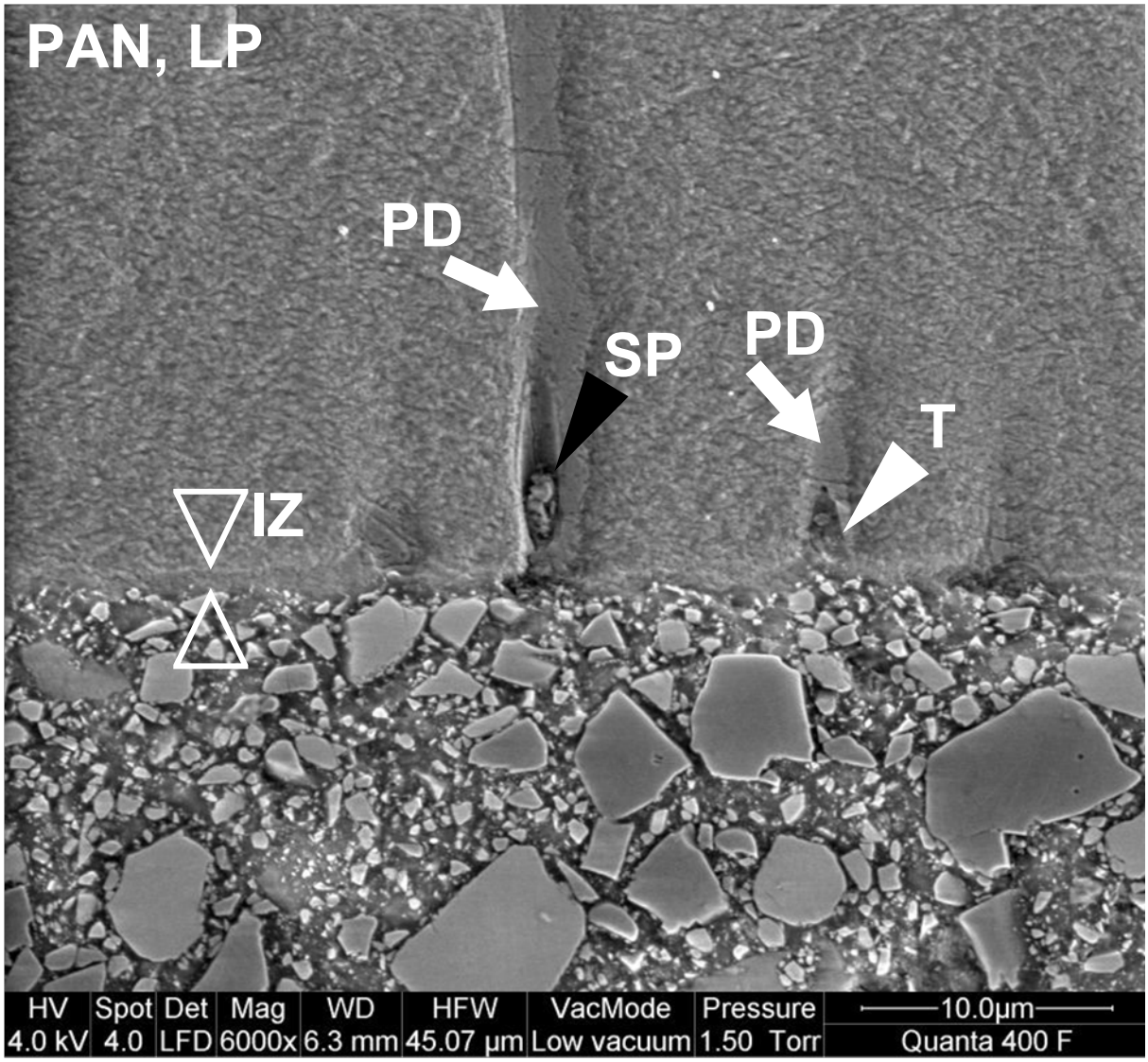


Fig. 8-c Characteristics of PAN, LP: enlarged depiction of the D1 image at x6000 magnification from Fig. 8. The intrerdiffusion zone (IZ; arrow heads) ~1 µm thick, smear plug (SP; black arrow head) and tag (T; white arrow head), as well as very distinct peritubular dentin (PD; white arrow) were noted.

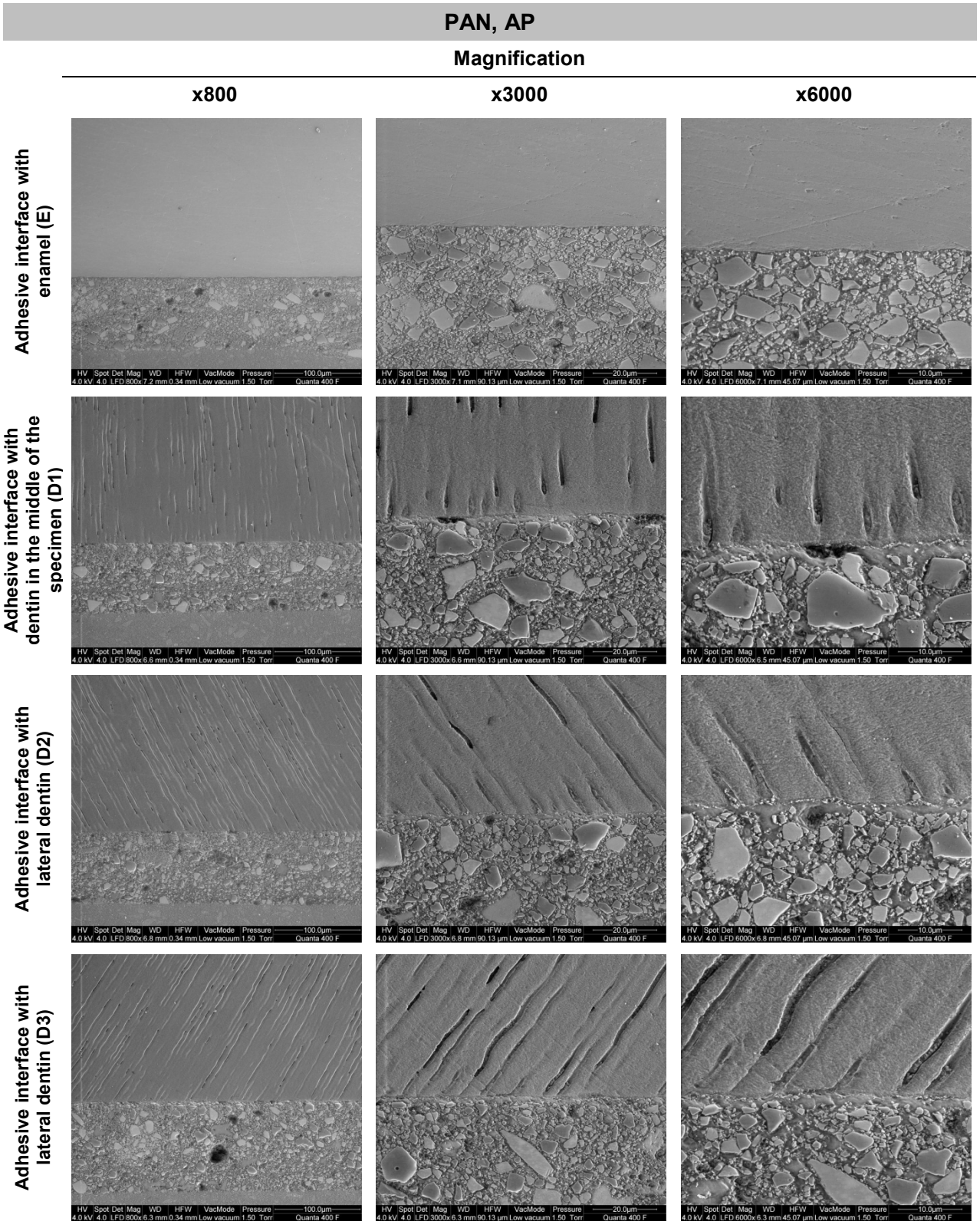


Fig. 9 Adhesive interface of the PAN, AP polished specimen No. S07. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

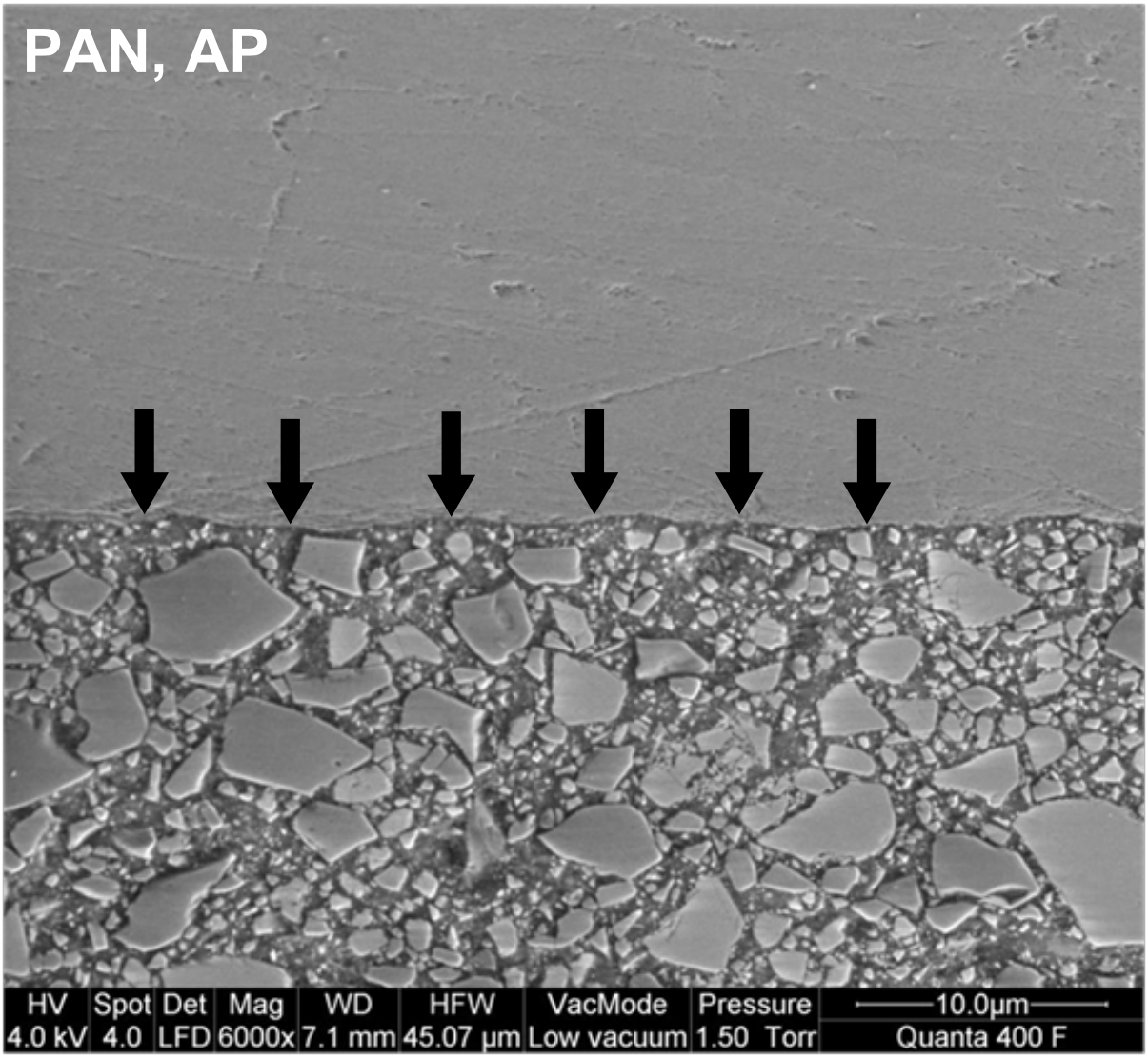


Fig. 9-a Characteristics of PAN, AP: enlarged depiction of the E image at x6000 original magnification from Fig. 9. In none of PAN, AP specimens could the microgaps be observed along the adhesive interface. The integrity of enamel-luting agent adhesive interface was perfect (black arrows) although no deeper interaction or etching pattern could be revealed.

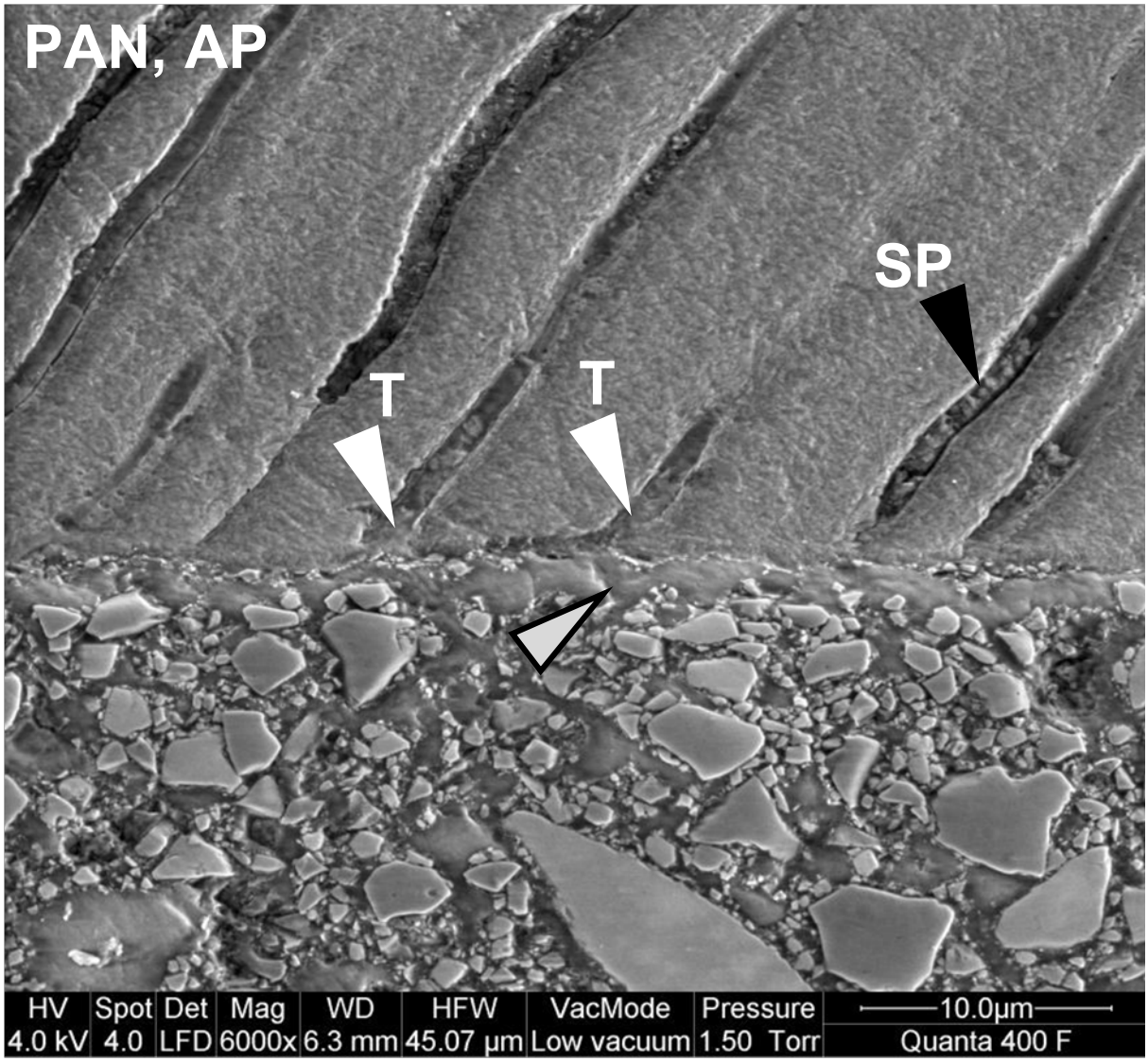


Fig. 9-b Characteristics of PAN, AP: enlarged depiction of the D3 image at x6000 magnification from Fig. 9. Tags (T; white arrow head), smear plugs (SP; black arrow head) and the layer of primer/adhesive mixed with composite resin (grey arrow head).

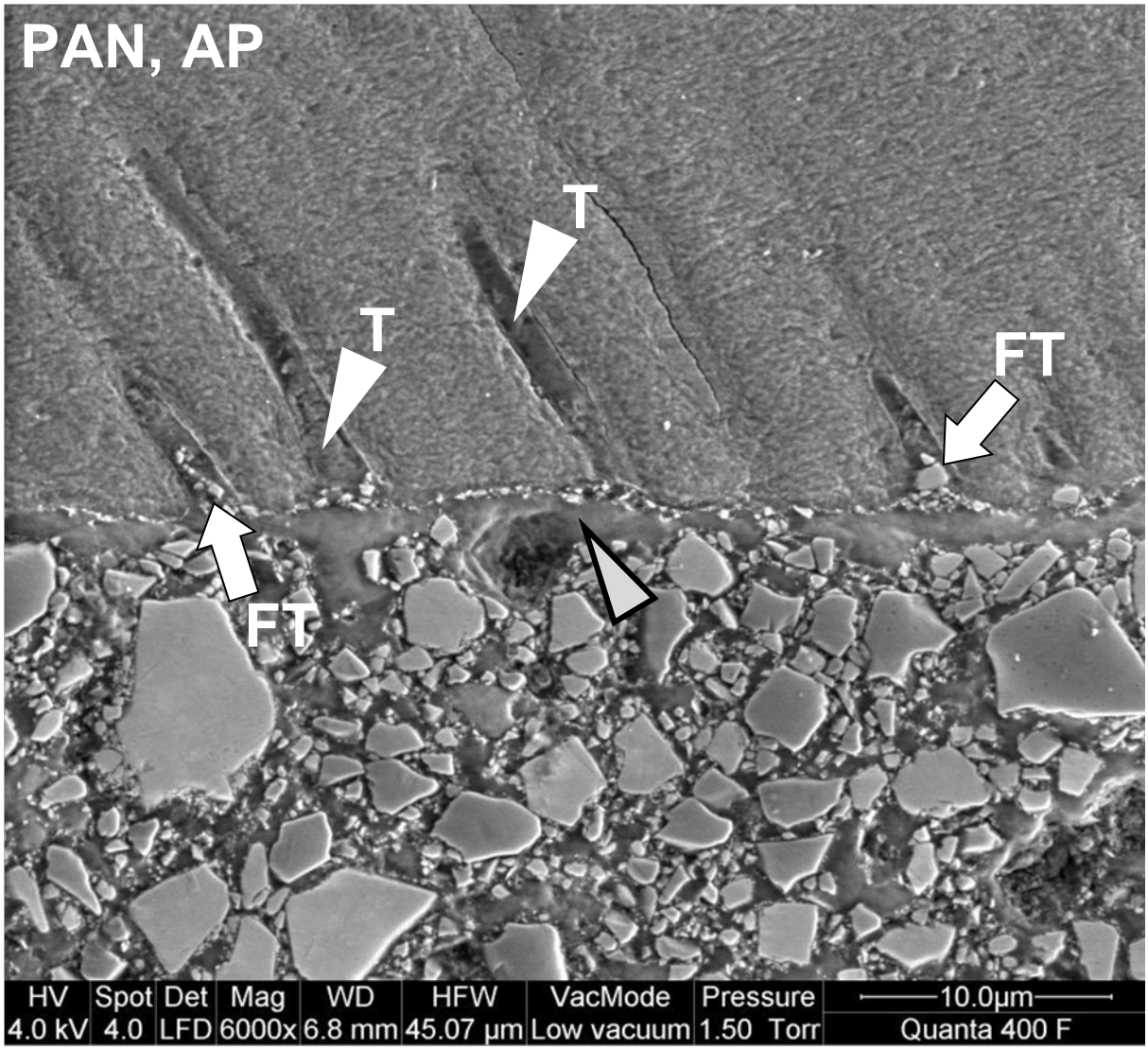


Fig. 9-c Characteristics of PAN, AP: enlarged depiction of the D2 image at x6000 magnification from Fig. 9. The resin tags in dentinal tubules (T; white arrow heads), filler reinforced tags (FT; white arrow) and the layer of ED primer mixed with resin composite (grey arrow head).

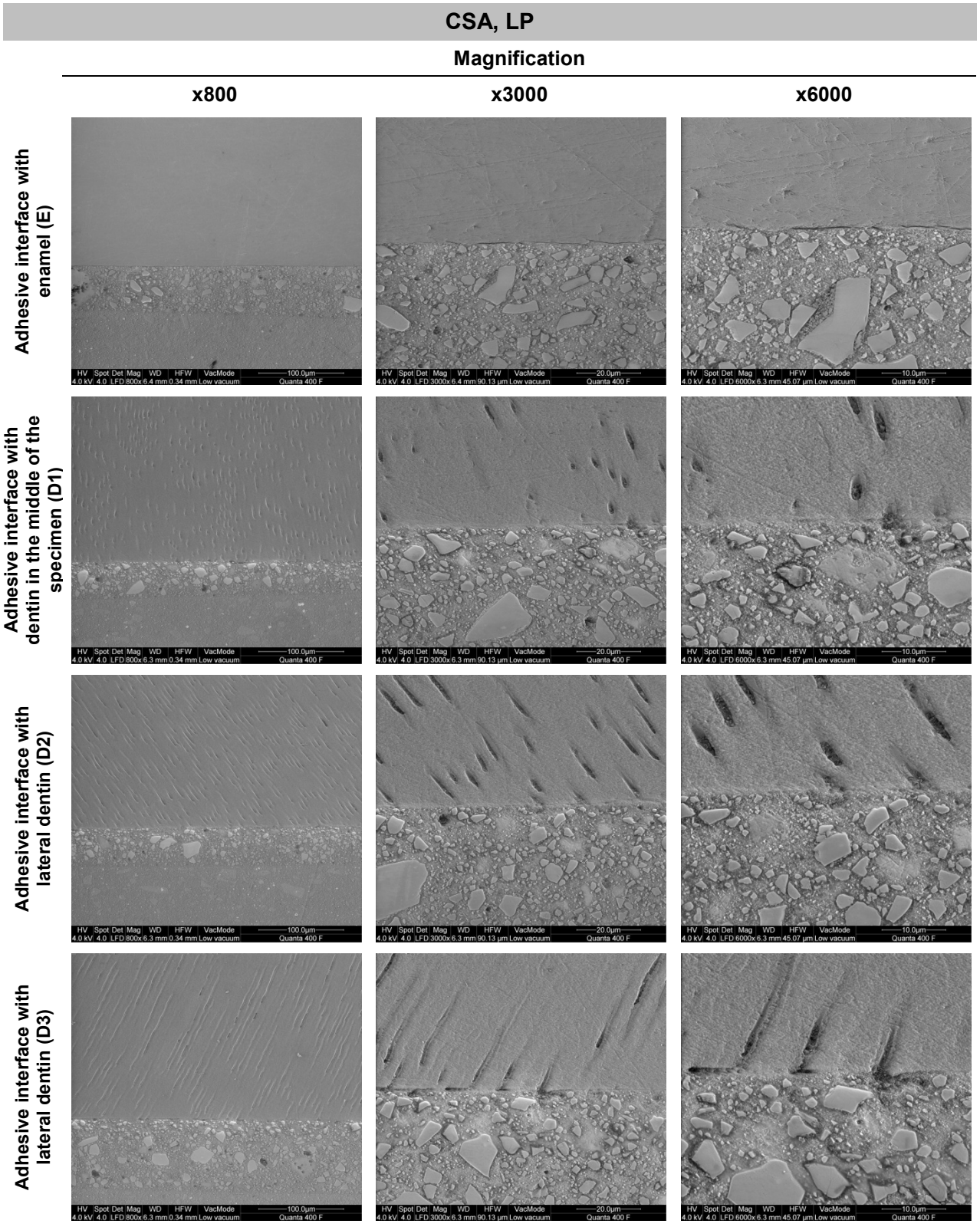


Fig. 10 Adhesive interface of the CSA, LP polished specimen No. S10. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

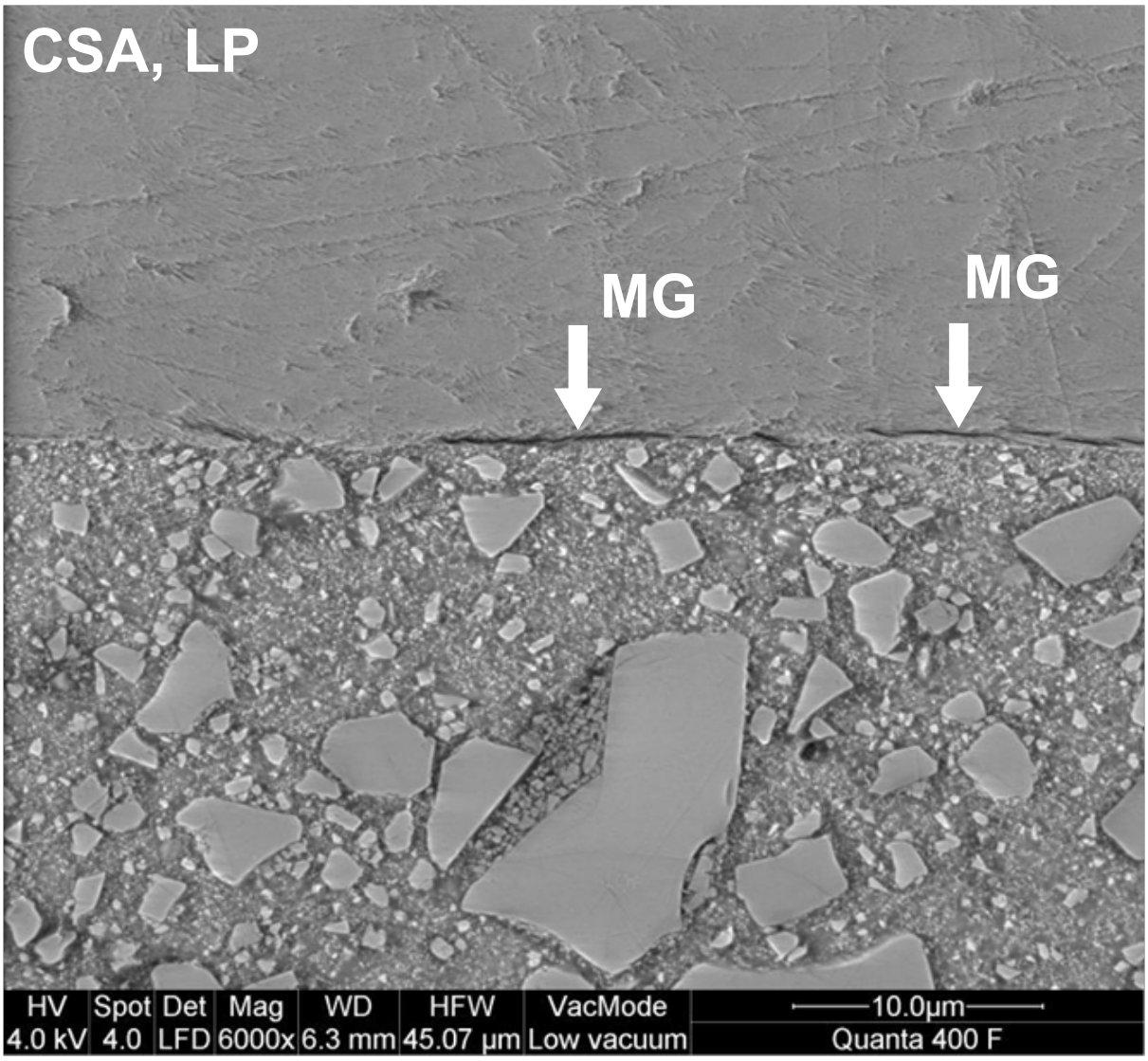


Fig. 10-a Characteristics of CSA, LP: enlarged depiction of the E image at x6000 magnification from Fig. 10. The microgaps (MG; white arrows) proceeded mostly adhesively but not along entire adhesive interface.

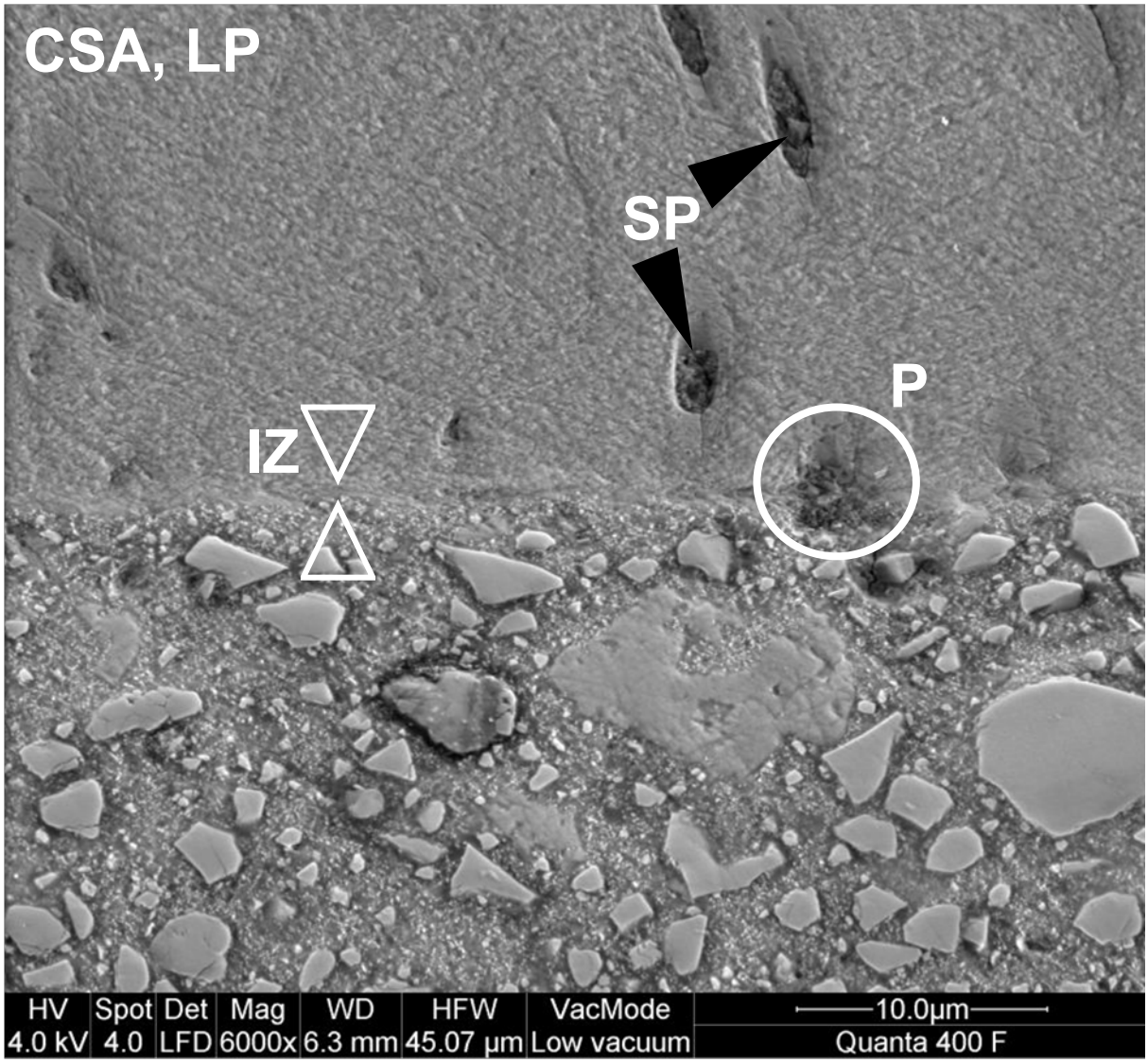


Fig. 10-b Characteristics of CSA, LP: enlarged depiction of the D1 image at x6000 magnification from Fig. 10. The thin resin-dentin interdiffusion zone could be barely seen (IZ; arrow heads). Typical pore at the orifice of dentinal tubule (P; in the circle) and smear plugs (SP; black arrow heads).

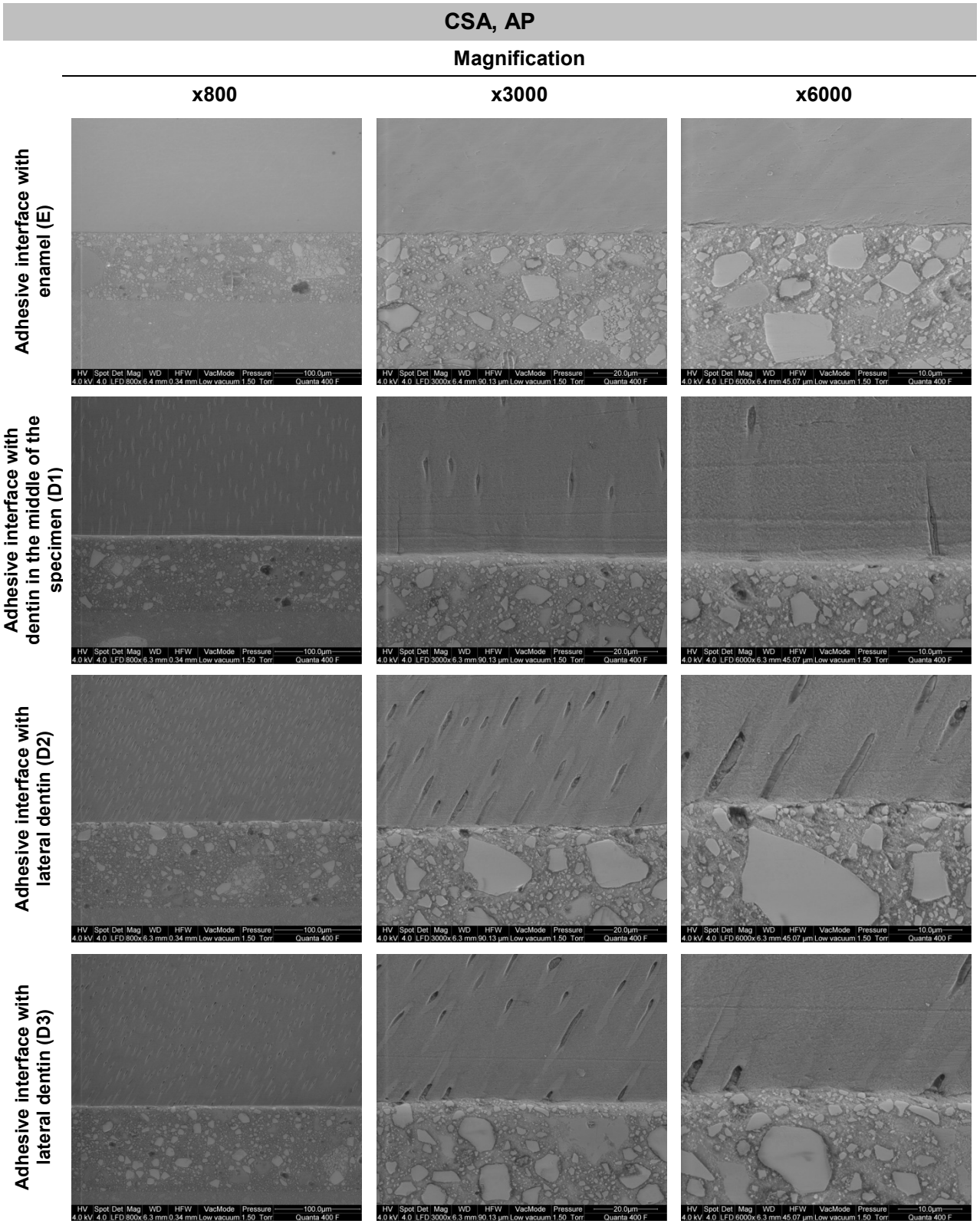


Fig. 11 Adhesive interface of the CSA, AP polished specimen No. S02. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

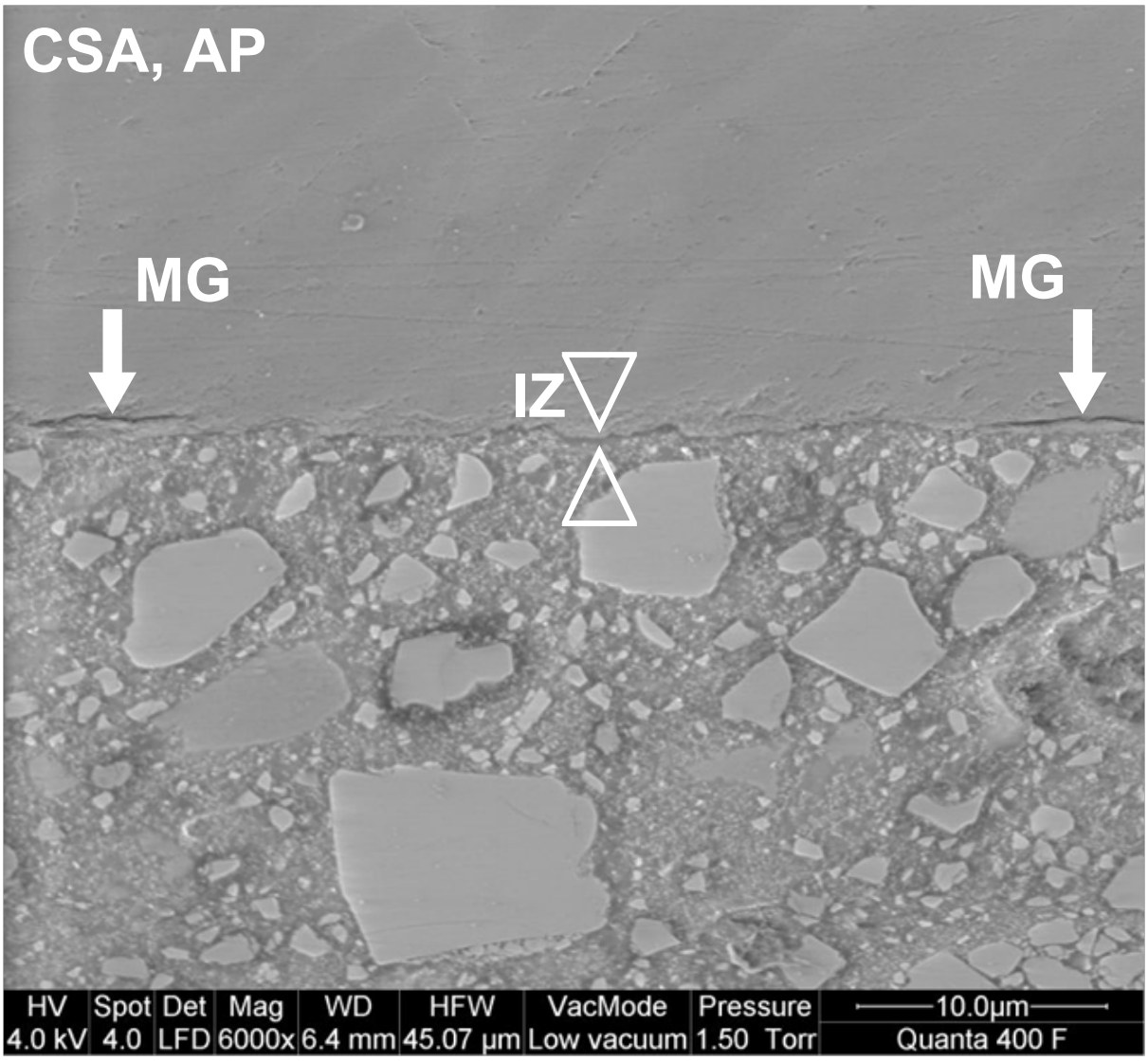


Fig. 11-a Characteristics of CSA, AP: enlarged depiction of the E image at x6000 original magnification from Fig. 11. Here the observed microgaps (MG; white arrows) proceeded cohesively in the enamel near to the adhesive interface, however, the interdiffusion zone is noticeable (IZ; arrow heads).

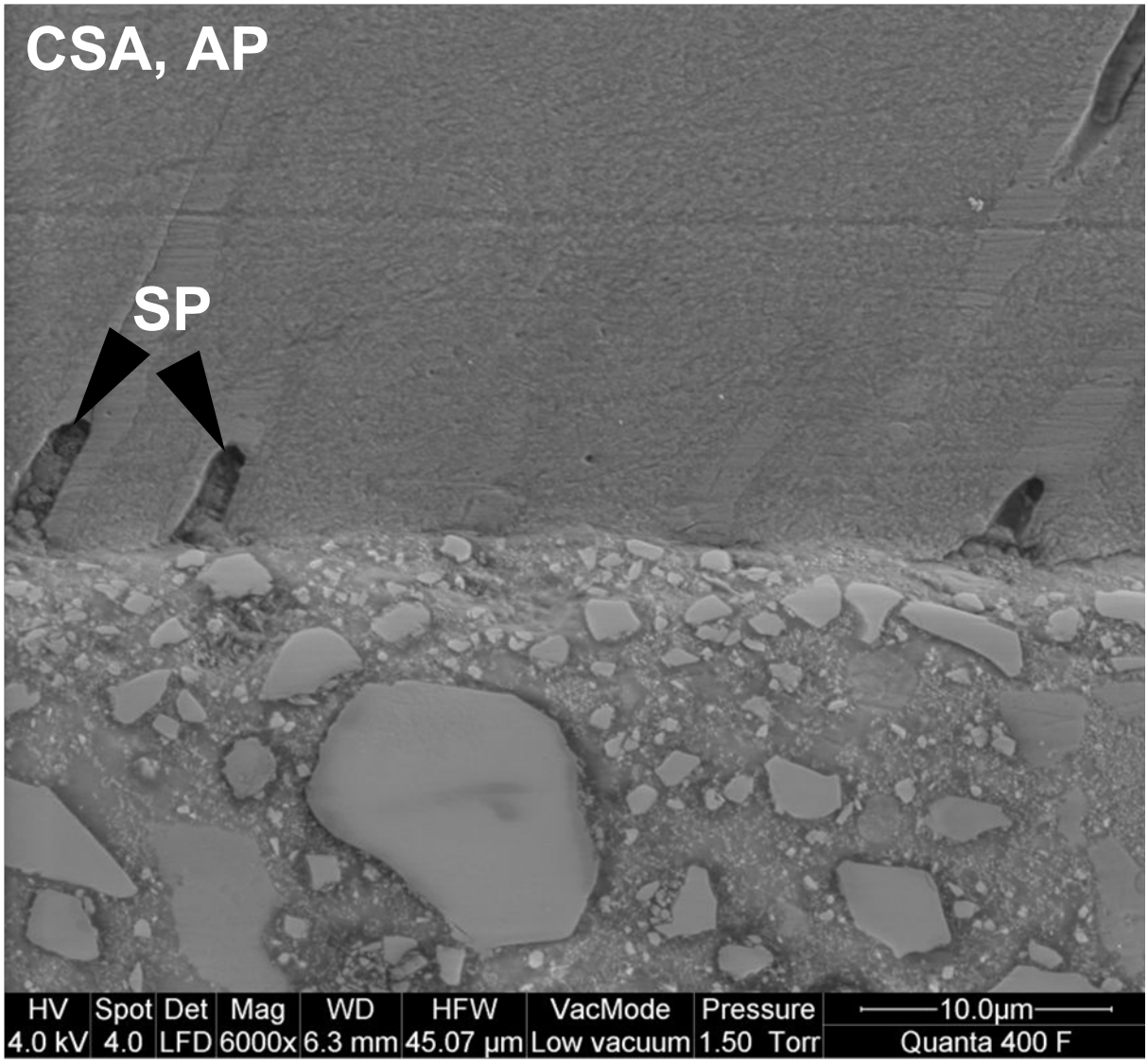


Fig. 11-b Characteristics of CSA, AP: enlarged depiction of the D3 image at x6000 magnification from Fig. 11. No interdiffusion zone could be observed. Smear plugs (SP; black arrow heads) in dentinal could be distinguished.

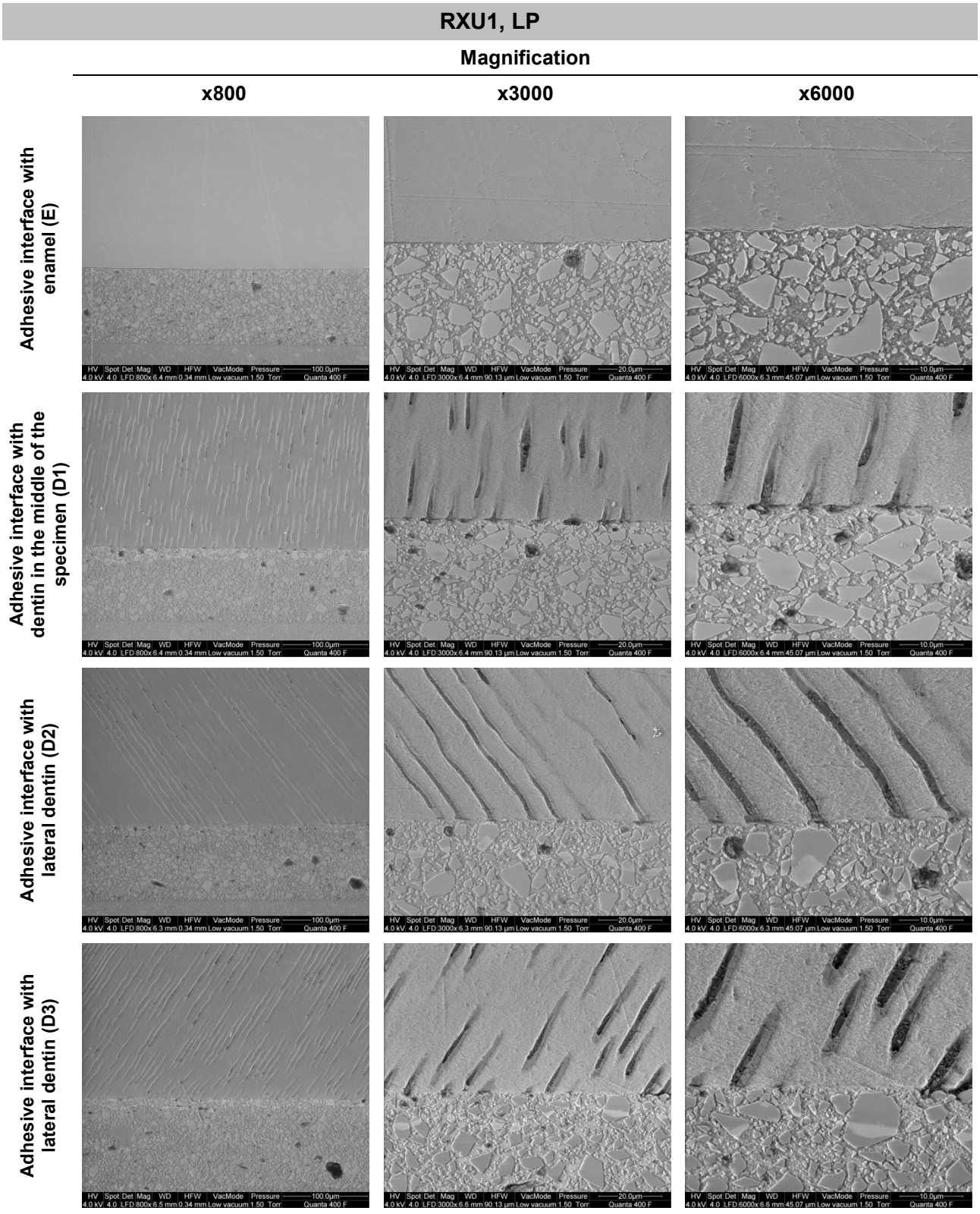


Fig. 12 Adhesive interface of the RXU1, LP polished specimen No. S09. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

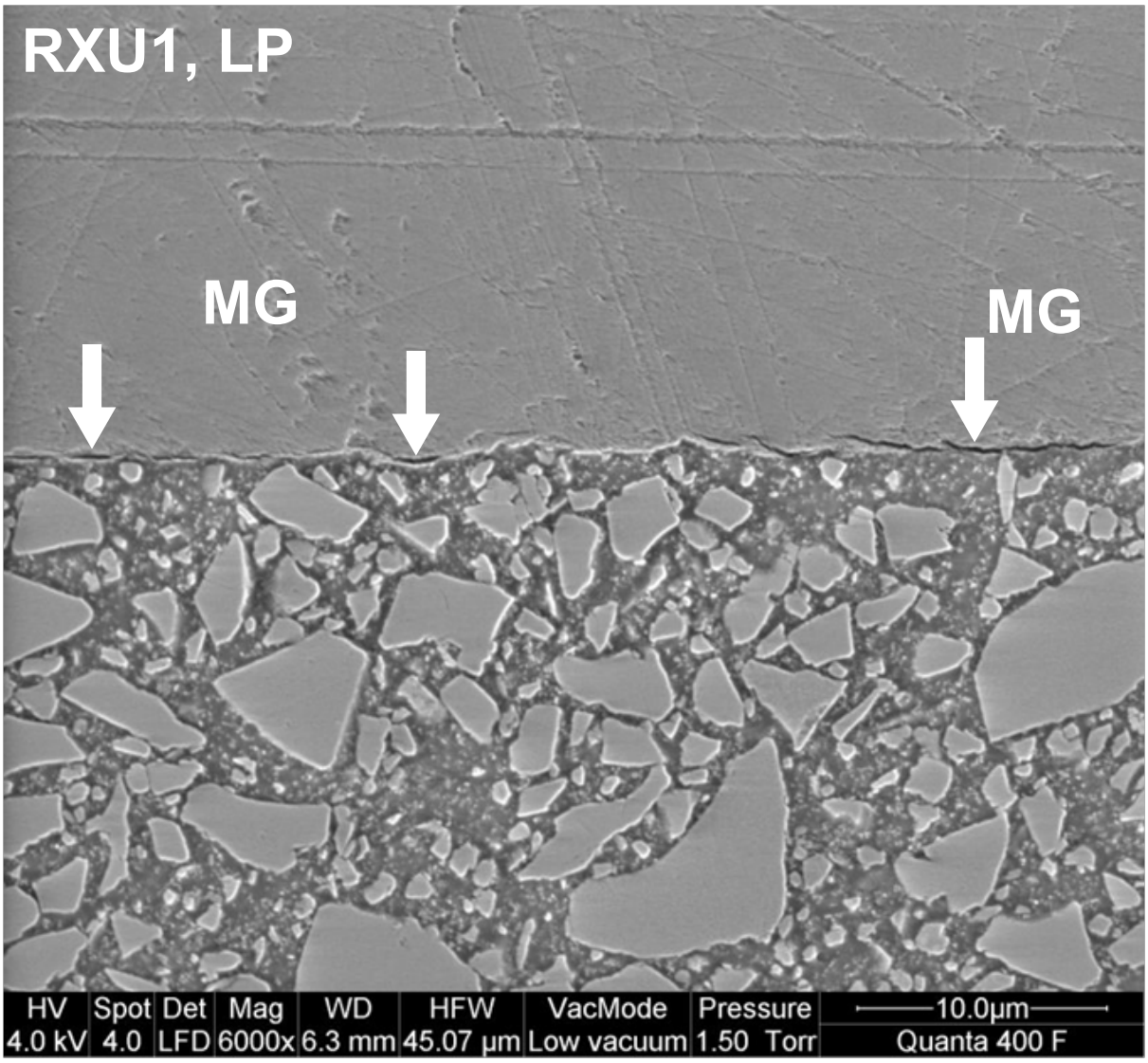


Fig. 12-a Characteristics of RXU1, LP: enlarged depiction of the E image at x6000 original magnification from Fig. 12. The microgaps (MG; white arrows) proceeded mostly adhesively.

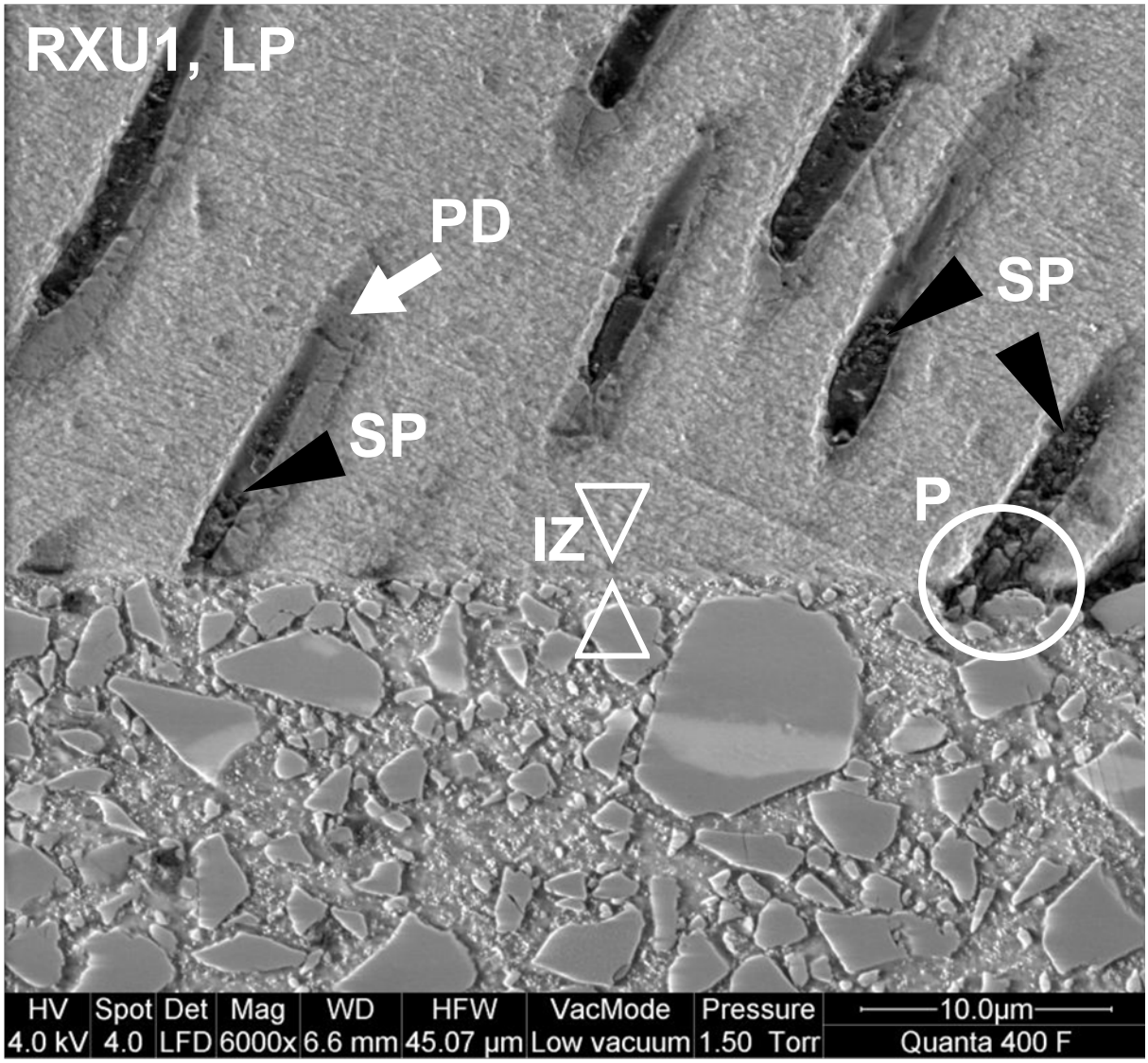


Fig. 12-b Characteristics of RXU1, LP: enlarged depiction of the D3 image at x6000 magnification from Fig. 12. Very thin but clearly detectable interdiffusion zone (IZ; arrow heads) and smear plugs filling dentinal tubules (SP; black arrow heads). At the orifices of dentinal tubules pores could be observed also (P; in the circle). Very distinct peritubular dentin (PD; white arrow) was observed in this specimen.

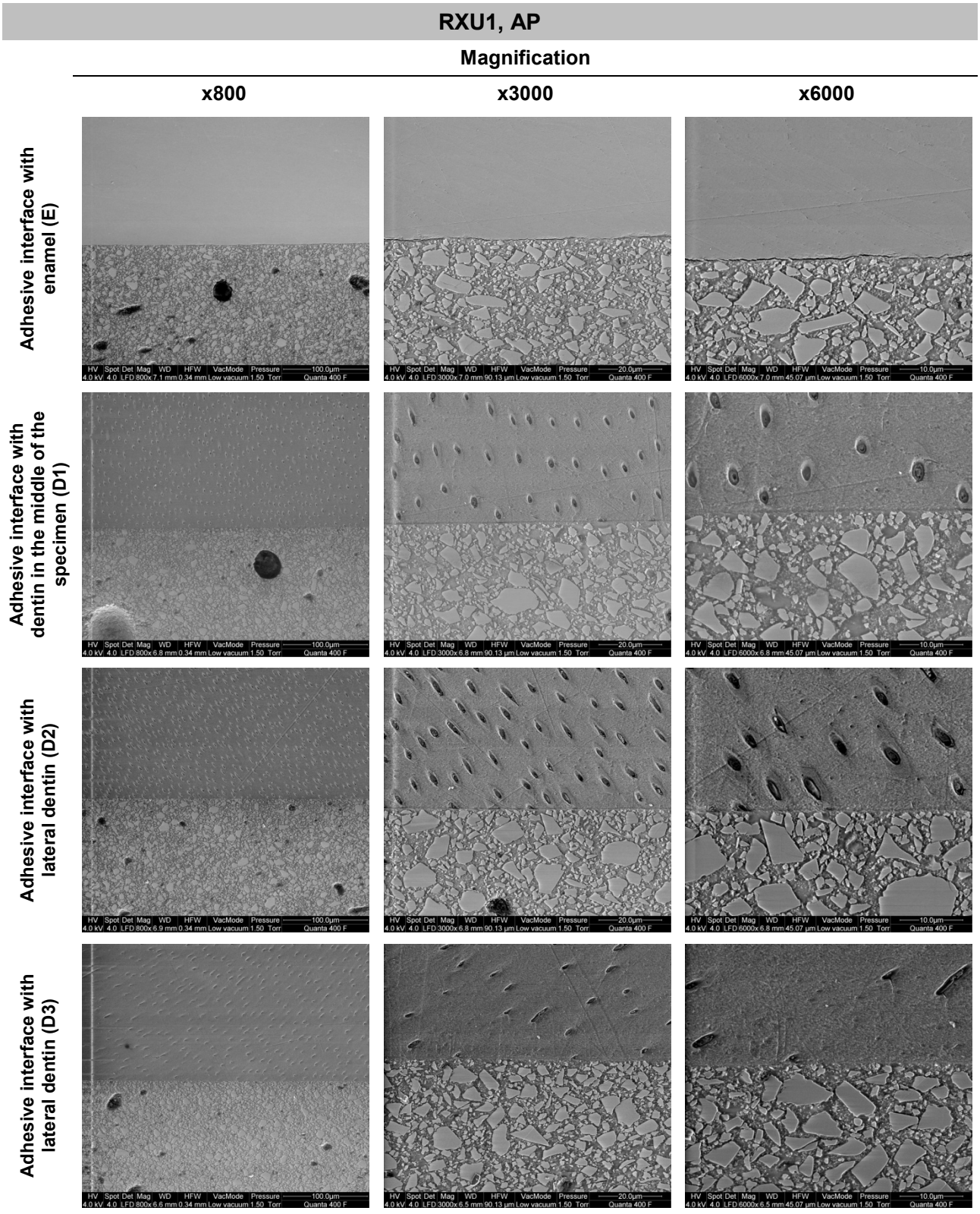


Fig. 13 Adhesive interface of the RXU1, AP polished specimen No. S04. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

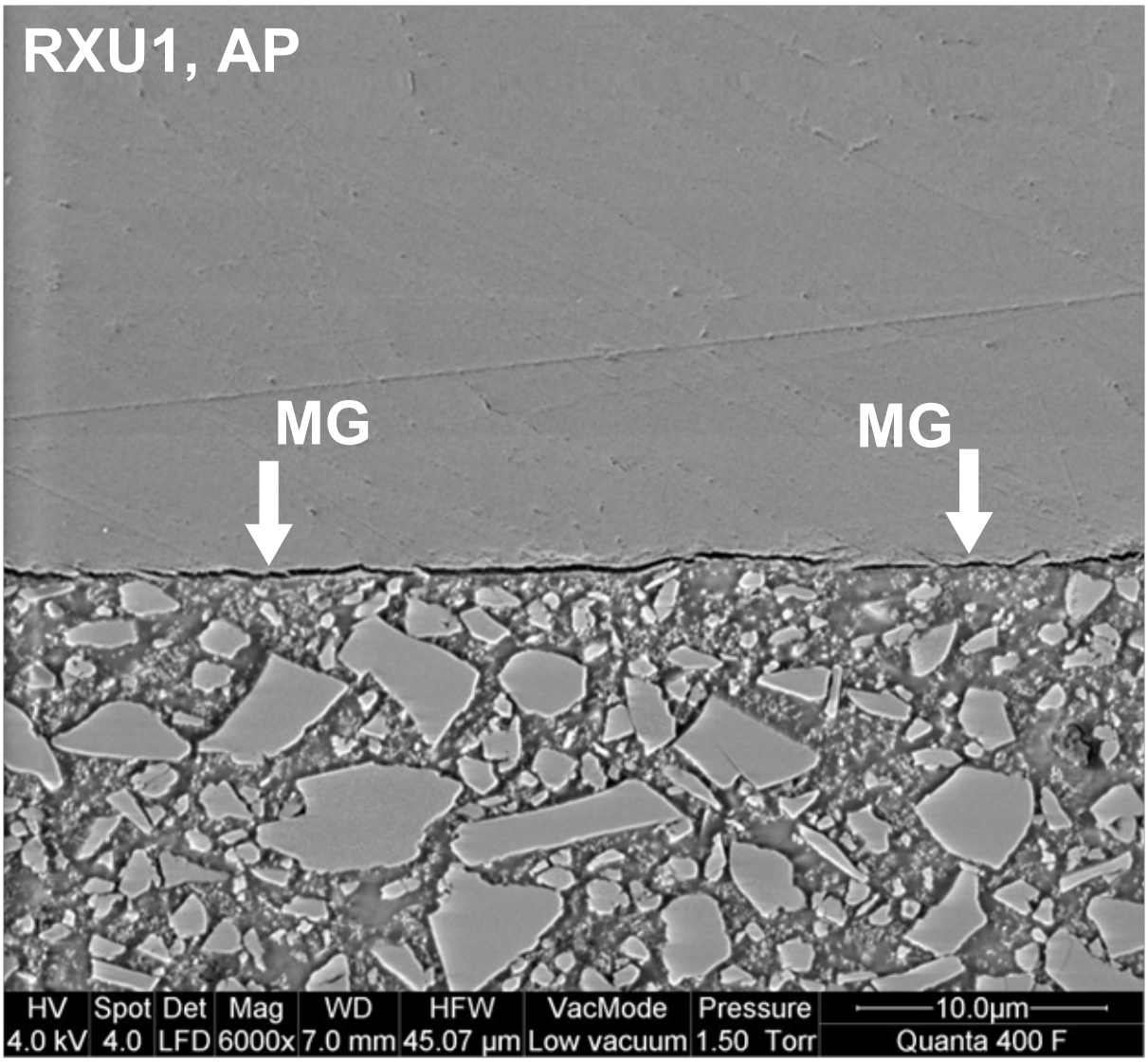


Fig. 13-a Characteristics of RXU1, AP: enlarged depiction of the E image at x6000 original magnification from Fig. 13. The microgaps (MG; white arrows) proceeded adhesively along almost entire adhesive interface.

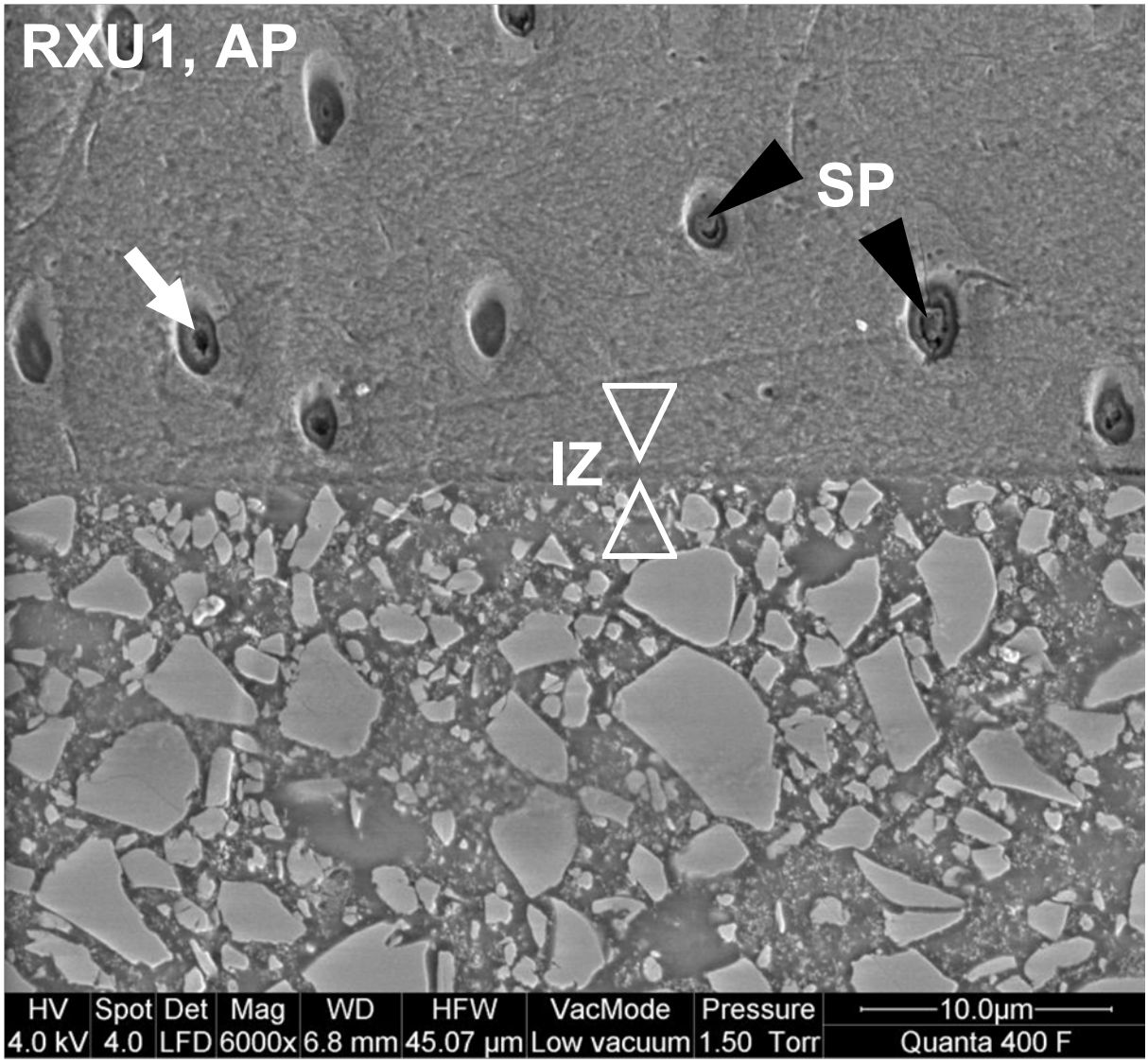


Fig. 13-b Characteristics of RXU1, AP: enlarged depiction of the D1 image at x6000 magnification from Fig. 13. The interdiffusion zone (IZ; arrow heads) is noticeable. Note the structures in dentinal tubules - the sheath covering dentinal tubules from inside (white arrow) could be *lamina limitans*, occluded with smear plugs or polishing debris (SP; black arrow heads). However, differentiation of the *lamina limitans* from tags in this case is difficult due to the cut being almost perpendicular to dentinal tubules.

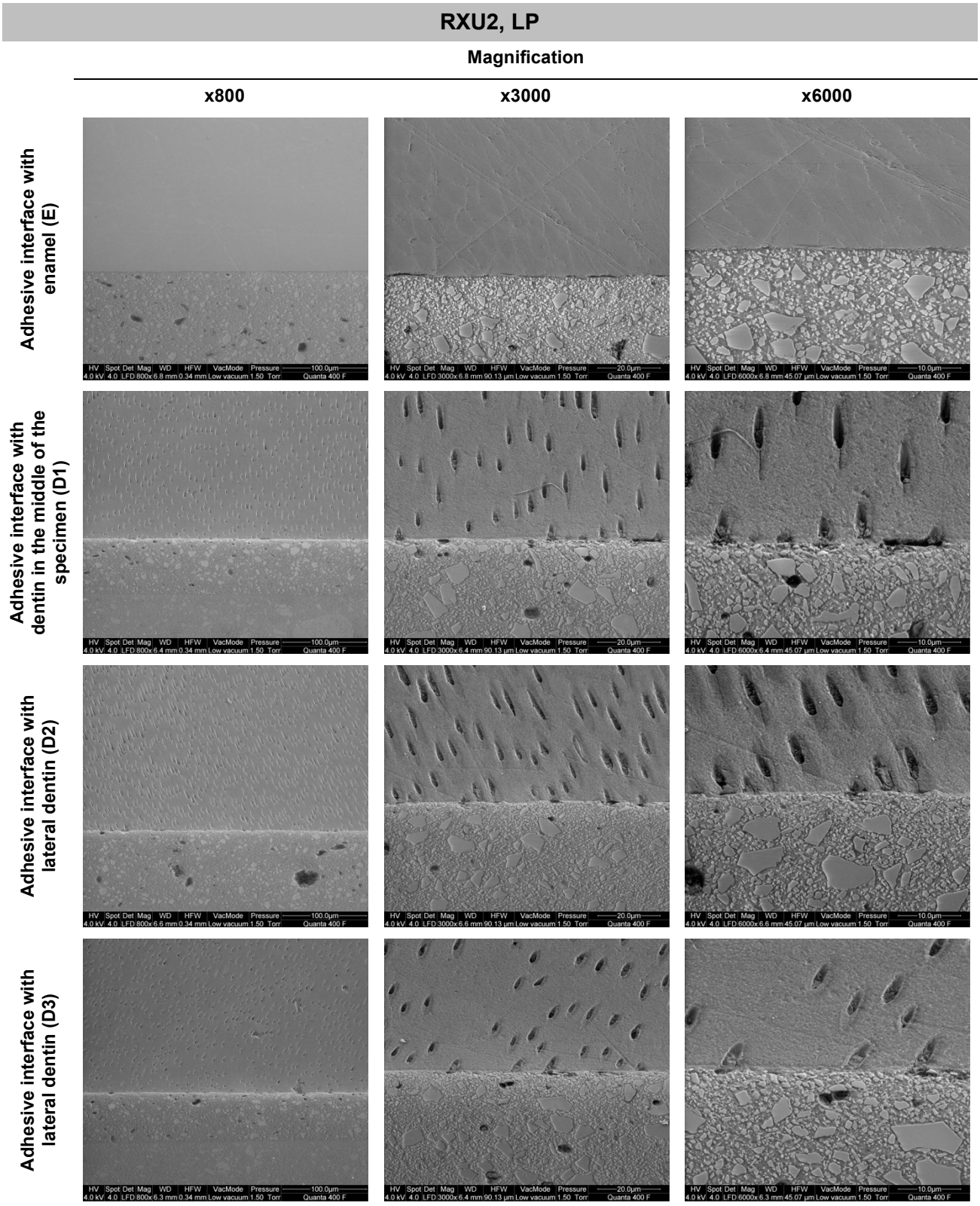


Fig. 14 Adhesive interface of the RXU2, LP polished specimen No.S09. The horizontal rows indicate locations on the specimen and the columns represent the different original magnifications of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

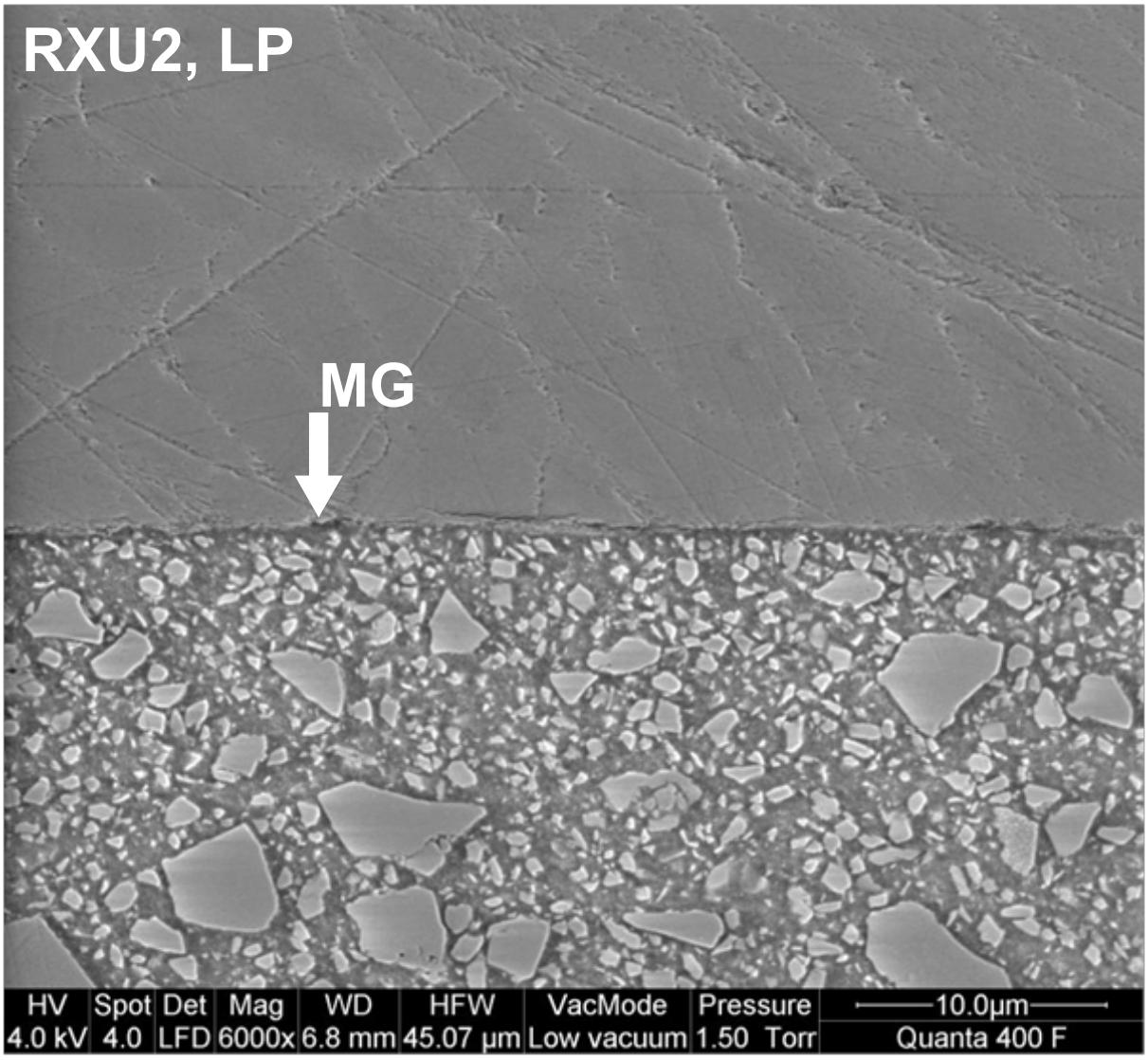


Fig. 14-a Characteristics of RXU2, LP: enlarged depiction of the E image at x6000 original magnification from Fig. 14. Only in one place the microgap (MG; white arrow) appeared in this SEM image.

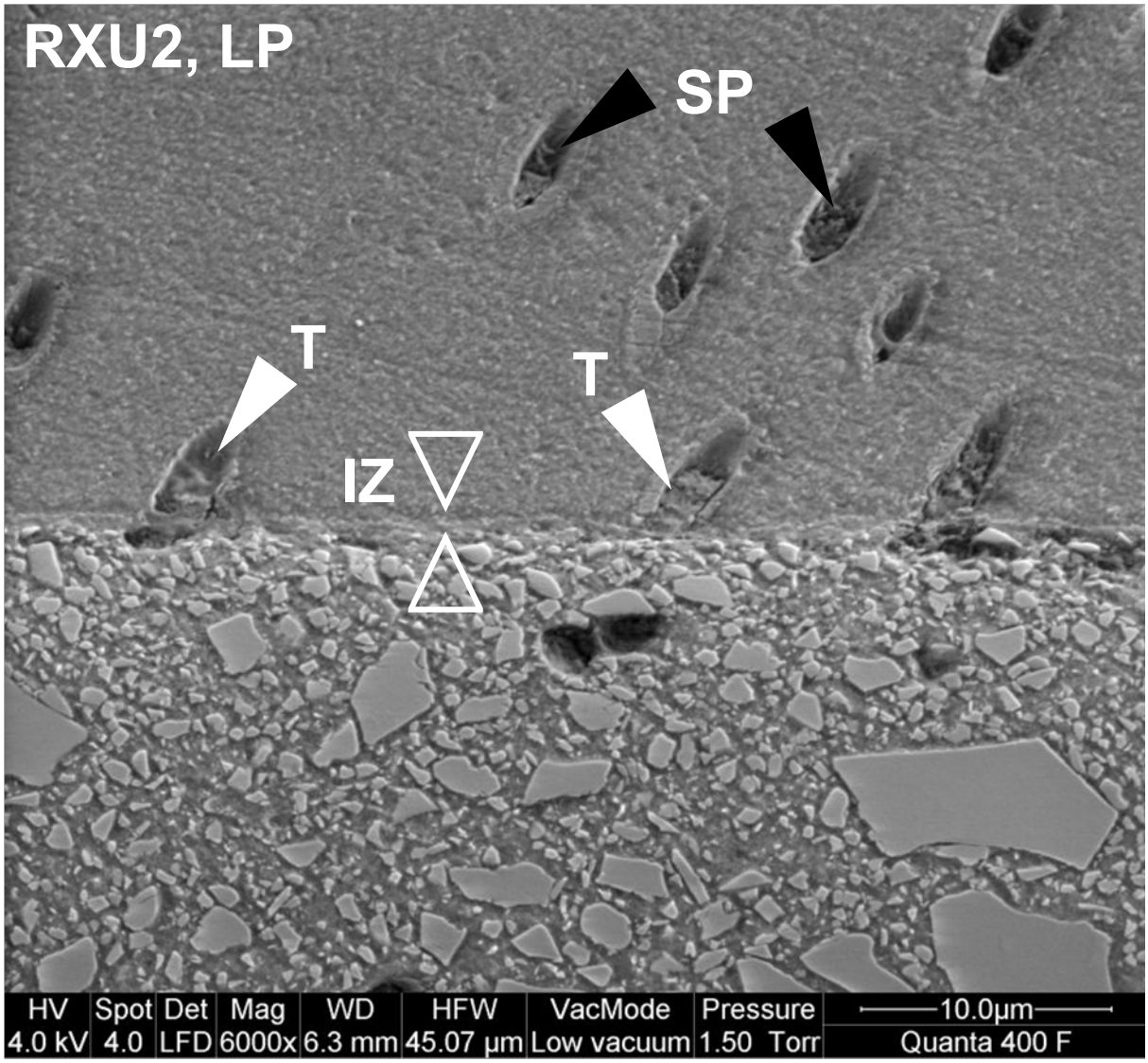


Fig. 14-b Characteristics of RXU2, LP: enlarged depiction of the D3 image at x6000 magnification from Fig. 14. The resin matrix of RXU2 is infiltrated in the dentinal tubules and reacted but not dissolved the smear plug (SP; black arrow heads) forming tiny resinous tags (T; white arrow heads). The presence of interdiffusion zone (IZ; arrow heads) could be observed.

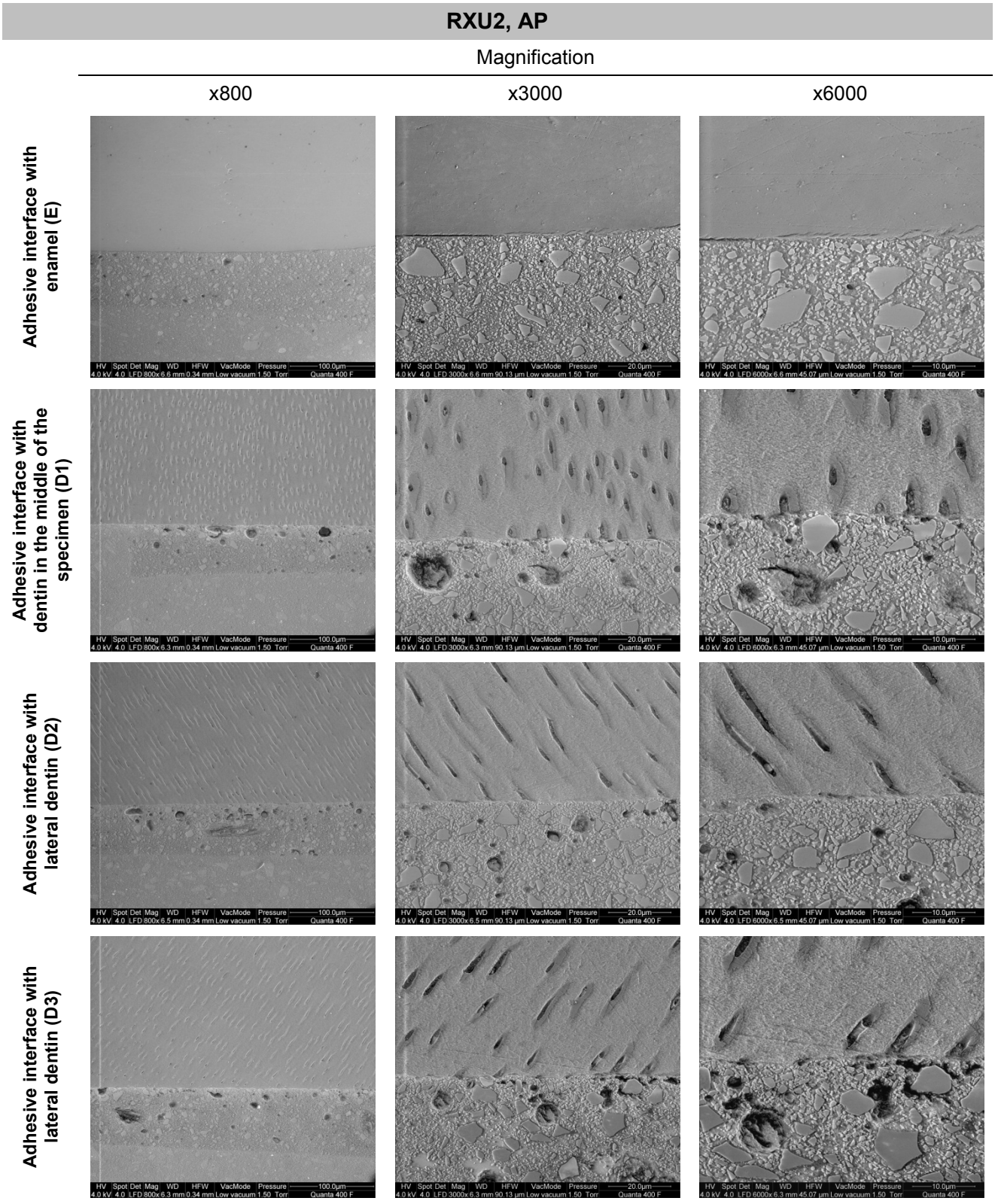


Fig. 15 Adhesive interface of the RXU2, AP polished specimen No. S09. The horizontal rows indicate the locations on the specimen and the columns represent the different original magnification of the SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm.

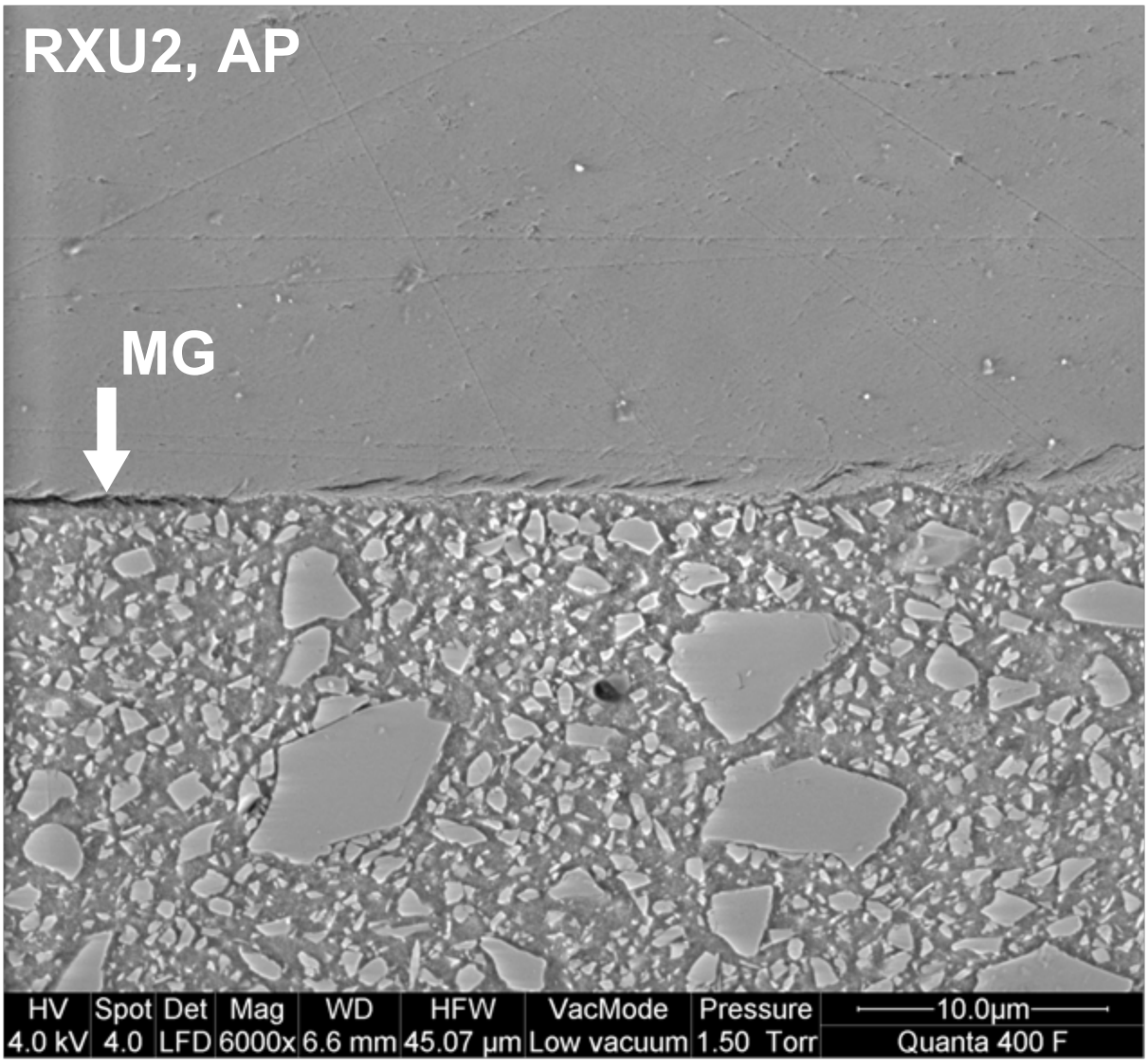


Fig. 15-a Characteristics of RXU2, AP: enlarged depiction of the E image at x6000 original magnification from Fig. 15. Microgap (MG; white arrow) proceeded both adhesively and cohesively within the enamel.

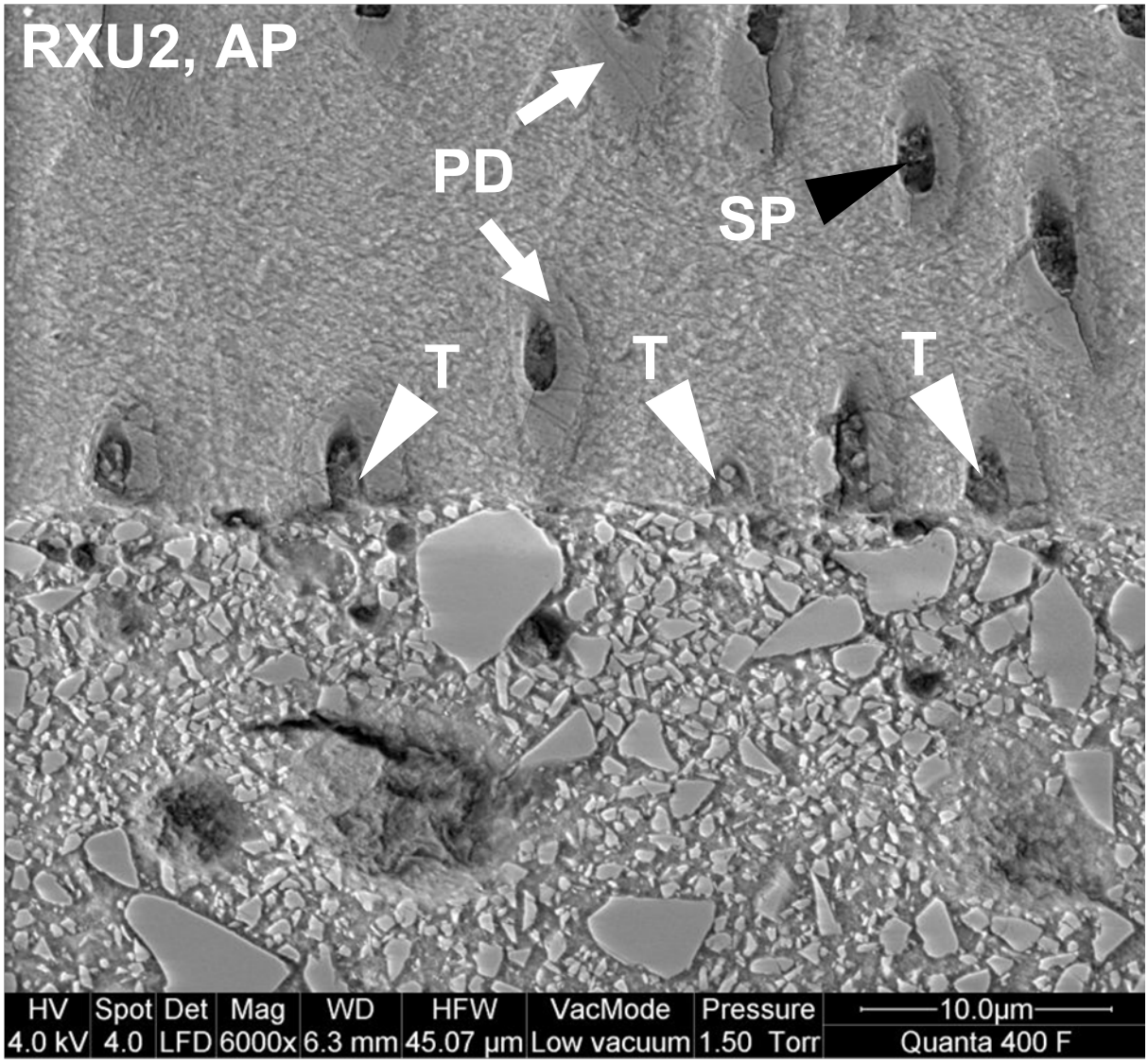


Fig. 15-b Characteristics of RXU2, AP: enlarged depiction of the D1 image at x6000 magnification from Fig. 15. Penetration of RXU2 into dentinal tubules formed tags (T; white arrow heads), the presence of smear plugs (SP; black arrow heads) and peritubular dentin (PD; white arrows) could also be observed.

9.2.2. Semi-quantitative evaluation of dentin-luting agent adhesive interfaces of polished specimens

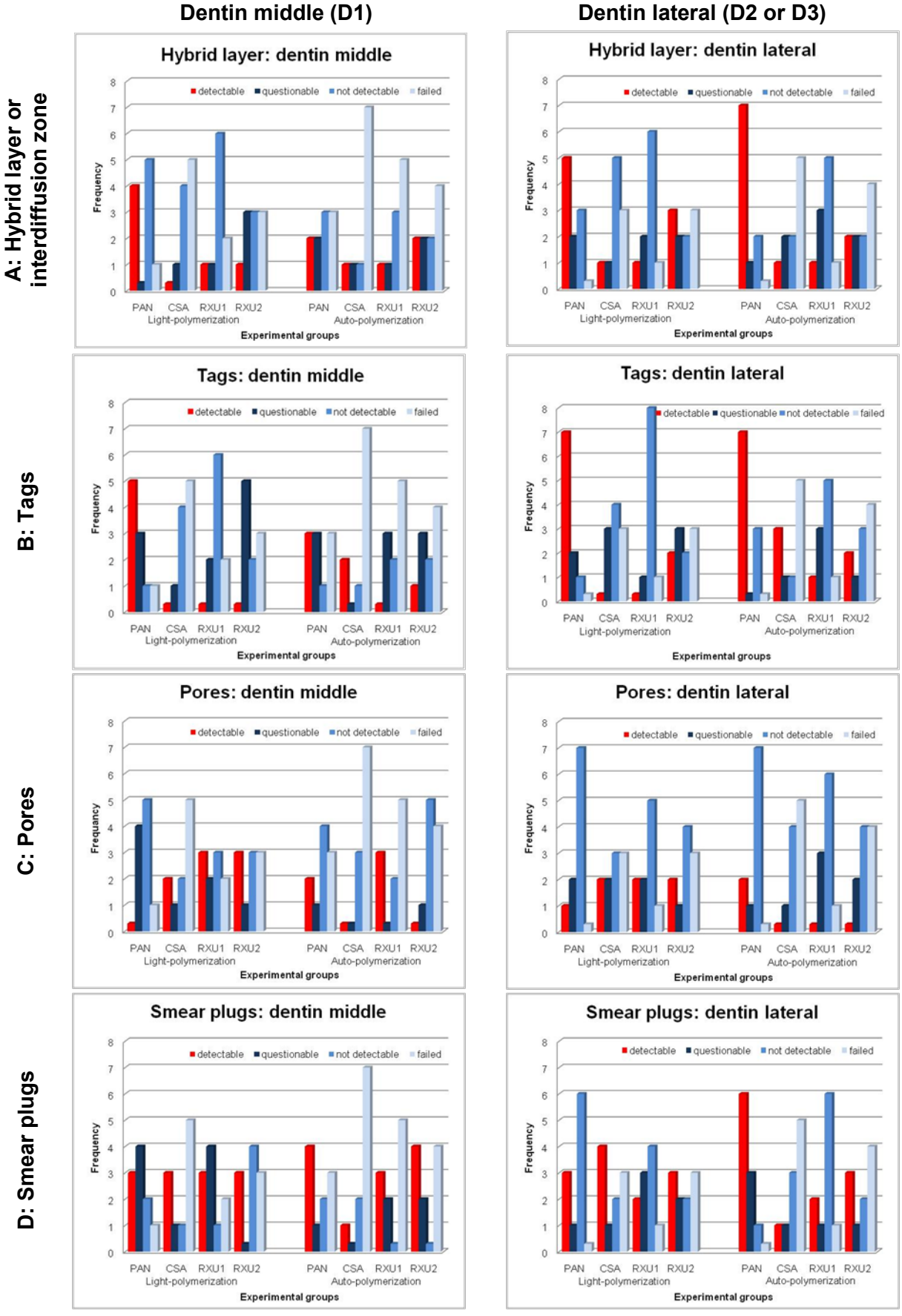


Fig. 16 Graphical depiction of semi-quantitative evaluated criteria (hybrid layer or interdiffusion zone, tags, pores and smear plugs) at the dentin-luting agent adhesive interface of polished specimens.

9.2.3. Qualitative evaluation of adhesive interfaces of demineralized/deproteinized specimens

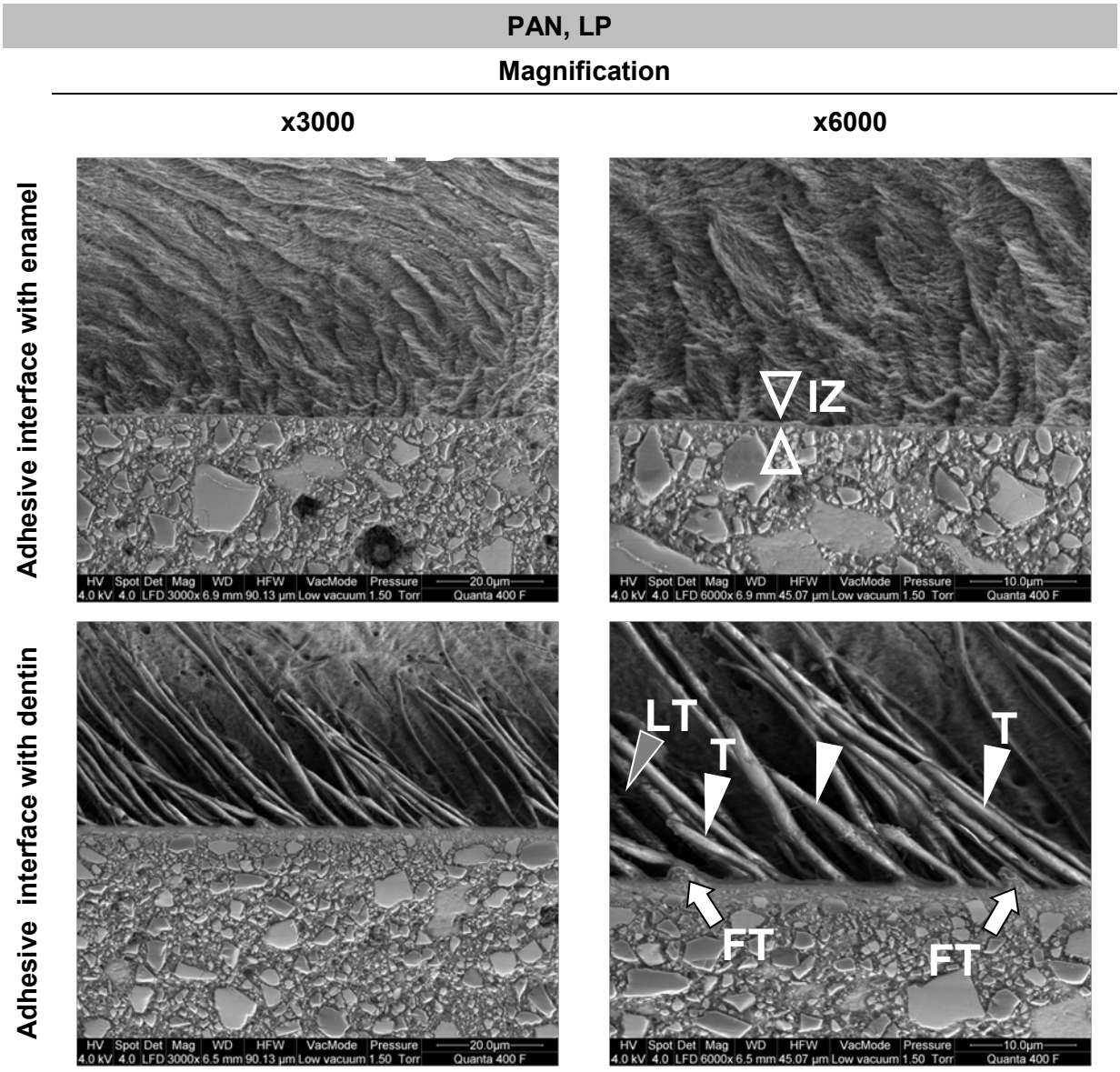


Fig. 17 Etched and deproteinized PAN, LP specimen No. S03. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnifications of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. Typical etched pattern of enamel and dentin, ultrathin interdiffusion zone (IZ; arrow heads) at enamel-luting agent adhesive interface, the appearance of resin tags (T; white arrow heads), lateral tags (LT; grey arrow head) and filler at the base of the tags (FT; white arrows) at the dentin-luting agent interface.

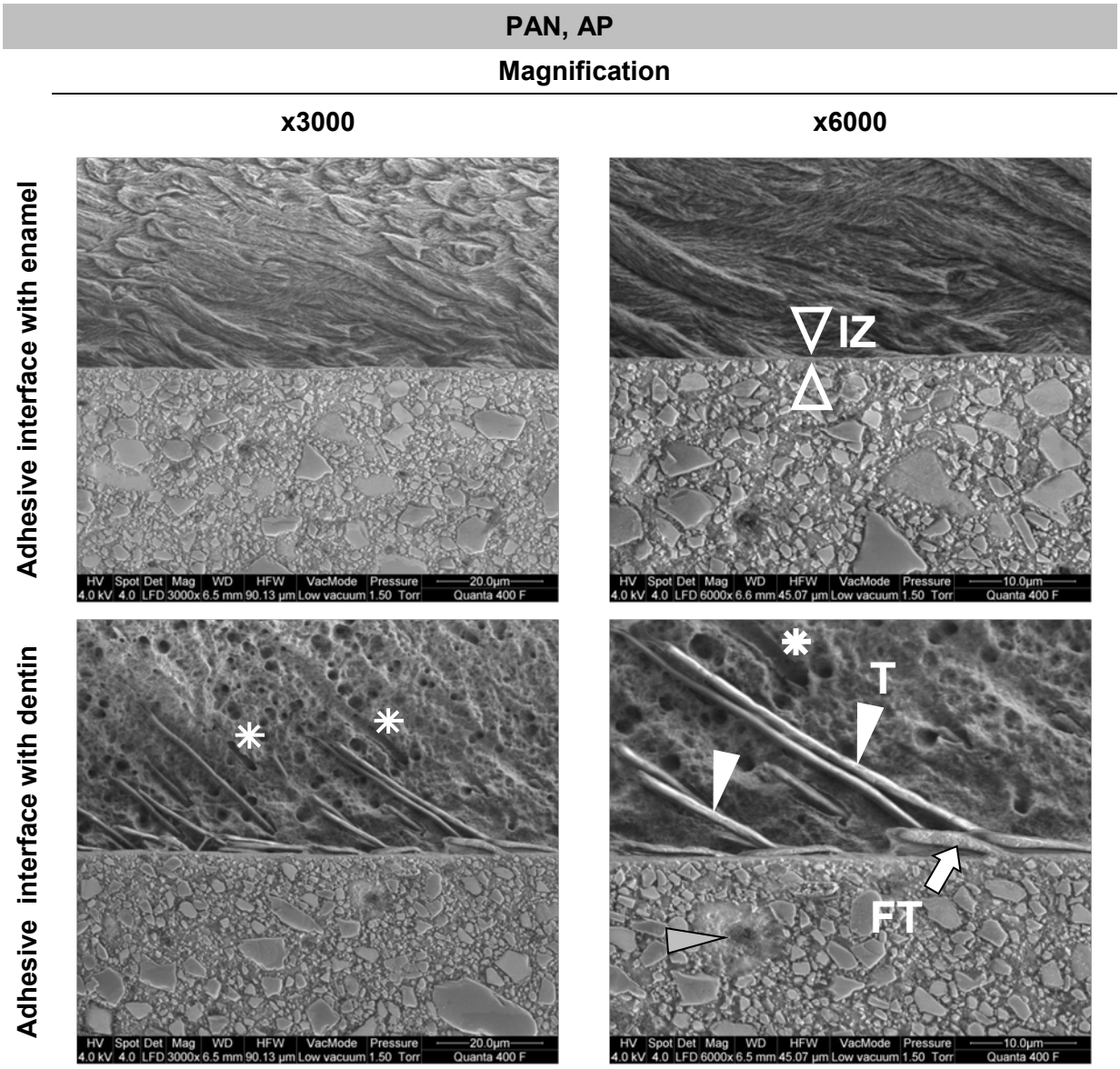


Fig. 18 Etched and deproteinized PAN, AP specimen No. S08. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. Typical etched pattern of enamel and dentin, ultrathin interdiffusion zone (IZ; arrow heads), resin tags (T; white arrow heads) and filler-reinforced resin tags (FT). Asterisks possibly indicate the mineralized *lamina limitans* which lines some dentinal tubules. The gray arrow indicates the specific type of filler in PAN luting agent with porous centre.

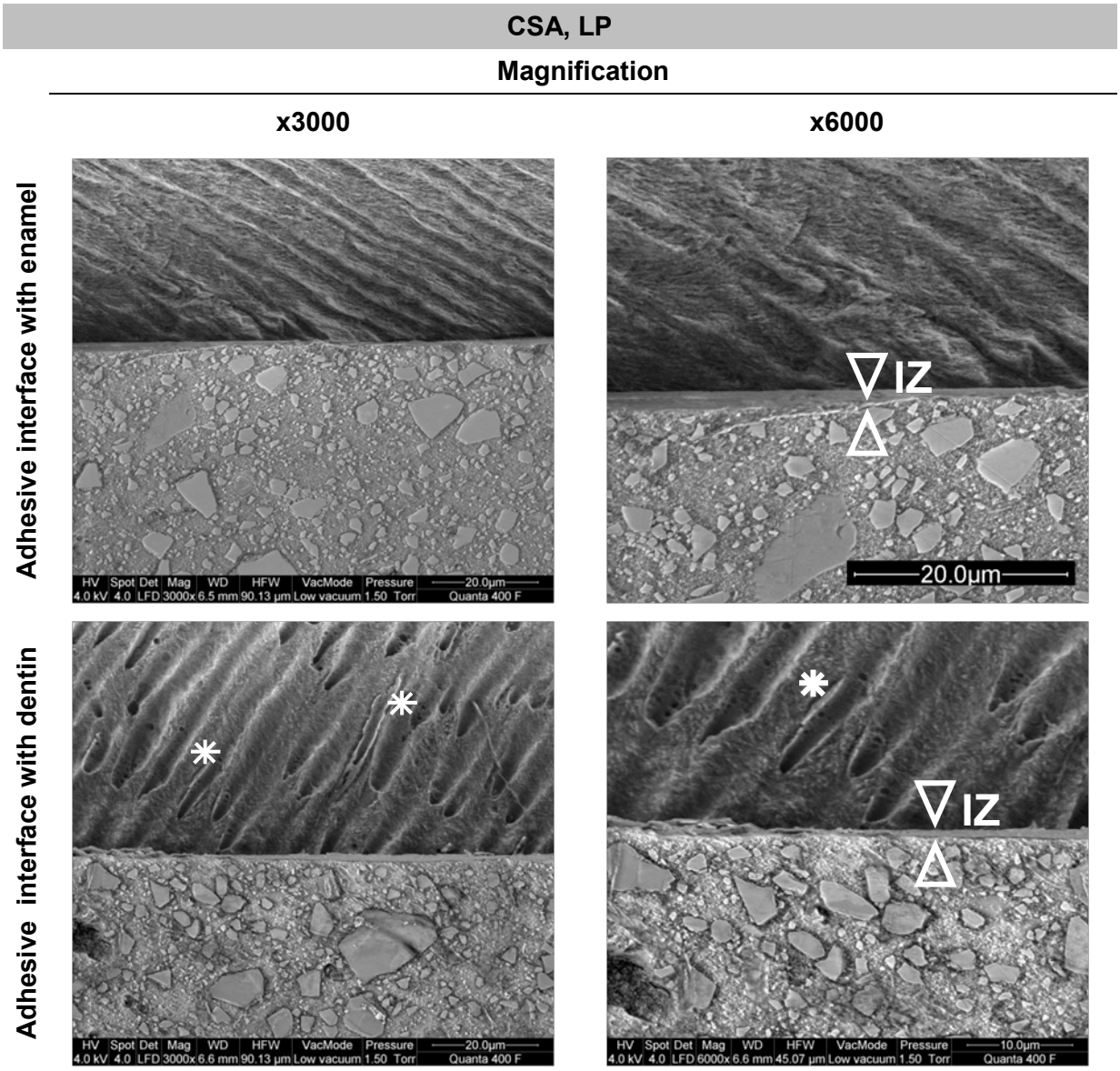


Fig. 19 Etched and deproteinized CSA, LP specimen No. S06. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. The interdiffusion zone (IZ; arrow heads) as a thin film covers the material both at the enamel-resin interface and at dentin-resin adhesive interface. The asterisks indicate the structure which could be mineralized *lamina limitans* occurring sometimes in dentinal tubules.

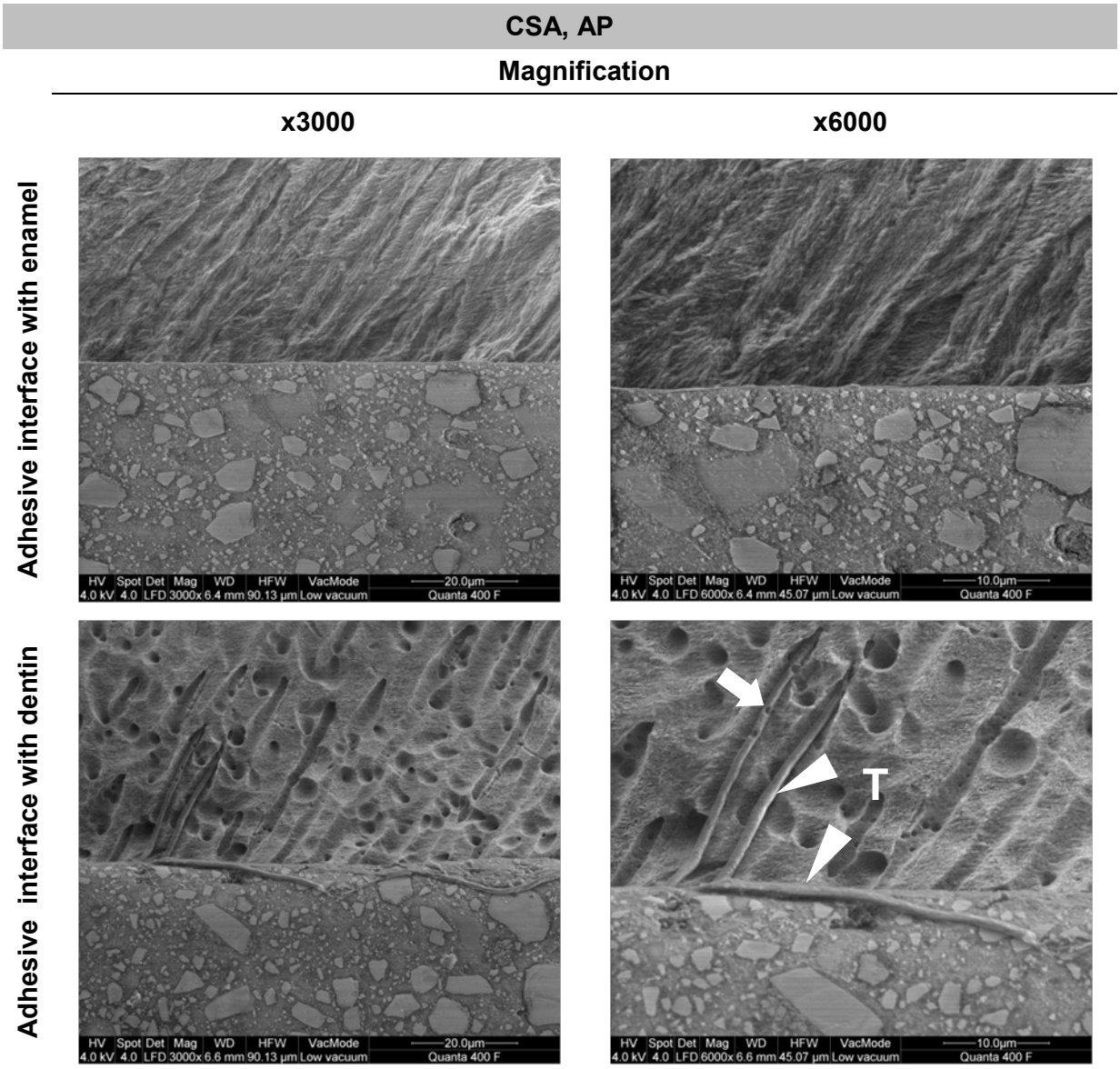


Fig. 20 Etched and deproteinized CSA, AP specimen No. S02. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. No interdiffusion zone could be detected at the enamel-luting agent adhesive interface. The resin tags (T; white arrow heads) were found. Note the porous appearance of the resin tags (white arrow).

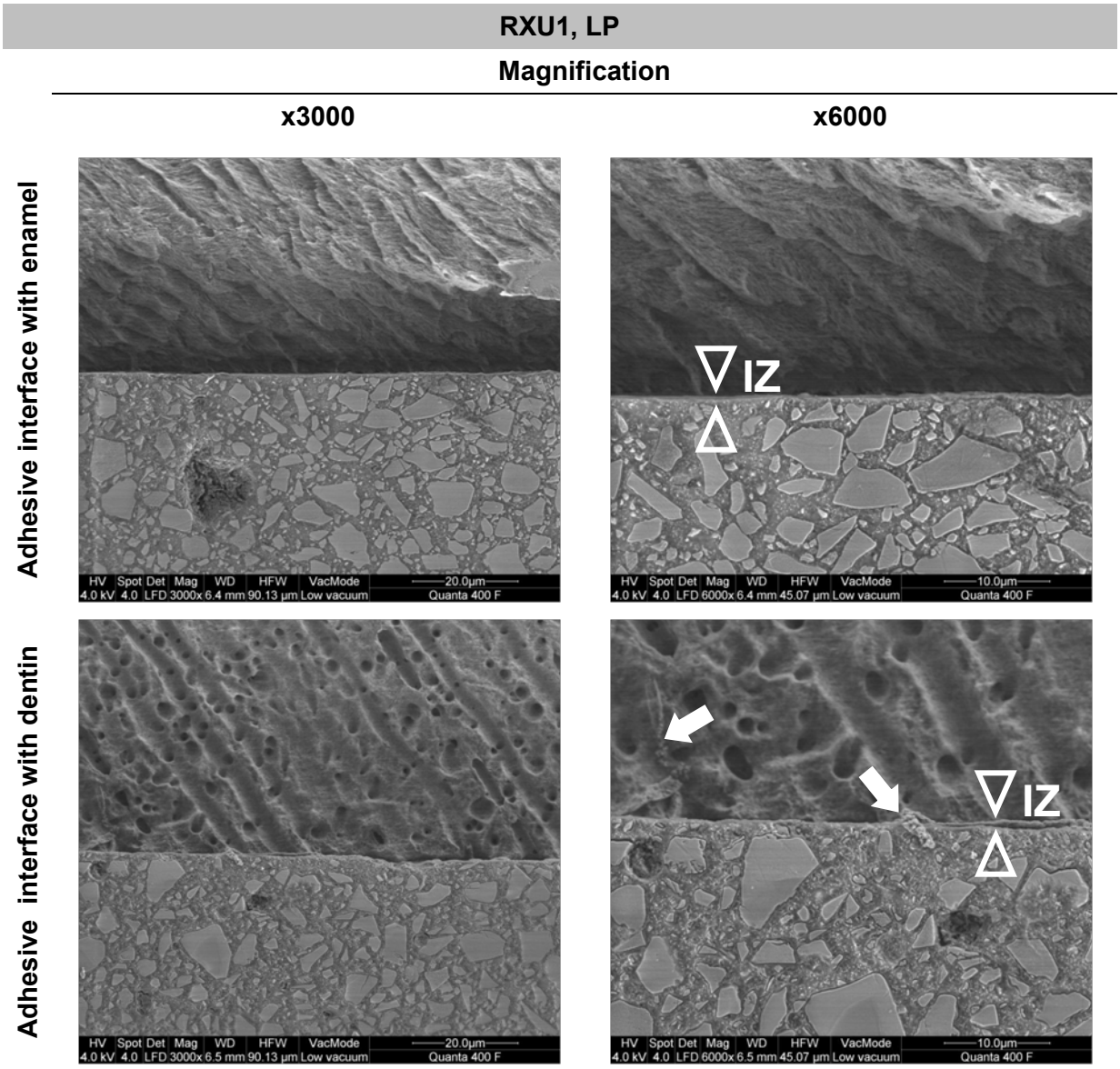


Fig. 21 Etched and deproteinized RXU1, LP specimen No. S09. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. The irregular interdiffusion zone (IZ; arrow heads) could be observed in this specimen. The white arrows indicate the remnants of smear plugs retained on the luting agent or dentin surface.

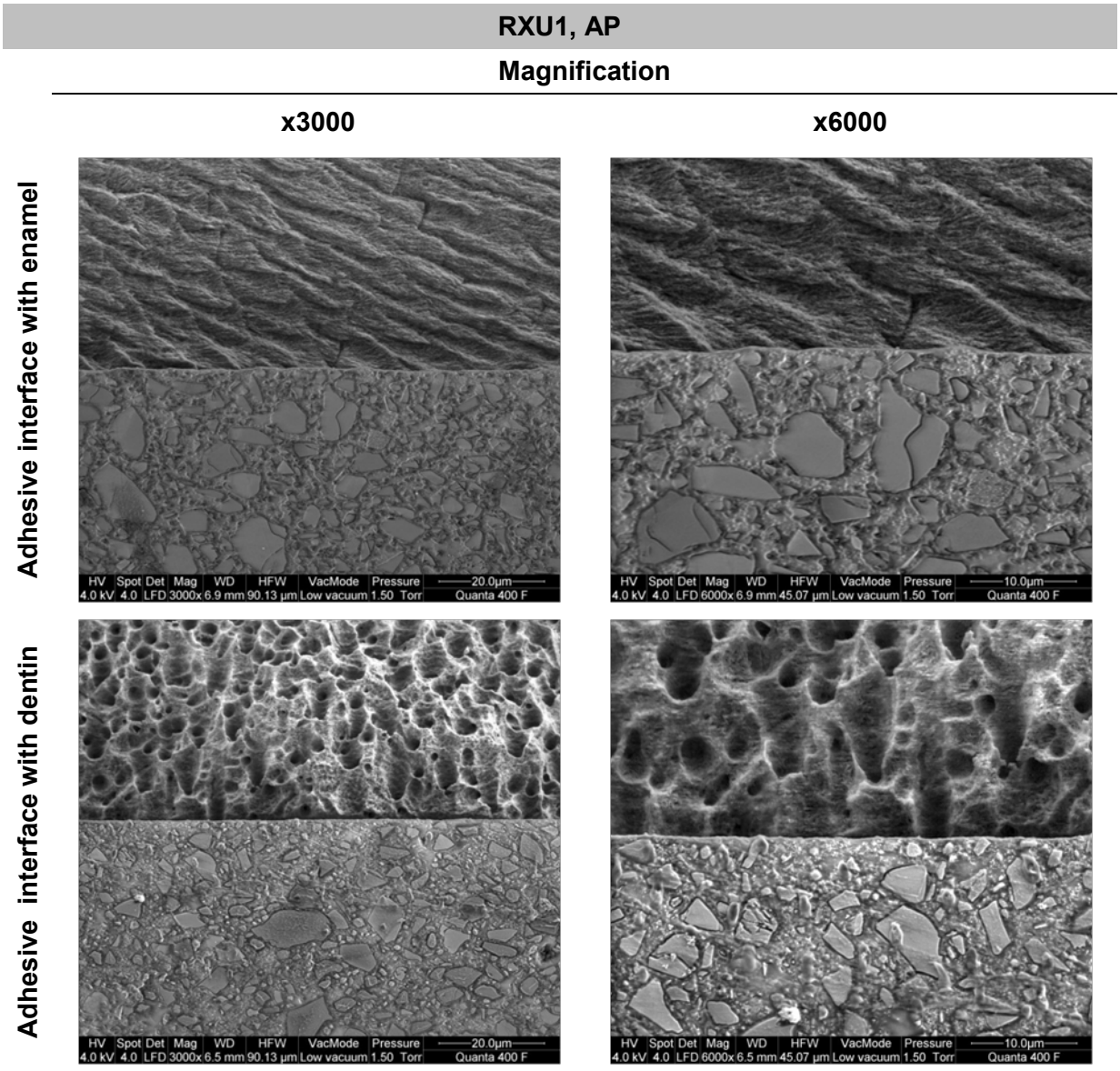


Fig. 22 Etched and deproteinized RXU1, AP specimen No. S04. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. No noticable interdiffusion within tooth hard tissue could be found in this specimen.

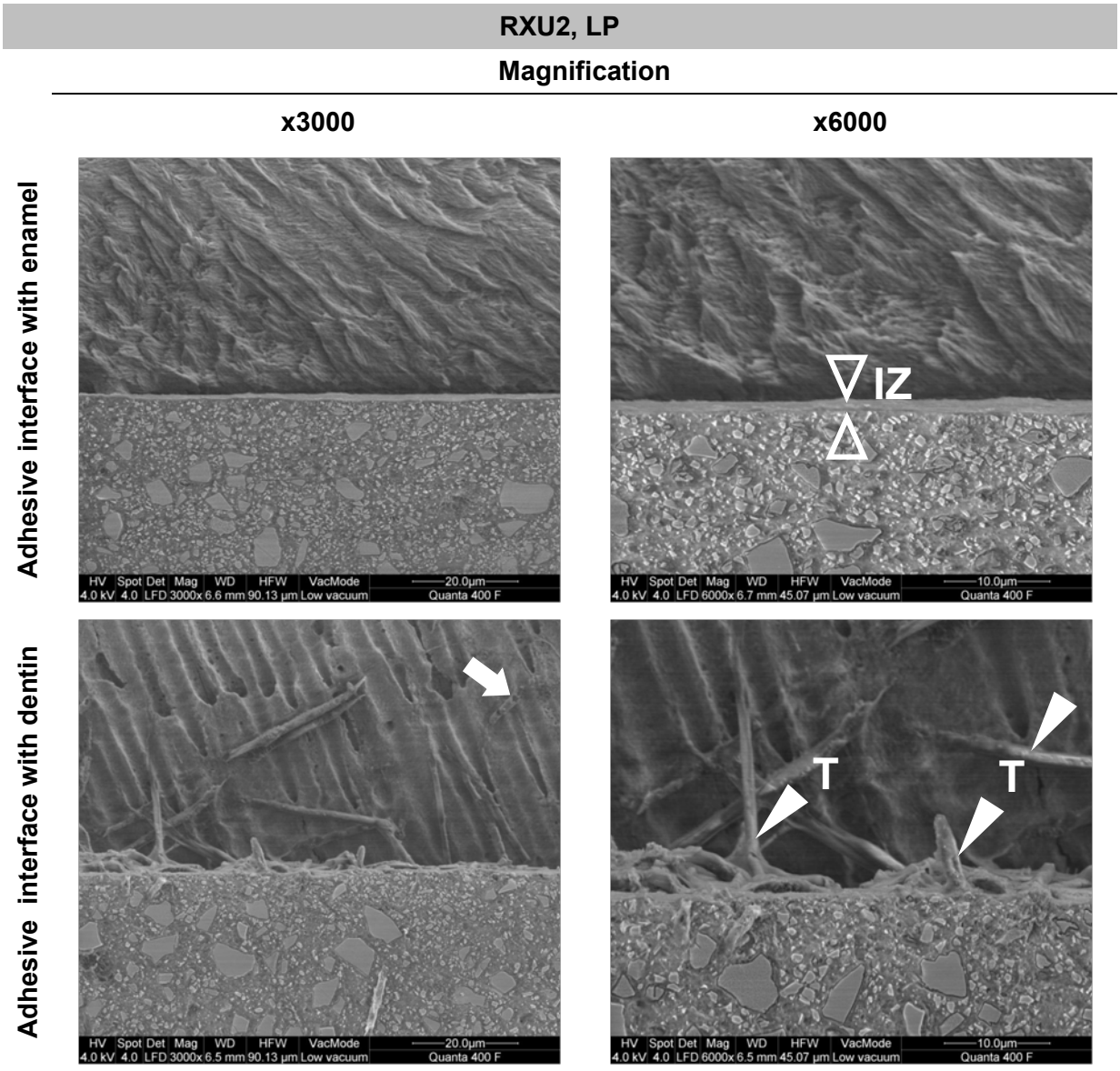


Fig. 23 Etched and deproteinized RXU2, LP specimen No. S09. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. The interdiffusion zone (IZ; arrow heads) was observable at the enamel-luting agent interface and collapsed resin tags (T; white arrow heads) could be seen lying on the dentin. Note the hollow structure of these tags (white arrow).

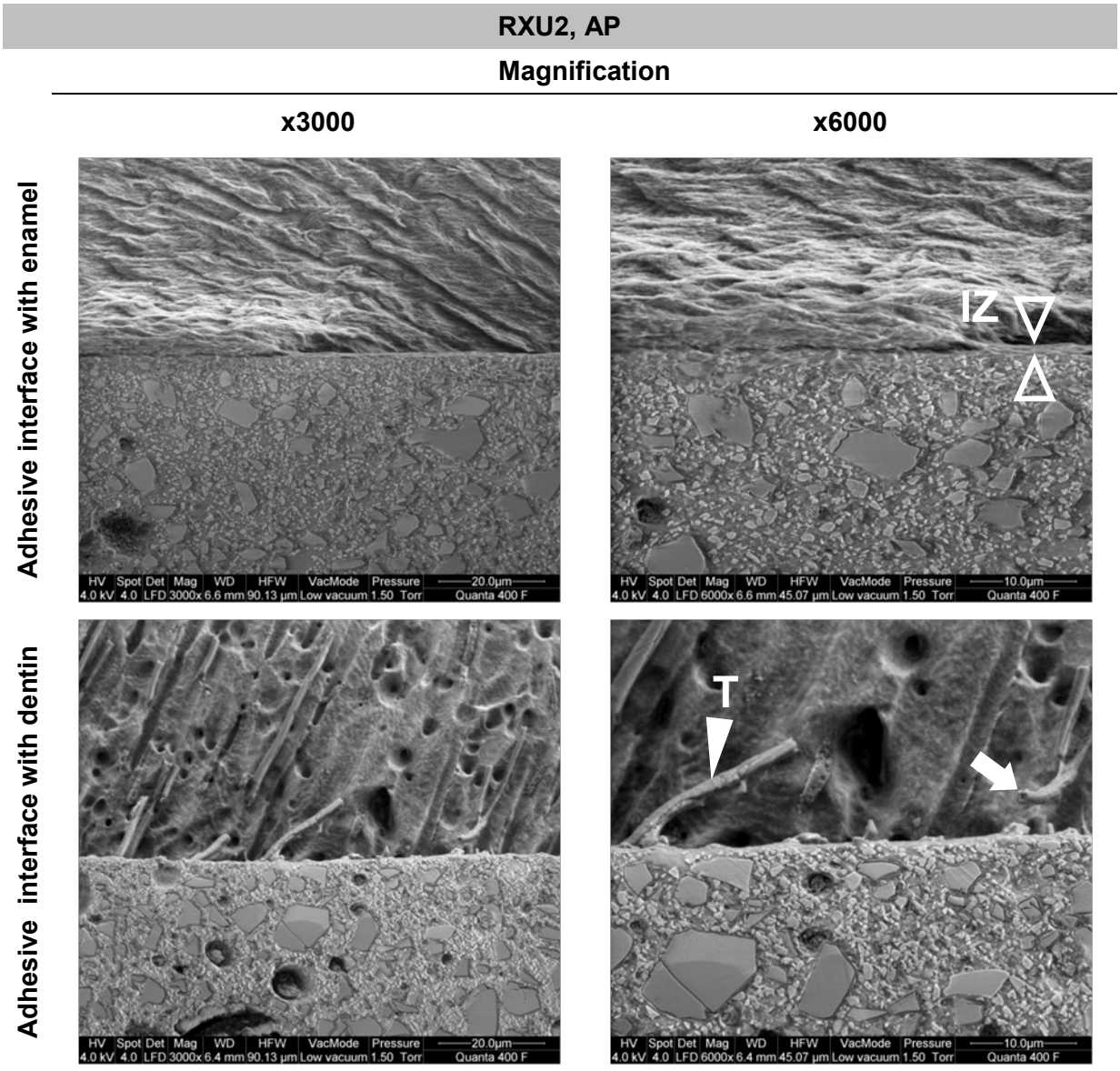


Fig. 24 Etched and deproteinized RXU2, AP specimen No. S08. The horizontal rows indicate the adhesive interface with enamel or dentin, the columns – original magnification of SEM images. SEM settings: LV mode, 1.5 Torr, 4 kV, Spot 4, working distance ~6.5 mm. At the enamel adhesive interface a thin interdiffusion zone (IZ) could be observed. Several resin tags (T; white arrow heads) were detected in this specimen. Note the hollow structure of the resin tag (white arrow).

9.2.3.1. Comparison: polished vs. demineralized/deproteinized specimens

Polished specimen **Demineralized/deproteinized specimen**

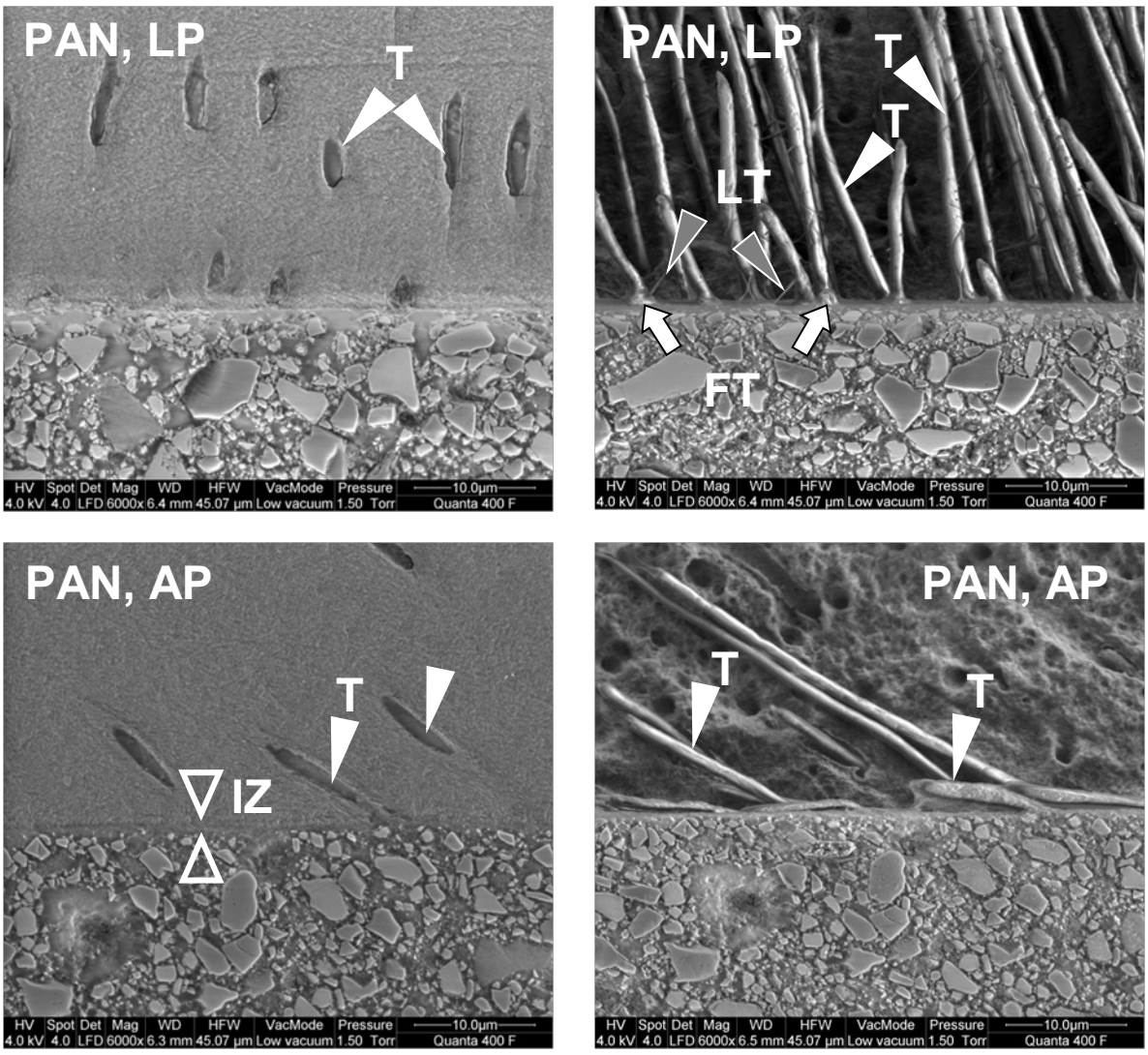


Fig. 25 Comparison of dentin-luting agent adhesive interface in polished (left) and identical but later demineralized/deproteinized specimen (right). PAN, LP (specimen No. S03) imaged sites are not identical. Due to artefacts the image could not be made at the same site. PAN, AP is specimen No. S08 and the image sites are identical. Tags (T; white arrow heads) occurred in both states, the demineralized/deproteinized specimen revealed additionally the lateral tags (LT) and the possible presence of fillers were seen at the base of tags (FT). The tags were not hollow. In PAN, AP the information on the interdiffusion zone (IZ) was lost after demineralization/deproteinization procedure, but the tags (T) could be seen better.

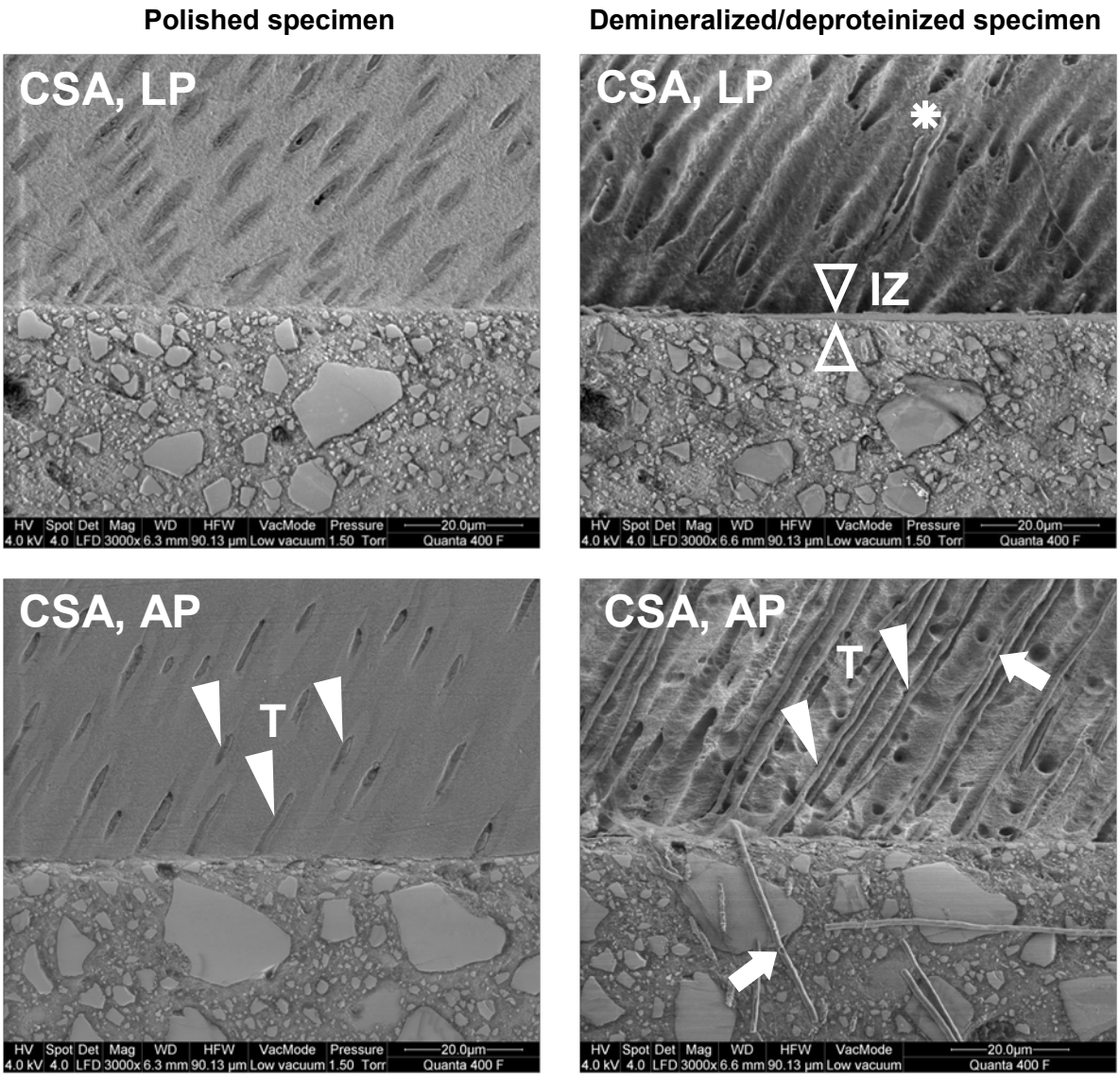


Fig. 26 Comparison of dentin-luting agent adhesive interface in polished (left) and identical but later demineralized/deproteinized specimen (right). CSA, LP is specimen No. S06, CSA, AP – specimen No. S02, the image sites are identical. Note the partially or completely occluded dentinal tubules of the polished CSA, LP specimen. However, in demineralized/ deproteinized specimen only the sheath (asterisk) at the tubule wall is left. It could be a mineralized version of *lamina limitans* (asterisk) left in the specimen after the demineralization/deproteinization procedure. The interdiffusion zone (IZ) became visible because it separated from luting agent. In the dentinal tubules of the polished CSA, AP specimen the resin tags (T) could be observed and were also confirmed after demineralization/deproteinization procedure. The tags were hollow and porous (white arrows).

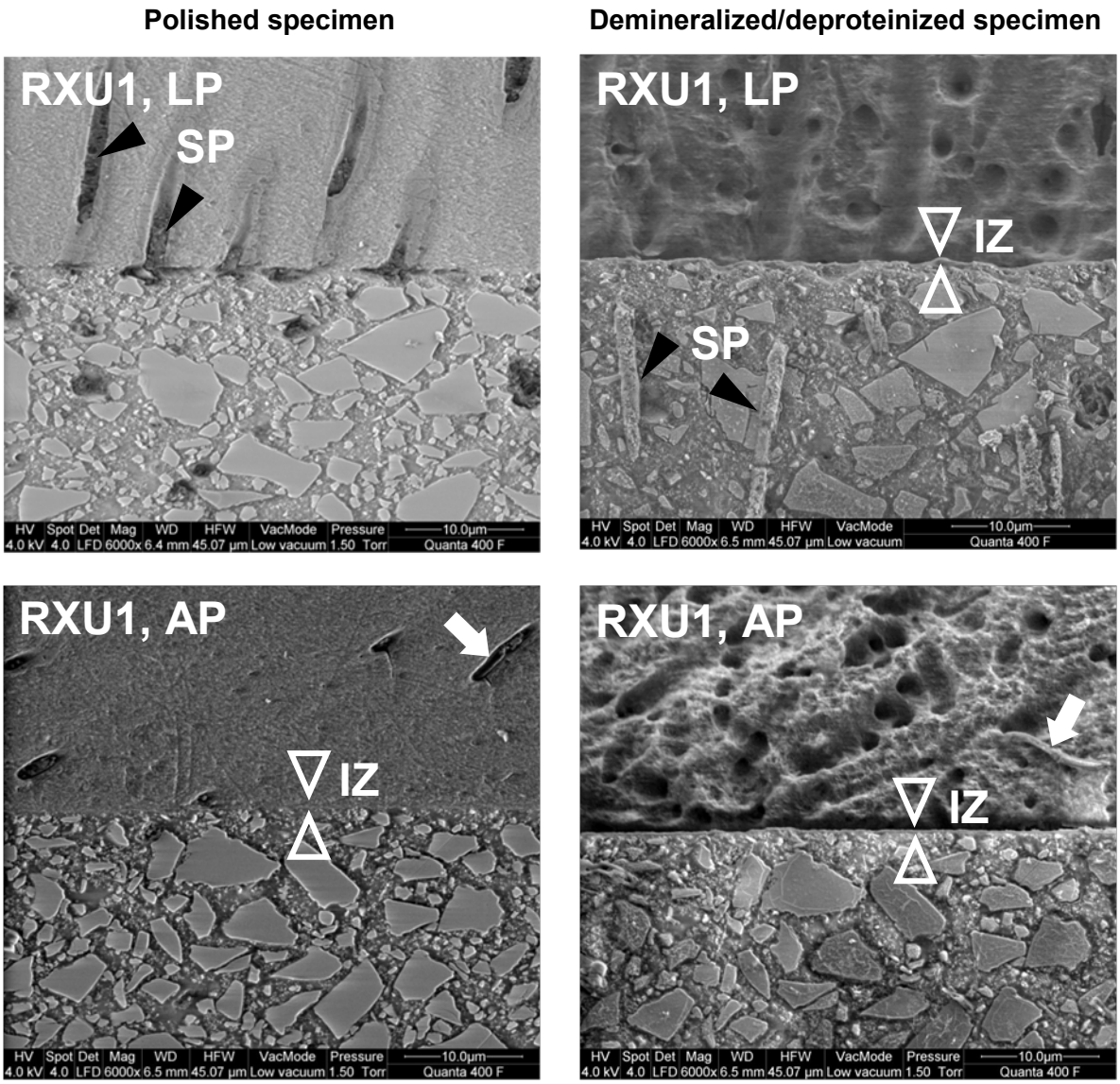


Fig. 27 Comparison of the dentin-luting agent adhesive interface in polished (left) and identical but later demineralized/deproteinized specimen (right). RXU1, LP is specimen No. S09, RXU1, AP – specimen No. S04, the image sites are identical. Smear plugs in the dentinal tubules (SP) of the polished RXU1, LP specimen after demineralization and deproteinization persisted on the surface of the luting agent. The ultrathin interdiffusion zone (IZ) appeared irregularly on the interface of the luting agent. A barely noticeable interdiffusion zone (IZ) is present in RXU1, AP specimen. The structure indicated with white arrow could be the *lamina limitans,* a thin sheath lining the dentinal tubules, which has resisted the demineralization/deproteinization.

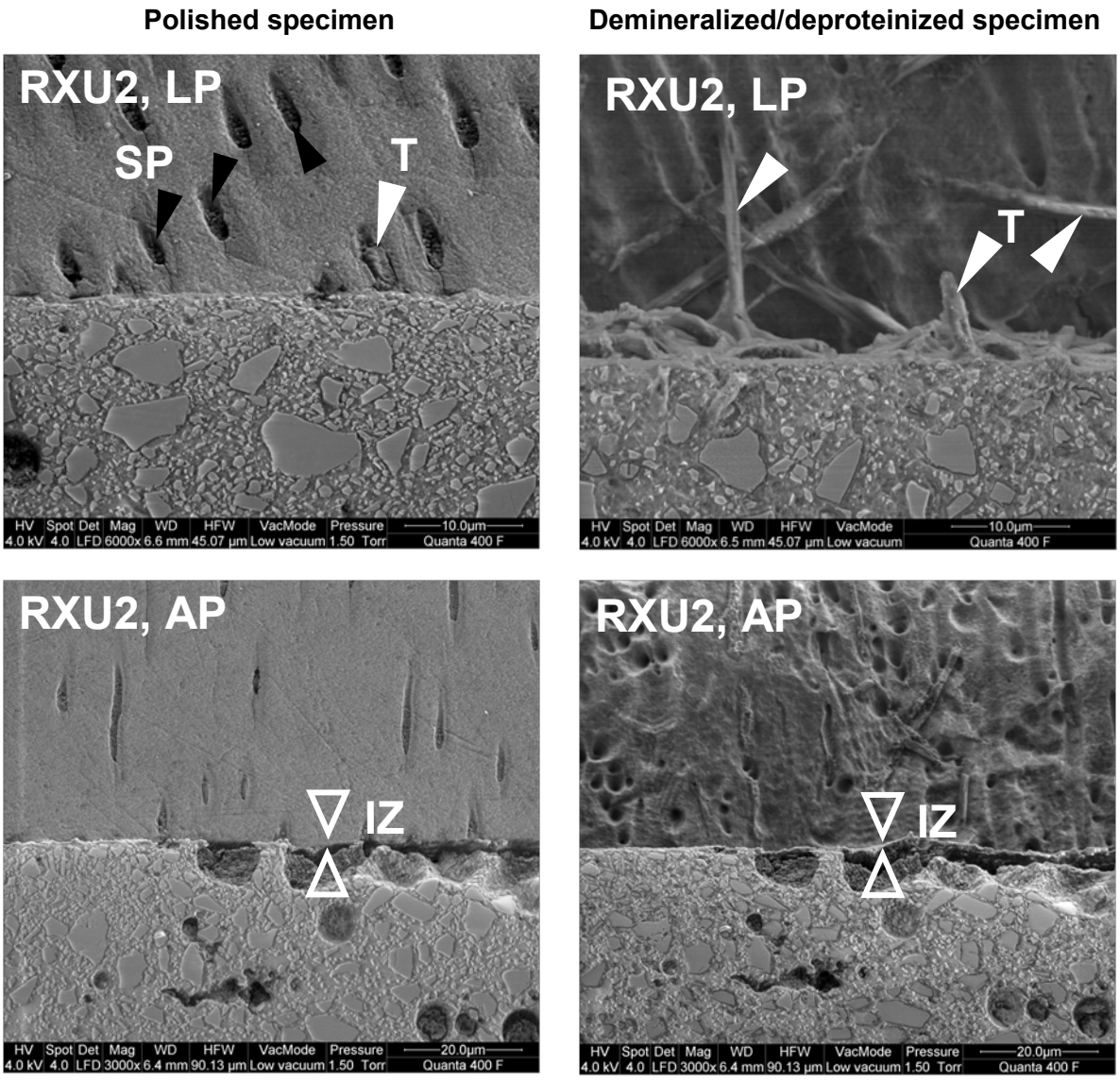
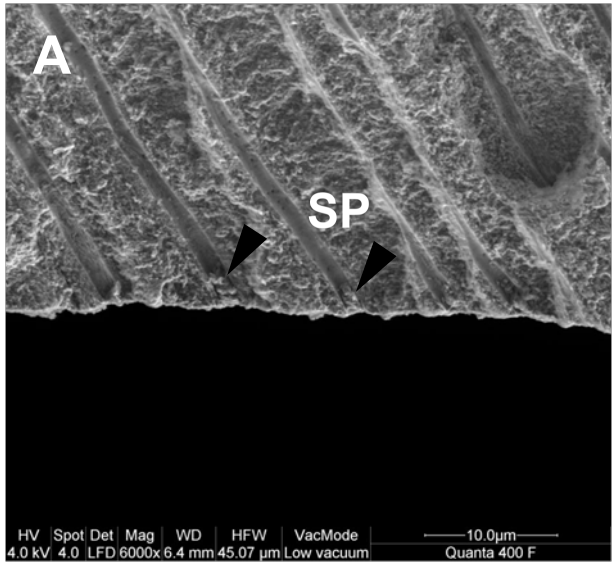


Fig. 28 Comparison of the dentin-luting agent adhesive interface in polished (left) and identical but later demineralized/deproteinized specimen (right). RXU2, LP is specimen No. S09, RXU2, AP – specimen No. S08, the image sites are identical. The tag (T) and smear plugs (SP) are shown filling the dentinal tubules in polished specimen of RXU2, LP. The tags in the demineralized/deproteinized state appeared disorderly and collapsed. Note, the interdiffusion zone (IZ) appears as a very thin layer adhering to the dentin in the polished RXU2, AP specimen and was more clearly exposed by the demineralization/deproteinization of the specimen.

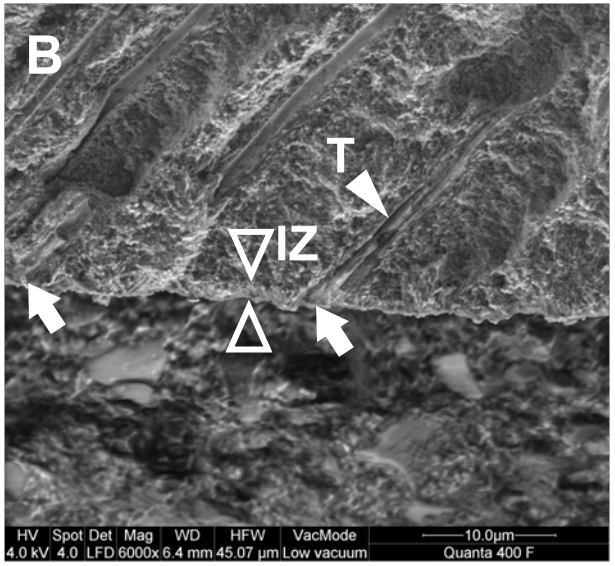
9.2.4. Qualitative evaluation of adhesive interfaces of fractured specimens

PAN, LP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

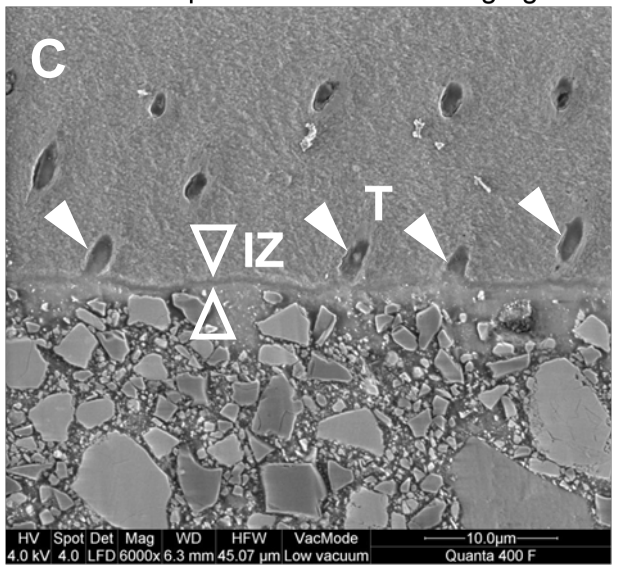
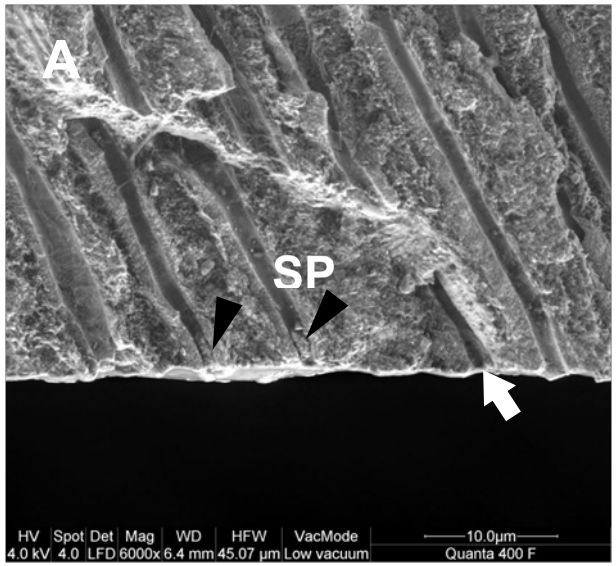


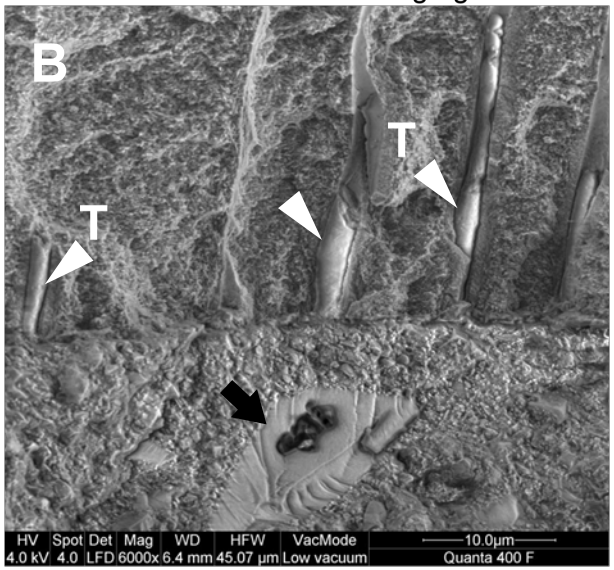
Fig. 29 The fractured and later polished specimen of PAN, LP. This shows the smear plugs (SP; black arrow heads) in the dentinal tubules and the unchanged orifice of the dentinal tubule (image A). The interdiffusion zone (IZ; arrow heads) on fractured surface is shown in image B. Note the modified dentin surface and widened orifice of the dentinal tubule (white arrows) as well as the resin tag (T; white arrow head) in the dentinal tubule. In image C the tnterdiffusion zone (IZ; arrow heads) and resin tags (T; white arrow heads) are clearly visible.

PAN, AP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

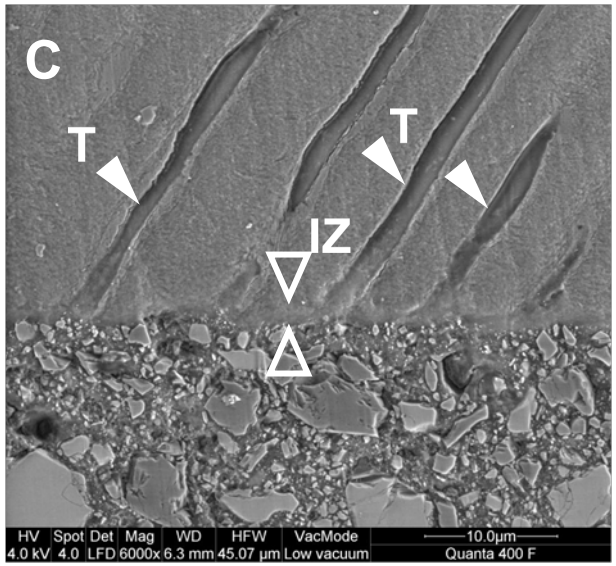
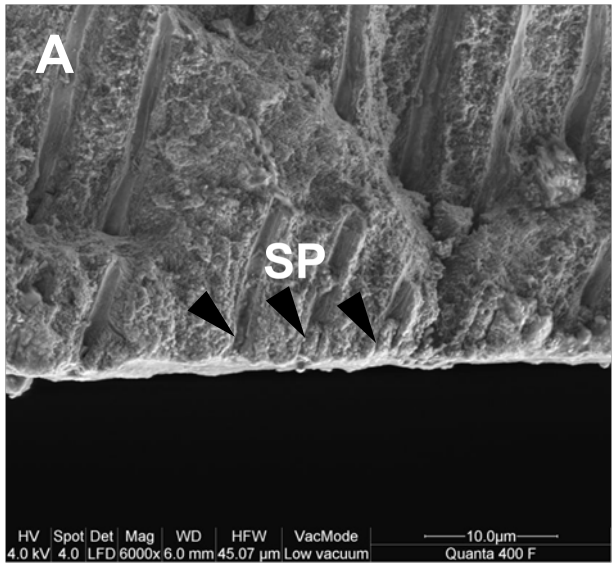


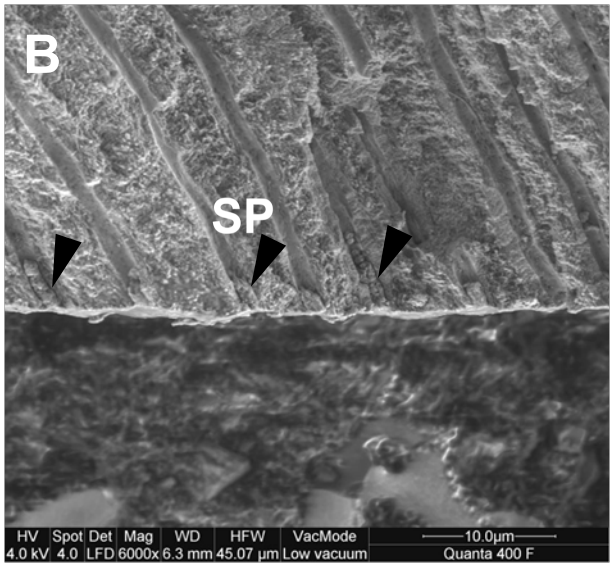
Fig. 30 The fractured and later polished specimen of PAN, AP. Several smear plugs (SP; black arrow heads) and smooth walled dentinal tubules from which smear plugs have probably been lost during fracturing (image A, white arrow).The dentin and luting agent (image B) has broken in one plane, allowing better observation of adhesive interface. Very distinct resin tags (T; white arrow heads) could be observed in image B and C. The interdiffusion zone (IZ; arrow heads) is noticeable in image C. In self-curing mode the interdiffusion zone (IZ) seems to be more diffuse (in comparison to PAN, LP, Fig. 26). The black arrow in image B indicates the specific filler type of PAN and CSA luting agents with hollow centre.

CSA, LP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

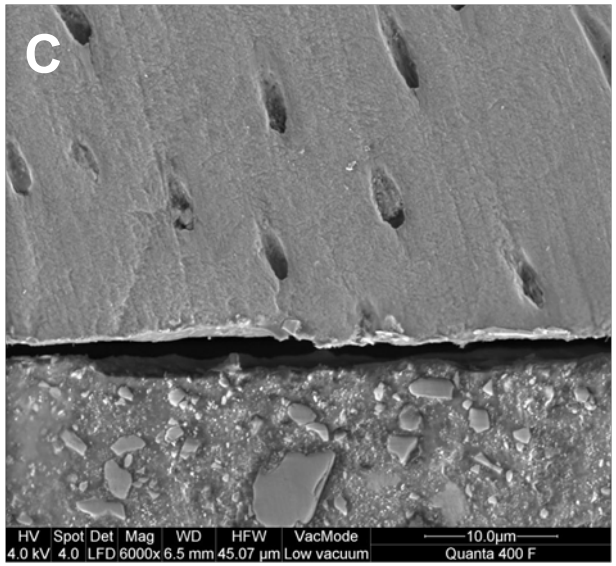
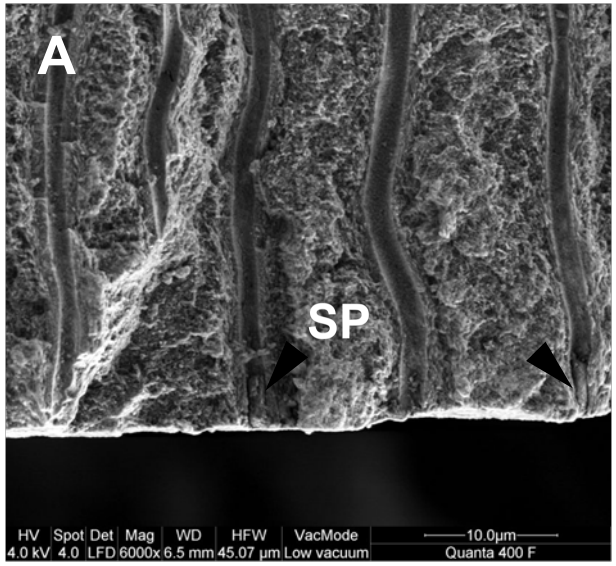


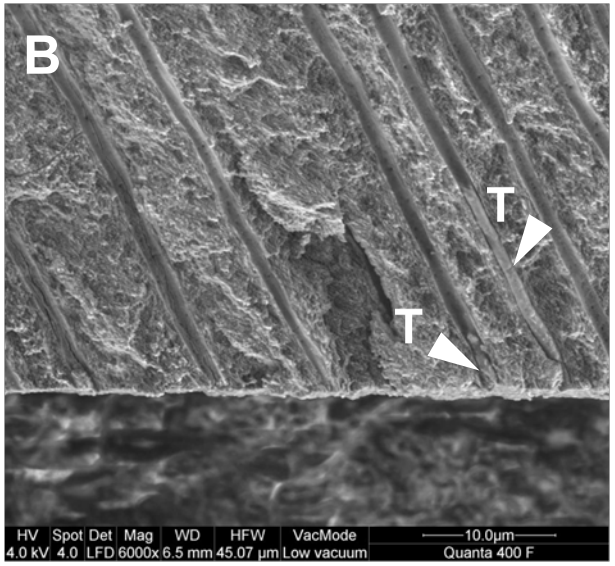
Fig. 31 The fractured and later polished specimen of CSA, LP. Only smear plugs (SP; black arrow heads) in the dentinal tubules of this specimen both in image A and image B could be observed. After polishing, a gap formed along the adhesive interface during the SEM examination. No interaction with the luting agent or surface modifications of the dentin could be detected in this specimen.

CSA, AP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

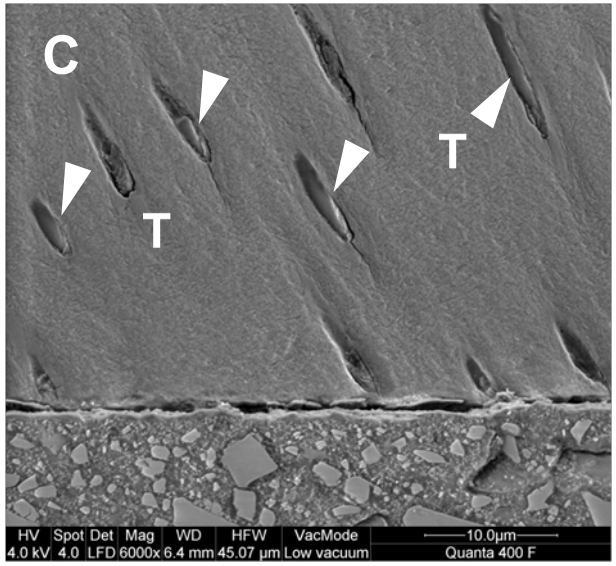


Fig. 32 The fractured and later polished specimen of CSA, AP. Smear plugs in dentinal tubules (SP; black arrow heads) appear in image A indicating that the dentinal tubules are not occluded with resin tags or similar structures. Irregular resin tags (T; white arrow heads) could be observed in the fractured state and more distinct resin tags appeared in the polished state although a gap fotmed along the adhesive interface during SEM examination. The length of these resin tags reached up to 100µm therefore the specimen underwent EDX chemical analysis to determine whether they have resin origin.

CSA, AP

Fractured + polished: dentin + luting agent

EDX analysis of chemical elements: carbon

EDX analysis of chemical elements: silicon

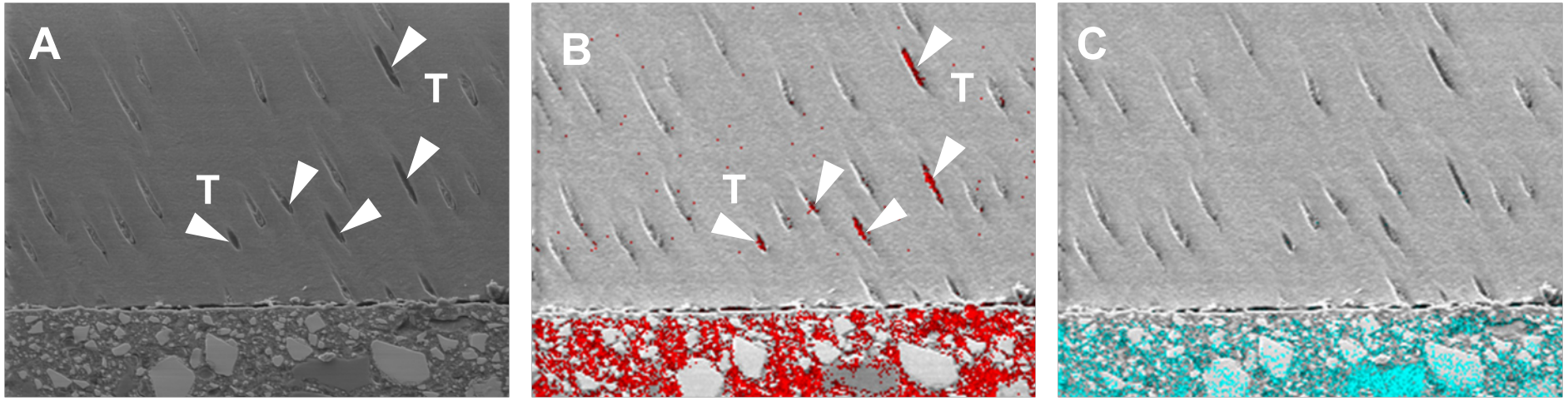


Fig. 32-a Qualitative EDX analysis of polished CSA, AP specimen. The aim of EDX examination was to determine the chemical origin of the structures considered to be resin tags and observed in the specimen in Fig. 32 (T; white arrow heads; SEM image A). EDX live-spectrum element mappings revealed carbon (red-colored) content in the tags indicating their resin origin (image B). Silicon (turquoise-colored) as the representative for fillers shown in the image (C) could be not observed in the resin tags.

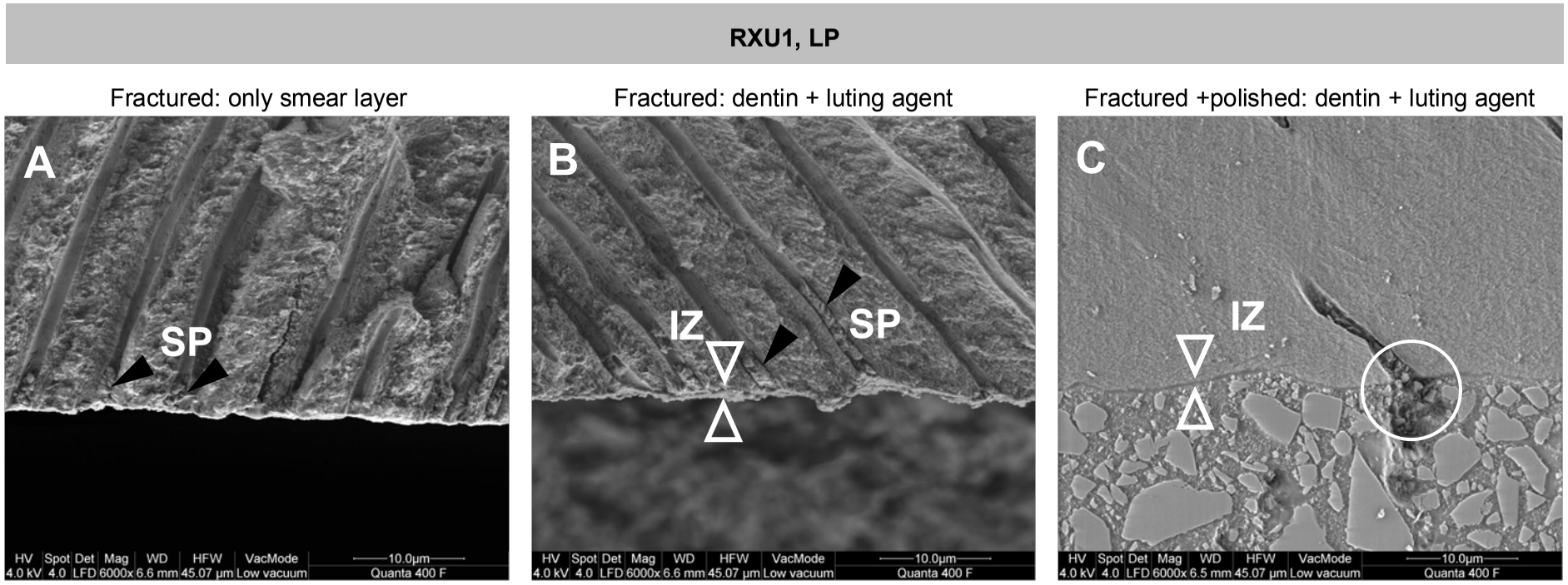
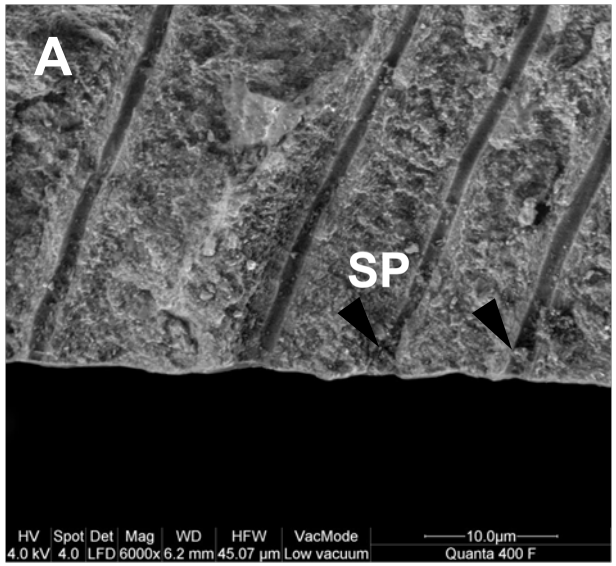


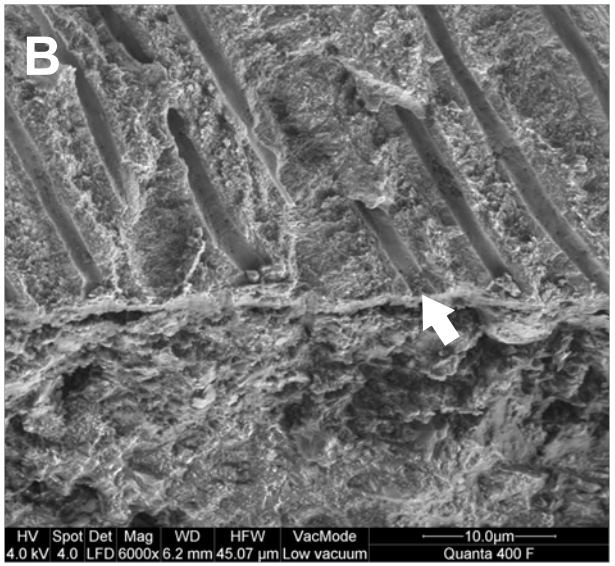
Fig. 33 The fractured and later polished specimen of RXU1, LP. Smear plugs filled the dentinal tubules (SP; black arrow heads) in image A and B. A thin interdiffusion zone (IZ; arrow heads) could be detected in the fractured and the polished state. Luting agent has not infiltrated into the dentinal tubules and the smear plugs (SP; black arrow heads) were left there unchanged. In the polished state there could be seen pores typical of SARC's pores at the orifice of the dentinal tubule (in the circle).

RXU1, AP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

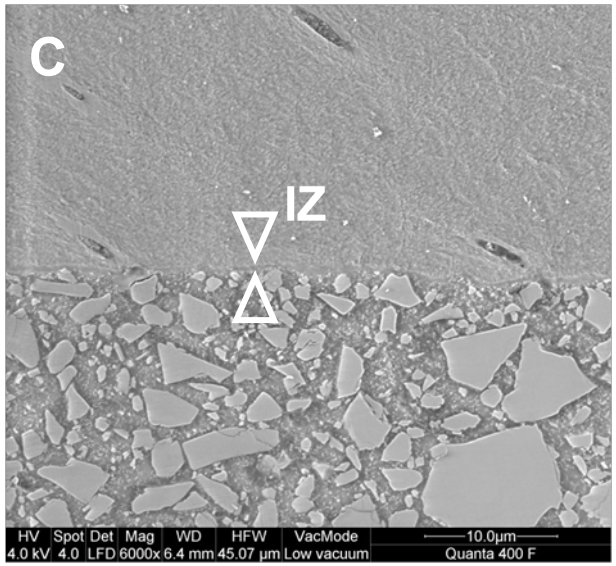
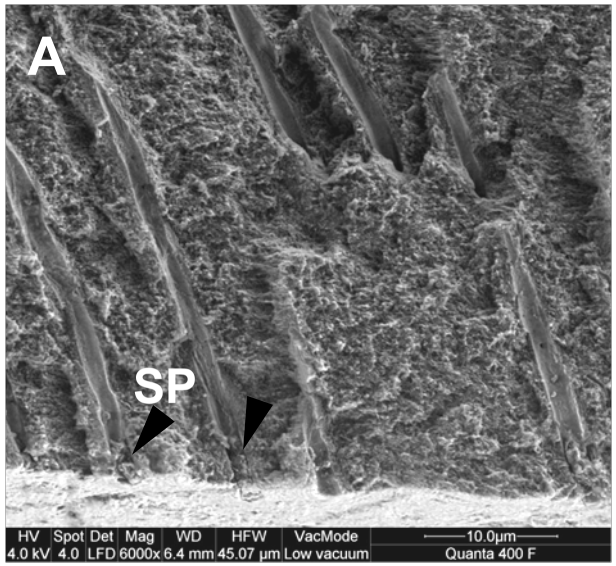


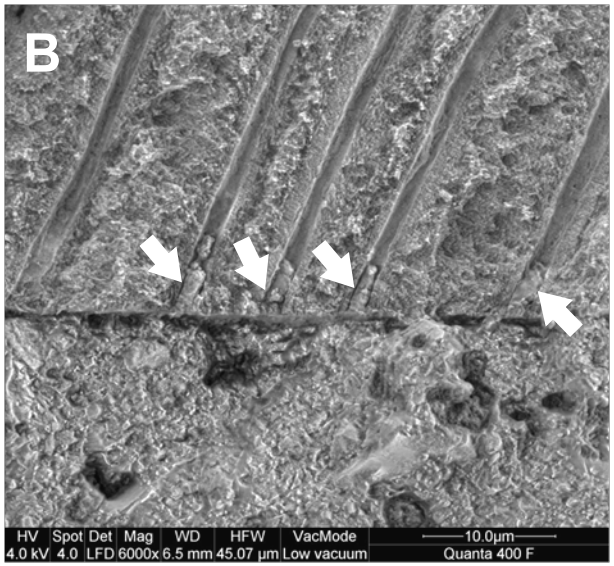
Fig. 34 The fractured and later polished specimen of RXU1, AP. Irregular smear plugs filled the dentinal tubules (SP; black arrow heads) as shown in image A. The luting agent has interacted with the smear layer, the ultrathin interdiffusion zone appears in image C, but deeper interaction or infiltration into dentinal tubules was not observed (white arrow, image B).

RXU2, LP

Fractured: only smear layer



Fractured: dentin + luting agent



Fractured +polished: dentin + luting agent

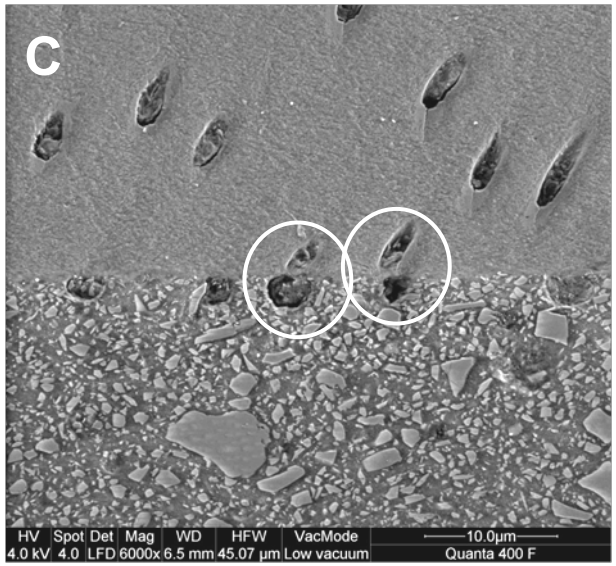


Fig. 35 The fractured and later polished specimen of RXU2, LP. The smear plugs in the dentinal tubules in image B seem to be modified, as though infiltrated with the resin but not dissolved (white arrows), by comparison to the smear plugs in the image A (SP; black arrow heads). Typical pores in the orifices of dentinal tubules demonstrate the insufficiency of the luting agent in this region (circled, image C).

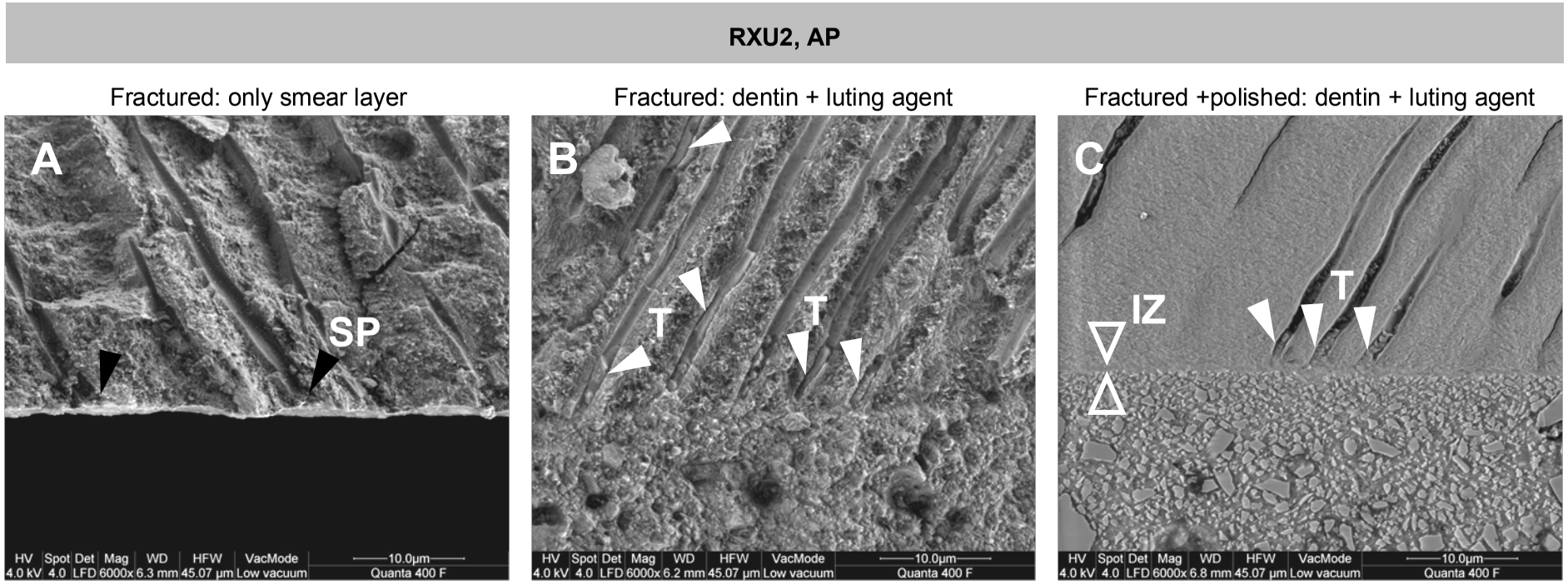


Fig. 36 The fractured and later polished specimen of RXU2, AP. Image A: smear plugs in the dentinal tubules (SP; black arrows) in this specimen were hardly visible. Resin tags (T; white arrow heads) could be observed in the image B and C. Although the dentin and the luting agent have broken in one plane no visible interdiffusion zone is present but an intimate adaptation of the material to the dentin could be detected. However, in the image C a thin interdiffusion zone could be observed (IZ; arrow heads).

10. Tables and abbreviations

Abbreviation	Explanation
4-META or MET-4	4-methacryloxyethyl trimellitic anhydride
5-NMSA	N-methacryloxyl-5-aminosalycyclic acid
Bis-GMA	Bis-phenol-A-diglycidylmethacrylate
BPEDMA	Bis-phenol-A-polyethoxy dimethacrylate
HAp	Hydroxyapatite
HEMA	2-hydroxyethyl methacrylate
MDP or 10-MDP	10-methacryloxydecyl dihydrogen phosphate
Phenyl-P	2-methacryloxyethyl phenyl hydrogen phosphate
SARC	Self-adhesive resin cement
TEGDMA	Triethyleneglycol dimethacrylate
TEM	Transmission electron microscopy
UDMA	Urethane dimethacrylate
CSA	Clearfil SA Cement
PAN	Panavia F2.0
RXU1	RelyX Unicem
RXU2	RelyX Unicem 2
SEM	Scanning electron microscopy
CLSM	Confocal laser scanning microscopy
HV SEM	High vacuum scanning electron microscopy
LV SEM	Low vacuum scanning electron microscopy
PLA	Pressure limiting apperture
LFD	Large field detector
LP	Light-polymerization
AP	Auto-polymerization
FE	Field emission
PE	Primary electron(-s)
SE	Secondary electron(-s)
GDD	Gaseous detection device
BSE	Backscattered electron(-s)
HL	Hybrid layer
IZ	Interaction zone
T	Tag
LT	Lateral tag
SP	Smear plug
P	Pore
EDX	Energy-dispersive X-ray spectroscopy
DEJ	Dentin-enamel junction
S01 – S10	Specimen No. 1 - 10
NaOCl	Sodium hypochlorite
HCl	Hydrochloric acid

Table 1 Abbreviations used in the present study.

SARC	Manufacturers
Bifix SE	Voco, Cuxhaven, Germany
BisCem	Bisco, Schaumburg, IL, USA
Breeze	Pentron Clinical Technologies Wallingford, CT, USA
Clearfil SA Cement (CSA)	Kuraray, Okayama, Japan
Embrace Wet Bond	Pulpdent, Watertown, MA, USA
GCem	GC, Tokyo, Japan
iCem	Heraeus Kulzer, Hanau, Germany
Maxcem Elite	Kerr, Orange, CA, USA
MonoCem	Shofu, San Marcos, CA, USA
Multilink Sprint	Ivoclar Vivadent, Schaan Liechtenstein
RelyX Unicem (RXU1)	3M ESPE, Seefeld, Germany
RelyX Unicem 2 (RXU2)	3M ESPE, Seefeld, Germany
SeT	SDI, Bayswater, Australia
SmartCem 2	Dentsply Caulk, Milford, CT, USA
SpeedCEM	Ivoclar Vivadent, Schaan, Liechtenstein
TotalCem	Itena, Tremblay-en-France, France

Table 2 Commercially available self-adhesive resin cements (SARC).

10.1.1. Materials and methods

Material	Main composition
ED Primer II and Panavia F2.0 (PAN)	
• **Primer A**	HEMA, MDP, 5-NMSA, water, accelerator
• **Primer B**	5-NMSA, accelerator, water, sodium benzene sulphinate
• **Paste A**	silanized and colloidal silica, BPEDMA, DMA, MDP, camphoroquinone, catalysts, initiators
• **Paste B**	silanized barium glass, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, catalysts, accelerators, pigments **Inorganic filler amount:** 78 wt% **Particle size:** 0.04 – 19.00µm
Clearfil SA Cement (CSA)	
• **Paste A**	MDP, Bis-GMA, TEGDMA, hydrophobic aromatic dimethacrylate, dl-camphorquinone, benzoyl peroxide, initiator, silanated barium glass filler, silanated colloidal silica
• **Paste B**	Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic dimethacrylate, accelerators, pigments, surface treated sodium fluoride, silanated barium glass filler, silanated colloidal silica **Inorganic filler amount:** 66 wt% **Particle size:** mean 2.5µm, 0.04 – 20.00µm
RelyX Unicem (RXU1)	
• **Powder**	Alkaline (basic) fillers, silanated fillers, initiator components, pigments
• **Liquid**	Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, initiator components, stabilizers **Inorganic filler amount:** 72 wt% **Particle size:** <9.5µm
RelyX Unicem 2 (RXU2)	
• **Base paste**	Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, silanated fillers, initiator components, stabilizers, rheological additives
• **Catalyst paste**	Methacrylate monomers, alkaline (basic) fillers, initiator components, stabilizers, pigments, rheological additives **Inorganic filler amount:** 67 wt% **Particle size:** 12.5µm

Table 3 Chemical composition of the resin luting agents, used in the present study (information from manufacturers).

Material	Manufacturer	Shade	Storage temperature	Batch Nr.
PANAVIA F 2.0 • Paste A • Paste B **ED Primer** • Liquid A • Liquid B	Kuraray Medical Inc.,Okayama, Japan	TC	2 – 8^{0}C	041304 00444A 00226A 00284B 00159A
Clearfil SA Cement	Kuraray Medical Inc.,Okayama, Japan	Universal (A2)	2 – 8^{0}C	0023AA 0026BA
RelyX Unicem (Aplicap)	3M ESPE, Seefeld, Germany	Universal (A2)	15 – 25^{0}C	406588
RelyX Unicem 2	3M ESPE, Seefeld, Germany	TR	15 – 25^{0}C	403918
Tetric EvoFlow	Ivoclar Vivadent, Schaan, Liechtenstein	A2	2 - 28^{0}C	M46631
Vita Mark II	VITA Zahnfabrik, Bad Säckingen, Germany		-	23920
Liquid Strip	Ivoclar Vivadent, Schaan, Liechtenstein		2 - 28^{0}C	K18931
Oxyguard II	Kuraray Medical Inc.,Okayama, Japan		2 – 8^{0}C	

Table 4 The list of materials used in the present study.

Step	Duration	Polishing procedure
1.	1min.	Wet 600-Grit silicon carbide sandpaper (twice if necessary)
2.	1min.	Rinsing with water/air spray.
3.	1min.	Wet 1200-Grit silicon carbide sandpaper
4.	1min.	Rinsing with water/air spray.
5.	1min.	Wet fabric tissue with alumina powder 1.0µm grain size
6.	1min.	Rinsing with water/air spray.
7.	1min.	Wet fabric tissue with alumina powder 0.3µm grain size
8.	1min.	Rinsing with water/air spray.
9.	1min.	Wet fabric tissue with alumina powder 0.05µm grain size
10.	1min.	Rinsing with water/air spray.
11.	1min.	Wet fabric tissue
12.	1min.	Rinsing with water/air spray.
13.	storage	At 100% humidity up to 4 hours maximum until SEM evaluation
14.	1min.	Wet fabric tissue, just before SEM examination
15.	1min.	Rinsing in water

Table 5 Sequence of the polishing procedure and materials used.

Step	Duration	Procedure
1.	15secs	**Demineralization** in 1N HCl solution (Merck, 9057, Darmstadt, Germany)
2. - 4.	5min.	**Rinsing** (3 times) in *aqua bidest.*
5.	10min.	**Deproteinization** in 2% NaOCl solution (Speiko, 1047, Münster, Germany)
6. – 8.	5min.	**Rinsing** (3 times) in *aqua bidest.*

Table 6 Processing of the demineralized/deproteinized specimens: the sequence of demineralization/ deproteinization procedure.

		Scores			
		Detectable (+)	**Questionable (+/-)**	**Not detectable (-)**	**Failed (X)**
Criteria	**1. Hybrid layer or interdiffusion zone**	The dark layer of interdiffusion zone is clearly discernible	The interaction zone is irregular or the dentin interface is modified and the luting agent has tight contact to dentin	Hybrid layer or interdiffusion zone is not discernible, but the luting agent has tight contact to dentin	The specimen is impossible to evaluate due to gaps or fractures or polishing remnants on the adhesive interface
	2. Tags	Clearly visible unfilled or filler reinforced resin tag(s) connected to dentinal tubule walls, sprouting from bulk of the luting agent material	Resin tag(s) are either not fully connected to tubule walls but are connected to the bulk of luting agent material or are not clearly discernible	Resin tags are not discernible	
	3. Pores	Pores at the adhesive interface at the orifices of dentinal tubules	Pores not directly opposite the tubule orifice or porosities along entire interface	The intimate adaptation of luting agent at or into the dentinal tubule orifice region is present	
	4. Smear plugs	Clearly discernible loose debris content in dentinal tubules, different in color from the bulk of the luting agent	Tubules have content or are filled with something which does not have a loose structure	Dentin tubules are empty	

Table 7 Evaluation criteria and explanation of the scoring for the dentin-luting agent adhesive interface in polished specimens.

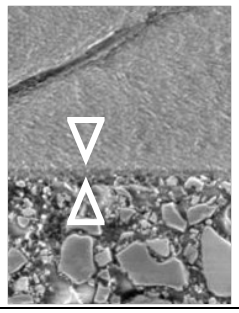
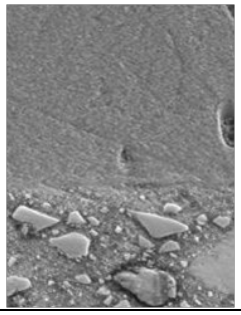
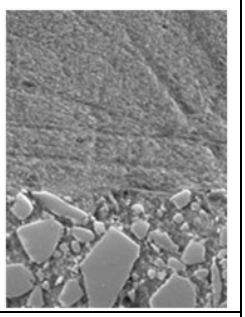
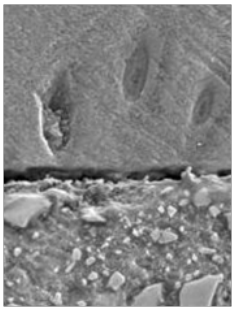
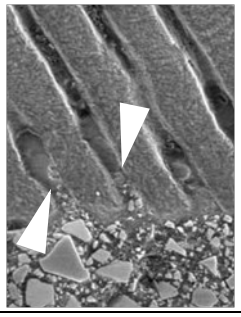
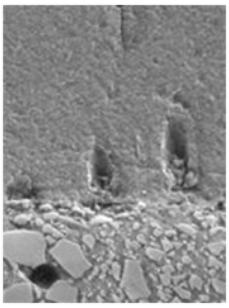
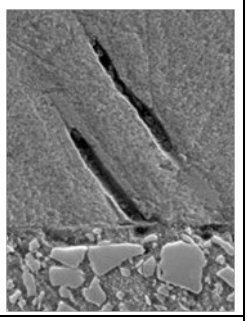
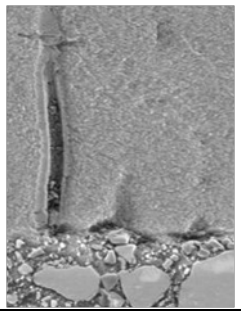
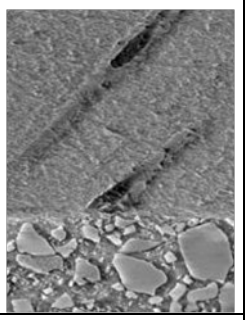
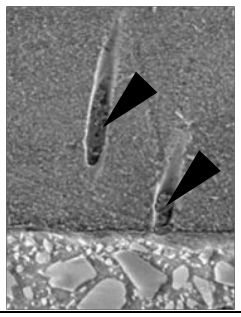
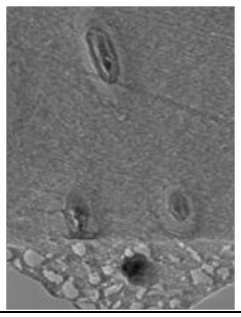
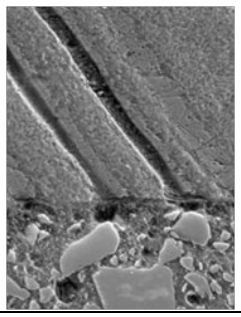
Criteria	Scores: Detectable (+)	Questionable (+/-)	Not detectable (-)	Failed (X)
1. Hybrid layer or inter-diffusion zone				
2. Tags				
3. Pores				
4. Smear plugs				

Table 7-a Evaluation criteria and scores of the dentin-luting agent adhesive interface in polished specimens in SEM images with applied marks.

10.1.2. Results

10.1.2.1. Semi-quantitative evaluation of adhesive interface of polished specimens: enamel-luting agent interface

Experimental groups	Microgaps along the enamel-luting agent adhesive interface	Intact enamel-luting agent adhesive interface
PAN, LP	0	10
PAN, AP	0	10
CSA, LP	10	0
CSA, AP	8	2
RXU1, LP	9	1
RXU1, AP	10	0
RXU2, LP	7	3
RXU2, AP	9	1

Table 8 The frequency of specimens with evaluation criterion "microgaps" by experimental group (visible at x3000 magnification) at the enamel-luting agent adhesive interface (n=10). (PAN = Panavia F2.0+ED Primer, CSA = Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2, LP = light-polymerization, AP = auto-polymerization.)

10.1.2.2. Semi-quantitative evaluation of adhesive interface of polished specimens: dentin-luting agent interface

PAN, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (+)	S04 (-)	S05 (+)	S06 (+)	S07 (-)	S08 (+)	S09 (-)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (+/-)	S04 (-)	S05 (+)	S06 (+)	S07 (+)	S08 (+)	S09 (+)	S10 (+/-)
PAN, AP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (+)	S06 (X)	S07 (+/-)	S08 (+)	S09 (-)	S10 (+/-)
	Dentin lateral, x3000									
	S01 (-)	S02 (+/-)	S03 (+)	S04 (+)	S05 (+)	S06 (+)	S07 (+)	S08 (+)	S09 (-)	S10 (+)
CSA, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (-)	S05 (X)	S06 (-)	S07 (X)	S08 (-)	S09 (X)	S10 (+/-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (+/-)	S04 (-)	S05 (X)	S06 (-)	S07 (X)	S08 (-)	S09 (X)	S10 (+)
CSA, AP	Dentin middle, x3000									
	S01 (X)	S02 (-)	S03 (X)	S04 (+)	S05 (+/-)	S06 (X)	S07 (X)	S08 (X)	S09 (X)	S10 (X)
	Dentin lateral, x3000									
	S01 (X)	S02 (-)	S03 (X)	S04 (+/-)	S05 (+/-)	S06 (+)	S07 (X)	S08 (-)	S09 (X)	S10 (X)
RXU1, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (-)	S05 (-)	S06 (+)	S07 (-)	S08 (-)	S09 (+/-)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (+/-)	S05 (+/-)	S06 (-)	S07 (-)	S08 (+)	S09 (-)	S10 (-)
RXU1, AP	Dentin middle, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (+)	S05 (-)	S06 (X)	S07 (+/-)	S08 (-)	S09 (X)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (X)	S03 (-)	S04 (+/-)	S05 (-)	S06 (+/-)	S07 (+)	S08 (+/-)	S09 (-)	S10 (-)
RXU2, LP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (+)	S07 (+/-)	S08 (+/-)	S09 (+/-)	S10 (-)
	Dentin lateral, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (-)	S05 (-)	S06 (+)	S07 (+/-)	S08 (+/-)	S09 (+)	S10 (+)
RXU2, AP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (+)	S07 (+)	S08 (+/-)	S09 (+/-)	S10 (X)
	Dentin lateral, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (X)	S05 (-)	S06 (+)	S07 (+/-)	S08 (+/-)	S09 (+)	S10 (X)

Table 9 Assignment of scores in polished specimens at x3000 magnification for the criterion "Hybrid layer" in the middle and lateral part of dentin. S01 – S10 are the index numbers of the specimens. The scores used: criterion detectable (+), criterion questionable (+/-), criterion not detectable (-) and failed specimen (X). (PAN = Panavia F2.0+ED Primer, CSA = Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2, LP = light-polymerization, AP = auto-polymerization.)

PAN, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (+)	S04 (+)	S05 (+/-)	S06 (+)	S07 (+/-)	S08 (+)	S09 (+)	S10 (+/-)
	Dentin lateral, x3000									
	S01 (+/-)	S02 (-)	S03 (+)	S04 (+)	S05 (+)	S06 (+)	S07 (+)	S08 (+)	S09 (+)	S10 (+/-)
PAN, AP	Dentin middle, x3000									
	S01 (+)	S02 (+/-)	S03 (X)	S04 (X)	S05 (-)	S06 (X)	S07 (+/-)	S08 (+)	S09 (+/-)	S10 (+)
	Dentin lateral, x3000									
	S01 (-)	S02 (+)	S03 (-)	S04 (+)	S05 (+)	S06 (-)	S07 (+)	S08 (+)	S09 (+)	S10 (+)
CSA, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (-)	S05 (X)	S06 (-)	S07 (X)	S08 (+/-)	S09 (X)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (-)	S04 (-)	S05 (X)	S06 (+/-)	S07 (X)	S08 (+/-)	S09 (X)	S10 (+/-)
CSA, AP	Dentin middle, x3000									
	S01 (X)	S02 (-)	S03 (X)	S04 (+)	S05 (+)	S06 (X)	S07 (X)	S08 (X)	S09 (X)	S10 (X)
	Dentin lateral, x3000									
	S01 (X)	S02 (+)	S03 (X)	S04 (+)	S05 (-)	S06 (+)	S07 (X)	S08 (+/-)	S09 (X)	S10 (X)
RXU1, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (+/-)	S05 (+/-)	S06 (-)	S07 (-)	S08 (-)	S09 (-)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (-)	S05 (+/-)	S06 (-)	S07 (-)	S08 (-)	S09 (-)	S10 (-)
RXU1, AP	Dentin middle, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (-)	S05 (+/-)	S06 (X)	S07 (+/-)	S08 (-)	S09 (X)	S10 (+/-)
	Dentin lateral, x3000									
	S01 (+/-)	S02 (X)	S03 (-)	S04 (+/-)	S05 (-)	S06 (-)	S07 (+)	S08 (+/-)	S09 (-)	S10 (-)
RXU2, LP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (+/-)	S07 (+/-)	S08 (+/-)	S09 (+/-)	S10 (+/-)
	Dentin lateral, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (+/-)	S05 (-)	S06 (+/-)	S07 (+)	S08 (+/-)	S09 (+)	S10 (-)
RXU2, AP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (+/-)	S07 (+/-)	S08 (+/-)	S09 (+)	S10 (X)
	Dentin lateral, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (X)	S05 (-)	S06 (-)	S07 (+/-)	S08 (+)	S09 (+)	S10 (X)

Table 10 Assignment of scores in polished specimens at x3000 magnification for the criterion "Tags" in the middle and lateral part of dentin. S01 – S10 are the index numbers of the specimens. The scores used: criterion detectable (+), criterion questionable (+/-), criterion not detectable (-) and failed specimen (X). (PAN = Panavia F2.0+ED Primer, CSA = Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2, LP = light-polymerization, AP = auto-polymerization.)

PAN, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (+/-)	S04 (-)	S05 (-)	S06 (+/-)	S07 (+/-)	S08 (-)	S09 (+/-)	S10 (-)
	Dentin lateral, x3000									
	S01 (+/-)	S02 (+/-)	S03 (-)	S04 (-)	S05 (-)	S06 (-)	S07 (+)	S08 (-)	S09 (-)	S10 (-)
PAN, AP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (-)	S06 (X)	S07 (+)	S08 (+/-)	S09 (-)	S10 (+)
	Dentin lateral, x3000									
	S01 (-)	S02 (+/-)	S03 (+)	S04 (-)	S05 (+)	S06 (-)	S07 (-)	S08 (-)	S09 (-)	S10 (-)
CSA, LP	Dentin middle, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (-)	S05 (X)	S06 (+)	S07 (X)	S08 (+/-)	S09 (X)	S10 (+)
	Dentin lateral, x3000									
	S01 (-)	S02 (+/-)	S03 (-)	S04 (+/-)	S05 (X)	S06 (-)	S07 (X)	S08 (+)	S09 (X)	S10 (+)
CSA, AP	Dentin middle, x3000									
	S01 (X)	S02 (-)	S03 (X)	S04 (-)	S05 (-)	S06 (X)	S07 (X)	S08 (X)	S09 (X)	S10 (X)
	Dentin lateral, x3000									
	S01 (X)	S02 (+/-)	S03 (X)	S04 (-)	S05 (-)	S06 (-)	S07 (X)	S08 (-)	S09 (X)	S10 (X)
RXU1, LP	Dentin middle, x3000									
	S01 (+)	S02 (X)	S03 (X)	S04 (+/-)	S05 (+)	S06 (-)	S07 (-)	S08 (+/-)	S09 (+)	S10 (-)
	Dentin lateral, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (-)	S05 (+/-)	S06 (-)	S07 (+/-)	S08 (+)	S09 (+)	S10 (-)
RXU1, AP	Dentin middle, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (-)	S05 (-)	S06 (X)	S07 (+)	S08 (+)	S09 (X)	S10 (+)
	Dentin lateral, x3000									
	S01 (-)	S02 (X)	S03 (-)	S04 (-)	S05 (+/-)	S06 (+/-)	S07 (-)	S08 (-)	S09 (-)	S10 (+/-)
RXU2, LP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (+)	S07 (+)	S08 (+/-)	S09 (+)	S10 (-)
	Dentin lateral, x3000									
	S01 (X)	S02 (X)	S03 (X)	S04 (-)	S05 (-)	S06 (+/-)	S07 (-)	S08 (-)	S09 (+)	S10 (+)
RXU2, AP	Dentin middle, x3000									
	S01 (-)	S02 (-)	S03 (X)	S04 (X)	S05 (X)	S06 (-)	S07 (+/-)	S08 (-)	S09 (-)	S10 (X)
	Dentin lateral, x3000									
	S01 (-)	S02 (X)	S03 (X)	S04 (X)	S05 (+/-)	S06 (-)	S07 (-)	S08 (-)	S09 (+/-)	S10 (X)

Table 11 Assignment of scores in polished specimens at x3000 magnification for the criterion "Pores" in the middle and lateral part of dentin. S01 – S10 are the index numbers of the specimens. The scores used: criterion detectable (+), criterion questionable (+/-), criterion not detectable (-) and failed specimen (X). (PAN = Panavia F2.0+ED Primer, CSA = Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2, LP = light-polymerization, AP = auto-polymerization.)

PAN, LP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(-)	(X)	(+/-)	(-)	(+/-)	(+)	(+/-)	(+)	(+)	(+/-)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+)	(+/-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(-)
PAN, AP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(-)	(+/-)	(X)	(X)	(-)	(X)	(+)	(+)	(+)	(+)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+)	(+)	(+/-)	(+)	(+)	(-)	(+/-)	(+/-)	(+)	(+)
CSA, LP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+)	(X)	(X)	(+/-)	(X)	(+)	(X)	(-)	(X)	(+)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+)	(-)	(-)	(+/-)	(X)	(+)	(X)	(+)	(X)	(+)
CSA, AP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(X)	(-)	(X)	(+)	(-)	(X)	(X)	(X)	(X)	(X)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(X)	(+)	(X)	(-)	(-)	(-)	(X)	(+/-)	(X)	(X)
RXU1, LP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+/-)	(X)	(X)	(+)	(+)	(+/-)	(-)	(+/-)	(+)	(+/-)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(-)	(+)	(X)	(-)	(+/-)	(-)	(+)	(-)	(+/-)	(+/-)
RXU1, AP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(X)	(X)	(X)	(+)	(+)	(X)	(+)	(+/-)	(X)	(+/-)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+)	(X)	(-)	(+/-)	(-)	(-)	(-)	(+)	(-)	(-)
RXU2, LP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(-)	(-)	(X)	(X)	(X)	(-)	(+)	(+)	(+)	(-)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(X)	(X)	(X)	(+)	(-)	(+/-)	(+/-)	(-)	(+)	(+)
RXU2, AP	Dentin middle, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(+/-)	(+/-)	(X)	(X)	(X)	(+)	(+)	(+)	(+)	(X)
	Dentin lateral, x3000									
	S01	S02	S03	S04	S05	S06	S07	S08	S09	S10
	(-)	(X)	(X)	(X)	(-)	(+)	(+)	(+)	(+/-)	(X)

Table 12 Assignment of scores in polished specimens at x3000 magnification for the criterion "Smear plugs" in the middle and lateral part of dentin. S01 – S10 are the index numbers of the specimens. The scores used: criterion detectable (+), criterion questionable (+/-), criterion not detectable (-) and failed specimen (X). (PAN = Panavia F2.0+ED Primer, CSA = Clearfil SA Cement, RXU1 = RelyX Unicem Aplicap, RXU2 = RelyX Unicem 2, LP = light-polymerization, AP = auto-polymerization.)

11. References

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