

SCIENTIFIC REPORTS



OPEN

Investigations on the adhesion of new composites for restoring cervical lesions using energy dispersive X-ray analysis and scanning electron microscopy

Alexandra Roman¹, Stefan Ioan Stratul², Darian Rusu², Marius Boariu³, Andrada Soanca ¹, Robert Balazsi⁴, Maria Suciu^{5,6}, Mărioara Moldovan⁷ & Adriana Elena Bulboacă⁸

Restoration of noncarious cervical lesions with resin composites is one of the treatment options, but the retention of the restorations located at the crown-root junction is still a cause of clinical concern. The aim of this study was to evaluate the adhesive properties of three experimental resin composites and two commercial materials used to restore cavities prepared on extracted teeth as well as to determine the relative elemental composition of these materials. We tested the null hypothesis, which considered that the adhesive behaviours of different resin composites did not differ. The microleakage test using tracers showed that all tested materials exhibited some degree of dentinal microleakage, although they all had good dentinal adhesion. The results failed to reject the null hypothesis. The scanning electron microscopy revealed completely adapted adhesive interfaces underneath the restorations along with well-developed hybrid layers depending on the adhesive system. Energy dispersive X-ray analysis analyses showed that the restorative materials have similar chemical compositions, with some differences between the samples from the same material. The results support the implementation of experimental resins in clinical settings.

Modern dentistry techniques have improved human dentation with increased longevity of teeth through improved personal oral hygiene, fluoridation, and advanced restorative procedures¹. However, performing thorough daily oral hygiene for extended periods in association with erosive aggressions, and tooth flexure will invariably wear the tooth substance away from a cement-enamel area (the neck of the tooth) and induce cervical non-carious cervical lesions (NCCLs)². Gingival consequences (e.g., gingival recession) of aggressive personal oral hygiene procedures also have been described³. When NCCLs are associated with gingival recessions and consequent root surface exposure a combined restorative-surgical treatment is needed⁴.

¹Department of Periodontology, Faculty of Dental Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, 15V. Babeș St., 400012, Cluj-Napoca, Romania. ²Department of Periodontology, Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy, 9 Bulevardul Revolutiei din 1989, 300070, Timisoara, Romania. ³Department of Restorative Dentistry and Endodontics, Faculty of Dental Medicine, Victor Babes University of Medicine and Pharmacy, 9 Bulevardul Revolutiei din 1989, 300070, Timisoara, Romania. ⁴Department of Psychology, Babes-Bolyai University, 7 Sindicatelor St, 400029, Cluj-Napoca, Romania. ⁵Electron Microscopy Integrated Laboratory (LIME), National Institute for Research and Development of Isotopic and Molecular Technologies, INCDTIM, 67-103 Donath St., 400293, Cluj-Napoca, Romania. ⁶Department of Molecular Biology and Biotechnologies, Faculty of Biology and Geology, Babeș-Bolyai University, 5-7 Clinicilor St., 400006, Cluj-Napoca, Romania. ⁷Raluca Ripan Institute for Research in Chemistry, Babes-Bolyai University, 30 Fântânele St., 400294, Cluj-Napoca, Romania. ⁸Department of Pathophysiology, 4-6 Victor Babeș St., Iuliu Hațieganu University of Medicine and Pharmacy, 15V. Babeș St., 400012, Cluj-Napoca, Romania. Alexandra Roman, Stefan Ioan Stratul, Darian Rusu and Marius Boariu contributed equally. Correspondence and requests for materials should be addressed to A.S. (email: andrapovici@gmail.com)

If ignored, NCCLs invariably enlarge¹. Identification of the putative etiological factors mediating NCCLs allows control of the extrinsic abrasive and erosive factors⁵. Subsequent restoration of crown-located NCCLs strengthens the tooth and prevents fracture, pulp involvement, caries, acid dissolution, further abrasion from tooth brushing; eliminates cervical hypersensitivity; improves aesthetics and gingival health; and eases oral hygiene maintenance for the patient¹. A good adhesion of restorative materials to dental substrates is mandatory for the long term retention of fillings restoring NCCLs. Even small marginal gaps of 1–3 μm width allow the penetration of cariogenic bacteria and/or pigments that lead to marginal discoloration. The infiltrating saliva eventually leads to debonding and retention loss of NCCL restorations⁶.

Resin-modified glass ionomer cements have the highest chance of survival for restoring NCCLs^{7,8}. Conventional composites placed with two-step etch-and-rinse adhesives also have good outcomes for restoring NCCLs^{7,9}. When restoring NCCLs with resin composites, the material adhesive properties severely impact the stability of the filling and consequently its biological effects on periodontal and dental tissues¹⁰. However, the adhesion of resin composites is a complex phenomenon that depends on the intrinsic adhesive capabilities of the material, the setting reaction, the type of adhesive system, the clinical application protocol⁵, moisture control¹¹, and the type and quality of dental substrates^{2,12}.

Conventional investigations are performed to test the adhesive properties of resin composites^{13,14}. Supplemental investigations have been proposed recently to determine specific details related to the adhesion phenomenon and the material structure. Scanning electron microscopy (SEM) provides details of the adhesive interface, and energy dispersive X-ray analysis (EDX) enables a semi-quantitative evaluation of the chemical elements on the substratum surface. EDX has been used extensively in engineering and chemistry, but is rarely used in dentistry^{15,16}.

Our team evaluated the solubility and cytotoxicity of three experimental resin composites¹⁷ that were designed by an autochthonous research institute to restore NCCLs. The aim of this study was to evaluate the adhesive properties of these experimental resin composites and two commercial materials using optical microscopy and SEM. We tested the null hypothesis, which considered that the adhesive behaviours of different resin composites and commercial materials did not differ. We also determined the relative composition of these dental materials and of their adhesive interface by performing EDX analyses.

Results

Microleakage test. Cervical class V cavities were prepared on extracted teeth and restored with three experimental (P2S, P14M, and PM) and two commercial (Ge and En) resin composites. After storing in dye solution, the teeth were sectioned and the dye penetration along the restoration-tooth interface was evaluated using 17 samples for each material. Optical microscopy was used to investigate the marginal microleakage of restorations, which was highlighted as a pink infiltrating line of various lengths along the tooth-restoration interface (Fig. 1A). The ratio of marginal microleakage and total length of adhesion interface was calculated, and a value of zero was reported whenever no leakage was recorded (a very good adhesion was present). The summary of the dye penetration values for the sections of tested materials and their ratios related to the adhesive interface length are presented in Table 1.

The effect of material type (P2S, P14M, PM, Ge and En) on the ratio of dentin microleakage length (μm) to adhesive interface length (μm) was evaluated using Kruskal-Wallis ANOVA. The material type had a non-significant effect on the measured variable at the adjusted $p < 0.01$ level for the five conditions [$F(4, 85) = 12.623, p = 0.0133$]. This result suggests that the observed differences of the ratios of dentin microleakage length of the five material types could result from random variation. The optical micrographs revealed some degree of marginal microleakage in dentin for all tested composite resins, but no dye penetration was observed for the majority of samples (Fig. 1B). No microleakage was observed into enamel.

Scanning electron microscopy results. SEM analysis showed that the PM experimental resin composite contained microfillers that were randomly distributed in the resinous matrix. A thick adhesive layer of inconstant dimensions (10–70 μm) was observed underneath the PM restorations. The granular aspect of the two-step etch-and-rinse adhesive is due to the filler content. The hybrid layer was a thin, homogenous, and more electron-dense structure of relatively uniform thickness (~2 μm) that closely followed the tooth surface and formed resinous tags ranging from 5–10 μm penetrating into the dentin (Fig. 2A). The adhesive interface underneath the P14M experimental material had a 30–90 μm thick adhesive layer, a 5 μm hybrid layer, and 2–3 μm long tags (Fig. 2C). The adhesive interfaces associated with P2S restorations had well-developed resinous tags and continuous adhesive layers: a 20–45 μm thick adhesive layer, a 5 μm hybrid layer, and tags at least 13 μm long (Fig. 2E). The hybrid filler of P14M and P2S experimental materials generally was densely packed in the resinous matrix of the restorations and had marbled appearances. Continuous adhesive interfaces were observed for all experimental materials (Fig. 2B,D,F).

The uniform tooth-restoration interface associated with Ge and En suggested good adhesion and was consistent with the microleakage test results. The hybrid layer was 10–35 μm thick, with tags at least 24 μm long. SEM observations confirmed the inhomogeneous microhybrid structure of the commercial composite Ge, which was described by the manufacturer as prepolymerized filler particles (16 nm silica particles or 400 nm strontium glass and lanthanoid fluoride) of approximately 16–30 μm diameter (larger particles also are present), submicron inorganic filler (silica glass), and randomly dispersed small particles of fumed silica. SEM observations of En restorations revealed a homogenous matrix containing rarely dispersed 10–30 μm filler particles, a single homogeneous structure for the hybrid layer of ~10 μm thickness, and resinous tags at least 50 μm long expanding into the dentinal tubuli (Fig. 3).

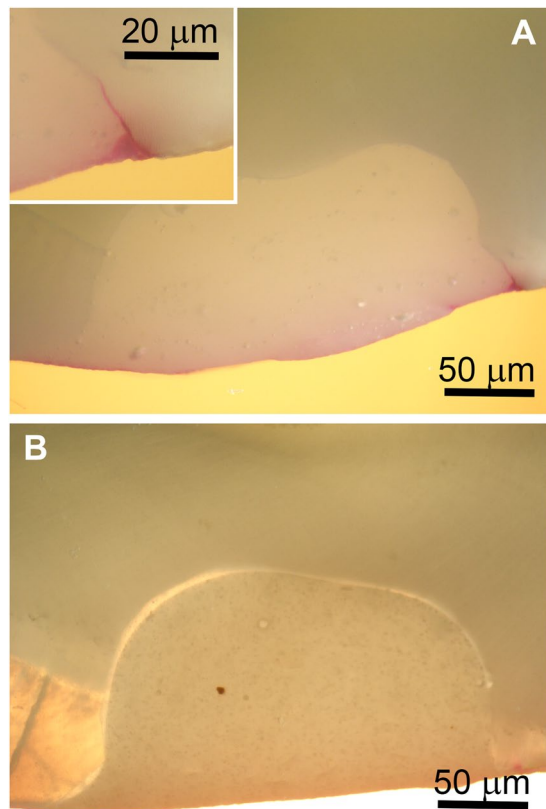


Figure 1. Microleakage test; optical microscopic images of two samples from a tooth restored with P2S composite. (A) Microleakage in dentin (40 \times). Inset, dye penetration (100 \times). (B) No leakage around restoration.

Energy-dispersive X-ray analysis. EDX analysis was performed using 10 samples for each resin composite material. Element content was determined as percent weight (%wt) in three regions of interest (ROIs) for each sample (the material layer, adhesive layer, and dentinal area) (Figs 4 and 5).

Supplementary Table S1 presents the mean values \pm SD for relative elemental content of the resin composite materials. As expected, the resin composites had different compositions. For example, silicon (Si) ranged from 18.84%wt in P2S to 12.28%wt in P14M. Barium (Ba) contents were similar in the three experimental resin composites because all contained barium oxide in the glass-based fillers. Oxygen, carbon, and silicon were the predominant elements in all composite resins. Strontium (Sr) was not detected in P14M and P2S, was detected at low levels in PM and Ge, and was detected at four times higher levels in En. Fluoride (F) had the highest content in P14M, and was not detected in En.

Discussion

The success of resin composites for restoring NCCLs depends on adhesion to the tooth structures via adhesive systems, a conservative cavity preparation approach, and very good aesthetics⁵. Shrinkage during polymerization still remains as an important shortcoming of composite restorations, leading to bonding failure and dental and periodontal consequences^{18,19}. New materials have been developed to increase the performance of restorations in cervical areas. This study investigated the adhesive capabilities of three new composite resins that were designed to restore NCCLs.

The microleakage test showed that all tested materials exhibited some degree of dentinal microleakage, although they all had comparable adhesion. The SEM micrographs of many samples revealed completely adapted adhesive interfaces underneath the restorations along with well-developed hybrid layers depending on the adhesive system. The restorative materials showed no significant differences of the adhesive performances. Therefore, these results failed to reject the null hypothesis. The data could be appreciated beyond the fact that we could not show sufficient evidence against null hypothesis. It can be observed that PM material has the best adhesion since only three samples present dentinal leakage (see Table 1) in comparison with eleven and eight samples for P2S and P14M, respectively. There are nine and fourteen samples with leakage for Ge and En, respectively. There is an important variability of the data that may be due to the differences of the intrinsic properties of dental substrates and to some errors due to material dislocation during tooth sectioning.

Our findings are consistent with those of other studies on microleakage around composite restorations²⁰. The adhesive capacity of the resin composite is one factor that may influence the microleakage of directly placed composite restorations, although the substrate structure also has an important role^{18,21}. NCCLs are associated with a heterogeneous hypermineralized dentin layer that resists etching^{22,23}, obliteration of the majority of dentinal

Material	Dentin microleakage length (μm)	Adhesive interface length (μm)	Ratio of dentin microleakage length (μm): adhesive interface length (μm)
P2S			
Median (Q1 to Q3)	103 (81 to 847)	1903 (1616 to 2060)	0.059 (0.044 to 0.495)
Mean (SD)	494 (652)	1832 (305)	0.268 (0.351)
Min to max	47 to 1639	1341 to 2202	0.021 to 0.828
n	11	17	11
P14M			
Median (Q1 to Q3)	140 (121 to 164)	1977 (1905 to 2084)	0.068 (0.059 to 0.085)
Mean (SD)	156 (79)	2001 (253)	0.079 (0.043)
Min to max	64 to 331	1411 to 2389	0.032 to 0.175
n	8	17	8
PM			
Median (Q1 to Q3)	87 (85 to 89)	2080 (2062 to 2195)	0.0411 (0.0407 to 0.0415)
Mean (SD)	87 (4)	2062 (234)	0.041 (0.001)
Min to max	83 to 90	1406 to 2248	0.0403 to 0.0420
n	3	17	3
Ge			
Median (Q1 to Q3)	123 (100 to 214)	2091 (2043 to 2257)	0.055 (0.045 to 0.104)
Mean (SD)	223 (202)	2112 (269)	0.105 (0.096)
Min to max	75 to 575	1477 to 2439	0.035 to 0.272
n	9	17	9
En			
Median (Q1 to Q3)	77 (59 to 273)	2158 (1938 to 2370)	0.040 (0.030 to 0.115)
Mean (SD)	165 (151)	2165 (263)	0.075 (0.067)
Min to max	20 to 466	1832 to 2612	0.010 to 0.216
n	14	17	14

Table 1. Summary of data corresponding to microleakage test according with material type. Q1 is the 25th percentile; Q3 is the 75th percentile; n is the number of reported samples whenever different by zero; SD is the standard deviation.

tubules that generates fewer resin tags¹², and an altered composition and microstructure of the enamel and dentin consecutive to aging process². From this point of view, one of the limitations of the present study was adhesion to the freshly cut dentin, which is an ideal substrate that does not generally reproduce the clinical circumstances of NCCLs.

A three-step etch-and-rinse adhesive system was used for the experimental resin composites because its efficacy is well established^{24,25} and superior to other adhesive systems⁷. A two-step self-etch adhesive and a one-step self-etch adhesive was used for the commercial resin composites, which were recommended by the manufacturers, even if they had a lower probability of being the best combination in NCCLs^{7,8,26}. It seems that the combination of primer and adhesive in one product reduces the bond strength and durability of the hybrid layer²⁴, thereby affecting survival of the restorations. Qualitative SEM analyses confirmed a less well-developed hybrid layer associated with one-step self-etch adhesive, although it had a well-adapted adhesive layer and good adhesion. However, the thickness of the hybrid layer is not correlated with the bond strength or the clinical performances¹⁴.

No enamel leakage was recorded in this study. Class V cavities are considered an advantageous research model to test the effectiveness of bonding systems⁸ because they offer no mechanical retention and are located primarily in dentin. This facilitates evaluation of the resin-dentin bond, which is less stable than the resin-enamel bond²⁴. In this study, the coronal cavity bevel enhanced adhesion and “protected” the dentinal adhesive interface. Further experiments omitting bevel preparation would more rigorously test the adhesion to dentin, and would provide clinically realistic information on the adhesive capacities of the materials.

Some authors question the effectiveness of the microleakage test using tracers because a correlation between dye penetration and clinical outcomes has not been proven¹⁴. However, ISO standard No.11405/mentions this type of investigation, which has otherwise been used by recently published investigations^{27–29}. The microleakage test using dye penetration was used in our experiment as it seemed to be the only method to evaluate new composite resin variants in the laboratory in order to gain some safety before clinical trial. The present experiment was standardized as much as possible in order to reduce the variability (a single operator for each important experimental step of the study).

EDX analyses determined the elemental compositions of three different structures; the restorative materials have similar chemical compositions, with some differences between the samples from the same material. This variation might be explained by the fact that EDX provide semi-quantitative results as well as by the technical limitations of EDX, which analyses surfaces with a maximum depth of 6 μm ³⁰. Some variations were related to the presence of inorganic elements in filler content of experimental materials in comparison with commercial resin composites. Barium derived from barium oxide-based glass fillers was included in experimental composites

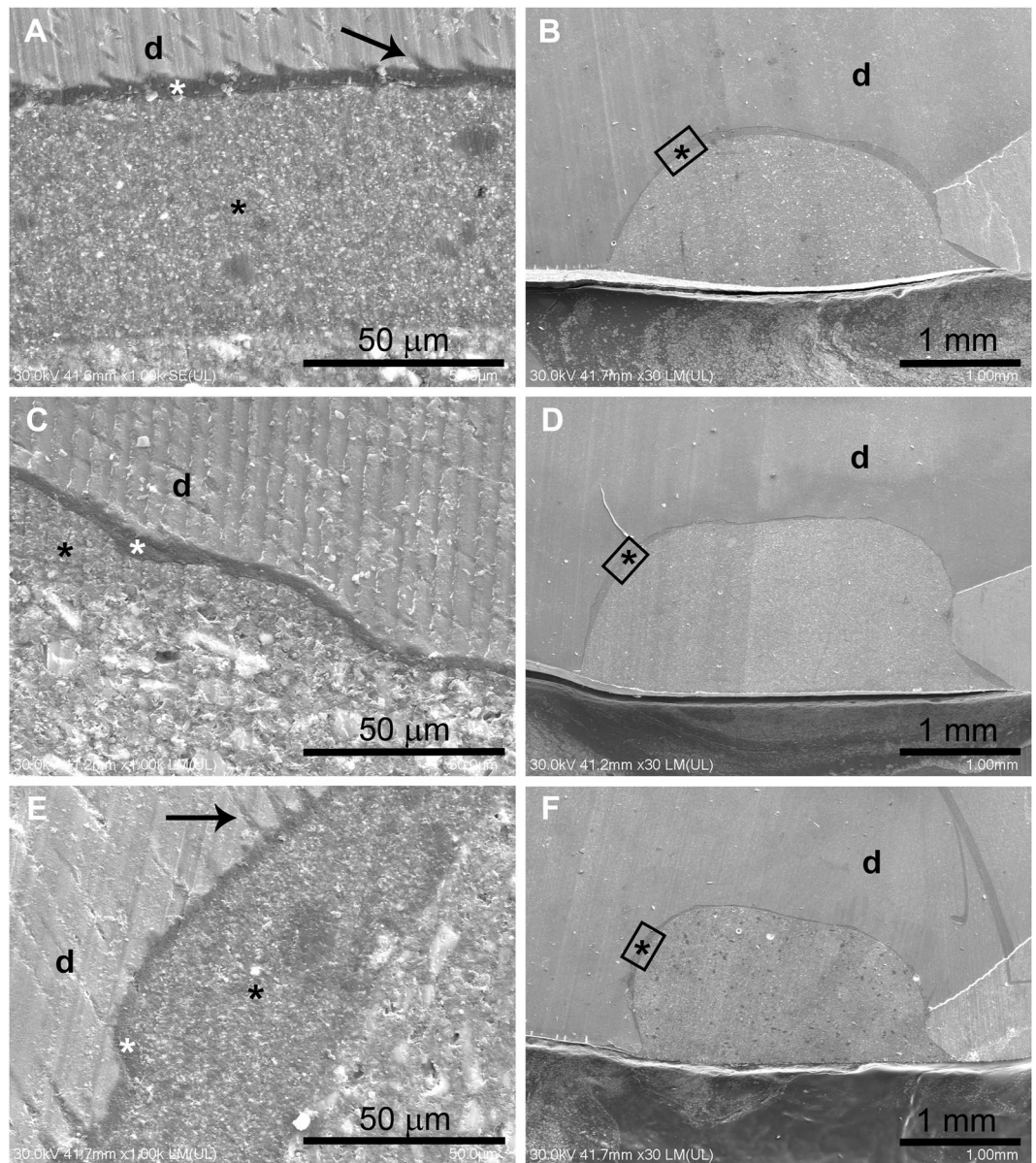


Figure 2. Scanning electron microscopy of experimental material restorations. (A) PM material structure and well-designed hybrid layer. (B) Continuous adhesive layer associated with PM material. (C) P14M material structure and adhesive interface. (D) Continuous adhesive layer associated with P14M material. (E) P2S material structure and well-designed hybrid layer. (F) Continuous adhesive layer associated with P2S material. Black arrow = resin tags; black asterisk = adhesive interface; white asterisk = hybrid layer; d = dentin. Label = the selected regions in the low magnification SEM images (B,D,F) corresponding to the magnified images (A,C,E).

to integrate into the silica network and increase the X-ray radiopacity of the materials, which reduced the need to add a separate radiopacity agent such as ytterbium fluoride³¹. For this reason, PM and P2S materials do not contain ytterbium. Barium was also derived from barium fluoride included in P14M material in order to induce a possible cariostatic effect³² through fluoride release so important in cervical areas. Levels of 0.095–0.190 ppm fluoride in saliva can be sufficient for tooth decay prophylaxis³³. Barium and strontium glasses are used to tune the refractive index of the filler particles to obtain a more transparent composite because they have a higher refractive indices than silica, which improves esthetics without altering mechanical properties³⁴. Commercial composites do not contain barium. Strontium compounds were detected at low levels in Ge; ytterbium was added in this material to provide radiopacity.

Another modification of the composition of P2S and PM filler was the introduction of small quantities of hydroxyapatite - zirconium in order to make composites take advantage of the biocompatibility of hydroxyapatite and higher strength of zirconia³⁵; an improved radiopacity was also targeted.

Based on the EDX analyses of material and adhesive compositions, we achieved consistent qualitative results. EDX results sustain the qualitative compositional references as provided by the manufacturers and which are responsible

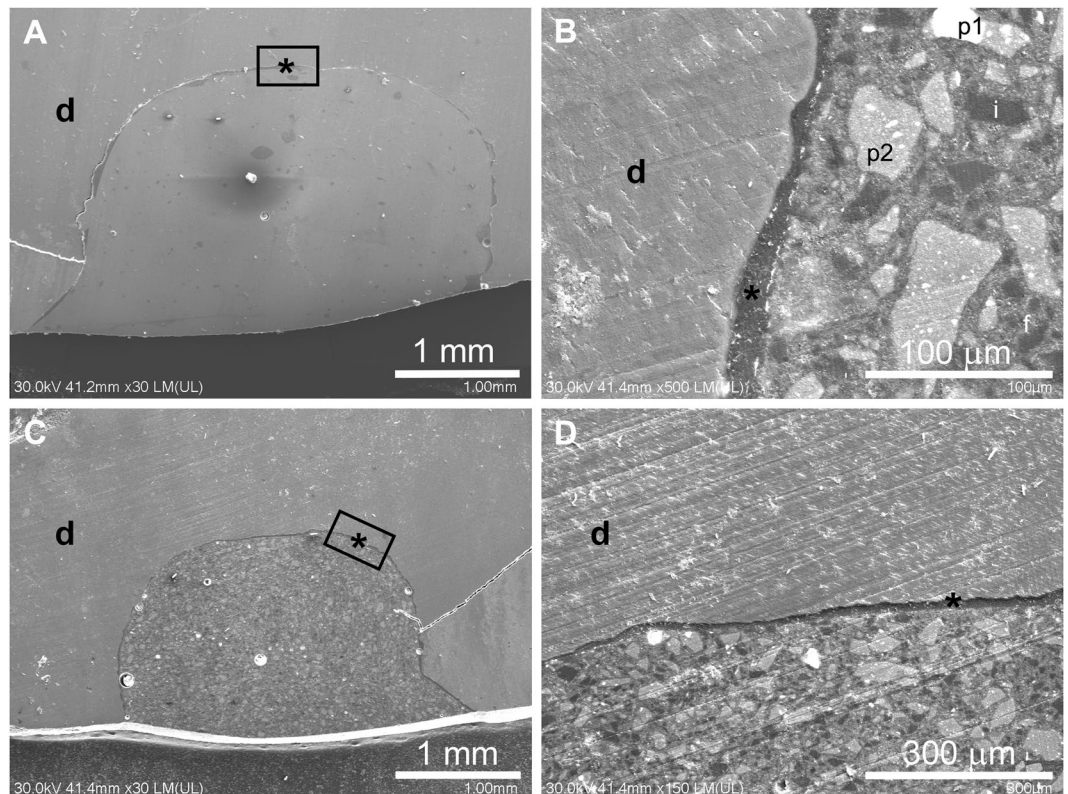


Figure 3. Electron microscopy of commercial resin composites. (A) Ge has a uniform adhesive interface (overall aspect). (B) Ultrastructure of Ge (prepolymerized filler silica based, p^2 = prepolymerized filler containing strontium glass and lanthanoid fluoride, i = condensed silica particles, f = submicron silica particles). (C) En has a uniform adhesive interface (overall aspect). (D) Ultrastructure of En and the associated adhesive interface. Black asterisk = adhesive interface; d = dentin. Label = the selected regions in the low magnification SEM images (A,C) corresponding to the magnified images (B,D).

for the materials' behaviour. Not only the filler composition is important but also the filler load highly influences the adhesive properties of the resin composites. High filler load produces less contraction on setting and less stress at the level of the adhesive interface¹⁹. The filler load is comparable for experimental and commercial materials.

The improvement of the organic component of the experimental materials was also targeted. Polycaprolactone - a biodegradable and biocompatible polymer- was introduced in PM material to enhance interfacial interaction between the polymer and the reinforcement material (filler)³⁶ in order to better resist to flexural stress developed in tooth cervical areas. Unfortunately, the presence of polycaprolactone in the organic phase was probable responsible for PM instability (lower performance at sorption/solubility tests)¹⁷ as this molecule may be degraded through a hydrolytic mechanism by oral microorganisms³⁷.

The behavior of experimental materials is the natural consequence of their composition. The present study proved comparable adhesive capacities of experimental and commercial resin composites. Previous data showed that experimental P14M and P2S composites had a good stability as proved by sorption and solubility tests; all experimental materials had a good biocompatibility in relation with oral mesenchymal stromal cells¹⁷. P14M and P2S materials possess properties that could be considered at least as good as the properties of commercial materials and could be manufactured at a more convenient price.

An interesting observation is related to some compositional differences detected by EDX in dentinal areas, which may be explained by the interindividual variability. The composition of apatites was reported to vary significantly³⁸. In the ideal hydroxyapatite composition, some Ca ions could be substituted by similar chemical elements such as Ba or Mg, and the hydroxyl groups could be substituted by F, which modifies apatite properties³⁸. This could explain the traces of these ions in dentin specimens.

The restoration of NCCLs still faces some clinical challenges². Our study provides information on good adhesive behavior (under ideal *in vitro* conditions) of experimental resin composites designed to restore NCCLs. The results support the implementation of these resins in clinical settings. EDX analyses suggest that dental substrate variability is responsible in part for differences in clinical outcomes of cervical restorations.

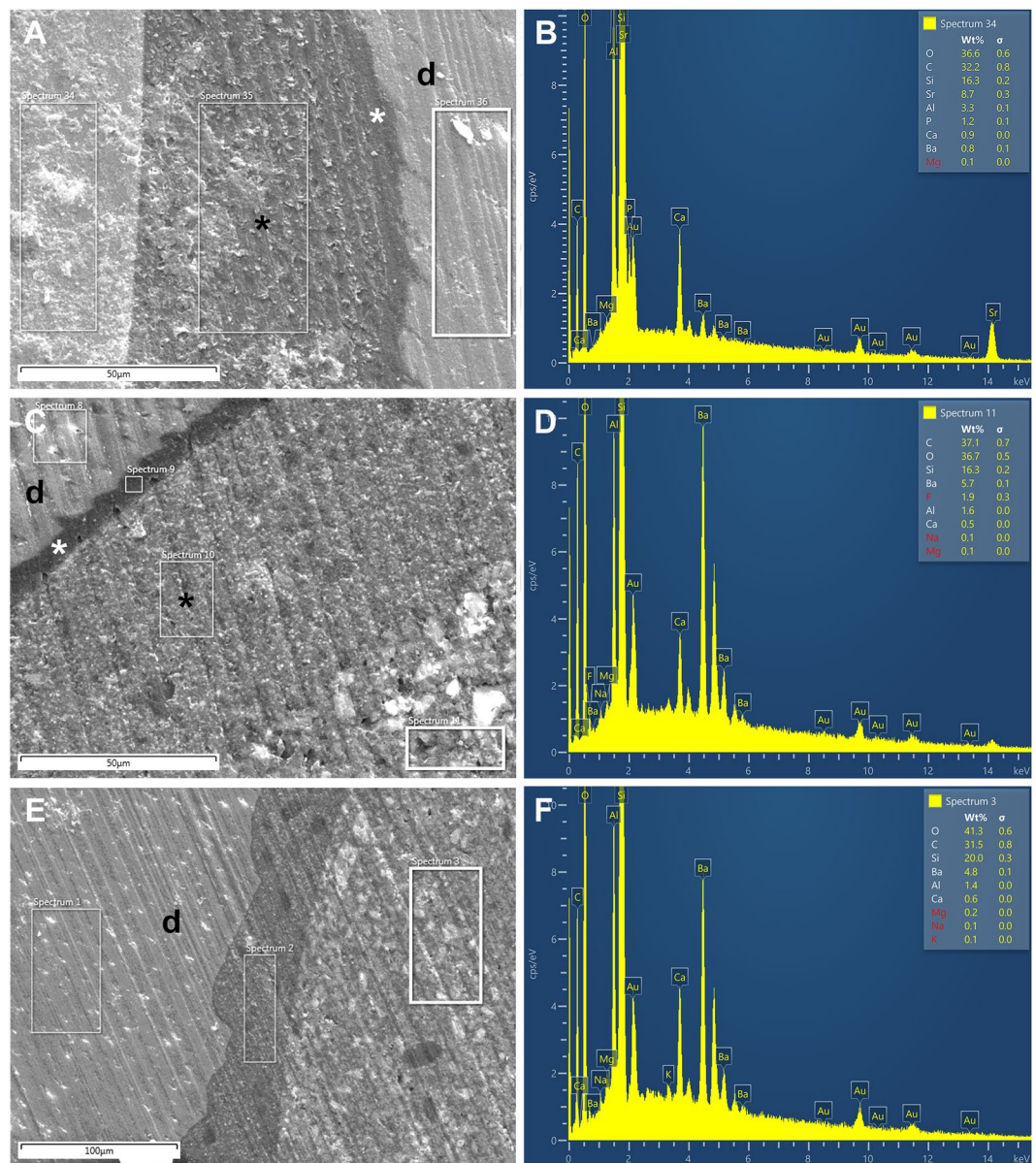


Figure 4. Scanning electron microscopy and EDX analysis of the samples. Restorations with PM (A), P14M (C), and P2S (E) materials and EDX results of the resin composites PM (B), P14M (D), and P2S (F). Black asterisk = adhesive interface; white asterisk = hybrid layer; d = dentin.

Methods

Ethics. Extracted teeth were obtained with informed consent from patients according to a protocol approved by the Ethical Board of the *Iuliu Hațieganu* University (Nos 359/13.10.2014). Tooth collections were performed in accordance with relevant guidelines and regulations. Original clinical indications for tooth extractions were not related to the present study.

Resin composites and adhesive systems. Three experimental and two commercial light-curing composite resins, shade A3, were used in this *in vitro* study to restore NCCLs (see Supplementary Table S2).

Cavity preparation, restoration, and sample preparation. Premolars that were extracted for orthodontic reasons and free of any lesions or previous restorations were used in the present experiment. They were cleaned to remove any debris, attached soft tissues, and calculus, and stored in 4% chloramine-T until use. The teeth were used within one month after extraction¹³. A total of 55 teeth were used for the experiments, with 6 teeth used for each material for microleakage test and 5 teeth for each material used for SEM + EDX investigation.

Standard class V cavities were prepared with a mesiodistal width of 3 mm, occluso-gingival height of 3 mm, and axial depth of 1.5 mm, with the gingival margin located 1 mm below the cement-enamel junction (CEJ). The cavities were prepared on the buccal surface of teeth using diamond-coated burs (Komet S6801.314.014; Komet, Lemgo, Germany) mounted on a high speed hand-piece with air and water cooling (see Supplementary Fig. S1).

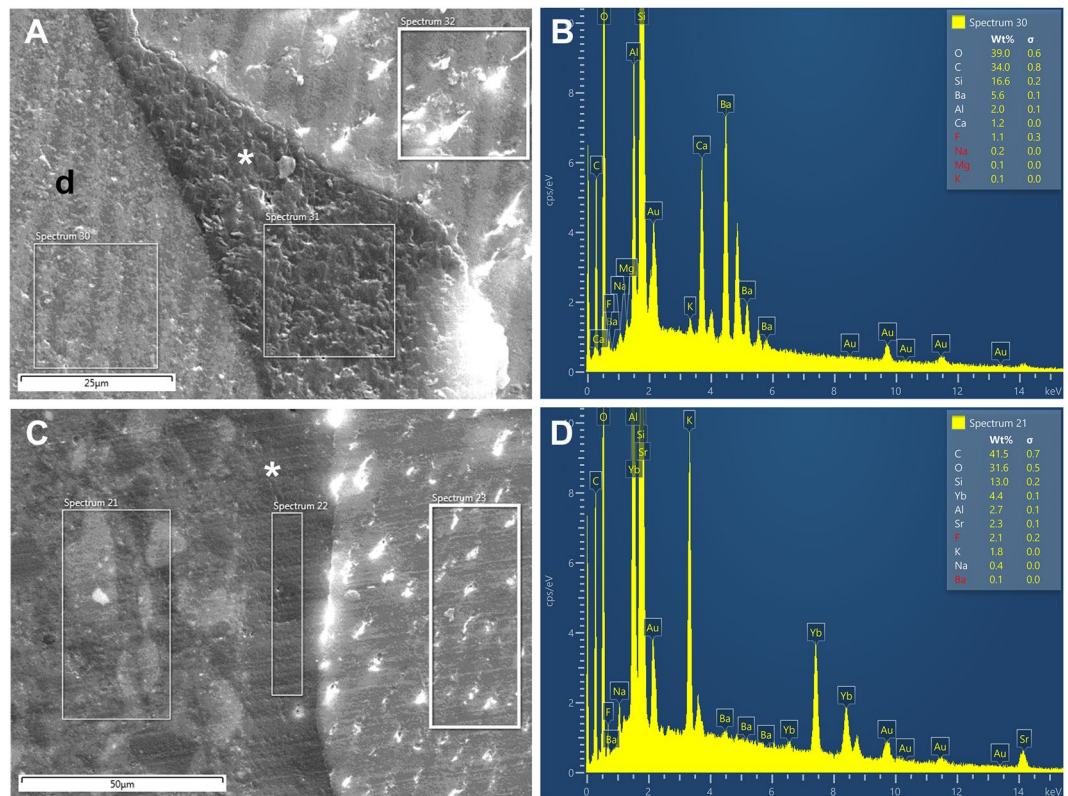


Figure 5. Scanning electron microscopy of samples restored with En (A) and Ge (C), and EDX analysis of the resin composites En (B) and Ge (D). White asterisk = hybrid layer; d = dentin.

Each bur was discarded after preparing five cavities. The prepared teeth were subsequently stored in distilled water as described previously³⁹.

Each group of teeth was restored according to the manufacturer's specifications, including adhesive applications. The adhesives were light-cured for 20 seconds. The cavities were restored using bulk technique, and the composite resins were light-cured for 40 seconds (Demetron A2 light-curing unit, Kerr Corporation, wavelength 450–470 nm, and light intensity of 1000 mW/cm²). After finishing and polishing all restorations, the restored teeth were kept in distilled water at 37 °C for 24 hours before microleakage and SEM analyses.

Microleakage test. The root apices of the restored teeth were sealed with utility wax. The entire surface of each tooth was coated with two layers of varnish, except for a 1 mm width around the margins of the restorations. The teeth were immersed in 0.5% basic fuchsin solution for 24 hours, then rinsed thoroughly in running water for approximately 10 minutes. The teeth were sectioned in the mesiodistal direction using a low-speed diamond saw (Isomet, Buehler Ltd. Illinois, USA), resulting in three sections of 1 mm width through the middle of the restoration (see Supplementary Fig. S1). A total of 85 sections were evaluated.

Dye penetration at the gingival margin along the tooth-resin interface was evaluated with an inverted microscope (Olympus KC301, Olympus America, Inc.) at 40× magnification. Microleakage values were recorded using QuickPhoto Micro 2.2 software (Olympus, Inc). The extent of microleakage at the tooth-resin interface was quantified using a method previously developed by our research team^{20,39} as variant of a gap measurement approach previously described⁴⁰.

Microleakage measurements (in µm) included the length of dye (pink) penetration (marked with a green line) relative to the total length of the tooth-restoration interface (marked with a yellow line). The microleakage proportion was calculated by the ratio (% µm) of the dye penetration length and the tooth-restoration interface length (see Supplementary Fig. S1).

Scanning electron microscope (SEM) and energy-dispersive X-ray (EDX) analyses. Two dentin specimens from each tooth, which represents 10 specimens from each tested material, were prepared for SEM and EDX analyses. The same specimens were used for both analyses. The samples were dried in a graded ethanol series (50–100%), and then sputter-coated with gold. SEM was performed under high-vacuum conditions (Hitachi SU8230 STEM). Two photomicrographs were captured for each section to observe details at the tooth-restoration interface (the intimate adaptation of the adhesive-resin layer to the dentine, the hybrid layer) and the materials' structures. The photomicrographs were analyzed by a single examiner (LB) using a blinded protocol, and measurements of the hybrid layers and resinous tags penetrating the dentin were performed using ImageJ software. EDX was performed using a Hitachi SU8230 STEM under the following conditions: 30 kV acceleration voltage, 10 µA extraction current, 15 mm working distance, Oxford Instruments EDS detector placed inside the sample

chamber, and AZtec Software. Measurements were performed at the same magnifications of similar surfaces (three ROIs) for all samples: the healthy tooth, the added restorative material, and the transition material between the healthy tooth and the restorative material. Ten measurements for each material were averaged to provide a single mean value for each parameter for each specimen.

Data analysis. The microleakage, adhesive lengths, and the ratio of dentin microleakage length (adhesive interface length) were reported as median and associated quartiles (Q1 as the 25th percentile and Q3 and the 75th percentile), average and standard deviation, and minimum and maximum calculated whenever measurements were different by zero. The ratios of dentin microleakage lengths (μm) were compared using Kruskal-Wallis ANOVA, with the material type (P2S, P14M, PM, Ge, and En) as independent variable. Results were considered as significant at $\alpha^* = 0.05/5 = 0.01$. For EDX analysis, the average concentrations of elements and standard deviations were calculated for each material.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

References

- Grippio, J. O. Noncarious cervical lesions: the decision to ignore or restore. *J. Esthet. Dent.* **4**(Suppl), 55–64 (1992).
- Lai, Z. Y., Zhi, Q. H., Zhou, Y. & Lin, H. C. Prevalence of non-carious cervical lesions and associated risk indicators in middle-aged and elderly populations in Southern China. *Chin. J. Dent. Res. Off. J. Sci. Sect. Chin. Stomatol. Assoc. CSA* **18**, 41–50 (2015).
- Heasman, P. A., Holliday, R., Bryant, A. & Preshaw, P. M. Evidence for the occurrence of gingival recession and non-carious cervical lesions as a consequence of traumatic toothbrushing. *J. Clin. Periodontol.* **42**(Suppl 16), S237–255 (2015).
- Santamaria, M. P. *et al.* Resin composite plus connective tissue graft to treat single maxillary gingival recession associated with non-carious cervical lesion: randomized clinical trial. *J. Clin. Periodontol.* **43**, 461–468 (2016).
- Perez, C. D. R. *et al.* Restoration of noncarious cervical lesions: when, why, and how. *Int. J. Dent.* **2012**, 687058 (2012).
- Heintze, S. D., Blunck, U., Göhring, T. N. & Rousson, V. Marginal adaptation *in vitro* and clinical outcome of Class V restorations. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **25**, 605–620 (2009).
- Schwendicke, F. *et al.* Directly Placed Restorative Materials: Review and Network Meta-analysis. *J. Dent. Res.* **95**, 613–622 (2016).
- Peumans, M., De Munck, J., Mine, A. & Van Meerbeek, B. Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **30**, 1089–1103 (2014).
- Albuquerque, N. L. G. *et al.* Four-year randomized clinical trial of oxalic acid pretreatment in restorations of non-carious cervical lesions. *Clin. Oral Investig.* **20**, 199–205 (2016).
- Leknes, K. N. The influence of anatomic and iatrogenic root surface characteristics on bacterial colonization and periodontal destruction: a review. *J. Periodontol.* **68**, 507–516 (1997).
- Perez, C. R. Alternative technique for class V resin composite restorations with minimum finishing/polishing procedures. *Oper. Dent.* **35**, 375–379 (2010).
- Zhou, L., Tan, J., Hu, B. & Feng, H. Ultrastructural study of sclerotic dentin in non-carious cervical lesions disposed by a total-etching dentin adhesive. *Beijing Da Xue Xue Bao* **36**, 319–321 (2004).
- ISO/TS 11405:2003. ISO Available at, <http://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/03/14/31486.html>. (Accessed: 8th January 2019)
- Heintze, S. D. Clinical relevance of tests on bond strength, microleakage and marginal adaptation. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **29**, 59–84 (2013).
- Paradella, T. C., Koga-Ito, C. Y. & Jorge, A. O. C. Ability of different restorative materials to prevent *in situ* secondary caries: analysis by polarized light-microscopy and energy-dispersive X-ray. *Eur. J. Oral Sci.* **116**, 375–380 (2008).
- Mathew, S. P., Pai, V. S., Usha, G. & Nadig, R. R. Comparative evaluation of smear layer removal by chitosan and ethylenediaminetetraacetic acid when used as irrigant and its effect on root dentine: An *in vitro* atomic force microscopic and energy-dispersive X-ray analysis. *J. Conserv. Dent. JCD* **20**, 245–250 (2017).
- Roman, A. *et al.* Cytotoxicity of Experimental Resin Composites on Mesenchymal Stem Cells Isolated from Two Oral Sources. *Microsc. Microanal. Off. J. Microsc. Soc. Am. Microbeam Anal. Soc. Microsc. Soc. Can.* **22**, 1018–1033 (2016).
- Duquia, Rde. C., Osinaga, P. W., Demarco, F. F., de V Habekost, L. & Conceição, E. N. Cervical microleakage in MOD restorations: *in vitro* comparison of indirect and direct composite. *Oper. Dent.* **31**, 682–687 (2006).
- Gonçalves, F., Kawano, Y. & Braga, R. R. Contraction stress related to composite inorganic content. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **26**, 704–709 (2010).
- Popovici, A. *et al.* A microleakage comparative study for indirect composite restorations on posterior teeth. *J. Optoelectron Adv Mater.* **11**, 490–493 (2009).
- Raskin, A., D'Hoore, W., Gonthier, S., Degrange, M. & Déjou, J. Reliability of *in vitro* microleakage tests: a literature review. *J. Adhes. Dent.* **3**, 295–308 (2001).
- Karan, K., Yao, X., Xu, C. & Wang, Y. Chemical profile of the dentin substrate in non-carious cervical lesions. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **25**, 1205–1212 (2009).
- Palamara, J. E. A., Palamara, D., Messer, H. H. & Tyas, M. J. Tooth morphology and characteristics of non-carious cervical lesions. *J. Dent.* **34**, 185–194 (2006).
- De Munck, J. *et al.* A critical review of the durability of adhesion to tooth tissue: methods and results. *J. Dent. Res.* **84**, 118–132 (2005).
- Chee, B., Rickman, L. J. & Satterthwaite, J. D. Adhesives for the restoration of non-carious cervical lesions: a systematic review. *J. Dent.* **40**, 443–452 (2012).
- Heintze, S. D., Rousson, V. & Hickel, R. Clinical effectiveness of direct anterior restorations—a meta-analysis. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **31**, 481–495 (2015).
- Pedram, P., Hooshmand, T. & Heidari, S. Effect of Different Cavity Lining Techniques on Marginal Sealing of Class II Resin Composite Restorations. *In Vitro. Int. J. Periodontics Restorative Dent.* **38**, 895–901 (2018).
- Politi, I., McHugh, L. E. J., Al-Fodeh, R. S. & Fleming, G. J. P. Modification of the restoration protocol for resin-based composite (RBC) restoratives (conventional and bulk fill) on cuspal movement and microleakage score in molar teeth. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **34**, 1271–1277 (2018).
- McHugh, L. E. J., Politi, I., Al-Fodeh, R. S. & Fleming, G. J. P. Implications of resin-based composite (RBC) restoration on cuspal deflection and microleakage score in molar teeth: Placement protocol and restorative material. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **33**, e329–e335 (2017).
- Mota, E. G., Hörlle, L., Oshima, H. M. & Hirakata, L. M. Evaluation of inorganic particles of composite resins with nanofiller content. *Stomatologija* **14**, 103–107 (2012).
- Watts, D. C. Radiopacity vs. composition of some barium and strontium glass composites. *J. Dent.* **15**, 38–43 (1987).

32. Duggal, M. S., Chawla, H. S. & Curzon, M. E. A study of the relationship between trace elements in saliva and dental caries in children. *Arch. Oral Biol.* **36**, 881–884 (1991).
33. Zero, D. T. *et al.* Fluoride concentrations in plaque, whole saliva, and ductal saliva after application of home-use topical fluorides [published erratum appears in *J Dent Res* 1993 Jan;72(1):87]. *J. Dent. Res.* **71**, 1768–1775 (1992).
34. Habib, E., Wang, R., Wang, Y., Zhu, M. & Zhu, X. X. Inorganic Fillers for Dental Resin Composites: Present and Future. *ACS Biomater. Sci. Eng.* **2**, 1–11 (2016).
35. Evis, Z. Reactions in hydroxylapatite–zirconia composites. *Ceram. Int.* **33**, 987–991 (2007).
36. Griffith, L. G. Polymeric biomaterials. *Acta Mater.* **48**, 263–277 (2000).
37. Deng, C. *et al.* Preparation and mechanical property of poly(ϵ -caprolactone)-matrix composites containing nano-apatite fillers modified by silane coupling agents. *J. Mater. Sci. Mater. Med.* **21**, 3059–3064 (2010).
38. Kunin, A. A., Evdokimova, A. Y. & Moiseeva, N. S. Age-related differences of tooth enamel morphochemistry in health and dental caries. *EPMA J.* **6**, 3 (2015).
39. Soană, A. *et al.* Microscopic evaluation of the interface between composite biomaterials and dentin biostructure. *Digest Journal of Nanomaterials and Biostructures* **6**, 383–392 (2011).
40. Mixson, J. M., Richards, N. D. & Mitchell, R. J. Effects of dentin age and bonding on microgap formation. *Am. J. Dent.* **6**, 72–76 (1993).

Acknowledgements

This research was supported by a grant from the Romanian Ministry of National Education and Scientific Research [grant PNII-PT-PCCA-2013-4-1474, no. contract: 127/2014]. The SEM and EDX analyses were supported by the POS CCE 2.2.1 623/11.03.2014-CETATEA project. We are grateful to *BioMed Proofreading LLC* for their help with the manuscript, and Dr. Adrian Florea for his assistance in preparing the illustrations.

Author Contributions

A.R. planned the study, principal investigator- cavity preparation, data acquisition, wrote the manuscript; S.I.S. and D.R. planned the study, checked the manuscript, documentation, data interpretation; M.B. principal investigator- cavity restorations, documentation, correction to the manuscript; A.S. principal investigator- sample preparation, microinfiltration analysis and measurements; R.B. planning data acquisition, statistical analysis; M.S. SEM observations, EDX measurements. M.M. experimental material preparation; A.E.B. study coordinator, supervised data acquisition, wrote the manuscript, tables. All authors reviewed the manuscript.

Additional Information

Supplementary information accompanies this paper at <https://doi.org/10.1038/s41598-019-46340-3>.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2019