

Ph.D Thesis

**INVESTIGATIONS ON RESIN-BASED RESTORATIVE MATERIALS FOCUSING ON
THEIR POLYMERIZATION AND BIOCOMPATIBILITY**

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INTRODUCTION

The resin-based restorative materials, also known as composite materials are essential in today's dentistry. Composites have an extremely wide field of indication by excellent mechanical properties and aesthetics. Since their introduction, several enhancements have gone through the composites. These developments aimed to synchronize the clinical demands and the material's properties. However, despite a lot of developments, inadequate polymerization is still an unsolved problem. Moreover, insufficient polymerization can cause associated mechanical and chemical degradation of composites, which call into question the biocompatibility of the material. Many factors affect the polymerization rate of the resin, such as composition, type, and polarity of the monomer molecules contained; the filler volume fraction and the type; concentration of photoinitiators; and the light and energy properties delivered on the material and the applied resin layer thickness so the depth of cure. The degree of conversion (DC) is generally accepted to correlate with the elution of unreacted monomers; however, it is not known how big a difference in DC is required to produce a noticeable change in monomer elution. Further unsolved problem is the polymerization shrinkage and its consequent phenomena which can reduce the longevity of the resin-based restorations. Investigations of composites target the broader understanding of the material polymerization kinetics, biological effects, and physical properties. Besides the several *in vitro* experiments, the number of the *in vivo* tests is negligible, although *in vivo* experiments provide relevant information on changes in the composites wear. Short- and long-term assessments provide data for the most commonly occurring defects. These experiences guide the further developments and help the dentist to reduce the failures to a minimum and increasing the lifetime of the restoration and the patient's satisfaction.

OBJECTIVES

The Ph.D. thesis has three main objectives, which are as follows:

- The objective of our *in vitro* study was to analyze the correlation between the quantity of eluted monomers using reverse-phase High Performance Liquid Chromatography (HPLC) and the DC of resin-based composite using micro-Raman spectroscopy. Experiments were done to evaluate how the energy of the light used for illumination influences the polymerization and how the polymerization performed in layers with different thickness and depth are affected by the illumination.
- The purpose of our first retrospective study to assess the 5 years old Class II direct composite restorations – made from Filtek Z250 microhybrid composite - according to USPHS criteria. Further aims were to evaluate the most frequent defects after 5 years and determine their correlation with the size of the restoration and to compare the frequency of the defects in the molar and the premolar regions.

- The aim of our second retrospective study was to investigate the longevity of Class II posterior restorations, according to the USPHS criteria, in clinical practice using 4 microhybrid RCs with slightly different filler types and resin matrix characteristics. Further objectives were to evaluate the most frequent defects and determine their correlation with the size and material of the restoration and to compare the frequency of the defects in the molar and the premolar teeth. The null hypothesis stated that, when placed in Class II preparations in adults, the durability of the direct placement of RC restorations from 4 microhybrid RC materials in molars and premolars with different cavity types would not be significantly different after 10 years.

MATERIALS AND METHODS

Quantification of conversion degree and monomer elution from dental composite using HPLC and micro-Raman spectroscopy

For the evaluation of the effect of light exposure time Filtek Z250 (3M ESPE, St. Paul, MN, USA, A2 shade) composite resins were poured into a stainless steel mold with a size of 2 mm in diameter x 2 mm in thickness (n = 20) and positioned on a glass slide. The specimens were irradiated with Light Emitting Diode (LED) curing unit (LED.C, Woodpecker, Guilin, China) with 20 s (n = 15) and 40 s (n = 5) exposure time at light intensity of 1000 mWcm⁻² with an irradiated diameter of 10 mm. The light intensity of the LED light source was monitored before and after curing with a radiometer (SDS, Kerr, Danbury, CT, USA). 5 specimens, cured with 20 s exposure time, were post-cured in a xenon polymerization furnace (Dentacolor XS, Heraeus Kulzer GMBH, Hanau, Germany) for 90 s and further 5 specimens for 180 s. The light intensity of the furnace was 250 mWcm⁻², the wavelength was 320-520 nm. After polymerization, the specimens were analyzed with micro-Raman spectroscopy and afterwards stored in 1 mL of 75 % ethanol/water solution for 72 h in darkness at room temperature for further dissolution of the unreacted monomers. The resins were removed after 72 h, and the ethanol solutions containing dissolved unpolymerized monomers were analyzed with reverse-phase high-performance liquid chromatography (RP-HPLC). BisGMA (Bisphenol A diglycidil ether dimethacrylate, 98%), UDMA (Urethane-dimethacrylate, ≥ 97%) and TEGDMA (Triethylene glycol dimethacrylate, 95%) (Sigma-Aldrich, Steinheim, Germany) were used as standard materials for the identification of the monomer peaks in the chromatograms. Acetonitrile (ACN) (VWR International, Leuven, Belgium) was used for the preparation of the mobile phase for the HPLC separation.

For the evaluation of the depth of polymerization uncured Filtek Z250 composite was placed in a cylindrical mould with 4 mm in diameter and 4 mm in height. The material was covered with Mylar strip and pressed with a glass slab to force the composite to adapt to the confines of the mould. Photo-activation was performed using LED unit (with the above mentioned parameters) with 20 s exposure

time. After photo-activation, the cylinder of cured composite was fixed to an acrylic base with soft wax, and transversally cut with a rotary saw (model 650, South Bay Technology Inc., San Clemente, CA, USA), using a diamond disc under water spray. Sections provided specimens representing the 1-4 mm layer thicknesses. After sectioning the specimens were analyzed with the same procedures that were described above.

The polymerized composite samples were examined using Labram HR 800 Confocal Raman spectrometer (HORIBA Jobin Yvon S.A.S., Longjumeau Cedex, France). The following sets of parameters were applied during the micro-Raman measurements: 20 mW He-Ne laser with 632.817 nm wavelength, spatial resolution $\sim 1.5 \mu\text{m}$, spectral resolution $\sim 2.5 \text{ cm}^{-1}$, magnification $\times 100$ (Olympus UK Ltd., London, UK). Three point spectra were taken on the top surface of the composite specimens at random locations with 10 s integration time and six acquisitions were averaged for each geometrical point. Spectra of uncured composite were taken as reference. Post-processing of spectra was performed using the dedicated software LabSpec 5.0 (HORIBA Jobin Yvon S.A.S., Longjumeau Cedex, France) and including the band fitting procedure using the Levenberg-Marquardt method of non-linear peak fitting for the best fit [15, 16]. The ratio of double-bond content of monomer to polymer in the composite was calculated according to the following equation:

$$DC\% = (1 - (R_{\text{cured}} / R_{\text{uncured}})) \times 100$$

where R is the ratio of peak intensities at 1639 cm^{-1} and 1609 cm^{-1} associated to the aliphatic and aromatic (unconjugated and conjugated) C-C stretching in cured and uncured composites, respectively.

The RP-HPLC instrument consists of a Dionex P680 gradient pump, Rheodyne 8125 injection valve, and a Dionex UVD 170U UV-Vis detector (Dionex GmbH, Germering, Germany). Data acquisition was completed using Chromeleon software (version: 6.60 SP3 Build 1485). The separations were performed on a Kovalis (particle size: $6 \mu\text{m}$, pore size: 11 nm , Zeochem, Uetikon, Switzerland) ODS (C_{18}) column with gradient elution. Eluent "A" was 40 % *v/v* ACN in bidistilled water, whereas eluent "B" contained 95 % *v/v* ACN. The applied gradient required 30 min, during which time the "B" eluent content increased from 20 to 100 %. The flow rate was 1.2 mLmin^{-1} . The separation was followed by regeneration. After 30-31 min, the "B" eluent content decreased from 100 % to 20 %, and after 31-46 min, the system was washed with 100 % "A". Chromatograms were monitored at 205 nm, 215 nm, 227 nm, and 254 nm, and among these, 205 nm was found to be optimal; therefore, the evaluation relied on the data collected at this wavelength. The residual monomers of BisGMA, UDMA and TEGDMA were calculated from the calibration curve ($R^2 \approx 0.998$) using the areas under the curve of peaks produced by BisGMA, UDMA and TEGDMA. The TEGDMA, UDMA and BisGMA standard solutions had retention times of 7.59 min, 11.34 and 13.50 min respectively, whereas the peaks were well separated from each other. All measurements of residual monomers were performed at room temperature.

The statistical analysis was performed using SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, USA) software for Windows. The values for degree of conversion and for residual monomers between the studied test groups were compared by a one-way analysis of variance (ANOVA) test followed by post-hoc test (Tukey's and Dunnett's T3).

5 year retrospective evaluation of direct Class II. composite restorations using USPHS criteria

The study protocol was approved by the Regional Research Ethics Committee of University of Pécs (3410.1./2009). For this retrospective study, 85 adult patients (52 female, 33male) were selected according to pre-determined inclusion criteria from the registers, from Apryl 2004 to May 2005. 65 restorations placed in premolars and 175 restorations placed in molars – total n=240 – mesio-occlusal (MO), occluso-distal (OD) and mesio-occluso-distal (MOD) were evaluated. Each patients got 1-4 restorations (1 filling n=22, 2 fillings n=6, 3 fillings n=22, 4 fillings n=35) from Filtek Z250 (3M ESPE, St Paul, MN, USA) microhybrid resin composite with successive cusp build-up technique. The 5 years old restorations were evaluated using USPHS quality criteria. The following characteristics of the restorations were estimated: secondary caries, fracture, color match, marginal discoloration, anatomic form, marginal integrity and surface texture. The characteristics were assessed according to the following criteria:

Alpha (A) – restoration without changes or clinical remarks.

Bravo (B) – restoration with changes that are clinically acceptable and without need for replacement.

Charlie (C) – restoration with major changes that require the replacement of the restoration, which were clinically unacceptable.

The data collection and the statistical analysis were performed using SPSS for Windows 17.0 (SPSS, Chicago, IL, USA). Descriptive statistics were used to describe the frequency distributions of the evaluated criteria and the reasons for failure. Qualitative analysis based on the USPHS criteria was analyzed independently for each of the 7 evaluated clinical characteristics. Differences in the qualitative criteria were analyzed using Fisher's Exact Test. Furthermore, Pearson's Chi-Square Test was applied to evaluate the influence of the tooth type and cavity size on the results. The hypothesis was rejected at the 5% level.

Retrospective evaluation of four microhybrid posterior direct resin composite restorations: 10-year findings

For this retrospective study, 225 adult patients were selected according to pre-determined inclusion criteria from the registers, from January 2001 to December 2003. The inclusion criteria were the following: good oral hygiene, absence of any pulpal and periodontal disease from the tooth to be

restored, absence of known allergic symptoms for dental resins, being able to control the moisture during the restorative procedure. Furthermore, patients who were selected for the study had full dentition and normal occlusion, as verified by the clinical and radiographic records, and these patients had remained in continuous clinical follow-up for the last 9-11 years, including at least 1 annual recall without attending other dentists. Reasons for placement of composite were primary caries and the choice of using resin-based composite and not amalgam was requested by the patients because of esthetic or non-metallic reasons. Further requirements had to be fulfilled in order for the placement of composite restoration: the oro-vestibular size of the cavity should not be bigger than the 1/3-2/3 of the oro-vestibular cusp-cusp distance; the margins are placed on enamel; there were no missing cusps. The restorations were placed using one of the 4 microhybrid RCs (Filtek Z250 - 3M ESPE, St Paul, MN, USA, Gradia Direct - GC America, Inc., Alsip, IL, USA, Herculite XR - Kerr, Orange, CA, USA, Renew - Bisco Inc., Schaumburg, IL, USA) composed of slightly different material properties. The patients gave their written, informed consent prior to the start of the clinical evaluation, and 2 authors (EL and TF) carried out the clinical examinations. The study protocol was approved by the Regional Research Ethics Committee of University of Pécs (3410.1./2009). The patients had 701 Class II RC restorations in their permanent molars and premolars. The patient group consisted of 86 male and 139 female patients, with ages ranging from 21 to 55 years old. 342 restorations were made in molar teeth and 359 in premolars. All restorations were placed by one operator (EL) between 2001 and 2003 with successive cusp build-up technique. Each steps of the restorative procedure were performed according to the manufacturer's instructions. The history of the restorations was initially investigated from the dental records. If a restoration had failed, resulting in either replacement or repair, it was considered a failure, and both the data and the reason for failure were recorded. Replacements or repairs due to caries in a non-filled surface of a tooth with an acceptable RC were not considered reasons for failure. The restorations were then clinically evaluated by two examiners between October 2012 and December 2012 using an explorer and a dental mirror. The dentists were trained and calibrated before the start of the evaluation. When disagreements arose during the evaluations, a consensus was obtained among the examiners. The evaluation was performed according to the USPHS guidelines. The following characteristics of the restorations were assessed: secondary caries, fracture, color match, marginal discoloration, anatomic form, marginal integrity and surface texture. The characteristics were assessed according to the above mentioned criteria (A, B, C).

The surfaces were dried with an air stream before evaluation, except for color scoring. Approximal surface control was performed with the help of dental flosses and with a Gottlieb probe. Radiographs were only made in those cases when the clinical examination indicated (by the patient's complaints, marginal gap formation especially gingivally, shadow under the sound enamel near the restoration, food retention interproximally, high level of plaque accumulation especially interproximally) that it

was necessary for the completion of the examination, in order to avoid unnecessary radiation exposure.

The data collection and the statistical analysis were performed using SPSS for Windows 17.0 (SPSS, Chicago, IL, USA). Descriptive statistics were used to describe the frequency distributions of the evaluated criteria and the reasons for failure. Qualitative analysis based on the USPHS criteria was analyzed independently for each of the 7 evaluated clinical characteristics. Differences in the qualitative criteria between the 4 materials were analyzed using Fisher's Exact Test. Furthermore, Pearson's Chi-Square Test was applied to evaluate the influence of the material, tooth type and cavity size on the results. The hypothesis was rejected at the 5% level. The analysis of the survival of the restorations was performed with the Kaplan-Meier method.

RESULTS

Quantification of conversion degree and monomer elution from dental composite using HPLC and micro-Raman spectroscopy

The degree of conversion for the composite material after different exposure time showed the following order: 46.28 % after illumination at 20 s with LED, 53.99 % after exposure at 40 s with LED polymerization, 54.87 % after illumination at 20 s LED followed by 90 s polymerization in the furnace and 55.3 % after 20 s LED, followed by 180 s polymerization in the furnace. On the Raman spectra the polymerization effect can be followed by decreasing the intensity of the Raman peak at 1639 cm^{-1} , while considerable increase of the Raman peak located at 1609 cm^{-1} can be observed. This spectral change is associated to the aliphatic to aromatic conversion of the material and reflects the formation of polymeric structure. Investigating the effect of exposure time on rate of polymerization the increase of obtained Raman peak intensity at 1609 cm^{-1} happens rapidly, while the intensity of the other peaks decreases stepwise. The difference in DC % was significant ($p < 0.05$) between 20 s and 40 s exposure times, however post-polymerization in the furnace did not provide significantly increased DC %.

The mean values of the degree of conversion for four increments of composite after illumination at 20 s with LED curing unit showed the following order: in the uppermost 1 mm 56.68%, at 2 mm 50.42%, in 3 mm deep 45.13% and in the 4 mm bottom 17.78%. Evaluating the depth of cure, the change of obtained Raman peaks can be followed continuously. As the layer thickness of the composite increased the degree of polymerization decreased. Statistically significant differences in DC % ($p < 0.05$) associated to the different layer thicknesses were obtained.

The exposure time affect the amount of extractable monomers subjected to 1 mg composite, as follows:

at 20 s illumination 0.05 µg TEGDMA, 0.25 µg UDMA and 0.5 µg BisGMA were eluted; at 40 s exposure 0.04 µg TEGDMA, 0.19 µg UDMA, 0.37 µg BisGMA were extracted; after 20 s LED + 90 s polymerization in furnace 0.04 µg TEGDMA, 0.22 µg UDMA, 0.42 µg BisGMA were leached; after 20 s LED + 180 s polymerization in furnace 0.03 µg TEGDMA, 0.18 µg UDMA and 0.34 µg BisGMA were eluted from the specimens. The amount of eluted UDMA was the highest in case of each exposure time. Half amount of BisGMA was leached than UDMA and the amount of eluted TEGDMA was the least. There was statistically significant difference in the amount of eluted UDMA and BisGMA between the 20 s and 40 s exposure times ($p < 0.05$). Further increase of curing time did not affect significantly the elution of monomers. The release of unreacted TEGDMA monomer was not influenced by the time of polymerization.

The amount of eluted monomers from different depth showed the following order: from 0-1 mm layer thickness 0.07 µg TEGDMA, 0.82 µg UDMA and 0.43 µg BisGMA; from 1-2 mm thick layer 0.23 µg TEGDMA, 2.57 µg UDMA, 1.33 µg BisGMA were eluted; from 2-3 mm depth 0.67 µg TEGDMA, 6.28 µg UDMA, 3.21 µg BisGMA were leached; from 3-4 mm layer thickness 1.71 µg TEGDMA, 11.67 µg UDMA and 5.81 µg BisGMA were extracted from the composite samples. 50 % more amount of leached UDMA monomer was detected than BisGMA in each depth, meanwhile, very low amount of released TEGDMA was observed. The difference in the amount of each monomer was statistically significant among the investigated depths and was approximately two times higher between each depth ($p < 0.05$).

5 year retrospective evaluation of direct Class II. composite restorations using USPHS criteria

240 class II composite restorations in 85 patients – 65 premolars (27.0 %), 175 molars (73.0 %) – were involved in the study. Control examinations were carried out five years after placement of restorations, according to the USPHS criteria. Namely, anatomic form, marginal integrity, marginal stain, color stability, surface smoothness and the presence of secondary caries or fractures of the restorations. 52.9 % of the restorations were accepted without any change. In 35.0 % change in one, in 10.4 % change in two and in 1.7 % change in three characteristics was observed. From the 240 cases, 3 composite restorations (1.3 %) were rejected as clinically not acceptable fillings. In 0.8% of the fillings, secondary caries and in 0.4% of the cases fracture was found as a failure. The frequency of adjacent deficiencies were found as follows: color instability, 12.5%; marginal stain, 20.8%; anatomic deformity, 15.0%; failure of marginal integrity, 8.8% and surface roughness, 2.5%. Color instability was significantly more frequent in premolar teeth, than in molars ($p = 0.031$). Color instability ($p = 0.015$), marginal stain ($p < 0.001$) and anatomic form malformation ($p = 0.002$) occurred more frequently in MOD restorations than in MO/ OD fillings. Our results suggest that class II restorations are correct both functionally and esthetically in 98.8% of the cases, even after a 5-year-period.

Retrospective evaluation of four microhybrid posterior direct resin composite restorations: 10-year findings

In the present study, a total of 701 posterior RC restorations were evaluated. The date of the placement and the date of the failure were obtained from the dental records. Of the 701 restorations, 15 (2.1 %) were determined to be unacceptable. The reasons for the failure included secondary caries, fracture of the restoration and endodontic treatment. All restorations given a “Charlie” rating were regarded as failed. Endodontic treatment was recorded as a reason for the failure of the restoration, and then the restoration was excluded from the following evaluation, because the USPHS criteria does not contain this clinical characteristic. The overall success during the registration period was 97.86 %. The failure rates for Filtek Z250 and Herculite XR were constant, 0.9 % and 1.36 %, respectively, after 11 years; however, an increasing failure rate was observed for Renew (7.81 %) and Gradia Direct Posterior (8.57 %), and these increased failure rates were significantly higher ($p < 0.05$) than the rates for Filtek Z250 and Herculite XR. A comparison between the RCs according to the USPHS criteria is presented in Table 4. A total of 349 (50.2%) restorations were accepted without changes and clinical remarks (A score), and in 346 (49.8%) cases, at least 1 deficiency was found (B or C score). The incidence of deficiencies (B and C codes) was 60% when the restorations were made with Filtek Z250, 72% with Renew, 81% with Herculite XR and 82% with Gradia Direct Posterior. The differences in the numbers of B and C codes between Filtek Z250 and Herculite XR ($p = 0.015$) or Filtek Z250 and Gradia Direct Posterior ($p = 0.013$) were statistically significant over the total observation period. Pearson’s Chi-Square test revealed a significantly higher rate of B and C scores in the color matching of Gradia Direct Posterior ($p = 0.02$) and in the fracture of Renew ($p = 0.005$); however, it should be mentioned that fractures were found in only 2 cases of the 61 Renew fillings. Considering the number of surfaces, in Filtek Z250, significantly more B and C scores were found in cases of 3 surface (MOD) restorations than in 2 surface (MO, OD) fillings ($p < 0.001$). Similar tendencies were observed in the other materials; however, these differences were not significant. Additionally, significantly greater marginal discolorations ($p = 0.001$) and anatomic form deficiencies ($p = 0.02$) were found in 3 surface RC fillings, independent of the type of material. The most frequent deficiency was the marginal discoloration ($p = 0.027$) among the evaluated variables. Considering the type of tooth, restorations in premolars had similar survival rates as restorations in molars, although towards molars an increasing trend was observed.

DISCUSSION

Several factors influence restoration survival, including patient factors, as well as dentist factors and dental factors (the type of tooth, the tooth’s position in the dental arch, the cavity size, and the number of restored surfaces), adhesive and placement of the base material and the composition of the

restorative material. The resin matrix has an influence on the polymerization kinetics and, therefore, on polymerization stress formation and on their consequences, such as marginal leakage or the fracture of the tooth or restoration. Likewise, differences in filler features, especially the material, size and shape of the filler particles, have a direct impact on the properties of the restoratives, especially wear resistance, hardness and flexural strength. Degree of polymerization is a key element of the composite fillings durability because it can determine the parameters of mechanical, chemical and biological properties. Since the composition of each composite brands - if only slightly, but - different, so the material will be different even with the same degree of polymerization kinetics and conditions. The efficiency of the polymerization depends inter alia on the total amount of energy transmitted. The applied light intensity and exposure time are important factors in the rate of polymerization and the polymerization depth perspective. It has been shown that the same degree of conversion is produced by a fixed energy amount, independent of variations in light irradiance. Thus, a recommendation of 21 and 24 Jcm⁻² energy density has been made for the adequate polymerization (not more than 50-70 %) of 2 mm thick composite specimen. Light from the curing source must be able to adequately polymerize deeper composite regions than just the top, irradiated surface. However, as light passes through the composite, it is absorbed and scattered, reducing its effectiveness to initiate polymerization, and consequently resulting in variation of cure with depth. Consequently the structural stability of the composite deteriorates, which causes weakening of the mechanical properties. The DC is generally accepted to correlate with the elution of unreacted monomers which are released in the saliva. Leached monomers and their degradation products have proved negative effects on the human body.

In the present study, Filtek Z250 commercial resin composite samples were used to investigate the correlation between the elution of monomers and DC and the influence of the setup of light illumination delivery on the material and the depth of cure was evaluated on these properties. According to our results the total quantity of eluted residual UDMA was considerably greater than quantity of residual BisGMA and TEGDMA. The possible explanation is the difference in molecular weight of the monomers (BisGMA>UDMA>TEGDMA) and the total content of the monomer in the composite (BisGMA>UDMA>TEGDMA). In Filtek Z250, the TEGDMA content is very low; the matrix is based on mostly BisGMA and UDMA. Tanaka et al discovered that small-molecular-weight monomers could be extracted in considerably higher quantities than large-molecular-weight monomers. Small-molecular-weight monomers such as TEGDMA have higher mobility and will be eluted faster than large molecules such as BisGMA and UDMA, despite their low content in the composite resin. Comparing the molecular weight of BisGMA and UDMA, BisGMA has higher weight, thus the release of UDMA is faster and more in a certain time interval. Furthermore, both of these resins are of higher molecular weight and, therefore, have fewer double bonds per unit of weight which permits to reach a higher DC value.

To minimize the amount of residual monomer elution, resin-based restorative materials have to be polymerized to a high degree. Characteristics related to the light source, such as energy density and spectral flux, alter the final DC. According to Rueggeberg et al. and Emami et al., there is a close correlation between energy density and the DC. Peutzfeldt et al. also suggested that the higher the energy density, the higher the DC of a monomer. However, according to our findings there is a limit in total energy where further rise does not increase significantly the rate of polymerization. The DC values (46.28 % and 53.99 %) were in accordance with the delivered energy up to 40 Jcm^{-2} , however above this energy density the difference between the DC (53.99 %, 54.87 % and 55.83 %) was not significant. It means that the DC % of specimens cured with 40s exposure time was 7.71 % higher than DC % of samples cured for 20 s, however there was no significant increase in DC % of specimens polymerized further 90 s or 180 s in the furnace in spite of the higher energy density delivered on these samples.

In accordance with the DC % of each groups the UDMA and BisGMA monomer release showed the same pattern, however, the amount of eluted TEGDMA was not significantly influenced by the energy density, although a tendency was noticeable when the delivered energy density increased. The saturation of monomer conversion in Filtek Z250 at this energy density might be caused by the limited concentration of photoinitiator and / or the filler loading of the material.

Comparison among the four layer thicknesses showed significant reduction in DC between 56.68 % and 17.78 % from the top to the bottom of the specimens. This decrease is generally explained by light attenuation in the specimen due to reflection, absorption, and scattering of light. According to Obici et al. and Yap et al., the light is not significantly reduced in intensity in more shallow composites (~1 mm layer thickness); therefore, the maintained energy is sufficient to initiate the reaction. Conversely, in deeper regions (3-4 mm), as the light passes through the bulk of the restorative material, the light absorption and scattering by the resin composite greatly decrease the light intensity, thereby decreasing the potential for curing and increasing the elution of leachable monomers. According to our results the DC% of the top 1 mm is 56.68 %, in 2 and 3 mm layer thickness the DC % is 50.42 % and 45.13 % which means ~5% decrease in each mm from the top towards the bottom. However, in the bottom layer (4 mm) the DC % is only 17.78 % which is nearly 30 % drop in DC. Reporting on the elution of monomers with regard to the depth of cure each monomer had significantly higher elution when the depth was increased. When the depth was increased from 1 to 2 mm and from 2 to 3 mm the elution rate of each monomer was approximately 30-35 %. There was an increased elution rate (55 %) of BisGMA and UDMA when the depth was increased from 3 to 4 mm, however, the elution rate of TEGDMA was only 37% similarly to the shallower depths.

In vitro studies demonstrate the importance of proper curing and the use of thin layers of material in order to achieve a high degree of polymerization. With effective polymerization the dentist can

decrease the amount of leachable monomers and can improve the physical properties. However the question arises as to how these materials behave in vivo.

According to our study the success of the investigated Class II direct composite restorations is ~99% after 5 years. This result is similarly favorable comparing with other studies where the success rate is 94-99% after the same period. The restoration has failure when it does not fit the function or aesthetics and damage the surrounding tissues (C codes or B in case of secondary caries). Similar to the findings of other investigations, the main reasons for the failures in this study were secondary caries (0.83%) and fracture (0.42%); however, the occurrence of failures was low in the present study. Assessing the further characteristics minor deficiencies were found which did not require any correction. Considering the color match significantly more discoloration was found in MOD restorations and in premolar teeth. The color difference may be caused by chemical changes of photoinitiator, but could be caused by the penetration of organic matter degradation products as well. The extension and C-factor of MOD restorations are greater than MO or OD fillings, thus greater stress may manifest in these restorations. The food/beverage coloring agents or degradation products may penetrate in the resulting micro cracks of the fillings and causes discoloration. Significant result was obtained also in relation to the marginal discoloration. Although the gap can not be palpated with a probe at the restoration-tooth interface but it is permeable for a variety of staining substances. The most marginal discolorations occurred in molar teeth and large MOD restorations with disadvantageous cavity configuration, as well as exposed to high chewing force. Also in MOD restorations were found significantly more anatomic form differences with slightly opened contact points and occlusal under-contouring. 97.5 % of surface smoothness was impeccable thus the polish ability or the retention of polished surface of microhybrid composites after 5 years is appropriate. In our long-term – more than 10 years - retrospective clinical study, the clinical performance of 4 microhybrid composite restoratives applied in Class II cavities was analyzed over an extended period of time. The main objective of the study was to observe whether microhybrid resins with slightly different compositions of the resin matrix and different types, but similar sizes and volume fractions of filler particles showed distinct clinical performances. Considering the overall service time, this study reported a 97.86 % survival rate of posterior Class II composite restorations. The average annual failure rate for the 4 RC materials was 0.52 %, and it varied between 0.08 – 0.71 %. Opdam et al. concluded that a randomized clinical trial has the advantage to standardize methods and calibrate the operators. Among others this can explain why the results of randomized clinical studies are often better than those of retrospective studies. According to Frankenberg et al. the overall success rate of RC restorations after 8 years is 98.5 %, and 2.4 % cumulative failure rate was reported by van Dijken et al. after 12 years. However, our results show better survival rate than other retrospective studies owing to one operator performed all the evaluated restorations with the maximal adherence to the indications and steps of the adhesive technique. It is more likely that patient and operator factors (the

single and skilled operator and the high socioeconomic status and good oral hygiene of patients), both favorable in this study, are the main factors influencing restoration longevity, while material properties may have a secondary role. Similar to the findings of other investigations, the main reasons for the failures in this study were secondary caries, fracture and endodontic treatment; however, the occurrence of failures was low in the present study which may be explained by the above mentioned facts. Overall, the results showed that it is possible to place posterior composite restorations with considerable success and low failure rates, although a slightly increasing failure rate over the course of time was observed for 2 materials (8.37 % for Gradia Direct Posterior and 7.81 % for Renew). For example, when looking at the time interval, it was observed that Filtek Z250 (AFR 0.08 %) and Herculite XR (AFR 0.1 %) had a significantly better long-term survival. As one of the main reasons for restoration failure, fractures may indicate that Renew has lower long-term fracture resistance, most likely due to its lower *E*-modulus, which, in turn, is related to the slightly lower filler volume, which therefore increases the clinical effects of fatigue, compared to the other investigated materials. The clinical data also indicated that other minor differences in material composition and properties may affect the clinical behavior of RC restorations. Gradia Direct Posterior showed a significantly greater change in color match; this could be explained by its larger average particle size, which may lead to an increased rate of extrinsic discoloration. Furthermore, the amount of unreacted matrix monomers, photo-initiators and co-initiators has a considerable influence on the discoloration of the RC. In accordance with other studies, our results showed that the amount of restored surfaces has a considerable effect on the quality of the restoration. Significantly more B codes were found in the MOD restoration cases made using Filtek Z250 than in the 2 surface restoration cases or in cases using the other materials. Independent of the materials, the anatomic form deficiencies in the cases of 3 surface restorations were higher levels than in 2 surface restorations. This could be explained by the larger filling surface, which wears under abrasive attacks, leading to a loss of material. Similarly to Gordan et al., marginal discoloration was the most frequent defect observed in the restorations, independent of the composite used. In general, marginal quality decreases over time due to physiological and chemical interactions with the oral environment, and the onset of degradation could imply problems associated with the adhesive or the composite resin. There are inconsistent findings concerning the effect of tooth type. Some articles have reported that restorations placed in premolars exhibited significantly better survival rates than those placed in molars. This is suspected to be due to the greater occlusal forces on molar restorations compared with those in premolars. Another possible explanation is the decreased access to the operating field when restoring molars. However, our results cannot confirm these findings. Similar to the findings of Aoyama et al., our results showed no significant effect of tooth type on the longevity or quality of posterior composite restorations.

Ph.D. THESES

1. Quantification of conversion degree and monomer elution from dental composite using HPLC and micro-Raman spectroscopy

During polymerization of Filtek Z250 resin-based composite material the correlation between the quantity of eluted monomers and the degree of conversion was analyzed. Experiments were done to evaluate how the energy of the light used for illumination influences the polymerization and how the polymerization performed in layers with different thickness and depth are affected by illumination.

- In *Filtek Z250* microhybrid composite the higher degree of conversion and the lower amount of leached monomers was achieved with 40 J/cm^2 delivered energy density and in 1 mm layer thickness.
- *Direct correlation* was detected between the *quantity of eluted monomers and the degree of conversion*. 1% increase in degree of conversion led to 3 % decrease in the amount of eluted monomers (BisGMA, UDMA) in *Filtek Z250*.
- Since the data provided by the manufacturer does not include the amount of energy is necessary to proper polymerization, only the exposure time, it is recommended to change and provide the value of energy density for maximum conversion. Furthermore, it is recommended to decrease the layer thickness from 2.5 mm to 1 mm.

2. 5 year retrospective evaluation of direct Class II composite restorations using USPHS criteria

In our first retrospective study 5-year-old Class II direct composite restorations – made from Filtek Z250 microhybrid composite - were controlled according to USPHS criteria and the most frequent defects were analysed.

- The *success* rate for *Filtek Z250* composite after 5 years was 98.8 %.
- Analyzing the *color match* significantly *more differences* were found in *premolar teeth* compared to molar teeth.
- In *three-surface (MOD)* restorations *significantly more changes* occurred including *differences in color match, marginal discolorations and anatomical form deficiencies*.

3. Retrospective evaluation of four microhybrid posterior direct resin composite restorations: 10-year findings

In our second – 10-year - retrospective study the longevity of Class II posterior restorations was investigated according to the USPHS criteria, in clinical practice using 4 microhybrid RCs with slightly different filler types and resin matrix characteristics.

- *The average success rate of the Class II restorations was 97.86 %, which is a very good result for all the microhybrid composites were investigated.*
- *During the analysis according to the USPHS criteria more B and C codes were found in case of Renew (fracture) and Gradia Direct Posterior (color match).*
- *The marginal discoloration was the most frequent deficiency independently from the brand of the material.*
- *In MOD restorations significantly more deficiencies were detected in contrast with the two-surface restorations especially in change of anatomical form.*

PUBLICATIONS

Publications supporting the Ph.D. theses

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Textbook chapters

5 textbook chapters in hungarian and english and 4 chapters in english and 1 german:
 „A magyarországi fogorvosképzés módszertani és tartalmi modernizációja korszerű hosszanti

digitális tananyagfejlesztéssel három nyelven” for dental medicine students in topics of endodontics and esthetic dentistry:

- Az endodontia modern koncepciója (angol)
- Endodontiai vizsgáló módszerek, differenciál diagnózisok (angol)
- Pulpa megbetegedések és diagnosztikájuk (angol)
- A pulpa és a periapicalis tér pathológiája (magyar, angol)
- Step-back technika, step-down technika (angol)
- A kompozitok összetevői, szint befolyásoló hatásuk (magyar, angol)
- Polimerizációs stressz, rétegzéses technikák moláris fogakon (magyar, angol)
- Direkt héj készítése különböző technikákkal (magyar, angol, német)
- A kerámiák anyagtana (magyar, angol)

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Oral presentations

- **LEMPEL E, TÓTH Á, FÁBIÁN T, KRAJ CZÁR K, SZALMA J:** Mikrohibrid kompozit tömések 10 éves retrospektív követése – endodonciai kezelés, mint a sikertelenségek egyik vezető oka. MFE Magyar Endodontiai Társaság és Dento-Maxillo-Faciális Radiológiai Társaság III. Kongresszusa, Ráckeve, 2014.09.04-06.
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- **LEMPEL E, FÁBIÁN T, SZALMA J, KRAJ CZÁR K, JEGES S:** II. Osztályú kompozit tömések 10 éves retrospektív vizsgálata USPHS kritériumrendszer alapján. MFE Árkövy Vándorgyűlése, Pécs, 2012.09.20-22.
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