Ph.D. Thesis

Material science and biocompatibility examinations of pre-heated resin-based composites

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List of abbreviations

ANOVA = analysis of variance

BisEMA = ethoxylated bisphenol-A dimethacrylate

BisGMA = bisphenol A-diglycidil ether methacrylate

C = ceramic

D = dentin

DC = degree of conversion

DOC = depth of cure

DDMA = 1,12-dodecanediol dimethacrylate

DSC = Differential scanning calorimetry

LC = light-cured

LCU = light curing unit

LED = Light Emitting Diode

PTFE = polytetrafluoroethylene

RBC = resin-based composite

RP-HPLC = Reversed-phase High-Performance Liquid Chromatography

SPSS = Statistical Package for Social Science

TEGDMA = triethylene glycol dimethacrylate

UDMA = urethane dimethacrylate

UV = ultraviolet

VIS = visible

wt% = weight %

I. Introduction

Dental resin-based composites (RBC) are the most extensively employed restorative materials due to their manifold advantages, including excellent esthetics and mechanical properties. Some disadvantage of RBC materials may compromise the longevity of RBC restorations such as polymerization stress, mismatch in thermal expansion, marginal leakage, toxicity, to mention just a few of them. To overcome these problems, attempts have been made to improve the mechanical properties as well as to ameliorate the clinical procedure. During the restorative procedure, it is favorable to use an easy-to-handle, non-technique sensitive, durable, and esthetic restorative material with quick and efficient polymerization. In case of RBC restorations, expediting polymerization, increasing of maximum layer thickness and the degree of monomer conversion can be considered as the main objectives. In order to achieve a durable, successful RBC restoration, the most important factors, among others, include good mechanical properties and handling characteristics, reduced polymerization stress, good marginal adaptation and high degree of conversion (DC). According to the literature data, there is a clear correlation between the DC and the physico-chemical characteristics of RBCs. Meanwhile, DC is influenced by several factors, such as light exposure conditions, composition, shade, opacity and thickness of the RBC layer, pre-cure temperature also plays an important role in the polymerization process. As the success of RBC restorations depends on their polymerization and DC, the influence of temperature has become one of the central issue of several studies. The polymerization of RBCs is an exothermic reaction, and the released heat is mainly produced in the propagation phase. The process of monomer conversion and the properties of the set polymer are influenced by the polymerization temperature. Not only the exothermic reaction, but also the absorbed light energy contributes to the system temperature during the polymerization of light-cured RBCs. While some authors attribute a greater significance to the heat emitted by the light curing unit in the temperature rise, others regard the heat generated in the exothermic reaction as more important. As reported by several investigations, pre-heating may have a beneficial impact on marginal adaptation, gap formation and microleakage by reducing the viscosity of RBCs. Improved handling properties, such as flowability can facilitate the application of the filling material, consequently making the procedure less time-consuming. Reduced viscosity also improves marginal seal and surface microhardness, thereby contributing further to the overall clinical success. There may be a lack of efficiency associated with the use of conventional heating devices as some authors have shown a rapid decrease in RBC temperature after removal from the device, as well as during dispensing and handling. Moreover, during the cooling phase, the system bears a loss of energy, so vitrification takes place earlier and causes decreased DC.

Several types of RBC dental materials have been developed over the years, including bulk-fills, which can be placed in larger increments to reduce chairside time and technique sensitivity. The

primary advantage of bulk-fill RBCs over conventional ones is the increased depth of cure. The maximum layer thickness which still ensures adequate material characteristics as recommended by the manufacturers, is 4 mm or in some cases even 5 mm. Although most studies have confirmed the improved depth of cure for bulk-fill RBCs, some controversial data can still be found.

Besides the physico-mechanical properties, the chemical characteristics are also important determinants of the clinical performance and biocompatibility of an RBC. Although, there is a strong inverse correlation between DC and monomer elution, the amount of released monomers may be influenced further by other factors such as the quality of the monomer system, filler type and content, porosity as well as the employed solvent. Elution from bulk-fill RBCs was found to be comparable to that of conventional materials despite their increased increment thickness, since the monomer release is more dependent on the hydrophobicity of the base monomers and the final network characteristics of the resin-matrix. Detection of unreacted monomers from pre-heated RBCs is not a well investigated topic so far.

Besides the advocacy of pre-heating in direct restorative procedures, pre-warmed RBCs are also introduced in cementation of indirect restorations. Posterior indirect partial restorations (inlay, onlay, overlay) are widely used in dental clinical practice to overcome issues resulting from the use of direct RBCs. Adhesive cementation of ceramic inlays is recommended to improve the esthetic and mechanical properties of the restoration. An alternative innovation is the use of chairside pre-heated conventional restorative RBCs as a luting agent for indirect ceramic restorations. Owing to their reduced viscosity, low film thickness and good adaptation can be achieved. In addition to color stability, favorable mechanical and physical properties are further benefits as a result of their high filler load. An increased pre-polymerization temperature can improve the monomer-to-polymer conversion; however, this might be compromised during the luting procedure because of the rapid cooling of the luting RBC before it is light-cured. The durability of adhesively bonded restorations depends on the DC of the adhesive cement.

An increase in irradiance delivered from the curing unit or upon extending the exposure time as a strategy to enhance the polymerization degree might cause an unfavorable temperature rise within the pulp chamber. However, the polymerization of RBC materials is an exothermic reaction that leads to further heat generation. Although most of the heat generated during RBC polymerization is dissipated, the increase in pulpal temperature may exceed the putative pulpal damage threshold. Several studies have consistently reported that the remaining dentin thickness is a critical factor in relation to the intra-pulpal temperature increase due to the heat dissipating effect. Although heat transfer or heat flux occurs at a lower rate in dentin, inducing a thermal insulating effect, the potential for pulpal damage is expected to be great in deep cavities, where the tubular surface area increases, and the light attenuation effect is weak. Thus, clinically, it would be optimal for dentin preservation, or continuous high-energy output photo-curing should

be avoided to protect pulp tissues from thermal injury. A temperature rise in pulpal tissues of 5.5 °C may lead to irreversible changes. In support of the above observation, a recent *in vivo* study showed that increased pulpal temperatures may induce inflammatory reactions, even if the temperature rise does not exceed the previously defined 5.5 °C threshold. Although several investigations have been conducted on the effects of the light curing unit, RBC type, and remaining dentin thickness on the pulpal temperature rise, data are lacking in the dental literature regarding the effects of different dentin thicknesses on pulpal temperature change during cementation of different thicknesses of indirect ceramic restorations with adhesive resin cements or pre-heated restorative RBCs. Furthermore, reliable, comparative data on the thermophysical properties of dentin and ceramic are essential to obtain precise calculations of the thermal changes in teeth and provide safer dental procedures, such as ceramic inlay cementation.

II. Objectives

II.1. Investigation of the effect of pre-heating on monomer elution and degree of conversion of contemporary and thermoviscous bulk-fill resin-based dental composites

The purpose of this study was to examine the temperature changes during the application and polymerization of a relatively new thermoviscous (VisCalor Bulk) and a high-viscosity full-body bulk-fill RBC (Filtek One Bulk Fill Restorative) in relation to different pre-cure temperatures. Further aims were to evaluate the effect of pre-heating on the DC using micro-Raman spectroscopy as well as determine the amount of released monomers using Reversed-phase High-Performance Liquid Chromatography (RP-HPLC).

Two null hypotheses were formulated: (1) Pre-heating had no effect on RBCs' post-cure DC%, and (2) pre-cure temperature did not affect the amount of released unreacted monomers.

II.2. Investigation of the effect of ceramic and dentin thicknesses and the type of resin-based luting agents on intrapulpal temperature changes during the luting of ceramic inlays

The aim of our research project was to compare the intrapulpal thermal changes resulting from cementation of ceramic inlay with light- and dual-cured adhesive resin cements and pre-heated sculptable submicron restorative RBC. The aim was to assess the influence of simultaneously variable dentin and ceramic layer thicknesses on pulpal temperature rise, supplemented by a qualitative comparison of the thermal properties between dentin and ceramics.

The null hypotheses of the *ex vivo* study were threefold: (1) intrapulpal temperature change does not differ significantly using distinct luting agents during ceramic inlay cementation; (2) pulpal temperature rise is not influenced significantly by ceramic and dentin layer thicknesses; and (3) there is no significant difference between the thermal conductivity and heat capacity of dentin and ceramics.

III. Materials and methods

III.1. Investigation of the effect of pre-heating on monomer elution and degree of conversion of contemporary and thermoviscous bulk-fill resin-based dental composites

Two brands of high-viscosity bulk-fill RBCs – the VisCalor Bulk (VCB) and the Filtek One Bulk Fill Restorative (FOB) – were investigated in this *in vitro* study. The pre-cure temperature of the RBC samples was 25 °C (room temperature) (FOB_25 and VCB_25), 55 °C (FOB_55) and 65 °C (VCB_65). In case of VCB, pre-heating was performed by VisCalor Dispenser (VOCO, Cuxhaven, Germany). Pre-warming of FOB was undertaken by Ena Heat Composite Heating Conditioner (Micerium, Avegno, Italy). Five specimens were prepared in each group, from each material for both the micro-Raman spectroscopy measurements as well as for the monomer elution measurements (n=40).

The samples were prepared in a cylindrical polytetrafluoroethylene (PTFE) mold with an internal diameter of 5 mm and a height of 4 mm, placed on a thermostatically controlled (30 ± 1 °C) glass slide to represent an isolated tooth. All specimens were irradiated with Light Emitting Diode (LED) curing unit (LED.D, Woodpecker, Guilin, China; average light output given by the manufacturer 850-1000 mW/cm^2; λ = 420–480 nm; 8 mm exit diameter fiberglass light guide) in standard mode for 20 s.

Temperature measurements during the application and the polymerization of RBCs were recorded with a registration device (El-EnviroPad-TC, Lascar Electronics Ltd., Salisbury, UK) attached to 0.5 mm diameter Cu/CuNi thermocouple probes (K-type, TC Direct, Budapest, Hungary) — positioned at the bottom of the temperature regulated mold — with a frequency of one measurement per second and resolution of 0.1 °C.

RBC samples made to estimate the temperature change of the RBC during sample preparation were then used to measure the DC. Confocal Raman spectrometer (Labram HR 800, HORIBA Jobin Yvon S.A.S., Longjumeau Cedex, France) was used to evaluate the 24 h post-cure DC values of the polymerized RBC samples.

The amounts of the eluted monomers (BisGMA, TEGDMA, UDMA, DDMA) were evaluated with RP-HPLC system (Dionex Ultimate 3000, Thermo Fisher Scientific Inc., Sunnyvale, CA,

USA) consists of a Dionex LPG 3400 SD gradient pump, Rheodyne injector (Rheodyne, CA, USA), and a Dionex DAD 3000 RS UV–VIS detector (Dionex GmbH, Germering, Germany). Data acquisition was completed using Chromeleon software (version: 7.2.10). The separations were performed on a LiChrospher® 100 RP-18e (particle size: 5 μm, pore size: 100 Å) (Merck KGaA, Darmstadt, Germany) column (250 mm × 4.00 mm) with gradient elution.

The statistical analyses were performed with SPSS v. 26.0 (SPSS, Chicago, IL, USA). Levene's test was employed to test the equality of variance. This was followed by Paired Samples Test to analyze the differences in mean DC% between top and bottom surfaces and Two-tailed Independent Samples T-test to analyze the differences in mean DC% between the investigated materials polymerized at room temperature and with the application of pre-heating. The differences in monomer elution from the RBCs at the investigated temperatures were also compared with the Two-tailed Independent T-test.

Multivariate analysis (General Linear Model) and Partial Eta-Squared statistics were used to test the influence and describe the relative effect size for *Material* and *Temperature* as independent factors. p values below 0.05 were considered statistically significant.

III.2. Investigation of the effect of ceramic and dentin thicknesses and the type of resin-based luting agents on intra-pulpal temperature changes during the luting of ceramic inlays

All intra-pulpal temperature measurements were performed on a caries-free, freshly extracted, human mandibular third molar as a single-tooth model. The temperature was detected with a 0.5 mm diameter Cu/CuNi thermocouple probe (Type K thermocouple device; Ø = 0.5 mm; Cu/CuNi; TC Direct, Budapest, Hungary), placed into the pulp chamber. The thermocouple sensor was positioned on the dentin at the top of the pulp chamber and assessed radiographically. To replicate the pulp tissue, the pulp chamber and root canal were injected with ECG gel (Aqua Sound Basic, Ultra-gel Hungary 2000, Budapest, Hungary). The occlusal surface was prepared leaving dentin with 2.5, 2.0, 1.5, and 1.0 mm thickness from the top of the pulp chamber. The occlusal thickness to be removed was estimated and controlled using digital intraoral radiography. Highly translucent A2 shade lithium disilicate ceramic blocks (6 × 6 mm with thicknesses of 2.0 mm, 2.5 mm, 3.0 mm, and 3.5 mm) were fabricated (GC Initial LiSi Press; GC Europe, Leuven, Belgium) using the heat-pressed method and cemented with light-cured adhesive cement (Variolink Esthetic LC [VE_LC)]), dual-cured adhesive cement (Variolink Esthetic DC [VE_DC]), and a pre-heated restorative RBC (Estelite Sigma Quick [EQ_55 °C]). A single-dose capsule of the latter RBC was pre-heated to 55 °C in an RBC warming device (Ena Heat Composite Heating Conditioner, Micerium, Avegno, Italy) for 15 min. The resulting RBC

temperature was measured using a non-contact infrared digital thermometer (TESTO 845, Testo Magyarország Kft., Budapest, Hungary).

During each cementation a light-emitting diode (LED) light curing unit (LCU) (LED.D, Woodpecker, Guilin, China; Λ = 420–480 nm; 8 mm exit diameter fiberglass light guide) was used in the standard mode for 40 s of exposure time.

Microcalorimetric measurements were carried out using a MicroSC microcalorimeter (Setaram Instrumentation, Caluire, France) in the differential scanning mode to compare the heat flow of a new series of the above-described ceramic blocks and dentin slices.

The SPSS v. 26.0 (SPSS, Chicago, IL, USA) software was used to perform statistical analyses. The normality of the data distribution was tested using the Kolmogorov–Smirnov test, followed by the application of parametric statistical tests. The differences in temperature changes were compared using one-way analysis of variance (ANOVA). Tukey's post hoc adjustment was used for multiple comparisons for all the ANOVA models. To evaluate and explain the relative effect size on *dentin* and *ceramic thickness*, as well as *luting material* as independent variables, a General Linear Model (Multivariate analysis) and Partial Eta-Squared statistics were applied. Linear Regression was used to determine the correlation between dependent (*temperature*) and independent (*dentin thickness, ceramic thickness, and luting material*) variables. The statistical significance was set at $p < 0.05$.

IV. Results

IV.1. Investigation of the effect of pre-heating on monomer elution and degree of conversion of contemporary and thermoviscous bulk-fill resin-based dental composites

The measured maximum radiant exitance of the LED LCU was 1250 ± 15 mW/cm^2. The delivered radiant exposure was 25 J/cm^2. The LCU increased the temperature by an average of 7 °C when the thermocouple was irradiated through the empty 4 mm deep mold for 20 s. Meanwhile, the T2 setting of the heating device stated preset temperature is 55 °C in 55 s, the real temperature of the FOB was 46 °C in the capsule after the recommended pre-warming period. The VisCalor Dispenser T1 setting provides 65 °C pre-warming in 30 s, however, the actual temperature of VCB was 60 °C after the recommended duration of pre-heating. The temperature of the 46 °C pre-heated FOB decreased to 33.4 °C as it was removed from the warming device and started to be applied into the mold, and showed further temperature drop of 1 °C during the condensation. The total temperature decrease from the pre-heating until the start of polymerization was 13.6 °C in approximately 20 s. In the second phase, during polymerization, the exothermic reaction and the heat released from the curing unit elevated the RBC's temperature by 4 °C. Regarding the

thermoviscous VCB, its pre-heated temperature (60 °C) decreased to 36.6 °C during the initial phase of the application and continued to show a further drop of 2.6 °C during the condensation phase. The total drop of temperature for the pre-heated VisCalor Bulk from the pre-heating until the start of polymerization was 26 °C in approximately 20 s. The temperature rise caused by the light-curing and the exothermic reaction was 4.4 °C.

Considering the DC at the top and bottom surfaces in samples applied in 4 mm thickness, the mean percentages ranged between 54.2–64% and 45.0–51.8%, respectively.

The DC values at the bottom of the specimens showed a statistically significant decrease for both materials at both temperatures compared to the DC values measured at the top. When room temperature specimens were applied, the DC values were very similar to that of pre-heated samples, except on the bottom surface of FOB which was significantly lower when applied after pre-heating. In a comparison of the two bulk-fill RBCs, VCB showed a statistically significantly lower DC (~10% less) both on the top and bottom when applied at room temperature. Samples applied following pre-heating showed a significantly lower DC% only on the top. The lowest DC values were measured on the bottom surfaces of both investigated RBCs when they were applied with pre-heating.

A 2 (*Material*) x 2 (*Temperature*) mixed-model ANOVA revealed that the main effect for *Material* on DC values measured on top surfaces was significant and the Partial Eta-Squared was considered to be large. The effect of *Temperature* was found insignificant with medium effect size. The interaction had no effect on the monomer conversion at the top. Regarding the DC values at the bottom surfaces, a significant effect for both the *Material* and *Temperature* factor was obtained. The interaction between the two variables also significantly affected the monomer conversion at the bottom surfaces. The main effect for *Material* was significant at room temperature, meanwhile, the *Temperature* factor affected significantly only the FOB, but not the VCB.

The differences in the monomer elution were also significant between FOB and VCB both when applied at room temperature and with pre-heating in the case of all the evaluated monomers, except for DDMA. This latter was released in similar (statistically insignificant) amounts from the pre-heated RBCs. At room temperature, 30 and 2.5 times as much UDMA and DDMA were released, respectively from FOB, while 10.5 times more BisGMA was eluted from VCB. The latter was the monomer released in the largest amount. With the utilization of pre-heating, 7.5 times as much UDMA was found to elute from FOB, while 25 times more BisGMA was released from VCB. For FOB, pre-heating significantly reduced the amount of eluted monomers, while for VCB, the temperature did not affect the dissolution. The following order of mean monomer elution was detected from FOB for both room temperature and pre-heated samples from highest to lowest: UDMA < DDMA < BisGMA, meanwhile the amount of leached monomers was roughly half (UDMA, DDMA) or one-third (BisGMA) for pre-heated specimens. Regarding

VCB, both the order, as well as the amount of the released monomers were the same in the case of both the room temperature and the pre-heated samples (BisGMA < TEGDMA < DDMA < UDMA), except for TEGDMA, which showed a significantly lower elution from the pre-heated samples. 2 (*Material*) x 2 (*Temperature*) mixed-model ANOVA showed that the main effect for *Material* was significant on UDMA, BisGMA, DDMA release with a Partial Eta-Squared value which was considered to be large. The *Temperature* factor also influenced significantly the monomer elution; however, its effect was slightly weaker compared to the *Material*'s effect. TEGDMA was released only from VCB. The effect of the *Temperature* factor was calculated to be significant on the elution of this monomer. The interaction between the two independent variables had a significant effect on UDMA and DDMA elution, while the elution of BisGMA was independent of the *Material* x *Temperature* interaction.

IV.2. Investigation of the effect of ceramic and dentin thicknesses and the type of resin-based luting agents on intrapulpal temperature changes during the luting of ceramic inlays

The intrapulpal thermal changes induced by the 40 s light exposure of dentin adhesive through the 1.0 mm, 1.5 mm, 2.0 mm, and 2.5 mm dentin thicknesses, using the 2.0 mm, 2.5 mm, 3.0 mm, and 3.5 mm-deep empty molds ranged between 4.0-9.9 °C, representing the insulating effect of the dentin without the ceramic blocks and luting agents. The thermal effect of the LCU through the eight combinations of different thicknesses of dentin (D1.0, 1 mm; D1.5, 1.5 mm; D2.0, 2.0 mm; D2.5, 2.5 mm) and ceramic (C2.0, 2.0 mm; C2.5, 2.5 mm; C3.0, 3.0 mm; C3.5, 3.5 mm) assemblies without luting agents were tested. None of the combinations approached the critical 5.5 °C threshold. Increased dentin thickness showed a more pronounced insulating effect than ceramic thickness. Luting the ceramic blocks into cavities of different depths with light-cured and dual-cured adhesive cements, or with the restorative RBC pre-heated to 55 °C increased the pulpal temperature significantly. Subtracting the temperature rise caused by the LCU from the thermal change in the pulp chamber induced by the luting agent provides an estimation of the heat generated by the exothermic reaction. According to this calculation, which does not account for the thermal transfer between the thermodynamic system and its environment, the pre-heated RBC elevated the pulpal temperature to the highest value, although statistically significant difference was not detected between the luting materials. Dentin thickness below 1.5 mm is the most critical for the heat insulating effect.

The Multivariate General Linear Model revealed that the *dentin thickness* had the main effect on the pulpal temperature changes ($F (3, 96) = 6.02$, $p = 0.001$), followed by the effect of *luting material* ($F (2, 96) = 4.29$, $p = 0.02$). The effect size was considered to be large for the *dentin thickness* (Partial $\eta^2 = 0.16$) and medium for the effect of *luting material* (Partial $\eta^2 = 0.08$). The effect of *ceramic thickness* on the pulpal temperature rise was considered to be insignificant

according to the results of the General Linear Model (F (3, 96) = 2.28, p = 0.09), although the Eta-Squared indicated a medium effect (Partial η^2 was 0.07). However, there was no statistically significant three-way interaction between *luting material*, *dentin thickness*, and *ceramic thickness* [F (14, 96) = 0.06, p = 1.0; Partial η^2 = 0.009]. According to the linear curve fitting model, the data regarding the *dentin* and *ceramic thickness* and the tested *luting agents* allowed us to predict the behavior of the data series. The adjusted R-square statistics revealed higher values for *dentin* (R^2 = 0.47) and *ceramic thickness* (R^2 = 0.41), indicating a better fit; meanwhile, the R^2 value for the *luting materials* was 1%.

The thermal properties of the samples were evaluated by DSC measurements. The heat capacities were measured directly with the Calisto software by calculating the area of the curve, where the heat flow was plotted as a function of time until the curve reached the saturated region. Considering the mass of the samples and the area of the heating curves, the heat capacity of dentin was found to be 86% larger than the heat capacity of ceramic. To compare the heat conductivities, the time constant for the achievement of thermal equilibrium was applied. The average time constants of the dentin and ceramic samples were measured as 165.72 s and 133.33 s, respectively. The pre-exponential factors associated with the samples with increasing thickness and mass are −1.35 μW, −4.08 μW, −6.08 μW, and −6.39 μW, or −7.13 μW, −9.13 μW, −11.38 μW, and −12.02 μW in respect to the dentin or ceramic samples. Calculating the thermal conductivity difference using the ratio of time constants, it was found to be approximately 24% lower in the case of dentin samples compared to ceramic specimens. Three scans of each sample were averaged then fitted using the exponential saturation function.

V. Discussion

V.1. Investigation of the effect of pre-heating on monomer elution and degree of conversion of contemporary and thermoviscous bulk-fill resin-based dental composites

In our first *in vitro* study, the relation of DC and the elution of unreacted monomers of a thermoviscous and high-viscosity bulk-fill dental RBCs were assessed using micro-Raman spectroscopy and High-Performance Liquid Chromatography measurements. Additionally, the thermal change of the RBCs was also registered during the sample preparation with a K-type thermocouple to assess the temperature change of the room temperature and pre-heated RBCs during the manipulation and polymerization phases.

According to our results, the first null hypothesis, which stated that the pre-heating had no effect on post-cure DC% of VisCalor Bulk and Filtek One Bulk, was partially rejected, since pre-warming of RBCs neither increased nor decreased the DC on the top of both materials and the

bottom of VCB_65, however, the bottom DC was significantly decreased in the case of FOB_55. The second null hypothesis was also partially rejected, because the external heating of the investigated RBCs decreased the monomer elution in the case of FOB regarding all the investigated monomers and TEGDMA elution VCB, however, had no influence on the BisGMA, UDMA, and DDMA release from VCB. It has been reported that increased pre-cure temperature of RBC may result in a greater extent of monomer to polymer conversion. However, investigations, that have shown improvement in the degree of polymerization upon pre-warming generally maintained the RBC temperature constant during the experimentation. On the other hand, there are also results that found increases in DC at non-isothermal conditions to be material composition-dependent. Regarding the real-life clinical scenario, the RBC's temperature drops rapidly to the physiological level upon removal from the pre-heating device. Additionally, the pre-warmed RBC can reach a lower internal temperature than the maximum stated preset temperature of the heating device.

The measured temperatures for FOB_55 and VCB_65 were on average 32.5 °C and 34 °C, respectively, at the start of polymerization. The direct contact to the 30 °C molds and glass slab and also to the room temperature condensing instrument accelerated the cooling of the RBCs. The equilibration of the ambient and the pre-heated RBC's temperature resulted in faster cooling of the warmer RBC. During the photopolymerization, both exothermic reaction and the released heat from the curing device increased the RBC's temperature. The extent of temperature increase, however, seems to be influenced by the speed of the temperature drop before photocuring. Accelerated drop may hinder the exothermic temperature increase.

Adequate monomer to polymer conversion is crucial to the material's long-term clinical success. Although the minimum DC% for clinically acceptable restoration has not yet been precisely defined, DC values below 55% may be inadequate for occlusal restorative layers. The DC% on the top of FOB_25 and FOB_55 were 63% and 64%, respectively, which is a characteristic value for a well polymerized RBC. In comparison to the FOB values, the DC% on the top of the VCB_25 and VCB_65 samples were significantly lower, 54% and 55%, respectively. The lower DC values are presumably due to the material composition. The monomer system has a major effect on the DC, which increases in the following order: BisGMA < BisEMA < UDMA < TEGDMA. This might be one of the explanations for the significantly lower DC of the VCB, which is a BisGMA-based RBC. FOB is an UDMA-based bulk-fill RBC, containing both aliphatic and aromatic UDMA. In addition to the monomer system, the filler-matrix ratio is also decisive. VCB's filler loading is higher (83 wt%) compared to the filler content of FOB (76.5 wt%), which may restrict the light penetration and the mobility of monomers and radicals. Other investigations showed similar values. The pre-heating did not influence the DC% values of our investigated materials at the top of the samples, assuming that the RBCs on the top reached their maximum conversion degree already at room temperature.

Contrary to the values measured at the top of the room temperature samples, the DC% at the bottom of the 4 mm thick bulk-fill materials were lower by ~10% (FOB_25, 51.8%; VCB_25, 46.2%). The present research examined two, so-called full-body bulk-fill RBCs that have a higher filler load. Decreased DOC from the surface to the bottom may be a result of the increased filler ratio which may hinder light penetration due to the nano-sized particles despite the increase in translucency. On the other hand, high molecular weight monomers, such as BisGMA (in VCB) and aromatic UDMA (in FOB), also help to increase the viscosity, however, decreasing the reactive groups in the resin may negatively influence the DC. Clinically relevant, non-isothermal circumstances enhance the strong effect of RBC composition on the results. Several studies found the effect of pre-heating to vary on DC (decrease, no change, increase) depending on the composition of the investigated RBC. Confirming the above findings, our results also showed a dissimilar effect of pre- heating on the monomer conversion of the investigated RBCs. The rapid temperature drop of pre-heated RBC during handling results in excess heat loss which may deprive energy of the system and might prevent a sufficient increase in polymerization reactivity and consequent enhancement in monomer conversion. Considering the findings of the temperature measurements, it is visible, that the temperature increase during polymerization shows a direct correlation with the measured DC values. Since the reaction behavior of multifunctional monomer systems is very complex and highly dependent on the reaction conditions and composition, other possible explanations for DC decrease may arise. Although pre-heating did not increase the monomer conversion in many cases, several studies have shown that the mechanical properties and marginal integrity of RBCs (including FOB and VCB as well) are satisfactory or better than those applied at room temperature. As our results confirmed, RBCs do not have a complete monomer to polymer conversion. Incomplete conversion may result in the presence of unreacted monomer content within the polymer network which is partially or completely released short- or long-term. Released monomers may depress the biocompatibility of the RBC by stimulating bacterial growth around the restoration leading to secondary caries development and may promote allergic reactions. Additionally, cytotoxic effects of monomers have been demonstrated. Unreacted monomers can reduce the mechanical properties of the RBCs. During our experiment, aromatic (BisGMA) and aliphatic (TEGDMA, UDMA, and DDMA) dimethacrylate standard monomers were used to identify eluted monomers from the investigated RBCs. Regarding the monomer release, our results showed elution to be strongly dependent on the material. The measured unreacted monomer release is in line with our results regarding the degree of monomer conversion in VCB_25 and VCB_65 since pre-heating did not change the DC on the top or bottom of VCB. On the other hand, the observed relationship between DC and monomer elution from FOB is contradictory. While the DC of the bottom surface decreased after pre-heating, the detected elution of unreacted monomers from FOB_55 samples was also lower. Although several studies have shown that the extent of leached unreacted monomer is correlated

to the DC, the conversion degree does not necessarily correlate with the amount of free residual monomer, since the detected double bonds may remain as pendant groups bonded to the polymer structure and are not free to be released, however, may reduce the clinical success of the RBCs. Probably, the above issue is the explanation for the lack of the expected relationship between the DC and monomer elution in the case of the pre-heated FOB_55.

The monomer detected to be eluted in the highest amount was BisGMA from both VCB_25 and VCB_65, with the latter showing a significantly lower quantity. As it was previously mentioned, the extremely high viscosity of BisGMA limits the DC, leaving behind more unreacted monomers, which may release into the oral cavity. Admixing low molecular weight monomers, such as TEGDMA and DDMA, to BisGMA, can lower its viscosity, and via their synergistic effect can increase the rate of polymerization. The released quantity of the latter two was very small both from VCB_25 and VCB_65. FOB, on the other hand, is a UDMA-based RBC. At present, UDMA is the only commercial alternative to the bisphenol A-based dental methacrylates. Although, UDMA viscosity is lower than BisGMA, still high enough to require the addition of a reactive diluent, such as DDMA. Due to UDMA's lower molecular weight in comparison to BisGMA, it is expected to show higher DC and lower unreacted monomer elution.

The results of this study cannot be extrapolated to other room temperature and pre-heated RBCs, since the composition has a strong influence on both DC and monomer elution and can vary from RBC to RBC.

V.2. Investigation of the effect of ceramic and dentin thicknesses and the type of resin-based luting agents on intrapulpal temperature changes during the luting of ceramic inlays

To discover the thermal change in the pulp chamber using pre-heated restorative RBCs or light-cured and dual-cured adhesive resin cements a single tooth model was applied using different thicknesses of dentin and ceramic blocks imitating inlays. The results showed that in the case of investigated ceramic–dentin combinations, the cementing agents increased the intrapulpal temperature above the considered critical 5.5 °C. Furthermore, a qualitative comparison of thermal conductivity and capacity showed differences between dentin and ceramic. Therefore, all the tested null hypotheses were rejected. Our findings are consistent with the results of other studies regarding the shielding effect of the interposed ceramic and the remaining dentin thickness on pulpal temperature rise and the material-dependent temperature-increasing effect of the polymerization of RBCs. Thus, even though the interposed ceramic inlay and the remaining dentin thickness attenuate the light intensity of the curing unit and the delivered energy during the polymerization process, the exothermic temperature rise associated with the adhesive luting agent may jeopardize pulp health. The temperature changes measured in this study cannot be

directly applied under *in vivo* conditions. Despite the absence of blood circulation in vital tissues, this study provides important information. The clinical relevance of increased intrapulpal temperature is that it is a potential risk factor for thermal pulp damage. According to Zach and Cohen, a 5.5 °C temperature rise is critical and may cause irreversible pulpal damage. According to previous studies, the intensity and duration of the applied light were the most crucial factors for the pulpal temperature rise. The results of this study confirm the above statement because light curing without the interposition of a ceramic inlay and resin-based luting agent increased the pulpal temperature (ΔT = 4.0–9.9 °C) by a significant degree, depending on the dentin thickness, with an inverse correlation. Because the monomer-to-polymer conversion of an RBC is a function of the applied total energy during photocuring, it is advisable to increase the delivered radiant exposure for a higher degree of conversion. This is highly relevant to adhesive luting of indirect restorations. While the pulpal temperature in this study remained below the critical value of 5.5 °C without luting agents, the tested adhesive cements significantly increased it because of their exothermic reaction. The exothermic reaction was proportional to the amount of resin matrix, and it was found that the inorganic fillers have an impact on heat diffusion within the material by their capacity to absorb external and internal energy. The Multivariate General Linear Model revealed that the material factor had a medium impact on the pulpal temperature rise. The highest temperature was measured with the pre-heated RBC in all the tested groups. Although the temperature-raising effect was less than expected owing to the rapid cooling and heat absorption by the ceramic and dentin, these results suggest the potential hazard to pulp health owing to their higher thermal effect. Regarding the composition of the investigated light- and dual-cured resin cements, they had the same resin matrix/filler ratio; however, the dual-cured cement showed a slightly, but not significantly, lower temperature rise in each tested group compared to that of the light-cured resin cement. Dual-cured resin cements are supposed to compensate for decreased light transmission and may be more efficient at monomer-to-polymer conversion, even with increased ceramic thicknesses. It was found that dual-cured resin cements fail to achieve the same degree of conversion as light-cured cements with up to 2 mm of interposed ceramic thickness. Our results are indirectly in line with these findings because the polymerization process is proportional to the exothermic reaction, which results in a slightly lower pulpal temperature rise in the dual-cured resin cement.

Thermal transfer to the pulp is strongly dependent on the thickness of the remaining tooth structure. The thermal conductivities of enamel and dentin are ~0.81 W/mK and ~0.48 W/mK, respectively, which are considered low. Low thermal conductivity is equivalent to high insulating capability; thus, the pulp is protected from noxious thermal irritation if the tooth is intact. However, during cavity preparation the dentin thickness is reduced. Hard tissue removal during cavity preparation and several steps of the adhesive restorative procedure may cause thermal damage due to the weakened thermal insulation effect. For dentin thickness below 2.0 mm, the

heat transmitted by the curing unit increased the intrapulpal temperature above the 5.5 °C limit. These results are consistent with previous finding that showed a strong relationship between the thickness of the dentin and the intrapulpal temperature increase. In the present study, Partial Eta-Squared statistics revealed that the remaining dentin layer had the most pronounced effect on the pulpal temperature change values, and the effect size was considered to be large. The Linear Regression Model revealed a 47% value for the coefficient of determination and predicted a decreasing effect of thickness on temperature as the dentin thickness exceeded 2 mm. Even though the thinnest dentin was combined with the thickest ceramic, the highest intrapulpal temperature was detected in all measurements, regardless of the use of the luting agents.

In contrast, the effect of ceramic thickness on pulpal temperature rise was considered insignificant according to the results of the General Linear Model, although the Partial Eta-Squared indicated a medium effect. This result demonstrates that the shielding effect of the ceramic is not as great as that of dentin, although an inverse relationship between the ceramic thickness and temperature rise is evident, which is consistent with the findings of a previous study. The regression curve fit for temperature as a function of different ceramic thicknesses showed a linear energy loss with increasing ceramic thickness, which reflects the light attenuation occurring through an absorptive/scattering medium. The value of the coefficient of determination for ceramic thickness was found to be 41% in our Linear Regression Model. The differences in temperature changes caused by the thermal shielding effect of dentin and ceramic can be explained by their distinct thermal conductivities. It is higher for silica-based ceramics, which is approximately 1.7 W/mK, compared to the thermal conductivity of the dentin (~0.48 W/mK). Increasing the inlay thickness may result in a proportional removal of tooth hard tissues by decreasing the thickness of the remaining enamel and dentin. Although the shielding effect is proportional to increasing ceramic thickness, a decreasing dentin thickness has a stronger inversely proportional effect on the temperature increase within the pulp chamber. These results are supported by the DSC measurements in this study, which revealed a 24% lower thermal conductivity of the dentin than that of the ceramic. However, the thermal capacity of the dentin was calculated to be 86% more compared to the investigated lithium disilicate ceramic. The importance of this result lies in the ability of dentin to store large amounts of heat and then dissipate it slowly, reducing the sudden thermal effects on the pulp. However, considering the multiple thermal effects during adhesive restorative treatment, the gradually increasing temperature of the dentin may conduct more heat towards the pulp during heat dissipation. According to these findings, it is advisable that more dentin should be preserved during cavity preparation to protect the pulp from undesirable temperature increases. Furthermore, it should provide more time for heat dissipation between treatment steps, which can have a thermal effect on the pulp, to avoid heat accumulation in the dentin.

VI. Conclusion of novel results

VI.1. Investigation of the effect of pre-heating on monomer elution and degree of conversion of contemporary and thermoviscous bulk-fill resin-based dental composites

Within the limitations of this *in vitro* study, the following conclusions can be stated:

- Significantly higher DC values were achieved on the top of the room temperature and pre-heated investigated bulk-fill RBCs than on the bottom.
- Room temperature VisCalor Bulk has lower DC% values both on the top and bottom compared to Filtek One Bulk Fill Restorative.
- Pre-heating did not influence the DC of VisCalor Bulk, however, significantly decreased the DC at the bottom of Filtek One Bulk.
- Pre-heating had no effect on the monomer elution from VisCalor Bulk, but significantly decreased the monomer release from Filtek One Bulk.
- *Material* factor had a significant effect on each investigated variable, while *Temperature* factor and its interaction with *Material* is surface- (top vs. bottom) and monomer-dependent.

VI.2. Investigation of the effect of ceramic and dentin thicknesses and the type of resin-based luting agents on intra-pulpal temperature changes during the luting of ceramic inlays

Within the limitations of this *ex vivo* study, the following statements can be declared:

- The intrapulpal temperature rise may exceed the critical 5.5 °C threshold during ceramic inlay cementation, regardless of the dentin and ceramic thicknesses and the type of resin-based adhesive luting material used.
- The temperature values were predominantly influenced by the remaining dentin thickness, followed by the applied resin-based adhesive luting materials, and were least influenced by the ceramic thickness.
- The thermal conductivity of dentin was 24% less compared to the ceramic, while the thermal capacity was 86% higher.

VII. Publications

VII.1. Publications related to the topic of the Ph.D. dissertation

Kincses D, Böddi K, Őri Z, Lovász BV, Jeges S, Szalma J, Kunsági-Máté S, Lempel E. Pre-Heating Effect on Monomer Elution and Degree of Conversion of Contemporary and Thermoviscous Bulk-Fill Resin-Based Dental Composites. *Polymers* **2021**, 13, 3599. https://doi.org/10.3390/polym 13203599

Q1**; IF$_{2021}$: **4.967 *Citations: 20 (independent: 17, dependent:3)*

Kincses D, Jordáki D, Szebeni D, Kunsági-Máté S, Szalma J, Lempel E. Effect of Ceramic and Dentin Thicknesses and Type of Resin-Based Luting Agents on Intrapulpal Temperature Changes during Luting of Ceramic Inlays. *Int J Mol Sci* **2023**, 24,5466. https:// doi.org/10.3390/ijms24065466

Q1**; IF$_{2023}$:**5.6 *Citations: 1 (dependent: 1)*

Cumulative impact factor of dissertation related publications: **10.567**

VII.2. Publications independent from the topic of the Ph.D. dissertation

Lempel E, Őri Z, **Kincses D**, Lovász BV, Kunsági-Máté S, Szalma J. Degree of conversion and in vitro temperature rise of pulp chamber during polymerization of flowable and sculptable conventional, bulk-fill and short-fibre reinforced resin composites. *Dent Mater* **2021** Jun;37(6):983-997. doi: 10.1016/j.dental.2021.02.013. Epub 2021 Mar 10. PMID: 33714623.

Q1**; IF$_{2021}$: **5.687 *Citations: 29 (independent:23, dependent: 6)*

Lempel E, **Kincses D**, Szebeni D, Jordáki D, Lovász BV, Szalma J. Intrapulpal temperature changes during the cementation of ceramic veneers. *Sci Res* **2022** Jul 28;12(1):12919. doi: 10.1038/s41598-022-17285-x.

Q1**; IF$_{2022}$: **4.6 *Citations: 3 (independent: 2, dependent: 1)*

Cumulative impact factor of all publications: **20.854**

VII.3. Conference poster presentations related to the Ph.D. dissertation

Kincses D, Böddi K, Őri Z, Lovász BV, Jeges S, Szalma J, Kunsági-Máté S, Lempel E. Pre-Heating Effect on Monomer Elution and Degree of Conversion of Contemporary and Thermoviscous Bulk-Fill Resin-Based Dental Composites. Hungarian Association of Aesthetic and Restorative Dentistry (HAARD) Annual Conference, Budapest, **2022**. - Original research poster presentation

Kincses D, Jordáki D, Lempel E. Pulpal temperature change during the cementation of intracoronal ceramic restorations. 20th ESE Biennial Congress – Original research poster presentation. Abstracts. (R089) *Int Endod J*, **2023**. 56: 3-47. doi: 10.1111/iej.13875 *Q1;* ***IF$_{2023}$: 5.0***