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RESEARCH

Crack propensity of different direct restorative procedures in deep MOD cavities

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Abstract

Objective The purpose was to evaluate the crack formation associated with different direct restorative procedures of the utilized resin composites (RC) right after and 1 week later of the restoration.

Materials and methods Eighty intact, crack-free third molars with standard MOD cavities were included in this in vitro study and randomly divided into four groups of 20 each. After adhesive treatment, the cavities were restored either with bulk (group 1) or layered (group 2) short-fiber-reinforced resin composites (SFRC); bulk-fill RC (group 3); and layered conventional RC (control). Right after the polymerization and a week later, crack evaluation on the outer surface of the remaining cavity walls was performed with a transillumination method utilizing the D-Light Pro (GC Europe) with the “detection mode.” Between- and within-groups comparisons Kruskal-Wallis and Wilcoxon tests were used, respectively.

Results Post-polymerization crack evaluation showed significantly lower crack formation in SFRC groups compared to the control ($p<0.001$). There was no significant difference within SFRC groups and non-SFRC groups ($p=1.00$ and $p=0.11$, respectively). Within group comparison revealed significantly higher number of cracks in all groups after 1 week ($p\leq0.001$), however, only the control group differed significantly from all the other groups ($p\leq0.003$).

Conclusions Post-polymerization shrinkage induced further crack formation in the tooth 1 week after the restoration. SFRC was less prone to shrinkage-related crack formation during the restorative procedure; however, after 1 week, besides SFRC, bulk-fill RC also showed less prone to polymerization shrinkage-related crack formation than layered composite fillings.

Clinical relevance SRFC can decrease the shrinkage stress-induced crack formation in MOD cavities.

Keywords Enamel crack · Short fiber-reinforced composite · Deep MOD cavity · Direct restoration · Bulk-fill · Transillumination

Introduction

Currently, direct resin composite restorations are the primary choice for the rehabilitation of caries-related cavities in the posterior dentition, characterized by high clinical performance and durability [1–3]. However, polymerization shrinkage and related stress are still relevant issues.

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Polymerization shrinkage generates stress within resin composites at the interface between the composite restoration and the tooth substance, as well as within the tooth structure [4]. This can lead to various problems including marginal gap formation, micro-cracking (of the restorative material and/or the tooth itself), marginal staining, and cuspal movement [5]. These, in turn, may lead to postoperative sensitivity, pulpal complications, and restoration loss [6, 7]. If strong and stable adhesives are used, polymerization shrinkage is expected to cause cuspal deformation and enamel cracking on the external surface [8, 9]. Polymerization shrinkage and the stress it causes are determined by several factors, including the composition of the resin composite material [10], the C-factor (bonded surface to unbonded free surface ratio) [11], and the applied restorative technique, among others [12]. Cavities in the posterior region that correspond to Black's classification are characterized by a high C-factor, ranging from 3 to 5. While class II MOD cavities have a lower C-factor compared to class I occlusal cavities, the absence of both marginal ridges generates a problem from a mechanical point of view [13, 14]. It has been demonstrated that standardized MOD cavity preparation results in an average loss of 63% in relative cuspal stiffness due to the loss of marginal ridge integrity [15], simultaneously causing an approximately 54% reduction in fracture strength [16, 17]. The expected number of fatigue fractures is proportional to the magnitude of cuspal flexure.

A possible way to address these problems is to utilize an incremental technique during the filling procedure. This is done by applying the resin composite in horizontal or oblique increments of a maximum thickness of 2 mm. This technique can reduce the volumetric shrinkage of the material and minimize internal gap formation as a consequence [18]. However, this is a complex technique which requires a considerable amount of chair time, and voids may still be included between the increment layers [18, 19]. In response to a clearly felt demand for simple applicability and a more reasonable application time, so-called bulk-fill resin composites have been developed for restoring class I and class II posterior cavities [4]. These resin composites can be applied in a single increment of approximately 4–5 mm thickness [20]. The mechanical properties of bulk-fill resin composites promote lower polymerization shrinkage stress, better stress distribution, and good adaptation to the cavity walls [21, 22]. As a further improvement to ease application and adaptation, flowable bulk-fill composites with a lower filler content have been introduced. The first marketed flowable bulk-fill composite was SDR (Dentsply Sirona, Wien, Austria), which contains a stress-relieving additive in the monomer matrix. Due to this, SDR is considered to produce less polymerization shrinkage and shrinkage stress, thereby causing less cuspal deflection as compared to incrementally applied conventional composite [23–25]. Another way to control polymerization shrinkage in resin composite materials is the incorporation of fibers. Short fiber-reinforced composite resin (SFRC) has been recommended to reinforce direct composite restorations in high-stress areas like deep posterior cavities in vital and root canal-treated teeth [26–29]. As these materials are transparent, and their incorporated fibers scatter the light, they can be used as a bulk-fill restorative material to substitute the missing dentine up to 4–5 mm thickness [30–32]. SFRC are anisotropic materials: the orientation of the reinforcing fibers has a major influence on polymerization shrinkage. Shrinkage is controlled in the direction of the fibers, and the effect is never homogeneously distributed in all directions [26, 30]. Therefore, during polymerization, the material cannot shrink along the length of the fibers and retains its original dimensions horizontally, while the polymer matrix between the fibers can shrink [26]. Whether the application of SFRC (layered or bulk-fill) influences crack formation and/or marginal adaptation remains a question. Furthermore, the effect of hygroscopic expansion on crack formation in the case of different materials needs to be clarified.

Transillumination has long been suggested for crack detection in both direct and indirect restorations by Magne et al. [9, 33, 34] and Oliveira et al. [35]. Transillumination enables the operator to evaluate the effects of polymerization shrinkage in a more clinically relevant way than measuring isolated resin composite specimens or cuspal flexure [9]. However, Magne and co-workers only examined the number of enamel cracks 1 week after the restorative procedure and after fatigue testing. Thus, their results are more likely a result of the dynamic loading conditions during the accelerated fatigue testing procedure.

This study aimed to assess the crack formation associated with the different direct restorative procedures in deep class II MOD cavities. Three null hypotheses were formulated. The first one was that the number of cracks generated by the polymerization shrinkage stress right after the restorative procedure would not differ among the investigated direct resin composite restorations. The second one was that there would not be any difference in terms of crack formation 1 week after the restoration within the investigated groups. And the third null hypothesis assumes that there is no difference among groups in the 1-week crack number.

Materials and method

The study was approved by the Ethics Committee of the University of Szeged, and by the Regional Research Ethical Committee of the University of Pécs, and the study design conformed to the Declaration of Helsinki in all respects. A total of 110 mandibular third molars extracted for orthodontic reasons were used. All the selected teeth had similar coronal dimensions: orovestibular diameter 8–10 mm, mesiodistal diameter 9–11 mm, crown height measured from the cemento-enamel junction (CEJ) 6–7 mm. During the entire study period, the teeth were stored in 0.9% saline solution at room temperature.

Specimen preparation

Class II MOD cavities were prepared in all 110 teeth. The cavities were 5 mm deep and their oral and vestibular walls were 2.5 mm wide each, like in our previous studies [27, 28, 36]. The preparation was performed with a round end parallel diamond (881.31.014 FG – Brasseler USA Dental, Savannah, GA) bur initially positioned at the midline of the occlusal surface of the teeth (determined by dividing the distance between the buccal and lingual cusp tips). The thickness of the opposing walls at the cavity base were continuously checked during the preparation with a digital caliper (Mitutoyo Corp., Kawasaki, Japan) and adjusted to have a uniform 2.5 mm thickness at the base of the cavity. The cavity walls were prepared parallel to the axis of the tooth. The depth of the cavity was evaluated with a 15 UNC periodontal probe (Hu-Friedy Mfg. Co., Chicago, USA) measured from the corresponding cusp tip by touching the cavity wall with full length of the instrument. The cavity was one continuous cavity with the proximal box having exactly the same width (2.5 mm) and depth (5 mm) as the occlusal one. The cavosurface margins were prepared perpendicular to the tooth surface at the end of the preparation.

After cavity preparation, the teeth were screened for enamel cracks with D-Light Pro (GC Europe, Leuven, Belgium) in “detection mode,” at ×4.3 magnification. Teeth with enamel cracks were removed from the sample and replaced with ones that remained crack-free after cavity preparation. Finally, 80 crack-free third molars with MOD cavities were included to the study and were then randomly distributed into four groups of 20.

Restorative procedures

All teeth underwent the same adhesive treatment, as follows. A Tofflemire (1101C 0.035, KerrHawe, Bioggio, Switzerland) matrix was applied, and the enamel surrounding the cavity was etched with 37% phosphoric acid for 15 s, then rinsed with water. After drying the cavity, a one-step self-etch adhesive system (G-Premio Bond, GC Europe) was used according to the manufacturer’s instructions. The adhesive was light-cured for 60 s with an Optilux 501 quartz-tungsten-halogen light-curing unit (Kerr Corp., Orange, CA, USA). The average power density of the light source, measured with a digital radiometer, was 800 ± 40 mW/cm^2. The class II cavities were first modified to class I using the centripetal technique, building up the proximal walls with conventional resin composite (G-aenial Posterior A3, GC Europe). Then, the cavities were restored in either of the following ways:

Group 1 (n=20): The cavities were restored with SFRC (everX Posterior, GC Europe) in one 4-mm-thick bulk layer, according to the anatomy of the dentin, leaving 1 mm for occlusal covering with conventional composite. The SFRC was light-cured for 40 s.

Group 2 (n=20): The cavities were restored with SFRC with an oblique layering technique, according to the anatomy of the dentin. The approximately 2-mm-thick layers were placed overall in 4 mm depth, leaving 1 mm for occlusal covering with conventional composite. The deeper layers were light-cured for 40 s, and the ones closer to the surface were only for 20 s.

Group 3 (n=20): The cavities were restored with a bulk-fill resin composite (SDR Flow+, Dentsply Sirona, NC, USA) in one 4-mm-thick layer, according to the anatomy of the dentin, leaving 1 mm for occlusal covering with conventional resin composite. The bulk-fill composite was light-cured for 40 s.

Group 4 (control group, n=20): The cavities were restored with conventional resin composite (G-aenial Posterior A3, GC, Europe) with an oblique layering technique, according to the anatomy of the dentin and enamel. The layers were approximately 2 mm thick. The deeper layers were light-cured for 40 s, and the ones closer to the surface were only for 20 s.

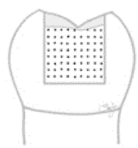
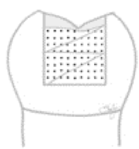
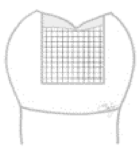
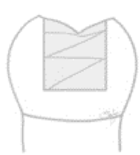
In groups 1, 2, and 3, the occlusal 1 mm was covered with conventional resin composite (G-aenial Posterior A3) and light-cured for 20 s.

The study groups, application methods, the investigated materials, and their composition are presented in Table 1. The restorations were finished with a fine granular diamond burr (FG 7406-018, Jet Diamonds, Ft. Worth, TX, USA, and FG 249-F012, Horico, Berlin, Germany) and aluminum oxide polishers (OneGloss PS Midi, Shofu Dental GmbH, Ratingen, Germany). The restored teeth were stored in physiological saline solution (Isotonic Saline Solution 0.9% B. Braun, Melsungen, Germany) in an incubator (mco-18aic, Sanyo, Japan) at 37°C until the start of the experimental procedures.

Screening for cracks in the restored teeth

Screening for cracks was performed with D-Light Pro (GC Europe) at ×4.3 magnification under transillumination with

Table 1 Study groups, materials, application methods, manufacturers, and composition of the investigated resin based composites

Group	Application method	Material	Manufacturer	Shade	Organic matrix	Filler	Filler loading (vol%/wt%)
Group 1* 	HV SFRC 4mm bulk layer	EverX Posterior	GC Europe, Leuven, Belgium	U	BisGMA, TEGDMA, PMMA	0.7µm barium glass (65.2%), 17µmx1-2mm short E-glass fibers (9%)	53.6/74.2
Group 2* 	HV SFRC in 2x2mm incremental layers						
Group 3* 	LV bulk-fill RBC in 4mm	Surefil SDR Flow+	Dentsply, Milford, DE, USA	U	Modified UDMA, TEGDMA, DMA, TMA	4.2µm Ba-Al-F-B silicate glass, Sr-Al-F silica, YbF	47.4/70.5
Group 4 (Control) 	HV conventional RBC in 2x2mm incremental layers	G-aenial Posterior	GC Europe, Leuven, Belgium	A3	UDMA, TCDDD, DMA	F-Al-silicate, Sr-glass, lanthanide-F	65.0/77.0

Abbreviation: *RBC*, resin-based composite; *SFRC*, short-fiver reinforced resin composite; *HV*, high viscosity; *LV*, low viscosity; *U*, universal; *vol%*, volume%; *wt%*, weight%; *BisGMA*, bisphenol-A diglycidil ether dimethacrylate; *TEGDMA*, triethylene glycol dimethacrylate; *PMMA*, polymethyl methacrylate; *UDMA*, urethane dimethacrylate; *DMA*, dimethacrylates; *TMA*, trimethacrylates; *TCDDD*, tricyclodecane dimethanol dimethacrylate

*1mm covering with G-aenial posterior RBC

the "detection mode," utilizing a protocol requiring two-examiner agreement (Fig. 1A and B). The light source was used in multiple positions searching for cracks on the external tooth surface for 1–2 min in order not to miss any. Only cracks reaching or exceeding the length of 2 mm were considered as shrinkage-induced cracks in our study. The length of the crack was measured with a 15 UNC periodontal probe (Hu-Friedy Mfg. Co., Chicago, USA) positioned parallel to the remaining coronal surface of the tooth next to the crack. The teeth were screened for cracks two times: first after the last polymerization phase and then 1 week later. Between the two sessions, the teeth were kept in physiological saline solution.

Statistical analysis

Sample size estimation was done in G*Power 3.1.9.7 (RRID:SCR_013726), with the following input parameters: $f = 0.35$; $\alpha = 0.05$; $1\text{-}\beta = 0.8$; number of groups = 4. The required sample size turned out to be n=96, but we chose to start out with 110 specimens, allowing for dropout.

Statistical analysis was performed in SPSS 26.0 (IBM, USA). For the descriptive characterization of the crack counts in each groups, means (with standard deviations) and medians (with minima and maxima) were calculated. For normality testing, the Shapiro-Wilk test was used. As the distribution of the data was non-normal in most cases, nonparametric tests were chosen for the hypothesis tests. Between-groups comparisons at one given time point were carried out with the Kruskal-Wallis test with post hoc pairwise comparisons, and for the within-group comparisons between the two time points, we used the Wilcoxon matched-pairs test. Where it was necessary because of the multiple comparisons, the level of significance was reduced to $p < 0.0125$.

Given that we managed to include fewer specimens than required for the 80% power, a post hoc power analysis was also performed with n=80. The power of the study was 72% this way.

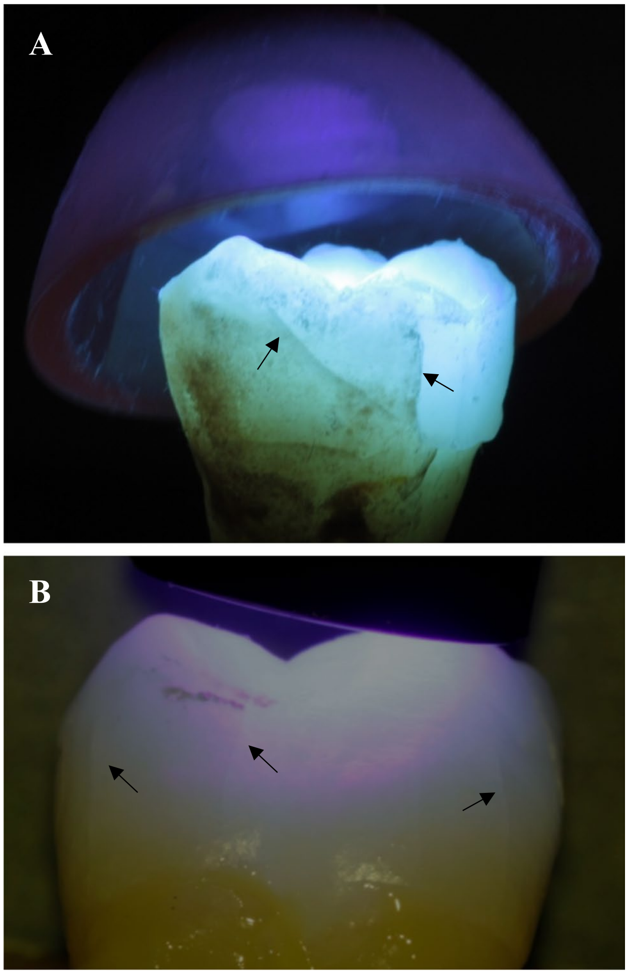


Fig. 1 **A** and **B** Examples of cracks (arrows) developing during the polymerization process

Results

Regarding the number of cracks right after the restorative procedure, a significantly lower number of polymerization-induced cracks were counted in the fiber-reinforced restorations (groups 1 and 2) than in the control group (layered composite filling, p=0.000 and p=0.000, respectively). Comparing either the non-fiber-reinforced groups (group 3 and the control group) or the SFRC groups (groups 1 and 2), the results did not indicate significant difference (Fig. 2 and Table 2). Thus, the first null hypothesis was rejected.

As for the comparison between the two timepoints (right after the restorative procedures and 1 week post hoc), a significantly higher number of cracks were counted in all groups after a week (group 1 p=0.000, group 2 p=0.000, group 3 p=0.001, control group p=0.000). Thus, the second null hypothesis was rejected too.

However, only the control group (layered composite filling) differed significantly from all the other groups (group 1 p=0.001, group 2 p=0.000, group 3 p=0.003) and there was no significant difference between the other groups (Fig. 3 and Table 3). Thus, the third null hypothesis was also rejected.

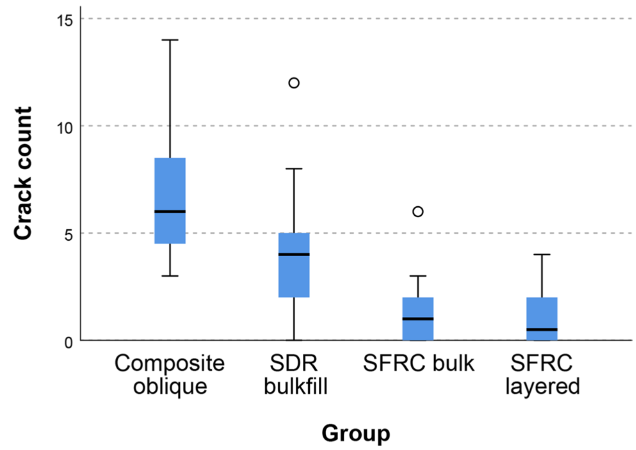


Fig. 2 Box plots of the crack counts immediately after restoration

Table 2 Pairwise comparisons between the study groups immediately after restoration (Kruskal-Wallis test, post hoc). Asterisk (*) indicates significant difference. The level of significance was adjusted to p=0.0125 according to Bonferroni because of the multiple comparisons

Comparison	Test statistic	Sig. (p)
SFRC-layered-SFRC bulk	4.750	1.000
SFRC-layered-SDR bulk-fill	26.125	0.002*
SFRC-layered-composite oblique	43.225	0.000*
SFRC bulk-SDR bulk-fill	21.375	0.020
SFRC bulk-composite oblique	38.475	0.000*
SDR bulk-fill-composite oblique	17.100	.113

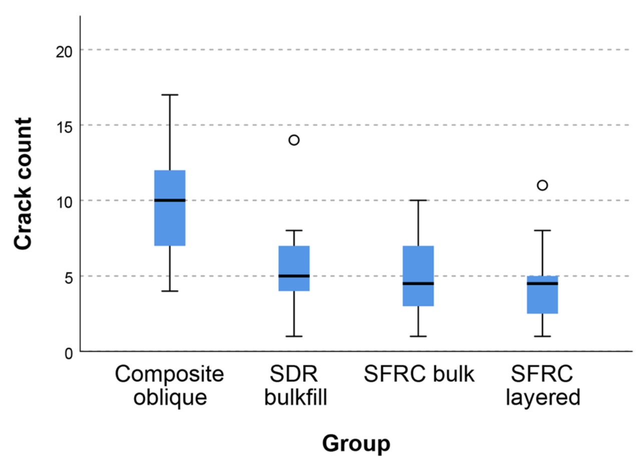


Fig. 3 Box plots of the crack counts 1 week after restoration

Table 3 Pairwise comparisons between the study groups 1 week after restoration (Kruskal-Wallis test, post hoc). Asterisk (*) indicates significant difference. The level of significance was adjusted to p=0.0125 according to Bonferroni because of the multiple comparisons

Comparison	Test statistic	Sig. (p)
SFRC-layered-SFRC bulk	4.975	1.000
SFRC-layered-SDR bulk-fill	7.725	1.000
SFRC-layered-composite oblique	33.300	0.000*
SFRC bulk-SDR bulk-fill	2.750	1.000
SFRC bulk-composite oblique	28.325	0.001*
SDR bulk-fill-composite oblique	25.575	0.003*

Discussion

As already known, polymerization shrinkage stress in resin composites may result in marginal disintegration, cuspal deflection, enamel crack formation, reduced bond strength, compromised mechanical properties, and interfacial gaps between the composite and the cavity walls, all of which are factors that contribute to the success or failure of direct composite restorations [5, 22, 37]. As a result, polymerization shrinkage-related stress is still deemed a clinically relevant problem in the field of dentistry [38]. Cuspal deflection and subsequent enamel crack formation is closely related to cavity dimensions, namely the volume factor (mainly the depth of the cavity) and cavity wall compliance (the continuity and thickness of the remaining walls) [35, 39]. As shown by Magne and colleagues, in the posterior region, deep MOD cavities show the highest amount cuspal flexure due to the missing marginal ridges [40]. According to Forster et al., in this clinical situation, 5 mm is the critical depth at which material-related disadvantages (such as suboptimal fracture toughness) start to show [36]. It is important to note that the number of direct posterior composite restorations is expected to increase in such deep cavities with the worldwide phasing out of the use of amalgam [41–43]. Therefore, in our study, we analyzed polymerization shrinkage-induced crack formation of different restorative materials in deep MOD cavities via transillumination.

In our study, when analyzing crack formation immediately after the restorative procedure, both the bulk-fill and the layered SFRC restorations (groups 1 and 2, respectively) were characterized by significantly fewer cracks compared to layered conventional composite restorations (control group) (p=0.000 and p=0.000, respectively) (Fig. 2). Thus, the first null hypothesis was rejected. So far, SFRC has not been compared to any direct restorative materials in this aspect. The superior results of SFRC might be attributed to its unique structure with short glass fibers of 0.3–1.9 mm embedded in the resin matrix [44]. As the fibers are randomly oriented in the material, they might be able to control the shrinkage and the resulting stress on the cavity walls as the material is not able to shrink in the direction of the fibers, only perpendicular to them [30, 45]. In contrary, Fronza et al. demonstrated higher shrinkage stress values of SFRC samples and their result was explained by high inorganic content and resultant high elastic modulus of the material [18]. In terms of fracture resistance and failure mode, direct restorations containing SFRC were superior to conventional layered composite restorations in deep MOD cavities [27, 28]. When comparing the layered SFRC group (group 2) with the bulk-fill SFRC group (group 1) regarding crack formation, no difference was found. This is contrary to the findings of Oliveira et al., who found that a significantly higher number of cracks were generated in layered composite restorations than in bulk-fill composite fillings [35]. However, they tested non-fiber-reinforced composites. So far, this is the first study to examine crack formation after restoration with fiber-reinforced and non-fiber-reinforced direct restorative materials applied according to different methods. In our previous studies, we found no difference between layered and bulk-fill direct SFRC restorations in terms of mechanical performance [27, 46]. However, crack formation was not evaluated in these studies. Interestingly, only the layered SFRC group (group 2) was superior to the SDR group (group 3) in this respect (p=0.002), while there was no difference between the two bulk-fill groups (groups 1 and 3). This is explained in part by the reduced volume of the 2 mm layers compared to the 4 mm bulk increment which may decrease the final polymerization volumetric shrinkage and consequently the shrinkage-induced cracks [47]. In contrast, the layering technique did not show this beneficial effect in the control group regarding the crack formation. Boaro et al. demonstrated strong relationship between normalized stress and specimen volume in favor of bulk-fill materials [48]; however, Fronza and co-workers refuted this proposition [18]. According to our findings and the above referred conflicting results, it is clear that not only the volume of the material but its composition as well plays an important role in shrinkage stress behaviors. Among the investigated materials, G-aenial Posterior—which served as control—has the highest inorganic filler content of 65 vol% and presented the most cracks, which were developed during and after the polymerization. Although the filler content reduces the volumetric shrinkage, it can increase the material stiffness and thus the elastic modulus [49]. Post-polymerization further can increase substantially the Young's modulus [50]; however, its opposite in wet storage was also demonstrated [51]. Our results support the former findings, since when comparing the number of cracks right after the restorative procedure and 1 week post hoc, a significant increase was observed in all groups. Thus, the second null hypothesis was rejected.

The third null hypothesis, which anticipated no significant difference among groups after 1-week water storage, was also rejected, because more prominent differences were observed in the number of cracks at the end of one week. All groups showed significantly fewer cracks compared to the control group (conventional layered composite restorations). Polymerization process and consequently the shrinkage are known to continue for more than 24 h after light-curing (known as post-cure polymerization) [52]. In fact, it has been observed as much as 1 month after photopolymerization [53]. As the occurrence of enamel cracks is known to be closely related to the polymerization shrinkage of composite resin materials [54], post-cure polymerization should be reflected in the number of cracks after the restorative treatment. The correlation between post-cure polymerization and crack formation is clearly confirmed in our study: the number of cracks significantly increased in all groups during the first week after the restorative procedure (Fig. 2). Interestingly, the effect of hygroscopic expansion could not counterbalance the effect of post-cure polymerization in this respect. Water storage, on the other hand, can plasticize the resin matrix that may also degrade the filler-matrix interface, leading to decrease in Young's modulus and thus in accumulated stress [55]. Under clinical conditions, restored teeth are continuously bathed with oral fluids, and thus, water absorption, hygroscopic expansion, and plasticizing effect can be expected to counterbalance polymerization contraction, and thus could even cancel out cuspal flexure and neutralize residual shrinkage stresses [56]. According to Suiter and colleagues, this stress-relaxation takes more than 4 weeks in the case of resin composite materials [57]. Thus, a possible explanation as to why water sorption could not effectively eliminate stress may be that we kept our specimens in water only for a week before the repeated examination. This is in line with the findings of Magne et al. [34]. It seems that both SFRC and bulk-fill resin composites are more resilient to polymerization shrinkage-related crack formation than layered conventional high-viscosity resin composite fillings.

A limitation of our study is that samples were not loaded after crack analyzing to see whether the cracks are directly correlated with fracture behavior and whether they propagate in case of the tested materials under loaded condition or not. In future, this aspect should be studied also.

Another limitation is that the direction of cracks (horizontal, vertical, or oblique) was not assessed. Furthermore, D-Light Pro using the "detection mode," specifically designed to detect cracks, was utilized in our study. However, the specificity and sensitivity of the device to detect cracks is not known as this device has not been used for such purposes in scientific literature. It is important to highlight that transillumination cannot give information on the depth-wise extension of cracks, which would hold great importance in clinical practice. This could also be a limitation of our study. Despite these known limitations, the current study is a good start point for future research in the field of material-related shrinkage-stress–induced crack formation.

Conclusions

Within the limitations, in conclusion, the current study has demonstrated that:

– Polymerization shrinkage stress induced material- and placement technique–dependent crack formation in tooth, which phenomenon further progressed 1 week after the restoration.
– SFRC was more resistant to shrinkage stress during the restorative procedure; however, after 1 week, besides SFRC, bulk-fill RC also showed higher resistance to polymerization shrinkage-related crack formation than layered composite fillings.
– SRFC can decrease the shrinkage stress-induced crack formation in MOD cavities.

Author contribution Conceptualization, E.L. and M.F.; methodology, E.L. and M.F.; software, G.B.; validation, G.B., T.S., and M.F.; formal analysis, G.B.; investigation, V.N., T.S., and L.F.Sz.; resources, M.F.; data curation, G.B.; writing—original draft preparation, M.F., E.L., T.S., V.N., and B.Sz.; writing—review and editing, S.G.; visualization, T.S. and B.Sz.; supervision, E.L. and M.F.; project administration, V.N. and T.S.; funding acquisition, M.F. All authors have read and agreed to the published version of the manuscript.

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Data Availability Data is contained within the article.

Declarations

Ethical approval and consent to participate The study was approved by the Ethics Committee of the University of Szeged (4029), and by the Regional Research Ethical Committee of the University of Pécs (3795), and the study design conformed to the Declaration of Helsinki in all respects. The study does not contain human subjects.

Conflict of interest The authors declare no competing interests.

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