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**ISPITIVANJE UTICAJA POLIMERIZACIONE KONTRAKCIJE DENTALNOG KOMPOZITA NA DEFORMACIJU KVRŽICA ZUBA DIGITALNOM HOLOGRAFIJOM**

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CUSPAL DEFLECTION CAUSED BY DENTAL COMPOSITE POLYMERIZATION SHRINKAGE ANALYZED BY DIGITAL HOLOGRAPHY

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Abstract

Background/Aim. The objective of this study was to measure tooth cusps deflection caused by polymerization shrinkage of a resin-based dental material (RDM), in real-time using digital holographic interferometry (DHI), in two groups of cavities restored with and without an additional wall. Simultaneously, internal tooth mechanical behavior was monitored. Methods. Standardized three class I cavities were prepared on third molar teeth. The teeth were cut in two halves in the longitudinal plane, obtaining six samples for the study (now with class II cavities), divided into two groups (group G1 – with the additional wall, group G2 – without it) and mounted in aluminum blocks. The cavities were filled with the RDM, cured with a light emitting diode (LED) for 40 s from the occlusal direction, and monitored during the curing and post-curing period using DHI. Data were analyzed using student's t-test for independent samples and Anderson-Darling test, with an alpha level of 0.05. Results. At the end of the examined period, the samples from group G1 showed significantly increased tooth cusps deflection (t (10) = 4.7; p = 0.001) compared to samples from group G2. Conclusion. Within the limitations of this study, it was concluded that the presence of the additional wall simulating a dental matrix-band, influenced increased and prolonged tooth cusps deflection during the examined RDM polymerization shrinkage.

Keywords: tooth cusps deflection; resin-based dental material; digital holographic interferometry.

Apstrakt

Uvod/Cilj. Cilj ove studije bio je da se detektuje naprezanje kvržica zuba izazvano polimerizacionom kontrakcijom dentalnog materijala na bazi smole, u realnom vremenu primenom digitalne holografske interferometrije (DHI), u dve grupe kaviteta restauriranih sa i bez dodatnog zida. Uporedo je praćeno mehaničko ponašanje zubnog tkiva. Metode. Na humanim trećim molariima pripremljena su tri standardizovana kaviteta klase I. Zubi su presečeni na dve polovine u uzdužnoj ravni, dobivši šest uzoraka (sada sa kavitetima klase II) podeljenih u dve grupe (grupa G1 - sa dodatnim zidom, grupa G2 - bez njega) i fiksiranih u aluminijumske modle. Kaviteti su potom ispunjeni materijalom izbora, prosvetljeni 40 s svetlosno-emitujućom diodom (eng. LED) iz okluzalne projekcije i
ispitivani tokom i nakon perioda prosvetljavanja primenom DHI. Podaci su analizirani studentskim t-testom za nezavisne uzorke i Anderson-Darlingovim testom, sa nivoom značajnosti alfa od 0,05. Rezultati. Na kraju ispitivanog perioda, uzorci iz grupe G1 pokazali su značajno veću deformaciju kvržica zuba (t (10) = 4,7; p = 0,001), u poređenju sa uzorcima iz grupe G2. Zaključak. U okviru ograničenja ove studije, zaključeno je da je prisustvo dodatnog zida simulirajući dentalnu matricu, značajno uticalo na povećano i produženo naprezanje kvržica zuba prilikom polimerizacione kontrakcije ispitivanog dentalnog materijala na bazi smole.

Ključne reči: naprezanje kvržica zuba; dentalni materijali na bazi smole; digitalna holografska interferometrija.

Introduction

Despite the continuous improvements in the field of resin-based dental materials (RDM), polymerization shrinkage of filling materials is one of the main disadvantages that remains of interest in adhesive restorative dentistry. During material setting, polymerization shrinkage stress (PSS) is generated at the tooth-restoration interface, which is considered responsible for several negative clinical effects that may arise including debonding, leakage, post-operative sensitivity, secondary caries, cusps deflection, and crack formation in enamel/dentin \(^1\). \(^2\). Regarded as a physical process, PSS is dependent upon several factors such as volume/weight percentages of the resin matrix, filler formulation, restorative procedure and cavity configuration (C-factor) \(^3\). Hence, PSS itself is a highly non-uniform and multifactorial phenomenon that cannot be measured directly \(^4\)–\(^6\). Consequently, experimental tests need to be carried out to investigate the constraints imposed on the bonded restorations \(^7\)–\(^10\), and to estimate interface problems in adhesive reconstructions caused by PSS \(^10\),\(^11\). On the other hand, direct monitoring of secondary phenomena, such as tooth cusps deflection, can provide indirect vision into PSS development \(^12\)–\(^16\). Digital holographic interferometry (DHI) is a laser optic technique suitable for non-destructive and contactless measurements of submicron changes in highly asymmetrical objects with micrometer precision \(^16\),\(^17\). The efficiency of classical holographic interferometry in the field of dental biomechanics was previously studied \(^18\),\(^19\), while DHI is a relatively new testing method in the field. Due to the digital nature of the method (digital camera, computer software) enabling fast and simple recording and reconstruction of
holographically generated interference images, DHI has become a valuable tool in different fields of science and technology.  
The bulk of the mineralized tooth tissue is composed of dentin which supports the overlying hard and brittle enamel in the part of the tooth crown. These two specific calcified tissues are joined by the dentin-enamel junction (DEJ), described as a natural multilevel interface that plays an important role in the accommodation of stress. Considering the anisotropic histological structure of enamel and dentin, it is of utmost importance for the clinical practice to appreciate the impact of PSS on each of the surrounding hard tooth tissues.  
On the other hand, it was previously identified that confinement imposed on the RDM by bonding to tooth cavity walls affects the level of PSS. This specific relationship described through the concept of C-factor and defined as the ratio of bonded to unbonded (free) surfaces of the restoration, still contributes to layering restorative procedures or bulk filling techniques. Meanwhile, during proximal tooth cavity reconstruction, the creation of the missing tooth part is built by using a metal band (matrix-band) to perform a proper tooth crown reconstruction. Therefore, the impact of this additional constraint on the RDM, caused by the matrix-band, was examined in this study.

The objective of this study was to measure tooth cusps deflection during the RDM polymerization shrinkage, in real-time using DHI, in two groups of cavities restored with and without an additional wall. Simultaneously, internal tooth mechanical behavior throughout the curing and post-curing period of the RDM was monitored. Following the aforementioned aim, the hypothesis that there is no significant difference in tooth cusps deflection at the end of the examined period between the two groups was presumed.

**Methods**

Ten third molar teeth extracted for pericoronitis, periodontal disease, or orthodontic reasons were collected before the beginning of the experiment at the Department of Oral Surgery, Dental Clinic of Vojvodina, Novi Sad, Serbia. The teeth were cleaned of residual periodontal ligament and debris and stored in distilled water (23±1°C). All clinical procedures were performed under the ethical guidelines of the Ethics Committee of the Faculty of Medicine, University Novi Sad. Due to numerous anatomical variances of third molar teeth, all gathered teeth were measured following the previously defined criteria by Politi et al. – a maximum buccal-palatal width (BPW) of 10.25-10.75 mm, and the
presence of four cusps (two buccal and two palatal). As a result, three third molar teeth were included in the study.

Sample preparation

Class I cavities were prepared on the three selected teeth using a high-speed hand-piece (300,000 rpm) with water spray, and a round diamond bur for cavity preparation with perpendicular walls to the pulp floor and rounded internal line angles. In the interest of better control of the biological variability of human teeth, cavity preparation was performed following relative rather than absolute measures as follows: the width was two-thirds of the BPW, the occlusal isthmus was prepared to half of the BPW, the mesial-distal extension was performed towards the end of the central groove preserving marginal ridge integrity, and the axial depth was set at 2 mm (measured from the end of the central groove). In that manner, the integrity of the tooth cusps and marginal ridges were preserved, avoiding potential inconsistency in tooth tissue mechanical behavior during PSS.

To estimate the mechanical behavior of internal tooth tissue, teeth were cut in half to expose dentin, enamel, and DEJ since DHI can only visualize surface changes of non-transparent objects for the wavelength of the selected light source. Samples were cut longitudinally (vestibule-oral direction) in two halves according to the study of Xia et al., resulting in six samples for the study with two tooth cusps each (buccal and palatal), permitting the internal tooth mechanical behavior evaluation. In the next step, the samples were mounted in aluminum blocks using dental gypsum (Marmorock 20; Dr.Böhme & Schöps™ GmbH, EN 26873/ ISO 6873, type IV) to the level of the enamel-cement junction, ensuring visibility of the tooth crown, and appropriate mechanical stability necessary for the experiment. Before application of the resin bonding adhesive system (Single bond universal adhesive® – 3M™ Deutschland GmbH, LOT No. 663414), the cavities were cleansed under copious water irrigation and etched with phosphoric acid (Gel etchant 37.5% phosphoric acid, Kerr Italia™ s.r.l, LOT No. 3596305) using the total-etch technique (30 s enamel, 15 s dentin), and then once again water-rinsed. The bonding system was then applied following the manufacturer’s instructions, and cured for 10 s with a LED light source (SmartLite IQ2® L.E.D. curing light, Dentsply®, Model No. 200, Serial No. B21581, 500 mW/cm²).

Two groups of three samples each were formed so that one half of every tooth was included in the first group (G1) and the second group (G2), respectively. On the G1 group
samples, a piece of a thin microscopic cover glass was added along the proximal side of the cavity during tooth fixation, simulating a matrix-band (normally used in clinical practice to restore class II cavities) and maintaining the visibility of internal tooth tissues, while the samples in G2 group were mounted without it serving as a negative control. The prepared cavities were restored by the bulk filling technique, using a single increment of material per cavity. As the RDM, a nano-filling resin composite was used (Filtek Ultimate Universal Restorative® , A2 body shade, 3M ESPE ™ USA, LOT No. N867954) (Table 1). Finally, samples were fixed in the DHI setup. After fixation, the polymerization process was activated with the LED light source, applied from the occlusal direction at a distance of 1mm from the sample surface, and using a continuous curing mode of 40 s. The study included the examination of the curing and post-curing period lasting a total of 320 s (~ 5 min).

Experimental setup

The tooth cusps deflection was directly monitored in real-time using a custom-made DHI setup with a single laser beam expanded by a diverging lens and a spherical mirror (Fig. 1). In that manner, observation and laser light illumination of the sample from both sides (front and rear) was enabled, while the region of interest was the cut side of the sample providing vision of the internal tooth tissues (Fig. 2a-3b). By generating all the necessary beams from the same input beam, excellent mechanical stability of the setup was obtained, required for the holographic experiment. In this experiment, a diode-pumped solid-state, frequency-doubled Nd: Vanadate (Nd: YVO₄) laser was used, that provided single-frequency green output (Coherent® Verdi V5, 532 nm wavelength, 5 W maximum power). The power output of 500 mW was enough for this experiment, while the linewidth of the laser was less than 5 MHz, guaranteeing a highly coherent beam. A digital single-lens reflex camera (Canon® EOS50d, 15.1 megapixels, 4752 x 3168 image size) recorded the resulting holograms (every 2 s during the first minute, and 10 s afterwards until the end of the observation period). The obtained images were transferred to a computer, and numerically reconstructed by parallel processing on a graphic card (NVidia® GeForce GTX 1060 6GB) The holographic experiment was based on the interference between the object beam (scattered from the object) and the reference beam (one that misses both the front and rear side of the object and continues to propagate) generated from the same radiation source.
Due to the existing movement of the sample (tooth cusps deflection), the reconstructed holographic interferograms showed a specific interference pattern presented in a form of series of interference lines (so-called “fringes”) whose number, shape, and orientation gave information about the resulting mechanical deformation. The exact amount of deformation was calculated by multiplying the number of fringes that appeared in the examined interval of time with the wavelength of laser light 17.

Statistical analysis

Statistical analysis was performed in Minitab® software (version 19.2020.1; 64-bit) using the student's t-test for independent samples (n=6 + n=6, G1 and G2 respectively) for testing of the presumed hypothesis. The distribution normality of the results was analyzed by Anderson-Darling tests. Power calculations were carried out for the student's t-test. Alpha level of 0.05 was used for all statistical tests.

Results

The resulting DHI images (interferograms) from both groups presented a specific interference pattern indicating tooth cusps deflection, with each fringe appearing corresponding to deformation of 0.532 µm (Fig. 2a-3b). At the end of the examined period, the G1 group samples restored with the cover glass (n = 6; M = 5.4; SD = 1.6) showed a significantly higher amount of cusps deflection per cusp (t (10) = 4.7; p = 0.001) than the samples from the G2 group restored without it (n = 6; M = 2.1; SD = 0.6). Table 2 summarizes the measured single values of cusps deflection. The cusps deflection reached a maximum of 7.8 µm and 2.7 µm per cusp in groups G1 and G2 respectively (Fig. 4).

Anderson-Darling test showed that data from both G1 (AD = 0.2; p = 0.6) and G2 groups (AD = 0.3; p = 0.4) followed normal distribution. Power calculations carried out for the student’s t-test, showed that the chosen sample size allowed registering differences between groups at < 3 µm (2.9 µm) with the power of 0.87 (87%). The results also provided qualitative information about the submicron movements of the examined samples: during the curing reaction of the RDM in some samples from group G2, a change in fringe appearance was noticed when moving along the DEJ projection (Fig. 3a).

Discussion

Results of this study contributed to the investigation of the biomechanics of tooth cusps displacement in class II adhesive restorations during PSS development. Utilizing DHI,
direct measurement of submicron tooth cusps displacement was performed, enabling indirect monitoring of the polymerization reaction kinetics. When the interferograms of the two groups were compared at the same moment of recording (end of the curing period and the whole examined period), it was observed that in the G1 group the interferograms presented more fringes than in the G2 group (Fig. 2a-3b). This result indicated increased tooth cusps displacement in the G1 group (on average 5.4 µm per cusp, versus 2.1 µm in G2). Assuming the same experimental conditions in the two groups such as standardized cavities, RDM, restorative technique and polymerization protocol, the occurrence of increased cusps deflection in the G1 group was associated with the cavity configuration (C-factor) variation due to the presence of the additional glass wall.

The results also showed that cusps deflection did not finish with the end of the curing period, but continued to increase in the post-curing period (Fig. 4). This indicated that the polymerization reaction continued after the photo-activation step, as already demonstrated by several studies. When the post-curing deformation per cusp in groups G1 and G2 was compared, it was evident that in group G1 it continued to gradually increase until the end of the examined period, unlike the absence of such progress in group G2 (Fig. 4).

Nevertheless, the research conducted by Germscheid et al. stated that examination of the post-curing period of contemporary RDMs, should cover a time interval longer than 1 h (up to 15 h), due to the significant amount of measured post-curing shrinkage. Further evaluation in a prolonged period could be a topic of another study, using a modified examination protocol by recording the interference images every 10-15 min i.e., thereby rationalizing hard-disk memory storage.

However, the marginal adaption of RDMs in class I and class II cavities reflects complex interactions between adhesive bonding on one hand, and PSS at the tooth-restoration interface on the other. The level of PSS and debonding are more probably dependent upon the shape and hence constraints of the cavity, as well as viscoelastic properties of RDMs, than other factors. It is well-known that cavity configuration (C-factor) is one of the main factors affecting the development of PSS, since greater confinement imposed on the RDM leaves a smaller number of free surfaces for resin composite shrinkage and PSS relaxation. According to the study of Han et al. the RDMs can shrink relatively free in a cavity with a larger number of unbonded surfaces. These findings have been shown using different tools in several researches where post-gel PSS was evaluated.
and not only tooth cusps displacement. Apart from the effect of the C-factor and RDM elastic modulus upon PSS, a relevant role was also found in adhesive filling techniques. In the present study, bulk-filling technique was used to avoid sample movement during examination, and preserve the mechanical stability of the setup necessary for the holographic experiment. Even though the samples in the G1 group were not bonded to the cover glass, this additional wall transformed their cavity configuration, which increased and extended cusps deflection (following PSS), imitating class I cavity configuration. Control of glass stability was performed manually for every sample in G1 group, before filling of the cavity. Based on the presented results, the first null hypothesis that there is no significant difference in tooth cusps deflection at the end of the examined period between the two proposed groups (with and without an additional wall) was rejected. On the other hand, qualitative assessment of the resulting interference images revealed the change in fringe appearance at the DEJ projection in some samples from group G2 during the curing reaction of the RDM (Fig. 3a). This finding could highlight the role of DEJ in the accommodation of internal forces such as PSS, as suggested by the results of several studies. However, the presence of a regular interference pattern and the influence of the additional wall in this sense, are yet to be explored. Given the aggravating circumstances of conducting a clinical study that would establish a direct link between the phenomenon of PSS with certain clinical outcomes, in vitro studies play a significant role in the field of RDM examination due to the need for constant improvement of materials on the market. Therefore, this study proved that DHI, as a non-destructive method with submicron precision, is able to directly investigate the tooth cusps deformation and to predict PSS influence on the behavior of adhesively restored molar teeth.

**Conclusion**

Based on the obtained results and within the limitations of this study, it was concluded that by changing cavity configuration, the presence of the additional glass cover wall simulating a dental matrix-band, influenced increased and prolonged tooth cusps deflection during the polymerization reaction of the RDM. Future perspectives would be to explore if any regular pattern in the behavior of tooth tissue under internal stress such as PSS could be found, especially in the presence of an additional matrix-band wall.
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Tables

| Name                  | Filtek Ultimate Universal Restorative® |
|-----------------------|----------------------------------------|
| Manufacturer          | 3M ESPE™, St. Paul, MN, USA            |
| Classification        | Nano-filled resin composite            |
| Lot No.               | N867954                                |
| Shade                 | A2 body                                |
| Matrix                | Bis-GMA, UDMA, TEGDMA, Bis-EMA(6), PEGDMA |
| Filler                | Non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4-11 nm zirconia filler, and aggregated zirconia/silica cluster filler (average cluster particle size 0.6 - 10μm). |
| Filler loading        | 78.5% by weight, 63.3% by volume       |

Legend: Bis-GMA- bisphenol A diglicidil ether dimethacrylate; Bis-EMA- bisphenol A polyethylene glycol diether dimethacrylate; UDMA- urethane dimethacrylate; TEGMA- triethylene glycol dimethacrylate; PEGDMA- polyethylene glycol dimethacrylate.

Table 1. Details of the RDM used in the study (data obtained from the manufacturer https://multimedia.3m.com/mws/media/629943O/filtek-ultimate-technical-product-profile-cee.pdf - date of access 03.06.2021.)
| Group | Sample number | Tooth cusp | Final cusps deflection (µm) | Standard deviation (SD) | Sample size (n) |
|-------|---------------|------------|-----------------------------|-------------------------|----------------|
| G1    | 1             | Buccal     | 4.79                        |                         | 6              |
|       |               | Palatal    | 3.72                        |                         |                |
|       | 2             | Buccal     | 5.85                        | 1.6                     | 6              |
|       |               | Palatal    | 3.72                        |                         |                |
|       | 3             | Buccal     | 7.80                        |                         |                |
|       |               | Palatal    | 6.39                        |                         |                |
| G2    | 4             | Buccal     | 2.66                        |                         | 6              |
|       |               | Palatal    | 1.06                        |                         |                |
|       | 5             | Buccal     | 1.6                         | 0.62                    | 6              |
|       |               | Palatal    | 2.13                        |                         |                |
|       | 6             | Buccal     | 2.13                        |                         |                |
|       |               | Palatal    | 2.66                        |                         |                |

Table 2. Measured single values for each tooth cusp, at the end of the whole examined period (final tooth cusps deflection)

**Figure legends**

Fig. 1 Custom made DHI setup (Las - laser, Len - lens, SF - spatial filter, FM – flat mirror, Sph - spherical mirror, Sam - sample, Ref – reference beam, Obj - object beam, Cam – camera)

Fig. 2 Interference pattern in group G1 (a) at the end of the curing period (42 s) (b) at the end of the whole examined period (320 s)
Fig. 3 Interference pattern in group G2 (a) at the end of the curing period (42 s) – white arrow indicating the change in fringe appearance (b) at the end of the whole examined period (320 s)
Fig. 4 Tooth cusps deflection (µm) during the examined period as a function of time (G1 – group 1 samples restored with the additional wall, G2 – group 2 samples restored without it)

Abbreviations
DHI – digital holographic interferometry
DEJ – dentin-enamel junction
LED – light-emitting diode
PSS – polymerization shrinkage stress
RDM – resin-based dental material
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