Effect of dentin desensitizing procedures on methyl methacrylate diffusion through dentin

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Abstract

Background: Acrylic and bisacryl resins are widely used both during the temporization phase as well as for provisional restorations and the effect of external agents on dentin sensitivity can be reduced by the obliteration of the tubules.

Objective: The purpose of this study was to evaluate diffusion of methyl methacrylate monomer through dentin by high performance liquid chromatography (HPLC) after three different desensitizing procedures during the fabrication of two different provisional crown materials.

Materials and Methods: Forty extracted restoration and caries free human premolar teeth were used in this study. Thermoplastic vacuum formed material was used as a matrix to fabricate provisional restorations for each tooth before crown preparation. Teeth were prepared for a metal supported ceramic crown with 1 mm shoulder margins and then crown parts were separated from cementoenamel junction with a carborundum disk perpendicular to the long axis of the teeth. To the cementoenamel junction of each tooth a polypropylene chamber was attached that contains 1.5 cm^3 of deionized distilled water. Prepared teeth were divided into four groups (n = 10) including control, desensitizing agent (DA) application, neodymium-doped yttrium aluminum garnet (Nd: YAG) laser irradiation (LI), and LI after DA application groups. After application of DA (except control) each group were divided into two subgroups for fabrication of provisional restorations (n = 5). Two autopolymerizing provisional materials (Imident (Imicryl) and Systemp C and B (Ivoclar, vivadent)) were used to fabricate provisional restorations using the strips. Water elutes were analyzed by HPLC at 10 min and 24 h.

Results: The monomer diffusion values varied statistically according to desensitizing procedures, provisional resin systems, and the time periods. Monomer diffusion through dentin surfaces desensitized with Nd: YAG LI after DA application was the lowest.

Conclusions: Nd: YAG LI in association with DA application is an effective combination to eliminate monomer diffusion through dentin to pulpal chamber.

Key words: Dentin hypersensitivity, dentin permeability, laser, monomer diffusion, provisional crown

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Introduction

Professional interest in the causes and treatments of dentinal hypersensitivity has existed for the last 2 century.[1] With removal of enamel or denudation of the root surface by loss of the periodontal tissues and overlying cementum, the dentin exposure may occur.[2] Desensitizers obstruct exposed dentin tubules with a chemical contents, block tubule fluid flow, and reduce the sensation of pain.[3] The movement of dentinal fluid is influenced by the number of open tubules, the tubule's configuration, and the diameter of tubules.[4]

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After crown preparation, millions of dentinal tubules may be exposed.[5] The amount of dentin reduction as well as the area of tooth surface prepared can lead to various degrees of dentin permeability and subsequent pulpal irritation.[6]

The concept of tubular occlusion as a method of dentin desensitization is a logical conclusion of the hydrodynamic theory.[7] Treatment methods for dentin hypersensitivity are numerous and diverse and mostly involve two principal therapeutic aims: Obstructing the dentinal tubules to prevent dentinal flow and/or desensitizing the nerve to make it less responsive to stimulation.[8] The most commonly used agents in the treatment of dentin sensitivity can be broadly classified by their modes of action: Anti-inflammatory drugs, protein precipitants, tubule occluding agents, tubule sealants, and laser treatment.[2] In the mid of 1980s, the use of lasers to decrease the level of dentin hypersensitivity was proposed.[9] One of the most approved theories for explaining the therapeutic effect of laser irradiation (LI) on dentin hypersensitivity is its sealing effect on dentinal tubules by melting and recrystallization.[10]

In prosthodontic treatment, provisional crowns takes part with an important place in protecting the prepared tooth and preventing the teeth migration and occlusal changes, and they restore function until cementation of the permanent prosthesis and decrease dentinal hypersensitivity.[11] Polyethylmethacrylates (PEMAs) and polymethylmethacrylates (PMMAs) have been popular choices as provisional materials for temporization of direct and indirect restorative procedures.[12] In polymerization reaction, transformation of monomers to polymers is not complete, and some unreacted methylmethacrylates (MMAs) are left in the denture base that are soluble in water and so into saliva and dentin tubules.[13] As an excellent barrier; although dentin protects pulp from both pathological and iatrogenic insults, diffusion of monomers through dentin may elicit inflammation and foreign body reactions.[14] Previous studies have shown the cytotoxic and allergic characteristics of MMA.[15,16] Kojima *et al.*,[17] examined cytotoxicity of PMMA-based dental temporary filling resin to dental pulp cells and they reported that PMMA-based temporary filling dental resin leads to functional suppression and critical levels of cell death *in vitro*.

The aim of this study was to evaluate diffusion of MMA monomers through dentin by high performance liquid chromatography (HPLC) during the fabrication of two different provisional restoration materials (PRMs) after the application of three different desensitizing procedures (desensitizing agent (DA) application, neodymium-doped yttrium aluminum garnet (Nd:YAG) LI, and LI after DA application) on dentin surface. One of the hypothesis of the current study was that, there would be diffusion of residual monomers from dentin tubules to pulp chamber after polymerization of PRMs. The second research hypothesis was that desensitizing procedures decrease this monomer diffusion.

Materials and Methods

The format of the study was reviewed and approved by the Ethical Committee of the Gaziantep University, Gaziantep, Turkey (protocol number: 05-2009/209).

The materials used for this study are summarized in Table 1. Forty extracted caries and restoration-free human premolar teeth (because of orthodontic and periodontal reasons) were used in this study. Apical thirds of the roots were embedded in acrylic blocks to facilitate manipulation. Thermoplastic vacuum-formed material (Umg, Uysal Medikal, Istanbul, Turkey) 0.5 mm in thickness was used as a matrix to fabricate provisional restorations for each tooth before crown preparation. Teeth were prepared for a metal-ceramic complete crown with 1-mm shoulder margins by the same prosthodontist. The prepared crown parts of teeth were separated perpendicular to the long axis of the teeth with a carborundum disk 2 mm under the cementoenamel junction. The remnant pulpal tissue was expanded and cleaned.

Dentin thicknesses were measured from five points (two at the cusp ridge, one each at the buccal and palatal cervical points, and one at the center of the occlusal surface) of prepared teeth with a caliper (Kumpas Metal Iwanson, Jensen, Metzingen, Germany) [Figure 1]. Average thickness was between 2 and 2.5 mm. Each tooth was attached from the cementoenamel junction with soft wax to a polypropylene chamber that contains 1.5 cm^3 of deionized distilled water [Figure 2]. Prepared teeth were categorized into four groups. In the first group, no dentin desensitizer was used as the control (C) group. In the second group, one layer of a DA (Smartprotect, Detax, Ettlingen, Germany) was applied on dentin with a brush in a uniform coating.

Table 1: Materials used for this study

Materials	Identification	Composition	Manufacturer
Desensitizer	Smartprotect	Chemical desensitizing agent	Detax, Ettlingen, Germany
Nd: YAG laser	Fidelis Plus 3	Nd: YAG laser	Fotona, Ljublgana, Slovenia
Provisional crown materials	Imident	Polymethylmethacrylate	Imicryl Dis Malzemeleri, Konya, Turkey
	Systemp C&B	Acrylates+Methacrylates+BisGMA	Ivoclar, Vivadent, Schaan, Liechtenstein

Nd: YAG=Neodymium-doped: yttrium aluminum garnet

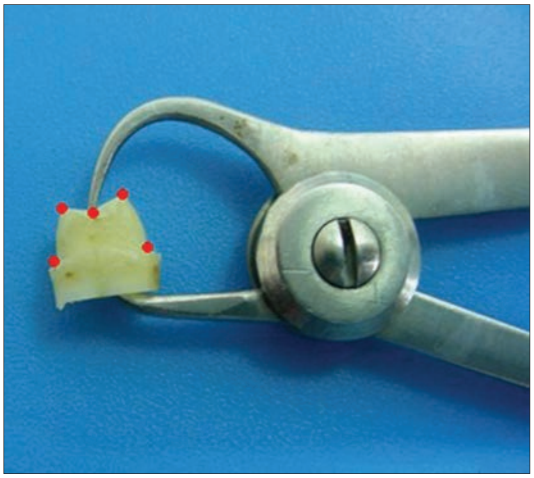

Figure 1: Measuring of dentin thicknesses from five points with caliper

In the third group, Nd: YAG LI (Fidelis Plus 3, Fotona, Ljublgana, Slovenia) was applied with 300-μm fiber probe at 1 W with 100 μs pulse duration. It operates at a wavelength of 1.06 μm, the repetition rate was 10 Hz. The pulse energy was 100 mJ. The beam was aligned perpendicular to the dentin at a distance of 1 mm and 1 mm^2 was irradiated for 1 s.

In the fourth group, Nd: YAG LI was applied with the same settings after DA application (DA + LI). After application of desensitizing procedures, each group (except the C group) was categorized into two subgroups for fabrication of PRMs. The PRMs (Imident (Imicryl) (PMMA) or Systemp C and B (Ivoclar) (acrylates + methacrylates + bis-GMA (AMB)) were mixed according to the manufacturer's instructions than filled in strip crowns and placed on prepared teeth with finger pressure. Specimens were stored in reverse position to contact the liquid in the pulpal chamber at 37°C. All of the deionized distilled water in the polypropylene chamber was taken at 10^{th} min, than filled with a new 1.5 cm^3 deionized distilled water and this one was taken after 24 h. Residual monomer (MMA) which diffused from PRMs to distilled water was analyzed by HPLC (Agilent 1100, Agilent Technologies, Santa Clara, CA, USA) at 10 min and at 24 h.

HPLC analysis

The analysis of extracts from the PRMs as well as reference solutions of the monomers in water/acetonitrile (25:75) was carried out by HPLC as previously described.[18] The linear calibration equation for MMA is shown in Table 2.

The data of eluted residual monomers from PRMs in periods were analyzed by three-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) tests. The interactions among groups were analyzed by least significant difference (LSD) test. The data of residual monomers eluted in different periods were analyzed by paired t-tests.

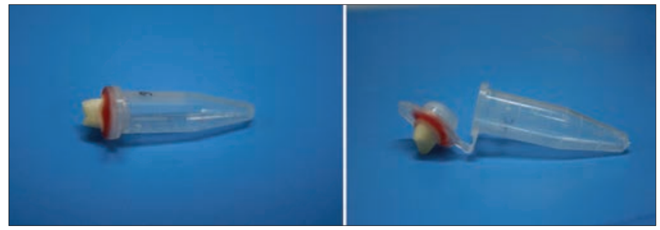

Figure 2: Attached tooth to a polypropylene chamber with soft wax

Table 2: Linear calibration equations for MMA

Monomer	l (nm)	r^2	Equation
MMA	208	0.997	y=7.97E+05x+3.71E+02

MMA=Methylmethacrylate

Scanning electron microscopy examinations

The surface morphology and tubular occlusion or potencies were observed by SEM (Jeol JSM-6400, Jeol Ltd., Tokyo, Japan). One dentin disk specimen prepared for SEM analyze for each group. The surfaces of the dentin specimens were polished with 1,000; 1,500; and 2,000 SiC paper and then polished by using 6-, 3-, 1-, and ¼ μm diamond suspensions and polishing cloths (DP Diamond Products, Struers A/S, Ballerup, Denmark). All specimens were cleaned in an ultrasonic bath for 5 min and dried using an oil-free air. After surface treatments, the specimens were sputter coated (Hummer VII SEM Sputtering System, Anatech LTD, Alexandria, VA, USA) with gold-palladium alloy under high vacuum and photomicrographs were taken.

Results

The three-way ANOVA results are shown in Table 3. Mean values and SDs of all groups are shown in Table 4. The monomer diffusion values varied statistically according to desensitizing procedures (C, DA, LI, DA+LI), PRMs, and the time periods (10 min, 24 h) ($P < 0.05$). There were significant interactions between desensitizing procedures and provisional resin systems ($P < 0.05$); desensitizing procedures and time periods ($P < 0.05$); PRMs and time periods ($P < 0.005$); and desensitizing procedures, PRMs, and time periods ($P < 0.05$) [Table 3].

When the interactions considered by LSD test, control group of PMMA for 10 min was significantly different from all groups ($n = 5$, $P < 0.005$).

When the desensitizing procedures were considered for monomer diffusion through dentin surfaces, monomer diffusion was the highest in the control group and the lowest in the DA+LI group ($P < 0.05$).

According to the results of the t-tests, there were statistically significant differences between PRMs ($P < 0.05$). The methacrylate diffusion of PMMA was higher than AMB. There were statistically significant differences between time periods ($P < 0.05$). The methacrylate diffusion for the first 10 min was higher than at 24 h.

For the first 10 min, the monomer diffusion was the highest for the control group and there were no statistically significant differences among other groups ($P < 0.05$). At 24 h, there were no statistically significant differences among all groups ($P = 0.647$) [Table 5].

In the control group, SEM analysis revealed numerous exposed, normally structured dentinal tubule orifices with no smear layer [Figure 3a]. The surface morphologies were almost the same for the LI group and the DA+LI group [Figure 3c and d]. In the Nd: YAG LI groups, the dentinal tubules were occluded and carbonization areas were absent in the irradiated dentin surface [Figure 3c].

Discussion

In the current study the effect of different desensitizing procedures on diffusion of MMA from dentin tubules to water solution with time was evaluated. The first research hypothesis was supported by the detection of residual monomers diffusion from dentin tubules to water solution after polymerization of PRMs and they also supported the second research hypothesis that desensitizing procedures decrease this monomer diffusion. The MMA monomer diffusion was highest in the control group (3.26E-05) and lowest in the DA+LI combination group (1.72E-06).

Table 3: Three-way analysis of variance for MMA diffusion

	Type III SS	df	Mean square	F	*P* value
Procedure	13.18E-09	3	4.392E-09	7.08	0.000
PRM	5.739E-09	1	5.739E-09	9.25	0.003
Time period	4.385E-09	1	4.385E-09	7.07	0.010
Procedure×PRM	9.896E-09	3	3.299E-09	5.31	0.002
Procedure×time period	9.244E-09	3	3.081E-09	4.96	0.004
PRM×time period	3.290E-09	1	3.290E-09	5.30	0.025
Procedure×PRM×time period	6.533E-09	3	2.178E-09	3.51	0.020

MMA=Methylmethacrylate, DF=Degrees of freedom, PRM=Provisional restoration material, SS= Sum of squares

After tooth preparation, the resultant formation of bacterial by-products and interim restoration microleakage may lead to dentin hypersensitivity.[19] When compared with nonsensitive teeth, the number of tubules per unit area is about eight times increased, and the tubular diameter is two times greater.[20] Therefore, reducing the number of open tubules or decreasing their diameter is one goal of therapy for sensitive teeth.[21] Many investigators have been applying different therapeutic agents or methods.[22] Laser desensitization has been introduced as an effective tool for rapidly eliminating or reducing dentin hypersensitivity.[23] In the last decade, some studies have demonstrated that the use of different lasers along with desensitizer may be a useful option for decreasing dentinal hypersensitivity.[7,24,25] In the current study, the combination of DA+LI gave the best result in MMA passage to the pulp chamber, and the diffusion of MMA through dentin was reduced with the use of desensitizing procedures.

In the results of the current study, the hypersensitivity treatments affected the monomer diffusion of MMA from dentin tubules. The utility of hypersensitivity treatments on the dentinal tubule orifice was examined by SEM observation in this study. It was observed that the surface photomicrographs of the DA+LI group and the LI group

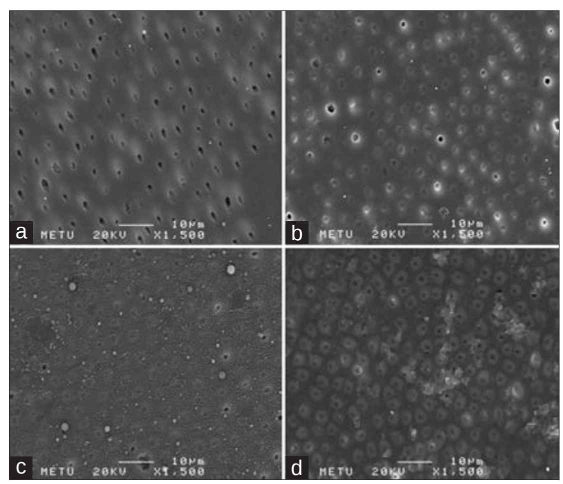

Figure 3: (a) Dentin surface of control group. The view of dentin surface presents opened dentinal tubules. (b) Desensitizer layer on dentin tubules of desensitizing agent group. (c) The view of surface irradiated Nd: YAG laser. Occluded dentinal tubules can be seen. (d) Dentin surface of the combination of laser and desensitizer

Table 4: Mean and SD values of all groups (mole) (*n*=5)

	Imident		Systemp	
	10 min	24 h	10 min	24 h
Control	1.08E-04 (9.46E-05)	1.23E-05 (2.53E-05)	8.92E-06 (1.25E-05)	1.16E-06 (2.61E-07)
Dentin desensitizer	9.84E-06 (8.63E-06)	3.17E-06 (4.85E-06)	1.30E-06 (4.12E-07)	1.06E-06 (8.94E-08)
Laser	9.88E-06 (8.34E-06)	3.02E-06 (4.52E-06)	1.38E-06 (3.63E-07)	1.24E-06 (3.36E-07)
Desensitizer+Laser	2.93E-06 (1.65E-06)	1.96E-06 (1.13E-06)	1.20E-06 (1.20E-06)	1.14E-06 (1.95E-07)

SD=Standard deviation

Table 5: Means and Tukey's HSD test results for desensitizing procedures and control group (n=20)

Desensitizing procedure	Mean (Mole)	SD	Tukey's HSD 10 min	Tukey's HSD 24 h
Control	3.26E-05	6.39E-05	A	C
DA	3.75E-06	5.87E-06	B	C
LI	3.72E-06	5.74E-06	B	C
DA+LI	1.72E-06	1.23E-06	B	C

Group means with same uppercase letters are not statistically different from each other for $P>0.05$. HSD=Honestly significant difference, DA=Desensitizing agent, LI=Laser irradiation, SD=Standard deviation

were similar [Figure 3]. The untreated dentin presents opened dentinal tubules with a smooth and homogeneous surface [Figure 3]. SEM confirmed that the Nd:YAG laser and DA had an occluding effect on the dentinal tubules [Figure 3].

Acrylic and bisacryl resins are widely used both during the temporization phase as well as for provisional restorations.[26] In fabrication of the provisional restorations two principal methods may be used; direct and indirect.[27] High pulpal damage risk, due to the light-activated resin composite and polymerization of autopolymerizing resin and has been equally well-documented. This problem is associated primarily with direct methods of fabrication[28] and the effect of external agents on dentin sensitivity can be reduced by the obliteration of the tubules with the use of DA.[19] This result is in accordance with the results of the current study in which the diffusion of MMA through dentin was reduced with the use of desensitizing procedures.

The applicability of resin materials in clinical practice depends on their chemical and physical qualifications, but also their biological safety is of importance. The dental resin material has organic matrix compounds that have the potential of causing adverse biological reactions. Related characteristics of these materials were previously evaluated by a number of studies.[29,30] Of these primarily epoxy resins and acrylic monomers, have been illustrated as important occupational sensitizers, with an established potential for cross-reactivity. The adverse effects such as allergic contact dermatitis, occupational skin disease, or irritant contact dermatitis have been frequently reported by clinicians.[31] As a primary irritant and sensitizer, MMA may lead to allergic reactions on the skin as well as on oral mucosa.[32] As a cytotoxic agent,[13] it is found to induce papilloma and fibroma in terms of sequential histopathological changes on hamster cheek pouches.[33] Nonetheless, the potential cytogenetic implications of MMA remains unclear.[34] In the present study, the highest amount of diffused residual MMA (108.4 μM) from PRMs (PMMA) is above the cytotoxic level[15,35] and may cause adverse reactions. The quantity of diffused residual MMA from PRMs was statistically different. This difference may depend on the different monomer contents of PRMs. When studying dentin permeability or diffusion trough dentin, dentin thickness is crucial and has to be carefully controlled. Most of the previous studies are usually performed in standard thickness of dentin.[36-38] However, in clinical conditions dentin thickness could not be homogenous. In the present study, prepared premolars for a metal-ceramic complete crown were used to simulate clinical situation.

In the previous studies, several time periods (10 min, 30 min, 90 min, 1 h, 3 h, 6 h, 24 h, 7 days, 14 days, and 21 days) were used to determine early and late elution of monomers from resins.[39-41] It takes approximately 10 min to fabricate a single provisional restoration under clinical situation, and self-cure resins complete polymerization reaction after 24 h. For this reason, in the current study the time periods 10 min and 24 h were determined. The diffusion of residual MMA from resins (PMMA, AMB) for time periods 10 min and 24 h was statistically different (P = 0.01). Monomer release of resins was decreased by time. For 24 h there were not significantly differences among groups. This may cause an effect of decreased methacrylate elution from PRMs.

Although this *in vitro* study was performed in well-controlled laboratory situations, it has several limitations. Firstly, the *in vitro* design is making it difficult to compare the results with the results of clinical studies. As only a limited number of PRMs were tested, the results may not be valid for other systems. Although concerning the correlation between *in vitro* and *in vivo* tests and also clinical usage is difficult, the *in vitro* residual monomer measuring test by using HPLC is valuable in understanding the leaching ability of organic leachables from these PRMs.[14] The polymerization degree and material properties are related to the amount of diffusion of residual monomers from PRMs.[42] Various factors may affect the elution process of residual monomers *in vivo*. One of these factors is related to clinician who apply PRMs. Moreover, the application and polymerization process of PRMs as per manufacturers' instructions gains importance. Moreover, evaluation of residual monomers and their effects needs to be evaluated in *in vivo* studies.

Conclusions

Within the limitations of this *in vitro* study, the following conclusions were drawn:

- The MMA monomer diffusion through dentin to the pulp chamber occurred during the fabrication of PRMs
- The highest monomer diffusion occurred in first 10 min.

Nd: YAG LI in association with DA application is an effective combination to eliminate monomer diffusion to the pulpal chamber.

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