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Concept of experimental preparation for treating dentin hypersensitivity

DOI 10.1515/med-2016-0070
received September 17, 2015; accepted August 8, 2016

Abstract: Background. Dentinal hypersensitivity (DH) is a diagnostic and therapeutic problem that is now appearing more frequently in modern dentistry. The aim of this work was to elaborate formulation of a new, original desensitizing preparation with prolonged action based on the knowledge of similar commercializations and to compare their performance in vitro.

Methodology. The analyses were performed with the aid of NMR spectroscopy. The experimental and commercial preparations were examined in vitro after thermocycling on human teeth by optical microscopy. The presence of the material on tooth tissue, its ability to penetrate into the tooth structure and its layer thickness were subjected to statistical analysis.

Results. A detailed knowledge on composition of commercial material was achieved from spectroscopic measurements. A new adhesive monomer was synthesized and incorporated into an experimental desensitizing formulation. The new monomer appeared to have comparable performance to the commercial one when regarding the affinity to tooth tissue and resistance to thermocycling.

Conclusions. The experimental formulation comprising a new adhesive monomer seems to be promising and could be applied in dental practice providing that biocompatibility is satisfactory.

Keywords: Dentinal hypersensitivity; Dental adhesive monomer; Dentin desensitizer; Nuclear magnetic resonance; Optical microscopy

1 Introduction

Dentinal hypersensitivity (DH) is a diagnostic and therapeutic problem now appearing more frequently in modern dentistry. The reasons for this ailment have not been explained until now. Dentin hypersensitiveness seems to be connected with irritation of nerve endings at a dentin/pulp boundary in response to thermal, chemical, mechanical, dehydrative and osmotic stimuli. As a result, an acute pain of various intensities arises which can not be explained by other teeth diseases [1]. Indicating the type of treatment is problematic in dentistry since establishing reasons behind DH is difficult.

DH may arise from teeth having dentinal tubules that are exposed at the surface and patent to the pulp. There are other theories of DH but the most widely accepted is the hydrodynamic one, proposed c.a. 100 years ago by Gysi, described in detail and modified by Brannstorn in the 1960s [2,3]. The theory assumes that irritation of nerve fibers neighboring to odontoblasts and in initial sections of dentinal tubules is caused by a sudden bulk flow of tubular fluid in the presence of an irritating stimulus. As shown by scanning electron miscroscopy (SEM) investigations, hypersensitive teeth have 8-fold number of dentinal tubules with twofold diameter compared to the “normal” tooth, which causes greater flow of tubular fluid and intensification of symptoms [2].

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Sensitivity to a thermal stimulus is the most distinct symptom of DH. The intensity of the pain felt depends on the psychic condition of the patient, their subjective reaction to the pain, and even on the season in the year. Pain occurs most frequently during brushing, consumption of either cold or hot drinks, and inhalation of cold air. The ailment may concern a single tooth or a number of teeth. Dentinal hypersensitivity happens mainly in adults, and most frequently in the cuspids, premolars, incisors and molars [4]. The factors and methods known to abolish DH may be divided into three following basic groups.

1. Physical factors act on the basis of destroying nerve conduction in some area of the dentin. Methods of treatment include application of laser radiation, where a high energy laser beam is used to partially melt and thus block dentin tubules. This reduces sensitivity to nerve impulses and decreases nerve conduction speed [5,6].
2. Physicochemical methods are based on introduction of some chemicals into dentinal tubules and application of an electric current. That can be ionophoresis with use of 1-2% NaF and a direct current of 1-5mA. The electric current enhances uptake of fluoride ions by a dentin which results in obliteration of dentinal tubules. When supplied electrophoretically into tubules, the fluoride ions penetrate deeper and are less susceptible to be washed out than those applied in a classic way [7].
3. Chemical factors – chemicals include various inorganic and organic compounds, such as fluorine compounds (sodium fluoride, sodium fluorosilicate, tin fluoride, amine hydrofluorides), calcium compounds (calcium phosphate), oxalates (ferric oxalate and aluminum oxalate), strontium compounds (strontium chloride), protein precipitating agents (glutaraldehyde), adhesive and non-adhesive resins, potassium compounds (potassium nitrate), etc. The mode of therapeutic action of some chemicals is given in Table 1 [3, 4].

As early as 1935 Grossman specified properties of preparations applied to abolish dentinal hypersensitivity. These properties should:

- be mild in respect to a pulp,
- not irritate a pulp,
- rapidly yield desired therapeutic effect,
- provide long-term therapeutic effect,
- be easy to apply,
- not cause pain during application,
- not discolor teeth [1].

A variety of desensitizing preparations are available on the dental materials market, including toothpastes, mouthwashes, gels and other dentifrices, to be used in a domestically, as well as more sophisticated materials to be applied in a dental clinic. Those include formulations designed to exhibit a long-term action, which is realized by incorporation of therapeutic ingredients into a polymeric matrix formed directly on to a tooth surface either via photopolymerization of (meth)acrylate resins, similarly as in the case of light-cured bonding agents and adhesive resins, or by evaporation of a volatile solvent in the case of varnish-type materials. Some of most known commercial materials of that type are listed in Table 2. The data on basic ingredients specified are derived from producers' leaflets and available material safety data sheets.

Table 1: Therapeutic action of selected ingredients of dentinal desensitizing preparations

Ingredient	Therapeutic action
Sodium fluoride (NaF)	Blocks dentinal tubules by reaction with calcium contained in tubular fluid and resulting formation of calcium fluoride deposits
Strontium chloride (SrCl2)	Prevents rapid flow of tubular fluid. Blocks nerve conduction biochemically
HEMA (2-hydroxyethyl methacrylate)	Facilitates diffusion of sodium fluoride and potassium nitrate, creates a barrier in dentinal tubules preventing flow of tubular fluid
hydroxyapatite $Ca10(PO4)6(OH)_2$	Blocks dentinal tubules
Cetylamine hydrofluoride	Increase dentition immunity to caries
Potassium nitrate (KNO3)	Generates K+ ions which influence biochemical transformations in nerve endings; as a result nerve conduction is destroyed and relief of pain is achieved
Aluminum lactate	Prevents gingival bleeding
Vitamin E, provitamin B5	Exhibit anti-inflammatory properties, facilitate regeneration of oral mucosa epithelium

Table 2: Composition of selected commercial dentinal desensitizing agents

Material (manufacturer)	Basic ingredients
Seal&Protect (Dentsply DeTrey GmbH, Konstanz, Germany)	di- and trimethacrylate resins, PENTA, functionalized amorphous silica, photoinitiators, BHT, cetylamine hydrofluoride, triclosan, acetone
Gluma Comfort Bond + Desensitizer (Heraeus KulzerGmbH, Hanau, Germany)	ethanol, HEMA, poly(methacrylic-oligo-acrylic acid), 4-META, glutaraldehyde
Admira Protect (Voco GmbH, Cuxhaven, Germany)	acetone, bis-GMA, acidic adhesive monomer, ormocer, HEMA, urethanedimethacrylate, catalyst
Fluor Protector (Ivoclar Vivadent, Liechtenstein)	difluorosilane, polyurethane varnish, ethyl acetate, isoamyl propionate
Bifluorid 10 (Voco GmbH, Cuxhaven, Germany)	ethyl acetate, cellulose nitrate, isopentyl propionate, sodium fluoride, clove oil

PENTA: dipentaerythriol pentaacrylate monophosphate; BHT: butylated hydroxytoluene (2,6-di-t-butyl-p-cresol); HEMA: 2-hydroxyethyl methacrylate;
4-META: 4-methacryloxyethyltrimellitic acid anhydride;
bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropyl)phenyl] propane

The objective of this work is to elaborate an original, experimental formulation designed to diminish dentinal hypersensitivity in the long-term. To aid the research we have estimated quantitative composition of selected commercial products using nuclear magnetic resonance spectroscopy (NMR). The main chemical novelty comprises introduction of a new adhesive monomer, pyromellitic methacrylate anhydride (PMMAn), having the structure shown below. From point of view of chemical structure, PMMAn looks similar to two well-known dental adhesive monomers: 4-META and PMDM, contained in many adhesive formulations (Figure 1) [8].

The formulation was tested in vitro to estimate affinity to human tooth structure. In the next step, immunological response on appropriate cell cultures was evaluated. If results appeared to be positive, behavioral tests on rats will be conducted, prior to clinical trials on humans.

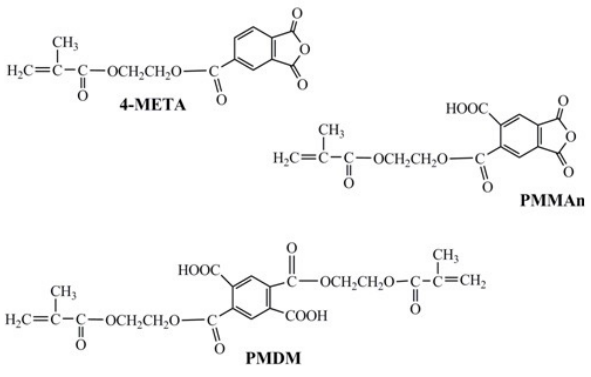

Figure 1: Structure of PMDM, 4-META and PMMAn adhesive monomers

2 Methods

Analyses: 1H NMR spectra were recorded using UNITY/INOVA 300MHz NMR spectrometer (Varian). The samples were tested as solutions in deuteriated acetone containing tetramethylsilane (TMS) as a chemical shift internal standard. Infra-red spectra were recorded using FT-IR Nicolet 6700 spectrophotometer. DSC analysis was performed using METTLER-TOLEDO (DSC822^e) instrument.

Chemicals: Bis-GMA resin was synthesized as previously reported [9]. PMDA (pyromellitic anhydride; 1,2,4,5-benzenetetracarboxylic anhydride, Aldrich) was recrystallized from acetic anhydride. HEMA (2-hydroxyethyl methacrylate, Sigma) and acetone (POCh) were dried over anhydrous magnesium sulphate. TEGDMA (triethylene glycol dimethacrylate, Fluka), HMDI (hexamethylene diisocyanate, Fluka), DBTDL (dibutyltin dilaurate, Fluka), HA (hydroxyapatite nanopowder, Aldrich), triclosan (irgasan; 5-chloro-2-(2,4-dichlorophenoxy)phenol, Sigma Aldrich), KF (potassium fluoride, UCB), DMAEMA (N,N-dimethylaminoethyl methacrylate, Merck), CQ (camphorquinone, Aldrich) as well as other auxiliary reagents and solvents were used as supplied.

Additionally, samples of commercial UDMA resin (urethane dimethacrylate; PLEX 6661, Röhm GmbH) and TMPTMA (trimethylolpropane trimethacrylate, Aldrich) were subjected to comparative NMR measurements.

Syntheses: Methacrylate resin: The modified patented recipe was applied. Thus, 6g of bis-GMA resin, 4g of TEGDMA and catalytic amount of DBTDL (0.05% by weight) were homogenized manually by stirring with a glass rod with gentle heating. After that, 0.3g of HMDI was added dropwise whilst the mixture was magnetically stirred at 40°C for 4hrs. The product was left to stand over-

night in a thermostat at 40°C. According to stoichiometry and our previous findings [9] the resin should contain ca. 20% by weight of bis-GMA/HMDI adduct as well as TEGDMA and unreacted bis-GMA.

2.1 PMMAn adhesive monomer

1.00g (0.00467mole) PMDA, 0.596g (0.00467mole) HEMA and 0.064g DMAEMA (catalyst, 4% by weight in respect to the reagents) were dissolved in 12.77g of anhydrous acetone and stirred magnetically at room temperature for 2hrs 15min. Evaporation of acetone yielded a semi-solid crude product. 1.14g of the product was dissolved in 1ml of acetone and resulting solution was added dropwise to 4ml of hexane/dichlorometane 1:1 (v/v) mixture. Precipitate formed and was filtered off and the filtrate was mixed with 5ml of hexane. Again precipitate appeared which was filtered off and the filtrate was shaken with 3 ml of hexane. After 3min a delicate, white precipitate appeared, which after filtering and drying under vacuum consisted of the pure product with final yield of 33%.

2.2 In vitro tests on human teeth

The experiments were performed on 28 random, undamaged human teeth that were extirpated due to prosthetic indications belonging to 2 patient groups, with 14 teeth in each group. Dentin was exposed by polishing with grade 1000 abrasive paper in a water stream. Seal&Protect and experimental desensitizing formulation (EDF), both tinted with eosin to enable visual observation, were applied on to the dentin surface and cured for 40s with HILUX 200 polymerization lamp. After that, the teeth were subjected to 1000 variable temperature cycles (5 and 55°C alternatively, for 30s each) using EMT-SYSTEMS thermocycler. Next, the teeth were cut along the axial plane with EVA type prosthetic cutting-off machine to visualize thickness of the preparation layers and its penetration into tooth tissues. Microsections and cross-sections were examined with the aid of KAPS Asslar/Wetzlar type SOM 62 optical microscope coupled with a Nikon digital camera.

2.3 Statistical analysis

Statistics was employed to compare the formulations by estimation of differences in population of particular features, i.e. the amount of material present at the tooth surface in the microsection and cross-sections of the teeth. Chi2 squared independence test with Yates correction and/or Fisher's exact test were applied. All the tests were carried out at a significance level a= 0.05 using the Statistica 6.0 software (SUM, Katowice, Poland).

3 Results and discussion

3.1 NMR analysis of Seal&Protect composition

Table 2 shows that manufacturers do not disclose precise information on the composition of commercial formulations. For instance, one does not know what particular species are di- and trimethacrylate resins contained in Seal&Protect or which adhesive monomer is used in Admira Protect. Quantitative composition is not revealed at all. Theretofore, we have analyzed composition of Seal&Protect using NMR spectroscopy. This technique enables identification and quantitative analysis of mixtures of organic compounds in solution. Thus, no information on the content of silica nanofiller, which is an inorganic compound, could be achieved from reading NMR spectra.

Figure 2 presents a set of 1H NMR spectra, recorded for the aliphatic resonances region for Seal&Protect and two methacrylate resins widely used in dentistry, i.e. UDMA and TMPTMA. We ascertained monomers contained in Seal&Protect to be di- and trimethacrylate resins since the spectra contained characteristic signals of both UDMA and TMPTMA. Characteristic UDMA spectral patterns are clearly seen at δ=2.8-3.2ppm and δ=0.9-1.0ppm, whereas for TMPTMA the most characteristic signal is the quartet at δ=1.64ppm and triplet at δ=0.98ppm. Methacrylate

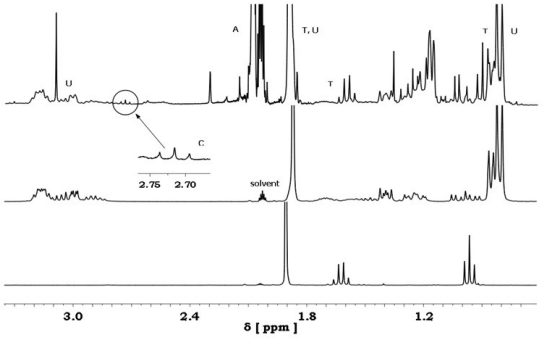

Figure 2: 1H NMR spectra of Seal&Protect (upper), UDMA (middle) and TMPTMA (lower run); aliphatics absorption range; A – acetone, C – cetylamine, T – TMPTMA, U – UDMA.

methyl groups from both UDMA and TMPTMA give overlapping signals at δ=1.92ppm. Additional signals identified are those of acetone solvent (singlet at δ=2.10ppm) and cetylamine moiety (triplet at δ=2.72ppm).

Another informative region of NMR spectra is that of vinylic and aromatic proton absorption, presented in Figure 3. Besides the signals of acrylate and methacrylate vinylic protons from PENTA (δ=5.8-6.5ppm) and methacrylate resins (δ=5.6-6.1ppm), a set of signals is seen in the range δ=6.8-7.4ppm matching exactly for those of a triclosan sample. Additionally, two doublets at δ=6.73 and 7.84ppm could be assigned to aromatic protons in ethyl 4-(dimethylamino)benzoate, a commonly used reducing agent in camphorquinone based photoinitiating system [10].

Based on the above assignments and integral intensities of particular signals we could calculate molar percentages for identified components, and using the respective molecular weights, recalculate those to weight percentages. The data are collected in the Table 3.

3.2 Synthesis of PMMAn

The new adhesive monomer, PMMAn, can be considered as an intermediate product in the synthesis of PMDM, a well known dental monomer (Figure 4). We have observed formation of such intermediates when synthesizing series of PMnEDM monomers [11]. To synthesize PMDM, two moles of HEMA must be reacted with one mole of PMDA [12]. When keeping equimolar HEMA to PMDA ratio, PMMAn is a main product. We have followed this reaction by 1H NMR spectroscopy – 2h 15 min conversion to PMMAn reaches a maximal value of 87% which enables separation of PMMAn from unreacted PMDA and HEMA, as well as small amount of PMDM formed, by the purification procedure, as described in the materials and methods part. Prolongation of the reaction time leads to decrease of PMMAn and increase of PMDM contents.

PMMAn is a new compound having the formal name: 5-(7-methyl-1,6-dioxo-2,5-dioxa-7-octenyl)trimellitate anhydride or, more briefly, 5-methacryloyloxyethyltrimellitate anhydride. The chemical structure is elucidated from

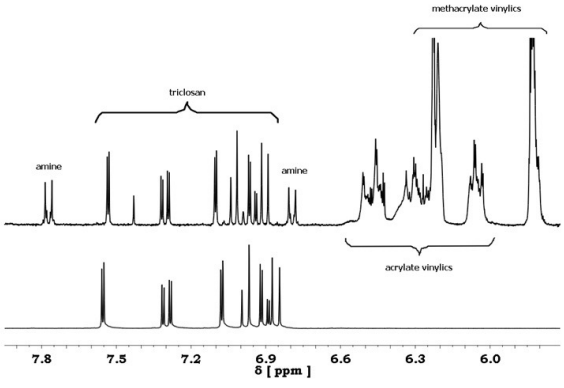

Figure 3: 1H NMR spectra of Seal&Protect (upper) and triclosan (lower run); aromatics and vinylics absorption range.

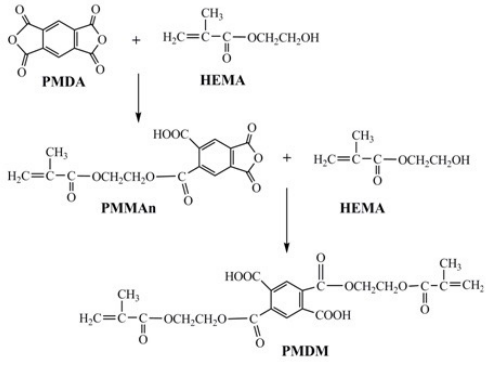

Figure 4: Reaction scheme in PMMAn synthesis.

Table 3: NMR based quantitative data on Seal&Protect composition (organics only)

Ingredient	Molar percentage	Molecular weight	Weight percentage
UDMA	5.5	470.56	24.3
TMPTMA	5.1	338.40	16.2
PENTA	1.3	604.52	7.4
Triclosan	1.2	289.54	3.3
Cetylamine hydrofluoride	0.4	261.48	1.0
Ethyl dimethylaminobenzoate*	0.5	193.24	1.0
Acetone	86.0	58.08	46.8

* Since this compound was found in the material, one could suspect also presence of camphorquinone as photoinitiator at the level of below 1%. However, the signals of the latter, if present, are hidden under intense signals of the monomers in the aliphatics region.

IR and NMR data. In IR spectra, bands at ν=1785 and 1745 cm^{-1} show the presence of an anhydride group. 1H NMR spectra is given in Figure 5, and the following signals were assigned: aromatic hydrogens at δ=8.36 and 8.45ppm (two doublets 1H each, J_{para}=0.73Hz); CH_2= narrow multiplets 1H each at δ=5.65 and 6.11ppm; CH_3- narrow multiplet 3H at δ=1.92ppm; $ArCOOCH_2$- triplet 2H at δ=4.67ppm; -CH_2-OCOC= triplet 2H at δ=4.51ppm. A melting endotherm was found by DSC at 153.99°, followed by polymerization exotherm at 163.13°.

3.3 Preparation of experimental desensitizing formulation (EPF)

The composition of Seal&Protect as revealed by NMR analysis, indicates that desensitizing formulation of prolonged action basically consist of two groups of ingredients: those responsible for formation of stable polymeric layer (methacrylates, adhesive monomer, photoinitiating system) and those responsible for desensitization (triclosan, cetylamine hydrofluoride, silica nanofiller). Additionally, a volatile solvent (to be evaporated after application) facilitates penetration of the composition into a tooth structure.

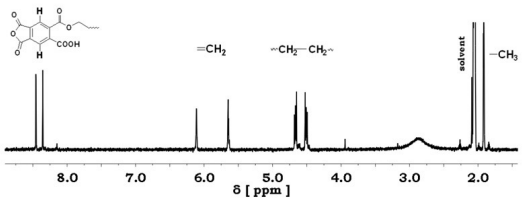

Figure 5: 1H NMR spectrum of PMMAn.

The formulation investigated in this work complies with such an idea. The basic differences to Seal&Protect are another methacrylate resin, new adhesive monomer PMMAn, incorporation of hydroxyapatite instead of silica, and potassium fluoride as a source of potassium and fluoride ions. Moreover, addition of HEMA – hydrophilic monomer of low viscosity, enabled reduction of acetone solvent content considerably. The proposed percentages of particular species are collected in Table 4.

3.4 In vitro experiments on human teeth

Application performance of the experimental and commercial materials was evaluated in vitro after intense thermocycling corresponding to long-term oral conditions. The main features observed were uniformity of the preparation layer at the surface of exposed dentin, penetration of the material into dentinal tubules and the layer thickness (Figure 6). The uniform layer was observed in 71% of samples coated with EDF and in 79% of those coated with the commercial product. Penetration of EDF into tubules was stated in 50% of samples coated with EDF and in 64% of samples coated with the commercial product. A uniform and considerably thick layer was found in 50% samples coated with EDF and in 21% of samples coated with the commercial product. However, differences in population of particular features when evaluated statistically appeared to be not significant. Thus, performance of both materials when tested in vitro appeared to be comparable (Figure 7).

Table 4: Composition of the experimental desensitizing formulation (EPF)

Ingredient	Function in the preparation	Weight percentage
Methacrylate resin	Formation of the crosslinked polymer matrix	47.5
HEMA	Hydrophilic monomer	8.4
PMMAn	Adhesive monomer	2.9
Hydroxyapatite nanopowder	Potentially bioactive filler	2.6
Triclosan	Antibacterial action	5.3
KF	Generation of potassium and fluoride ions	0.8
Camphorquinone	Photoinitiator	0.2
DMAEMA	Reducing agent for photoinitiator	0.6
Acetone	Solvent	31.7

Figure 6: Photograph of polished surface (A) and exposed dentin surface (B) both coated with the experimental material; micrograph of microsection (C), all after thermocycling.

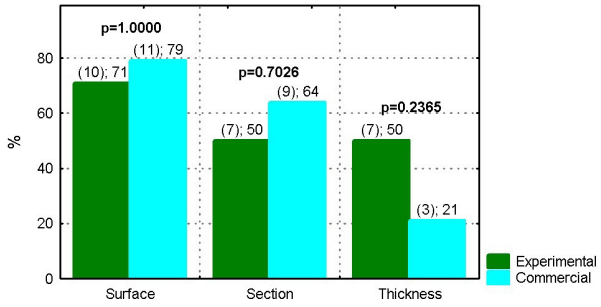

Figure 7: The statistics of the in vitro experiment.

4 Conclusions

Nuclear magnetic resonance spectroscopy is a powerful technique in determining constitution of methacrylate-based dental formulations which is especially important since manufacturers usually do not reveal full information on the constituents.

A new adhesive monomer PMMAn exhibited a good ability to adhere to dentin which was confirmed by in vitro experiments.

Durability of bonding to the tooth tissue of the experimental desensitizing formulation is comparable to that of a renowned commercial product.

New desentizing formulations of prolonged action based on adhesive systems should be exploited since they seem to be promising from clinical point of view.

The research should be continued towards evaluation of biocomatibility of the new material.

Conflict of interest: The authors declare that they have no conflict of interest.

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