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Investigation of the chemical profile and cytotoxicity evaluation of organic components eluted from pit and fissure sealants

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ABSTRACT

The aim of this study was to identify organic components eluted from five resin dental sealants using gas chromatography and mass spectrometry (GC/MS) after 1-day and 40-days storage and the effect of sealants on cell survival of cultured fibroblasts.

Five resin materials were studied: BeautiSealant (SHOFU), Clinpro (3M/ESPE), Conseal F (SDI), Grandio Seal (VOCO) and Helioseal Clear (Ivoclar/Vivadent). The organic monomers detected were butylated hydroxytoluene (BHT), bis-phenol-A (BPA), camphoroquinone (CQ), diethylenglycoldimethacrylate (DEGDMA), 4N, N-dimethylaminobenzoic acid butylethoxyester (DMABEE), hydroxyethylmethcrylate (HEMA), hydroquinone monomethylether (MEHQ), triethylene glycol dimethacrylate (TEGDMA), tetrabutylammonium tetrafluoroborate (TBATFB), triphenylstibane (TPSb). The main monomer detected was TEGDMA, whereas BHT and DEGDMA were detected at lower concentrations. Higher monomer concentrations were detected after 40 days storage. The eluting chemical profiles of the tested materials differ qualitative and quantitative.

For cytotoxicity evaluation, NIH/3T3 cells were exposed to eluates of sealants and cell viability was assessed by a quantitative technique at two observation periods. Decreased cell viability was observed.

The cytotoxicity and the release of monomers from dental materials examined depends on the type of material and the observation time point. Resin-based dental materials have raised public concerns regarding possible adverse biological effects, thus it is essential to evaluate possible side effects for human health.

1. Introduction

Use of pit and fissure sealant is essential in order to prevent caries in children and adolescents (Ahovuo-Saloranta and Forss, 2013; Beiruti et al., 2006; Llodra et al., 1993; Simonsen, 1991). The term "pit and fissure sealant" has been documented as *"a chemically-active liquid material that is introduced into the occlusal pits and fissures of caries-susceptible teeth, that after application, either cures chemically (auto-polymerizing), or is cured with a visible light source (light-cured), thus forming a micromechanically bonded protective layer that prevents the invasion of caries producing bacteria, and simultaneously cuts off the access of surviving caries-producing bacteria from their source of nutrients"* (Simonsen and Neal, 2011). The two main types of pit and fissure sealants are resin-based and glass ionomer cement sealants (Ahovuo-Saloranta and Forss, 2013). Resin-based sealants are preferable by clinicians, as they are easy to apply and handle clinically (Geurtsen et al., 1999).

The polymerizable matrix base monomers that are used in pit and fissure sealants are dimethacrylates such as diglycididimethacrylate (Bis-GMA) and derivatives of Bis-GMA (Komurcuoglu et al., 2005). This monomer is usually co-polymerized with TEGDMA. Other main compounds are urethane dimethacrylate (UDMA), benzoylperoxide and methylmethacrylates (Geurtsen et al., 1999; Moon et al., 2000; Müller et al., 1997). During light-curing of the pit and fissure sealant material, Bis-GMA, UDMA and TEGDMA form a three-dimensional network structure (Geurtsen et al., 1999; Moon et al., 2000). As the crosslinking proceeds diffusion inside the three-dimensional network is decreased,

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Table 1
Material composition according to Material Safety Data Sheets.

Material	Company	Batch number	MDS Synthesis	% by Wt
CLINPRO SEALANT	3M ESPE	N865322	TRIETHYLENE GLYCOL DIMETHACRYLATE (TEGDMA) (C.A.S No 109-16-0)	40–50
			BISPHENOL A DIGLYCIDYL ETHER DIMETHACRYLATE (BISGMA) (C.A.S No 1565-94-2)	40–50
			SILANE TREATED SILICA (C.A.S No 68611-44-9)	5–10
			TETRABUTYLAMMONIUM TETRAFLUOROBORATE (C.A.S No 429-42-5)	< 5
			DIPHENYLIODONIUM HEXAFLUOROPHOSPATE (C.A.S No 58109-40-3)	< 1
			TRIPHENYLANTIMONY (C.A.S No 603-36-1)	< 0.5
			ETHYL 4-DIMETHYL AMINOBENZOATE (EDMAB) (C.A.S No 10287-53-3)	< 0.5
			TITANIUM DIOXIDE (C.A.S No 13463-67-7)	< 0.5
			HYDROQUINONE (C.A.S No 123-31-9)	< 0.05
CONSEAL F	SDI	160139	URETHRANE DIMETHACRYLATE	50–70
			TRIETHYLENE GLYCOL DIMETHACRYLATE	30–40
HELIOSEAL CLEAR	IVOCLAR VIVADENT	V16218	BIS-GMA (C.A.S No 1565-94-2)	50–100
			TRIETHYLENE GLYCOL DIMETHACRYLATE (C.A.S No 109-16-0)	25–50
GRANDIO SEAL	VOCO	1713494	TRIETHYLENE GLYCOL DIMETHACRYLATE (C.A.S No 109-16-0)	10–25
			FUMED SILICA	5–10
			BIS-GMA (C.A.S No 1565-94-2)	2.5–5
BEAUTISEALANT	SHOFU	91676	Glass powder	30
			UDMA (C.A.S No 72869-88-4)	
			TEGDMA (C.A.S No 109-16-0)	

therefore complete polymerization is not possible (Michelsen et al., 2007). The residual monomers and additives that are not bonded to the network are free to elute in the oral cavity (Michelsen et al., 2007). Generally, the elution of substances follows two paths: firstly the short-term elution due to monomer-polymer conversion (elution of non-polymerized monomer) and secondly the elution due to erosion and degradation. As a result factors that influence elution of resin-based materials like pit and fissure sealants are the network's complexity, the extend of polymerization (Ferracane, 1994; Nathanson et al., 1997), the composition of the organic matrix (Polydorou, 2018), the characteristics of the elutable substances like the molecular weight, hydrophilicity and chemical composition and the characteristics of the extraction solution used (chemistry and polarity) (Al-Hiyasat et al., 2004; Sideridou and Achilias, 2005). Finally the porosity of the material and specimen thickness are additional factors that could influence elution of substances (Ferracane, 1994; Nathanson et al., 1997). According to the literature organic solvents like ethanol and methanol, are solvents that stimulate oral conditions (Ruyter, 1981; Yoshida et al., 1992).

In order to identify the eluted compounds from a dental resin material, different analytical methods are proposed. All methods seem to be suitable in order to identify eluted monomers, but there are differences among them as far as the molecular weight of the eluted substances, the identification of by-products and degradation products and the quantification of the substances concerns (Polydorou, 2018). Large molecular size monomers are preferably detected by the use of high-performance liquid chromatography (HPLC) or HPLC mass spectrometry (HPLC/MS) (Durner et al., 2015). Generally, HPLC is applied for the detection of base monomers such as Bis-GMA, UDMA, Bis-EMA (Durner et al., 2015; Geurtsen et al., 1999). On the other hand, gas chromatography/ mass spectrometry (GC/MS) is used to identify additives, smaller monomers, comonomers and other volatile compounds (Spahl et al., 1998), decomposition and fragmentation products (Polydorou, 2018).

The elution of monomers and degradation products from pit and fissures sealers to the oral cavity raise concerns about their biocompatibility and toxicity (Seiss et al., 2009). Some of the ingredients of the resin-based pit and fissure sealers exhibit cytotoxic (Becher et al., 2006; Bouillaguet et al., 1996; Geurtsen et al., 1999; Hanks et al., 1990; Heil et al., 1996; Schweikl et al., 2005) and genotoxic effects (Heil et al., 1996; Schweikl et al., 2005) or may cause allergic reactions (Hensten-Pettersen, 1998; Ortengren et al., 1999). Furthermore, some of them demonstrate estrogenic activity (Lewis et al., 1999; Olea et al., 1996). The cytotoxic effects of the eluted substances have been studied by tests estimating the damage to ribonucleic acid, the glutathione level in cells and the severity of apoptotic action (Małkiewicz et al., 2017). Studying the cytotoxicity of dental materials in cells populations simulates oral conditions (Małkiewicz et al., 2017). The cells that are usually used are gingival fibroblast, keratinocytes of the oral epithelium and standardized strains of mouse L-929 or 3T3 fibroblasts (Polydorou, 2018). Because of the fact that the composition of pit and fissure sealant materials differs from other dental resin-based materials (pit and fissure sealants contain more resin matrix and less filler particles) evaluation of residual monomers in sealant materials is an important issue nowadays (Komurcuoglu et al., 2005; Tarumi et al., 2000).

The aim of this study is the short and long-term evaluation of the residual organic eluates from five commonly used pit and fissure sealant materials after 1 and 40 days storage by the use of GC/MS and their effect on cell survival of NIH/3T3 mouse embryo fibroblasts.

2. Materials and methods

2.1. Materials and specimens preparation

Five commercially available resin-based pit and fissure sealants were investigated: BeautiSealant (SHOFU), Clinpro (3M / ESPE), Conseal F (SDI), Grandio Seal (VOCO) and Helioseal Clear (Ivoclar/ Vivadent). Detailed information about their composition according to manufacturers is shown in Table 1.

Teflon molds were filled with 100 mg of uncured material to produce disks with a diameter of 6 mm and thickness of 2 mm (according to ISO 6874:2015 and ISO 10993-1). The disks were polymerized using a curing LED light (Bluephase style, Ivoclar/Vivadent). The curing light was directly applied on the samples' surface. All disks were cured for 20 s according to the manufacturer's instructions. The power of the LED light ranged across all experiments in a range of 1100–1400 mW / cm2, as tested by a special power meter. Thirty-two samples from each material were prepared and four identical series of experiments were conducted.

2.2. Monomer elution evaluation

Two series of twenty glass tubes were prepared all with each glass tube containing solution of 1 ml of methanol (Methanol, HPLC gradient grade 99.9+ %, CHEM-LAB) contained 0.1 mg/ml caffeine (Caffeine 99%, Alfa Aesar) as internal standard. The disks were detached from the molds and the four parallel samples of each pit and fissure sealant were immediately immersed in methanol in the separate glass tubes. The glass tubes were secured with a ground glass stopper to prevent