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Evaluation of cell responses toward adhesives with different photoinitiating systems

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ABSTRACT

Objectives. The photoinitiator diphenyl-(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) is more reactive than a camphorquinone/amine (CQ) system, and TPO-based adhesives obtained a higher degree of conversion (DC) with fewer leached monomers. The hypothesis tested here is that a TPO-based adhesive is less toxic than a CQ-based adhesive.

Methods. A CQ-based adhesive (SBU-CQ) (Scotchbond Universal, 3 M ESPE) and its experimental counterpart with TPO (SBU-TPO) were tested for cytotoxicity in human pulp-derived cells (tHPC). Oxidative stress was analyzed by the generation of reactive oxygen species (ROS) and by the expression of antioxidant enzymes. A dentin barrier test (DBT) was used to evaluate cell viability in simulated clinical circumstances.

Results. Unpolymerized SBU-TPO was significantly more toxic than SBU-CQ after a 24 h exposure, and TPO alone (EC_{50} = 0.06 mM) was more cytotoxic than CQ (EC_{50} = 0.88 mM), EDMAB (EC_{50} = 0.68 mM) or CQ/EDMAB (EC_{50} = 0.50 mM). Cultures preincubated with BSO (L-buthionine sulfoximine), an inhibitor of glutathione synthesis, indicated a minor role of glutathione in cytotoxic responses toward the adhesives. Although the generation of ROS was not detected, a differential expression of enzymatic antioxidants revealed that cells exposed to unpolymerized SBU-TPO or SBU-CQ are subject to oxidative stress. Polymerized SBU-TPO was more cytotoxic than SBU-CQ under specific experimental conditions only, but no cytotoxicity was detected in a DBT with a 200 μm dentin barrier.

Significance. Not only DC and monomer-release determine the biocompatibility of adhesives, but also the cytotoxicity of the (photo-)initiator should be taken into account. Addition of TPO rendered a universal adhesive more toxic compared to CQ; however, this effect could be annulled by a thin dentin barrier.

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1. Introduction

During the past decade, most developments in the field of dental adhesive technology have been based on the simplification of multi-step systems. In line with this, so-called 'universal adhesives', which recently have been introduced onto the market, represent one further step in simplification. Typically, universal adhesive systems can be used for bonding not only to enamel and dentin, but also to ceramics, metal and composites. Universal adhesives are actually not new, but new is that the latest generation of universal adhesives come as one-component, one-bottle systems [1].

Even though application of adhesives on exposed pulp tissue is nowadays advised against [2,3], the biocompatibility of adhesives remains very important. There is ample evidence that adhesive ingredients such as monomers and additives may be toxic for pulp cells as they were shown to seriously disrupt vital cell functions [4].

The dentin substrate should be regarded as a permeable substrate, through which ingredients may permeate to the pulp. Self-evidently, the thickness of the remaining dentin after cavity preparation plays an important role [5], and a remaining dentin thickness of 300 μm is considered critical to maintain pulp health [6]. Permeation of monomers can occur during the application of the unpolymerized adhesive, but also after polymerization ingredients may be released [7]. In this regard, the degree of polymerization, often also called 'degree of conversion (DC)' is important. The higher the DC, the lower is the release of unpolymerized monomers [8].

The monomers in methacrylate-based adhesives polymerize thanks to a radical polymerization reaction, for which purpose photoinitiators are added in small amounts to the composition of adhesives [9]. Conventionally, the co-initiator camphorquinone/teriary amine is added to adhesives, but a major drawback of this photoinitiator system is its intense yellow color [10]. Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO) is an alternative photoinitiator belonging to the group of acylphosphine oxides, whose initiating system is based on photofragmentation [9]. In contrast to the camphorquinone/co-initiating system, which is characterized by a broad absorption spectrum with peak absorption around 468 nm, the absorption spectrum of TPO is situated more toward the UV spectrum (380–425 nm). Several studies showed that methacrylate composites obtained similar [11,12] or higher degree of conversion [13,14] when TPO was used as photoinitiator. It was also shown that TPO is more reactive than camphorquinone [15]. Significantly fewer monomers eluted from a TPO-based methacrylate resin compared to a CQ-based material in ethanol-based extraction solutions [16,17].

This specific finding is also of particular relevance considering biological effects of these two dentin adhesives. It has been clearly established that resin monomers disrupt the redox homeostasis in cells of the oral cavity through the generation of elevated levels of reactive oxygen species (ROS). As an adaptive response, cells modify the expression of enzymatic antioxidants like superoxide dismutase (SOD1), which eliminates superoxide anions, and glutathione peroxidase (GPx1/2) or catalase, which reduce increasing levels of hydrogen peroxide (H_2O_2) to water. In addition, an increased expression of the stress-responsive haem oxygenase (HO-1) supports antioxidant defense by the generation of the antioxidant bilirubin. Remarkably, the expression of these cytoprotective enzymes depends on the availability of glutathione (GSH), a non-enzymatic antioxidant [18]. Moreover, monomer-induced oxidative burden exceeding the cells antioxidant capacities to regain balanced intracellular redox homeostasis finally leads to cell death via apoptosis through the intrinsic mitochondrial pathway [4,19].

The objective of this study was to use these parameters for a detailed analysis of oxidative stress related cellular responses toward a CQ/amine or TPO based universal adhesive. To this end, cytotoxicity, generation of ROS and expression of enzymatic antioxidants were analyzed, and the raw photoinitiators were evaluated as well. It could be hypothesized that the TPO-based adhesive is biologically less active as it releases fewer monomers, but the initiator itself is a leachable compound whose biological activity should also be taken into account. The null hypothesis tested in the current investigation was that the TPO adhesive would be less cytotoxic than the CQ/amine-based adhesive.

2. Materials and methods

All chemicals and reagents have been listed in Table 1.

2.1. *Adhesives tested*

One commercial camphorquinone-based adhesive (Scotchbond Universal, 3M ESPE, Seefeld, Germany) and its experimental counterpart were included in this study, which were also used in the study by Pongprueksa et al. [16]. Their compositions can be found in Table 2. Both adhesives were identical in composition, except that they contained a different photoinitiator. Whereas the commercial adhesive contained camphorquinone and ethyl 4-(dimethylamino)benzoate (EDMAB) as co-initiator, the non-commercialized experimental version contained diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO). In the remainder of the text, they will be referred to as SBU-CQ and SBU-TPO, respectively.

Dissolved unpolymerized adhesive (i), 24h-extracts of polymerized adhesive (ii) and the raw photoinitators (iii) were used for further testing.

(i) *Unpolymerized adhesives*: the uncured adhesives were dissolved in pure ethanol (0.5 g/ml; w/v) at room temperature and stock solutions were prepared in culture medium at a concentration of 10 mg/ml following ISO standards [20,21]. Serial dilutions in cell culture medium were prepared. In a pilot study, it was found that the ethanol in the tested concentrations was not toxic for the cells used in following experiments.

(ii) *Extracts of polymerized adhesives*: Polymerized adhesive disks were prepared in a standardized teflon mold (diameter 5 mm and heigth 0.5 mm). After applying the uncured adhesive in the mold and gently air-blowing for 5 s (as per manufacturer's instructions), the adhesive was covered by a glass plate to prevent incomplete polymerization