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Early responses of human pulp to direct capping with resin adhesive systems and calcium hydroxide

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ABSTRACT

Objective. Early responses of human pulp to Prime&Bond/phosphoric acid, Clearfil SE Bond, Clearfil S3 Bond and Dycal were investigated *ex vivo*.

Materials and methods. The three adhesives, Dycal or buffer (DPBS) were applied directly onto the pulp of human teeth slices that were placed in culture for 4 days. Cell viability was monitored by the MTT assay during the culture period. After 4 days, tissue integrity was examined by hematoxylin–eosin staining. Vimentin levels were assessed by Western blotting. TUNEL assay was applied for apoptotic cell detection at specific pulp areas.

Results. Profound reduction of cell viability and tissue integrity was observed in adhesive-treated groups, while the impact of Dycal was found to be less harmful. Extended apoptosis was caused mostly by the Clearfil SE and Prime&Bond. All adhesives reduced Vimentin levels.

Significance. The study provides evidence that early pulp responses to direct capping with different adhesive systems or calcium hydroxide may vary significantly and underline the need for further studies in relevant *ex vivo* systems.

1. Introduction

The most common clinical approach in treating caries lesions involves the mechanical removal of the caries-affected tooth structure and the restoration of the resulting cavity. In case of very deep caries lesions, the pulp can be exposed. In order to prevent pulp necrosis and stimulate healing, a capping material is applied over the exposed area. Highly alkaline calcium hydroxide has been established as the most efficient direct pulp capping agent, due to its beneficial properties (low cytotoxicity, antimicrobial action and dentin bridge induction) [1,2]. Recently, both etch-and-rinse and self-etch resin adhesive systems have been proposed as alternatives to calcium hydroxide [3,4]. Their pulp repair potential as well as their biological safety has been investigated in a variety of experimental conditions.

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In vivo studies addressed the ability of individual adhesive system components to induce pulp repair by using either human teeth (scheduled for extraction) [5–8] or animal models [9–13]. The results of these studies were found to be contradictory. Some studies demonstrated that pulp inflammation was related to the presence of microorganisms while others showed that resin adhesive components caused pulpal toxicity [14–22].

Cell culture studies have already demonstrated that major adhesive monomers, co-monomers, and phosphoric acid are cytotoxic [23–32]. Individual adhesive components can disrupt the stable cellular redox balance. This imbalance may result in increased reactive oxygen species (ROS) and subsequent ROS-induced cellular damage, including cell cycle arrest, apoptosis and/or necrosis [33–38]. However, it has not been yet thoroughly investigated whether the aforementioned cellular responses are induced in the pulp upon the application of complete adhesive system/s or upon the application of their individual components. Furthermore, it is important to note that the interaction of adhesive materials with the pulp-dentin system is a complex phenomenon determined by plenty of integrated molecular events such as inflammation, ROS production and apoptosis. Therefore, the effect of adhesive systems on pulp capping can be evaluated by studying the pulp response indexes.

Pulp reactions, such as changes in the extracellular matrix components, following direct application of individual adhesive components, have been already studied using the *ex vivo* cultured human tooth slices system. This system partially allows tissue microenvironment and microvascular integrity maintenance and provides the advantage of studying cellular responses to biomaterials in a more natural context [39–42]. This is a useful alternative to the *in vivo* model, as it helps overcome ethical issues associated with *in vivo* studies and restrictions associated with animal models.

The aim of this study was to *ex vivo* investigate the short-term impact and early pulp responses to three different resin adhesive systems and calcium hydroxide by using cultured human tooth slices. The null hypothesis was that there were no differences among the three adhesive systems, calcium hydroxide and the control group, regarding pulp responses.

2. Materials and methods

2.1. Specimen selection and study design

Thirty (n = 30) sound mature impacted third molars, freshly extracted for orthodontic reasons, were selected from patients between 18–30 years old who visited the Department of Oral and Maxillofacial Surgery (School of Dentistry, National and Kapodistrian University of Athens, Greece). Informed consent was obtained from all individual participants included in the study in compliance with the protocol of the Ethics Committee of the School of Dentistry (National and Kapodistrian University of Athens, Greece).

Immediately after the extraction, 750 μm-thick tooth slices were cut with a hard tissue microtome (IsoMet™ Low Speed Saw, Buehler, Illinois, USA) equipped with a diamond disk, under constant flow of rinsing solution [DPBS (0.9 mM $CaCl_2$, 0.5 mM $MgCl_2 \cdot 6H_2O$, 2.7 mM KCl, 1.47 mM KH_2PO_4, 137.93 mM NaCl, 8.06 mM $Na_2HPO_4 \cdot 7H_2O$) containing Penstrep 3%, and Fungizone 0.3% (Gibco™, Life Technologies Ltd., Paisley, UK)]. Three to four longitudinal sequential slices with buccal-lingual direction were performed per tooth and transferred into the culture hood under sterile conditions.

The tooth slices were washed extensively with fresh rinsing solution and dentin was carefully dried using sterile paper. The slices used were selected to have intact pulp of comparable size. They were randomly sorted in five groups for the application of the capping materials (Table 1). The adhesive systems were applied with a fine adhesive system micro applicator (TPC Advanced Technology Inc., City of Industry, CA) according to the manufacturer's instructions and were polymerized with a LED light-curing unit (Radii Plus, SDI Limited, Victoria, Australia) (operation mode: 440–480 nm, 1500 mW/cm^2, no ramp function). The calcium hydroxide was applied with a special Dycal instrument. Shortly afterwards, the slices were placed in 6-well plates (Corning Incorporated, NY, USA) with culture medium (DMEM, FBS 10%, Pen-Strep 1%, Fungizone 0.1%). The cultures were maintained at 37 °C, 5% CO_2 and 95% air for 4 days without medium change. During the culture period, pulp vitality and metabolic activity was monitored *via* MTT assay immediately upon application (t = 0 h) and at 24 h (t = 24 h) and 48 h in culture. Four days after treatment, the tooth slices were collected for further analysis.

2.2. MTT assay

Cell viability and metabolic activity of the pulp, upon application of the three adhesive systems and Dycal, were assessed using the MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (Biovision Inc., Milpitas, California, US)] assay. Under this method, the purple color which results from the complete reduction of MTT to its insoluble salt formazan, is an index of cell viability. The tooth slices in culture medium were tested for MTT staining immediately after the materials' application (t = 0 h), and then again, 24 h (t = 24 h) and 48 h (t = 48 h) after the application. Tissue viability was visualized based on the purple color of the formazan formation.

2.3. Tissue processing

Following the 4 days incubation period, the tooth slices were fixed in 4% paraformaldehyde (PFA) solution for 48 h at 4 °C. Afterwards, tooth slices were demineralised in 10% EDTA solution (pH 7.0) for a period of 16 week. The EDTA solution was renewed every 2 days. After that period, the enamel around the tooth slices was removed. The remaining tissue was dehydrated through ascending ethanol series, embedded in paraffin and cut in 5 μm sections.

2.4. Hematoxylin–eosin staining

For histological analysis, three non-adjacent paraffin sections per tooth slice, per group were stained with hematoxylin–eosin, using a standard protocol. The sections were examined under an optical microscope, (Leica DM LS2; Leica Biosystems, Wetzlar, Germany).

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