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ORIGINAL ARTICLE

Effect of dentin bonding agent diffusing through dentin slices on the reactive oxygen species production and apoptosis of pulpal cells

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Background/Purpose: Dentin bonding agents (DBAs) are cytotoxic to dental pulp cells. This study aimed to evaluate the effects of three DBAs (Optibond Solo Plus, Op; Clearfil SE Bond, SE; and Xeno III, Xe) after diffusion through 0.2-mm or 0.5-mm dentin slices on reactive oxygen species (ROS) production and apoptosis in dental pulp cells.
Methods: The amounts of DBAs diffusing through 0.2-mm or 0.5-mm dentin slices were quantified using a UV-Vis spectrophotometer. The effects of diffused DBAs on ROS production and viability of dental pulp cells were investigated using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay on Days 1 and 2. Flow cytometric analysis and double staining of treated dental pulp cells with Annexin V-fluorescein isothiocyanate (V-FITC) and propidium iodide (PI) were performed on Day 2.
Results: Xe showed greatest diffusion through dentin slices after 8-hour period, followed by SE and Op. Dental pulp cells produced a lesser amount of ROS, when treated with DBAs diffusing through a 0.5-mm dentin slice than through a 0.2-mm dentin slice for the same period of time. A small proportion of cells were TUNEL-positive after treatment with any of the three diffused DBAs. Annexin V-FITC/PI staining identified apoptotic cells; cell survival was higher in those

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cells treated with DBAs diffusing through a 0.5-mm dentin slice than through a 0.2-mm dentin slice.
Conclusion: The three DBAs after diffusion through 0.2- or 0.5-mm dentin slice still exhibit cytotoxicity to dental pulp cells. However, the 0.5-mm dentin slice is found to be a better barrier than the 0.2-mm dentin slice to protect dental pulp cells from DBA-induced cytotoxicity.

Introduction

Dentists frequently perform indirect pulp capping during daily dental practice when the dental pulp is almost exposed. A 0.5—1-mm-thick calcium hydroxide liner is generally used as a barrier to protect the underlying pulp during indirect pulp capping procedures.[1] The direct use of dentin bonding agents (DBAs) instead of a calcium hydroxide liner has been suggested because calcium hydroxide liners reduce the area of dentin for bonding.[2] However, DBAs contain various monomers that exhibit cytotoxic effects and cause cell apoptosis.[3—6] Although dentin can serve as a barrier to reduce the toxicity of zinc oxide eugenol or acids during indirect pulp capping,[7] a previous study showed that monomers within the adhesives can diffuse through dentin to influence cell survival.[8] Therefore, we may need to know whether a thin layer of dentin can be used as a barrier to protect the underlying nearly exposed pulp.

Although the cytotoxic effects of DBAs have not yet been fully elucidated, DBAs have wide application in modern dentistry. Among the different generations of bonding agents, dentists popularly employ total-etch and self-etch dentin bonding systems for composite resin restorations. Total-etch dentin bonding systems remove the smear layer on the dentin surface, exposing the underlying collagen fibers. On the contrary, self-etch adhesive systems do not completely remove the smear layer. Demineralization and dentin deposition procedures occur almost simultaneously.[9] Comparison of total-etch and self-etch adhesive cytotoxicities revealed that the toxicity of self-etch adhesives was equal to, or less than, that of total-etch adhesives.[10] Another previous study found that the dental pulp capped with self-etch adhesives exhibits a moderate to severe inflammatory cell infiltrate in the area beneath the capping materials.[11]

Previous studies evaluated the cytotoxicity of DBA by using a dentin barrier to mimic the clinical situation.[8,12—16] In this study, we used 3-(4,5-dimethylthiazol -2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess whether a thin dentin barrier could decrease the cytotoxicity of DBAs. The advantages of MTT assay are its simplicity and rapidity, but it does not provide information on cell apoptosis. The cytotoxicity effects of DBA after diffusion through a thin dentin slice on reactive oxygen species (ROS) production and apoptosis in dental pulp cells remain unclear. This study aimed to measure the amounts of one total-etch [Optibond Solo Plus (Op)] and two self-etch [Clearfil SE Bond (SE) and Xeno III (Xe)] adhesives after diffusion through 0.2—0.5-mm dentin slice using a UV spectrophotometer. The cytotoxic effects of DBAs diffusing through dentin slices on the production of ROS and viability of dental pulp cells were investigated using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) and flow cytometric analysis after cells stained with both Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI).

Materials and methods

Specimen preparation

This study was performed according to a protocol approved by the Institutional Review Board of the National Taiwan University Hospital. Extracted human permanent molars from 16—40-year-old patients were collected and stored in distilled water containing 0.2% (w/v) thymol at 4°C. Slices of crown dentin (0.2 and 0.5 mm in thickness) were prepared using a low-speed diamond wafering blade (Isomet, Buehler Ltd., Lake Bluff, IL, USA) perpendicular to the long axis of the tooth. Each dentin slice was subsequently cut into a 4 mm × 4 mm square. The dentin specimens were wet-polished with 600 grit silica paper to create uniform flat surfaces and finely adjust specimen thickness. Specimen thickness was precisely determined to be 0.20 and 0.50 mm by an electronic vernier (CD-10CX, Mitutoyo Co., Ltd., Tokyo, Japan). Specimens were then treated with 10% (w/v) EDTA for 2 minutes and 2.5% (w/v) NaOCl for 1 min, followed by three washes with physiological saline solution for 2 minutes each to remove the smear layer. Specimens were observed using a scanning electron microscope (SEM) to confirm that the smear layer had been removed.

Evaluation of cytotoxicity using a dentin disc model

The tested materials (Table 1) were Optibond Solo Plus (Op, Kerr Corp., Orange, CA, USA), Clearfil SE Bond (SE, Kuraray Medical, Inc, Tokyo, Japan), and Xeno III (Xe, Dentsply Detrey GmbH, Konstanz, Germany). Dentin slices were placed on the bottom of transwell inserts (Costar Transwell Permeable Supports, Corning, NY, USA; 6.5 mm in diameter, with a pore size of 3.0 μm). The dentin surfaces were sealed with epoxy resin, leaving a 2.5 mm × 2.5 mm window for DBA application. The gap between the circumference of the dentin slice and the lateral wall of the transwell was also tightly sealed with epoxy resin. Tested materials could, therefore, only influence the viability of pulp cells by diffusing through the dentin slices. Materials were applied on the upper surfaces of the slices according to the relevant manufacturer's instructions. To compare the relative toxicities of different materials, the transwells were transferred into 24-well culture