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Apoptotic effect of different self-etch dental adhesives on odontoblasts in cell cultures

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ABSTRACT

Objectives: We aimed to evaluate the potential cytotoxicity (apoptosis-induction) of three types of self-etch dental adhesives: two-component one-step (Xeno III), two-component two-steps (Clearfil Protect Bond) and one-component one-step (Xeno V) on cultured odontoblasts.

Methods: Each adhesive was prepared to simulate its clinical manipulation. Cured sterile individual masses were immersed in DMEM and left at 37 °C for 24 h. Then a volume of 100 μL of the extract medium was added to the cultured odontoblasts and incubated for additional 24 h, 48 h and 72 h, respectively. Acridine orange–propidium iodide (AO–PI) labelling was employed to assess the proportion of dead to total number of cells. In addition, an *in situ* apoptosis detection kit was used to evaluate the DNA cleavage and chromatin condensation employing the immunohistochemical (IHC) technique. Statistical analysis of the data was performed using one-way ANOVA.

Results: Both apoptosis evaluation methods revealed comparable results with the exception that IHC showed 5–7% less number of dead cells when compared to similar groups evaluated by AO–PI. The percentages of dead to total cells after treatment with Clearfil Protect Bond, Xeno III and Xeno V, were significantly different from the percentage of dead cells after treatment with DMEM alone (−ve control), P value <0.05 and Xeno V dental adhesive had the weakest cytotoxic effect on odontoblasts followed by Xeno III especially after 24 h of incubation. Clearfil Protect Bond had the strongest cytotoxic effect on odontoblasts that was almost closer to that of Staurosporine in DMEM (+ve control).

Conclusion: All tested dental adhesives had remarkable adverse effect on the odontoblasts *in vitro*; this might be of concern when applied clinically in deep cavities where such cytotoxic chemicals become in close contact to dental pulp. Therefore, further *in vivo* studies on animal models are recommended to support or refute these *in vitro* findings.

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Abbreviations: HEMA, 2-hydroxyethyl methacrylate; 10-MDP, methacryloyloxydecyl dihydrogen phosphate; Bis-GMA, bis-glycidyl methacrilate; MDPB, methacryloyloxydodecylpyridinium bromide; Piro-EMA, phosphoric acid modified methacrylate; PEM-F, mono fluoro phosphazene modified; UDMA, urethane dimethacrylate; BHT, butylated hydroxytoluene; CQ, camphorquinone; EPD, ethyl-4-dimethylaminobenzoate; NaF, sodium fluoride.

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1. Introduction

Currently, bonded restorations are widely used owing to their conservative and aesthetic merits. Resin composites combined with dental adhesives serve as alternatives to dental amalgam particularly in aesthetic appealing zone. The last two decades witnessed marked changes in dental adhesives aiming to increase their efficiency and to enhance simplicity of their application. Self-etch adhesive systems were evolved in attempts to fulfil these objectives. They gained the users' attention due to the simplicity of application and acceptable sealing achieved by several produced types. Most of these brands include two components, which are applied separately in two steps or mixed immediately before being used in one step. Numerous efforts have been undertaken seeking further simplicity; thereafter, all in one self-etch adhesives have been evolved as one-component one-step application. These improvements necessitate incorporation of new monomers and solvents in their chemical formulation. However the biocompatibility of these components is still controversial. Following application of resin-based materials on deep cavities, residual components may diffuse through the dentinal tubules to the pulp.[1] The odontoblasts underlying the circumpulpal dentine, sending long cytoplasmic processes into dentine tissue are the first cell line affected by the residual components leached from the dental materials or chemical substances applied at the very deep dentine.[2] To evaluate the cytotoxic effects of dental biomaterials, cell culture analyses have been the recommended methodology employed. Particularly when performed with cells that are comparable to human cells/tissues.[3] Therefore, odontoblasts are the most appropriate target cells to evaluate the *in vitro* side effects of dental restorative biomaterials.[4]

Cytotoxic activity of a chemical compound is indicated by its ability to induce cell death. Two mechanisms of cell death, necrosis and apoptosis have been identified.[5] Both would be distinguished based on morphological and biochemical changes[6] and would be detected by analysis of those changes.[7] Necrosis is a passive process of cell death that results in disruption of the cell membrane and release of cell components to the extracellular matrix. In contrast, apoptosis (programmed cell death), as an active process, is stimulated by developmental signals or environmental factors.[8,9] It was reported that soon after exposure to an adhesive resin, most pulp cells showed certain apoptosis-related morphological changes that included rounding and detaching off the culture plate surface. This observation led to hypothesize that the mode of odontoblasts cell death induced by the adhesive resin might be apoptosis but not necrosis.[10] Recent studies have demonstrated that dental adhesive monomers like TEGDMA and HEMA have the potential to induce apoptosis *in vitro*.[11–13] Several methods were developed to study apoptosis in individual cells and specific tissue types. These methods focused on two key events in cells undergoing apoptosis; DNA fragmentation and alterations in plasma membrane.[14] Therefore, the purpose of this study was to evaluate the apoptotic changes induced in cultured odontoblasts after exposure to three types of self-etch dental adhesives; two-component one-step (Xeno III), two-component two-steps (Clearfil Protect Bond) and one-component one-step (Xeno V).

2. Materials and methods

The components and compositions of self-etch dental adhesive systems used in this study are presented in Table 1.

2.1. Odontoblast cell line

Immortalized mouse odontoblast cell line (MDPC-23 cells) was derived from dental papillae of CD-1 mice at 18–19 days of foetal life as explained by Hanks et al.[15] Briefly, foetal mice (18–19 days) from pregnant CD-1 females (Charles River Labs, Cambridge, MA), were decapitated and their mandibles were removed and placed in cold Hanks balanced salt solution (HBSS, pH 7.3). Teeth germs of first molars ($n = 8$) at bell stage were removed surgically and the mesenchymal tissue of the dental papillae was mechanically separated from beneath the inner enamel epithelium and placed in HBSS supplemented with 0.25% trypsin and kept for 30 min at 37 °C for tissue dissociation. Cells were collected by low speed centrifugation (300 rpm) for 5 min and cultured in 24-well plates (1×10^5 cell/cm^2) using Dulbecco's Modified Eagle Medium (DMEM, Sigma–Aldrich) supplemented with 10% foetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 1% glutamine, 3% penicillin/streptomycin and 50 μL/mL ascorbic acid (Gibco), each well received 1 mL of prepared medium and incubated at 37 °C in a humidified 5% CO_2 incubator. Cells had the medium changed every 2–3 days. Cells were trypsinized after 7 days of culture, then sub-cultured at half their density. After the 5th passage,

Table 1 – Compositions and components of dental adhesive systems used in the current study.

Material	Component	Composition
Clearfil Protect Bond (Kurary Europe GmbH, Dusseldorf, Germany)	Primer	MDPB, 10-MDP, HEMA, water solvent, and CQ.
	Bond	MDP, HEMA, silanated colloidal silica, Bis-GMA, NaF, and CQ.
Xeno III (Dentsply De Trey, Konstanz, Germany)	Liquid A	HEMA, purified water, ethanol, BHT, and highly dispersed silicon dioxide.
	Liquid B	Piro-EMA, PEM-F, UDMA, BHT, CQ, EPD, and p-dimethyl amino ethyl benzoate.
Xeno V (Dentsply De Trey, Konstanz, Germany)	One bottle	Acrylic amide resin, inverse functionalized phosphoric acid esters, CQ, butylated benzenediol, and tertiary butanol.