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Proceeding Paper

Effects of Adper™ Scotchbond™ 1 XT, Clearfil™ SE Bond 2 and Scotchbond™ Universal in Odontoblastic Activity †

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Abstract: This study aimed to assess in vitro cytotoxicity for Adper™ Scotchbond™ 1 XT (SB1), Clearfil™ SE Bond 2 (CSE) and Scotchbond™ Universal (SBU), using MDPC-23 cell cultures. The metabolic activity, protein content, cell death types and cellular morphology were evaluated. All extracts determined a significant reduction in cell metabolism and viability. CSE extracts significantly reduced cell's metabolic activity at its higher concentrations (50% and 100%). All adhesives determined a reduction in the number of viable cells. Changes were dependent on the adhesive, concentration and incubation time. CSE was the most cytotoxic and showed a higher degree of reactivity.

Keywords: dental adhesives; adhesive systems; cytotoxicity; odontoblasts; cell culture

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1. Introduction

Adhesive systems allow the adhesion of restorative materials to dental substrate [1,2]. These materials are evolving towards simpler clinical application protocols and better clinical performance [1,3]. With the increasing complexity of adhesive formulations, several substances present in these materials have been identified and studied, which can induce adverse biological reactions [4,5].

2. Materials and Methods

2.1. Adhesive Systems Extracts and Cell Cultures

Adper™ Scotchbond™ 1 XT (SB1, 3M ESPE, St. Paul, MN, USA), Clearfil™ SE Bond 2 (CSE, Kuraray Noritake Dental Inc., Tokyo, Japan) and Scotchbond™ Universal (SBU, 3M Deutschland GmbH, Neuss, Germany) were used in this study. Extracts were obtained by incubating cured adhesive pellets with Dulbecco's Modified Eagle's Medium culture medium (DMEM, 13.4 g/L—D-5648, Sigma Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, F7524, Sigma Aldrich, St. Louis, MO, USA), for 24 h [6]. For all studies, the odontoblast-like cell line MDPC-23 was used.

2.2. Metabolic Activity and Protein Content

MDPC-23 cells were incubated with the adhesive extracts at 6.25%, 12.5%, 25%, 50% and 100% concentration to assess metabolic activity and with extracts at 25% and 50% for protein content evaluation. Metabolic activity was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the protein content by the sulforhodamine B (SRB) assay.

2.3. Types of Cell Death

The cells were incubated with the adhesive extracts at 25% and 50% concentrations. The types of cell death were determined using double labelling with annexin V (AnV-FITC), and propidium iodide (PI).

2.4. Morphology and Qualitative Cytotoxicity Assessment

Cells were stained with May-Grünwald Giemsa for morphology evaluation, followed by optical microscopy analysis. The grading of reactivity described in the ISO 10993-5 [7] was applied.

2.5. Statistical Analysis

The statistical analysis was performed using GraphPad Prism 8® (GraphPad Software, San Diego, CA, USA). For the metabolic activity and protein content results, the Shapiro-Wilk test followed by the t-test or the Wilcoxon test were used (control cultures were normalized at 100%). Two-factor ANOVA or Kruskal-Wallis tests were used to compare the experimental conditions along the incubation periods. Multiple comparisons and corrections were performed using the Tukey or Dunn corrections. Regarding flow-cytometry results, one factor ANOVA or Kruskal-Wallis were used, and multiple comparisons with the corrections of Bonferroni or Dunn were performed as applicable.

3. Results and Discussion

3.1. Metabolic Activity and Protein Content

Incubation of the cells with the adhesive's extracts determined a metabolic activity reduction, significantly for the higher concentrations (Figure 1).

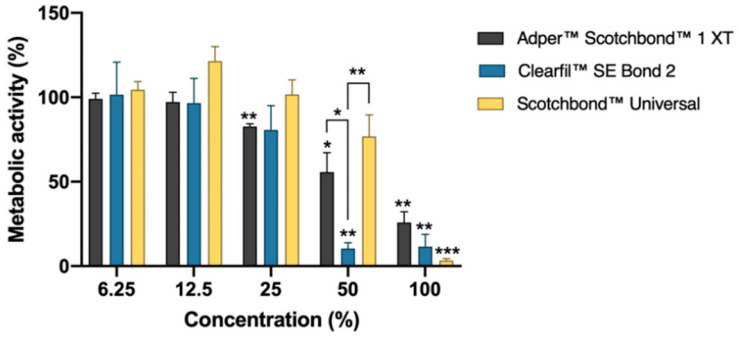

Figure 1. Metabolic activity of MDPC-23 cells after incubation with the adhesive extracts for 24 h. Results are presented in the form of mean and standard error of the mean of four independent experiments. Statistically significant differences are presented with *, where * means $p < 0.05$, ** means $p < 0.01$ and *** means $p < 0.001$.

Protein content was significantly reduced after the incubation of the cultures with the adhesive extracts at 25% and 50% concentrations. CSE extracts significantly reduced cell viability at both concentrations compared to SB1 and SBU extracts (Figure 2).

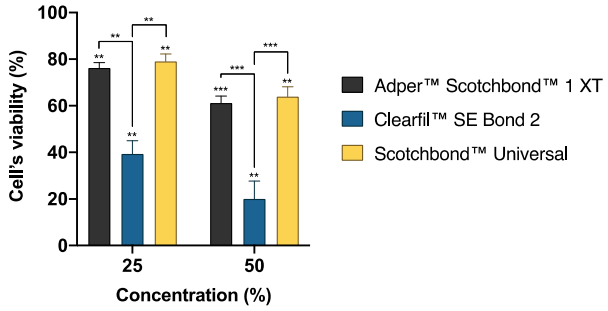

Figure 2. Protein content of MDPC-23 cells when submitted to the adhesive extracts for 24 h. Results are presented in the form of mean and standard error of the mean of three independent experiments. Statistically significant differences are presented with *, where ** means $p < 0.01$ and *** means $p < 0.001$.

3.2. Types of Cell Death

Cultures exposed to the extracts showed reduced numbers of live cells with a consequent increase of cells in apoptosis, late/apoptosis and in necrosis (Figure 3).

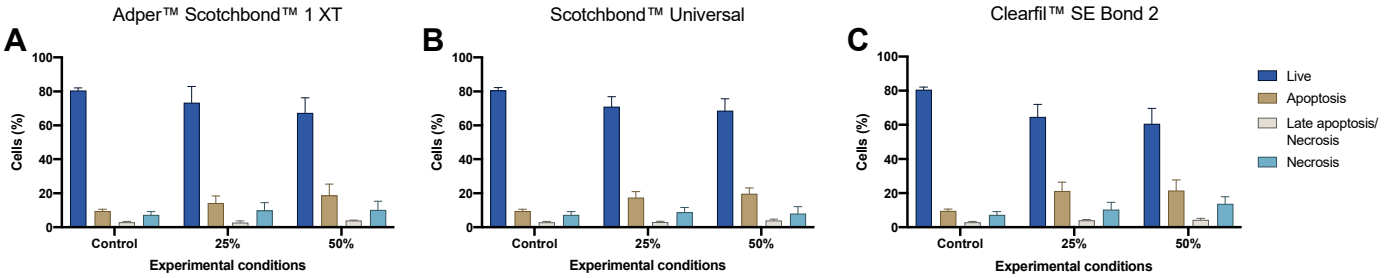

Figure 3. Cell death pathways of the MDPC-23 cells submitted to the extracts of Adper™ Scotchbond™ 1 XT (**A**), Clearfil™ SE Bond 2 (**B**) and Scotchbond™ Universal (**C**) after 24 h of incubation. Results are presented in the form of mean and standard error of the mean of three independent experiments.

3.3. Morphology and Qualitative Cytotoxicity Assessment

CSE extracts led to the greater inhibition of cell growth with the destruction of the membrane, being classified with a higher degree of reactivity among the adhesives under study.

4. Conclusions

Adhesive extracts determined changes in the cultures depending on the adhesive and its concentration. CSE extracts were the most cytotoxic. The clinical application of these materials has to be cautious, and the possibility of pulpal-induced cytotoxicity must be taken into account.

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