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Isolation and Identification of Cytotoxic Compounds Present in Biomaterial Life® †

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Abstract: Direct pulp capping consists of a minimally invasive procedure in which a material is placed directly over the exposed pulp, in order to maintain dental vitality. In previous studies, it was found that the biomaterial Life® presented high cytotoxicity and cell death in vitro. This study aims to identify which Life® constituents are responsible for the decrease in the proliferation and viability of odontoblast-like cells (MDPC-23). The aqueous media conditioned with Life® was submitted to liquid-liquid extraction with ethyl acetate. Cells were treated with both organic and inorganic fractions and further MTT assays were carried out to evaluate metabolic activity. The toxic compounds were determined by nuclear magnetic resonance spectroscopy (1HNMR and 13CNMR) and gas chromatography coupled with mass spectrometry (GC-MS). The organic phase showed a significant decrease in metabolic activity. However, no cytotoxic effect was observed with the inorganic fraction. From the spectroscopic analysis, the organic extract was identified as a mixture of isomers (ortho/para/meta) of *N*-ethyl-toluenesulfonamide. The toxicity of biomaterial Life® in MDPC-23 cells was confirmed and *N*-ethyl-toluenesulfonamide was identified as the toxic agent and, based on our results, its application is not recommended. Our findings may also allow the development of new materials with improved biocompatibility characteristics.

Keywords: direct pulp capping; biomaterial; calcium hydroxide; cytotoxicity; odontoblasts

1. Introduction

Direct pulp capping consists of a minimally invasive procedure, in which a biomaterial is placed directly over the exposed pulp, in order to maintain pulp vitality [1].

For decades, calcium hydroxide has been considered the gold standard biomaterial for pulp capping despite some limitations [2]. New compounds such as mineral trioxide aggregates or tricalcium silicates allow better results [2,3].

Since the biomaterial Life® is still widely used in clinical practice this study aims to identify which are the compounds responsible for its cytotoxicity.

2. Methods

2.1. Biomaterial

The biomaterial Life® was used in this study. Pellets were made by placing the material in polyvinyl chloride molds (3 mm × 1.5 mm) for 24 h, followed by sterilization. Conditioned medium or conditioned water was prepared by incubating the pellets with Dulbecco's Modified Eagle's Medium culture medium (DMEM, Sigma D-5648) supplemented with 10% fetal bovine serum (FBS Sigma F7524) or with water, respectively.

2.2. Liquid-Liquid Extraction, Chromatography Column Separation and Chemical Analysis Liquid-Liquid Extraction Was Performed with Extraction with Ethyl Acetate (2 × 20 mL)

Chromatography Column Separation was performed by "flash" chromatography on a silica column. Chemical analysis was performed by nuclear magnetic resonance spectra, infrared (IR) spectra and by gas chromatography coupled with mass spectrometry (GC-MS).

2.3. Cytotoxicity Studies

MDPC-23 cells were incubated with the conditioned medium, at 6.25%; 12.5%; 25%; 50% and 100% concentration.

Metabolic activity was evaluated by the MTT assay, and cell viability by the trypan blue exclusion assay.

2.9. Statistical Analysis

The statistical analysis was performed using the GraphPad software, Prism 8. The Shapiro- Wilk test followed by the t-test or the Wilcoxon were used. For cell viability results one-way ANOVA was used. For multiple comparisons the Bonferroni correction was performed.

3. Results and Discussion

3.1. Metabolic Activity and Cell Viability

Treatment with the conditioned medium with Life® determined a significant decrease in metabolic activity, for the incubation periods of 24, 72 and 120 h (Figure 1).

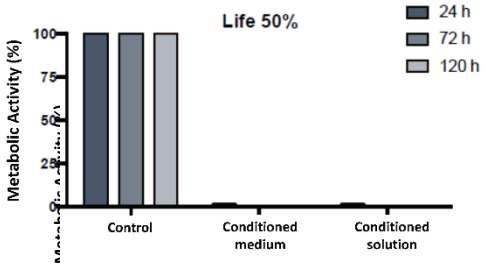

Figure 1. Treatment with the conditioned medium with Life® determined a significant decrease in metabolic activity.

Cell viability significantly decreased at 24 and even more at 120 h, for the 50% concentration. The aqueous phase did not induce changes in cell viability.

3.2. Chemical Analysis of Organic Extract

The GC-MS revealed the presence of three isomeric compounds, presenting different retention times, but with identical mass spectra. The 1H NMR confirm the presence of a mixture of three aromatic regioisomers, ortho, para and meta.

This study confirmed that Life® presents high cytotoxicity when compared to other biomaterials [2], shown by decrease in metabolic activity and cell death.

Spectroscopic methods were used in order to identify the constituents of the organic fraction, showing the presence of aromatic compounds, which were later investigated. compound *N*-ethyl-toluenesulfonamid (NETSA) was identified as the compound present, although not mentioned in the Life®'s European safety data sheet.

4. Conclusions

This study confirmed Life®'s toxicity in MDPC-23 cells, validating previous studies, as well as identifying *N*-Ethyl-o/p/m-toluenesulfonamide as the responsible for its cytotoxicity.

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Conflicts of Interest: The authors declare no conflict of interest.

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