

THE INFLUENCE OF BIOMATERIALS IN REPARATIVE DENTINOGENESIS

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Introduction

When exposure of the pulp tissue to external environment occurs, reparative dentinogenesis can be induced by performing a direct pulp capping, whose aim is to maintain the vitality and function of the pulp tissue. It is agreed in the scientific community that the treatment of these clinical situations requires the use of materials that induce the repair of the dentin tissue and, subsequently, the formation of a dentin bridge between the injured pulp tissue and the repair and restoration material of the dental structure. In this technique the calcium hydroxide cement has already been used as 'gold standard', but mineral trioxide aggregates are the recommended materials. More recently, other calcium silicate based materials have been developed with satisfactory clinical performance.

Objective

This *in vitro* study intends to evaluate the cytotoxicity of three materials with different compositions (calcium hydroxide, mineral trioxide and calcium silicate) with indication for direct pulp capping, since they induce reparative dentinogenesis, in a rat odontoblast type cell line (MDPC-23).

Materials and Methods

This *in vitro* study was developed based on ISO 10993-5, from which the substrate conditioned medium procedure (82.4 mm² of material per ml of culture medium) was selected.

The cell line used was a rat odontoblasts cell line, line MDPC-23. The materials used in this study were:

Materials	Trademark	Dimensions	Setting time	Sterilization
Life®	Kerr Hawe, Bioggio, Switzerland	Pellets with ø 3mm x 1,5mm alt.	24 hours	Sterilized by UV for 20 minutes
White ProRoot® MTA	Dentsply, Maillefer, Tulsa Dental Specialties, Switzerland		72 hours	
Biodentine™	Septodont, Saint-Maur-des-Fossés, France		24 hours	

Evaluation of metabolic activity

Metabolic activity was assessed by the test of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), (5mg / ml; Sigma® M2128), a colorimetric assay that is based on the ability of metabolically active cells to reduce intracellular soluble tetrazolium salt, giving rise to formazan crystals, a dark blue product, insoluble in water. The test was performed after 3 periods of incubation – 24, 72 and 120h.

Evaluation of cell viability

The SRB assay is a sensitive cytotoxicity marker, and is carried out with an anionic dye, sulforhodamine B, which will electrostatically bind to proteins. The fixed dye, measured photometrically after solubilization, correlates with protein content and consequently with cell viability. The test was performed after 3 periods of incubation – 24, 72 and 120h.

Evaluation of cell death by flow cytometry

To evaluate types cell death resulting from the incubation of dentin-inducing materials, double labeling with annexin V (AnV) linked to fluorescein isothiocyanate (FITC) and propidium iodide (PI) was used. The assay was performed after 120h of incubation.

Evaluation of cell cycle by flow cytometry

To evaluate the cell cycle was used the propidium iodide (PI), dye commonly used for DNA and cell cycle analysis. The evaluation was done through PI / Rnase Solution (Immunostep PI / Rnase). Detection was performed on the FACSCalibur cytometer at a wavelength of 488 nm. The assay was performed after 120h of incubation.

Evaluation of protein synthesis (alkaline phosphatase and dentin sialoprotein)

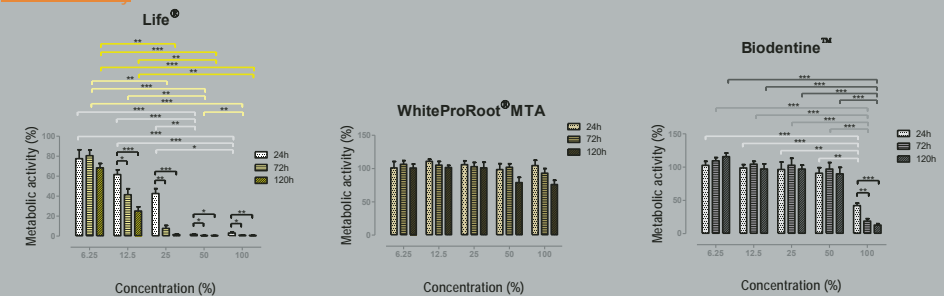
To evaluate expression of alkaline phosphatase was used a diluted solution from the 500x LIVE AP substrate (Life Technologies Corporation) and the Hoechst 33252 nuclear dye. The labeled colonies were visualized under fluorescent microscopy, using the standard FITC filters and the DAPI. The evaluation of dentin sialoprotein synthesis was assessed by immunocytochemistry (DSP (M-20) Antibody and Polyclonal Rabbit Anti-goat immunoglobulins / HRP) and observed under optical microscope. The assays were performed after 96h of incubation.

Evaluation of mineralization through calcium deposits

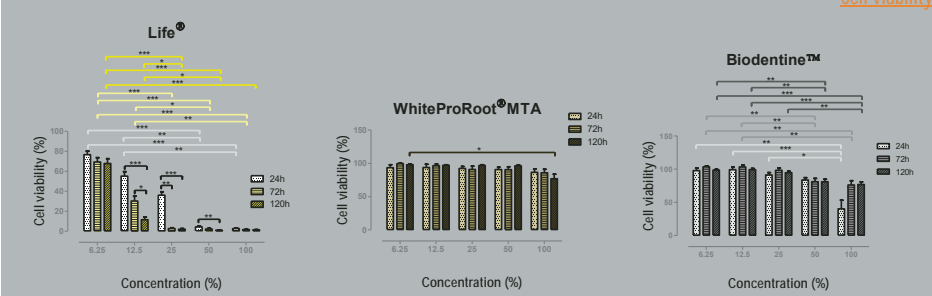
The Alizarin Red S (3,4-Dihydroxy-9,10-dioxo-2-anthracenesulfonic acid sodium salt - A5533 Sigma-Aldrich) dye assay is a cytochemical assay used to determine the degree of mineralization by visualization of calcium in cell cultures. The absorbance of the extracts was measured at wavelength 490nm on a FACSCalibur cytometer. The assay was performed after 120h of incubation.

Results

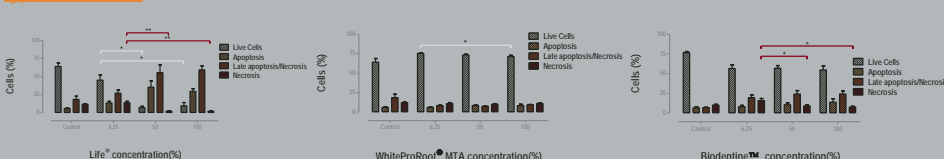
Metabolic activity



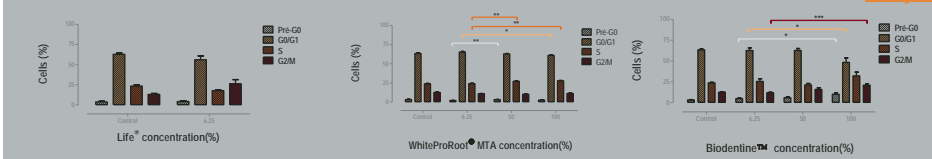
Cell viability



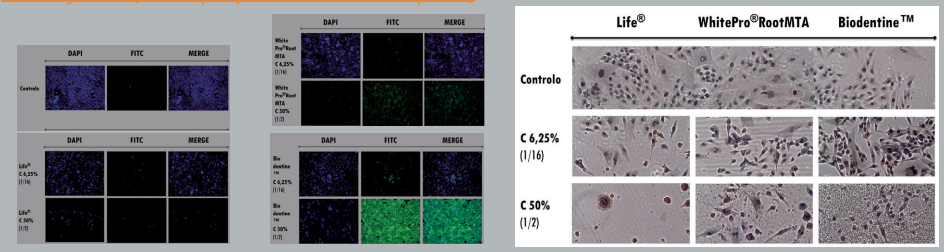
Types of cell death



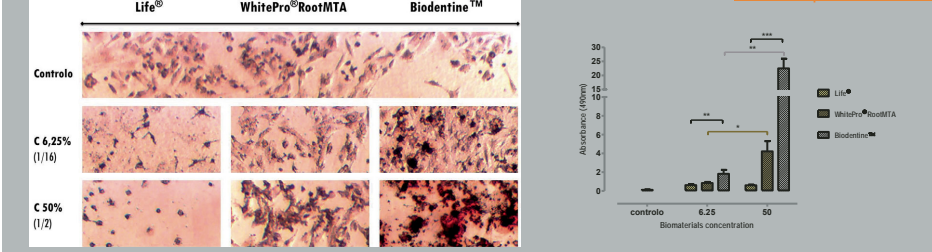
Cell cycle



Protein synthesis (alkaline phosphatase and dentin sialoprotein)



Calcium deposits formation



Discussion

The performance of Life®, especially at higher concentrations, induces a decrease in metabolic activity and cell viability. White ProRoot® MTA and Biodentine™ induced decreased metabolic activity and cellular viability after 24 hours of incubation, a trend that is completely reversed for longer exposures. Both apoptosis and necrosis were observed, particularly after exposure to Life®. White ProRoot® MTA shows a percentage of live cells similar to the control. Biodentine™ behaves similarly to White ProRoot® MTA at the lowest concentration. The MDPC-23 line synthesizes alkaline phosphatase and dentin-sialoprotein, and it produces calcium deposits, since it is an odontoblast-type cell line, and therefore dentin producing. The synthesis of dentin sialoprotein, and the labeling of calcium deposits is very high with Biodentine™, far superior to White ProRoot® MTA for the same concentration.

Conclusions

This *in vitro* study demonstrates that the induction of reparative dentinogenesis should be performed with a biomaterial that stimulates the regenerative potential of odontoblast cells. Life® has the worst results, especially at high concentrations, as it decreases metabolic activity and viability, with a marked increase in cell death and considerable changes in the cell cycle. As a consequence protein synthesis and the formation of calcium deposits is non-existent. For WhiteProRoot® MTA and Biodentine™, the results are very positive, demonstrating an increase in metabolic activity and cell viability, with a high percentage of live cells and no interference in the cell cycle. Regarding the stages of differentiation and mineralization, the results demonstrate a better performance of Biodentine™, with a marked increase in alkaline phosphatase, dentin sialoprotein and calcium deposit formation when compared to White ProRoot® MTA, for all concentrations studied.

Clinical implications

In vitro results indicate that Life® may influence the proliferation of pulp cells. The results of White ProRoot® MTA indicate and confirm its indication for this type of therapy, justifying its recognition as a 'gold standard'. Biodentine™ may be an alternative to White ProRoot® MTA, since its clinical handling is simplified by its short cure time.

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