Cytotoxicity Analysis of Pulp Capping Material on Dental Pulp Stem Cells

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Introduction

Vital pulp therapies, including direct/indirect pulp capping, are essential to preserve pulpal health during management of deep caries. Resin-modified calcium hydroxide [$Ca(OH)_2$] and calcium silicate cements (CSC) constitute recent advancements in pulp capping materials capable of inducing dentin bridge formation and promoting adhesion with overlying resin restorative material. However, there has been report of cytotoxicity and pulp inflammation related to these light-cured pulp liners. We hypothesize that leaching of unpolymerized monomers may lead to cytotoxicity in dental pulp cells. Herein we analyze and compare the cytotoxicity of major light-cured $Ca(OH)_2$ and CSC liners on dental pulp stem cells (DPSCs), as well as the effect of curing time on cytotoxicity.

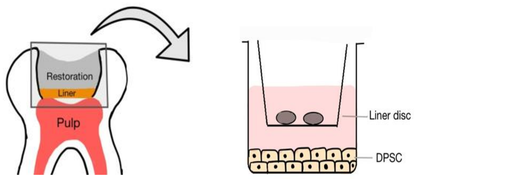
Materials & Methods

Self-cured (Control)

Light-cured $Ca(OH)_2$

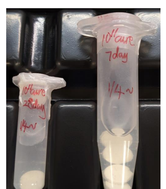
Light-cured CSCs

Experimental model to evaluate cytotoxicity mimicking clinical scenario



Pre-cured 3 mm x 1 mm liner discs were co-cultured with underlying DPSCs in a transwell plate to mimic clinical scenarios.

6 mm x 1 mm diameter discs were cured for 40" (OC) and 20". MTT assay was performed after 72 hours.



Liner discs were soaked in HBSS solution and serially diluted leachate was added to culturing media. After 3 days MTT assay and live/dead cell assay were performed.

Results

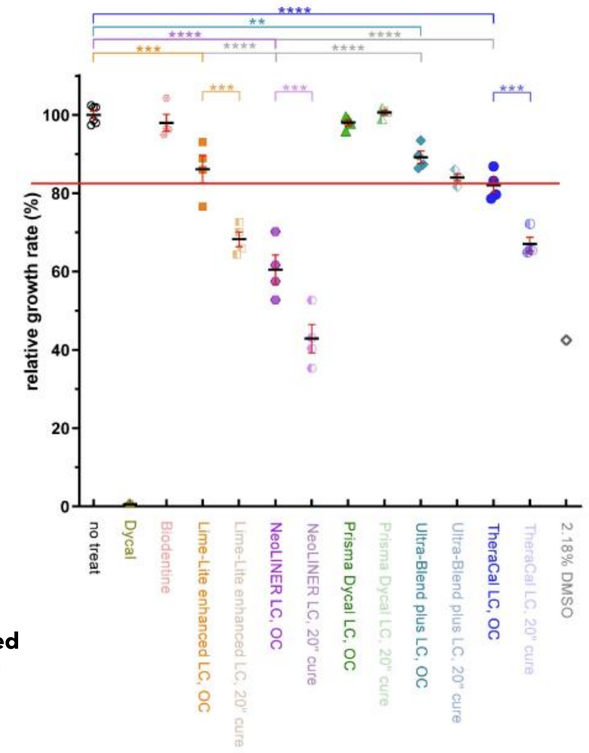


Figure 1: MTT assay after 3-day co-culture with various liner discs. 20s cure showed significantly higher cytotoxicity compared to 40s overcure in Lime-lite, Neoliner and TheraCal. ***: P<0.001 by two-way ANOVA.

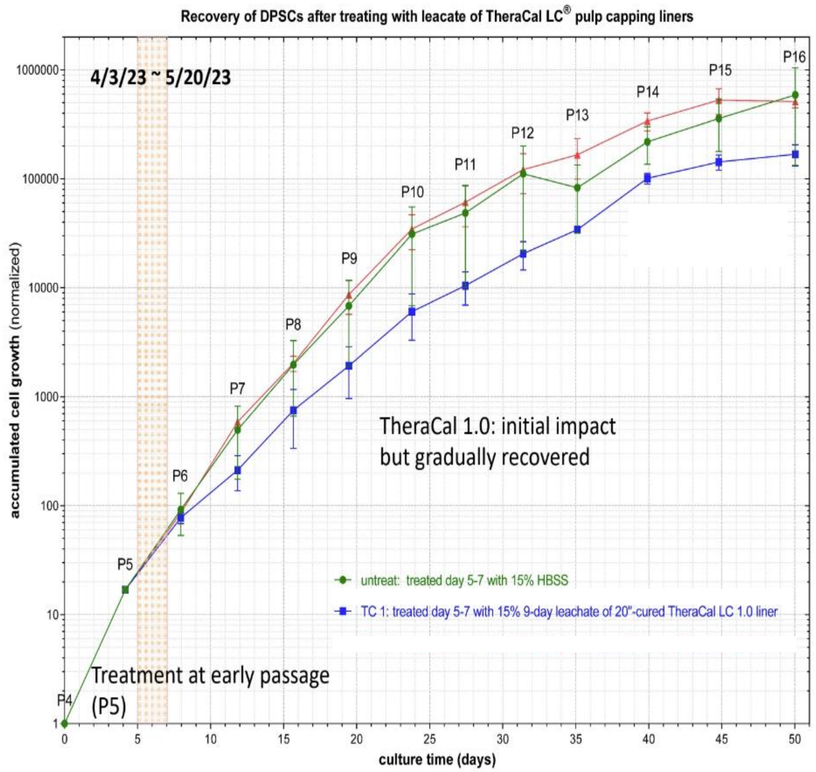


Figure 2: Recovery of DPSCs after treating of TheraCal LC pulp capping liners

Live/Dead Cell Staining

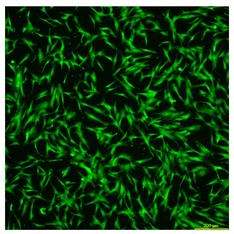


Figure 3A: No treatment live cell staining

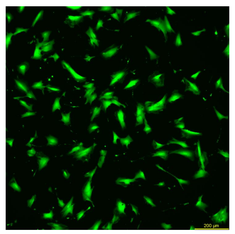


Figure 3B: TheraCal live cell staining

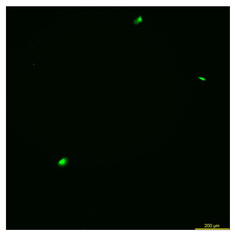


Figure 3C: NeoLiner live cell staining

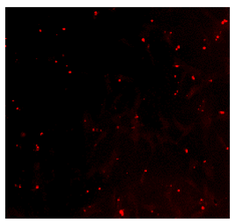


Figure 3D: No treatment dead cell staining

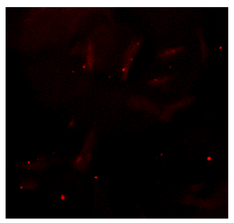


Figure 3E: TheraCal dead cell staining

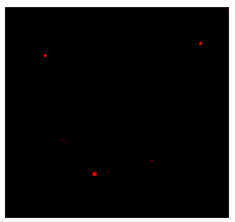


Figure 3F: NeoLiner dead cell staining

Figure 3: Live, viable DPSCs in no treatment, TheraCal, and NeoLiner groups are stained green, after culturing with leachate. Dead and unviable DPSCs from all three groups are stained red after similar culturing.

Experimental Setup

Discussion

- When fully cured, most light-cured CSC and [$Ca(OH)_2$] liners demonstrated acceptable biocompatibility both in short and long-term.
- Curing time has significant impact on cytotoxicity in light-cured CSCs (TheraCal and Neoliner), and one [$Ca(OH)_2$] liner (Limelite).
- Manufacturer's recommended curing time (20s) may not be sufficient.
- The decrease in proliferation observed is a combination of retardation of cell growth and increase in cell death.
- Dental pulp stem cells have the ability to recover from initial insult from the liner materials.

Future Directions

- Analyze the monomer and ion release profile in the leachates using UPLC to correlate with MTT assay results.
- Confirm effect of various liners on cell apoptosis of DPSCs.
- Evaluate the effect of light-cured liners on the induction of mineralization by DPSCs, to reflect efficacy in inducing dentin regeneration.

References

1) Bruins in Genomics (B.I.G.) Summer Program, Institute for Quantitative and Computational Biosciences, UCLA
2) Department of Biomedical Engineering, University of Arizona
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4) Division of Regenerative and Constitutive Sciences, School of Dentistry, UCLA

1. Antimicrobial and biological activity of leachate from light curable pulp capping materials. M.T. Arias Moliz et al., JOD 2006.
2. Current Status Of Direct Pulp-Capping Materials For Permanent Teeth, Takashi Komabayashi et al. Dent Mater J 2016