Dental Pulp Stem Cells' response to three different bioceramic root repair materials

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AAE in Salt Lake City
April 16, 2026
Poster: PR53

Introduction

Bioceramic materials, particularly calcium silicate–based cements, are widely used due to their bioactivity, sealing ability, and favorable biocompatibility (1). During endodontic interventions, including root-end filling, perforation management, and pulp capping, bioceramics engage in a direct biological interaction with dental pulp stem cells (DPSC). This interaction is critical, as these cell types are the primary drivers behind the healing and regeneration of both the pulp and the surrounding periapical tissues (2).

The purpose of this study was to investigate the cytotoxicity and biocompatibility profiles of three different endodontic bioceramic root repair materials and compare them to control: EndoSequence Root Repair Material (Brasseler, Savannah, GA), Neoputty (Avalon Biomed, Houston, TX), and C-Root BP (Beijing C-Root Dental Medical Devices Co., Ltd) on human dental pulp stem cells (DPSCs).



Figure 1. Different endodontic bioceramic root repair materials
a. EndoSequence, b. Neoputty, c. C-Root BP

Material & Methods

Premixed bioceramic root repair materials were allowed to set for 24 hours at 37 °C incubation under humidified conditions in sterile culture dishes. Material extracts were then prepared by incubating the set materials in culture medium at an extraction ratio of 0.1 g/mL for 24 hours. The resulting eluates were filtered through a 0.22-µm membrane and diluted to 50%, 25%, 12.5%, and 6.25% (3). Human DPSCs at P6, obtained from Chen's lab, were seeded (6×10^4 cells per well) in 12-well plates and incubated for 24 hours at 37 °C in 5% CO_2. Cells were subsequently exposed to equal amounts of diluted extracts for 24 hours, with untreated cells cultured in standard medium serving as the control.

After incubation, Toluidine Blue staining was performed, plates were scanned, and nuclear staining intensity was quantified using ImageJ software. Images were converted to 8-bit grayscale and split into RGB channels. The blue channel was selected for quantification due to its high sensitivity to Toluidine Blue absorption. Mean gray values were measured within defined Regions of Interest (ROIs); lower values (closer to 0) indicate higher staining intensity.

Statistical analysis was conducted using an unpaired Student's t-test, with $p < 0.05$ considered statistically significant.

Results

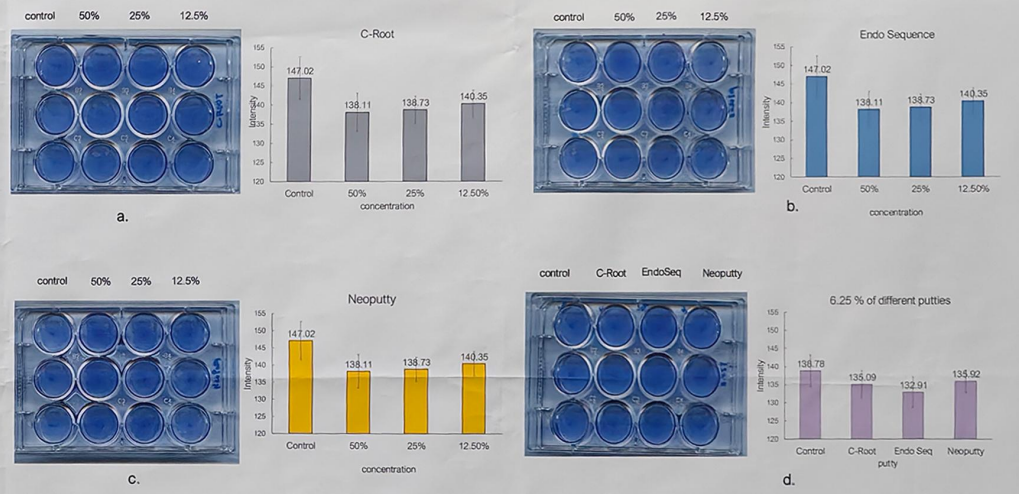


Figure 2. Toluidine blue staining of dental pulp stem cells (DPSCs) following exposure to eluates from different endodontic bioceramic root repair materials at varying concentrations. Quantitative analysis was performed using ImageJ software. (a) C-Root BP, (b) EndoSequence Root Repair Material, (c) NeoPutty, and (d) comparison of all bioceramic materials at 6.25% concentration.

Conclusion

Our data showed no significant differences in cell viability among EndoSequence, Neoputty, and C-Root BP treatments in DPSCs, and no differences between these treatments and the control group. All three bioceramic root repair materials showed superior biological compatibility in the presence of DPSCs. Results indicate these materials could be used in clinical practice settings. However, findings from in vitro studies do not always correspond with in vivo or clinical outcomes (4). Therefore, additional in vitro and in vivo studies are necessary.

References:
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