



Comparative Evaluation of the Cytotoxic Effects of AH Plus Sealer and BioRoot RCS on Periodontal Ligament Fibroblasts

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KEYWORDS

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AH Plus,
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MTT assay,
Biocompatibility

ABSTRACT:

Background: As sealers come in direct contact with periapical tissue their biocompatibility is crucial. Sealers cytotoxicity due to released components can induce inflammation, delayed healing and necrosis of human periodontal ligament fibroblasts.

Aim: To compare and evaluate the cytotoxic effects of AH Plus and BioRoot RCS on human periodontal ligament fibroblasts

Materials and Methods: AH Plus and BioRoot RCS mixed to prepare discs and allowed to set. After setting eluates were collected at 24 and 48 hours and human periodontal ligament fibroblasts cells were treated with elutes. Untreated cells are maintained as a negative control. Post incubation cell viability was assessed using MTT assay. Data were then analyzed using one-way ANOVA and Tukey's post-hoc test ($p < 0.05$).

Results: At both 24h and 48h BioRoot RCS demonstrated significantly higher cell viability than AH Plus with ($p < 0.001$). over the time AH Plus showed a progressive reduction in cell viability with ($p = 0.048$), whereas BioRoot RCS showed a slight increase in viability over time with ($p < 0.001$).

Conclusion: Superior biocompatibility and significantly lower cytotoxicity were observed with BioRoot RCS than AH Plus. The enhanced cellular response seen with BioRoot RCS is due to bioactivity and calcium ion release. Therefore BioRoot RCS is been considered a preferable endodontic sealer for achieving optimal periapical tissue healing.

Introduction

Endodontic sealers are essential components in root canal treatment, with main function of endodontic sealer is to establish a tight seal within the canal, to trap any remaining microorganisms, and prevent subsequent bacterial penetration. For optimal

performance, a sealer should exhibit sealing capability, dimensional stability, antimicrobial effects, and compatibility with surrounding periapical tissues in order to support proper healing. Biological safety of sealers plays significant role, as these materials are placed in close proximity to human periodontal ligament cells and



adjacent periapical tissues. During the setting reaction or material dissolution cytotoxic substances are leached out. They can trigger inflammatory responses, interfere with cellular function, and potentially delay tissue repair, thereby impacting treatment outcomes. Evaluating the effect of sealers on PDL fibroblasts provides meaningful information regarding their biological compatibility and safety profile.

AH Plus, is extensively used an epoxy-resin-based sealer. It has excellent sealing efficiency, favourable handling characteristics, and clinical reliability. However, some reports have indicated that certain components released during its polymerization phase proven to exert adverse cellular effects, which could compromise tissue response and healing.

In contrast, BioRoot RCS which is a bioceramic sealer based on calcium-silicate technology and has been developed to enhance biological performance. This material is known for its bioactive properties, ion release and alkaline environment induction. These properties facilitate fibroblast attachment, proliferation, and tissue repair processes, which overall contribute to enhanced periapical healing and favorable tissue response.

Thus, the aim of this study was to compare the cytotoxicity of AH Plus and BioRoot RCS sealers on human periodontal ligament fibroblasts using eluates collected at 24 and 48 hours.

Materials and Methods

Study Design

Cytotoxicity analysis using human periodontal ligament fibroblast culture.

Materials

- Dentsply AH Plus Root Canal Sealant (Epoxy resin-based)
- SafeEndo BioActive RCS Root Canal Sealant (Calcium-silicate based)
- DMEM culture media
- 96-well plate
- MTT assay kit
- light microscope

Cell Culture

Primary human periodontal ligament cells were cultured in sterile disc filled with DMEM and supplemented with 10% fetal bovine serum at condition of 37°C and 5% CO₂.

Preparation of Specimens

Sealers were manipulated per manufacturer instructions and formed into discs of 5mm diameter and 3mm thickness.

Eluate preparation

After setting, each sealer disc was placed individually into sterile tubes containing 1 mL of Dulbecco's Modified Eagle's Medium. Discs were then incubated in culture medium at 37 °C in 5% CO₂ to obtain eluates at:

- 24 hours
- 48 hours

Exposure of PDL cell cultures to eluates

Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and allowed to adhere for 24 hr.

The culture medium was then added with

- 24-h eluate (AH Plus / BioRoot RCS)
- 48-h eluate (AH Plus / BioRoot RCS)
- Negative control (fresh culture medium only no elutes added).

Cells from respective groups were incubated for 24 h and 48 h depending on groups under standard culture conditions i.e at 37 °C in 5% CO₂ atmosphere for 24 hr.

Group	Subgroup	Condition	Elution Time
A (BioRoot RCS Sealer)	A1	Bioroot RCS Discs Immersed in Dulbecco's Modified Eagle's Medium	24 hours
	A2	BioRoot RCS Discs immersed in Dulbecco's Modified Eagle's Medium	48 hours
B (AH Plus Sealer)	B1	AH Plus Discs immersed in Dulbecco's Modified Eagle's Medium	24 hours
	B2	AH Plus Discs immersed in Dulbecco's Modified Eagle's Medium	48 hours
C (Control - No Sealer)	C1	No discs Dulbecco's Modified Eagle's Medium	24 hours
	C2	No discs Dulbecco's Modified Eagle's Medium	48 hours

Assessment of cell viability using MTT assay



Once the sealer discs incubated with medium, the culture medium was removed from each well using filter paper and cells were collected.

20 μL of (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reagent added to cell containing wells and 96-well plate was placed in an incubator at 37°C for 4 hours, protected from light.

After incubation the optical density of each well was measured at 570 nm using a microplate spectrophotometer

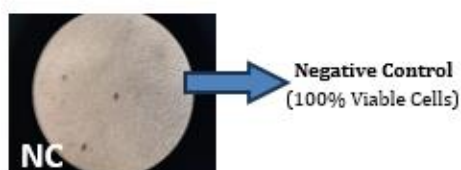
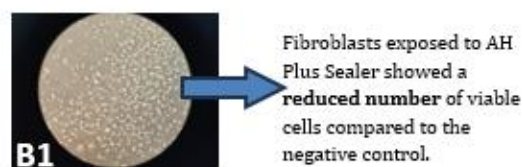
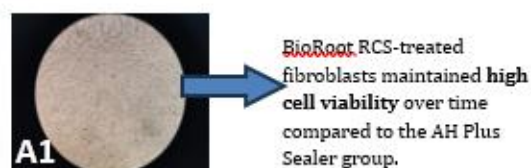
Cell viability was observed and expressed as percentage relative to the negative control.

The cytotoxicity of the tested materials was calculated as the relative absorbance of tested wells versus untreated (negative control) wells using following formula

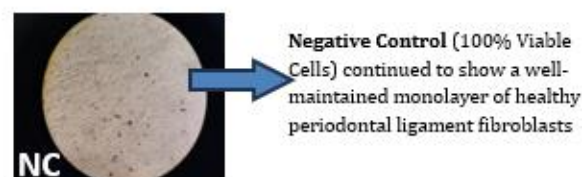
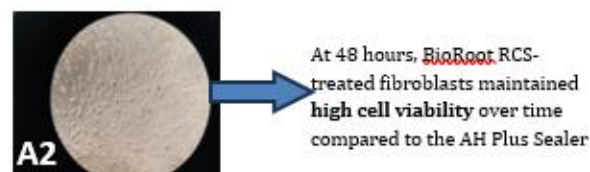
Percent (%) cell viability = $\frac{\text{Optical density of control group}}{\text{Optical density of test group}} \times 100$

Optical density of test group

MTT assay results after 24 hrs of treatment with elutes



MTT assay results after 48 hrs of treatment with elutes



Statistical Analysis

Data analyzed using ANOVA with Tukey's post-hoc test (SPSS/GraphPad). $P < 0.05$ considered as statistically significant.

Results

- BioRoot RCS when compared with control group showed significantly higher mean cell viability with p value ($p < 0.001$) at both 24 h and 48 h time intervals (Figure 1)

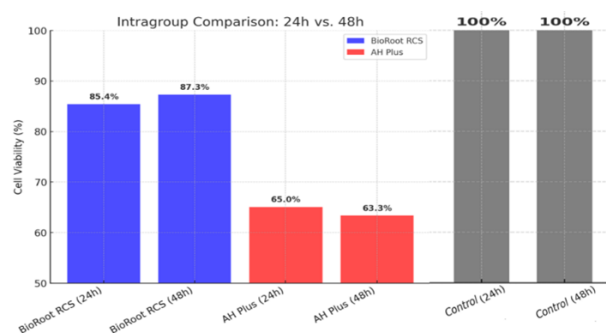
- AH Plus when compared with control group demonstrated significantly decrease in cell viability with p value ($p = 0.048$) at both 24 h and 48 h time intervals (Figure 1)

- A progressive decline in cell viability was seen in the AH Plus group from 24 h to 48 h ($p = 0.048$), whereas BioRoot RCS exhibited a slight but significant increase in viability over the same period ($p < 0.001$).

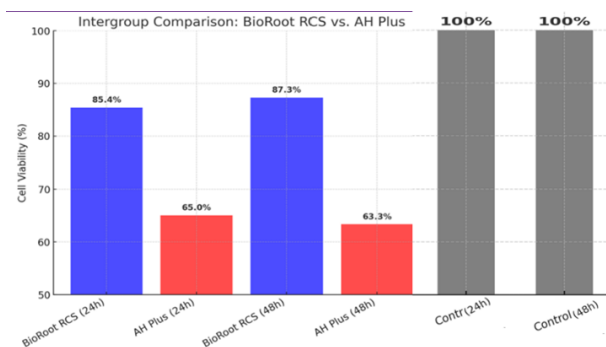
- This signifies both sealers Bioroot RCS and AH plus at both time interval has showed some effect on cell viability



- Cell viability changes for both group i.e BioRoot RCS & AH plus sealer with time i.e 48 hr (figure2)
- Cytotoxicity rate for BioRoot RCS is less than AH plus over 48 hr interval (Figure 2)



BioRoot RCS maintained high cell viability over time ($p < 0.001$), while AH Plus shows a slight decrease, indicating higher cytotoxicity.



BioRoot RCS maintained high cell viability and exhibited a consistently favorable cytocompatibility profile than AH Plus at both 24h and 48h ($p < 0.001$), confirming its superior biocompatibility

Discussion

The present study was designed to evaluate and compare the cytotoxic effects of two commonly used sealers in endodontics—AH Plus and BioRoot RCS on primary human periodontal ligament fibroblasts using elutes collected at 24 and 48 hours.

Since sealers can diffuse into periapical tissues during and after root canal filling, their cellular compatibility plays a important role in periapical healing mechanism. Making sure that these materials do not adversely affect living tissues, to minimize inflammatory reactions,

promote periapical healing, favorable endodontic outcomes and long-term clinical success.

Biocompatibility is particularly important when sealer extrusion occurs beyond the apex or in regenerative endodontic procedures where direct interaction with vital tissues is expected. Due to their wide clinical use and contrasting composition, BioRoot RCS, (a calcium-silicate-based sealer) and AH Plus (an epoxy-resin-based sealer) were selected.

Human periodontal ligament fibroblasts were used in this investigation because they closely resemble the primary cells which are located around the apical area of the tooth. Their behaviour provides valuable insight into how periapical tissues may respond to dental materials, making them a reliable test model. These cells provide a reliable platform for detecting cytotoxic effects. Compared with animal cell lines or immortalized cultures, periodontal ligament fibroblasts are more sensitive to material-induced changes and therefore allow for a more accurate evaluation of potential cytotoxic effects.

To reproduce the clinical scenario Eluate-based testing was selected. Uniform sealer discs were fabricated and then submerged in culture medium allowing soluble constituents to be released in surrounding tissue. Instead of placing the material directly on cultured cells, the eluate method allows assessment of the biological impact of substances released during the early phases of material setting and dissolution, providing a more realistic simulation of how periapical tissues would encounter these materials in vivo. This method of testing has been widely recommended in earlier biocompatibility studies on root canal sealers.

The time intervals of 24 and 48 hours were specifically selected to capture the critical initial period after sealer placement, during which the highest concentration of bioactive or potentially toxic compounds is generally released. Most significant chemical interaction between sealers and surrounding tissue occurs within the first 24 hours and continuous diffusion tendency was observed up to 48 hours, shown by previous investigations. Therefor assessing these eluates at these two intervals allows information about the initial cellular response to each sealer as well as changes that occur as the material stabilizes with time. It becomes possible to identify differences in cell behaviour related to early elution



mechanics and to distinguish between immediate and short-term biological effects of the tested sealers, by examining both time points

Cytotoxicity was assessed using the MTT assay, which is a sensitive method to evaluate mitochondrial enzyme activity as an indicator of viable cells. This assay provides a reliable evaluation of the metabolic activity of fibroblasts exposed to material eluates.

In present study when the eluate obtained from Group A (BioRoot RCS) was compared with the control group C, cell viability remained high at both 24 and 48 hours, showing minimal deviation from the control group. With p value ($p < 0.001$) the difference was statistically significant indicating that BioRoot RCS was well tolerated by human periodontal ligament fibroblasts and produced the least cytotoxic effect among the tested materials.

Our findings are in support with earlier reports given by Al-Hiyasat et al. 2017¹, Poggio et al. 2019², and Camps and About³ 2003 which demonstrated that calcium-silicate-based sealers demonstrate greater cellular tolerance and bioactivity compared to epoxy-resin-based (AH-Plus Jet) which.¹⁻³ BioRoot RCS has been shown to release calcium and hydroxyl ions, which induce an alkaline environment, and allows fibroblast proliferation and mineralization-related activity.⁴⁻⁵

In contrast, Group B (AH Plus sealer) showed a noticeable reduction in cell viability when compared to the control group at both time intervals of 24 and 48 hr with p value ($p = 0.048$) The decline in fibroblast viability was more pronounced after 48 hours, suggesting a time-dependent release of reactive components during polymerization.

Decreased cell viability observed with AH Plus correlates with findings by Lee et al.⁶ 2007 and Lee et al.⁶ and Geurtsen et al. 1998⁷, who stated that its transient cytotoxicity is due to the presence of unreacted epoxy resin monomers, amine hardeners, and trace formaldehyde released during polymerization.

Intergroup comparison between BioRoot RCS and AH plus confirmed that BioRoot RCS (Group A) exhibited significantly higher cell viability than AH Plus (Group B) at both 24 and 48 hours ($p < 0.001$), These findings indicate that BioRoot RCS has superior biocompatibility

and maintains a stable biological profile throughout its setting process.

The cytotoxicity increased for both groups, BioRoot RCS and AH Plus, with time i.e 48-hour interval compared to 24 hours, indicating a time-dependent rise in the release of reactive components. However, the rate of increase in cytotoxic effect of BioRoot RCS remained significantly lower than that of AH Plus at both time points i.e 24 and 48 hr

BioRoot RCS displayed superior biocompatibility and consistently exhibited high cell viability the reason behind it is its ability to release calcium ions, induce a sustained alkaline pH, and create a biologically favorable environment that supports fibroblast adhesion, proliferation, and differentiation.⁴⁻⁵ Benetti et al⁴ and Laurent et al⁵ reported that BioRoot RCS releases calcium and hydroxyl ions that create an alkaline environment supportive of fibroblast growth and mineral deposition, thereby promoting favorable cellular responses and periapical tissue regeneration.

In contrast, AH Plus demonstrated lower cell viability, which can be attributed to the release of unreacted epoxy-resin monomers, amines, and trace aldehydes during the setting phase.^{6,7} supported by Geurtsen & Leyhausen (1998) and Chang et al. (2001) These compounds cause oxidative stress, impair mitochondrial function, disrupt cell membranes, and interfere with cellular metabolism, leading to reduced fibroblast survival.^{6,8,9} supported by Huang et al. (2002) and Bouillaguet et al (2004). Both sealers have demonstrated acceptable levels of biocompatibility, yet BioRoot RCS consistently showed superior cellular response, which suggests as an advantage in clinical situations where material comes in contact with periapical tissues.

Conclusion

At both 24hr and 48hr tested time points BioRoot RCS showed markedly lower cytotoxicity and higher fibroblast viability compared to AH Plus. These findings support that the cases requiring enhanced periapical tissue healing, BioRoot RCS can be used as a biologically favorable endodontic sealer. Although AH Plus remains a widely used sealer with favorable mechanical properties, its relatively higher cytotoxic potential highlights the importance of case selection when biocompatibility is crucial.



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