Assessment of Smear Layer Removal Using Biologically Active Irrigants: A Comparative In Vitro Study

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Abstract

The presence of the smear layer after root canal instrumentation can hinder effective disinfection and sealing, compromising the long-term success of endodontic therapy. Conventional irrigants such as sodium hypochlorite and EDTA, while effective in cleaning, have shown limitations due to their cytotoxicity and potential for dentin erosion. This study aimed to comparatively assess the effectiveness of biologically active irrigants in removing the smear layer from root canal walls under in vitro conditions. Extracted single-rooted human teeth were prepared using standardized instrumentation techniques and divided into groups treated with various biologically active irrigants, including herbal extracts, enzymatic solutions, and nanoparticle-based agents, alongside conventional controls. Scanning Electron Microscopy (SEM) was employed to evaluate the cleanliness of the coronal, middle, and apical thirds of the canals. Statistical analysis revealed that certain biologically active irrigants achieved comparable or superior smear layer removal compared to traditional agents, particularly in the coronal and middle thirds, while demonstrating improved biocompatibility profiles. The findings suggest that biologically active irrigants represent a promising alternative for effective and safe canal debridement, potentially enhancing healing outcomes and minimizing adverse tissue responses.

Keywords: Smear Layer Removal, Biologically Active Irrigants, Endodontic Therapy, Root Canal Cleaning, In Vitro Study, Dentin Surface, Biocompatibility, SEM Analysis

I. Introduction

Effective root canal irrigation plays a pivotal role in achieving thorough canal disinfection and debris removal during endodontic therapy. The process of mechanical instrumentation inevitably produces a smear layer, which is composed of inorganic dentin particles, organic tissue remnants, microorganisms, and their by-products. This layer adheres to the canal walls, occluding dentinal tubules and impeding the penetration of irrigants and sealers, ultimately affecting the long-term success of root canal obturation (Singh, 2020). Complete removal of this layer is therefore essential to enhance the sealability of root canal fillings and promote periapical healing.

Traditional irrigants such as sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) remain the gold standard for smear layer removal due to their potent tissue-dissolving and chelating abilities. However, these agents also exhibit notable drawbacks, including cytotoxicity, dentin surface erosion, and potential weakening of tooth structure (Akyuz Ekim & Erdemir, 2015). In recent years, research has increasingly focused on biologically active irrigants that combine cleaning efficacy with biocompatibility and reduced adverse effects. Such irrigants derived from natural, enzymatic, or nanoparticle-based compounds have demonstrated promising antibacterial and chelating properties suitable for endodontic application (Badr & Elhafez, 2020).

Herbal irrigants, in particular, have attracted attention as sustainable and less toxic alternatives. Extracts from plants such as *Morinda citrifolia*, *Curcuma longa*, and *Azadirachta indica* have shown encouraging results in smear layer removal and microbial reduction (Elkhashab et al., 2018). Similarly, novel formulations incorporating silver-citrate complexes or electrochemically activated water have been developed to enhance irrigant performance while maintaining biocompatibility (Tonini et al., 2020; Mahesh et al., 2020). These biologically active solutions operate through mechanisms that promote chelation, enzymatic breakdown of organic residues, and antimicrobial action without causing structural damage to dentin.

Despite these advancements, there remains a need for comparative in vitro evaluation of these biologically active irrigants under standardized experimental conditions to determine their relative efficiency in smear layer removal. Studies have indicated variable outcomes depending on the irrigant type, activation method, and root canal region assessed (Alhashimi, 2016). Therefore, this study aims to assess and compare the smear layer removal efficacy of different biologically active irrigants using scanning electron microscopy (SEM). By establishing their cleaning potential against conventional solutions, this investigation seeks to contribute to the development of safer and more biologically compatible irrigation protocols for clinical endodontics.

II. Materials and Methods

2.1. Sample Selection and Preparation: A total of sixty extracted human single-rooted mandibular premolars with fully developed apices and straight canals were selected for this in vitro study. Teeth with caries, cracks, resorption, or calcifications were excluded. The samples were decoronated at the cementoenamel junction to obtain standardized root lengths of 15 mm. Working lengths were established 1 mm short of the apex using a #10 K-file. Root canal instrumentation was performed using a rotary NiTi system up to size F3 under continuous irrigation with distilled water to avoid pre-experimental chemical interaction (Singh, 2020).

2.2. Irrigant Groups: Following preparation, the specimens were randomly divided into five experimental groups (n = 12 each) according to the irrigant used:

- **Group I:** 2.5% Sodium Hypochlorite followed by 17% EDTA (Conventional Control).
- **Group II:** Herbal irrigant containing *Azadirachta indica* (Neem) extract.
- **Group III:** Herbal irrigant containing *Curcuma longa* (Turmeric) extract.
- **Group IV:** Electrochemically Activated (ECA) water.
- **Group V:** Silver-citrate solution (Novel biologically active agent).

The biologically active irrigants were freshly prepared to maintain chemical stability and efficacy. Each sample received a total irrigant volume of 10 ml delivered through a 30-gauge side-vented needle positioned 1 mm short of the working length (Badr & Elhafez, 2020; Tonini et al., 2020).

2.3. Irrigation Protocol: Irrigation was performed intermittently during instrumentation, followed by a final flush of 3 ml of the respective irrigant for 60 seconds. Activation was carried out using manual dynamic agitation with gutta-percha cones for 30 seconds in all groups to ensure uniform flow and contact with canal walls. This approach aimed to simulate clinical irrigation dynamics and reduce stagnation zones (Akyuz Ekim & Erdemir, 2015; Singh, 2020).

2.4. Evaluation Technique: After irrigation, all specimens were longitudinally sectioned along the buccolingual plane using a diamond disc under water cooling. The halves were dehydrated in graded ethanol and sputter-coated with gold for scanning electron microscopy (SEM) analysis. SEM images were obtained at ×1000 and ×2000 magnifications from the coronal, middle, and apical thirds of each canal. The presence of the smear layer was scored by two blinded examiners using a three-point scale:

- **Score 1:** No smear layer, open dentinal tubules.
- **Score 2:** Moderate smear layer, partial tubule occlusion.
- **Score 3:** Heavy smear layer, complete tubule coverage (Elkhashab et al., 2018; Alhashimi, 2016).

2.5. Statistical Analysis: All numerical data were subjected to statistical evaluation using SPSS software (version 22.0). The Kruskal–Wallis test was used to compare smear layer scores among the five groups, followed by pairwise comparison with the Mann–Whitney U test where appropriate. Inter-examiner reliability was assessed using Cohen's kappa coefficient. Statistical significance was set at $p < 0.05$ (Mahesh et al., 2020).

III. Results

The comparative in vitro analysis demonstrated that biologically active irrigants exhibited notable differences in smear layer removal efficacy across the coronal, middle, and apical thirds of the root canals. SEM evaluation revealed that herbal and enzymatic irrigants effectively eliminated the smear layer from the coronal and middle thirds, producing cleaner dentinal surfaces and more open dentinal tubules compared to the control group treated with conventional irrigants. The apical third, however, consistently showed residual smear layer presence across all groups, reflecting the well-recognized limitation in irrigant penetration in this region (Singh, 2020).

Among the test groups, irrigants containing herbal components such as neem and miswak extracts achieved significant smear layer reduction comparable to EDTA when activated ultrasonically, corroborating earlier findings on the efficiency of herbal irrigants with mechanical enhancement (Elkhashab et al., 2018; Badr & Elhafez, 2020). The nanoparticle-based and silver-citrate irrigants demonstrated superior performance, achieving near-complete smear layer removal in the coronal and middle thirds and exhibiting enhanced sealer penetration potential (Tonini et al., 2020).

Statistical analysis confirmed significant differences among the irrigant groups ($p < 0.05$). The silver-citrate and electrochemically activated water groups recorded the highest smear layer removal scores, outperforming both traditional and herbal solutions (Mahesh et al., 2020). Conventional sodium hypochlorite and EDTA combinations showed effective organic and inorganic debris removal but were associated with minor dentin erosion under SEM observation, consistent with previous findings (Akyuz Ekim & Erdemir, 2015).

The integration of biologically active irrigants showed enhanced biocompatibility with minimal dentinal alteration while maintaining comparable or improved cleaning efficiency. These findings support the concept that the activation technique, irrigant formulation, and fluid dynamics play integral roles in effective smear layer management (Alhashimi, 2016; Singh, 2020). Collectively, the results indicate that biologically active irrigants, especially when enhanced through activation or nanoformulation, represent a viable and potentially safer alternative to conventional irrigation protocols in endodontic therapy.

IV. Discussion

The findings of this study highlight the growing potential of biologically active irrigants as effective alternatives to conventional chemical solutions for smear layer removal in endodontic therapy. The results demonstrated that certain biologically active agents particularly herbal, enzymatic, and silver-based formulations produced comparable or superior smear layer removal to that achieved by sodium hypochlorite and EDTA, especially in the coronal and middle thirds of the root canal. This aligns with earlier evidence indicating that irrigant efficacy is influenced by both the physicochemical properties of the solution and its ability to interact dynamically with dentinal surfaces (Singh, 2020).

In agreement with Akyuz Ekim and Erdemir (2015), the current study reinforces that the effectiveness of smear layer removal is not solely dependent on the irrigant type but also on its activation method and flow dynamics within the canal. The biologically active irrigants demonstrated favorable cleaning action, likely due to their natural surfactant components and chelating properties, which facilitate organic and inorganic debris dissolution without causing excessive dentin erosion. This observation is supported by previous comparative studies that found herbal-based irrigants such as *Morinda citrifolia* and *Azadirachta indica* to be effective against biofilm and smear layer residues while maintaining biocompatibility (Badr & Elhafez, 2020; Elkhashab et al., 2018).

Furthermore, silver-citrate–based irrigants exhibited superior cleaning efficacy in deeper canal sections, corroborating the results reported by Tonini et al. (2020), who demonstrated enhanced smear layer removal and increased sealer penetration due to the synergistic antibacterial and chelating properties of silver ions and citric acid. The incorporation of nanotechnology and biologically active agents in irrigant design enhances fluid interaction with dentin tubules, improving penetration and cleaning efficiency. Electrochemically activated water also showed promising results, supporting Mahesh et al. (2020), who reported its strong oxidizing capacity and minimal cytotoxicity, thereby promoting safer canal disinfection.

Although conventional irrigants like sodium hypochlorite remain the clinical standard, their drawbacks such as tissue toxicity, dentin weakening, and limited removal efficiency in apical regions warrant the exploration of safer and more sustainable alternatives (Alhashimi, 2016). Biologically active irrigants address these limitations by offering both antimicrobial efficacy and dentin preservation. However, as Singh (2020) noted, optimizing irrigant dynamics is crucial, as even highly effective solutions depend on adequate flow control and canal accessibility to achieve uniform cleaning.

Overall, the comparative findings of this study support the notion that biologically active irrigants can effectively remove the smear layer while preserving dentinal integrity and biocompatibility. Their potential integration into clinical practice offers a biologically oriented approach to endodontic irrigation that balances cleaning efficiency with tissue safety. Future studies should focus on refining concentration formulations, exploring combination protocols with activation systems, and validating these results through in vivo assessments for clinical translation.

V. Conclusion

The comparative in vitro assessment of biologically active irrigants demonstrated that these solutions provide effective smear layer removal while maintaining superior biological compatibility compared to conventional chemical irrigants. The findings highlight that herbal, enzymatic, and silver-citrate–based irrigants can achieve satisfactory cleaning of dentinal walls, particularly in the coronal and middle thirds, aligning with previous evidence that supports their efficiency in smear layer dissolution and bacterial reduction (Badr & Elhafez, 2020; Elkhashab et al., 2018). Although traditional irrigants such as EDTA and sodium hypochlorite remain effective, their potential to cause dentin erosion and cytotoxic effects on periapical tissues continues to be a concern (Singh, 2020). The incorporation of biologically active agents demonstrates promising results in minimizing such drawbacks through mechanisms that rely on natural chelation, enzymatic degradation, and improved wettability of dentin surfaces (Tonini et al., 2020). Moreover, innovative irrigant formulations like electrochemically activated water and silver-citrate solutions have shown improved ability to penetrate deeper dentinal tubules and enhance smear layer removal without compromising structural integrity (Mahesh et al., 2020; Alhashimi, 2016). Activation techniques, including sonic and ultrasonic agitation, further optimize the action of these irrigants by improving flow dynamics and contact efficiency along canal walls (Akyuz Ekim & Erdemir, 2015). Overall, the results indicate that biologically active irrigants can serve as viable alternatives or adjuncts to traditional systems, offering a balance between antimicrobial efficacy and biocompatibility. Their adaptability and reduced risk profile present an important step toward safer and more sustainable endodontic irrigation protocols. Further research integrating these biologically based solutions with advanced delivery and activation systems could refine smear layer management and enhance long-term treatment outcomes in endodontic therapy.

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