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Evaluation of biofilm removal using different irrigation protocols

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Abstract:
Persistent microbial biofilms inside complex root canal systems remain the primary cause of endodontic treatment failure. Conventional syringe irrigation often fails to eliminate these resistant communities due to poor apical fluid exchange. Therefore, it is of interest to evaluate the efficacy of standard chemical irrigants combined with advanced fluid activation techniques. Ultrasonic and multisonic activation significantly enhance biofilm disruption via acoustic streaming and cavitation. Thus, Integrating targeted chemical regimens with advanced activation protocols offers a superior approach for optimal root canal disinfection.

Keywords: Root canal irrigation, biofilm removal, sodium hypochlorite (NaOCl), ultrasonic activation, endodontics

Background:
The main factor that causes failure in endodontic treatment is persistent microbial biofilms in the root canal system. These organized microbial networks are entrenched in an extracellular polymeric network that provides great resistance to antimicrobial agents and mechanical instruments. Traditional cleaning and shaping techniques do not tend to achieve full elimination, especially in complicated anatomy like isthmuses, fins and dentinal tubules. Root canal irrigation is also an important aspect in destabilizing and elimination of biofilms that cannot be reached using tools. Sodium hypochlorite, chlorhexidine and chelating agents are still the most used solutions, but cannot eliminate biofilms, especially when it comes to resistant species like *Enterococcus faecalis* **[1-4]**. To surmount these constraints, several irrigation timetables and activation plans have been proposed. Sonic, ultrasonic and laser-assisted forms of activation increase penetration of irrigants and mechanical disruption of biofilms, compared to syringe irrigation alone **[5-8]**. Recent studies also focus on the need to determine the effectiveness of cleaning of irrigants with the main canal and with lateral morphological features and dentinal tubules, the sites where biofilms are most resistant **[5]**. Therefore it is of interest to compare and evaluate the comparative evaluation of irrigation protocols is warranted to determine their effectiveness in disrupting root canal biofilms.

Conventional irrigants:
Mechanical instrumentation alone cannot be used to disinfect the root canal without the addition of chemical irrigants. Of these, sodium hypochlorite (NaOCl) is the most common solution due to its special dual capability of having antimicrobial effect and dissolving organic tissues. Nonetheless, its effectiveness is largely dependent on concentration, exposure duration and route of delivery and is not always capable of fully eliminating biofilms that are covered by dentinal tubules or lateral canals **[9, 10]**. Another widely used irrigant that has a broad-spectrum antibacterial effect and substantivity is Chlorhexidine (CHX) which permits the agent to last longer even after irrigation. Nevertheless, it is not as effective as NaOCl due to its inability to dissolve tissues as effectively; therefore, its application alone cannot be effective against multifaceted biofilms like those created by *Enterococcus faecalis* **[3, 4]**. Ethylenediaminetetraacetic acid (EDTA) and other chelating agents have been commonly used as adjunctive agents to remove the layer of smear produced by instrumentation, which allows the entry of antimicrobial solutions. However, chelators alone report minimal antibacterial activity **[5]**. Recently, *in vitro* models have explored irrigant combinations and new formulations, with a particular emphasis on the synergistic effect of sodium hypochlorite (NaOCl) and EDTA in combination and on the discovery of alternative irrigants- including herbal or nanoparticle-based solutions. Although the results are encouraging, NaOCl is still the standard of chemical disinfection and other solutions are used as supplements to increase the overall effectiveness **[11, 12]**.

Irrigant delivery systems:
Irrigant delivery into the root canal system is as important as the choice of the irrigant. The most widely used technique is conventional syringe irrigation, typically using side-vented needles, which still form the most used method of the technique in clinical practice. Despite its simplicity and low cost, this method fails to guarantee proper exchange of irrigants in the apical third and lateral anatomical deviations and thus results in remaining biofilms **[5]**. In order to counter such shortfalls, apical negative pressure systems have been developed to provide irrigants to the working length as well as evacuate debris coronally. This technique enhances the replacement of irrigants in the apical zone and minimizes the chances of extrusion out of the apex **[2]**. Experimental models have shown that the efficacy of irrigation relies not just on the irrigant itself, but on the fluid mechanics which act in the lateral canals and in dentinal tubules. Experimental studies affirm that biofilms in inaccessible sites are

characterized by high resistance hence requiring sophisticated delivery and activation mechanisms **[10]**. Therefore, despite the continued use of syringe irrigation as the standard of clinical care, advancements in system delivery methods have significantly increased the penetration of irrigants in previously inaccessible canals making biofilm disruption more advantageous.

Irrigant activation techniques:

The traditional syringe irrigation method proves to have limited ability to reach into the complexity of the root canal system, thus driving the emergence of a wide variety of activation strategies aimed at increasing irrigant dynamics and biofilm elimination. Sonic activation causes low-frequency agitation that enhances the movement of irrigant without causing overheating or mechanical strain on the canal walls. Empirical evidence has shown that sonic activation can significantly lessen dual-species biofilms compared to passive irrigation, but it is less effective than ultrasonic systems **[6]**. Ultrasonic activation (Passive Ultrasonic Irrigation, PUI) makes use of high-frequency oscillations to create acoustic streaming and cavitation, which subsequently enable fluid movement and biofilm structure disruption. Numerous studies validate that ultrasonic activation produces better penetration in lateral canals and dentinal tubules than syringe or sonic activation **[8, 10]**. Multisonic activation devices have recently been introduced, employing simultaneous multiple frequencies to generate greater fluid agitation. Systematic reviews indicate that multisonic systems can outperform conventional ultrasonics in multispecies biofilm removal, though evidence is still emerging **[7]**. EDDY, a polymer-based sonic device, has been proposed as an alternative to ultrasonics. Meta-analyses suggest that while both EDDY and ultrasonic improve cleaning efficacy compared with syringe irrigation, ultrasonic activation often demonstrates slightly superior performance in biofilm disruption **[9]**. Overall, activation techniques substantially enhance the antimicrobial and cleaning potential of irrigants, with ultrasonics and multisonic devices currently providing the most consistent results.

Comparative effectiveness:

Comparative investigations highlight that no single irrigation approach can fully eliminate biofilms across all canal complexities, but activated techniques consistently outperform syringe irrigation **[6, 8]**. Ultrasonic activation has been widely validated for its ability to disrupt dual- and multispecies biofilms and to achieve superior cleaning in simulated lateral and curved canals **[10]**. Recent systematic reviews comparing multisonic versus ultrasonic systems suggest that multisonic activation may achieve better outcomes against multispecies biofilms due to broader energy distribution, though ultrasonic irrigation remains highly effective and better studied **[7]**. The meta-analytical comparisons of EDDY and ultrasonic irrigation systems indicate that despite their similarity in the effectiveness of biofilm removal, ultrasonic activation always exhibits better efficiency in the removal of smear layer and disruption of biofilm, especially in the anatomically complex root canals **[9]**. Recent evidence shows that activated irrigation systems, be they sonic, ultrasonic or multisonic, attain significantly better canal cleanliness than traditional syringe irrigation. However, ultrasonic and multisonic instruments seem to provide the strongest of gains, particularly in morphology that is anatomically difficult to access **[11]**. Recent evidence further supports activated irrigation over syringe irrigation for biofilm disruption and smear layer removal **[12]**. A concise overview of the comparative features of conventional irrigants, delivery methods and activation systems is presented in **Table 1**, highlighting both their advantages and inherent limitations. A schematic overview of irrigation protocols, delivery systems and activation strategies in biofilm removal is shown in **Figure 1**, illustrating how these approaches integrate to enhance root canal disinfection.

Table 1: Comparative features of irrigation protocols and activation methods in root canal biofilm removal

Protocol/Technique	Mechanism of Action	Key Advantages	Main Limitations
Sodium hypochlorite (NaOCl)	Tissue dissolution, broad antimicrobial activity	Gold standard, strong biofilm effect	Cytotoxicity, dentin weakening
Chlorhexidine (CHX)	Antimicrobial, substantivity	Sustained antibacterial effect	No tissue dissolution
EDTA / Chelators	Smear layer removal, tubule opening	Enhances irrigant penetration	Limited antimicrobial action
Syringe irrigation	Direct delivery with needle	Simple, inexpensive	Poor penetration into apical/lateral areas
Apical negative pressure	Irrigant exchange at apex	Reduces extrusion risk, better apical cleaning	Requires special equipment
Sonic activation	Acoustic streaming, low-frequency agitation	Safe in curved canals, improves irrigant flow	Less effective than ultrasonics
Ultrasonic activation (PUI)	Cavitation, acoustic streaming	Strong biofilm disruption, better penetration	Technique sensitive
Multisonic activation	Multiple frequencies, enhanced agitation	Promising for multispecies biofilms	Limited clinical data, costly
EDDY (polymer tip sonic)	High-frequency vibration, hydrodynamic agitation	Comparable to ultrasonics, flexible	Slightly less effective in smear removal
Laser-assisted irrigation	Photothermal and photomechanical effects	Strong disruption of biofilms, deep tubule penetration	Cost, limited availability

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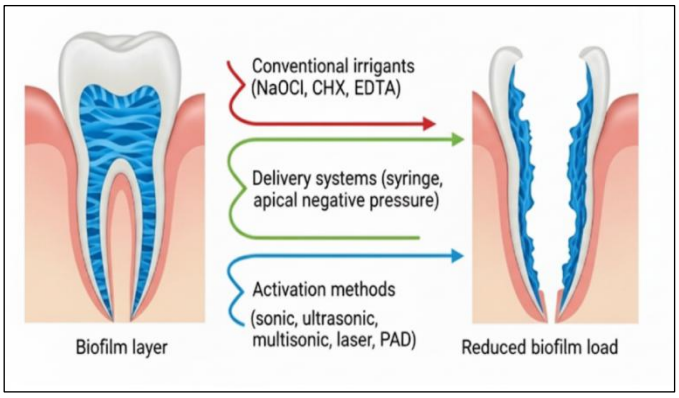


Figure 1: Irrigation strategies for root canal biofilm removal

Current trends:

Recent literature has demonstrated significant shift between traditional syringe irrigation towards activated and multimodal irrigation practices. Preliminary studies showed the inability of traditional irrigants, including sodium hypochlorite and chlorhexidine, to reach sheltered niches of the root canal system **[1, 3 and 5]**. As a result, the use of chelating agents and irrigant mixtures has been suggested as a way of increasing penetration and elimination of the smear layer **[4]**. As biofilm architecture has become recognized as a driver of treatment resistance, more recent modalities, such as sonic, ultrasonic and laser-assisted activation, have been attracting increased interest. In vitro studies have shown that such activation strategies significantly increase the flow of irrigants and are better at destabilizing complex biofilm structures than traditional syringe irrigation **[2, 6]**. Among these, ultrasonic activation has become the benchmark, supported by consistent evidence of improved cleaning efficacy in both straight and curved canals **[8, 10]**. However, multisonic systems are emerging as promising alternatives, with systematic reviews suggesting enhanced performance in multispecies biofilm models compared to traditional ultrasonics **[7]**. Similarly, devices such as EDDY have attracted attention for their polymer-based activation, offering results comparable to ultrasonics in certain scenarios **[9]**. Current trends therefore emphasize the integration of irrigant chemistry with activation techniques. Combinations such as NaOCl with EDTA activated ultrasonically or sonically, are increasingly reported as producing synergistic effects against biofilm **[11]**. The focus of ongoing research is to refine these approaches, balancing efficacy, safety and cost-effectiveness **[12, 13-15]**.

Limitations and future directions:

Despite promising laboratory results, several limitations persist. Much of the available evidence is derived from *in vitro* or *ex vivo* models, which cannot fully replicate clinical root canal infections. Biofilm diversity and patient-specific canal morphology introduce variables that controlled laboratory models often overlook. Moreover, the performance of ultrasonic and multisonic activation may vary in severely curved or anatomically complex canals. Comparative studies often demonstrate incremental advantages of one system over another, but these differences may not translate into clinically meaningful outcomes. A further constraint is the lack of protocol standardization; studies differ in irrigant concentration, activation time and outcome measures, limiting direct comparison. Safety concerns, including irrigant extrusion and dentin demineralization associated with high-concentration sodium hypochlorite, may also restrict broad adoption. Future research should prioritize biocompatible biofilm-targeted irrigants, optimized activation technologies and well-designed clinical trials with long-term follow-up to establish evidence-based protocols for routine practice.

Conclusion:

Activated irrigation enhances root canal biofilm disruption beyond conventional syringe irrigation. Thus, Ultrasonic and multisonic systems currently provide the most consistent adjunctive cleaning benefit. However, Standardized clinical trials are needed to define safe, reproducible protocols for routine endodontic use.

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