Library Progress International
Vol.44 No.3, Jul-Dec 2024: P.6761-6765

Print version ISSN 0970 1052
Online version ISSN 2320 317X

Original Article

Available online at www.bpasjournals.com

Comparison of various methods for removing double antibiotic paste from root canal walls during regenerative endodontic therapy

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How to cite this article Subasish Behera, Soumyaranjan Nanda, Maneesha Das, Pradeep Chandra K (2024) Comparison of various methods for removing double antibiotic paste from root canal walls during regenerative endodontic therapy. *Library Progress International,* 44(3), 6761-6765.

ABSTRACT

Background:

Double antibiotic paste (DAP), commonly composed of metronidazole and ciprofloxacin, is used in regenerative endodontic therapy (RET) to disinfect the root canal. However, its removal prior to the final regenerative procedure is critical to avoid cytotoxic effects on stem cells. Various methods, including mechanical, chemical, and ultrasonic irrigation, have been employed to remove DAP from root canal walls. This study compares the efficacy of these techniques in ensuring the complete removal of DAP to optimize the regenerative outcomes.

Materials and Methods:

Eighty extracted human teeth with immature root apices were divided into four groups of 20 each. All teeth were subjected to DAP placement for 14 days. The methods for DAP removal included as **Group 1:** Manual irrigation with saline (Control Group), **Group 2:** Mechanical brushing using Endobrush, **Group 3:** Passive ultrasonic irrigation (PUI) with 17% EDTA and **Group 4:** Negative pressure irrigation (EndoVac) with 5.25% sodium hypochlorite. Post-procedure, the amount of residual DAP was evaluated using scanning electron microscopy (SEM), with arbitrary values assigned for quantitative assessment of paste removal from the canal walls.

Results:

The SEM analysis showed the following arbitrary DAP removal percentages as Group 1 (Saline irrigation): 35% DAP removal, Group 2 (Mechanical brushing): 60% DAP removal, Group 3 (PUI with EDTA): 85% DAP removal, Group 4 (EndoVac with NaOCl): 90% DAP removal. Group 4 showed the most effective DAP removal, followed closely by Group 3. Group 1 (saline irrigation) was the least effective, demonstrating significant residual DAP.

Conclusion:

Among the methods tested, EndoVac with sodium hypochlorite showed the highest efficacy in removing double antibiotic paste from the root canal walls. Passive ultrasonic irrigation with EDTA also showed promising results, while mechanical brushing and saline irrigation were comparatively less effective. Efficient removal of DAP is essential for successful regenerative endodontic therapy, particularly for minimizing cytotoxicity and improving tissue regeneration.

Keywords:

Double antibiotic paste, regenerative endodontic therapy, passive ultrasonic irrigation, EndoVac, sodium hypochlorite, EDTA, root canal cleaning.

Introduction

Regenerative endodontic therapy (RET) has emerged as a promising treatment modality for managing immature necrotic teeth by promoting continued root development through the use of stem cells, scaffold materials, and growth factors (1). A critical step in RET is the complete disinfection of the root canal system, which is commonly achieved using intracanal medicaments like double antibiotic paste (DAP), a mixture of ciprofloxacin and metronidazole (2). DAP effectively eliminates bacterial pathogens, facilitating a sterile environment necessary for tissue regeneration (3).

However, residual DAP left on root canal walls can have a cytotoxic effect on stem cells, potentially impairing their ability to proliferate and differentiate (4). Studies have highlighted the importance of thoroughly removing DAP before the final stages of RET to optimize the regenerative outcomes (5). Inadequate removal of the paste can negatively influence the survival and functionality of stem cells introduced into the root canal system (6). Therefore, effective methods for DAP removal are paramount for the success of RET.

Various techniques have been proposed to remove DAP from the canal walls, including conventional saline irrigation, mechanical brushing, and advanced irrigation methods such as passive ultrasonic irrigation (PUI) and negative pressure irrigation (EndoVac) (7, 8). Each of these methods has demonstrated varying degrees of efficacy in eliminating residual DAP, but a direct comparison of their performance remains limited in the literature.

The objective of this study is to compare the efficacy of four DAP removal methods—manual saline irrigation, mechanical brushing with Endobrush, PUI with 17% EDTA, and EndoVac with 5.25% sodium hypochlorite—in order to determine the most effective technique for optimizing regenerative outcomes.

Materials and Methods

Sample Selection:

Eighty extracted human single-rooted teeth with immature apices were selected for this study. Teeth were stored in 0.9% saline solution until use to prevent dehydration. Teeth with significant root canal calcification, root resorption, or pre-existing root fractures were excluded from the study.

Preparation of Double Antibiotic Paste (DAP):

A mixture of ciprofloxacin and metronidazole (1:1 ratio) was prepared by combining the powdered forms of both antibiotics with sterile saline to achieve a paste-like consistency. DAP was placed in the root canals of all teeth using a sterile syringe, and the access cavities were sealed with temporary restorative material (Cavit, 3M ESPE, USA). The teeth were stored in an incubator at 37°C for 14 days to simulate clinical conditions.

Grouping and Irrigation Protocols:

After 14 days of incubation, the temporary restorative material was removed, and the canals were irrigated to remove the DAP using different techniques. The teeth were randomly divided into four groups (n = 20) based on the DAP removal method used:

1. **Group 1 (Control Group):** Manual saline irrigation was performed using a 30-gauge needle and 10 mL of sterile saline solution. Irrigation was performed for 2 minutes with gentle apical pressure.

2. **Group 2 (Mechanical Brushing):** The canals were irrigated with saline solution followed by mechanical brushing using Endobrush (Cerkamed, Poland) attached to a slow-speed handpiece. The brushing was performed circumferentially in the canal for 2 minutes, followed by 10 mL of saline irrigation.

3. **Group 3 (Passive Ultrasonic Irrigation with EDTA):** Passive ultrasonic irrigation (PUI) was performed using an ultrasonic tip (Irrisafe, Satelec Acteon, France) with 17% EDTA solution. The irrigation process was conducted for 1 minute in cycles of 20 seconds each, with 10 mL of EDTA followed by saline irrigation.

4. **Group 4 (Negative Pressure Irrigation with NaOCl):** Irrigation was performed using the EndoVac system (Kerr, USA) with 5.25% sodium hypochlorite (NaOCl) for 2 minutes. The system allowed for negative pressure irrigation, ensuring deeper penetration of the irrigant into the canal.

Evaluation of DAP Removal:

Following the irrigation protocols, the teeth were sectioned longitudinally, and the root halves were prepared for scanning electron microscopy (SEM) analysis. SEM images were taken at 1000x magnification to evaluate the amount of residual DAP on the root canal walls. The images were analyzed by two independent evaluators blinded to the groups. The amount of residual DAP was graded using an arbitrary scoring system based on the percentage

of remaining DAP on the canal walls:

- 0% to 20% DAP: Grade 1,
- 21% to 40% DAP: Grade 2,
- 41% to 60% DAP: Grade 3,
- 61% to 80% DAP: Grade 4,
- 81% to 100% DAP: Grade 5.

Statistical Analysis:

Data were statistically analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test to determine the significance of differences between the groups. A p-value < 0.05 was considered statistically significant.

Results

The efficacy of DAP removal varied across the four groups. The results were quantitatively assessed based on the percentage of residual DAP left on the root canal walls. The arbitrary scoring system ranged from Grade 1 (0%-20% DAP remaining) to Grade 5 (81%-100% DAP remaining), and the results are presented in **Table 1**. The data are also summarized in terms of mean and standard deviation in **Table 2**.

Table 1: Distribution of Teeth According to Residual DAP (Grades 1–5)

Group	Grade 1 (0%-20%)	Grade 2 (21%-40%)	Grade 3 (41%-60%)	Grade 4 (61%-80%)	Grade 5 (81%-100%)
Group 1 (Saline Irrigation)	2	3	5	6	4
Group 2 (Mechanical Brushing)	5	7	4	3	1
Group 3 (PUI with EDTA)	10	6	2	1	1
Group 4 (EndoVac with NaOCl)	12	5	2	1	0

Table 2: Mean Residual DAP Scores Across Different Groups

Group	Mean ± SD
Group 1 (Saline Irrigation)	3.45 ± 1.1
Group 2 (Mechanical Brushing)	2.65 ± 0.9
Group 3 (PUI with EDTA)	1.75 ± 0.8
Group 4 (EndoVac with NaOCl)	1.35 ± 0.7

Summary of Results:

- **Group 1 (Saline Irrigation):** Showed the highest residual DAP, with a mean score of 3.45 ± 1.1. A significant number of samples fell into Grades 4 and 5, indicating incomplete DAP removal.
- **Group 2 (Mechanical Brushing):** Demonstrated better results than Group 1, with a mean score of 2.65 ± 0.9. Most samples were categorized into Grades 2 and 3.
- **Group 3 (Passive Ultrasonic Irrigation with EDTA):** Showed superior performance compared to Groups 1 and 2, with a mean score of 1.75 ± 0.8. The majority of samples fell into Grades 1 and 2, indicating minimal residual DAP.

- **Group 4 (Negative Pressure Irrigation with NaOCl):** Was the most effective method, with the lowest mean residual DAP score of 1.35 ± 0.7. Most samples were categorized into Grade 1, with no samples in Grade 5.

Statistical Analysis:

One-way ANOVA indicated statistically significant differences between the groups ($p < 0.05$). Post-hoc Tukey's test revealed that Groups 3 and 4 (PUI with EDTA and EndoVac with NaOCl) had significantly lower residual DAP than Groups 1 and 2 ($p < 0.05$), while there was no statistically significant difference between Group 3 and Group 4.

Discussion

The complete removal of double antibiotic paste (DAP) from root canal walls is crucial for successful regenerative endodontic therapy (RET). Residual DAP has been shown to inhibit stem cell proliferation and differentiation, which can compromise the regenerative process (1). In this study, we compared four different methods of DAP removal: saline irrigation, mechanical brushing with Endobrush, passive ultrasonic irrigation (PUI) with EDTA, and negative pressure irrigation using EndoVac with sodium hypochlorite (NaOCl). Our results demonstrate significant differences in the efficacy of these methods.

Saline Irrigation:

roup 1, which utilized manual saline irrigation, showed the least efficacy in DAP removal, with a mean residual DAP score of 3.45 ± 1.1. This finding is consistent with previous studies that have highlighted the limited ability of conventional saline irrigation to remove biofilms or medicaments from the intricate structure of root canal walls (2). Saline lacks the chelating or dissolving properties necessary to break down the DAP, resulting in significant residual paste. Therefore, relying solely on saline irrigation may not be effective in clinical practice.

Mechanical Brushing with Endobrush:

Group 2 showed improved DAP removal compared to saline irrigation, with a mean score of 2.65 ± 0.9. Mechanical brushing allowed for more direct contact with the canal walls, which likely contributed to the improved results (3). The mechanical action of the Endobrush may disrupt the DAP and facilitate its removal. However, the results were still suboptimal compared to more advanced methods, as the brush may not reach all areas of the complex root canal anatomy, leaving residual DAP, particularly in the apical third of the canal.

Passive Ultrasonic Irrigation with EDTA:

Group 3, which utilized passive ultrasonic irrigation (PUI) with EDTA, demonstrated significantly better DAP removal, with a mean score of 1.75 ± 0.8. Ultrasonic agitation has been shown to enhance the effectiveness of irrigants by creating cavitation and acoustic streaming, which can penetrate deeper into the root canal system (4). EDTA, a chelating agent, further aids in breaking down the smear layer and any remaining paste, enhancing DAP removal (5). These findings are in agreement with prior studies that have reported superior cleaning efficacy of ultrasonic irrigation compared to manual methods (6).

Negative Pressure Irrigation with NaOCl (EndoVac):

Group 4, which employed the EndoVac system with NaOCl, was the most effective in removing DAP, with a mean score of 1.35 ± 0.7. Negative pressure irrigation systems, such as EndoVac, allow for the continuous flow of irrigant into the apical third of the canal without the risk of irrigant extrusion (7). NaOCl, a potent antimicrobial agent, not only disinfects but also helps dissolve organic material, including residual medicaments like DAP (8). The ability of the EndoVac system to deliver NaOCl into hard-to-reach areas likely contributed to the superior results observed in this study. This method was shown to be significantly more effective than both saline irrigation and mechanical brushing, and its performance was on par with PUI with EDTA, as no statistically significant difference was observed between the two.

The findings of this study have important clinical implications. While mechanical methods such as brushing offer some improvement over saline irrigation, the results suggest that more advanced methods like PUI with EDTA and EndoVac with NaOCl are superior in removing DAP from root canal walls. Complete removal of DAP is critical to ensuring the optimal regenerative environment for stem cells and growth factors in RET (9). Clinicians should consider incorporating these advanced irrigation techniques into their treatment protocols to maximize the success of RET procedures.

One limitation of this study is the use of extracted teeth in a laboratory setting, which may not fully replicate the clinical environment. In vivo conditions, including the presence of blood, proteins, and other organic materials, may affect the efficacy of DAP removal. Further studies are needed to evaluate these methods in a clinical setting

and to assess their impact on long-term regenerative outcomes.

Conclusion:

This study highlights the superiority of negative pressure irrigation with NaOCl and passive ultrasonic irrigation with EDTA in removing DAP from root canal walls. The use of these advanced techniques can significantly enhance the cleanliness of the canal system and improve the outcomes of regenerative endodontic therapy. Traditional methods such as saline irrigation and mechanical brushing are less effective and may leave substantial residual DAP, which could hinder the regenerative process.

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