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Antibacterial Efficacy and Discoloration Potential of Topical Antibiotics Used in Regenerative Endodontic Therapy

Beenish Khalil Rana[1], Kanwal Sohail[2], Amber Kiyani[3]

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ABSTRACT

Objective: The present study was conducted to determine the minimum inhibitory and bactericidal concentrations and discolouration potential of various topical antibiotics that include triple antibiotic paste (TAP), Augmentin and Tetracycline against the pathogens that cause endodontic lesions in an ex-vivo study evaluation.

Materials and Methods: Minimum bactericidal and inhibitory concentrations of the concerned antibiotic were evaluated by Epsilometer test method against various pathogens that included Porphyromonas, Fusobacterium, Enterococcus and Streptococcus Intermedius. 104 extracted single-rooted teeth were selected for study purpose and the biofilm for the selected species were grown in extracted teeth for 21 days under strict anaerobic condition. TAP, Augmentin and Tetracycline were then infused in the root canal in the concentration of 0.1 mg/ml, 1 mg/ml in degradable hydrogel scaffold and pure TAP at the concentration of 1 g/ml for 7 days initially.

Results: The minimum inhibitory concentration/minimum bactericidal concentration of TAP at 1g/ml was highest, while comparing the efficacies of three antibiotics at the concentration of 1 mg/ml the following order was observed TAP>Augmentin>Tetracycline. No bacterial growth was found for TAP at 1 g/ml whereas at the concentration of 1 mg/ml the least growth was observed with TAP followed by Augmentin and Tetracycline. The log 10 colony-forming unit of all experimental groups show significant differences ($p<0.05$). The greatest discolouration was observed by TAP at the conc. of 1g/mL and the change of colour with Augmentin/hydrogel scaffold at the concentration of 1mg/ml was minimal.

Conclusion: The hydrogel scaffold of TAP, Augmentin and Tetracycline shows a significant reduction in bacterial growth with minimal change in colour at the concentration of 1 mg/ml.

Keywords: Discoloration, Regenerative Endodontics, Triple Antibiotic Paste

[1]Demonstrator, Department of Periodontology, Islamic International Dental College, Riphah International University, Islamabad, Pakistan

[2]Assistant Professor, Department of Oral Medicine, Margalla College of Dentistry, Margalla Institute of Health Sciences, Rawalpindi, Pakistan

[3]Assistant Professor, Department of Oral Medicine, Islamic International Dental College, Riphah International University, Islamabad, Pakistan

Corresponding author:

Beenish Khalil Rana, Department of Periodontology, Islamic International Dental College, Riphah International University, 7th Avenue, G-7/4, Islamabad, Pakistan, Email: beenish.khalil@riphah.edu.pk, Mobile: +923335877462

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INTRODUCTION

The ultimate goal of endodontic therapy is to retain natural dentition. The most exciting development that has been made to conserve natural dentition is regenerative endodontic therapy the concept of tissue engineering has been employed to restore the canals of tooth to a healthy state promoting the development of root and the surrounding periodontal tissues. The treatment is ideal for necrotic immature permanent teeth resulting in root development and apical closure for traumatized teeth.[1] Regenerative endodontic therapy (RET) has a promising impact on the efforts to retain natural dentition. RET involves disinfecting the root canal with the use of antibiotics. Disinfection of the canal is a critical step because multiple anaerobic and aerobic bacteria's harbour the canal and cause infection hence disinfecting a canal with only one antibiotic is not sufficient.[2] Disinfection of the canal determines the success of the regenerative endodontic procedure.[3] One of the most ways employed to achieve disinfection of the root canal is irrigation with sodium hypochlorite and placing a dressing of triple antibiotic paste (TAP).[1] TAP is a preferred choice for RET because it comprises minocycline, metronidazole and ciprofloxacin.[4] Since the root canal consists of diverse flora; gram-positive, gram-negative, aerobic and anaerobic bacteria, TAP provides good infection control.[5] TAP is however linked to discolouration of the crown in almost 50 % of the studies conducted on endodontic procedures.[6] This discolouration affects esthetics and may impact patient satisfaction.[7] The cause of discolouration is described as a reaction of the antibiotics with the dentinal walls.[8] Minocycline is implicated as the primary staining agent. It appears that if dentinal walls are sealed before the introduction of minocycline, staining can be prevented.[7] Even slightly higher concentrations can be toxic to the stem cells in the apical papilla that are crucial for RET.[9] The literature on the optimum concentration of TAP is deficient. There is also limited information regarding the introduction of TAP into the root canal within scaffolds used during other tissue generation procedures.[10] We believe that use of scaffolds would acts as carriers thus minimizing contact with dentinal tubules. The purpose of our study was to investigate the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Augmentin, TAP and Tetracycline, evaluate the antibacterial efficacy of Augmentin, TAP and Tetracycline in slow releasing hydrogel scaffolds, and to determine the discoloration potential *ex-vivo*.

MATERIALS AND METHODS

This study was approved by the Ethical Review Board at Riphah International University [Ref. No. IIDC/IRC/2018/10/001]. Five common organisms were selected commonly encountered in the root canal system for our investigation. These included Streptococcus Intermedius, Enterococcus Faecallis, Fusobacterium Nucleatum and Porphyromonas Gingivalis.

Anaerobic blood agar plate culture was used for the initial growth of bacterial strains. Every attempt was made to keep the environment strictly anaerobic. The temperature was maintained at 37^0C. Initial growth was re-cultured on the organism's recommended broth. These were Todd Hewitt for Streptococcus intermedius, tryptic soy with hemin and vitamin k for porphyromonas gingivalis, chopped meat for fusobacterium nucleatum and brain-heart infusion for enteroccocus faecallis. An epsilometer test (e-test) was used to determine the MIC and MBC of all the drugs.

One hundred and four intact permanent single-rooted human teeth with no carious lesions, restorations or anomalies were collected from the Orthodontic Department and kept under ideal conditions. The teeth were extracted for orthodontic purposes. The gross

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debris and visible blood were cleansed from all extracted teeth, and they were stored in saline followed by placement in a 1:10 diluted solution of sodium hypochlorite to water. The teeth were then autoclaved to eliminate bacterial accumulation during storage. Secure containers with airtight lids were used for storing the sample.

The teeth were autoclaved again for 40 minutes at 121°C and 15 Psi before intervention. Access cavities were prepared using a handpiece. The canals were prepared up to file size 60. The canals were irrigated with saline and autoclaved again before inoculation of bacterial cultures with a 30-gauge needle along the working length. This was then sealed with cavet. The teeth were incubated in anaerobic conditions for three weeks under weekly supervision. The antibiotic was incorporated into prepared hydrogel scaffolds. Two different concentrations of medicaments; 1 mg/ml and 0.01 mg/ml, were used. Two different mixtures at each concentration were all three antibiotics were mixed with oxidized alginate solution to form hydrogel microbeads.

The teeth were randomly divided into 8 groups having, each with 13 teeth. Root canals were filled with hydrogel scaffold with antibiotics. Group one had teeth with an empty canal and acted as negative controls. The canals of the teeth of group 2 were filled with hydrogel and Augmentin in the concentration of 0.1 mg/mL, group 3 has hydrogel and Tetracycline in conc. of 0.1mg/mL, group 4 had hydrogel and TPA at 0.1 mg/mL, group 5 consisted of hydrogel plus Augmentin in conc. of 1mg/mL, group 6 is comprised of hydrogel plus tetracycline in 1 mg/mL, group 7 composed of TPA and hydrogel 1 mg/mL, group 8 consisted of TPA 1g/ml mixed with the saline and was considered the positive control group.

These teeth were incubated for 1 week after packing. After one week of dressing, the canals were evaluated. Irrigation was done with 30 ml saline for 5 minutes for removal of canal contents. Instrumentation was done again with a 60 K file to disrupt the biofilm and the content was transferred to a labelled vial with normal saline for sampling. Glidden size 4 was used to prepare the canal up to the length of 100 to 200 micrometres. Five consecutive sterile paper points were used in each tooth and transferred to the respectively numbered saline vial. These samples were placed on an anaerobic blood agar plate. A thin, inert, and non-porous plastic strip with a MIC reading scale in µg/mL on one side, and the other side, a predefined antibiotic gradient. It has been then applied to the inoculated agar surface, to form a stable, continuous exponential gradient of antibiotic concentrations underneath the strip.

Vita pan classical farbscala Germany shade card was used to determine the colour of crowns. The colour measurements were performed in anaerobic conditions and were measured at weekly intervals for 3 weeks consecutively. E- test (BioMérieux, USA) was used to test the antimicrobial resistance by exponential gradient method as per manufacturer's instructions. This test provided direct quantification of antimicrobial susceptibility of microorganisms. A one-way ANOVA test was applied to compare the samples in SPSS software version 22.0.

RESULTS

The mean MBC and MIC of all the antibiotics tested are shown in Figure 1 and Figure 2. The highest concentration of MIC and MBC was shown by TAP at 1mg/mL, followed by Augmentin at 1mg/mL and Tetracycline at 1mg/mL.

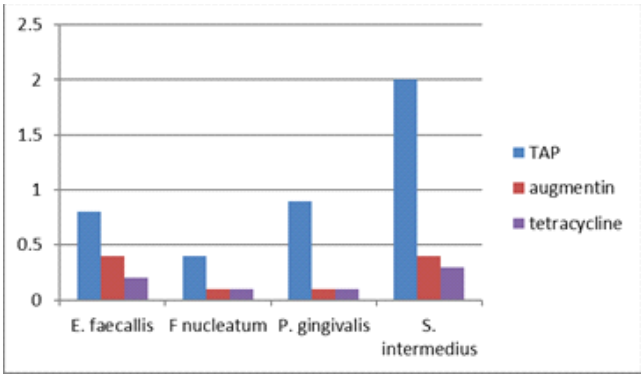

Figure 1: MIC of TAP, Augmentin and Tetracycline

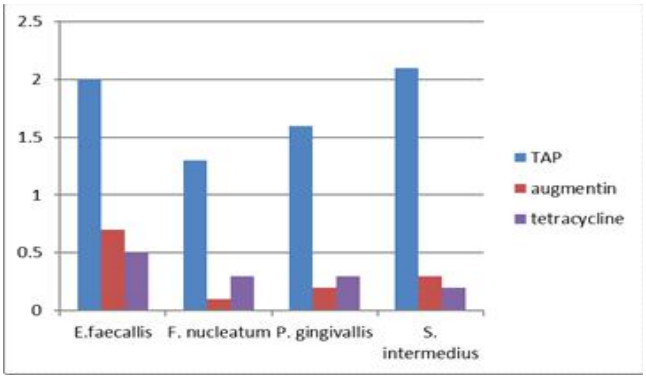

Figure 2: MBC of TAP, Augmentin, and Tetracycline

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Concerning the bacterial growth, the best results were shown by TAP at the concentration of 1 g/mL resulting in no bacterial growth followed by Augmentin at 1mg/mL and Tetracycline at 1mg/ml. Bacterial growth was observed in the control group with mean colony-forming units of 1.2 x 10^8 cells/mL. A reduction of the only 3-fold log was observed by using all the antibiotics in the concentration of 0.1mg/ml. However a significant reduction in the bacterial populations was observed when antibiotics were used in the concentration of 1 mg/mL (p<0.05) as shown in Table 1.

Table 1: Log10 CFUs in samples of the teeth after 1 week of therapy with various antibiotic/hydrogel groups

Group	Log_{10}	SD	*p*-value
Control group	8.4[a]	0.97	0.005
TAP (0.1mg/mL)	4.25[b]	0.83	
Tetracycline (0.1mg/mL)	3.40[b]	1.29	
Augmentin (0.1mg/mL)	4.00[b]	1.03	
TAP (1mg/mL)	0.73[c]	1.20	
Tetracycline (1mg/mL)	0.94[c]	1.36	
Augmentin (1mg/mL)	1.04[c]	1.34	
TAP (1g/mL)	**0.00[c]**	**0.00**	

SD, standard deviation; TAP, triple antibiotic paste. Lowercase superscript letters indicate groups that were statistically similar (analysis of variance, P< 0.05)

The discolouration value for all antibiotics is shown in Figure 3. The highest discolouration was noted with TAP at a concentration of 1g/ml while the tooth treated with Augmentin 1mg/ml had the lowest colour change at 1 week and 3 weeks follow up. TAP/hydrogel 1mg/ml had a slightly higher potential of discolouration than Tetracycline 1mg/ml and Augmentin 1mg/ml as shown in Figures 3 and 4.

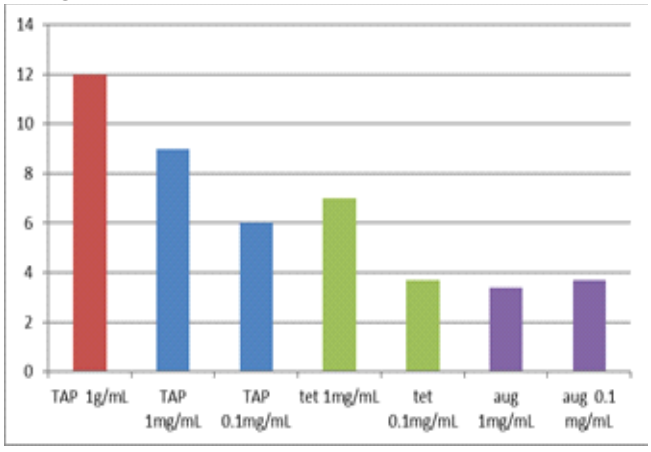

Figure 3: Comparative analysis of mean colour change 1-week post-treatment

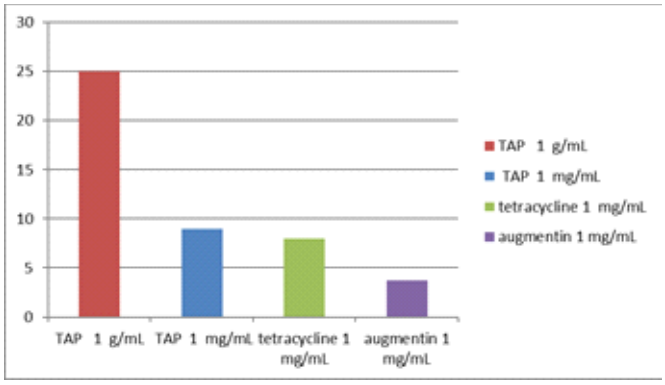

Figure 4: Comparative analysis of mean colour change 3 weeks post-treatment

DISCUSSION

Extracted teeth were used to determine the antimicrobial efficacy of Augmentin, Tetracycline and TAP and their discolouration potential. The bacterial growth was restricted despite the antibiotics being used in hydrogel scaffolds.

The oral cavity is the locale of hundreds of microbial taxa that have changed to coexist in multispecies

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communities.[11] Prior studies have considered only E. Faecallis in their investigations, which may not be characteristics because the root canal flora is polymicrobial.[9] In primary root canal infections, gram-negative bacterial species have been identified in the necrotic pulpal tissue however several studies have shown obligatory anaerobic bacteria in root canal infections, which comprise 90% of all bacterial species. To overcome the limitations of the previous studies we used organisms from both categories, gram-positive, gram-negative. Anaerobic organisms were selected to mimic the root canal environment.[4]

In regenerative endodontic procedures, effective sterilization of the root canal is essential and the most way used to disinfect the canal are antibiotics.[12,13] The most frequently used antibiotic in RET is TAP. It is also considered to be the most effective.[12-14] Studies have shown that the efficacy of two or more two antibiotics always performs better than using one drug. This is because the use of more than one medicament prevents the development of resistance.[1,5,15] A prior study showed that a combination of metronidazole and ciprofloxacin (double antibiotic paste-DAP) against 0.125mg/ml of TAP. TAP had superior performance.[16,17] The superior efficacy of TAP is replicated in our study. The second most effective antimicrobial agent appears to be Augmentin. It showed good efficacy against gram-positive bacteria as shown in different studies.[18,19] Similar results have been replicated in our study. Followed by Augmentin is the efficacy of Tetracycline as mimicked in our results.

Our results verify this premise. Another antibiotic that is used other than TAP and Augmentin was tetracycline all of these antibiotics have a different effects on the root canal flora and discolouration of the crown.[20,17]

According to a study concentration of medicine can influence its performance.[21] Moreover when the double antibiotic paste is used at a higher concentration that is 500mg/ml is proved to be more efficacious than when used in a lower concentration of 0.1mg/ml or 1 mg/ml.[22]

The antibiotics can be mixed with saline and introduced with a file or paper point. Another way to introduce the medicament is using a scaffold. This process is easier and ensures uniform delivery.[14] We preferred this technique because we hoped that the scaffold would prevent contact of the medicament against the dentinal walls and prevent discolouration.

The discolouration potential and its effect on hue, chroma and value of teeth are unique for each antibiotic.[17-23] In all prior studies, minocycline is the most common antibiotic linked with tooth discolouration.[24] Our findings with intracanal medicaments were similar to prior studies, TAP was linked with the greatest discolouration when compared with individual antibiotics.[25] According to the vita shade card guide, TAP discolouration 1 mg/mL showed 4 shade changes, while TAP/hydrogel at 1 mg/mL showed a 3 shade change. This indicates that the hydrogel barrier did not affect the contact of the medicament with the tooth. Tetracycline showed a 2 shade change while Augmentin was not associated with any significant colour changes.

Within the limitations of this *ex-vivo* study, TAP at a high concentration (1 g/mL) was the most efficacious antibiotic against common endodontic bacterial biofilms but caused the greatest tooth discolouration. Although 1 mg/mL TAP, Tetracycline, and Augmentin were as efficacious as 1 g/mL TAP in removing the bacterial biofilm, they caused minimal discolouration. Clinical studies are needed to confirm these *ex-vivo* findings.

CONCLUSION

In conclusion, while TAP at 1mg/mL is most effective against the bacterial populations of the root canal, it is also associated with the most discolouration, even with the hydrogel barrier. Augmentin at a concentration of 1mg/ml is second in line in terms of MIC and MBC values, followed by Tetracycline. No colour change in the tooth was seen with the use of Augmentin only indicating that it can be medicament of choice when esthetics are a priority.

DISCLAIMER

None to declare.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ETHICAL STATEMENT

The ethical approval is provided by the Ethical Review Board at Riphah International University [Ref. No.

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AUTHORS CONTRIBUTION

Conception and design of the study: B.K. Rana, A. Kiyani

Acquisition of data: B.K. Rana

Analysis and interpretation of data: K. Sohail

Drafting of the manuscript: B.K. Rana, K. Sohail

Critical review of the manuscript: A. Kiyani

Approval of the final version of the manuscript to be published: B.K. Rana, A. Kiyani, K. Sohail

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