



## ANTI MICROBIAL EFFECTIVENESS AND SMEAR LAYER REMOVAL OF DOXYCYCLINE WHEN USED AS A ROOT CANAL IRRIGANT : AN IN VITRO STUDY.

### Dental Science

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### ABSTRACT

**Objective:** The aim of the present study is to assess the effect of doxycycline hydrochloride (DH) on smear layer removal on intracanal walls and antimicrobial agent against selected microorganisms.

**Methodology:** Twenty freshly extracted human teeth were collected, the teeth were cleaned and washed to remove any debris, calculus attached to it. The crowns of the teeth were sectioned at the cemento enamel junction. Isolated 24 hr colonies of pure cultures grown on mixture of 10% sheep blood agar and Brain Heart Infusion (BHI) agar plates were collected with the help of a wired loop and suspended in sterile 0.85% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 BaSO<sub>4</sub> standard. Thereafter the suspension was poured in T C plate 24 wells with the micropipettes and the specimens were dipped in the suspension and kept in the incubator for 7 days at 37°C. The canals were dried by sterile paper points and the swab taken with the help of sterile paper point which is placed in a sterile appentoff filled with .5 l of saline and then vortexed for maximum leaching of bacteria from the paper point

**Scanning electron microscopic evaluation:** The specimens were scanned as a whole initially and representative photomicrographs were obtained. Each stub of the scanning electron microscope was given a code number to keep record for reference. The apical two-third area was scanned at the 2000x magnification and photomicrographs were taken. The photomicrograph were coded and evaluated in terms of evaluation of the number of open dentinal tubules. The teeth were subjected to SEM analysis to check the effect of the irrigant on the smear layer. Data's thus obtained were subjected to the statistical analysis using ANOVA test.

**Results:** This study supports that doxycycline is the irrigating solution for root canals. As it can open the dentinal tubules and penetrate the surface of dentinal tubule to the deepest layer of dentine and makes the environment aseptic and definitely increase the success rate of root canal treatment.

**Conclusion:** Doxycycline was found to have better effectiveness against streptococcus pyogenes and enterococcus faecalis whereas it was found less effective against both the staphylococcus aureus, and MRSA (methicillin resistant staphylococcus aureus).

### KEYWORDS

Doxycycline. Smear layer, , scanning electron microscopy,

### INTRODUCTION:

A critical objective in endodontic therapy is complete elimination of microorganism from the root canal system. In order to accomplish this it is essential to use an irrigant or combination of irrigants during the biomechanical preparation of the canal system.

Root canal systems are irregular and complex with many cul-de-sacs, fins, and lateral canals. Microorganisms present in the root canal can invade these irregularities as well as penetrate the dentinal tubules opening onto the root canal surface to establish infection.

Gram-positive facultative anaerobes such as streptococcus, lactobacillus and enterococcus can survive root canal treatment readily than any other anaerobic species of original micro biota.

The smear layer was first referred to by Pashley in 1984. Mc Comb et.al observed this layer on the walls of the instrumented root canals. It consist of a superficial layer and a layer loosely attached to the underlying dentin.

The smear layer has a amorphous irregular and granular appearance when viewed under the scanning electron microscope.

The several authors regard this smear layer to be beneficial because it fills the orifices of dentinal tubules thereby reducing the dentine permeability. On the other hand it is also desirable to remove the smear layer because it prevents its adaptation on the root canal sealers to the canal walls. Microorganisms also have the ability to penetrate the smear layer. Therefore, it is believed to be advantageous to remove it.

Weine suggested that open tubules were considered to be desirable because they allowed the penetration by medicaments and better adhesion for the sealers and that alternate irrigant gives a cleaner canal with less organic debris and more desirable walls.

The purpose of this study was to test the anti microbial effectiveness and smear layer removal efficacy of doxycycline in combination when used as a root canal irrigants.

### MATERIAL AND METHODS:

The root canal irrigants used in this study were Doxycycline ,Sodium hypochlorite, Saline

- The study was conducted in two different steps:

1. Doxycycline was used as a irrigant in different concentration of 50mg/ml and 100mg/ml in combination with sodium hypochlorite and saline and divided into the different groups to check the efficacy to remove the smear layer and SEM analysis was done
  - Group A – 50mg/ml + Naocl
  - Group B – 100mg/ml +Naocl
  - Group C – 50mg/ml + saline
  - Group D – 100mg/ml + saline
2. Doxycycline was used as a irrigant against the selected microorganisms and the antimicrobial study was done in the laboratory. In this study four bacteria were selected against the irrigants and divided into the following groups:
  - B1- Staphylococcus aureus – ATCC 29212
  - B2- Staphylococcus aureus methicillin resistant – ATCC 29213
  - B3- Streptococcus pyogenes – MTCC 442
  - B4- Enterococcus faecalis – MTCC 439

Twenty freshly extracted human teeth were collected , the teeth were cleaned and washed to remove any debris, calculus attached to it .The crowns of the teeth were sectioned at the cemento enamel junction. The samples were stored in distilled water till further experiment was taken up. Isolated 24 hr colonies of pure cultures grown on 10% sheep blood plus Brain Heart Infusion (BHI) agar plates were collected with the help of a wired loop and suspended in sterile 0.85% NaCl solution. The cell suspension was adjusted spectrophotometrically to match the turbidity of a McFarland 0.5 BaSO<sub>4</sub> standard. Thereafter the suspension was poured in T C plate 24 wells with the micropipettes and the specimens were dipped in the suspension and kept in the incubator for 7 days at 37°C. Access cavity was prepared, 10 no, k-flex file was placed in the root canal until it was just visible at the apical foramen to establish the working length. BHI agar was poured in the

Petri dish and allowed to cool. The canals were dried by sterile paper points and the swab taken with the help of sterile paper point which is placed in a sterile appentoff filled with .5 l of saline and then vortexed for maximum leaching of bacteria from the paper point After one minute of vortexing the plating was done over BHI agar plates and kept for incubation at 37c in the incubator for 24 hours. After 24 hrs the Petri dish was observed for growth in the number of colony forming units. The Petri dish was then divided into four compartments to count the number of colony forming units.

**Preparation of samples for scanning :**

**Grooves preparation:**

A single longitudinal groove was cut on the labial and lingual surface of the sample by diamond disc. The grooves were deep but did not disturb the canal lining.

**Coating of samples:**

The specimens were coated with gold- palladium under vaccum pressure. Gold – palladium coating is based on the phenomenon of ion sputtering by establishment of electromagnetic field under vaccum between gold – palladium disc in the centre and to the conductive silver paint on the mounted aluminium stub attached all around the disc.

**Scanning electron microscopic evaluation:**

The specimens were scanned as a whole initially and representative photomicrograph were obtained . each stub of the scanning electron microscope was given a code number to keep record for reference. The apical two-third area was scanned at the x2000 magnification and photomicrograph were taken.

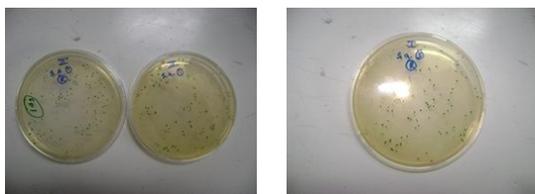
The photomicrograph were coded and evaluated in terms of evaluation of the number of open dentinal tubules.

The teeth were subjected to scanning electron microscope to check the effect of the irrigant on the smear layer. The data obtained was submitted to the analysis of variance (ANOVA.P=0.05).

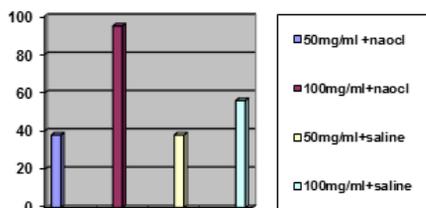
**RESULTS:**

Table 1 shows the antimicrobial effectiveness of doxycycline against the selected bacteria.

The results of the irrigant tested shows that the irrigant ( doxycycline) was found to be effective against both the staphylococcus aureus (80%), and MRSA (60%) reduction, where as it has shown less effectiveness against streptococcus pyogenes(20%) and enterococcus faecalis. (40%) reduction. The tested irrigant ( doxycycline) was also seen very much effective in removing the smear layer at all levels tested (coronal , middle and apical thirds). Among the tested irrigants the hypochlorite group irrigate with 100mg/ml of doxycycline showed 95% effective in opening the dentinal tubules, whereas 50mg/ml of doxycycline showed 38% effective in opening the dentinal tubules In the saline group teeth irrigated with 100mg/ml of doxycycline showed 57% effective in opening the dentinal tubules whereas teeth irrigated with 50mg/ ml of doxycycline showed 38% effective in opening the dentinal tubules.



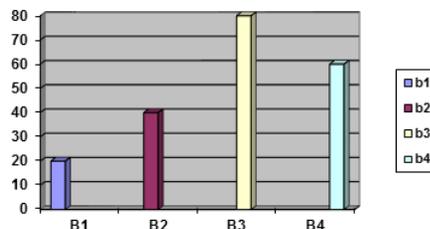
**Fig 1 And Fig 2 Showing The Reduction In The Number Of Colony Forming Units**



**Graph -1 Showing The Amount Of Opening Of The Dentinal Tubules**

**Table 1:-**

Doxycycline	B1 (s.aureus)		B2 (s.aureus methicillin resistant)		B3 (streptococcus pyogenes)		B4 (E.faecalis)	
	N	%	N	%	N	%	N	%
Present	1	20	2	40	4	80	3	60
Absent	4	80	3	60	1	20	2	40
Total	5	100	5	100	5	100	5	100



**GRAPH :-2 showing the reduction in the number of colony forming units**

**DISCUSSION :**

The primary goal of endodontic treatment is to optimize root canal disinfection and to prevent reinfection.

Microorganisms remaining within the root canal space after treatment or recolonizing the filled canal system are the main cause of endodontic failure thus microbial control by chemo mechanical procedures is very important for the success of root canal treatment.<sup>10</sup>

Marina George; et.al observed that there was a difference in the bacteria in the teeth with open pulp chambers and those in the root canals. It was also observed that only some were cultivable when compared to the numerous microbes seen under the microscope, and that the flora in the coronal, middle and apical parts of the root canal differed.

The ideal irrigant or combination of irrigants kills bacteria, dissolves necrotic tissue, lubricates the canal, removes the smear layer, and does not irritate healthy tissues The wide use of doxycycline in periodontal therapy has led to its evaluation as an adjunct in endodontic therapy. Results of this study indicate that a solution of 100 mg/ml doxycycline-HCl is highly effective for removal of smear layer and other debris from the root canal system. The effects of 50mg/ml and 100mg/ml doxycycline with sodium hypochlorite are shown in fig 1 and fig 2 respectively.



**Fig 3.Hypochlorite group irrigated by 50mg/ml of doxycycline at x2000**



**Fig 4.Hypochlorite group irrigated by 100mg/ml of doxycycline at x2000**



**Fig 5.Saline group irrigated with 50mg/ml of doxycycline observed at x2000**



**Fig 6.Saline group irrigated with 100mg/ml of doxycycline observed at x2000**

The findings from this study for intracanal dentin irrigation are in agreement with results obtained with tetracycline treatment of external dentin surfaces.<sup>6</sup>

The acidic property (pH = 2) of doxycycline- HCl is probably

responsible for the breakdown of both the superficial and intratubular smear layers attached to dentin surfaces. These smear layers are loosely attached to dentin following instrumentation. Root surface conditioning by topical application of acidic solutions in some bonding systems eliminates contaminants and smear layer.<sup>2</sup>

Doxycycline seems to be as effective as acidic conditioners in removing smear layer. Tetracycline's, including doxycycline, readily attach to dentin and are subsequently released without losing their antibacterial activity. This property creates a reservoir of active antibacterial agent, which is released from dentin surfaces in a slow and sustained manner.<sup>5</sup>

A further benefit of doxycycline is that prolonged treatment with the drug does not facilitate bacterial mutation to generate tetracycline-resistant microorganisms.<sup>2</sup>

The lower concentrations of doxycycline-HCl were significantly more effective in the presence of sodium hypochlorite than when used with saline (Table I). This raises the possibility that a combination of doxycycline- HCl and sodium hypochlorite could be a more effective irrigant. Higher doses of doxycycline are toxic and inhibit fibroblast adherence and spread along a substratum. The use of doxycycline-HCl solution is an effective irrigant in combination with sodium hypochlorite and has potential in root canal treatment procedures. The solution is easily delivered to the root canal system and adheres to the canal wall. Although no adverse effects of hypochlorite on doxycycline antibacterial activity are anticipated, further studies are needed to evaluate the in vivo efficacy of doxycycline in endodontic systems.

#### REFERENCES :-

1. Ana Carolina Silveira Cardoso de Menezes et.al : Smear layer removal capacity of disinfectant solutions used with and without EDTA for the irrigation of canals: a SEM study; *Pesqui Odontol Bras* 2003;17(4):349-55.
2. Baumgartner JC, Brown CM, Madar CL. A SEM evaluation of root canal debridement using saline, hypochlorite, and citric acid. *J Endodontol* 10:525-531, 1984.
3. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of NaOCl for root canal irrigation. *J Endodontol* 18(12):605-12, 1992 Dec.
4. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. *Dental clinics of north America* 34(1):13-25, 1990 Jan.
5. Carlos ESTRELA; et.al; Mechanism of Action of Sodium Hypochlorite; *Braz Dent J* (2002) 13(2): 113-117.
6. C. E. Radcliffe; et.al; Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*; *International Endodontic Journal*, 37, 438-446, 2004.
7. C. M. Sedgley; et.al; Survival of *Enterococcus faecalis* in root canals ex vivo; *International Endodontic Journal*, 38, 735-742, 2005.
8. Faruk Haznedaroglu; et.al; Efficacy of various concentrations of citric acid at different pH values for smear layer removal; *(Oral Surg Oral Med Oral Pathol Oral Radiol Endod)* 2003;96:340-4.
9. H. Ayhan; et.al; antimicrobial effect of various endodontic irrigant on selected microorganisms; *international endodontic journal*; 32:99-102; 1999.
10. Isabel Mello; et.al; Influence of different volumes of EDTA for final rinse on smear layer removal; *(Oral Surg Oral Med Oral Pathol Oral Radiol Endod)* 2008;106:e40-e43.
11. Mahmoud Torabinejad; A New Solution for the Removal of the Smear Layer; *journal of endodontics*; vol 29; no 3; march 2003.
12. Marina George; et.al; Root Canal Microflora; *ACTA MEDICA (Hradec Králové)* ;50(1):7-15; 2007.
13. Rahmat A. Barkhordar; et.al; Removal of intracanal smear by doxycycline in vitro; *(Oral Surg Oral Med Oral Pathol Oral Radiol Endod)* 1997;84:420-3.
14. R. Di Lenarda; et.al; Effectiveness of 1 mol L<sup>-1</sup> citric acid and 15% EDTA irrigation on smear layer removal; *International Endodontic Journal*, 33, 46±52, 2000.
15. Sidney Ricardo Dotto; et.al; Evaluation of ethylene diamine tetra acetic acid (EDTA) solution and gel for smear layer removal; *Aust Endod J* 2007; 33: 62-65.
16. Weine FS. *Endodontic therapy*, 4<sup>th</sup> Ed. St. Louis, Mosby 302-307, 1989.
17. YAVARI H; et.al; Determination the Best Concentration of Intracanal Irrigant Made of Citric Acid, Doxycycline and a Detergent for Suppression the Growth of *Enterococcus faecalis*; *Medical Journal Tabriz University of Medical Sciences* Vol. 29 No. 4 Winter 2008.
18. Z. Mohammadi; et.al; Antibacterial Substantivity of Three Concentrations of Doxycycline on Bovine Root Dentin Infections: an In Vitro Study; *[Dental Research Journal (Vol. 4, No. 1, Spring-Summer 2007): 48-52]*.
19. Zahed Mohammadi; An update on the antibiotic-based root canal irrigation solutions; *international endodontic journal*; vol-3; no-2; 2008.