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Bactericidal effect of nd: yag laser irradiation on enterococcus faecalis – *ex vivo study*

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Abstract: *The aim of this study is to define the role of NdYAG laser in root canal disinfection with minimally invasive concept.*
The hypothesis was tested ex vivo that NdYAG laser irradiation has a bactericidal effect on E. faecalis inoculated in instrumented root canals.
Methodology includes observation of bacterial cell structural changes using CSEM and ESEM on inoculated dentin surfaces following direct and indirect NdYAG laser irradiation respectively along with resultant colony forming unit count. Results showed NdYAG laser irradiation of E. faecalis inoculated canals resulted in significant reduction ($p<0.005$ wilcoxon signed rank test) of E. faecalis which meant 97.3% eradication of microbes (both conventional SEM and environmental SEM)
also proves the NdYAG laser has a bactericidal effect on E. faecalis(cell structural changes in E.faecalis and disruption of biofilm) upto 1mm of thickness of dentin and dentinal tubules.
To conclude Nd:YAG laser irradiation is an adjunct and not an alternative to various disinfectant protocols particularly in repeated failure of nonsurgical endodontic treatment and in persistent infections of pathogen like E. faecalis which resists many antimicrobial treatment, however E. faecalis that grow as biofilm are quite difficult to eradicate totally even on direct laser treatment.

Keywords: *Culture, environmental scanning electron microscopy, laser irradiation, bacterial invasion, cell structural changes*

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I. Introduction

The main objective of root canal therapy is to eradicate all microbes from infected root canal and moreover prevent reinfection. Complete chemo mechanical preparation remains the most important step in canal disinfection [1]. Inspite of thorough BMP and irrigation complete elimination of microbes is difficult in all cases (Byston and Sundquist 1983). Complex anatomy of root canal often makes it difficult to complete debridement almost impossible.

The choice of an irrigant is most significant regarding their potential to kill micro organisms, Despite diverse irrigants used copiously during BMP, certain notorious bacterial species like E. faecalis survive persistently in root canal[2]. E. faecalis survives in a high PH of 11 due to functioning proton pump which is (Events et all 2002)[3], the influencing factor contributing to its survival as a single organism. It is stubbornly alive in oxygen depleted, ecological complex environment (Fett et at 1994), and it can invade deep into dentinal tubules[4] (Alcapata and Blechman 1982, Haepeseto and Orrtavik 1987).

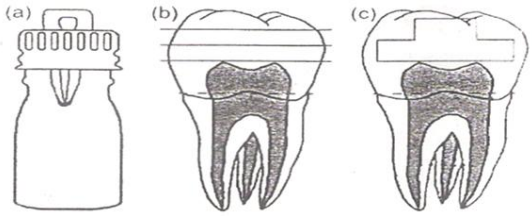
E. faecalis recovered from retreatment cases (Sundquist et al 1998, Siquiera and Rocas 2004), is proved by deep invasion into dentinal tubules [5], makes it to survive in thorough BMP, copious irrigation and intracanal medicaments (Love 2001, Peters et al 2001), get lodged, colonized deep in tubules and reinfect the obturated root canal (R.M. Love).

The important behaviour of this organism is contributed by its biofilm formation. Amidst multifamous irrigants in endodontists office, lasers are perhaps the most, innovatory, trailblaming though expensive. Amongst numerous type of lasers used Nd:YAG lasers are gaining popularity and the antibacterial effects of irradiation from Nd:YAG lasers consistently reduced the number of bacteria[6]. A marked reduction by a factor 10^2 to 10^4 was common in many studies using Nd:YAG laser.

The aim of this study is to emphasise the role of Nd:YAG laser as root canal disinfectant with minimally invasive hypothesis. This hypothesis was tested ex vivo that Nd:YAG laser irradiation has a bactericidal effect on E.faecalis inoculated in root canals. Resultant colony forming unit counts were associated with observation of bacterial cell structural changes using Conventional scanning Electron Microscope and ESEM following Nd:YAG laser irradiation.

II. Materials and methods

Microbiological analysis



a) Prepared maxillary incisor teeth mounted in bijou bottles
b) For CSEM molar crowns sectioned in occlusal planes near pulp extension, thrice to get one mm dentin disc
c) For ESEM molar crowns sectioned for mounting in specimen stub with flat surface positioned horizontally ,near pulp extensions

Thirty freshly extracted maxillary incisor teeth stored in 0.5% solution of chloramines in water, at 4^oC were used in this study after the root surfaces were cleaned with ultrasonic scalers at the time of use.

Access opening was done in all the specimens. Pulp remnants were removed and preflaring was done with GG_{1-4} .Each canal was instrumented using protaper rotary system in a crown down sequence upto MAF size 30. Throughout instrumentation irrigation with 5.25% NaOCl was performed using 27 guage needle and patency was assured with a10kfile,1 mm beyond apical constriction. Apical foramen were sealed with GIC, root canals and were irrigated with 17% EDTA for 2 minutes followed by tap water.

Prepared teeth specimens were mounted in bijou bottles and assembled specimens were autoclaved (134^oC for 15 min) to obtain sterilized systems. Bijou bottles were then filled under asepsis with sterile BHl broth $37gL^{-1}$ till the roots were surrounded.

A bacterial suspension of Enterococcus faecalis [standardized to $4x10^8$ CFU mL^{-1}) in BHI broth was inoculated into twenty root canals of prepared specimens using sterile syringes and samples were incubated for two days under anaerobic conditions (80% $N_2$10% Co_2+10%H_2) at 37^oC in isolated boxes. Ten remaining samples were not infected.

The specimens were divided into 3 groups [n=10]
Group 1 – Not infected with E.faecalis (negative control)
Group II – Infected with E.faecalis but not irradiated with laser – [positive control]
Group III – Infected with E.feecalis and irradiated with laser – [experimental group]

In the experimental group, laser irradiation without water spray, air cooling or photosensitizing dye was performed in wet canals with Nd : YAG laser.(Wave length – 1064nm, power output 1.5w, energy 100mj, time 150µs, frequency 15Hz. 300µm flexible thin fibre).

During laser treatment fibre tip was applied with a spiral movement starting one mm below the apex and moving coronally, four times for 5s with 20s recovery intervals between each application.

After laser treatment liquid contents of root canals of all groups were absorbed with sterile paper points without intentionally touching the walls. Root canals were filled with reduced transfer fluid (RTF) and gently filed in a circumferential way using sterile 25 K files to working length. Again the contents were absorbed with sterile paper points, transferred to 2 ml of RTF and immediately processed. After vortexing for 30 seconds, the contents of the test tubes were serially diluted in log steps and were applied to Blood agar plates [BA, Haemin (5mg mL^{-1}), menandione (1mgML^{-1}) 5% sterile sheep blood 0.8% bacto agar] and incubated under anaerobic conditions at 37^oC. After 3-7 days, colonies of E.faecalis were counted using microscope at 20x magnification and recorded as number of CFUml^{-1}.

Conventional scanning electron microscopy
Forty dentin discs were used for this study

Preparation of Dentin Disc

Twenty carious free molar teeth were stored in 0.5% chloramine in water at 4^oC. At the time of use teeth were sectioned in occlusal plane near pulp horn using a low speed diamond saw under water cooling. Each crown was To ensure smear layer removal discs were immersed in ultrasonic bath with 5.25 NaOCl for 4 min and later 17% EDTA for 4 min and three washes with saline for a period of 2 min each. Samples were stored in 0.9% saline at 4^oC. Until use. Before inoculation the discs were autoclaved and placed in sterile bijou bottles with flat pulpal oriented side up.

Bijou bottles filled with BHI broth inoculated with E.faecalis suspension. After 2h of incubation (discs were held upside down using sterile forceps to evaluate effect of laser energy through tubular dentine). Specimens were randomly assigned to form groups with 10 specimen each [n=10]
Group 1 – No treatment
Group 11 – One cycle of indirect laser treatment (1.5w, 15Hz, for 5 seconds)
Group 111 – Two cycles with recovery interval of 20s
Group IV – Thee cycles with recovery interval of 20s
During Nd : YAG laser irradiation hand piece was held to form an angle of C10^{o} between the fibre and dentinal surface whilst the tip was slightly touching (contact mode) the moistened (0.9% sterile saline) disc. The movement was in a zigzag manner from one to other corner to imitate the treatment of a canal.

Fixation of Dentin discs : Following laser treatment the discs were fixed in 2.5% gluteraldehyde in 0.1M sodium caccodylate buffer (pH7.4) at 4^{o}C for 12h and prepared for CSEM, in ascending series of low concentration aqueous ethanol, air drying followed with sputter coating with gold. Observations were done with SEM (Philips) with different magnifications at 10KV.

Specimen stub preparation for environmental scanning electron miscroscope

Ten molar teeth stored in 0.5% of chloramines in water at 4^{o}C were selected. Specimen stub was prepared with a flat surface (3mmx6mm) by cutting in a occulusal plane horizontally near the pulpal horns using slow speed diamond saw under water cooling. In this manner one could achieve an image of coronal dentine approximating the morphology of cervical radicular dentine. (Carrigen et al 1984). Whilst flat surface simplifies focusing with ESEM.
To ensure smear layer removal, the specimens were cleaned ultrasonically with 17% EDTA and stored in saline at 4^{o}C.

Before inoculation samples were autoclaved and placed in sterile bijou bottles with flat surface up. Now the bottles were filled with BH1 broth (E.faecalis suspension). After 2 days of incubation the samples and 20µl tests were spread on supplemented BA plates and anaerobically incubated for 7 days to confirm viability of microorganisms in solution at the point of laser treatment. The ESEM samples were directly viewed in wet mode in the ESEM (4^{o}C, 2.9-4.9 torr gas pressure, 80-85% relative humidity, 10 KV). Four to six interesting spots were selected, scanned and saved in stage memory. Next the samples were directly laser treated.

Two cycles of laser treatment (15W, 15Hz short pulsed mode for 5S with a recovery interval of 20s). After Nd : YAG laser exposure, identical spots on the specimen were reanalyzed with ESEM for morphological changes in bacterial configuration.

III. Discussion

For the disease process of primary interest of the endodontist is apical periodontitis. Since bacteria cause apical periodontitis the reduction or elimination of bacteria seems a logical aim for successful endodontic treatment[7]. Of certain microbes, the common recovery of e.faecalis from the root canals of teeth in which previous treatment has failed is noteable. E.faecalis was isolated in 38% of teeth failed[2] in root canal treatment which suggests that it is an important organism in endodontic failure[8].The use of e.faecalis in this study is justified because of its repeated resistance to chemo mechanical procedures, resistant to various irrigants& medicaments [Ca(oH)2, KI, NaOCl, chlorhexidine, saline][9]E.faecalis survives in dentinal tubules despite long periods of $Ca(oH)_2$therapy. E faecalis is chosen as an organism of eradication, because apart from its various resisting abilities to all irrigating agents it occurs primarily in retreatment scenarios, it is often considered in various endodontic studies as it is an early to grow bacterium in laboratory[10].

Moreover E.faecalis is highlighted in this study, regarding mode of in vasion into dentinal tubules and formation of bio films. Virulence of e.faecalis is attributed to its ability to survive as a single organism without support of other organism (fabricius et al 1982).E.faecalis can potentially invade dentinal tubules (hacpasalo&orsterik 1987) and can adapt to altered nutrient supply and able to grow within the tubules. Virulence factors which allow this to occur are adherence to host cell, (kreft et al 1992) expression of proteins to ensure cell survival in altered nutrient supply (giord et al 1996, 1997), ability to compete with bacterial cells, (gallery et al 1991), alter hosts response (miyazaki et al 1993) & environment

Bacterial invasion into dentinal tubules is attributed to cell adhesion to collagen induced morphological growth response[11]. Synergetic effect between serum and binding to immobilized collagen, promotes in failed rc treated teeth. In obturated teeth it is speculated that (siqueira 2001) bacteria "entombed" by filling material usually die, but ability of E.faecalis to survive in "entombed" environment for 90-180 days (neely &maley 2000) is a causative factor for reinfections. E.faecalis has the capacity to survive in various environmental stresses by cell wall alterations and maintained adhesive properties in human cells (purzzoetal 2002) and could provide a long term nidus for frequent infection if opportunity arises.

The antimicrobial efficacy of various disinfecting agentsused in chemomechanical protocol is very much influenced by their ability to penetrate in depths into infected dentinal tubules and eradicate the bacterial colonies and biofilms which has penetrated far into the tubules[12] and other virulence factors which facilitates E.faecalis in reinfection is formation of bio films. A bio film is defined as an aggregation of bacteria associated with a surface embedded in an extra cellular matrix of poly saccharide[13] barrier properties of biofilms is given by four mechanisms[14].

I. Barrier properties of extra cellur polymeric substance (eps) matrix – extracellular enzymes like b. Lactamase become entrapped and concentrated in the matrix and inactivates b .lactam antibiotics.

II. Bacterial cells within biofilm take up antimicrobials very slowly and depletion of nutrients forces bacteria into dormant stage where they are protected from stresses.

III. Biofilm experience metabolic heterogenicity. O_2 can be deleted from thebiofilm surface leaving anaerobic niches. Certain antibiotics like aminoglyco sides are effective in aerobic conditions, so these cells survive these antibiotics.

IV. These biofilms constitute a small percentage of original population and exists as "persisters".

E.faecalis to survive in root canals, it has to adhere to root canal dentin. Type 1 collagen is the main component of dentin organic matrix. The E.faecalis collagen binding protein targets the extracellular matrix proteins of host cells and allows adherence to type 1 collagen[15]. Ace is an adhesion to extracellular matrix proteins. Literature shows evidence that collagen is the attachment site for e.faecalis and ace can mediate this attachment to particulate dentin. The above mentioned complexity of organism to survive in various procedures and antimicrobials has shown that commonly used NaOCl & $Ca(oH)_2$ failed to eradicate E.faecalis[16] . Resistance to $ca(oh)_2$ is attributed to the functioning "proton pump" mechanism of e.faecalis, chlorhexidine, NaOCl, dermecyn were less effective on E.faecalis it survives the most meticulous biomechanical preparation of root canals[17]

Penetration of chemicals used various irrigants into dentinal tubules is questionable.

Moreover it is difficult to to eradicate residual biofilm in lateral canals, grooves, where usual irrigating solutions are unreachable. Thus it becomes mandatory to use innovative control techniques against infected root canal associated biofilms, like lasers which will be a good choice for depth of penetration. Irradiation with lasers reduced significant number of viable cells in biofilms and moreover atrophic changes in bacterial cell wall were seen in SEM. Er YAG lasers was found suitable for for removal of biofilms in retreatment cases[18].

literature reviews shows chlorhexidine liquid more effective antimicrobial action than gel but not eradicated, e.faecalis totally[19]. Newer treatment strategies that are designed to eliminate this microflora includes laser technology, which can penetrate dentinal tubules and destroys them, as they are located in area beyond host defence mechanisms and antimicrobials[20]. Laser treatment on e.faecalis in this study is justified by the fact that lasers are antimicrobials, invade dentinal tubules, act on biofilms (dobron& wilson 192) and reach anatomical complexities of root canal.

Of various lasers – Co_2,Nd: YAG, Er;YAG, Nd:YAG used Nd:YAG laser showed antibacterial effects[21]. The antibacterial effects of Nd:yag laser used in this study is proved in various literatures. Eradication of bacillus stearothermophilus.p.intermediastreptococcoussanguis is done with Nd:YAG lasers[22]. Commercially available Nd: YAG lasers are gaining popularity in endodontia, modern Nd: yag lasers are pulsed ie radiation is produced intermittently through a pulse energy input, each pulse has a very short & high energy peak upto 1000w, whereas average energy out put is low. Unlike CO_2 laser, Nd: YAG laser beam can be delivered through an optical fiber quartz for better accessibility to different complex anatomies in root canals[23]. Quartz optic fibre is used to deliver nd:YAG lasers which could reach canal irregularities, curvatures, fins, anastomoses (michael, hardee, et al).

In this study for bacteriologic testing maxillary incisor teeth were chosen as they have lesser areas inaccessible to sampling (siquire& hoper 1999).

1. Kept at standard pressures of 10 – 10 torr. In this way, if water vapour is the gas be imaged in their "native" state.
2. Insulators need not be coated with a metallic layer as in CSEM before imaging. Because gas is present in the chamber, a mechanism exists to help dissipate the build up of charge injected by the incident electron beam.

ESEM is based on integration of efficient differential pumping with a new design of electron optics and detection systems (l.bergmens, p.lambrechts etal). Enterococusfaecalis covered most of the dentinal surface creating a matrix embedded multileveled structure, that frequently hid the entrance of a dentinal tubule before application of laser. Two cycles and three cycles caused destruction of organism of deep layer and biofilms. As the thickness of biofilm was not constant.

Observation at the diverse locations and on different samples varied. (group i, ii, iii) were used for microbiological analysis. The results clearly reveal that elimination of E.faecalis is 97.3% in group iii after Nd YAG irradiation. The various above mentioned properties of Nd: yag lasers in literatures is attributed to the reduction of E.faecalis in gp iii. In this group Nd yag laser has wave length of (1064nm), power output (1.5w) energy (100mj) for150us and frequency 15 hz was used without water spray or dye. Coronal preflaring with gg,(1-4)irrigtion with NaOCl, EDTA, saline and using 300**u**m thin flexible fibre is a spiral movement has led to complete elimination of e.faecalis which is proved by colony counts. The negative control gp 1 yielded no cultivable cells as the teeth were autoclaved. The teeth those received no laser treatment – gpll showed a median number of cfu ml and some increase in bacterial strains.

The second approach of using dentin discs of 1mm thickness was used to prove the potential of laser energy to act deep into dentinal tubules in which beam was emitted indirectly at a distance of through 1mm dentin disc whilst observing the opposite side of inoculation using csem.in gp i convention scanning electron microscopic view provided evidence of persistence of e.faecalis in dentinal tubules. The structure of e.faecalis a gram positive facultative anaerobe, which occurs as pairs could be observed in detail.

CSEM also provides evidence that E.faecalis had different thresholds of sensitivity to radiation, whilst the extent of damage was inline with the total amount of laser energy. Group ii (one cycle) was well tolerated by e.faecalis,many cells showed shriveled and perforated but most maintained their typical shape and size as in control group. Group iii (two cycles) showed susceptibility to radiation. Cells showed protruberences& fragmentation. Most of the cells were completely disrupted and cell bodies were completely fused together coagulated amorphous masses were seen. In gp iv (after 3 cycles) disintegrated cells, no intact bacteria, altered dentinal surface showed coagulated amorphous masses surrounded by dentin.

The third approach was use of environmental SEM (danilaton 1993). ESEM differs from CSEM in two crucial aspects. Instead of sample under high vaccum in csem it is held under gaseous pressure while imaging is carried out, and electron itself is

1. Kept at standard pressures of 10 – 10 torr. In this way, if water vapour is the gas be imaged in their "native" state.
2. Insulators need not be coated with a metallic layer as in csem before imaging. Because gas is present in the chamber, a mechanism exists to help dissipate the build up of charge injected by the incident electron beam.

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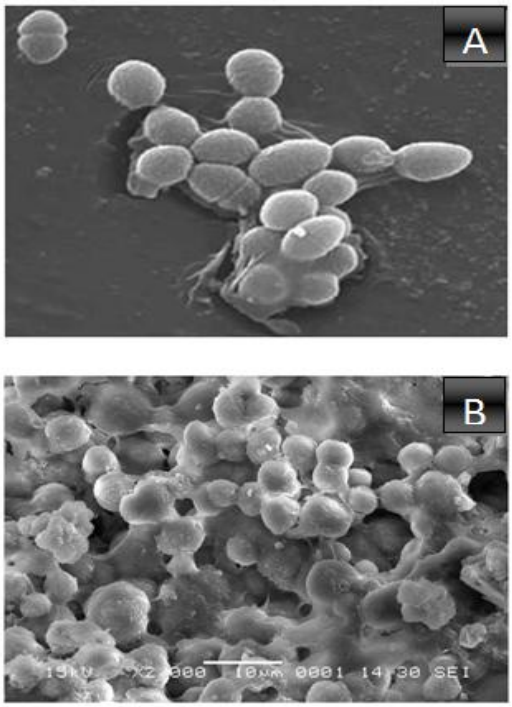


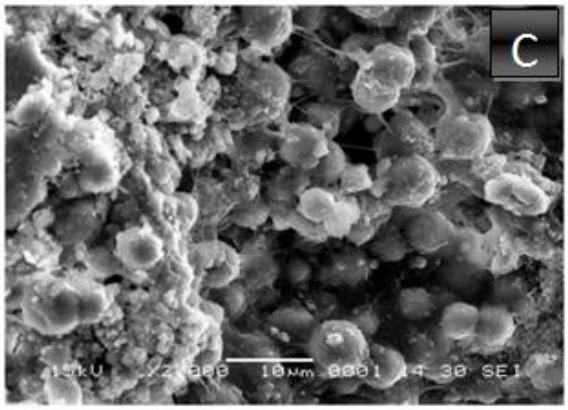


FIG:02

FIG:02 (A) enterococcus faecalis in ovoid pairs group I: (B) e.faecalis invading dentinal tubules, Group II after one cycle of laser irradiation: (C) group III, after two cycles of laser irradiation

FIG:03

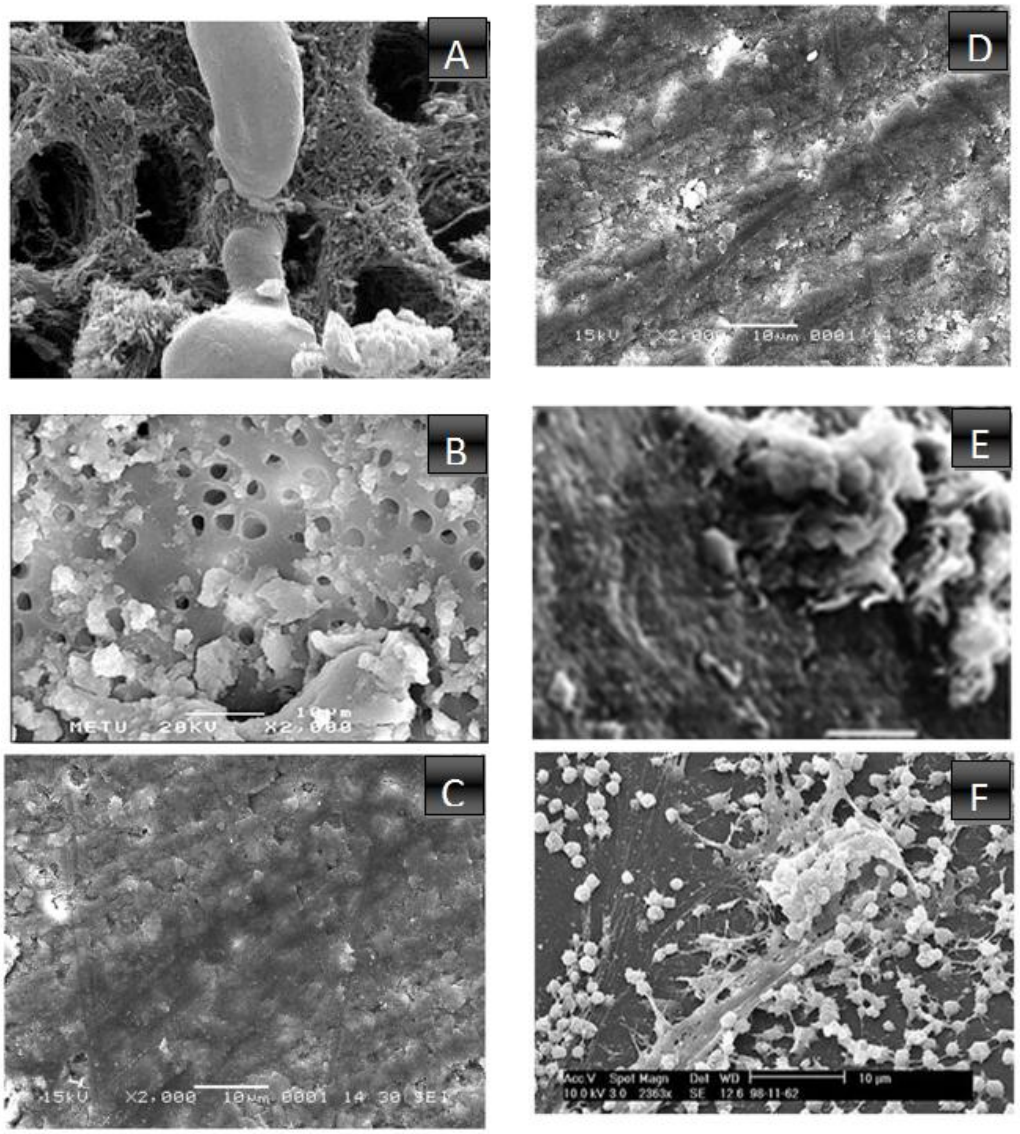


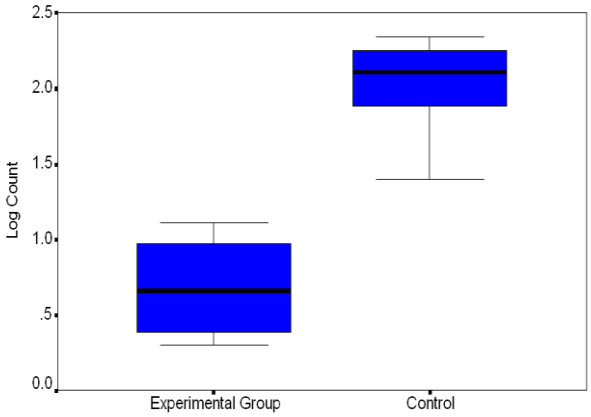
FIG:03 (A)&(B) group iv after three cycles of laser irradiation: (C)&(D) selected spots viewed in ESEM: (E) structural changes in particular spot viewed: (F) A view of bio film

IV. Statistical Analysis

A $\log_{10}$ transformation of each colony forming unit (CFU) count was performed to normalize the data before statistical evaluation. Any 'O' value was replaced by 'I' before log transformation. Paired t test were used to determine whether the difference between the mean $\log_{10}$ values within the the root canal before and after laser irradiation are presented in table 2. All the values obtained were analysed statistically, & a statistically significant difference detected within the groups. (G_1, G_2, G_3)

Wilcoxon signed rank test (non parametric paired t-test) were used to Determine the difference between the mean $\log_{10}$ values between the groups (G_1, G_2, G_3). The $\log_{10}$ value mean and standard derivation (SD) for G_1, G_2, G_3 are presented in table 3 and Box plot representing the number of CFU Ml-1 Of E.faecalis present in the experimental and positive control group after log transformation is represented in fig.1.

Fig – 1: Box plot representing the number of cfu ml-1 of e.faecalis present in the experimental and positive control group after log tranformation.



V. Results

Group I THE NEGATIVE CONTROL
Yielded no cultivable cell .
Group II THE POSITIVE CONTROL
Colony forming units presented in Table IA.
Group III EXPERIMENTAL
Colony forming units before & after laser irradiation is presented in Table IB.

VI. Conclusion

Results of this study indicates that Enterococus faecalis which persists in prepared root canals and retains in dentinal tubules as biofilms can be eradicated using Nd : YAG lasers in one or two cycles.Nd :YAG laser irradiation is not an alternative but a possible supplement to protocols for disinfection when repeated failures are observed in root canal treated teeth and cause of failure is found to be persistent infection of some endodontic pathogens like E.faecalis which resists many antimicrobial treatment.With microbial analysis it is proved eradication of E.faecalis is done with Nd:YAG laser (97.3%) and SEM study (Conventional and Environmental) also proves that Nd : YAG laser has a bactericidal effect on E.faecalis upto 1 mm of thickness of dentin and dentinal tubules.

TABLE II

Groups	Initial (log numbers)	Final (log numbers)	Reduction % (Mean of log nos.)
I	0	0	0
II	2.176	2.082	4.44
III	2.195	0.398	97.3

TABLE III

Groups	Mean	Std.Deviation
I	0	-
II	2.082	1.844
III	0.398	0.636

TABLE IA GROUP II

S. no	Before	After
1	180	179
2	90	77
3	195	173
4	200	187
5	160	140
6	37	25
7	273	18
8	187	118
9	93	87
10	88	11

TABLE IB GROUP III

S. NO.	Before	After
1	95	Nil
2	190	Nil
3	217	Nil
4	273	2
5	187	Nil
6	93	Nil
7	179	7
8	66	3
9	175	Nil
10	93	13

References

[1]. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. *Int. Endod. J.* 2004;37(3):193-8.
[2]. Love RM. Enterococcus faecalis--a mechanism for its role in endodontic failure. *Int. Endod. J.* 2001;34(5):399-405.
[3]. Berkiten M, Berkiten R, Okar I. Comparative evaluation of antibacterial effects of Nd:YAG laser irradiation in root canals and dentinal tubules. *J. Endod.* 2000;26(5):268-70.
[4]. Nagaoka S, Miyazaki Y, Liu HJ, Iwamoto Y, Kitano M, Kawagoe M. Bacterial invasion into dentinal tubules of human vital and nonvital teeth. *J. Endod.* 1995;21(2):70-3.
[5]. Sedgley CM, Lennan SL, Appelbe OK. Survival of Enterococcus faecalis in root canals ex vivo. *Int. Endod. J.* 2005;38(10):735-42.
[6]. Fegan SE, Steiman HR. Comparative evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation: an in vitro study. *J. Endod.* 1995;21(8):415-7.
[7]. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. *J. Endod.* 2000;26(12):751-5.
[8]. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 1998;85(1):86-93.
[9]. Baker NE, Liewehr FR, Buxton TB, Joyce AP. Antibacterial efficacy of calcium hydroxide, iodine potassium iodide, betadine, and betadine scrub with and without surfactant against E faecalis in vitro. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 2004;98(3):359-64.
[10]. Moqbel NM, Ibrahim SM, Eid GE, Ibrahim HK. Effect of intracanal medicament gel materials separate and in combination in the elimination of Enterococcus faecalis biofilm. *Tanta Dent. J.* 2014;11(3):199-205.
[11]. Pathways of the Pulp, 9th edition Cohen S, Hargreaves K. M : p-279,589,9
[12]. Wang Z, Shen Y, Haapasalo M. Effect of smear layer against disinfection protocols on Enterococcus faecalis-infected dentin. *J. Endod.* 2013;39(11):1395-400.
[13]. Seal GJ, Ng Y-L, Spratt D, Bhatti M, Gulabivala K. An in vitro comparison of the bactericidal efficacy of lethal photosensitization or sodium hyphochlorite irrigation on
[14]. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. *J. Endod.* 2006;32(6):527-31.
[15]. Kowalski WJ, Kasper EL, Hatton JF, Murray BE, Nallapareddy SR, Gillespie MJ. Enterococcus faecalis adhesin, Ace, mediates attachment to particulate dentin. *J. Endod.* 2006;32(7):634-7.
[16]. Siqueira JF, de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. *J. Endod.* 1996;22(12):674-6.
[17]. Noiri Y, Katsumoto T, Azakami H, Ebisu S. Effects of Er:YAG laser irradiation on biofilm-forming bacteria associated with endodontic pathogens in vitro. *J. Endod.* 2008;34(7):826-9.
[18]. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. *Int. Endod. J.* 2001;34(6):424-8.
[19]. Oguntebi BR. Dentine tubule infection and endodontic therapy implications. *Int. Endod. J.* 1994;27(4):218-22.
[20]. Fegan SE, Steiman HR. Comparative evaluation of the antibacterial effects of intracanal Nd:YAG laser irradiation: an in vitro study. *J. Endod.* 1995;21(8):415-7.
[21]. Berkiten M, Berkiten R, Okar I. Comparative evaluation of antibacterial effects of Nd:YAG laser irradiation in root canals and dentinal tubules. *J. Endod.* 2000;26(5):268-70.
[22]. *Ingle's Endodontics*. 5th ed.; :P-764.

Amudhalakshmi. "Bactericidal effect of nd: yag laser irradiation on enterococcus faecalis – ex vivo study." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), Vol. 18, no. 02, 2019, pp. 18-26.