REVIEW OF LITERATURE

3.1 – Endodontic Biofilm

Siqueira et al (47) have discussed an overview of biofilm’s lifestyle. They have emphasized on the relationship between biofilm and apical periodontitis, the theory regarding dynamics of endodontic biofilm formation and microbial diversity in endodontic biofilms. Bacterial biofilms are very prevalent in the apical part of the root canals of teeth with primary and post-treatment apical periodontitis. Existing evidence has proven that apical periodontitis is caused by biofilms due to arrangement of the bacterial community within the root canal.

Duggan JM, and Sedgley CM (48) have defined biofilm as ‘sessile’ microbial communities composed of cells irreversibly attached to a substratum and interface or to each other. Ultrastructurally, biofilms form distinct tower or mushroom shaped ultra structures, through which fluid movement takes place by convection through interspersed channels that are distinctly separate from the external environment. They have tested the hypothesis that the source of E. faecalis strains determines the ability to form biofilms. E. faecalis strains recovered from root canals, the oral cavity and non-oral/non-endodontic sources were studied and several virulence factors were determined. They have proved that there were no significant associations between biofilm formation
Dufour et al (49) have discussed the fundamental biology of microbial biofilm and how biofilms impact the pathogenesis of human infections. The review details on the various mechanisms involved in reduced antimicrobial susceptibility of microorganisms present within pathogenic biofilms. Possible approaches in eradication of biofilms by adopting new anti-biofilm strategies have also been presented. Microbiologists have traditionally focused on free floating bacteria growing in laboratory conditions; yet they have recently come to realize that in the natural world, more than 99% of all bacteria live in biofilm communities. Antibiotics have not been specifically developed to target microbial biofilm infections. Despite extensive efforts, no antimicrobial drug has been found that completely eradicates adherent microbial populations. New information is required on physical factors that affect biofilm formation within the body and the genetic basis of how pathogenic microorganisms persist in biofilms. Further, research is also required to relate this to tolerance mechanisms, which in turn is essential in the development of novel antibiofilm strategies.

Nair et al (5) have studied 6 cases that had undergone root canal treatment or re-treatment but demonstrated persistent periapical radiolucent lesions. These 6 teeth with nonresolving
periapical radiolucencies underwent periapical surgery. Biopsies were attained and compared with light and transmission electron microscopy for general features and microbial findings. The results were as follows: periapical lesions with persisting infection in the apical root canal system (2 cases); a cyst (1 case); and periapical healing by scar tissue formation (2 cases). The result confirms that the factors in the failure of endodontic treatment include persistent intraradicular infection and periapical cysts. In addition, unresolved periapical radiolucencies might occasionally be due to healing by scar tissue, which might be mistaken as a sign of failed endodontic treatment.

Vieira et al (50) have shown the relationship between recurrent post-treatment apical periodontitis and late failure after endodontic retreatment performed in a single visit. The most common reason for the persistent infection would be due to the presence of microorganisms located within the dentinal tubules. Bacteria causing persistent infections are usually located in areas unaffected by instruments and antimicrobial substances, including lateral canals, apical ramifications and isthmuses. In addition, bacteria may remain even in the main canal, especially on dentinal canal walls that remained untouched by instruments. Bacterial invasion of dentinal tubules has also been regarded as a potential source of persistent infection.
Sundqvist G (10)- The root canal represents a special environment containing restricted group of oral flora, which is established due to the selective pressures that exist within the canal. Population shifts occur over time with the ultimate domination of obligate anaerobes. Bacterial interrelationships and the nutritional supply are key factors in determining the outcome of the infection. Endodontic treatment not only eliminates bacteria directly but also completely disrupts the delicate ecology and deprives persisting bacteria of their nutritional source.

Sundqvist et al (8) have identified the microbial flora present in endodontically failed cases. They have also established the outcome of re-treatment procedures. The microbial flora was found to be composed of single species of predominantly, gram-positive organisms. The isolates most commonly recovered were bacteria of the species *E. faecalis*. They have concluded that the overall success rate of re-treatment was 74%. They have also stated that microbial flora in endodontically failed teeth was completely different from the untreated teeth. Infection at the time of obturation and size of the periapical lesion were factors that influenced a negative prognosis. Three of four endodontic failures were successfully managed by re-treatment procedure.

Siqueira JF (20) has highlighted the aetiology for endodontic failure, particularly in cases of well-treated root canals. Indications
for treatment of endodontic failures have also been discussed in his article. Failure in root canal treatment usually occurs when treatment falls short of acceptable standards. The reason for many teeth that do not respond to root canal treatment is procedural errors that prevent the control and prevention of intracanal endodontic infection. Microorganisms colonizing the root canal system play an essential role in the pathogenesis of periradicular lesions. The author has suggested that nonmicrobial factors may be implicated in endodontic treatment failure with suggestions that persistent intraradicular or secondary infections, and in some cases extraradicular infections, are the major causes of failure of both poorly treated and well-treated root canals.

Sedgley et al (51) have compared the culture & real-time quantitative polymerase chain reaction reports (qPCR) to detect and quantify the presence of *E. faecalis* in the root canals. *E. faecalis* was detected in 10.2% and 79.5% of samples by culture and qPCR, respectively. This study highlighted the importance of qPCR than culture method. *E. faecalis* was detected in more retreatment than primary infection samples. QPCR reported significantly higher prevalence of *E. faecalis* in endodontic samples than cultures.

Love RM and Jenkinson HF (14) have suggested that the bacterial invasion of dentinal tubules commonly occurs when dentin is exposed following a breach in the integrity of the overlying
enamel or cementum. Normally, the dental pulp is sterile and is primarily involved in the production of dentin. The overlying enamel and cementum protects the pulp dentin complex from exogenous substances. The knowledge of the mechanisms involved in dentinal tubule invasion by bacteria should allow development of new control strategies. Some of these could be in the form of inhibitory compounds incorporated into oral health care products or dental materials, which would assist in the practice of endodontics.

Sundqvist G & Figdor D (52) have described in their review, the type of microbial flora present in the untreated and root-filled canals with persistent infection. The major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth. Relationship exists between the size of the periapical lesion and the number of bacterial cells and species present in the root canal. More bacteria have been identified in the samples from teeth with large, long standing lesions. Ecological and environmental factors have been considered as the major reasons for differences in the microbial flora in these distinct habitats.

Hancock HH et al (9) have evaluated the microbial flora present in teeth after failure of root canal treatment in a North American population. These results were compared with those of previous studies in a Scandinavian population. The microbial flora was mainly composed of 1 to 2 strains of predominantly gram-
positive organisms. *E. faecalis* was the most commonly identified bacterial species. Bacteria were cultivated in 34 of the 54 teeth examined in the study. *E. faecalis* was identified in 30% of the teeth with a positive culture. This study proved the high prevalence of *E. faecalis* in failed root canals.

Ricucci D and Siqueira JF (53) have studied the occurrence of bacterial biofilms in untreated and treated root canals of teeth, showing evidence of apical periodontitis. They associated biofilms with clinical conditions, radiographic size and the histopathologic type of apical periodontitis. They have proven that there is a high prevalence of bacterial biofilms in the apical portion of root canals of both untreated and treated teeth with apical periodontitis. The bacterial community within the canal adhered to or was at least associated with the dentinal walls. The cells were encased in an extracellular amorphous matrix and often surrounded by inflammatory cells. They have suggested that this bacterial arrangement is consistent with acceptable criteria to include apical periodontitis in the set of biofilm-induced disease. The morphologic structure of biofilm varied from case to case. No unique pattern of biofilms for endodontic infections was determined. This study proved the presence of biofilms in association with longstanding pathologic processes, including large lesions and cysts.
Stuart et al (32) have detected the presence of *E. faecalis* in asymptomatic, persistent endodontic infections. Its prevalence in endodontic infections ranges from 24% to 77%. This might be due to various survival and virulence factors possessed by *E. faecalis*, including its ability to compete with other microorganisms, invade dentinal tubules and resist nutritional deprivation. There are several survival and virulence factors of *E. faecalis* with the most common ones being: (a) Endures prolonged periods of nutritional deprivation, (b) binds to dentin and proficiently invades dentinal tubules, (c) alters host responses, (d) Suppresses the action of lymphocytes, (e) possesses lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid, (f) utilizes serum as a nutritional source, (g) resists intracanal medicaments (i.e. Ca(OH)2), (h) maintains pH homeostasis, properties of dentin reduces the effect of calcium hydroxide, (i) competes with other cells and forms a biofilm. They have proved that the prevalence of *E. faecalis* is low in primary endodontic infections and high in persistent infections. *E. faecalis* is also more commonly associated with asymptomatic cases than symptomatic ones. In the above mentioned articles, the authors have proved that *E. faecalis* was the most common organism. Hence, we selected *E. faecalis* as test organisms in our study.
3.2 - Importance of disinfecting protocols

Happasalo M and Shen YA (54) have emphasized the importance and reasons for proper root canal preparation: (i) Enlarge the canals to an adequate geometry; (ii) clean the root canal system by increasing the access for regular irrigants like NaOCl, EDTA and CHX followed by placing intracanal medicament and (iii) make it possible to place a high-quality root filling material without presence of bacteria. They have proved the effect of various irrigating solutions on endodontic biofilms. The major challenges for root canal instrumentation come in the form of complex anatomy like isthmuses and C shaped canals. In addition, the complex chemical environment of the root canal prevents antimicrobial irrigating solutions and intra canal medicaments from exerting their full potential against the microorganisms found in endodontic infections. The success of root canal treatment is mainly based on complete eradication of micro organisms by instrumentation and disinfection protocols followed during the procedure.

Peters et al (38) have compared the effects of four preparation techniques on canal volume and surface area using three-dimensionally reconstructed root canals. In addition, micro CT data was used to describe morphometric parameters related to the four preparation techniques. Instrumentation of canals increased volume and surface area. Prepared canals were significantly more
rounded, had greater diameters and were straighter than unprepared canals. However, there were few differences between the four canal instrumentation techniques used. More than 35% of the canals’ surface area was unchanged, irrespective of the instrumentation technique used. Hence, it could be understood that shaping is not enough to completely eradicate the bacteria; cleaning plays a key role in the success of endodontic treatment.

3.3 Various Strategies to eradicate Endodontic Biofilms

Anil Kishen (25) has explained the various challenges in root canal disinfection in his research paper. The article also explains the advanced therapeutic strategies against endodontic biofilm. The strategies outlined were antibacterial nanoparticles, antimicrobial photodynamic therapy, laser-assisted root canal disinfection, ozone and herbal/enzyme alternatives. The resistance mechanisms in a bacterial biofilm to antimicrobial agents may generally include the following: (i) resistance associated with the extracellular polymeric matrix; (ii) resistance associated with growth rate and nutrient availability; or (iii) resistance associated with the adoption of a resistance phenotype. Endodontic disease is a biofilm-mediated infection. The current focus is on elimination of bacterial biofilm from the root canal system. It remains the primary focus in the management of endodontic disease. But, the root canal environment is a challenging locale for eliminating biofilm. Hence, various antimicrobials agents from irrigants (NaOCl, CHX),
intracanal medicaments, nanoparticles, lasers, advanced non invasive methods and photoactivated disinfection are used to eradicate the biofilm from infected root canal systems.

Saber SM and El-Hady SA (55) aimed to develop a mature *E. faecalis* biofilm inside the root canals and to test its susceptibility to some antimicrobial medicaments. In their study, Scanning electron Microscope examination confirmed the formation of a mature biofilm at the end of the incubation period. All the chemotherapeutic agents used were significantly better than calcium hydroxide in eliminating the biofilm. They proved that, the protocol followed (development of biofilm and maturation) by them is reliable and can be used to assess the efficacy of antibacterial agents.

Ozdemir HA et al (35) have suggested that as age increases, the alterations in dentin tissue might cause different adhesion capability of bacteria to dentin, yielding differences in clinical approaches regarding root canal irrigation. This study aimed to evaluate the effects of ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite on *E. faecalis* biofilm growth in root canal dentin of young and old individuals. Dentinal tubules become obliterated, resulting in narrowing of the tubules to approximately, 2.5 µm diameter near the pulp and 0.9 µm in diameter near the enamel. Thus, a tubule is normally larger in diameter than the
average *E. faecalis* cell diameter of approximately 0.8–1 µm. They proved that root canals from elderly population are more susceptible to canal infection. However, combined application of EDTA and NaOCl significantly reduces the amount of intracanal biofilm.

Evans et al (29) have explained the mechanisms that enable *E. faecalis* to survive against the high pH of calcium hydroxide. *E. faecalis* was resistant to calcium hydroxide at a pH of 11.1, but not at pH 11.5. Pre-treatment with calcium hydroxide at pH of 10.3 induced no tolerance to further exposure at a higher pH of 11.5. Cell survival showed no difference when protein synthesis was blocked during stress induction. However, addition of a proton pump inhibitor resulted in a dramatic reduction of cell viability of *E. faecalis* in calcium hydroxide. They concluded that survival of *E. faecalis* against calcium hydroxide appears to be unrelated to stress induced protein synthesis. However, a functioning proton pump is critical for survival of *E. faecalis* at high pH.

Estrela et al (56) determined the antibacterial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine against *E. faecalis*. The outcome of root canal treatment in the presence of apical periodontitis is directly influenced by the use of acceptable clinical procedures under strictly aseptic conditions, in addition to the immunological response of the host. They concluded that *E. faecalis* biofilm was
not completely eradicated by irrigation of root canals with ozonated water, 2.5% sodium hypochlorite, 2% chlorhexidine and the application of gaseous ozone for 20 min.

Hems et al (57) have studied the ability of ozone (O$_3$) as an antimicrobial agent against *E. faecalis*. O$_3$ is a powerful oxidizing agent. Ozone is a blue gas containing three oxygen atoms. It is an irritant, toxic and unstable and it is very reactive. It has also been tested in medicine to decontaminate hospital side rooms, rooms contaminated with methicillin resistant Staphylococcus aureus and in auto-haemotherapy. O$_3$ had an antibacterial effect on planktonic *E. faecalis*. The authors have also proved that gaseous ozone had no effect on the *E. faecalis* biofilm.

Zehnder et al (58) studied the role of bioactive glass S53P4 and calcium hydroxide when used as an intracanal medicament against *E. faecalis*. Ca(OH)$_2$ in suspension generates a high pH which is responsible for its antimicrobial action. Ca(OH)$_2$ is poorly soluble in water and an effective disinfectant in human teeth. The bioactive glass material appears to be an inferior root canal antiseptic in comparison with calcium hydroxide.

Mohammadi and Abbot (59) have discussed the structure and mechanism of action of CHX, its antibacterial and antifungal activity, its effect on endodontic biofilm, its substantivity, its
interaction with Ca(OH)$_2$ and other irrigants, its anticollagenolytic activity, its effect on coronal and apical leakage of bacteria, its toxicity and allergenicity and the modulating effect of dentine and root canal components on its antimicrobial activity. The elimination of microorganisms from infected root canal systems is a complicated task involving the use of various instrumentation techniques, irrigation regimens and intracanal medicaments. Due to complex anatomy of the root canal, it is not possible to achieve a bacteria-free root canal system with just mechanical instrumentation. Concentrations of 1 to 2% chlorhexidine combined with Ca(OH)$_2$ have also demonstrated efficacy in killing *E. faecalis*.

George S and Kishen A (60) have aimed to evaluate the cytotoxicity and selectivity of an advanced, noninvasive, light-activated disinfection (ANILAD). Simultaneous evaluation was also conducted to study the efficacy of light activated therapy (LAT) against *E. faecalis*. An irradiation dose producing 97.7% bacterial killing showed only 30% fibroblast dysfunction. This study indicated that ANILAD produced an insignificant effect on mammalian cells. In principle, LAT involves the killing of microorganisms, when a photosensitizer selectively accumulated in microbial cells is activated by a specific wavelength of light to produce oxygen-based free radicals. This technique could achieve disinfection without significant root canal enlargement.
Cwikla et al (61) have evaluated the antibacterial efficacy of three Ca(OH)$_2$ formulations against *E. faecalis* in infected tooth. The three formulations were Ca(OH)$_2$ mixed with water, Ca(OH)$_2$ mixed with iodine-potassium iodide and Ca(OH)$_2$ mixed with iodoform and silicone oil. Metapex was the most effective dentinal tubule disinfectant and the results were statistically significant.

Siqueira JF Jr and Lopes HP (62) have reported that microorganisms that persist after endodontic treatment would have entered the root canal during treatment, survived disinfection procedures and persisted after root canal obturation. Mainly, Ca(OH)$_2$ can be used as an intracanal medicament, to reduce the bacteria inside the root canals. However, Ca(OH)$_2$ is not effective against all bacterial species found in root canal infections. Hermann used Ca(OH)$_2$ in the field of endodontics in 1920. His review article consists of mechanisms of antimicrobial activity and how this material helps in disinfection of root canals. It also explains how the vehicle influences its efficacy in reducing the bacteria inside the root canal system.

Kandaswamy et al (63) investigated the antimicrobial activity of 2% chlorhexidine gel, propolis, Morinda citrifolia juice (MCJ), 2% povidone Iodine (POV-I), and Ca(OH)$_2$ against *E. faecalis* at two different depths (200 µm and 400 µm) and at three time
intervals (days 1, 3 & 5). The number of colony-forming units was statistically significant in all groups compared to the control group (Saline). Group V (chlorhexidine gluconate) (100%) produced better antimicrobial efficacy followed by 2% POV-I (87%), propolis (71%), MCJ (69%), and calcium hydroxide (55%). There was no significant difference between propolis and MCJ at two different depths. It was also proved that propolis and MCJ were effective against *E. faecalis*.

Zapata RO et al (64) have used confocal laser scanning microscopy to observe the percentage of remaining live bacteria after treating an infected dentin biofilm model with calcium hydroxide, 2% chlorhexidine gel and triantibiotic paste (ie, metronidazole, minocycline and ciprofloxacin). They have mentioned that Ca(OH)$_2$ cannot be used for revascularization procedure. Disinfection of immature teeth can be considered as a challenge that needs specific disinfection procedures when compared with conventional endodontic treatment. They have concluded that triple antibiotic paste reduces the live bacteria in a better manner compared to CHX and Ca(OH)$_2$.

Vagehla et al (65) have evaluated the efficacy of Ca(OH)$_2$ combined with propylene glycol and iodoform in silicone oil compared to 2 % chlorhexidine gel. Chlorhexidine gel (2 %) was found to be effective against both *E. faecalis* and *C. albicans*. 
The vehicle used in the study (propylene glycol and methylcellulose) altered the ability of Ca(OH)$_2$ as an intracanal medicament.

3.4 - Nanotechnology in Endodontics

Kishen et al (66) aimed to study the role of cationic nanoparticulates for root canal disinfection. Antibacterial particulates prepared by nanotechnology were shown to have higher antibacterial efficacy than antibacterial powders. Nanoparticles are microscopic particles that have at least one dimension less than 100 nm. The higher surface area and charge of nanoparticulates help to attain a greater degree of interaction with the negatively charged bacterial cell surfaces. Chitosan (CS) is a nontoxic biopolymer derived by the deacetylation of chitin. Chitin is a natural polymer occurring in the exoskeleton of the crustaceans. It is a bioadhesive, which means it readily binds to negatively charged surfaces and has excellent antimicrobial and antifungal activities. The CS exerts antibacterial properties because of the zeta potential or surface charge of nanoparticles that greatly influence its stability in suspension through the electrostatic repulsion between particles. The interaction between positively charged CS and negatively charged bacterial cell leads to leakage of intracellular components, which ultimately leads to cell lysis. This study focuses the advantage of nanoparticles in root canal disinfection, which helps in reducing the persistent root canal infection.
Shrestha et al (67) have investigated the role of High-intensity focused ultrasound (HIFU) collapsing cavitation bubbles to deliver antibacterial nanoparticles into dentinal tubules to improve root canal disinfection. The concept is applied clinically to create collapsing cavitation bubbles in fluids and tissues, which collapses with high-speed and can be used for drug delivery. Hence, by using this principle, nanoparticles can be pushed into the dentinal tubules to a greater depth. Treatment using HIFU resulted in significant penetration up to 1,000 µm of nanoparticles into the dentinal tubules. Hence they proved that the cavitation bubbles produced using HIFU can be used as a potential method to push the nanoparticles deeper into the dentinal tubules to improve the root canal disinfection. Hence, it can eradicate the bacteria present in deeper part of dentinal tubules.

Pagonis TC et al (68) have evaluated the in vitro effects of poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with the photosensitizer methylene blue (MB) and light against E. faecalis. Methylene blue is generally used as photosensitizer in photodynamic therapy. Transmission electron microscopy was used to study the distribution of nanoparticles in E. faecalis in suspension after incubation with PLGA complexed with colloidal gold particles. They concluded that PLGA nanoparticles encapsulated with photoactive drugs may be used in endodontic treatment.
Upadya et al (69) evaluated the role of efflux pumps in altering the susceptibility of E. faecalis biofilms to Ca(OH)\textsubscript{2}, chitosan nanoparticles and light activated disinfection. Antibacterial resistance can follow many mechanisms, with efflux pumps being a prominent contributor. The 4 day old E. faecalis have been treated by these medicaments. They have concluded that E. faecalis were more susceptible to killing by LAD, when compared with chitosan nanoparticle and calcium hydroxide. Chitosan nanoparticle required longer interaction time to eradicate biofilm compared to Ca(OH)\textsubscript{2}.

Wu et al (70) evaluated the antibacterial efficacy of AgNps. The biofilms were irrigated with 0.1\% AgNP solution, 2\% sodium hypochlorite and sterile saline for 2 minutes. The biofilms were treated with AgNP gel (0.02\% and 0.01\%) and Ca(OH)\textsubscript{2} for 7 days to test the efficacy as a medicament. Scanning electron microscopy was used to observe one half of the samples from each group. The other half of the specimens was assessed with CLSM for the structure and distribution of viable bacteria. Syringe irrigation with AgNP solution did not disrupt the biofilm structure and the proportion of viable bacteria in the biofilm structures was not different from that of the saline. 0.02\% AgNP gel, as a medicament significantly disrupted the structural integrity of the biofilm and resulted in the least number of post-treatment residual viable E. faecalis cells compared with 0.01\% AgNP gel and calcium hydroxide groups. From the results of their study, they have
concluded that the efficacy of AgNPs depends on the mode of application. AgNp gel performed better compared AgNp liquid in the eradication of the *E. faecalis* biofilm.

### 3.5 - Potential use of AgNps

Sondi I and Salopek-Sondi B (71) have studied the antimicrobial activity of AgNps against bacteria. The synthesis of nanoparticles with specific shape, size, chemical and physical properties have been of great interest. The application of nanotechnology in the field of medicine and engineering has been increasing rapidly with the release of newer materials in the nanoscale level. The silver ions and silver-based compounds show strong bactecidal effects on various microorganisms. Hence, silver ions; have been used in the composites and ion exchange fibers and in coatings of medical devices. Antibacterial mechanisms might be due to the following reasons: i) DNA loses its replication ability and cellular proteins become inactivated on silver ion treatment; ii) Ag+ has the ability to bind the functional groups of proteins, which in turn leads to protein denaturation. Few authors have shown that electrostatic attraction exists between negatively charged bacterial cell wall membrane and positively charged nanoparticles However, silver particles used in this study were negatively charged. The metal damages the cell wall membrane, and creates pits in membrane. Hence, progressive release of LPS molecules and
membrane proteins could account for the bactericidal action of these negatively charged nanoparticles.

Lara HH et al (72) have highlighted the role of silver. However, in the case of AgNps, the currently available data only superficially reveals the potential benefits and the wide range of applications. Currently, these nanoparticles help in prevention of infection and antiviral therapies. Recently, it has been suggested that AgNPs bind with external membrane of lipid enveloped virus to prevent the infection. The antimicrobial activity of AgNPs on Gram-negative bacteria depends on the concentration of AgNPs and is closely associated with the formation of pits in the cell wall of bacteria. The metal depletion may cause the formation of pits in the outer membrane and change membrane permeability. Another mechanism of bactericidal action of AgNps is based on the inhibition of cell wall synthesis, protein synthesis mediated by the 30s ribosomal subunit and nucleic acid synthesis.

Rai et al 46) have shown that the size of the nanoparticle plays a key role in antibacterial efficacy. The nanoparticles possessing a larger surface area will have a higher percentage of interaction than bigger particles. The reactivity of nanoparticles will be enhanced if the size is smaller than 10 nm which produce electronic effects. Hence, antibacterial efficacy is based on the size of particles. The AgNps shows the antimicrobial activity compared
to other salts due to their extremely large surface area, which provides better contact with microorganisms. The nanoparticles penetrate inside the bacteria and binds to the cell membrane. When AgNps enter the bacterial cell wall, it forms a low molecular weight region in the centre of the bacteria to which the bacteria conglomerates, thereby protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain and cell division finally leading to cell death.

Durán et al (73) reviewed the potential use of AgNps to control pathogens with emphasis on their action against bacteria, their toxicity and possible antibacterial mechanisms of action. Various types of nano materials like magnesium, gold, zinc and titanium have been shown to possess antibacterial efficacy. AgNps have shown better antimicrobial efficacy against viruses, bacteria and other microorganisms.

Prasad et al (74) have evaluated the antimicrobial activity of AgNps synthesized from Psidium guajava (P. guajava) against human pathogens. UV Vis and TEM were used for the formation and stability of AgNps. Agar well diffusion assay was used to study the antimicrobial activities of the AgNps. UV Vis showed strong absorbance peak at 410 nm which proved the formation of AgNps. TEM showed the formation of AgNps with an average size of 59 nm.
The formed AgNps showed good antimicrobial activity against E. coli, Bacillus cereus and Candida tropicalis.

Raffi et al (75) evaluated the antibacterial activity of AgNps against gram-negative bacterium E. coli, as a function of particles concentration. The experiment was carried out in liquid as well as solid growth media. SEM and TEM studies proved that AgNps have adhered to and entered into the bacterial cell membrane of the E. coli. Antibacterial properties of AgNps are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity.

Lotfi et al (76) have compared the antibacterial efficacy of nanosilver, chlorhexidine gluconate and NaOCl against E. faecalis. The agar diffusion and serial dilution methods were used in this study. In agar diffusion test, the effectiveness of antibacterial material against bacteria is measured in a grown culture. MIC method uses serial dilutions of a solution to determine the lowest concentration of medicaments that would still show antibacterial properties. E. faecalis was selected because this bacterium is the most commonly isolated organism in endodontically treated cases with apical periodontitis. They proved that 2% CHX showed larger zones compared to nanosilver and NaOCl. It was also observed that 0.0005% concentration of NaOCl could eliminate bacteria in 3 min.
They concluded that nanosilver could be safely used as an intracanal medicament in the concentration of 0.0005%.

Sotiriou et al (77) investigated the antibacterial activity of nanosilver against Gram negative bacteria. They closely controlled Ag content and size of the nanoparticles used. The antibacterial activity of nanosilver was dominated by Ag+ ions when fine Ag nanoparticles (less than about 10 nm in average diameter) were employed. It was observed that smaller nanoparticles released high concentrations of Ag+ ions. In contrast, with relatively larger Ag nanoparticles, the concentration of the released Ag+ ions was lower.

3.6 – Thermosensitive agent

Wannachaiyasit and Phaechamud (78) used lutrol as a thickening agent with chlorhexidine to produce a thermosensitive gel based mouth antiseptic. Due to its thermo sensitive properties, lutrol has sol-gel transition ability. It changes from solution stage at low temperature to the viscous gel at high temperature. The chlorhexidine thermosensitive gel showed antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*. Lutrol is a useful polymeric carrier for many agents and can be used as a thickening agent. Hence, in the present study, lutrol was used as a thickening agent with AgNps.
3.7 – Role of Passive Ultrasonic irrigation

Bhuva et al (79) have compared the efficacy of passive ultrasonic irrigation with 1% NaOCl, with that of conventional syringe irrigation with 1% NaOCl on E faecalis. Passive ultrasonic irrigation has recently led to a renaissance in the use of ultrasonics during root canal treatment. Following root canal preparation, the technique utilizes an ultrasonically activated file or smooth wire within the root canal space. The term ‘passive’ means where the file is used in non cutting manner. This technique allows the ultrasonic instrument to operate in the most effective way.

Harrison et al (80) have studied the ability of an ultrasonically activated irrigating system to eliminate bacteria from the root canal wall and dentinal tubules. Following canal preparation, passive ultrasonic irrigation enables the ultrasonic tip to oscillate freely in the root canal. Various studies have shown that inserting an ultrasonic tip into root canal filled with irrigant performed better in canal cleanliness and reduction in bacterial load. Reason might be due to acoustic streaming that occurs and creates shear forces that can cause physical disruption of bacterial aggregations, such as biofilm.

Huffake et al (81) have investigated the ability of a new passive sonic irrigation system (EndoActivator) to eliminate cultivable bacteria from root canals in vivo and compared it with
that of standard syringe irrigation. They placed an intervisit calcium hydroxide after the first session of treatment. Sampling results from the first session of treatment were then compared with results obtained after second session of treatment. They showed that there was no significant difference in the ability of sonic group to eradicate the bacteria from canals as compared to control group. The study results concluded a second session and intervisit calcium hydroxide disinfection were able to eliminate cultivable bacteria from significantly more teeth than a single visit of treatment.

Nóbrega et al (82) have evaluated both smear layer removal and reduction of *E. faecalis* after instrumentation with ultrasonic irrigation. They concluded that the level of disinfection and cleanliness of root canals achieved with ultrasonic irrigation is comparable to that obtained by conventional methods. Removal of smear layer and antibacterial efficacy is improved when root canal irrigants are used with ultrasonic agitation. Ultrasonic irrigation should be carried out passively without the file touching the root canal walls. Free-oscillating files are preferably used because they promote adequate agitation of the irrigation solution.

3.8 - Methodology Aspects

Zapata et al (83) have showed the ability of confocal laser scanning microscope (CLSM) to identify the live and dead *E. faecalis* in dentin. Micro organisms might penetrate into the
dentin due to caries or fracture. This leads to inflammation, necrosis of the dental pulp, root canal infection, apical periodontitis and abscess. Procedures such as histologic sections, SEM, TEM and microbiological analyses like PCR and Real Time PCR at different levels of the root canal can be routinely done. The bacteria’s ability to penetrate into the dentin can be expressed as number of colony-forming units (CFUs) through microbiological analyses, the number of tubules infected in the histologic sample or the presence of bacteria in the root canal walls. The authors proved that the viability of bacteria in infected dentin can be determined by using CLSM. FDA/PI dyes and acridine orange dyes are used for this technique. Hence, CLSM can be used as an accurate methodology to evaluate the antibacterial efficacy of various disinfectants by giving a ratio of live and dead bacteria.

Wang et al (84) investigated the antibacterial effects of different disinfecting agents like NaOCl, CHX and Q Mix on both young and old *E. faecalis* biofilms in dentin by using CLSM. The bacteria were inoculated for 1 day and 3 weeks. The proportions of dead and live bacteria inside the dentinal tubules after exposure to these disinfectants were assessed by CLSM using a LIVE/DEAD bacterial viability stain. Significantly fewer bacteria were killed in the 3-week-old dentin biofilm than in the 1-day-old biofilm. Hence, in the present study study, *E. faecalis* was inoculated for 3 weeks (21 days).
Ma et al (85) designed a study to develop a standardized protocol for quantification of the ability of dentin disinfection by different disinfectants. They centrifuged semicylindrical dentin samples infected with *E. faecalis* bacterial suspension. CLSM and viability staining were used to quantify the live bacteria. SEM was used to confirm the presence of bacteria in dentin. The new model made it possible to compare the effectiveness of various disinfectants by using CLSM. Centrifugation helped to create a heavy, evenly distributed infection deep into the dentinal tubules, which was confirmed by SEM.

Wang et al (86) have used a novel dentin infection protocol. They have tested regular disinfecting solution after adding detergents to them and evaluated their antibacterial efficiency by using CLSM. The addition of detergents in the disinfecting solutions increased their antibacterial effects against *E. faecalis* in the dentinal tubules. The authors proved that CLSM is an efficient method to differentiate live and dead *E. faecalis*.

George S et al (36) highlighted the effect of different growth conditions on the characteristics of *E. faecalis* biofilm formation on root canal and depth of penetration of *E. faecalis* into dentinal tubules. Samples were experimented under nutrient-rich, nutrient-deprived, aerobic, and anaerobic conditions for a period of 21 days. SEM with Energy Dispersive X-ray microanalysis, CLSM and Light
microscopic examinations were done. They concluded that the depth of penetration of *E. faecalis* was higher in nutrient-rich conditions.

Haapasalo M and Orstavik D (13) used an in vitro model for dentinal tubule infection of root canals. Freshly extracted bovine incisors were taken and cylindrical dentin specimens were prepared with dimensions of about 4 mm height, 6 mm diameter and 2.3 mm width. The cementum was completely removed from all dentin blocks. Teeth were irrigated with 17% EDTA for 4 minutes to open dentinal tubules followed by 5.25% NaOCl, before being infected with *E. faecalis*. Bacteria rapidly invaded the tubules. After three weeks of incubation, a heavy infection was found at 400 µm from the canal lumen. They evaluated the antibacterial efficacy of camphorated paramonochlorophenol (CMCP) and a Ca(OH)\(_2\) compound, Calasept, against *E. faecalis*. The method used in bacteriological sampling allowed for sequential removal of 100-micron-thick zones of dentin from the central canal towards the periphery. They gave a better methodology to assess the efficacy of disinfectants. The model proved to be quite sensitive and suitable for in vitro testing of root canal medicaments.

Maurya et al (87) have investigated the antifungal activity of two antimicrobial peptides, VS2 and VS3, incorporating unnatural amino acid dehydrophenylalanine. The peptide induced cell membrane permeabilization was assessed using a PI dye uptake
assay. Intracellular localization of the FITC-labeled peptides in Candida albicans was observed by CLSM and FACS. They showed that entry of the peptide in Candida cells resulted in accumulation of reactive oxygen species (ROS) leading to cell lysis. Killing of fungal cells by both natural and synthetic cationic peptides could either be by the disruption of the structure of fungal cell membrane (e.g., melittin), ROS formation (histatin 5) or depolimerization of actin cytoskeleton or ultrastructure damage (magainin-2). VS2 and VS3 showed rapid killing of C. Albicans.

Yoldas et al (88) have evaluated the effect of a mix of calcium hydroxide and glycerine and a mix of Ca(OH)₂ and water on the microhardness of human root dentin. The reduction in dentin microhardness following the use of a Ca(OH)₂ –glycerine combination was significantly greater than the Ca(OH)₂ –distilled water combination after 3 and 7 days. The use of Ca(OH)₂ combinations for intracanal dressing softens dentin. Even though various methodologies are available to evaluate the microhardness of tooth, we selected this methodology because the microhardness of dentin may vary considerably with other teeth. Hence, comparison of dentin hardness values before and after treatment with medicaments was made within the same root dentin sample. This was performed to minimize the effect of structural variations of different teeth and to establish a reasonable baseline evaluation.
Kinney et al (89) have discussed that many fractures occur in teeth that have been altered, for example restored or endodontically treated. It is therefore essential to evaluate the structure and mechanical properties of these altered dentins. One such altered form of dentin is transparent dentin, which forms gradually with ageing. This study highlights the differences in the structure and mechanical properties of normal dentin versus transparent dentin. The mineral concentration, as measured by X-ray computed microtomography, was significantly higher in transparent dentin, the elevated concentration being consistent with the closure of the tubule lumens.

Akcay I and Sen BH (90) have evaluated the effect of different concentrations of cetrimide with or without 5% EDTA solution on the microhardness of human root dentin. The reference microhardness values of untreated specimens were initially measured with a Vickers indenter under a 50-g load and a 10-second dwell time at the midroot level of the root dentin. All solutions significantly decreased the microhardness of root dentin. Microhardness was able to give an indirect evidence of mineral loss or gain in dental hard tissues.