Fig 1: Anatomy of tooth

2.2 - Need for Root Canal treatment

The pulp tissue can be diseased by one of the following ways: Decay (caries) or trauma to the tooth or gum disease. Dental caries if left untreated can progress deeper causing destruction of the enamel and dentin, which further results in bacterial invasion into the pulp which leads to infection and inflammation of the pulp tissue. A sequel of diseases affects the pulp, if left untreated, which includes pulpitis (may be reversible or irreversible pulpitis), apical periodontitis (microorganism from root canal space migrate to the periapical tissues through apical foramen), followed by abscess, granuloma, cyst and osteomyelitis. In such situations, the tooth can be treated and salvaged with the advent of root canal treatment. Root canal treatment is the removal of the tooth’s pulp tissue that is encompassed within the tooth. Once the damaged, diseased or dead
pulp is removed, the remaining space is cleaned, shaped and filled to create a healthy tooth and surrounding tissues.

The speciality of dentistry that specializes in treating the diseases of the pulp in a conservative and regenerative approach is known as Endodontics. Endo in Greek means “inside” and odont means “tooth”. The field Endodontics involves the procedures performed inside the tooth. Further, Root canal treatment is one of the most common therapies performed by an endodontist.

Root canal treatment involves the following steps:

1) Access cavity preparation: Gaining access to the pulp space
2) Cleaning and shaping of the canal space: Shaping of the canals is done mechanically with hand and engine driven specialized instruments. Endodontic instruments remove the infected dentin along with the bacteria from the pulp space. Cleaning of the canal space is done using different chemical and mechanical methods. The remaining bacteria would be eradicated by the irrigants and intracanal medicaments.
3) Obturation: This involves filling of the cleaned canal spaces to maintain the sterility of the pulpal space for a long time, which in turn can prevent recontamination of the canal spaces.
Though each and every step mentioned above has an important role in achieving a successful treatment outcome in root canal treatment, one step which is very critical from a biological point of view is cleaning the canal space. The treatment outcome of endodontic treatment is hugely dependent on how well this step can be performed. If this step is not done properly, ultimately it leads to recontamination.

2.3 - Endodontic Microbiology

W D Miller was a pioneer in demonstrating the bacterial invasion of dentinal tubules of both carious and non-carious dentin. He reported that the tubule microflora consisted of cocci and rods (2). In 1965, Kakehashi et al proved that pulp and periapical disease occurred in surgically exposed rat molar pulp, only when bacteria were present in the oral cavity (3). Factors that may contribute to a persistent periradicular infection after root canal treatment include intraradicular infection, extraradicular infection, foreign body reaction, cysts containing cholesterol crystals (4) and intrinsic or extrinsic nonmicrobial factors (5, 6). The composition of microflora of root canals has been the focus of considerable research over the years. Various studies have proved that the major cause of endodontic failure is the survival of microorganisms in the apical portion of the root-filled tooth (4,7). Unlike primary endodontic infections, which are polymicrobial in nature and dominated by gram-negative anaerobic rods, the microorganisms involved in
secondary infections are composed of single or few bacterial species (8, 9). Intraradicular infections are characterized by the presence of microorganisms within the root canal system. Microorganism plays a causative role in the pathogenesis of pulp and periradicular disease (10, 11). They are further classified into primary intraradicular, secondary intraradicular and persistent intraradicular infections. Oral cavity consists of more than 500 different kinds of microorganisms. The main function of enamel is to not allow this microorganism to enter into the dentin – pulp complex. Once the bacteria gains access to the root canal system, they rapidly invade into the dentinal tubules and may be responsible for persistent root canal infections (12, 13, 14). The chances of a favorable outcome following root canal treatment are significantly higher if the bacteria are eradicated effectively before the root canal system is filled. However, if microorganisms persist in the root canal at the time of root filling or if they penetrate into the canal after filling, there is a higher chance that the treatment will fail (15, 16).

Various studies have proved the occurrence of extraradicular infections in both treated and untreated root canals (17,18,19). Microorganisms established in the periradicular tissues are inaccessible to cleaning and shaping procedures. So, the extraradicular infections may be the reason for endodontic failure. Pathogens have developed mechanisms that allow them to survive in an inhospitable environment. Few oral microorganisms have the
ability to overcome these host defence mechanisms and thereby induce extraradicular infections (20). One of the most significant mechanisms of bacterial resistance from the host defence system is the microbial arrangement in the form of endodontic biofilms in the infected root canal systems (20).

2.4- Role of Biofilms in Endodontic Infections

Biofilm is a state of bacterial existence that is extremely difficult to eradicate. By definition biofilm is a sessile multicellular microbial community. It is characterized by cells that are firmly attached to a surface and encompassed in a self produced matrix of extracellular polymeric substances (21). The organization of a polymicrobial biofilm is an extremely dynamic process that involves a sequel of steps. The foremost step is the attachment of the bacterial cells to the particular abiotic or biotic surface. Bacteria usually adhere to a conditioning film typically composed of organic molecules (e.g. nutrients, salivary proteins, large macromolecules) which can enhance the adhesion of bacteria to the surface. Initially the attachment is mediated by weak reversible van der Waals interactions between the cell surface and the substratum, which can further lead to a stronger adhesion receptor mediated attachment (22). The bacterial structures like flagella, fimbriae, LPS and exopolysaccharides take part in irreversible interactions. They can be dipole, hydrogen, ionic or hydrophobic. The next step is the development of micro-colonies promoted by the growth and
division of the cells attached earlier (primary colonizers). The micro-colonies progressively multiply, enlarge in number and coalesce to form the first layer of cells covering the surface. When multiple layers of cells pile up on the surface, the third step of the formation occurs by the presence of a mature biofilm characterized by the presence of macro-colonies surrounded by water channels that aid in distributing nutrients and signaling molecules. Finally, to survive when nutrients become limited or to simply spread and colonize to other niches, some biofilm cells can detach individually or in clumps. In general, biofilm dispersion occurs in response to environmental changes and is dependent on growing conditions (23, 24). 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 (25). Many studies have proved that apical periodontitis is a biofilm induced disease and arises in situ (26, 27). Ricucci & Siqueira (28) have shown the prevalence of endodontic biofilms in 64 untreated teeth and 42 treated teeth with apical periodontitis. They have looked for associations between endodontic biofilms and clinical conditions, radiographic size and the histopathologic type of apical periodontitis. They have concluded that intraradicular biofilm was formed in 80% of untreated canals and 74% of treated canals. The prevalences of intraradicular biofilms in teeth
associated with apical cysts, abscesses and granulomas were 95%, 83%, and 69.5%, respectively. Biofilms were visualized in 62% and 82% of the root canals of teeth with small and large apical periodontitis lesions, respectively. Further, intraradicular bacterial biofilms were usually thick and composed of several layers of bacterial cells. Hence, the morphology of endodontic biofilms can be expected to differ from case to case. Thus a unique pattern for endodontic infection cannot be determined. Thirdly, the study stated that biofilms were also commonly seen covering the walls of apical ramifications, lateral canals and isthmuses. The access to instruments, irrigants and intracanal medicament will be difficult to these areas. Finally, the study had reported that extraradicular biofilms were found only in 6% of the cases.

2.5 - *E. faecalis*: Prevalence, Survival and Virulence factors

*Enterococcus faecalis (E. faecalis)* is a normal inhabitant of the oral cavity. *E. faecalis* is a persistent organism plays a major role in the etiology of persistent periradicular lesions after root canal treatment, despite constituting a small proportion of the flora in untreated canals. It is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora (29). *Enterococci* are gram positive cocci that can occur singly, in pairs, or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen (30). There
are currently 23 *Enterococci* species and these are divided into five groups based on their interaction with mannitol, sorbose, and arginine. *E. faecalis* belongs to the same group as *E. faecium*, *E. casseliflavus*, *E. mundtii*, and *E. gallinarum* (31, 32). *E. faecalis* is found in 4 to 40% of primary endodontic infections (33). *E. faecalis* prevalence in root-filled teeth with periradicular lesions ranges from 24 to 77%. (32). Survival and virulence factors (32) of *E. faecalis* have been listed below:

- Endures prolonged periods of nutritional deprivation
- Binds to dentin and travel inside the dentinal tubules
- Alters host responses
- Suppresses the action of lymphocytes
- Possesses lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid
- Utilizes serum as a nutritional source
- Resists endodontic disinfectants
- Competes with other cells and has the ability to form a biofilm.

### 2.6 - Need for study

The occurrence of persistent root canal infections can be attributed to the bacterial invasion of dentinal tubules (14). Dentin structure is characterized by the presence of dentinal tubules, which contains dentinal fluid traversing the entire bulk. Tubule diameter plays an important role in bacterial penetration into the tubuli.
Tubules that are sclerotic or obliterated can physically obstruct the bacterial penetration. Dentinal sclerosis occurs as a result of an increase in peritubular dentin. Dentinal tubules become obliterated, resulting in narrowing of the tubule to approximately 2.5 µm in diameter near the pulp and 0.9 µm in diameter near the enamel/cement. Thus, a tubule is normally larger in diameter than the average *E. faecalis* cell diameter of approximately 0.8–1 µm (14, 34, 35). *E. faecalis* can penetrate into dentinal tubules to a maximum of 1483.33 µm (36). Instrumentation and irrigation are effective in removing major amount of tissue in the root canals, but complete debridement of bacteria from dentinal tubules is usually very difficult (37, 38). Bacteria causing persistent infections are usually present in dentinal canal walls (including the lateral canal, isthumuses and apical ramification.) that remain untouched by instruments (39, 40, 41) and antimicrobial agents, (42). Unfortunately, the bacteria within the dentinal tubules are inaccessible to the conventional root canal irrigants and intracanal medicaments, because they have limited penetrability into the dentinal tubules.

2.7: Nanotechnology

Recent days have seen an interest in the development of new pharmaceutical products, particularly, the synthesis of nanoparticles with specific shape, size, chemical and physical properties. The application of nanotechnology in the field of medicine and
engineering has led to the introduction of newer materials at the nanoscale level (43). Different types of nanomaterials like copper, zinc, titanium (44), magnesium, gold (45) are shown to have antibacterial efficacy. Silver nanoparticles (AgNps) have also proven to be highly effective, as they possess good antimicrobial efficacy against bacteria and other organisms (46).

The success of the root canal treatment mainly depends on the complete elimination of bacteria from root canal system to prevent further reinfection. *E. faecalis* has the ability to penetrate deeper into dentinal tubules and it is the most common reason for endodontic failure. Nanotechnology may prove to be an advanced option to eliminate the biofilm and help endodontists overcome the above mentioned lacunae. Ag⁺ ions and Ag compounds have strong biocidal effect but are non toxic to the human body. AgNps may possess better antibacterial efficacy with no deleterious effects on dentin. It may used as an intracanal medicament to improve the disinfection of root canal system. Therefore, we selected AgNps as an intracanal medicament against *E. faecalis*. Also, to our knowledge, no study has been done earlier to compare the efficacy of AgNps with CHX against *E. faecalis* biofilm in the tooth model.

**2.8 Aim of the study**

To investigate the potential use of AgNps as an intracanal medicament to improve the root canal disinfection.
2.9 Specific objectives

I) To evaluate the antibacterial efficacy of AgNPs against *E. faecalis* biofilm formed on root dentin.

1. To synthesise and characterize the AgNps by using ultra violet spectroscopy and high resolution transmission electron microscopy.

2. To evaluate the minimum inhibitory concentration of silver nanoparticles against *E. faecalis* by using agar diffusion method and spectropotometric method.

3. To compare the antibacterial efficacy of AgNps with chlorhexidine against *E. faecalis* in tooth model at two different depths (200µm and 400µm).

4. To investigate the bacterial viability (live and dead *E. faecalis*) after AgNps and chlorhexidine treatment for 1 day and 3 days by using confocal laser scanning microscope (CLSM).

5. To improve the antibacterial efficacy of AgNps by replenishing, thermosensitive agents and combination of both thermosensitive agents followed by ultrasonic by using CLSM.

6. To investigate the mode of antibacterial mechanism of AgNps: Membrane damage by using Scanning electron microscopy, Propidium iodine uptake and Reactive oxygen species generation by using flow cytometry.
II) To study the biocompatibility of AgNps by using haemolytic assay.

III) To investigate the effect of AgNps on the microhardness of human root dentin in vitro.