INTRODUCTION

1.1 ENDODONTIC BIOFILM AND COAGGREGATION OF BACTERIA:

Biofilm is defined as a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other and are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription (1). In most biofilms, less than 10% are bacterial populations compared to 90% EPS matrix (2). Invasion of bacterial biofilm into the dentinal tubules is responsible for primary and persistent root canal infection (3,4). Collagen-binding protein production, withstanding nutritional deprivation, utilizing serum as nutritional source, bind to dentinal tubules, produce collagen-binding protein, serine proteases and lytic enzymes are the survival and virulence factors of a biofilm causing infection. Biofilm maintains pH homeostasis, invades into dentinal tubules and also resists the activity of antimicrobial agents (5,6,7). Microbial coggregation and autoaggregation have been associated with intraradicular and extraradicular endodontic disease. Autoaggregation (bacterial aggregation between cells of the same strain) and coaggregation (between different species and strains) is of considerable importance in several ecological niches. Recognition between a suspended cell type and one already attached to a substratum is termed coadhesion (8). Oxygen protection, cell-cell communication, and genetic exchange between cells are few physical interactions between coaggregating bacteria. (9,10).

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. Enterococci can withstand harsh environmental conditions. Enterococci can grow at 10°C and 45°C; at pH 9.6, in 6.5% NaCl broth, and survive at 60 °C for 30 minutes (11). It is a predominant bacteria implicated in root canal failures and persistent infections (12). It is found as 40% in primary endodontic infections and 24% to 77% in post treatment apical periodontitis (13).
Fusobacterium nucleatum is a non-spore forming, non-motile and gram-negative microorganism, which belongs to the family bacteroidaceae. Fusobacterium nucleatum participate in both adhesion and coaggregation reactions. In intrageneric, intergeneric, multigeneric coaggregation fusobacterium plays a key role in biofilm network formation. It is proposed that fusobacterium acts as a bridge between early and late colonizers (14). Rocas et al. have found F. nucleatum to be one of the most prevalent species found in asymptomatic primary endodontic infections (15). A study done by Johnson et al in 2006, investigated the coaggregation interactions between E. faecalis clinical isolates and four bacterial species Porphyromonas anaerobius, Porphyromonas oralis, Fusobacterium nucleatum, and Streptococcus Anginosus, and concluded that the coaggregation interactions observed between E. faecalis and F. nucleatum play a potential role in endodontic infection (16). Penetration of E. faecalis biofilm into the dentinal tubules is to the depth of 1483.33 μm (nutrient-rich aerobic condition), 1166.66 μm (nutrient-rich anaerobic condition) and 620μm (nutrient-deprived anaerobic condition) (17).

Coaggregation is defined as cell-to-cell adhesion in which cells of one microbial species adhere more or less specifically to those of a different species. Dominant cell-cell interactions are between Lectin-type (protein) adhesins on one of the cells and oligosaccharide moieties on the other (18). Coaggregation can be inhibited by denaturation of the lectin or, by the addition of sugars and aminoacids (19). Disruption may be mediated by sugars (Galactose, lactose (Galβ1-4Glc), and fucose (6-deoxygalactose) etc, which are used for inhibiting coaggregation (18). The coaggregation between gram-positive and gram-negative bacteria (B. gingivalis and S. mitis) was inhibited by two basic amino acids, L-Arginine and L-Lysine, and was not inhibited by any sugars tested (20). Exposure to dual species film (gram-positive S.Sanguis & E.faecalis) (gram-negative P.gingivalis & F.nucleatum) to NaOCl, CHX and Iodine resulted in CHX not as effective as NaOCl in disruption of dual biofilm (21). Fusobacterium nucleatum exhibits different types of adhesions and harbors several adhesins to coaggregate between bacteria and fungi. The outer membrane protein of F. nucleatum is the major protein, which helps in coaggregating to other bacteria. High-concentration NaOCl (6%) showed the strongest antibacterial effect among the solutions tested for both young and old E. faecalis biofilms (22). Shen et al studied
the susceptibility of multispecies biofilms to chlorhexidine (CHX) at different phases of growth from 2 days to several months, found that bacteria in mature biofilms are much more resistant to being killed by CHX than bacteria in young biofilms (23).

1.2. JUSTIFICATION OF THE PROBLEM:

Various root canal irrigants have been used to eradicate mono and dual species film. Nair et al have shown that instrumentation and irrigation with NaOCl eliminate biofilms in only 12% of root canals. Biofilms dwell in the unreachable parts of root canal systems like isthumuses and ramifications (24). A study done in 2007 by Ahmet Rifat et al concluded synergy in growth and increased resistance to antimicrobials in mixed biofilms of F.nucleatum and P.micros. The lowest number of viable cells in a biofilm and cell killing was observed after immersion with sodium hypochlorite in a dual film of F.nucleatum and S.sanguinis. Ozok et al concluded in his study that mixed-species biofilms of F.nucleatum and P.micros showed a time-dependent synergy in growth and resistance to NaOCl and thus none of the irrigants completely eradicated biofilm from root canal (25).

1.3. LACUNAE IN KNOWLEDGE:

From the above-mentioned studies, we have come to a conclusion that no single medicament or irrigant can completely disrupt and eradicate a dual species biofilms completely from the root canal because of the complexity of the root canal and adhesion and coaggregation of microorganisms. Studies on biofilms are mostly done using mono species and a very few studies on dual species. Research work done with biofilms is done mostly in membrane models, which may not correlate well with the clinical reality. Studies have to be done in root canal, which contains the complexity of dentinal tubules, accessory canals and lateral canals. There are no studies on sugars, amino acid inhibition on coaggregation of endodontic pathogens in root canal. The antimicrobial activity and disruption of coaggregation against endodontic pathogens have not been studied.
1.4. AIMS AND OBJECTIVES:

The aim of our study was to determine both the disruptive and bactericidal effect of various irrigating solutions with and without the pretreatment of coaggregation inhibitors against dual species biofilm of Enterococcus faecalis and Fusobacterium nucleatum.

1.5. OBJECTIVES:

Bacteria in root canal are always mostly present in a biofilm form:

1. To evaluate the total and dead bacterial biovolume after disinfection of root canal containing mono and dual species biofilm by root canal irrigants 3% sodium hypochlorite and 2% chlorhexidine.

2. To evaluate the biovolume of remaining bacteria and dead bacterial biovolume after pretreatment with coaggregation inhibitors (sugars and aminoacids) which was followed by disinfection of root canal with 3% sodium hypochlorite and 2% chlorhexidine.

3. To quantify the remaining bacteria after pretreatment with coaggregation inhibitors (sugars and aminoacids) which was followed by disinfection of root canal with 3% sodium hypochlorite and 2% chlorhexidine.

1.6. HYPOTHESIS:

**Phase I Aa:** There is no significant difference in the depth of penetration of bacterial invasion into dentinal tubules between mono and dual species biofilm groups of Enterococcus faecalis and Fusobacterium nucleatum.

**Phase I Ab:** There is no significant difference in the biovolume of bacteria into dentinal tubules between mono and dual species biofilm groups of Enterococcus faecalis and Fusobacterium nucleatum.

**Phase I B:** There is no change in the turbidity or coaggregation between Enterococcus faecalis and Fusobacterium nucleatum.

**Phase II A&B:** There is no significant difference in the bacterial biovolume and quantity of Enterococcus faecalis and Fusobacterium nucleatum in dentinal tubules before and after treatment with 3% sodium hypochlorite and 2% chlorhexidine.
Phase III A: There is no significant difference in the bacterial biovolume and quantity of bacteria in dentinal tubules pretreated with coaggregation inhibitors (sugars and aminoacids) followed by 3% sodium hypochlorite.

Phase III B: There is no significant difference in the incidence of bacterial biovolume and quantity of bacteria in dentinal tubules pretreated with coaggregation inhibitors (sugars and aminoacids) before treatment with 2% chlorhexidine.