

Development of Dental Implants with Antimicrobial Activity

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Background

Planktonic bacteria are capable of forming biofilms, which are described as microbial aggregates embedded within a matrix of extracellular polymeric substances (EPS) and are found attached to abiotic or biotic surfaces ^(1,2). The EPS contains exopolysaccharides, nucleic acids, proteins and lipids forming a protective matrix surrounding bacterial cells ⁽³⁾. Bacteria attach to the EPS facilitating cell-cell communication and cell-surface interactions ⁽⁴⁾. The biofilm matrix is important in maintaining the biofilm lifestyle, it has an influence on microbial behaviour, virulence and tolerance to antimicrobials ^(5,1).

Significance of biofilms

Biofilms are responsible for many infections ranging from chronic wounds, cystic fibrosis, urinary tract infections, dental caries, periodontitis, medical device implications, orthopaedic implant failure and dental implant failure. Biofilms possess more bacteria per gram compared to planktonic states and have the ability to withstand 500 to 1000 times the concentration of biocides and antibiotics killing their planktonic counterparts ^(6,7,8). Biofilms within the oral cavity are responsible for initiation of gingivitis, dental caries and the progression of periodontal disease ⁽⁹⁾.

Oral Biofilm Formation

The oral microbiome is a complex environment inhabited by a range of bacteria, archaea, fungi, protozoa and viruses ^(10,11). The oral cavity represents the second largest microbiome in the human body ⁽¹²⁾. There are now 775 prokaryotic taxa identified in the oral cavity according to the eHOMD database ⁽¹³⁾.

The oral cavity offers a perfect location for the growth of microorganisms. It provides a warm environment without temperature fluctuations. Saliva maintains a pH of 6.5-7, provides moisture and transports nutrients as well as containing adhesive components constituting the acquired pellicle to which bacteria can attach ⁽¹⁴⁾.

Oral biofilms can form on the many niches such as soft tissues including the gums, cheeks, gingival sulcus, tongue, hard and soft palate. Hard surfaces such as teeth and dental restorative materials can also be colonised by biofilms alternatively called “Dental Plaque”⁽¹⁵⁾. Biofilm formation in the oral cavity displays organisation, microbial succession and are formed in a sequential manner^(16,17). Biofilm development on a substratum involves four stages, initial attachment of cells onto the surface, early development, maturation and finally dispersal of planktonic cells^(18,4).

Tooth surfaces are coated by the acquired enamel pellicle to which primary colonisers bind such as *Streptococci spp.*, *Actinomyces spp.*, *Veillonella spp.* and *Neisseria spp.* on tooth surfaces (5). When microcolonies adhere EPS is produced and secondary colonisers such as *Streptococci*, *Fusobacterium nucleatum* and *Porphyromonas gingivalis* populate the biofilm. Biofilms mature and cell-cell communication known as quorum sensing (QS) occurs. Finally biofilms often breakdown and bacteria are released back into their planktonic state.

Project Overview

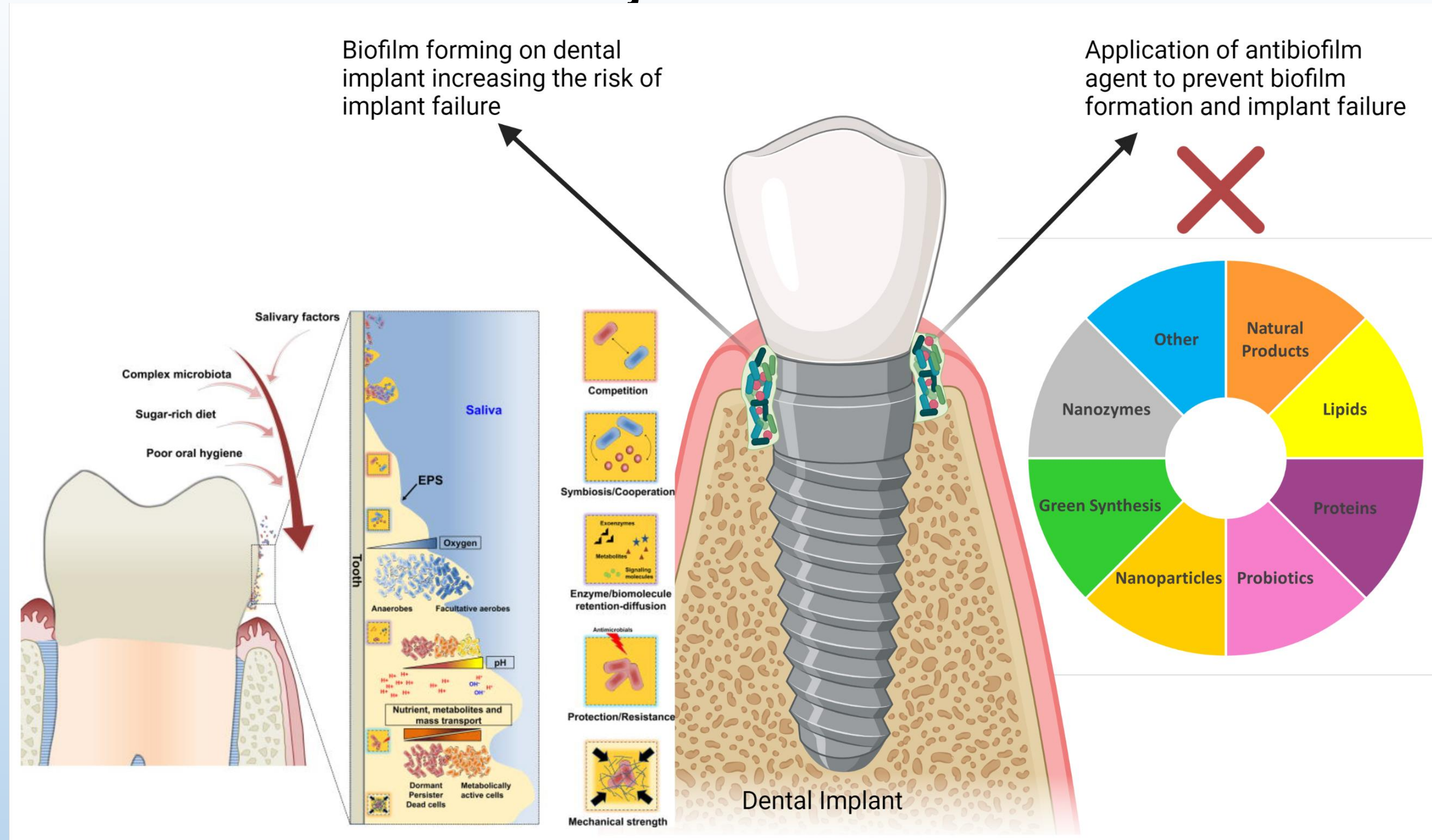


Figure 1.2. Project overview

Biofilm Formation

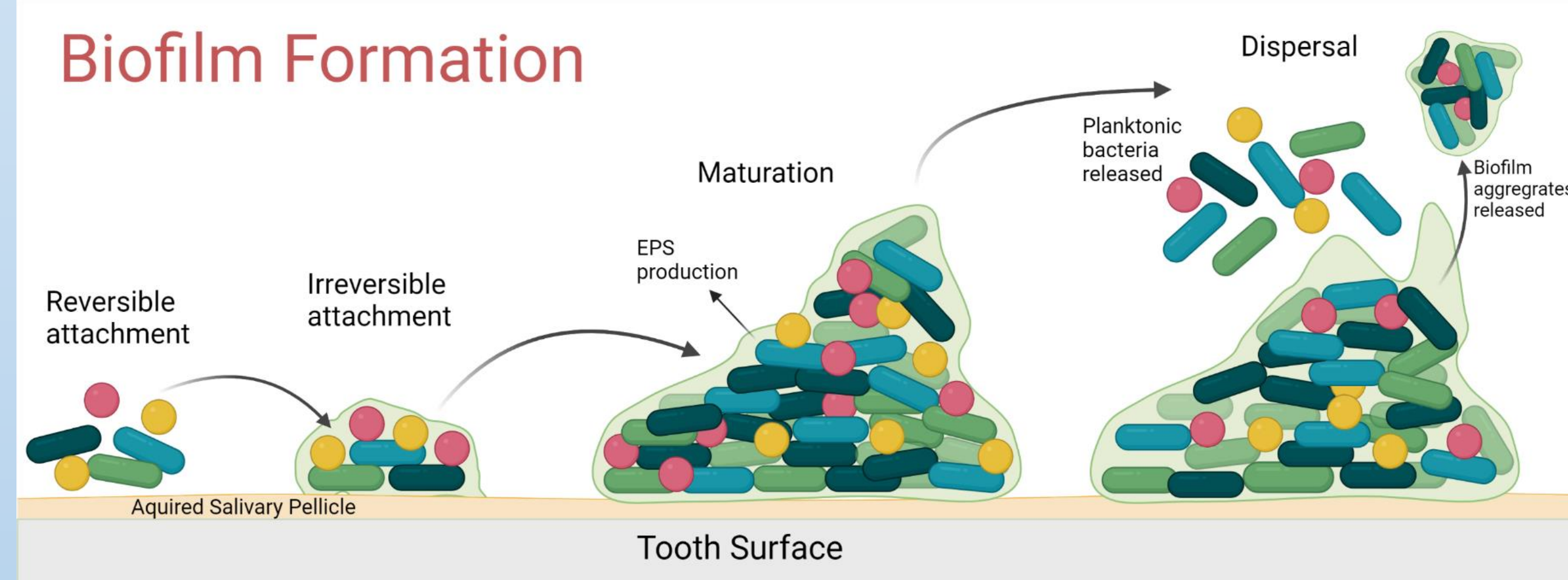


Figure 1.1. Biofilm formation

Materials and Methods

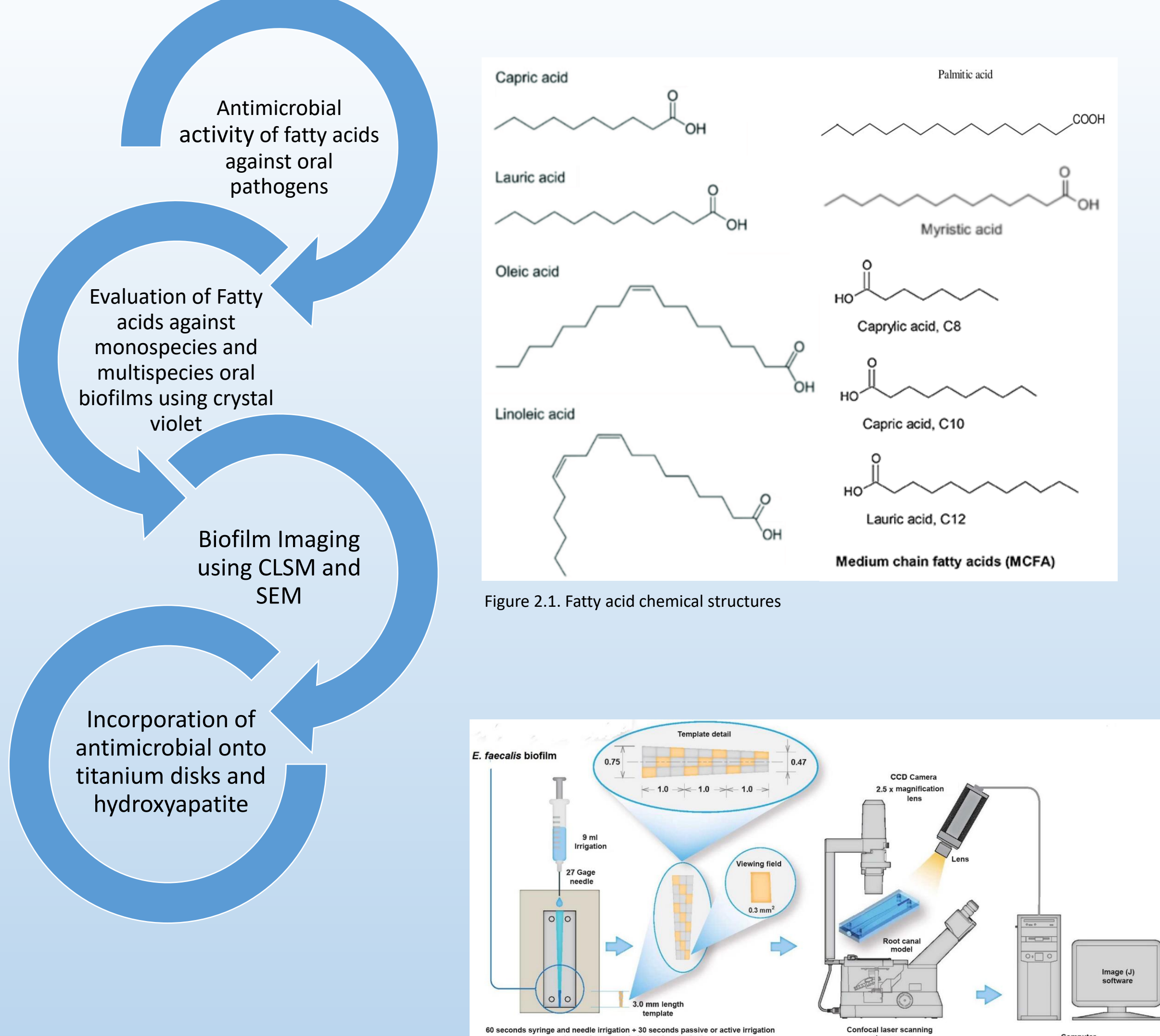


Figure 2.2. Confocal Laser Scanning Microscopy method

Results

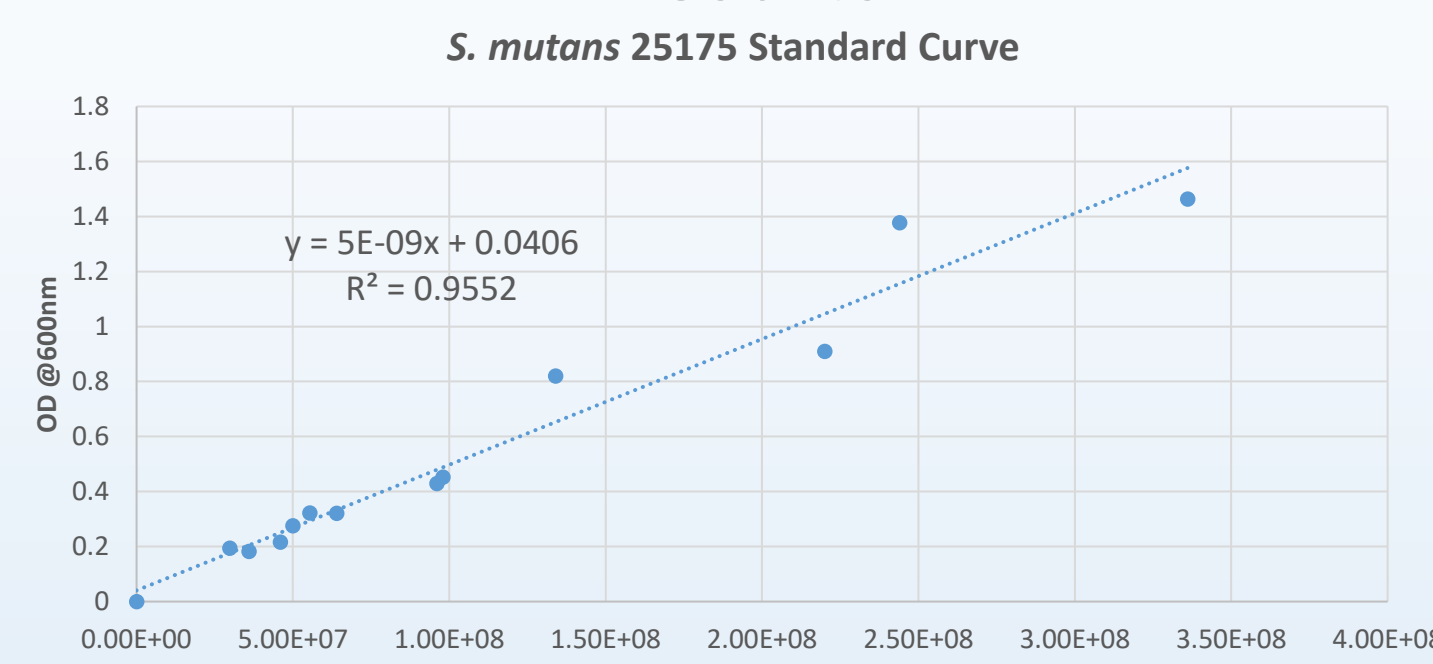


Figure 3.1. Standard curve used to determine CFU/ml *S. mutans*.

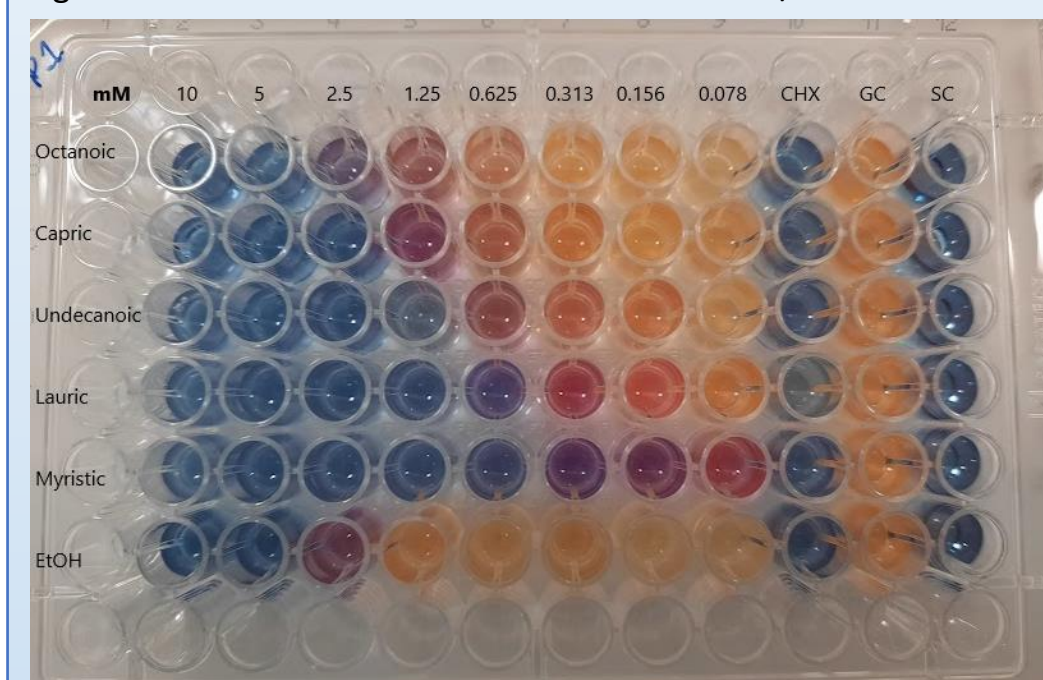


Figure 3.2. MIC of Octanoic, Capric, Undecanoic, Lauric, Myristic, 10% EtOH



Figure 3.3. Kirby Bauer 100mM Linoleic acid

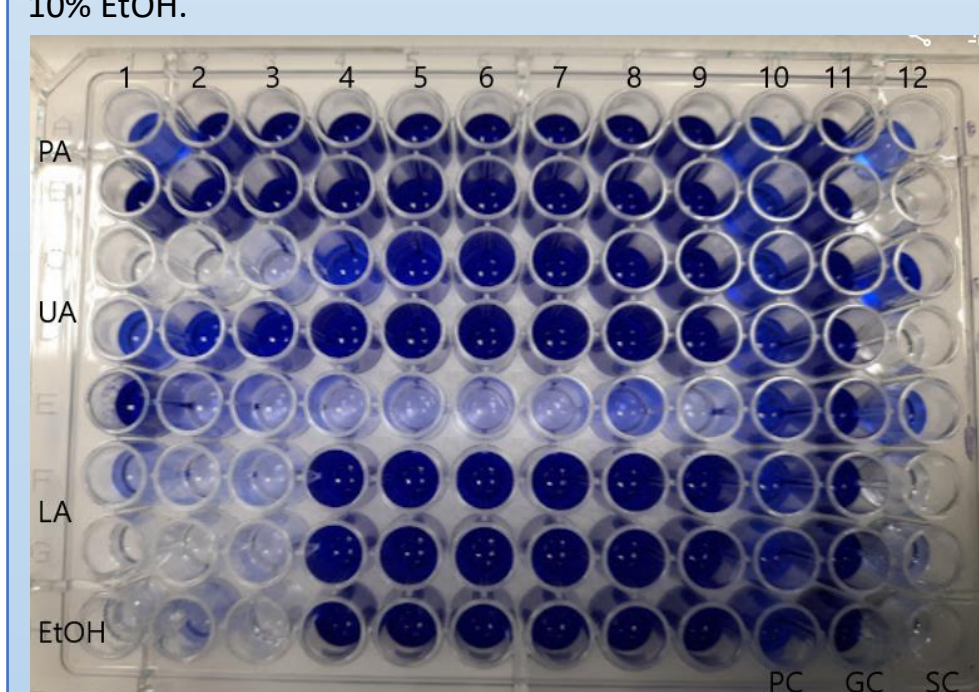


Figure 3.4. Effect of Palmatic acid, Undecanoic acid and Linoleic acid on *S. mutans* biofilm development

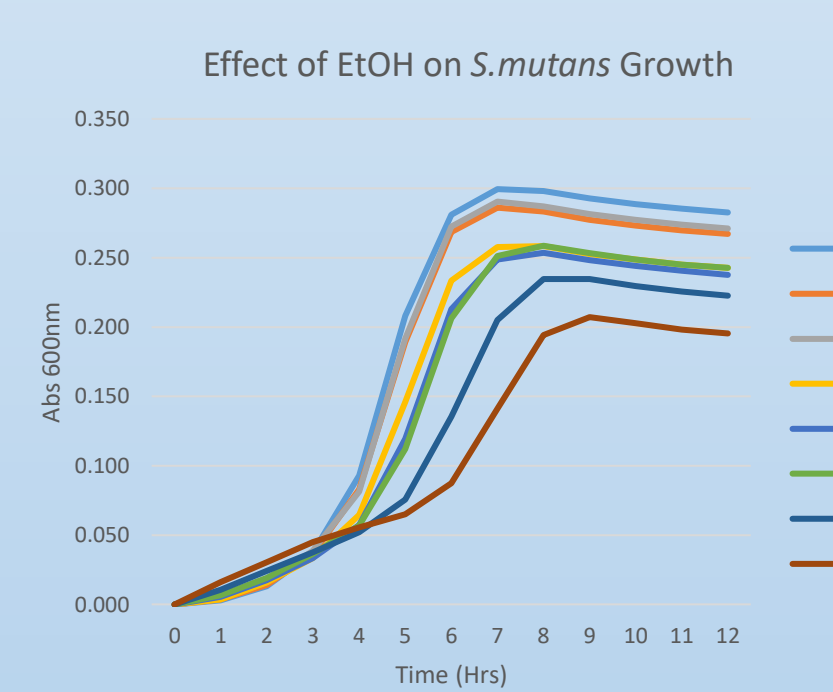


Figure 3.5. Effect of EtOH at varying concentrations on *S. mutans*

Discussion

- Preliminary results suggests that with increasing carbon chain length antimicrobial activity improves.
- LCFA's have displayed adequate antimicrobial effects.
- Polyunsaturated LCFA Linoleic and Monounsaturated LCFA Oleic acid have displayed most promising antibacterial properties with MIC's <0.078 mM and zones of inhibition being displayed for Kirby Bauer test.
- This may be due to the chemical formula containing double bonds.
- Linoleic acid has also revealed antibiofilm properties.
- EtOH at a concentration of 4% does not have significant effect on bacterial growth.

Challenges

- Fatty acids are not soluble in H_2O therefore an alternative solute without antimicrobial effects must be chosen.
- EtOH at 4% does not have a significant impact on viability and normal growth kinetics, however incomplete solubility of FAs is observed at this concentration.
- Incorporation of fatty acids onto dental materials.

Future work

- Alternative solutes such as DMSO, Ethyl acetate will be evaluated.
- Efficacy of fatty acids against monospecies and multispecies biofilms.
- Combining fatty acids to determine synergistic antimicrobial and antibiofilm effects.
- Addition of antimicrobial fatty acids onto titanium disks to evaluate biofilm inhibition.

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