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Antibiofilm of a Tooth Paste on Cariogenic Bacteria

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Abstract Caries is an infectious diseases of the oral cavity in which oral biofilms play a causative role. Moreover, oral biofilms are widely studied as model systems for bacterial adhesion, biofilm development, and drugs acting on biofilm to, due to their widespread presence and accessibility. The aim of the present in vitro study was to investigate the efficacy of specific fluoridated toothpaste on the biofilms formed by the test microorganisms (*Streptococcus mutans*, *Facklamia homins* and *Streptococcus saliviurus*). The colonizations of the three test bacteria on four surfaces (two natural; permenant and deciduous tooth surfaces and two artificial ;composite and amalgam filling surfaces) were observed with scanning electron microscopy (SEM).SEM detected the anti-biofilm effects on the same four surfaces medicated with a tooth paste. **Results**: The used tooth paste declined the formed biofilms on four surfaces efficiently. The natural tooth surfaces responded in same manner to the tooth paste while composite responded more efficiently than amlagam surfaces. **Conclusion**s: Flourdated toothpaste (0.454%) effectively can remove formed biofilms of the three test bacteria on natural surfaces and composite filling surfaces. It removed formed biofilm on amalgam surfaces but not as from other three surfaces.

Keywords: *Toothpaste, Flouride, Biofilm, Streptococcus mutans, Facklamia homins, Streptococcus saliviurus, Scanning electron microscope, permanent and deciduous tooth , composite and amalgam filling*

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1. Introduction

More than 700 unique bacterial species have been detected in the oral cavity. [1] Most of the them are harmless bacteria, but some have the ability to cause damage. [2] Dental caries is an infectious, multi-factorial disease caused by bacteria which aggregated in pathogenic biofilm. [3,4] A biofilm can be defined as a complex bacterial structure formed on a solid surface that provides protections to related bacteria from the host's immune defense and from antimicrobial agents. [5] Maintenance of good oral hygiene is the cornerstone for prevention of many dental diseases. [6] Toothpaste is considered as one of the most important means used to promote oral hygiene [7]. It is classified as drugs not cosmetics as it contains an ingredient to achieve the desired pharmacological effect. [8] The efficiency of any toothpaste in part, lies on its ability to remove pathogenic oral micro-flora [9] or by decreasing the accumulation of oral biofilms which is responsible for development of dental caries. Fluoride which is the mostly widely applied in tooth paste, has been the key of caries prevention for over many years [10,11].

2. Methods

1. Isolation and Identification of Oral Bacteria

Saliva samples were taken from several patients with dental caries at college of dentistry/University of Mousl-Iraq. After suitable mixing, dilution and inoculation on the three selective media, Mitis Salivirus Bactracin agar (Himedia, India), Rogosa SL agar (Himedia, India) and m Enterococcus agar (Himedia, India), colonies from each media were checked by automated microbial identification instrument (Vitek 2, BioMerieux, France) at Vin medical centre (Douhok, Iraq). Only three bacteria were selected. They were *Streptococcus mutans*, *Facklamia hominis* and *Streptococcus salivarus* [12].

2. Biofilm Formation Assay

In this experiment, attachment surfaces were :-

A-Natural surfaces: - which included both permanent and deciduous teeth surfaces.

Freshly extracted sound permanent and deciduous molars were manually scaled to remove calculus and surface-adhered debris. Sterilization was done by autoclaving with steam1psi, typically at 121°C for a minimum of 15minutes. A perpendicular cuts to long axis of buccal surfaces of permanent and deciduous teeth were made. At end, enamel slabs of 5mm×5mm measurements were obtained [12,13].

B. Artificial surfaces: - which included amalgam and composite fillings surfaces.

Amalgam capsules (high silver 69%, Ventura, Spain) were mixed with amalgam mixer according to manufacture instructions, allowed to set in 5mm×5mm measurements mold and polished with polishing paste. Composite slabs were made by putting the composite material (Estelite alpha, Tokuyama, Japan) in 5mm×5mm measurements transparent mold, light curring according to manufacture instructions (20 sec) and polishing with polishing paste.

2.1. Biofilm Formation Procedure

A-The three identified bacterial strains(*Streptococcus mutans, Facklamia hominis* and *Streptococcus salivarus)* were cultivated overnight in 20 ml sterile BHI broth at 37°C anaerobically for the first strain and aerobically for the others and their growth were compared visually to McFarland Standerd No.2 which equaled to 6×10^8 bacteria cells.

B-The four tested surfaces(natural and artificial) were separately put in new 20ml BHI broth and sterilized at 121°C for 15 min to insure sterile broth and surfaces.

C-One tenth milliliter from the two identified bacterial BHI broths (*Streptococcus mutans* with *Facklamia hominis* and *Streptococcus salivarus* with *Facklamia hominis*) were put aseptically in the sterile BHI broth contained the tested surfaces and incubated according to the bacterial cultural requirements.

D-Each tested surfaces was washed with phosphate buffer saline(PBS) ,fixed in 2% glutteraldehyde for 1h at 37°C then washed again with PBS twice times and left to dry at room temperature [14].

E-Samples were sent for examination using scanning electron microscope in Ministry of Sciences and Technology-Baghdad/Iraq.

2.2. Anti-Biofilm Formation Assay

The same procedure in 2.1 was repeated except fourth step was done as follow:-Each tested surfaces was washed with PBS and before fixation with in 2% glutteraldehyde, 0.1 ml of tested tooth paste (Sensodyne repair and protect, USA contain stannous fluoride 0.454 % as active ingredient) covered the entire tested surface for 30 min at 37°C, washed with PBS, fixed in 2% glutteraldehyde for 1h at 37°C then washed again with PBS twice times and left to dry at room temperature sent for using scan electron microscope [15].

3. Results

Figure 1, revealed the effects of test tooth paste on biofilm of single test bacteria (*Streptococcus mutans)* to investigate the bacterial cellular changes individually. Scanning electron micrographs showed the biofilms of two test bacteria (*Streptococcus mutans* with *Facklamia hominis* and *Streptococcus salivarus* with *Facklamia hominis*) on the permentant tooth surfaces, deciduous tooth surfaces, amalgam filling surfaces and composite filling surfaces before and after application of flourdated tooth paste, respectively(Figure 2 - Figure 5).

4. Discussion

The ordinary oral hygiene method of tooth brushing is, by itself, usually inadequate over a long period to provide an acceptable level of plaque control. Consequently, the addition of chemical agents with anti-plaque or antimicrobial activity into dental products has been used as a potential prophylactic method of reducing plaque-mediated disease [16,17].

Today's toothpastes have two purposes: to clean the tooth surface and to add a therapeutic effect. The therapeutic effect may either an antiplaque or anti-inflammatory [18].

Fluoride is considered to be an efficient anticaries agent because of the several supposed mechanisms of action; inhibition of the demineralization, facilitation of the remineralization and suppression of the bacterial metabolism (i.e. antibacterial effect) – by binding and inactivating many of the enzymes involved in different bacterial metabolic pathways.An indirect effect on the biofilm development can be also done by inhibiting the growth of oral bacteria [19].

Fluoride at 0.05%, is considered as a type of antimicrobials used clinically in toothpaste and mouthwashes to decrease plaques and prevent caries. [20] Watson *et al*, 2005, founded that mean biomass fluoride concentrations in biofilms exposed to flouride for 30 min were significantly higher than biomass fluoride concentrations (30 sec, 120 sec, 30 min) [21].

In Figure 1, it showed the effects of test tooth paste on mono biofilm of *Streptococcus mutans.* The application of fluoridated tooth paste not only remove the formed biofilm but cause morphological changes of the bacterial cells.

The bacterial cocci appeared elongated, swollen, scattered and change in their orientations .These changes were due to the pharmacological effects of fluoride. These results came with the same results reached by Abd El-Baky R. M (2012) who treated *S. aureus* with ciprofloxacin-N-acetylcystein [14].

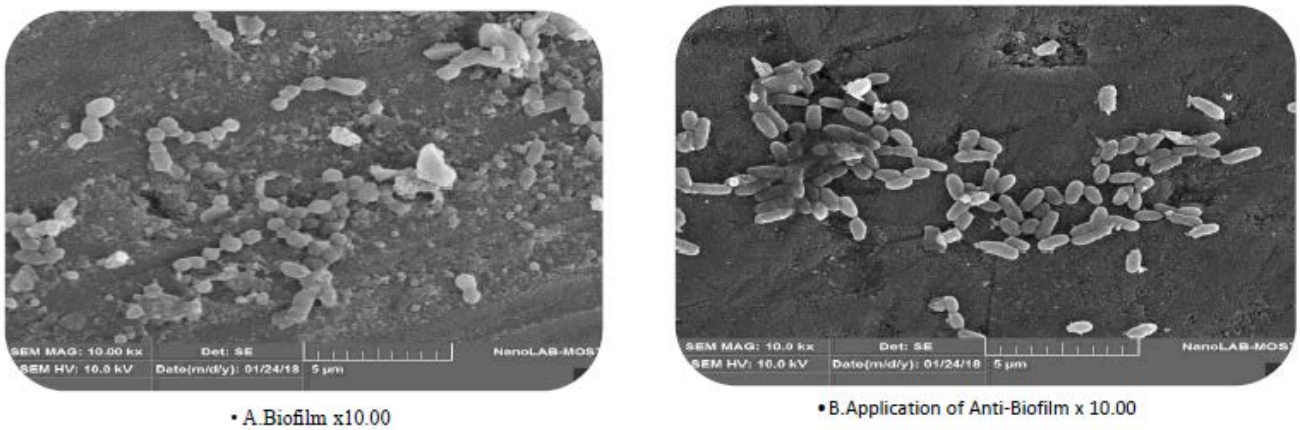

Figure 1. Application of Biofilm and Anti-Biofilm on *Streptococcus mutans* on Permanent Tooth

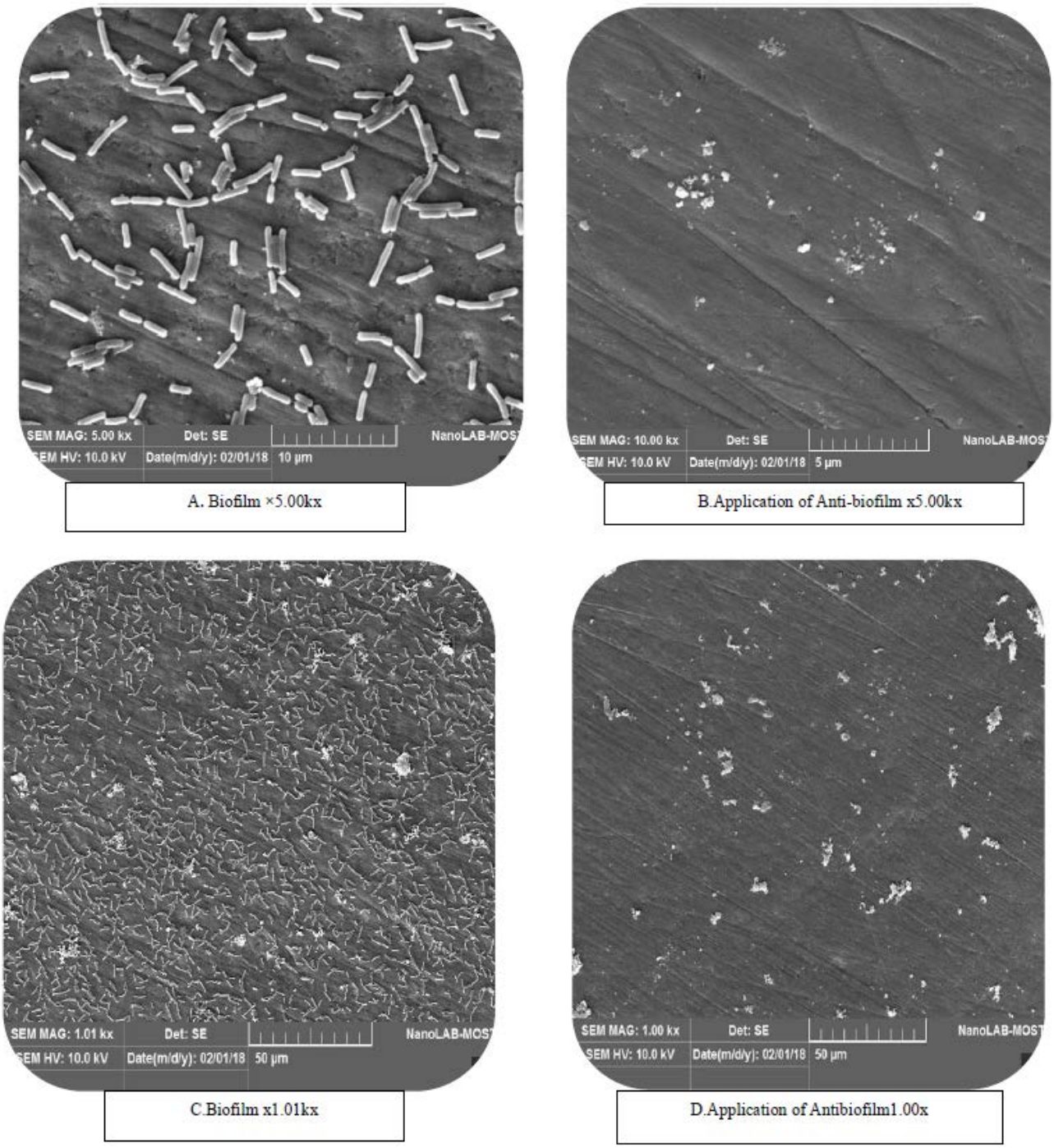

Figure 2.:Biofilms and Application of Antibiofilms of *Facklamia hominis* and *Streptococcus salivarius* on Deciduous Teeth surfaces

As it was observed using SEM, dramatic decline in formed biofilms of the three tested strains was observed on the four tested surfaces after the application of tooth paste contained 0.45% for 30 min (Figure 2 – Figure 5). The decline included both bacterial cells and exopolysacchaides.

In relation to the two natural surfaces, the tested tooth paste inhibited the formed biofilms efficiently in the same manner. This may be explained by two factors; similar enamel topography and similar plaque bacterial species composition of permanent and deciduous teeth.

Although microstructures and mineral compositions of dental enamel was different between permanent teeth and deciduous teeth, enamel rods density was higher in the deciduous teeth and the percentage of calcium and phosphorus was higher in the permanent teeth enamel [22,23,24]. Maria Ange *et al*, 2010 concluded that the morphological analysis of the enamel rod diameter in deciduous teeth was statistically similar to that of permanent teeth enamel. [11]

Microbiologically, although different bacteria can attach to solid surfaces with different strengths, there was no statistically significant difference of bacterial species composition isolated from supragingival plaques with deciduous and permanent teeth [25,26].

In relation to the two artificial surfaces, the tested tooth paste inhibited the formed biofilms more efficiently on composite than amalgam filling surfaces. This can be explained by differences in material roughness and composition.

The composite filling material used in this study was Estelite alpha, Tokuyama, Japan. The producer claimed to provide high gloss with little polishing. In Figure 3 and Figure 4, even the polishing process was done with the same manner ,composite surface appeared more smooth and gloosy than amalgam surfaces which had rough and cracked appearances.

Microbial adhesion on filling material surfaces(which is important step of biofilm formation) depends on the surface topography, chemical composition of these materials, and on the physicochemical properties of the bacterial cell surface(its surface charge and hydrophobicity) [27,28].

1-Roughness of Dental Material Surfaces

Bacterial initial attachment on roughened surfaces is aided by surface irregularities, where bacteria are protected from salivary flow(in this study, bacteria were protected from washing with PBS) so,bacteria can attach to pits and grooves in a way that reduce the influence of shear forces on them [29,30].

Einwag *et al.* 1990 examined the influence of the surface roughness of dental filling materials on plaque accumulation and found that *S. mutans* adhered more efficiently to rough surfaces than to high polished filling materials. [31] This approach agreed by Mei *et al.* 2011 who evaluated the streptococcal adhesion forces with composite resins with different surface roughness. They confirmed that adhesion forces of streptococcal bacteria to composite resin materials increase directly with increasing roughness of its surfaces [32].

However, some researchs were some-what confused. Yamauchi *et al*. 1990 stated that the influence of surface roughness was strain dependent. They founded that some strains (*S. oralis, P. intermedia, and P. gingivalis*) were higher in amounts on rough surfaces, whereas others (*S. sanguis, S. mutans, S. mitis and P. gingivalis*) were higher in amounts on smooth surfaces. [33]

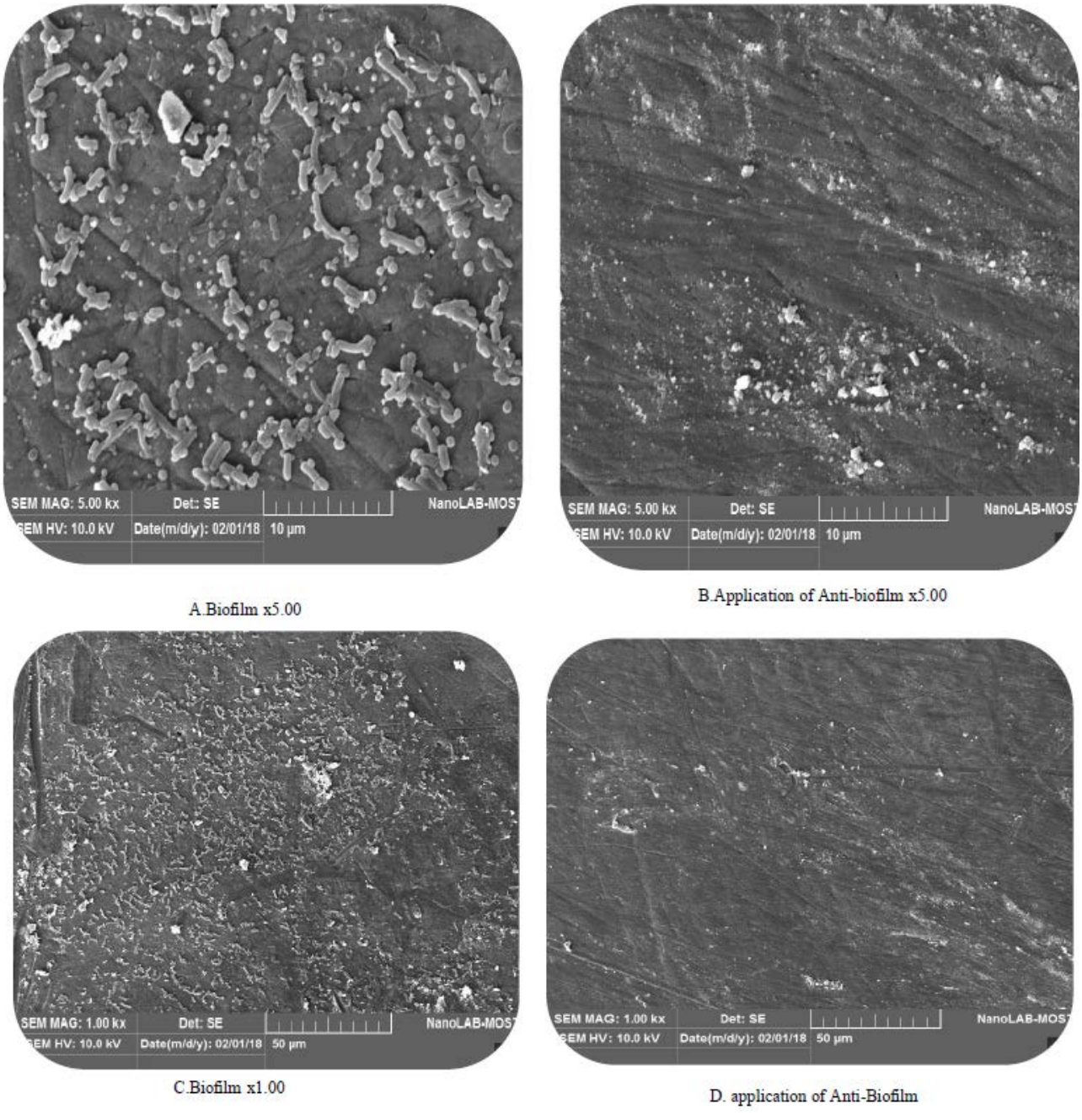

Figure 3. Biofilms and Application of Antibiofilms of *Fakclamia hominis* and *Streptococcus salivarius* on Permanent Teeth surfaces

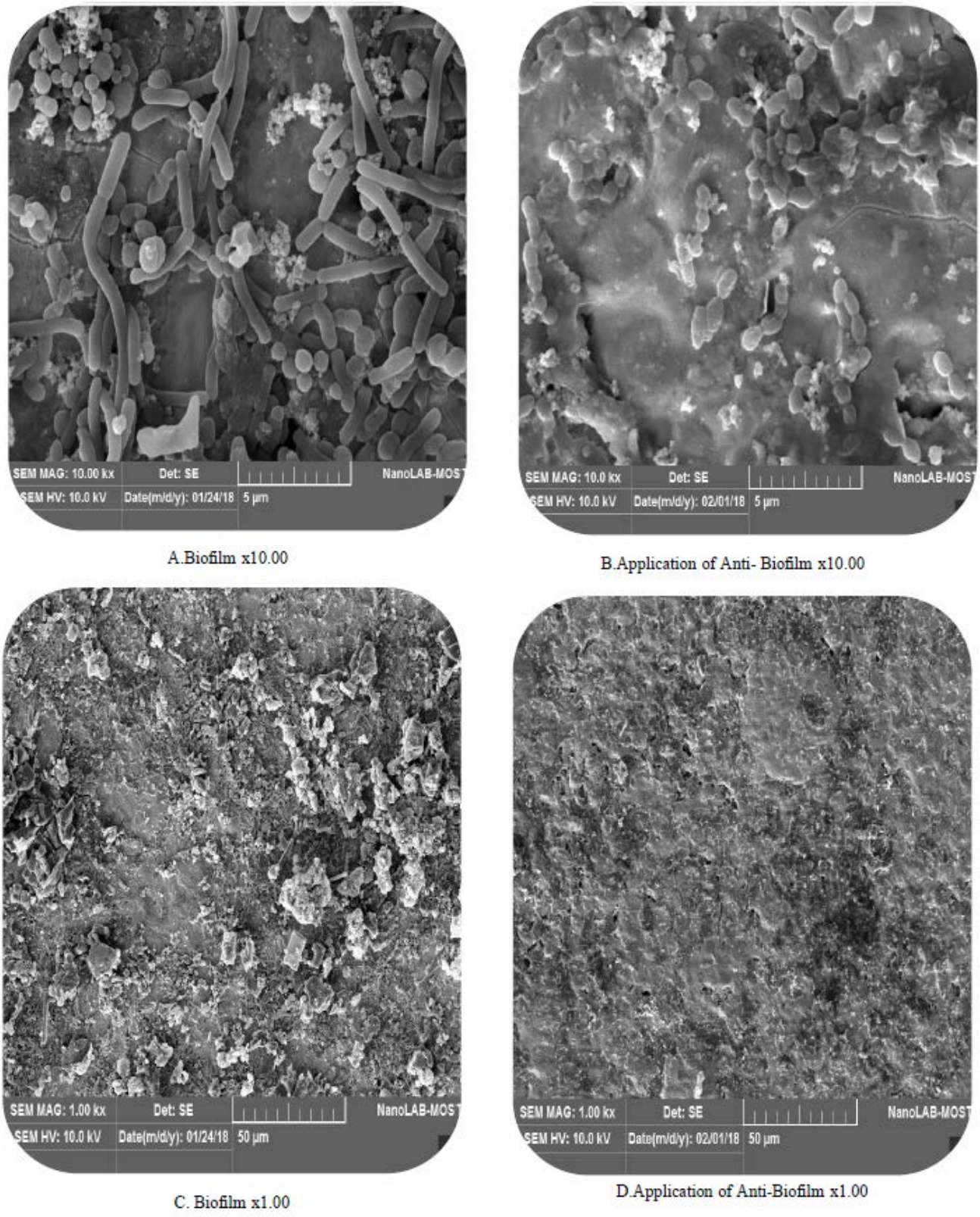

Figure 4. Biofilms and Application of Antibiofilms of *Facklamia hominis* and *Streptococcus mutans* on Amalgam surfaces

2. Hydrophobicity and Surface Charge

Bacterial colonization to a specific surface is determined by surface characteristics such as its hydrophobicity and surface charge. [34]

Bacterial cells, which have hydrophobic cell surfaces, can be attracted to many materials surfaces leads to increased adherence and subsequent biofilm formation. [35] A decrease in the surface hydrophilicity of any material can lead to strong hydrophobic interaction between infective bacteria and the filling material and then increase adherence [36].

The bacterial cell wall have many structures and properties that aid in bacterial adhesion (teichoic acid in gram-positive bacteria). These characteristics influence the surface charge and hydrophobicity of the bacterial cell, thereby directly affecting adherence. [37]

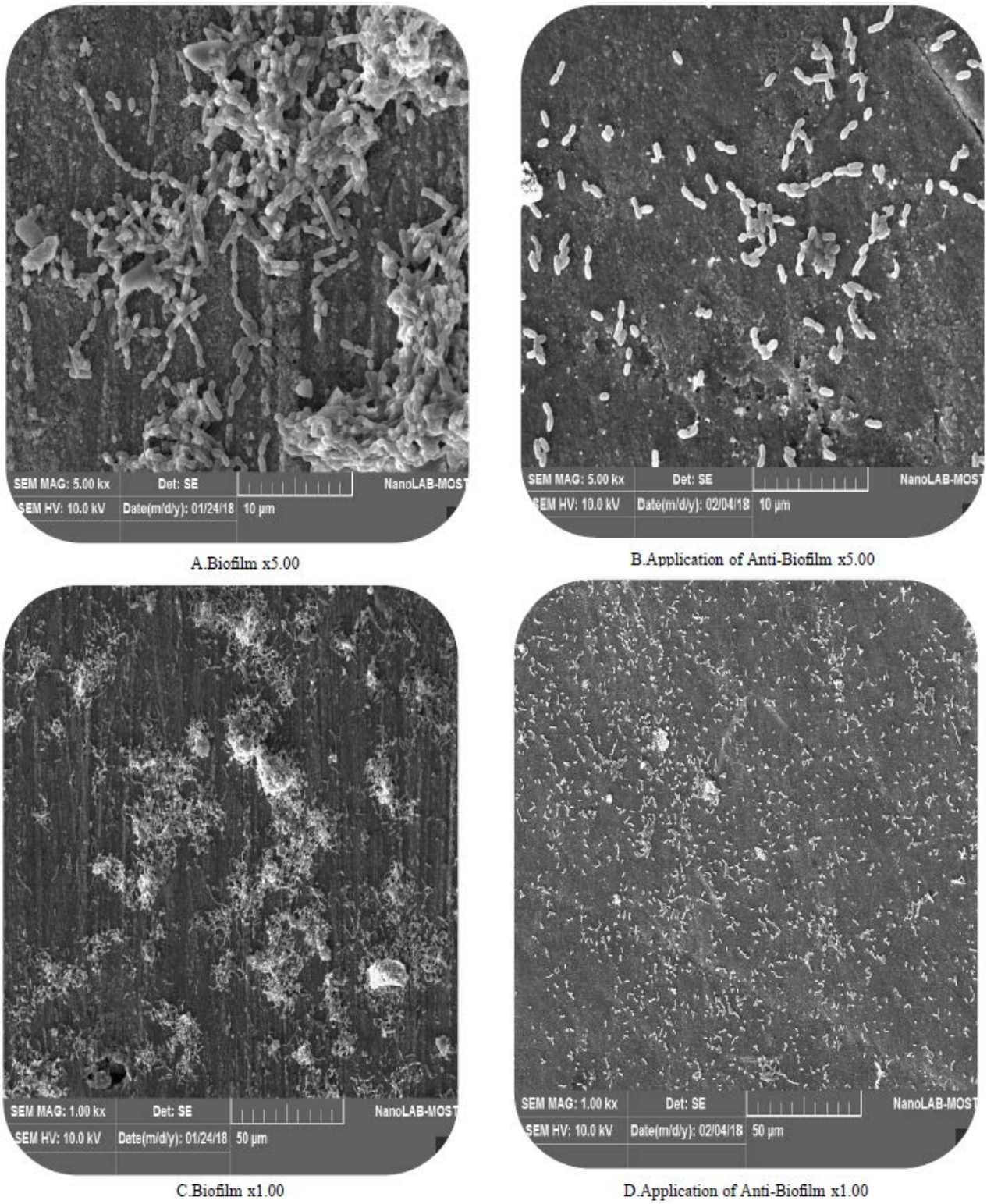

Figure 5. Biofilms and Application of Antibiofilms of *Facklamia hominis* and *Streptococcus mutans* on Composite surfaces

Filling material surface charge greatly influences bacterial adherence. Most bacteria exhibit a negative surface charge in an aqueous environment. Therefore, a negatively charged filling material surface should lead to decreased adherence of microorganisms due to a repulsion effect between both negatively charged surfaces [38].

In conducting materials, like amalgam, electron-transfer plays an important role in bacterial adhesion. [39] This can be due to attractive forces between the negatively charged bacteria and positive charges in the conducting material. This cannot occur in a non-conducting material (e,g, resin composite) [40].

3. Anti-bacterial Effects of Dental Materials

A. Leonhardtt, *et al* 1995 proposed that the different kinds of materials may not have any antibacterial properties in vivo, and the colonizing bacteria has a similar composition regardless of material type [41].

This opinion was refused by another school who claimed that dental materials have different effects on bacteria. Several studies have shown that amalgam alloys

have a bacteriostatic effect [42,43,44]. However, it is possible that bacteria develop resistance against mercury (which is part of dental amalgam). *In vitro*, more bacteria resistant to mercury were found in oral biofilms grown on amalgam than on enamel. [41] According to study that performed by Ready *et al* 2007, 98% of mercury resistant bacterial strains isolated, were streptococci [45].

Opposite to our study, Bernardo *et al* 2007, proposed that certain component of resin (methacrylate polymers) encourages the growth of microorganisms. [44] Hansel *et al* 1998 suggested that the release of ethyleneglycol dimethylacrylate and triethyleneglycol dimethacrylate from composite resins may enhance the growth of mutans streptococci and lactobacilli which were found mostly along the margins of composite fillings [45].

5. Conclusions

The focus of the current study was antibiofilm activity which represents an important outcome of good oral hygiene control. It may be particularly advantageous when mechanical oral hygiene measures are not enough. Results from this study have proved that flourdated toothpaste formulation remains a gold standard as far as antimicrobial efficacy is concerned. This paste can remove the biofilms associated with the bacteria of dental caries so, it can prevent primary caries on permanent and deciduous tooth surfaces. Notably, it can also prevent secondary caries on composite filling material surfaces and to lesser extent on amalgam surfaces.

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