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SeNPs/PLGA Inhibits Biofilm Formation by Cultivable Oral Bacteria Isolated from Caries-Active Children

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A B S T R A C T

Total of 523 bacterial isolates were collected from studied children groups (7-10 years); caries-active (*n=50*), caries-free (*n=50*). From caries-active children, 325 isolates have belonged to 22 bacterial species, whereas, in caries-free children, 198 bacterial isolates have belonged to 18 species. All isolated bacterial species were fit in 6 different bacterial genera included *Actinomyces*, *Lactobacillus*, *Porphyromonas*, *Prevotella*, *Streptococcus* and *Veillonella*. The predominant bacteria were *Streptococcus mutans*(96%) and *Str. orali*(56%) in caries-active children. In caries-free children, the most frequently detected species included *Lactobacillus acidophilus* (72%) and *Str. oralis* (36%). Partial sequence of the 16S rRNA gene was determined for confirming the identification of the 22 strong-biofilm bacterial isolates. The minimum inhibitory concentration (MIC_{90}) of Selenium nanoparticles (SeNPs) was 25 µg/mL against most of strong-biofilm bacteria. Sub-MIC_{90} (25 µg/mL) of SeNPs were incorporated in Poly lactic-co-glycolic acid (PLGA) to fabricate coating surface. PLGA/SeNPs coating surface showed potential reduction effect against biofilm formation by the 22 strong-biofilm strains. In conclusion, SeNPs can be used as a promising agent for effectively preventing biofilm formation by strong-biofilm bacteria related to dental caries.

Introduction

The oral cavity of healthy individuals contains hundreds of different commensal bacterial species that can become pathogenic as a result of the environmental variations and hygienic status of the individuals (Avila *et al*., 2009). Dental caries and periodontal disease are two of the most troublesome deceases of people worldwide. The disease process may involve enamel, dentin and cement, causing decalcification of these tissues and disintegration of the organic substances (Karpiński *et al*., 2013). Caries can be caused by different species of bacteria including *Streptococci* group such as *Streptococcus mutans, S. mitis, S. anginosus, S. salivarius, S. intermedius, S. gordonii* etc. in addition to

Enterococcus faecalis, *Actinomyces naeslundii*, *A. viscosus*, *A. gerencseriae*, *A. odontolyticus*, *Rothia dentocariosa*, *Propionibacterium*, *Prevotella* , *Veillonella*, *Bifidobacterium* and *Scardovia* (Karpiński *et al*., 2009; Liljemark *et al*., 1993; Tahmourespour *et al*., 2013; Tang *et al*., 2003). In preschool children, *A. odontolyticus*, *A. naeslundii* and *A. gerencseriae* have been reported to play an important role in supragingival plaque formation with other bacteria (Tang *et al*., 2003). Examine bacterial diversity of oral microbiota in saliva and supragingival plaques from 60 children aged 3 to 6 years old with and without dental caries from China (Ling *et al*., 2010). They added that, the genera of *Streptococcus* , *Veillonella*, *Actinomyces* , *Granulicatella*, *Leptotrichia*, and *Thiomonas* in plaques were significantly associated with dental caries. In children under 3 years old, Tanner *et al.*, (2002) indicated that a wide range of species, including *S. mutans* and putative periodontal pathogens can be detected. They suggested also that, the tongue serves as a reservoir for tooth-associated species, where species detection frequencies were higher in tongue of the younger children compared with tooth samples. In Saudi Arabia, high prevalence of dental caries amongst the 6-year-olds has been reported (Bhayat *et al*., 2013). This high prevalence of dental caries had a strong correlation with high mutans *Streptococci*, high lactic acid bacteria, and low saliva buffering capacity.

In general, it is believed that the initiation of caries is mainly caused by mutans *Streptococci*, especially, *S. mutans*, whereas the genus *Lactobacillus* is implicated in the further development of caries, especially in the dentin (Klein *et al*., 2015). *Streptococci* and *Actinomyces* are the major initial colonizers of the tooth surface, and the interactions between them and their substrata help establish the early biofilm community (Kolenbrander *et al*., 2000). Bacteria responsible for initiation of caries need to attach to and colonize on tooth surface. These bacteria are characterized by their ability to produce extracellular polymers such as exopolysaccharides (EPS), eDNA, and lipoteichoic acid (LTA) that help cells to attach to tooth and to form biofilm. This biofilm provides several advantages to the bacteria involved in its formation and for the other bacteria in the same environment. More information about bacterial interactions in dental biofilm and different strategies for control this biofilm are available in elsewhere (Huang *et al*., 2011; Chandki *et al*., 2011; Jhajharia *et al*., 2015). In this respect, nanoparticles of metals (*i.e.*, selenium, silver and zinc) and antimicrobial polymers have gained significant interest over the years due to their remarkable antimicrobial and antibiofilm properties (Melo *et al*., 2013).

Because of its biocompatibility, biodegradability, flexibility and minimal side effects, the polymer poly lactic-co-glycolic acid (PLGA) can be engineered to suit a range of medical applications (Xiong *et al*., 2014). Specifically, PLGA materials are also developed for the dental field in the form of scaffolds, films, membranes, microparticles or nanoparticles (Virlan *et al*., 2015). The recent uses of PLGA in the dental field and the relation between different dental fields have been described in the work of Virlan *et al*., (2015). The aim of the present work is to (i) isolate and identify the cultivable bacteria from caries-active and caries-free children, (ii) assay biofilm forming ability of the isolated bacteria, (iii) use of selenium nanoparticles incorporated in PLGA as antibiofilm agent against strong-biofilm strains.

Materials and Methods

Sample collection: To increase the accuracy of utilized methodologies to achieve the project aim, two extreme phenotypes (with

and without caries experience) were included in the study groups of the present work. First study group (Group I) consisted of unrelated 50 Saudi children (7-10 years old) with caries experience in comparison with equal number without caries experiences as control group (Group II)) with similar demographic and social characters. The clinical examination was processed for all subjects, using a sterile dental probe and mirror, in light of a dental lamp. Revised infection control guideline was followed to protect the research team and patients. Subjects with no clinical signs of oral mucosal disease were included in the study and had not used antibiotics for the last 6 months. Bacterial samples for microbiological analysis were collected by gently rubbing epithelial and dental surfaces for1 min with sterile cotton-swabs moistened with sterile distilled water. Immediately after sample collection, the swabs were stored in sterile test tubes containing 1 mL of phosphate buffered saline. Using a sterile pipette, 0.1 mL of each sample was spread onto Tryptic soy (Difco, Detroit, MI, USA) agar surface using a sterile bent glass rod. After seeding, the Petri dishes were incubated at 37 °C for 48 h in anaerobic jars(Sigma-Aldrich; with anaerobic atmosphere generation bag, product No. 68061).

Identification of bacteria

Colonies that developed were preliminary characterized by some physiological and biochemical tests according to the criteria of Bergey's Manual of Systematic Bacteriology. The studied characteristics were morphology of colony, cell shape, Gram reaction, catalase and oxidase activity, sporulation, and cell motility. According to the first screening, total of 523 bacterial isolates were subjected for phenotypic identification using BIOLOG™ GEN III system (Hayward, CA, USA) according to the manufacture's constructions. New GEN III MicroPlate™ test panel of the Biolog system provides a "Phenotypic Fingerprint" of the microorganism, which can then be used to identify them to a species level. This method enables testing of Gram-negative and Gram-positive bacteria in the same test panel. The test panel contains 71 carbon sources and 23 chemical sensitivity assays. GEN III dissects and analyzes the ability of the cell to metabolize all major classes of compounds, in addition to determining other important physiological properties such as pH, salt and lactic acid tolerance, reducing power, and chemical sensitivity. All the reagents applied were from Biolog, Inc. Fresh overnight cultures of the pure isolates were tested. Bacterial suspensions were prepared by collecting bacterial colonies from the plate surface with a sterile cotton swab and agitating it in 5 ml of 0.85% saline solution. Bacterial suspension was adjusted in IF-0a to achieve a 90–98% transmittance (T90). 150 µL of the suspension was dispensed into each well of a Biolog GEN III microplate. The plates were incubated at 37°C in the presence of 7.5% CO_2 for 20 h. After incubation, the phenotypic fingerprint of purple wells is compared to the Biolog's extensive species library (GEN III database, version 5.2.1).

Working stock cultures were maintained at -70°C in 15% v/v glycerol/tryptone soy broth (TSB). For routine work, strains were cultivated on TSB agar (in the presence of 7.5% CO_2) and stored at 4°C on slants.

Identification by 16S rRNA gene sequencing

Partial sequence of 16S rRNA gene was determined for confirming the identification of isolates that displayed strong-ability to form biofilm (22 isolates, as described below). Isolation of genomic DNA from was done by QIAGEN FlexiGene DNA Kit. The PCR-amplified 16S rDNA fragments were

amplified using two universal primers; forward: 5' agagtttgatcctggctcag 3'; reverse: 5' acggctaccttgttacgactt 3' (19). The reaction mix was composed of × µl Template DNA, 2 µlBigDye-Mix, 1 µl primer (10 µmol l^{-1}), and HPLC water to a final volume of 10 µl. The amount of template DNA applied was dependent on the concentration of target sequences to obtain about 10 ng DNA in the final mix. The PCR program was as follows; initial denaturation at 96 ºC for 2 min (1 cycle), denaturation at 96 ºC for 10 s (30 cycles), annealing at 45 ºC for 5 s (30 cycles), extension at 60 ºC for 4min (30 cycles), and then cooling at 4 ºC. The PCR product was purified as recommended by the manufacturer and then sequenced. PCR fragments were analyzed by cycle sequencing, using the BigDye terminator cycle sequencing kit (Applied Biosystems, UK). These fragments were sequenced in both directions using universal primers 518F and 1513R. The consensus sequences were then used to compare with online databases (NCBI BLAST-http://blast.ncbi.nlm.nih.gov/Blast. cgi). The obtained partial sequences were deposited to EMBL/ GenBank /DDBJ databases.

Biofilm formation

The commonly used microtiter plate method for determining bacterial adhesion to plastic surface was applied in the present study (Rode *et al.*, 2007). Briefly, the wells of sterile 96- well polystyrene microtiter plates (Falcon plastics; Becton Dickinson Labware) were filled with 230 mL of TSB. About 20 mL of each cell culture was added into each well (eight wells for each strain). Plates were incubated under static conditions at 30°C for 48 h. The negative control wells contained TSB only. The contents of the microtiter plates were poured off and the wells were washed three times with 300 µL of phosphate-buffered saline (PBS, pH 7.2). The remaining attached bacteria were fixed with 250 µL of methanol per well. After 15 min, microtiter plates were emptied and air-dried. The microtiter plates were stained with 250 µL per well of 1% crystal violet used for Gram staining (Merck) for 5 min. The excess of stain was rinsed off by placing the microtiter plates under running tap water. After drying the plates, absorbance at 570 nm (A_{570}) was measured by using ELISA reader. Based on the absorbance (A_{570} *nm*) produced by bacterial films, strains were classified into four categories according to the classification of Christensen *et al.*, (1985), with modification by Stepanović *et al.*, (2000). Briefly, the cut-off absorbance (Ac) was the mean absorbance of the negative control. Strains were classified as follows: $A = Ac$ = non-biofilm producer (0); $Ac < A \leq (2 \times Ac)$ = weak-biofilm producer (+); $(2 \times Ac) < A \leq (4 \times Ac)$ = moderate-biofilm producer (++); $(4 \times Ac) < A$ = strong-biofilm producer (+++). All tests were carried out in triplicates and the results were averaged. For statistical analysis, three independent experiments were carried out.

Antimicrobial activity of SeNPs

SeNPs were tested for minimum inhibitory concentration (MIC_{90}) by microtiter broth dilution method as described by (Khiralla *et al.*, 2015). The final tested concentrations of SeNPs were 0, 10, 15, 20, 25 and 30 µg/ mL. The MIC_{90} was defined as the lowest concentration of SeNPs, which inhibited 90% of the growth when compared with that of the positive control. All tests were carried out in triplicate ($n=3$) and the results were averaged.

Control of biofilm using SeNPs

Selenium nanoparticles (SeNPs) have been previously biosynthesized and characterized in laboratory of molecular and applied microbiology at Taif University (15). It has

been characterized as spherical shape with diameter range of 10–50 nm and a well-defined absorption peak at 263 nm in UV–vis spectra.

In the present study, SeNPs was used as antibiofilm agent in combination with Poly (lactic-co-glycolic acid) (PLGA; MW, 40 000 Da; Sigma-Aldrich, St Louis, MO, USA) against the strong biofilm forming bacteria. Briefly, SeNPs (final concentration of 20 µg/mL) were mixed into 2% PLGA dissolved in chloroform.

For fabricate a coating containing SeNPs as biofilm inhibitor, the 96-wells microtiter plates were filled with the mixture of SeNPs/PLGA. Microtiter plates were converted on paper to remove the excessive mixture and then were air-dried for 1 h and sterilized by UV exposure for 4 h.

Biofilm assay was carried out as mentioned above. Control experiment was done using microtiter plate coated with PLGA without SeNPs. All 22 strong biofilm strains were tested in triplicate (3 wells). Values were averaged and categorized as described above to no-, weak-, moderate- strong- biofilm producer.

Statistical analysis

The data obtained from three replicates were analyzed by a one-way ANOVA using 'Proc Mixed' available in SAS, version 8.2 (SAS Institute Inc., Cary, NC, USA) to investigate the antimicrobial effect of different SeNPs concentrations on each bacterial strain.

The same analysis method was used to study the antibiofilm effect of SeNPs and SeNPs/PLGA on each strain. In all cases, these tests were followed by Duncan's multiple range test at probability level = 0.05 to compare the significant differences between the mean numbers.

Results and Discussion

Cultivable microbiota of caries-active and caries-free children

Two children groups aged 7-10 years were subjected to determine the microbiota in the present study. These groups are: caries-active (Group I, *n=50*), caries-free (Group II, *n=50*). Total of 523 bacterial isolates were collected from studied children groups (Table I) and identified using Biolog system. From caries-active children, 325 isolates have belonged to 22 bacterial species, whereas, in caries-free children, 198 bacterial isolates have belonged to 18 species (Table I). All isolated bacterial species were fit in 6 different bacterial genera included *Actinomyces* , *Lactobacillus, Porphyromonas, Prevotella* , *Streptococcus* and *Veillonella* (Figure 1). The predominant bacterial genera detected in caries-active children were *Streptococcus* (171 isolates), *Actinomyces* (78 isolates) and *Prevotella* (52 isolates), whereas in caries-free children were *Streptococcus* (80 isolates), *Actinomyces* (44 isolates) and *Lactobacillus* (44 isolates). Moreover, in both studied groups, *S. oralis* was detected as the second predominant bacterium (Table 1). In general, the detection level of all isolated bacterial species in caries-active group was higher than those of active-free children, except *Lactobacillus acidophilus* and *Prevotella denticola* (Table.1). Four bacterial species, *Prevotella intermedia, P. melaninogenica, P. nigrescens* and *Streptococcus pneumoniae*, have not been isolated from caries-free children.

Isolation frequency was detected and calculated as positive percentage of the studied subjects in each group (*n=50*) and presented in figure 2. In caries-free children, the most frequently detected species included *Lactobacillus acidophilus* (72%) *Str. oralis* (36%) and *Str. mutans* (24%). The lowest isolation frequency (4%) was recorded for *Porphyromonas gingivalis* and *Str.*

pneumoniae in caries-active children and for *P. gingivalis*, *S. constellatus* and *S. gordonii* in caries-free children (Fig. 2).

Biofilm formation

Biofilm forming ability of 325 bacterial species isolated from caries-active children (7–10 years) was evaluated using 96-well microtiter plate technique (Table.3). According to the obtained results, the 325 isolates were classified into non- (40 isolates), weak- (139 isolates), moderate- (124 isolates) and strong- (22 isolates) biofilm producers (Table.2). Partial sequence of the 16S rRNA gene was determined for confirming the identification of the 22 strong-biofilm bacterial isolates. The obtained sequences of the 22 strains were deposited to the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries. The accession numbers in addition to coverage and similarity percentage of the submitted sequences were illustrated in table.3.

Out of 78 *Actinomyces* spp. only 3 *A. naeslundii* showed strong ability to form biofilm and the other *Actinomyces* isolates were ranged from weak- to moderate- biofilm producers (Table II). Among 52 *Prevotella* spp. one isolate of *P. denticola* and two of *P. intermedia* were recognized as strong biofilm producers (Table.2).

Table.1 Numbers of bacterial species isolated from caries-free and caries-active children aged 7-10 years

Bacterial species	Caries-active *n=50*	Caries-free *n=50*	Total
Actinomyces georgiae	16	6	22
Actinomyces gerencseriae	18	8	26
Actinomyces meyerii	14	12	26
Actinomyces naeslundii	12	10	22
Actinomyces odontolyticus	18	8	26
Lactobacillus acidophilus	4	44	48
Porphyromonas gingivalis	8	2	10
Prevotella denticola	14	20	34
Prevotella intermedia	10	0	10
Prevotella melaninogenica	8	0	8
Prevotella nigrescens	20	0	20
Streptococcus constellatus	14	2	16
Streptococcus gordonii	8	2	10
Streptococcus intermedius	16	10	26
Streptococcus mitis	22	8	30
Streptococcus mutans	48	12	60
Streptococcus oralis	30	22	52
Streptococcus pneumoniae	2	0	2
Streptococcus salivarius	12	12	24
Streptococcus sanguinis	11	8	19
Streptococcus sobrinus	8	4	12
Veillonellaparvula	12	8	20
Total isolated species	325	198	523

Table.2 Biofilm forming ability of bacterial species isolated from caries-active children aged 7-10 years

Bacterial species	Counts	Biofilm formation			
		Non	Weak	Moderate	Strong
Actinomyces georgiae	16	0	9	6	0
Actinomyces gerencseriae	18	0	12	6	0
Actinomyces meyerii	14	0	6	8	0
Actinomyces naeslundii	12	0	5	4	3
Actinomyces odontolyticus	18	0	11	7	0
Lactobacillus acidophilus	4	0	2	1	1
Porphyromonasgingivalis	8	2	2	4	0
Prevotella denticola	14	4	5	4	1
Prevotella intermedia	10	0	5	4	2
Prevotella melaninogenica	8	1	3	4	0
Prevotella nigrescens	20	7	9	4	0
Streptococcus constellatus	14	2	4	8	0
Streptococcus gordonii	8	0	3	5	0
Streptococcus intermedius	16	4	6	6	0
Streptococcus mitis	22	0	11	8	3
Streptococcus mutans	48	10	17	15	6
Streptococcus oralis	30	6	12	11	1
Streptococcus pneumoniae	2	0	1	0	1
Streptococcus salivarius	12	0	6	3	3
Streptococcus sanguinis	11	0	4	6	1
Streptococcus sobrinus	8	0	2	6	0
Veillonella parvula	12	4	4	4	0
Total species	325	40	139	124	22

Figure.1 Bacterial genera numbers detected in caries-active and caries-free children

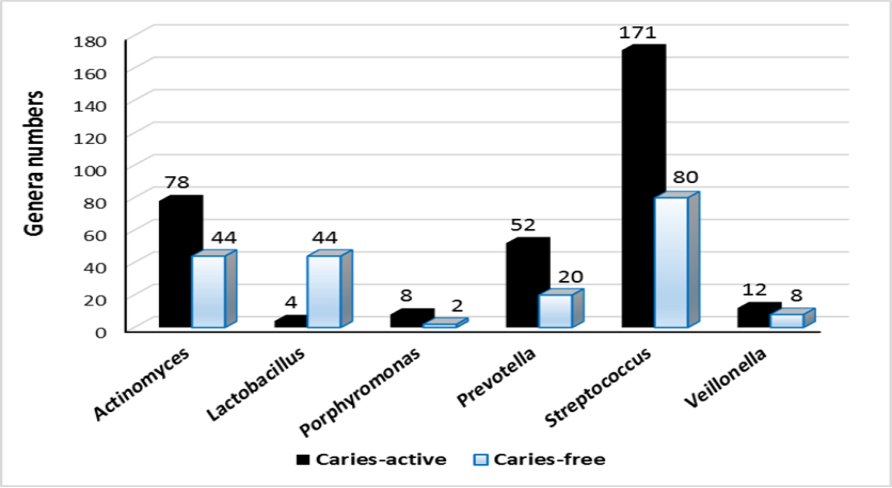


Table.3 Molecular identification* and accession numbers of the strong biofilm forming bacterial species isolated from caries-active children aged 7-10 years

Strains*	Sequence length (nt)	% coverage	% identity	Accession number
Lactobacillus acidophilus HAC01	1184	99	99	LT707607
Streptococcus oralis HAC02	1108	99	99	LT707608
Streptococcus mutans HAC03	1120	99	99	LT707609
Streptococcus mutans HAC04	1295	99	99	LT707610
Streptococcus mutans HAC05	1198	99	99	LT707611
Streptococcus mutans HAC06	1125	99	98	LT707612
Streptococcus mutans HAC07	1087	100	99	LT707613
Streptococcus mutans HAC08	1301	99	99	LT707614
Streptococcus mitis HAC09	1143	99	99	LT707615
Streptococcus mitis HAC10	1096	100	100	LT707616
Streptococcus mitis HAC11	1256	99	99	LT707617
Streptococcus salivarius HAC12	898	99	99	LT707618
Streptococcus salivarius HAC13	1166	100	100	LT707619
Streptococcus salivarius HAC14	1237	99	99	LT707620
Streptococcus sanguinis HAC15	1251	99	100	LT707621
Streptococcus pneumoniae HAC16	1108	99	100	LT707622
Prevotella denticola HAC17	971	99	99	LT707623
Prevotella intermedia HAC18	1260	99	99	LT707624
Prevotella intermedia HAC19	1312	99	99	LT707625
Actinomyces naeslundii HAC20	1103	100	99	LT707626
Actinomyces naeslundii HAC21	1382	99	99	LT707627
Actinomyces naeslundii HAC22	1168	99	99	LT707628

* identified by determining the partial sequence of 16S rRNA gene

Figure.2 Percentage of positive detection of isolated bacterial species in caries-active and caries-free children

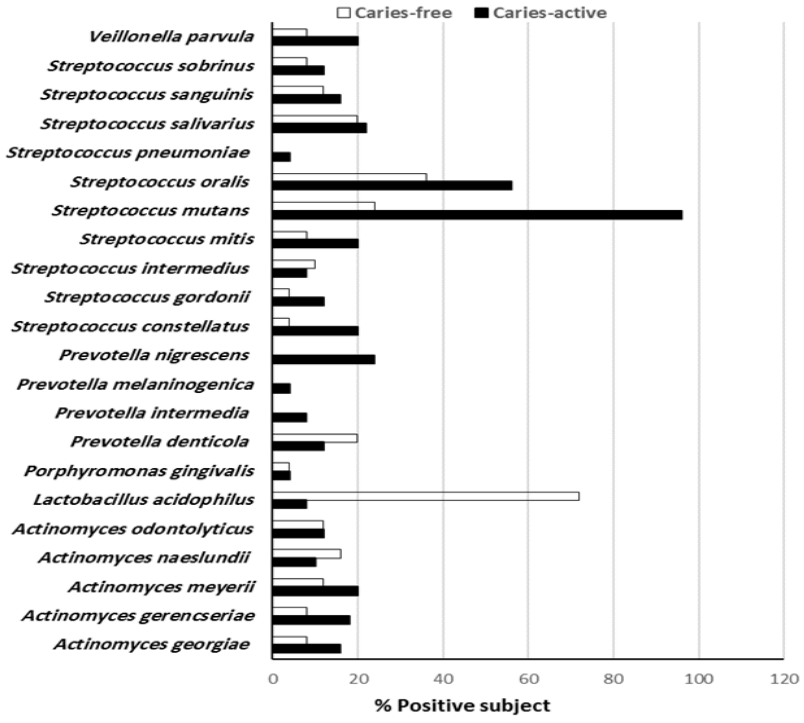


Figure.3 Antimicrobial effect and MIC_{90} of SeNPs against strong-biofilm forming bacterial strains. Values are means ±SD, *n = 3*. * denotes MIC_{90}. Columns with the same letter within each group are insignificantly different (*P > 0.05*)

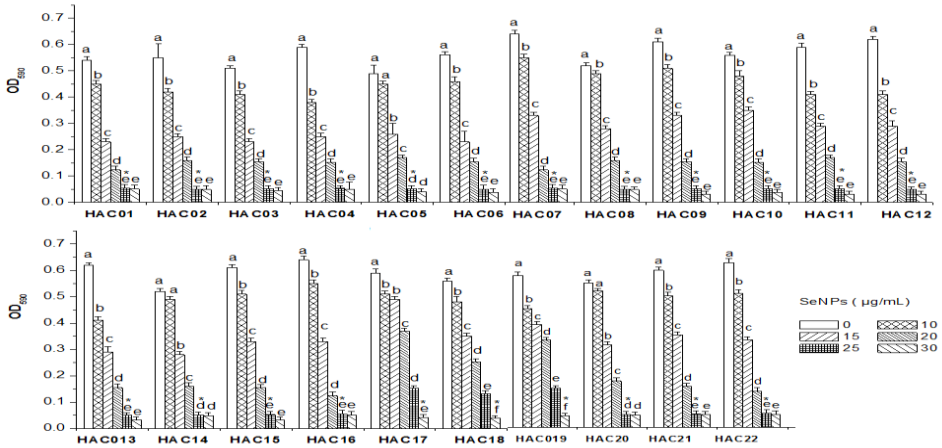
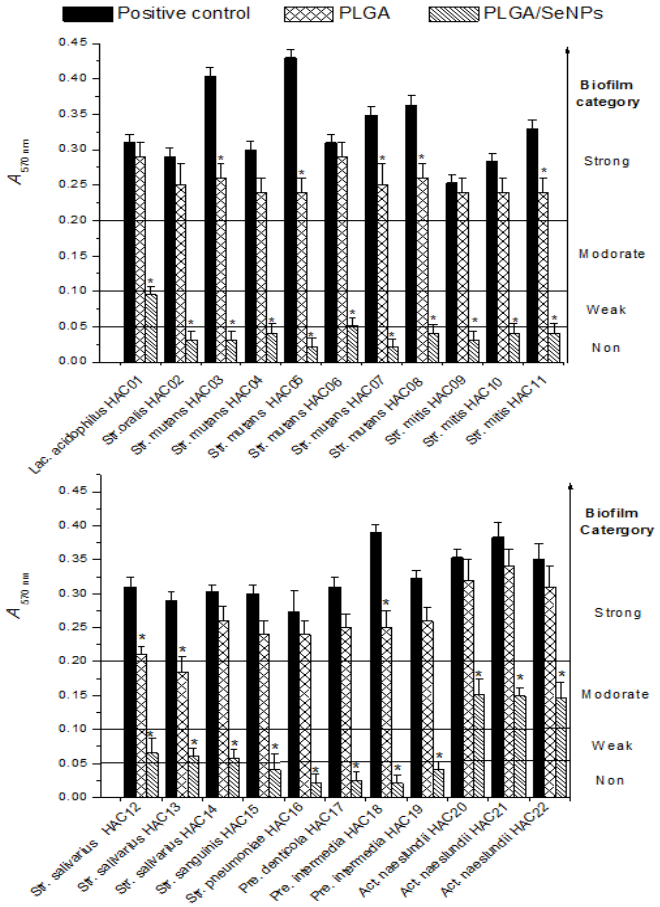


Figure.4 Antibiofilm effect of PLGA and PLGA/SeNPs against 22 strong biofilm forming bacteria isolated from caries-active children. Data represents mean ± SD, *n=3*. * denotes *p< 0.05*) within the same column-group



Concerning with *Streptococci* group, most *S. mutans* and *S. oralis* showed weak to moderate ability to form biofilm. Indeed, 6 isolates of *Str. mutans*; 3 isolates of *Str. mitis*, and *Str. salivarius*; 1 isolate of *Str. oralis*, *Str. pneumoniae* and *Str. sanguinis* displayed strong potential for forming biofilms (Table II).

Antimicrobial and antibiofilm effect of SeNPs

Selenium nanoparticles (SeNPs) have been previously biosynthesized and characterized in laboratory of molecular and applied microbiology at Taif University. The minimum inhibitory concentration (MIC_{90}) of SeNPs was 25 µg/mL against 19 Gram-positive strains (belonged to *Lactobacillus*, *Streptococcus* and *Actinomyces*) of the tested strong-biofilm bacteria (Fig. 3).

However, MIC_{90} of SeNPs was 30 µg/mL against the 3 Gram-negative strains belonged to the genus *Prevotella* (HAC17, HAC18 and HAC19). To avoid the effect of SeNPs on the growth of tested strains, sub- MIC_{90} dose (20 µg/mL) was selected to investigate the antibiofilm effect of SeNPs. The effect of PLGA and PLGA/SeNPs on the biofilm forming ability of the 22 strong-biofilm producers was studied. Although, PLGA had significant ($p<0.05$) effect on biofilm formation by some strains (8/22), these strains still in the category of strong-biofilm producer (Fig. 4). These strains were *L. acidophilus* HAC01, *S. mutans* HAC03, HAC05 and HAC08, *S. mitis* HAC11, *S. salivarius* HAC12 and HAC13, and *P. intermedia* HAC18. Dramatic reduction in the biofilm forming ability of all tested strains was noticed when SeNPs has been incorporated in PLGA coating material (Fig. 4). Out of 22 strong-biofilm producers, 15, 4 and 3 strains became non-, weak-, and moderate-biofilm producers, respectively.

Cultivable microbiota of caries-active and caries-free children

In accordance with the obtained results, several previous researches demonstrated that *Streptococcus* , *Actinomyces* and *Prevotella* have been associated with dental caries and played important roles in development of carries (Karpiński *et al*., 2013; Ling *et al*., 2010). Moreover, these genera have been commonly distributed not only in populations with moderate or high caries incidence but also in populations having no or low caries experience (Tahmourespour *et al*., 2013). By comparing the isolated bacterial species in caries-active and caries-free groups (Table 1), the predominant bacteria were *Streptococcus mutans* and *Lactobacillus acidophilus* in caries-active and caries-free children, respectively. In harmony with this finding, *S. mutans* strains had considerable interest and have been considered the main etiological agent of dental caries in humans (Huang *et al*., 2011). On the other hand, Corcuera *et al.*, (Corcuera *et al*., 2013) stated that, *Str. oralis* could be isolated from both health and infected mouth. This statement was in accordance with our finding in both studied groups, where *S. oralis* was detected as the second predominant bacterium (Table 1). In contrary of our finding, *P. gingivalis* are among the organisms that have been detected in subgingival plaque and have been implicated as the principal aetiological agents of periodontitis (Tompkins *et al*., 1997). This may be because we concern with the cultivable microbiota and this bacterium is uncultivable or extremely difficult to cultivate (Benkirane *et al*., 1995). The obtained results indicated that, 96% of caries-active children were positive for isolation of *Streptococcus mutans* followed by *S. orali*with percentage of 56%. Tanner *et al.,* reported that detection frequency of *Streptococcus* spp. were ranged from 40 to 78% in children aged 18–36 months. They added also that, *S. mutans* (with detection frequency of 70%) was the bacterial

species associated with caries in these children. The antagonistic effect of *L. acidophilus* against *Streptococcus* spp., especially *Str. mutans*, noticed in the present study could be explained by those mentioned by Tahmourespour *et al.,* (2011). They suggested that *L. acidophilus* can produce a biosurfactant that may interfere with adhesion processes of *S. mutans* to teeth surfaces. Moreover, Tahmourespour and Kermanshahi, (2011) demonstrated high effect of *L. acidophilus* against adherence of mutans *Streptococci* than non mutans *Streptococci*. They explained this adhesion-inhibitory effect as a result of bacterial interactions and colonization of adhesion sites with *L. acidophilus* strain before the presence of *Streptococci*.

Biofilm formation

Different bacterial species are distributed in the oral cavity and colonize certain sites over others according to the particular local environment those sites provide. *Streptococcus mutans* is well characterized by its ability to form different extracellular polymeric substances such as exopolysaccharides, eDNA, and lipoteichoic acid (Klein *et al*., 2015). These substances enable this bacterium to be one of the early colonizers on teeth and initiate the formation of biofilms. In the present study, most of isolated *S. mutans* (38 of 48 isolates) exhibited different degree of biofilm formation. Numerous studies have demonstrated biofilm formation in different sites of oral cavity by *S. Mutans* (10, 27-29). On the other hand, 20% of *S. oralis* isolated in the present work have no ability to form biofilm. In agreement with this finding, Corcuera *et al.,* (2013) stated that 12.1% of *Str. oralis,* isolated from gingival sulcus samples taken from patients with periodontal disease, have been considered as non-biofilm producers.

In a previous work, *A. gerencseriae, A. naeslundii* and *A. odontolyticus* have been reported to involved in the plaque formation on supragingival of primary teeth of children aged 3 to 4 years. Moreover, *A. naeslundii,* which displayed different biofilm formation patterns in the present study, has been described as one of the microbial flora that participate in the early forming and in maturation of microbial biofilms on tooth surfaces in human (Zijnge *et al*., 2010; Henssge *et al*., 2009). *Prevotella denticola* and *P. intermedia* have been previously isolated from subgingival biofilms of the infected sites (Socransky *et al*., 1998), however, there is no recent report on their role in early stage of biofilm formation. The synergy biofilm formation by *Prevotella* spp. and other bacteria such as *Fusobacterium nucleatum* (Okuda *et al*., 2012) and *Porphyromonas* (Henry *et al*., 1996) has been previously reported.

Antimicrobial and antibiofilm effect of SeNPs

Metal nanoparticles have different pattern of antimicrobial potential against Gram-positive and Gram-negative bacteria because of the differences in the cellular structure of both bacterial groups (Azam *et al*., 2012). The MIC_{90} of SeNPs obtained in the present study against Gram-positive bacteria was in agreement with the previously recorded MIC_{90} of the same nanoparticles. However, MIC_{90} of SeNP sagainst the 3 Gram-negative strains belonged to the genus *Prevotella* was higher than those was recorded against Gram-positive in the present study. This may be due to the presence of an extra layer of lipopolysaccharide and proteins (outer membrane), as a part of cell wall of Gram-negative bacteria (Guisbiers *et al*., 2016). In general, SeNPs applied in the present study showed high inhibition potential against Gram-positive bacteria compared with their

efficiency against Gram-negative bacteria. Similar statement has been previously described.

Thomas *et al.,* (2016) studied the efficacy of PLGA micro- and nanoparticles of ciprofloxacin against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms. They stated that these particles improved the efficiency of ciprofloxacin, however and in agreement with our results the blank PLGA particles did not affect the biofilm formation by both investigated bacteria. Incorporation of SeNPs in PLGA coating material displayed remarkable reduction in the biofilm forming ability of all tested strains. This finding demonstrated the antiadhesive and antibiofilm effects of SeNPs against caries-related bacteria, which had strong-biofilm forming potential. Different nanoparticles such as silver, copper-oxide, titanium-oxide, zinc-oxide, silicon-dioxide, etc. have been applied for controlling oral biofilm formation (Mohamed, 2012; Melo *et al*., 2016). For the best of our knowledge, there is no available scientific study on application of selenium nanoparticles to control of biofilm formation on dental field. Here, we suggested incorporating low concentration of SeNPs (20 µg/mL) in PLGA polymer for effective reduction of biofilm forming potential by all isolated bacteria. This reduction can be explained by the catalyse potential of selenium to form superoxide radicals and subsequently inhabitation of bacterial adhesion and viability (Tran *et al*., 2011). In addition, coating polymers (particularly, polycarbonate used for medical catheters) with nanostructured selenium is a fast and effective way to reduce formation of bacterial biofilm that lead to medical device infections (Wang *et al*., 2012).

Conflict of interest

The authors declare no conflict of interest.

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