Microbial Biofilm Formation as a Dental-fouling Microorganisms Attachment Phenomenon in Oral Cavity Environment

Hussein Ali Sabtie, Inaam Noori Ali and Yassmin Raad Humudat

Department of Biological Treatment Researches/Water Researches Centre/Environment and Water Directorate/Ministry of Science and Technology, Baghdad 10001, Iraq

Received: January 16, 2014 / Accepted: January 28, 2015 / Published: January 30, 2015.

Abstract: DMFP (Dental microbial fouling phenomenon) of OCE (oral cavity environment) was studied in the local dental labs. Multi-smears of mature supra gingival plaques were randomly collected from fifty persons in dental labs that had different cultures, ages, sex and different scientific levels. Rapid microbial identification method was used to identify of these multi-smears by using vitek-2 compact instrument. The most frequent and appearance species of bacteria was *Pantoeae* spp., then *Klebsiella pneumonia*. Total species of microbial fouling in supra gingival plaques were included sixteen species: *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pantoeae* spp., *Aerococcus viridians*, *Leuconostoc mesenteroides cremorise*, *Granuli.adiacens*, *Streptococcus sanguinis*, *Staphylococcus sciuri*, *Stenotrophomonas maltiphilia*, *Kocuria varians*, *Gememlla morbillorum*, *Granulicatella adiacens*, *Nonreac biopattern*, *Kocuria kritinae*, *Kocuria rosea* and *Nonreac.biopattern* while six fungal species were *Trichoderma viride*, *Aspergillus flavus*, *Trichoderma harzianum*, *Cladosporium macrocarpum*, *Penicillium citrinum* and *Verticillium lecanii* in addition to two yeast species were recorded *Candida dubliniensis* and *Candida glabrata*. The results were detected the most appearance of bacteria *Pantoeae* sp., then *Klebsiella pneumonia*, then *Staphylococcus aureus*. These results of study suggest that adhesive strength conjugated with the biofilm layers presence in OCE may vary across different from one to other persons and mark a new path of inquiry for dental Microbial-fouling phenomenon research.

Key words: Dental, microbial, biofilm, plaques, fouling.

1. Introduction

The OCE (oral cavity environment), like other parts of the gastrointestinal tract, possesses normal microflora and other invasive microbes from both clean and polluted external environment. The OCE is the gate of the gastro-intestinal tract, many diseases of OCE may affect the integrity of mucous cover of OCE and the remaining portions of the gate of the gastro-intestinal tract [1, 2]. It has a number of features that makes it a unique microbial habitat. Teeth surfaces characteristically provide hard non-shedding surfaces that allow accumulation of

large microbial communities of microorganisms and attached on it (dental plaque), especially in stable areas in the OCE. Such accumulation is restricted on mucosal surfaces due to continuous epithelial desquamation, the only exception is the dorsum of the tongue that is highly papillated and thus supports higher densities of microbes [3]. Another important feature is that the oral cavity is continuously bathed with saliva, which has a profound effect on the ecology of the mouth. Saliva has a pH range (6.75-7.25) that favors growth of many microorganisms. Salivary components influence oral microbes by one of four mechanisms: aggregating microbes to facilitate their clearance from the mouth, adsorbing to teeth surface to form an acquired pellicle

Corresponding author: Hussein Ali Sabtie, Ph.D., Chairman of this scientific team work, research field: ministry science and technology. E-mail: sabtie_59@yahoo.com.

to which microorganisms can attach, serving as a primary source of nutrients, and mediating microbial inhibition or killing [4].

The oral cavity is not a homogenous environment due to personality of main changes. There are differences among sites in key ecological factors like adhesion ligands, pH, nutrients, redox potential, oxygen and temperature metabolism of the species that occupy a particular habitat may modify the surrounding environment, make it suitable for other species to colonize. For example, early colonizers will deplete oxygen, lowering the redox potential and therefore providing a suitable environment for anaerobes [3].

Oral microbes are predominantly bacteria but fungi, viruses, mycoplasmas and even protozoa and archaea can also be found [3, 5]. Dental plaque can be defined as the diverse community of microorganisms found on the tooth surface as a biofilm, embedded in an extracellular matrix of polymers of host and microbial origin [6]. The new definition of a biofilm by Donlan et al. [7] can be adopted to redefine dental plaque as a microbially derived sessile community characterized by cells that are irreversibly attached to the tooth surface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. Dental biofilm is primarily composed of microorganisms; one

gram of wet plaque contains approximately $2 \times 1,011$ bacteria. The intercellular matrix account for 20-30% of the biofilm mass, and is principally made up of polysaccharides of microbial origin (glucans and fructans) [8]. This study aimed to detect Dental microbial fouling phenomenon presence in oral cavity environment that continuously adhering on teeth inner and outer surfaces as a result of inner microbial pollution adherence due to dental plaque and teeth grooming.

2. Materials and Methods

2.1 Specimens Sampling

Swabs were taken from the teeth wildly by fifty samples of employees of the ministry of science and technology in this study during 28/11/2011 to 15/4/2012. Both sexes were included the numbers of sixteen males and thirty-four females. The person's ages were between twenty to fifty-six years (Fig. 1). Characterized samples taken from associate of being taken from people who have different scientific levels as well as the personal characteristics which are included different types of drinks per day, the state of mouth teeth decay , the daily of washing processes and number times cleaning teeth per day.

2.2 Microbial Identification

2.2.1 Bacterial and Yeasts Identification

Bacterial species (species gram positive and gram

Fig. 1 The sampling ratio of men to women specimens of OCE.

negative) and yeasts species were identified by using the Vitek 2 Compact equipment to apply the rapid microbial microorganisms detection that attached to the hard surfaces of teeth enamel and make dental plaque. Examination cards were used as which this device is considered one of the best devices to identify the types of microorganisms that diagnoses microbiology duration of two to eight hours a detection method fast compared with conventional methods old consume a lot of material and circles and directories as well as the effort to complete, and is considered one of the best devices to identify the types of microorganisms within a short period and accurately, the device development company Biomerieux French will select the type of microorganisms through using the specific kits within a short period and accurately from company Biomerieux French. Fungal species were identified by traditional methods according to Alexopoulos, et al. [9].

2.2.2 Environmental Factors

Some of environmental factors were measured that included pH, oral temperatures and DO (dissolved oxygen) (mg/L).

2.3 Media Culturing

Bacterial and yeasts species were identified by using the Vitek 2 Compact equipment to know the microbial organisms attached to the hard surfaces of the dental enamel and dental plaque. Most of microbial organisms, bacterial species gram positive and gram negative and yeast cells were tested by this equipment.

3. Results and discussion

Oral cavity environment especially the dental habitats have many microbial types that recorded in this study included different bacterial species, fungi species and yeast species (Tables 1-3). Nowadays the study suggesting the inflammation of the oral cavity caused by different in its prevailing microbes which recording frequent bacteria, fungi and yeast species [1]. Two *Candida* species were recorded in oral cavity that

adhere on teeth which consider the prevalent species in it (Table 3) [10].

The best three serials of bacterial species that recorded *Pantoeae* spp. and *Klebsiella pneumonia* which recorded in most times for different samples while the frequent of other bacterial species ranged from 1-6 times (Table 1). While some of the bacteria in their mouths are harmful and can cause serious diseases, much of their oral bacterial species are actually beneficial in preventing diseases. Bacterial *Streptococci* make up a large part of oral bacteria that are recorded in an international studies. There are four main species within streptococci: the mutans, salivarius, anginosus, and mitis groups. *Streptococcus mutans* make up a large majority of what affects their teeth in oral cavity. The polluted environment of oral cavity for people has many epidemic types of numerous microbes such as bacteria, fungi, protozoa and yeasts. Most of the oral microbial population is bacteria, some of these produce acid from dietary sugar, which results in teeth cavities and some of them are involved in the formation of tartar and calculus deposits on teeth. In this study, the predominant microbial species from dental sites are reflected different healthy sites and diseased sites, although these states related to personality status either putative pathogens or healthy culture sites. Table 1 showed the percent frequency for bacteria in teeth of persons which appeared that the higher frequency rate of bacteria is *K. pneumonia* at percent (12%) then *Aero. viridians* at percent (8%), while *Staph. aureus* and *G. adiacens* recorded 6% frequency rate.

Microbes fouling attachment recorded their different presence on the mina hard surface of person's teeth. These results are distributed on main three microbial groups included bacteria, fungi and yeasts. Numerous factors of microbe's distribution depend on the differences of the persons' nature, teeth roughness, cultures, scientific levels, daily smoking, types of drinking, daily teeth cleaning and other environmental factors of OCE. Microscopic examination of early

Table 1 Bacterial species that isolated from oral specimens.

No	bacterial species	GP, GN	repetition No.	percent frequency (%)
1	<i>Pantoeae</i> spp.	GP	3	20
2	<i>Klebsiella pneumonia</i> ,	GN	6	12
3	<i>Staphylococcus aureus</i>	GP	10	6
4	<i>Aerococcus viridians</i>	GP	4	6
5	<i>Leuconostoc mesenteroides cremorise</i>	GP	2	4
6	<i>Staphylococcus sanguinis</i>	GP	1	4
7	<i>Granuli.adiacens</i>	GP	3	4
8	<i>Slashline</i>	GP	2	4
9	<i>Stenotrophomonas maltiphilia</i>	GN	1	4
10	<i>Staphylococcus sciuri</i>	GP	1	2
11	<i>Kocuria varians</i>	GP	1	2
12	<i>Gememlla morbillorum</i>	GP	1	2
13	<i>Granulicatella adiacens</i>	GP	1	2
14	<i>Kocuria kritinae</i>	GP	1	2
15	<i>Kocuria rosea</i>	GP	1	2
16	<i>Nonreac.biopattern</i>	GP	1	2
17	No presence infection state	*	11	22
Total	17		50	100

Table 2 Fungal species that isolated from oral specimens.

No	fungi species	repetition No.	percent frequency (%)
1	Non-microbial infections presence	28	56
2	<i>Tricoderma viride</i>	1	2
3	<i>Aspergillus flavus</i>	3	6
4	<i>Tricoderma harzianum</i>	7	14
5	<i>Cladosporium macrocarpum</i>	2	4
6	<i>Penicillium citrinum</i>	2	4
7	<i>Verticillium lecanii</i>	2	4
Total	7	50	100

Table 3 Yeast species that isolated from oral specimens.

No	yeast species	age	sex	percent frequency (%)
1	<i>Candida dubliniensis</i>	42	*	6
2	<i>Candida glabrata</i>	43	*	4

plaque formation on teeth showed the adhesion of the initial bacterial colonizing along cracks and pits in enamel, basically shares the microbial aggregation seriatim on teeth surfaces which caused the microbial adhesion on teeth surfaces of oral cavity environment. Twenty-four female's specimens were not recorded any adhered fungal pollution that reflex the DMFP presence on the hard teeth surfaces while also six male specimens were not recorded any fungal pollution which reflected healthy OCE due to personality culture and their care in teeth cleaning and their daily

brushing resulting in no pits and cracks (Table 2).

Two species of candida were recorded in this study, *Candida dubliniensis* and *Candida glabrata* in five hard teeth surface specimens only while non-existence in other specimens (Table 3) that attached on enamel of dental patients which are in conformity with part of candida yeasts recorded in the international studies, the genus *Candida* consist of 150-200 species , which were prevalent on dental oral cavity environment and recorded white coated the tongue by this yeast species [10].

Streptococcus sanguis was recorded on two specimens (male and female) (Table 2) attached on teeth surface which considered as initial colonizer in different materials it becomes a normal inhabitant of the human mouth [12]. Red spots and white coated due to the present of the three species *E. coli*, *Candida albicans* and *Pseudomas aeruginosa* recorded in the oral cavity environment (Tables 1, 3 and Fig. 1).

Nine of female specimens weren't recorded any microbial pollution adhering on the hard surfaces while two male specimens weren't recorded any microbes which reflected the healthy oral cavity due to their care in teeth cleaning and their daily brushing resulting in no pits and cracks.

This study pointed out that the absence of the daily care of teeth cleaning and non-maintaining or teeth treatment this case became respond to the internal microbial biofouling phenomenon on the external teeth surfaces of OCE (Fig. 3).

Most repetition of bacterial species in all smear specimens were *Pantoeae* spp. while the most repetition of fungal and yeast species were *Tricoderma harzianum* and *Candida dubliniensis* respectively in the oral cavity environments (Tables 5 and 6).

Triple main existed of microbial species were recorded (bacteria, fungi and yeasts) through an oral cavity environment of their different mechanisms that evolve depending on the host environment solid teeth

Fig. 1 Red spots and white coated of human tongue.

Fig. 2 Showed the cleaned teeth status and weren't microbial pollution.

Fig. 3 Showed the microbial fouling teeth pollution.

(a)

(b)

Fig. 4 Pits, cracks and niches in an infected microbial teeth: a-Tooth Pit; b- Cracks and Niches in Mandible Teeth.

surfaces and gums status in the environment of oral that reflect the health status in OCE for individuals were subjected to microbial examination. These microbes have evolved to survive in the environment of the teeth surfaces in OCE, (gingival epithelium, and oral cavity), while the microbial species repetition presence case of other is given the privacy of persons subjected to examination process of the DMFP in the

Table 5 Bacterial species identification repetition in OCE.

Bacterial isolates	Repetition No.	Repetition ratio(%)
<i>Staphylococcus aureus</i>	3	6.0
<i>Klebsiella pneumonia</i>	6	12.0
<i>Pantoeae spp.</i>	10	20
<i>Aerococcus viridans</i>	4	8.0
<i>Leuconostoc mesenteroides cremorise</i>	2	4.0
<i>Streptococcus sanguinis</i>	1	2.0
<i>Granulicatella adiacens</i>	3	6.0
<i>Slashline</i>	2	4.0
<i>Staphylococcus sciuri</i>	1	2.0
<i>Stenotrophomonas maltiphilia</i>	2	4.0
<i>Staphylococcus sanguinis</i>	1	2.0
<i>Gem. Morbillorum</i>	1	2.0
<i>Kocuria kritinae</i>	1	2.0
<i>Kocuria rosea</i>	1	2.0
<i>Nonreac.biopattern</i>	1	2.0
<i>Kocuria varians</i>	1	2.0

Table 6 Microbial identification of fungal and yeasts species repetition in OCE.

Fungal Isolates	Repetition No.	Repetition Ratio (%)
<i>Trichoderma harzianum</i>	7	14.0
<i>Aspergillus flavus</i>	2	4.0
<i>Candida dubliniensis</i>	3	6.0
<i>Candida glabrata</i>	2	4.0
<i>Trichoderma viride</i>	1	2.0
<i>Cladosporium macrocarpum</i>	2	4.0
<i>Penicillium citrinum</i>	2	4.0
<i>Verticillium lecanii</i>	2	4.0

OCE for people. Aquatic and residual foods biofilms, which are well-organized microbial communities of fungi, yeasts and bacteria microorganisms, are widespread in OCE [13]. This study shown the dental plaque is soft gelatinous mass adhering to teeth surfaces and solid surfaces in the OCE and formed from numerous microbial species that embedded in organic matrix in addition to this study that detected the presence of biofilm layer facilitates the existence of the microbial biofouling phenomenon on teeth.

References

- [1] Panahi, O., Rezaei, S., Marzi, M., and Asgharisana, F. 2011. "*Helicobacter pylori* and Oral Cavity Inflammation." *JPCS* 2: 1-15.
- [2] Windsor, H. M., and O'Rourke, J. 2000. "Bacteriology and Taxonomy of *Helicobacter pylori*." *Gastroenterol Clin. North Am.* 29 (3): 633-648.
- [3] Marsh, P. D. 2000. "Oral Microbial Diversity." In *Oral Bacterial Ecology: the Molecular Basis*, edited by Ellen, R. P., and Kuramitsu, H. K. Wymondham: Horizon Scientific Press.
- [4] Scannapieco, F. A. 1994. "Saliva-Bacterium Interactions in Oral Microbial Ecology." *Crit Rev. Oral Biol. Med.* 5 (3-4): 203-248.
- [5] Kulik, E. M., Sandmeier, H., Hinni, K., and Meyer, J. 2001. "Identification of Archaeal rDNA from Subgingival Dental Plaque by PCR Amplification and Sequence Analysis." *FEMS MICROBIOL LETT* 196 (2): 129-133.
- [6] Marsh, P. D. 2004. "Dental Plaque as a Microbial Biofilm." *Caries Res.* 38 (3): 204-211.
- [7] Donlan, R. M., and Costerton, J. W. 2002. "Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms." *Clinical Microbiology Reviews* 15 (2): 167-193.

- [8] Carranza, F. A., and Newman, M. G. 1996. *Periodontal Microbiology. Clinical Periodontology*. Philadelphia: Saunders.
- [9] Alexopoulos, C. J., Mims, C. W., and Blackwell, S. 1996. *Introductory Mycology*. New York: John Wiley & Sons, Inc.
- [10] Meurman, J. H., Siikala, E., Richardson, M., and Rautemaa, R. 2007. "Non-*Candida albicans* *Candida* Yeasts of the Oral Cavity." *Communicating Current Research and Educational Topics and Trends in Applied Microbiology* Méndez-Vilas, A. (Ed.).
- [11] Lee, B. C., Jung, G. Y., Kim, D. J., and Han, J. S. 2011. "Initial Bacterial Adhesion on Resin, Titanium and Zirconia *in vitro*." *J. Adv. Prosthodont* 3: 81-148.
- [12] Szymanska, J. 2003. "Biofilm and Dental Unit Waterlines, Review Articles." *Ann. Agric. Environ. Med.* 10: 151-157.