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IN VITRO STUDY ON THE ADHESION AND COLONIZATION OF CANDIDA ALBICANS ON METAL AND ACRYLIC PIERCINGS

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Summary. Oral/perioral piercing may provide an ideal environment for adhesion and colonization of microorganisms. The aim of this study is to perform an "in vitro" research on the capabilities of adhesion of Candida albicans on oral piercings made of plastic and metal. Acrylic and metal piercings were incubated with Candida albicans and then were observed using scanning electron microscopy under different magnifications. A lot of irregularities and roughness were observed on the surface of the plastic piercing unlike the surface of the metal one, which is not so rough. Nevertheless, the number of Candida albicans colonies was considerably larger on the scanned metal surface in comparison to the plastic surface. In vitro the metal surface of the piercing creates better environment for the adhesion and colonization of microorganisms than the acrylic. This could be attributed to the electrostatic forces that most likely attract Candida albicans to the metal piercing in the early stages of biofilm formation.

Key words: *candida albicans, oral, piercing, adhesion, colonization, microorganisms*

INTRODUCTION

Body piercing has been well known in many civilizations. It had religious, cultural and sexual significance indicating tribal identity [7]. In developed countries, body piercing became fashionable with the punk movement and was a part of "body art" [7]. First, body and ear piercing gained popularity and with the increase of social tolerance, people started piercing their tongues, lips, cheeks, uvula, etc.

From a medical perspective, the use of body jewellery is not a harmless fashion trend as it can produce undesired local and systemic effects. With the increasing popularity of oral piercings, dental professionals are being confronted with many oral and dental complications associated with this practice. There are a lot of healthy risks and complications associated with the use of intraoral and perioral piercings [23, 24]. This jewellery may provide an ideal environment for adhesion and colonization of microorganisms. The piercing channel is similar to the anaerobic conditions of the subgingival area, and may thus harbour increased concentrations of pathogenic microorganisms [8, 22, 25].The relationship between intraoral piercings and local bacterial colonization is not well documented in the literature. However, oral and perioral piercing may provide an ideal environment for microorganisms.

AIM/OBJECTIVE OF THE STUDY

The aim of the study is to evaluate in “in vitro” conditions the rate of adhesion of Candida albicans on oral piercings made of different materials.

MATERIAL AND METHODS

Ten piercings were bought from a piecing studio. Five of them were made of plastic and the other five were made of metal. The piercings were autoclaved at the temperature of 134oC and pressure of 210.0 kPa for 40 minutes to obtain sterility (Fig. 1). Afterwards the piercings were incubated in broth with Candida albicans for 7 days at the temperature of 37oC in LBG media (Fig. 1).

Fig. 1. Autoclaved plastic and metal piercing and incubation of piercings with Candida albicans for 7 days at T 37°C in LBG media

The piercings were immersed for one hour in a fixative 4% buffered solution of glutaraldehyde (0,075 M, pH = 7,3). In order to fix the organic structure, the piercings were rinsed with distilled water and put in a chilled buffered solution of sodium cacodylate (0,02M, pH = 7,2, 660 mOsm).

This was followed by dehydration which was performed in ascending series of 30%, 50%, 70%, 80%, 95% and 100% alcohol for an hour for each step, and the consequent drying of the piercing was performed by the CPD (Critical Point Drier) method in a desiccator. The dried samples were fixed to a metal tripod and were covered with nanogold (200-250 nm) layer.

The scanning electron microscopy was performed with a Philips (Holland) electron microscope model SEM 515 with accelerating high voltage 25kV in a mode of secondary electron emission. Photos of the observed samples were taken at different magnifications.

RESULTS

The SEM images gave an overview of the surface topography and morphology of the piercings made of different materials (Fig. 2 and 3). A lot of irregularities and roughness were observed on the surface of the acrylic piercing unlike the surface of the metal piercing, which was not so rough.

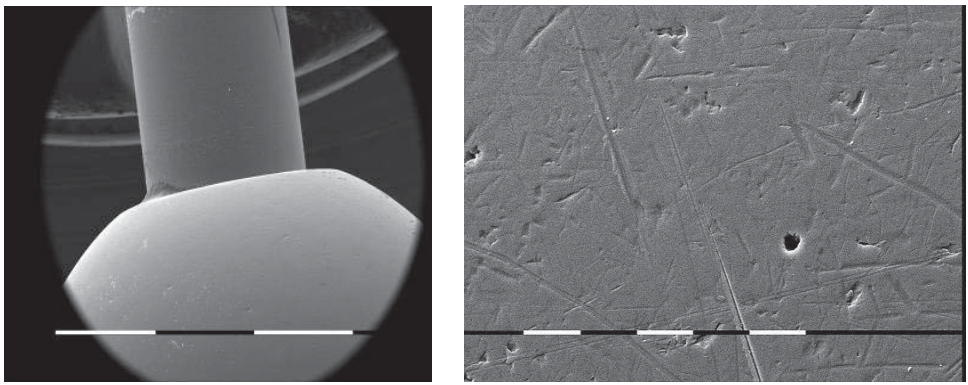


Fig. 2. SEM image of metal piercing (magnification X 30 и X 400)

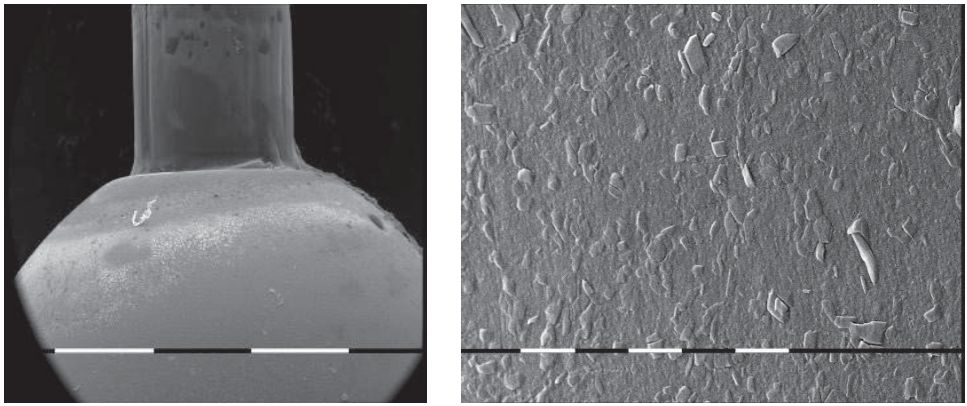


Fig. 3. SEM images of acrylic piercing (magnification X 30 и X 400)

A few dense colonies of Candida albicans were observed on the SEM image of metal piercing in Fig. 4.

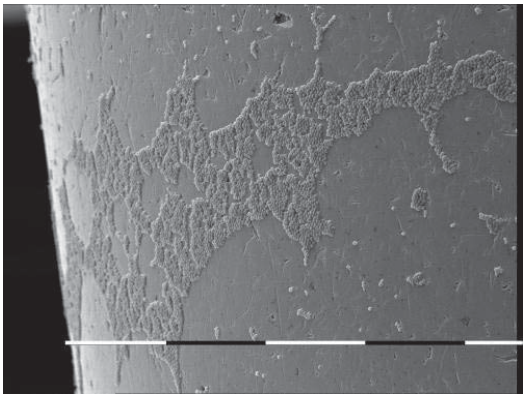
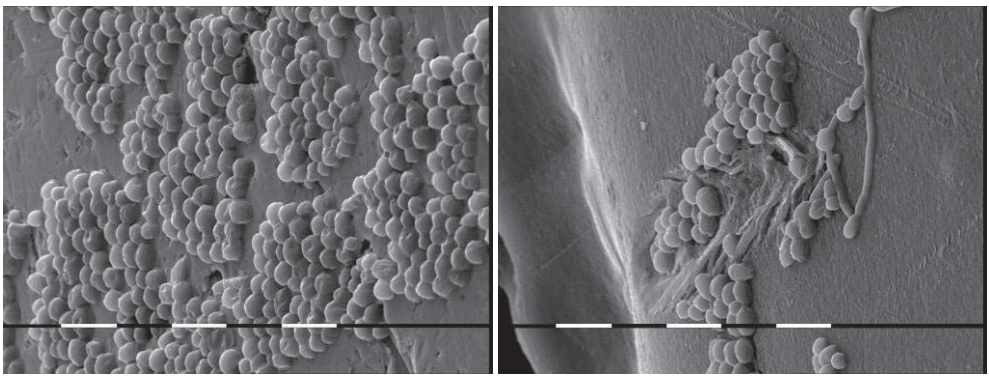


Fig. 4. SEM images of metal piercing (magnification X 250)

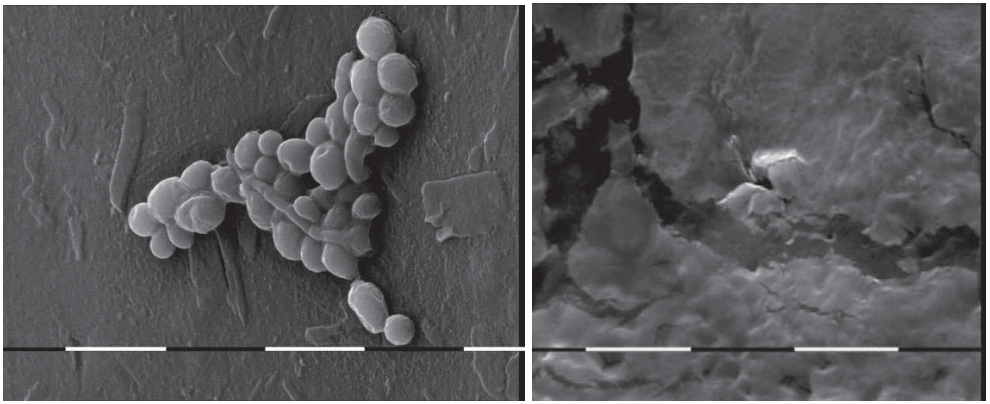
The number of Candida albicans is considerably larger on the scanned metal surface (Fig. 5 A) in comparison to the acrylic surface (Fig. 5 B).



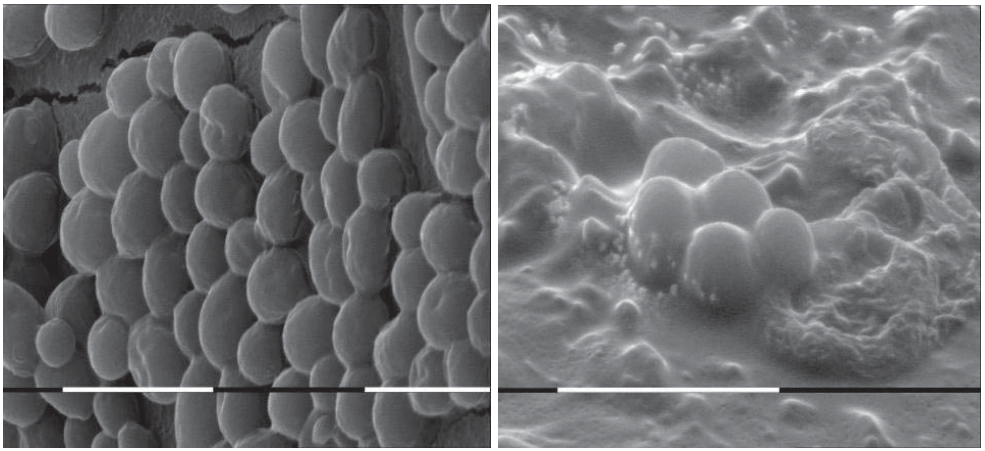
A) Metal piercing (magnification X 1500) **B)** Plastic piercing (magnification X 1500)

Fig. 5. SEM images of piercing surface

On X 2500 magnification, the Candida albicans and the roughness of the acrylic surface are observed very clearly (Fig. 6 A). In a definite area in magnification X3000 a lot of irregularities in the plastic can be seen and there are no Candida albicans (Fig. 6 B). On X 4000 magnification you can see an area with many microorganisms (Fig. 6 C). It is interesting that there is a crack on the upper area of the SEM image №6 A3 and there is no Candida albicans on this retentive area. On X 6000 magnification we can see Candida albicans and the roughness of the surface in details (Fig. 6 D).



A) Magnification X 2500 **B)** Magnification X 3000



C) Magnification X 4000 **D)** Magnification X 6000

Fig. 6. SEM images of acrylic piercing

DISCUSSION

According to some authors who performed a SEM research, initial colonization of the enamel surfaces starts from surface irregularities such as perikymata, cracks, grooves, or abrasion defects, and subsequently spreads out from these areas [12, 13, 14, 16].

At surface irregularities, attached bacteria can survive longer because they are protected against natural removal forces and oral hygiene measures. Moreover, roughening of the surface increases the area available for bacterial adhesion.

The surface of the metal piercing is smoother than the surface of the plastic one, which should be one of the reasons for the less adhesion and colonization of microorganisms. Nevertheless, that was not established in our research.

The characteristics of the surfaces of different dental materials, which help or prevent the adhesion and colonization of different microorganisms, are also subject of scientific research by a number of scientists [3, 4, 11, 19, 21].

In a review article by Quirynen (1994), he discusses the theories of the bacterial adhesion on the hard surfaces of dental materials. He concludes that the colonization with bacterial dental plaque in vitro and in vivo is associated with the high superficial free energy, but the greatest influence of colonization is the roughness of the material surface compared to that of the superficial free energy of plaque accumulation [18].

Yamauchi et al. (1990) revealed that the influence of surface roughness was strain dependent. Some strains as S. oralis, P. intermedia, and P. gingivalis were found in higher amounts on rough sites, whereas some strains as S. sanguis, S. mutans, S. mitis and P. gingivalis were found in higher amounts on smooth surfaces [27].

Increased levels of Candida species in biofilms formed on dentures can cause stomatitis and Streptococcus mutans accumulation on restorative materials is associated with secondary caries. The adhesion of microorganisms depends on the surface structure and composition of biomaterials, and on the physicochemical properties of the microbial cell surface, its surface charge and hydrophobicity [2, 4, 20].

The negative effect of Candida albicans on oral health is known since 1936, associating it with candidal stomatitis [6, 19]. Nevertheless, there is not much research on the mechanisms of adhesion and colonization of Candida albicans on the variety of materials in the oral environment and there is no research regarding oral piercings. A lot of in vitro studies suggest that Candida albicans is the initial colonizer but there is a little research [26, 27] analyzing in vivo the capabilities of the microorganisms to be primary or secondary colonizers. That was the reason for performing our study with inoculation of Candida albicans using two different piercing materials with the aim to establish if the material could influence this hardly curable oral infection.

The considerably larger amount of Candida albicans on the metal surface of the piercing in comparison to the plastic one is probably due to the electrostatic and van der Waals forces of interaction, as well as the probable cytostatic effect of acrylic monomers on the microorganisms. In conducting materials, like gold and amalgam, electron-transfer plays a role in bacterial adhesion [17]. The reason for this is the attraction between the negatively charged bacteria and their positive image charges in the conducting material, which cannot develop in a nonconducting material or in the presence of a nonconductive protein layer on the stainless steel surface [15]. In-Hye Kim et al. (2014) in an in vitro study tests the hypothesis that there is no difference in the adhesion of Streptococcus mutans and Streptococcus sobrinus between esthetic (plastic/silicon) and metal orthodontic wires, based on their superficial characteristics. The authors proved that the values of roughness of the esthetic wires are considerably different depending on the method of covering ($p < 0,05$). In their study the esthetic (plastic) wires show considerably lower adhesion of the inoculated microorganisms than the nickel-titan wires ($p < 0,05$) and these results are similar to

our findings. The researchers suggest that the nickel-titan alloy should be cover with plastic in order to reduce the adhesion of microorganisms in vivo [9].

In another research the authors observed in vitro the capability of adhesion of Candida albicans on 7 commonly used implants and restorative materials. They reported results different from our findings. In their study Candida albicans adhere less on titan materials than on plastic materials. They conclude that there should be a scientific approach on the choice of materials that are used for implant dentures regarding the microorganisms [10].

Arai et al. (2009) investigated the effect of coating denture base acrylic resin with titanium dioxide in order to prevent microbial adhesion considers that this treatment method inhibited biofilm formation [1].

CONCLUSION

In “in vitro” conditions the metal surface is more likely to be adhered and colonized by Candida albicans than the acrylic surface of the piercing. The electrostatic and van der Waals forces most likely cause the colonization of Candida albicans on the metal surface of the piercing.

In “in vitro” conditions the acrylic surface is less likely to be adherent colonized by Candida albicans than the metal surface of the piercing. We can speculate that the specific content (acrylic monomers) at the surface of the plastic material most likely inhibits the adhesion and colonization of microorganisms during the early stage of biofilm formation.

Wearing tongue jewellery over an extended period of time may result in the colonization of Candida albicans and other microorganisms at the piercing site in the absence of appropriate oral hygiene practices. Prospective and current pierces should be informed about possible side-effects and oral health hazards, and about the necessity of cleaning their piercing jewellery regularly with a CHX solution or another appropriate oral disinfectant.

Because of the limitations of the present study we would recommend additional researches to be performed regarding this topic.

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