Materials Science and Engineering C 71 (2017) 111–117

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Effect of preconditioning cobalt and nickel based dental alloys with *Bacillus* sp. extract on their surface physicochemical properties and theoretical prediction of *Candida albicans* adhesion

Mounyr Balouiri [a,*], Samira Bouhdid [b], Moulay Sadiki [a], Wessal Ouedrhiri [a], Hassan Barkai [a], Omar El Farricha [a], Saad Koraichi Ibnsouda [a,c], El Houssaine Harki [a]

[a] *Faculté des Sciences et Techniques, Université Sidi Mohamed Ben Abdellah, BP 2202, 30007 Fez, Morocco*
[b] *Faculté des Sciences de Tétouan, Université Abdelmalek Essaadi, Avenue de Sebta, Mhannech II, 93002 Tétouan, Morocco*
[c] *Cité de l'innovation, Université Sidi Mohamed Ben Abdellah, BP 2626, 30007 Fez, Morocco*

ARTICLE INFO

Article history:
Received 21 June 2016
Received in revised form 14 August 2016
Accepted 29 September 2016
Available online 30 September 2016

Keywords:
Bacillus extract
Dental alloy
Physicochemical property
Anti-adhesive
Candida albicans

ABSTRACT

Biofilm formation on dental biomaterials is implicated in various oral health problems. Thus the challenge is to prevent the formation of this consortium of microorganisms using a safe approach such as antimicrobial and anti-adhesive natural products. Indeed, in the present study, the effects of an antifungal extract of *Bacillus* sp., isolated from plant rhizosphere, on the surface physicochemical properties of cobalt and nickel based dental alloys were studied using the contact angle measurements. Furthermore, in order to predict the adhesion of *Candida albicans* to the treated and untreated dental alloys, the total free energy of adhesion was calculated based on the extended Derjaguin-Landau-Verwey-Overbeek approach. Results showed hydrophobic and weak electron-donor and electron-acceptor characteristics of both untreated dental alloys. After treatment with the antifungal extract, the surface free energy of both dental alloys was influenced significantly, mostly for cobalt based alloy. In fact, treated cobalt based alloy became hydrophilic and predominantly electron donating. Those effects were time-dependent. Consequently, the total free energy of adhesion of *C. albicans* to this alloy became unfavorable after treatment with the investigated microbial extract. A linear relationship between the electron-donor property and the total free energy of adhesion has been found for both dental alloys. Also, a linear relationship has been found between this latter and the hydrophobicity for the cobalt based alloy. However, the exposure of nickel based alloy to the antifungal extract failed to produce the same effect.

1. Introduction

Dental base metal alloys are often used for dental restorations and implants [1]. Nowadays, cobalt-chromium and nickel-chromium base metal alloys are the most prevalent types, mostly in the developing countries [2–4]. They are well known for their tarnish resistance, stainless properties, strength and hardness [3]. Regardless of noble metal alloys that have a high density and a low durability, base metal alloys are more durable and have a low density, in addition to their lower price [3]. Prosthetic devices are commonly used in the dental care. Nevertheless, they can provide suitable surfaces for the biofilm development, mostly by the common fungal agent, *Candida albicans* [5–8]. Therefore, device associated infections due to biofilms represent serious problems to oral health [9,10].

Microbial adhesion is the first and the key step of the biofilm development. On abiotic surfaces, it is mainly governed by non-specific physicochemical interactions such as hydrophobic, Van der Waals, electrostatic and Lewis acid-base interactions. The specific adhesion can occur only when the outcome of the non-specific interactions is attractive [11]. In the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, only the long-range interactions (Van der Waals and electrostatic interactions) were considered. Nonetheless, in aqueous media the Lewis acid-base forces represent around 90% of the total non-covalent interaction forces. Therefore they have been included in the extended version of DLVO theory (XDLVO) [12]. These theories provide theoretical equations predicting microbial behavior on material substrata based on the superficial physicochemical properties [13].

Due to the various concerns related to the biofilm formation on dental biomaterials, the challenge is to improve the materials in use [14]. One of the approaches adopted to achieve biomaterials with antimicrobial properties is based on reducing the initial attachment of microorganisms by altering the substratum surface properties, known as antibiofouling [15].

Bacillus strains are known for their ability to produce a wide range of antimicrobial molecules [16,17], some of which are microbial surface-

* Corresponding author.
E-mail addresses: b.mounyr@gmail.com, mounyr.balouiri@usmba.ac.ma (M. Balouiri).

http://dx.doi.org/10.1016/j.msec.2016.09.083

active compounds also known as biosurfactants [18]. In our previous study, the effect of an antifungal extract of rhizospheric *Bacillus* sp. isolate on the cell surface free energy of *Candida albicans* has been studied [19].

To our knowledge, the physicochemical properties of cobalt and nickel based dental alloys and the effect of microbial extract on their surface physicochemical properties was not well documented compared to acrylic resin and other biomaterials. Therefore, the present study aims to investigate the effect of the antifungal extract from *Bacillus* sp. on the physicochemical properties of cobalt-chromium and nickel-chromium dental alloys and to predict the adhesion of *Candida albicans* on their surface.

2. Material and methods

2.1. Bacterial strain

The strain Cp-LMA-9 was isolated from *Calotropis procera* Ait. rhizosphere, and identified as *Bacillus* sp. based on the 16 S rRNA gene sequence analysis [20]. The bacterial strain was maintained on agar slants at 4 °C and in glycerol 20% at −20 °C.

2.2. Solid-state fermentation and extract preparation

Colonies from 24 h-old culture of *Bacillus* sp. isolate were used to streak yeast extract-malt extract-agar (YMA) (yeast extract 1 g/L, malt extract 20 g/L, and agar 20 g/L at pH 7 ± 0.2). Plates were then incubated at 30 °C for 48 h. After incubation, biomass was discarded and solid medium was extracted with methanol at room temperature during 2 h by a classical maceration method. Then, the mixture was filtered and methanol was evaporated under vacuum. Finally, the extract obtained was weighed, resuspended in sterile distilled water (1 mL) and stored at 4 °C until use. The antifungal effect of the extract obtained against *Candida albicans* was confirmed by disk-diffusion and broth microdilution methods.

2.3. Yeast strain, growth conditions and fungal layer preparation

Colonies from 48 h-old culture of *Candida albicans* ATCC 10231, grown on YPG agar (yeast extract 10 g/L, peptone 10 g/L, glucose 20 g/L, agar 20 g/L, pH = 7 ± 0.2) at 30 °C, were used to inoculate 500 mL of sterile YPG broth. After incubation at 30 °C for 48 h in an orbital incubator with shaking at 125 rpm, the cells were harvested by centrifugation and washed three times with sterile solution of KNO_3 (0.1 M). The final pellet was resuspended in 10 mL of sterile KNO_3 (0.1 M). The obtained suspension was used for contact angle measurements as described by Hamadi & Latrache [21]. Briefly, the prepared fungal cell suspension was deposited onto a 0.45 μm cellulose acetate filter (Sartorius) by first washing the filter with 10 mL of distilled water for wetting, and then 10 mL of the cell suspension were added to obtain a thick lawn of the fungal cells (blastospore form) after filtration. The wet filter was placed prudently on a glass support with double-sided sticky tape and allowed to air dry before the contact angle measurements.

2.4. Preparation of metal alloy surfaces

Two metal alloys were used in this study: cobalt-chromium (Co-Cr) dental alloy (Mesa, Magnum H60, Italia) and nickel-chromium (Ni-Cr) dental alloy (Argeloy N.P. (V), Predominantly BASE (PB), USA). According to the data provided by the manufacturer, the elemental composition of each alloy expressed in weight percentage (wt%) was 63% Co, 29% Cr, 6.5% Mo and 1.5% (C, Si, Fe and Mn) for Co-Cr dental alloy and 72% Ni, 15%Cr, 9% Mo, 2% Al, 1.8% Be and < 1% (Fe, Si and C) for Ni-Cr dental alloy. Each specimen has a length of 10 mm, thickness of 1 mm, height of 10 mm and a test surface that was polished at the final surface roughness of Ra = 0.04 μm measured with a rugosimeter (Mitutoyo SJ 301, Aurora IL, United States). Each sample was cleaned with pure acetone and rinsed in sterile distilled water.

2.5. Treatment of dental alloys

The effect of the antifungal extract on surface physicochemical characteristics of dental alloys was performed as follows: The extract was applied on the surface of each alloy to a final concentration of 30 mg/cm^2. Different exposure times at room temperature (25 ± 2 °C) were studied: 30, 60, 120, 180 and 240 min. Before the contact angle measurements, each sample was washed three times with sterile KNO_3 (0.1 M) and adequately dried. The untreated alloys were only rinsed three times with sterile KNO_3 (0.1 M) and dried. All experiments were conducted in triplicate.

2.6. Contact angle measurements

Contact angle measurements were performed using a goniometer (GBX Instruments, France) by the sessile drop method [22]. Three measurements of contact angles were made on the fungal layer and each surface of the metal alloys by using two polar liquids (water and formamide) and one non-polar liquid (diiodomethane) with known energy characteristics (Table 1).

2.7. Calculation of surface free energy

All the surface physicochemical parameters for *C. albicans* cells and dental metal alloys (the Lifshitz-Van der Waals (γ^{LW}), the electron-donor (γ^-) and electron-acceptor (γ^+) components), were calculated by Young's equation [23].

$$\gamma_L(\cos\theta + 1) = 2\left(\gamma_S^{LW}\gamma_L^{LW}\right)^{1/2} + 2\left(\gamma_S^{+}\gamma_L^{-}\right)^{1/2} + 2\left(\gamma_S^{-}\gamma_L^{+}\right)^{1/2} \quad (1)$$

Where the terms S and L denote solid surface and liquid phases respectively and θ denotes the contact angle. The Lewis acid-base component was obtained by the following formula:

$$\gamma_S^{AB} = 2\left(\gamma_S^{-}\gamma_S^{+}\right)^{1/2} \quad (2)$$

Finally, the degree of hydrophobicity of each surface was evaluated through contact angle measurements and by applying the approach of Van Oss [23]. According to this approach, the degree of hydrophobicity of a given material is expressed as the free energy of interaction between two entities of that material when immersed in water (w): ΔG_{iwi}. This latter was evaluated through the surface tension components of the interacting entities, according to the following formula:

$$\Delta G_{iwi} = -2\,\gamma_{iw} = -2\left[\left(\left(\gamma_i^{LW}\right)^{1/2} - \left(\gamma_w^{LW}\right)^{1/2}\right)^2 + 2\left(\left(\gamma_i^{+}\gamma_i^{-}\right)^{1/2} + \left(\gamma_w^{+}\gamma_w^{-}\right)^{1/2} - \left(\gamma_i^{+}\gamma_w^{-}\right)^{1/2} - \left(\gamma_w^{+}\gamma_i^{-}\right)^{1/2}\right)\right] \quad (3)$$

Table 1
Surface energy properties of pure liquids used to measure contact angle[a].

Liquid	γ^{LW}(mJ/m^2)	γ^+(mJ/m^2)	γ^-(mJ/m^2)
Water (H_2O)	21.8	25.5	25.5
Formamide (CH_3NO)	39.0	2.3	39.6
Diiodomethane (CH_2I_2)	50.5	0	0

[a] Results found by Van Oss [12].