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The role of nicotine, cotinine and caffeine on the electrochemical behavior and bacterial colonization to cp-Ti

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ABSTRACT

Although smoking promotes deleterious effect to bone healing, there is a lack of study investigating its role on the implant structure and biofilm growth. We hypothesized that nicotine, cotinine and caffeine would impair the corrosion resistance of commercially-pure titanium (cp-Ti) and would enhance *Streptococcus sanguinis* biofilm growth. Neither the smoking products nor the caffeine affected the corrosion tendency ($P > .05$) and the oxide layer resistance ($P = .762$) of cp-Ti. Lower capacitance values were noted in the presence of nicotine ($P = .001$) and cotinine ($P = .0006$). SEM showed no pitting corrosion, and the EDS spectra did not differ among groups. Nicotine (300 μg/mL) induced higher surface roughness ($P = .03$) and greater surface change of cp-Ti. Nicotine at 3 μg/mL, and cotinine at 0.3 and 3 μg/mL increased the number of viable cells ($P < .05$). Biofilm exposed to nicotine (0.3, 3 and 30 μg/mL) ($P = .025, .030, .040$, respectively) and cotinine (3 and 30 μg/mL) ($P = .027, .049$, respectively) enhanced carbohydrate content. Biofilm biomass and protein content were similar among groups ($P > .05$). These findings suggest a greater biofilm accumulation in smokers, a risk factor that may lead to peri-implantitis.

1. Introduction

Smoking has long been indicated as a risk factor for dental implant survival [1,2]. From a total of 2194 implants inserted in 540 patients, 4.76% and 11.28% failure in nonsmokers and smokers, respectively [1]. A meta analysis and systematic review study demonstrated that smokers have also greater periimplant complications and bone loss than nonsmokers [3].

According to the American Heart Association, among adults over 18 years of age, 21.3% of men and 16.7% of women in the United States smoke cigarette [4]. Cigarette smoke has more than 4000 harmful substances in which the nicotine is its key constituent, and the cotitine is the main metabolite of the nicotine [5,6]. Nicotine is a potent vasoconstrictor which reduces blood flow and nutrient supply of the implant at the surgical site and inhibits the proliferation of fibroblasts, red blood cells and macrophages [7], affecting the bone regeneration [8]. Additionally, smoking is highly linked with coffee consumption which is rich in caffeine [9]. In a review of six epidemiological studies, authors observed that nearly 86.4% of smokers consumed coffee versus 77.2% of nonsmokers [9]. The concentration of nicotine in the saliva range from 96 ng/mL to 1.6 mg/mL [10], while the concentration of cotinine in the saliva and crevicular fluid varies from 158 ng/mL to 1054 ng/mL, and from 206 to 8056 ng/mL, respectively [11]. However, some studies use even higher concentrations of such compounds in order to induce a cellular effect [12]. Caffeine concentration in the saliva and crevicular fluid remains unknown [5].

Titanium and its alloy have been used for dental implant due to their good strength, corrosion resistance, and biocompatibility [13–16]. Nevertheless, the complex oral environment can potentially damage the longevity of dental implants due to the presence of chemical, microbial and physical agents such as saliva, acid, fluoride, bacteria and mastication load [13,17,18]. Such conditions can impair the electrochemical stability of titanium which may be a factor contributing to the failure of the implant–bone interface [19]. Additionally, microorganism attachment and biofilm formation over implants and abutments are risk factors for the development of peri-implantitis [16].

Streptococcus oralis, *Streptococcus sanguinis*, and *Actinomyces naeslundii* are the first colonizers into the oral cavity [20]. An in vivo study showed that *Streptococcus* spp. are the most predominant

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microorganism on implant materials [21]. Grobner-Schreiber et al. [22] observed in vivo that 45% of bacteria onto titanium discs with different surface treatments are *Streptococcus* spp., followed by *Neisseria* (8%), *Haemophilus* (7%), *Pseudomonas* (7%) and *Gemella* (6%).

Previous studies have investigated the influence of smoking products and caffeine on biofilm development. Huang et al. [23] showed that nicotine increased the biofilm and metabolic activity of *S. mutans* in in vitro model. This data was also confirmed by Li et al. [24]. Others demonstrated a dose-dependent effect of nicotine and cotinine on the growing of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* biofilms over epithelial cells [25]. On the other hand, Cogo et al. [5] observed no influence of nicotine, cotinine and caffeine on the viability of single-species biofilms of *Streptococcus gordonii*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and dual-species biofilms of *Streptococcus gordonii–Fusobacterium nucleatum* and *Fusobacterium nucleatum–Porphyromonas gingivalis* in hydroxyapatite discs.

At present, controversial results concerning the effect of smoking products and caffeine on the attachment of oral periodontal pathogens onto titanium are noted. Additionally, although attention has been closely paid to the adverse result of nicotine on the bone healing [26,27]; and the effect of saliva, fluoride, human plasma, osteoclast, biofilm and lipopolysaccharide on the corrosion behavior of titanium [13,14,28–30], to the best of the authors' knowledge, there have been no study to date that have evaluated the influence of smoking compounds and caffeine on the electrochemical stability of titanium.

This study aimed to evaluate the role of different concentrations of nicotine, cotinine and caffeine on the corrosion stability of commercially-pure titanium (cp-Ti). We also tested the influence of such compounds on the *S. sanguinis* biofilm growth to cp-Ti. Our hypothesis was that the presence of smoking products and caffeine would impair the corrosion resistance of cp-Ti and would increase biofilm development to cp-Ti surfaces.

2. Material and methods

2.1. Specimen preparation for electrochemical assay

Discs of cp-Ti (grade II) with 15-mm diameter and 2-mm thickness (MacMaster Carr, Elmhurst, IL, USA) were ground with silicon carbide paper (#320, 400 and 600) (Carbimet 2, Buehler, Lake Bluff, IL, USA) and mirror finished with diamond paste (9 μm) (MetaDi 9-micron, Buehler) and colloidal silica suspension (0.05 μm) (MasterMed, Buehler) (roughness arithmetic mean value (R_a) = 31.2 ± 3.4 nm and quadratic mean (R_q) = 39.6 ± 3.1 nm). Samples were cleaned in distilled water and 70% propanol in ultrasonic bath for 10 min each, and finally hot air dried.

2.2. Electrochemical tests

The electrochemical measurements were carried out using a potentiostat (Interface 1000, Gamry Instruments, Warminster, PA, USA) in a 3-electrode standard method according to the guidelines of the American Society for Testing of Materials (G61-86 and G31-72). The exposed area of the titanium disc (1.77 cm^2) was used as a working electrode. The potentials were measured against a saturated calomel electrode (SCE) (reference electrode) and a graphite counter electrode. Corrosion tests were performed in artificial saliva (0.4 g/L KCl, 0.4 g/L NaCl, 0.906 g/L $CaCl_2 \cdot 2H_2O$, 0.690 g/L $NaH_2PO_4 \cdot 2H_2O$, 0.005 g/L $Na_2S \cdot 9H_2O$, and 1 g/L urea [13] at 37 ± 1 °C).

Discs were randomly divided into 13 groups (n = 3) as a function of nicotine, cotinine and caffeine (Sigma-Aldrich, St Louis, MO, USA) concentrations (0.3, 3, 30 and 300 μg/mL) diluted in artificial saliva. These concentrations were based in a previous study investigating the effect of nicotine on attachment and proliferation of human osteoblast-like cells [31]. Artificial saliva was used as a control.

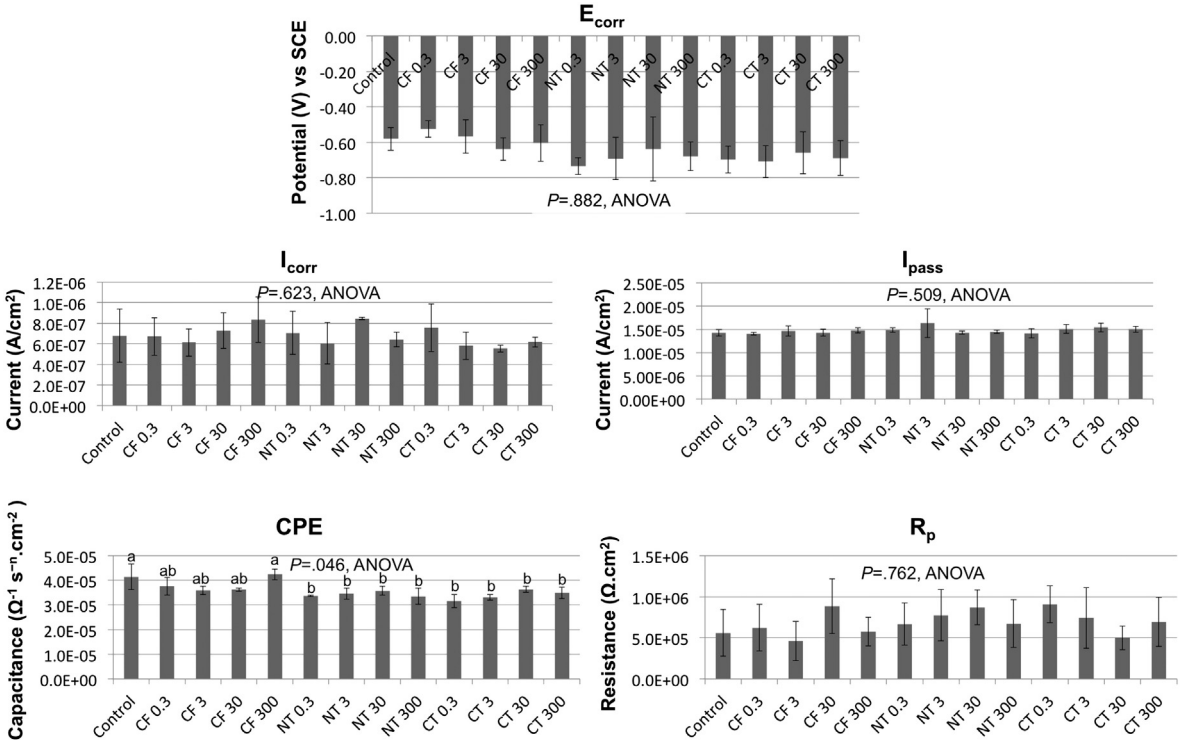

Fig. 1. Electrochemical data evolution. Mean and standard deviation of corrosion potential (E_{corr}), corrosion current density (I_{corr}), passivation current density (I_{pass}), capacitance of the oxide layer (CPE) and polarization resistance of the oxide layer (R_p) for cp-Ti in artificial saliva (control) with different concentrations of caffeine (CF), nicotine (NT) and cotinine (CT). Different letters indicate significant difference among groups (α = .05).