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Corrosion potential in artificial saliva and possible genotoxic and cytotoxic damage in buccal epithelial cells of patients who underwent Ni-Cr based porcelain-fused-to-metal fixed dental prostheses

Gülce Alp[a,*], Gonca Çakmak[b], Murat Sert[c], Yavuz Burgaz[d]

[a] Department of Prosthodontics, Faculty of Dentistry, Okan University, 34959, Akfırat, Istanbul, Turkey
[b] Department of Toxicology, Faculty of Pharmacy, Gazi University, 06330, Hipodrom, Ankara, Turkey
[c] Department of Medical Laboratory Techniques, Yildirim Beyazit University, 06760, Cubuk, Ankara, Turkey
[d] Department of Prosthodontics, Faculty of Dentistry, Gazi University, Emek, 06510, Ankara, Turkey

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The authors dedicate this article in the loving memory of Prof. Dr. Yavuz Burgaz, Professor of Department of Prosthodontics at Gazi University, who passed away on November 15, 2017.

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ABSTRACT

Nickel–chromium(Ni–Cr) based alloys account for the majority of the porcelain-fused-to-metal fixed dental prostheses(PFM-FDPs) on account of their superior properties despite both nickel and chromium being known as human carcinogens. Understanding the genotoxicity and the cytotoxicity alongside the characteristics of corrosion behavior of the alloy is vital for understanding their biocompatibility. This study has evaluated whether the Ni-Cr based alloys corroded in artificial saliva by analyzing alloy decomposition at different pH levels and immersion durations(7, 14, 21, and 28 days) using inductively coupled plasma-optic emission spectrophotometry(ICP-OES). The principal aim of the study was to determine the possible genotoxicity and cytotoxicity using micronucleus(MN) and other nuclear anomaly frequencies [nuclear bud(NBUD), binucleated (BNC), condensed chromatin(CC), karyorrhectic(KhC), pyknotic(PC) and karyolytic(KC) cells] and various cytome parameters [basal cells(BC), differentiated cells(DF)] with the buccal epithelial cell(BEC) micronucleus cytome assay(BMCyt). This test was administered at 1 pre- and 3 post-treatment time points to 40 patients who underwent installation of PFM-FDPs made of Ni-Cr based alloy. Furthermore, at the final post-treatment time point, saliva cotinine levels were measured with salivary cotinine quantitative enzyme immunoassay(EIA) kit and information obtained by questionnaire prior to the first pre-treatment time point was confirmed. The highest greatest release of Ni and Cr ions were seen at pH 2.3. MN and micronucleated cell frequencies, and BNC cell frequencies were significantly elevated at post-treatment time points($p < 0.03$). BC, CC, KhC, PC and KC cell frequencies however were not significantly different between pre-and post-treatment time points($p > 0.05$). MN frequency was significantly lower in non-smokers than in current and former smokers($p < 0.001$) at the pre-treatment time point. There was no significant correlation between the unit number of PFM-FDPs and MN frequencies. Our results revealed that Ni-Cr based alloys are prone to corrosion and that PFM-FDPs fabricated with Ni-Cr based alloys may induce genotoxic effects rather than cytotoxic effect.

1. Introduction

Porcelain-fused-to-metal fixed dental prostheses (PFM-FDPs), which combine strength of metal and esthetic of porcelain, have been accepted as a “gold standard” for many years and predominantly have been used as an esthetic option in the restoration of teeth [1,2]. PFM-FDPs exhibited good clinical performance characteristics with low failure rates in follow up clinical studies over a minimum 5-year period [3,4]. The success of the PFM-FDPs largely depends on the physical properties of the metallic framework [5]. Metallic frameworks can be fabricated by several types of dental casting alloys through improvements in materials and techniques [5]. Consequently, good mechanical and physical properties, casting techniques, cost, ease of workability, biocompatibility, and corrosion properties are the factors which affect the selection of the alloy used [6,7].

Biocompatibility properties have an important role in the selection of appropriate alloys for PFM-FDPs because these are placed in the mouth and are in long-term contact with oral epithelial cells [8]. Undesired coloring, porosity and degrading of the mechanical properties of the alloy occur as a result of corrosion [9]. The host response depends on the type and the amount of the released elements as a result of the process of degradation and the duration of exposure to these

* Corresponding author.
E-mail addresses: gulce165@hotmail.com (G. Alp), msert06@gmail.com (M. Sert).

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components [10]. Corrosion of alloys could be in constantly changing amounts and affected by different factors [11,12]. In particular, the mouth provides a conducive environment for the corrosion of metals through occlusal load, pH and temperature alterations, bacterial activity, dental plaque, diet, presence of moisture and saliva flow [13]. The corrosion of the alloy leads to the development of adverse biological effects such as cytotoxicity, mutagenicity, carcinogenicity, and allergic reactions [8,10]. Hence, apart from its mechanical and esthetical properties, the corrosion resistance of an alloy is very important for its biocompatibility [5,8].

Among the most used alloys, nickel-chromium (Ni-Cr) based alloys are favored as the metallic framework material of PFM-FDPs due to their low cost and good clinical performance [14]. Nickel and chromium are metals capable of modulation of the immune response [15]. Moreover, both of the metals are known to either be human carcinogens or possible carcinogens (IARC Group 1) [16,17]. Therefore, as they are prone to corrosion, the safety of nickel-chromium (Ni-Cr) based alloys in regard to the biocompatibility in the oral cavity is the subject of toxicological evaluations, [11,12,18–21]. It has also been reported that Ni-Cr based alloys have different corrosion potentials depending on the type and pH of the corrosive medium, the immersion durations and the chemical composition of the alloy [11]. Although there are in vitro studies concerning the cytotoxicity and genotoxicity of this alloy [16,22], to our knowledge, none have explored the cytogenic effects of Ni-Cr based alloys used for PFM-FDPs construction.

Micronucleus assay as the measure of genotoxicity can be used in several cell types from peripheral blood lymphocytes to the epithelial cells such as buccal and nasal ones and in several areas from environmental to clinical and to the occupational ones [23–26]. The technique also allows for evaluating nuclear anomalies other than the micronucleus frequency. As a result, it was named the cytome assay [27].

One of the aims of this study was to evaluate whether the Ni-Cr based alloys were corroded in artificial saliva by analyzing decomposition at different pH levels and immersion durations (7, 14, 21, and 28 days). A further aim was to determine the possible resultant genotoxicity and cytotoxicity by examining increases in the frequencies of micronucleus (MN) and other nuclear anomalies [nuclear bud (NBUD), binucleated (BNC), condensed chromatin (CC), karyorrhectic (KhC), pyknotic (PC) and karyolytic (KC) cells] and cytome parameters [basal cell (BC), differentiated cell (DF)] in buccal epithelial cell (BEC) micronucleus cytome assay (BMCyt) at 4 selected time points, pre- and post- treatment, among a cohort of 40 patients who underwent procedures to install PFM-FDPs made of Ni-Cr based alloy.

2. Materials and methods

2.1. Determination of elemental release in artificial saliva samples

A total of 36 Ni-Cr (Prestige KN, Adentatec Lagerei und Warenvertriebs GmbH, Germany) rectangular samples with the dimensions of 32 mm × 15 mm × 1.2 mm were cast, grounded, and polished according to the manufacturer recommendations. All samples were prepared from new alloy without any surplus alloy. The samples were immersed for 7, 14, 21 and 28 days at 37 °C in 3 different artificial saliva samples with different pH values; sodium chloride and lactic acid solution, pH 2.3 [10], Fusayama solution, pH 5.3 [28] and Ringer's solution, pH 7.0 [11]. The concentration of elemental release (Ni, Cr, and molybdenum (Mo); ppm) was analyzed with static immersion test method according to International Standards Organization (ISO) 10271:2001 [29] by using inductively coupled plasma-optic emission spectrophotometry (ICP-OES) (Spectro Genesis, Kleve, Germany). All measurements were repeated three times and the mean values were calculated.

2.2. Study design

2.2.1. Subjects

The subjects of this study comprised 40 healthy adult volunteers (20 men and 20 women) with a mean age of 34.2 ± 7.5 years, each of whom had applied for prosthodontic treatment at the Department of Prosthodontics, Gazi University, Turkey. Subjects completed a questionnaire scoring information on their age, sex, smoking status (current smokers, former smokers, and non-smokers), alcohol consumption, dietary habits, daily mobile phone usage time, X-ray exposure (over a period of least one month previous to questionnaire completion), and occupational and medical history. Current smokers were defined to be those who had been smoking for at least 1 year before biological sampling was undertaken. Former smokers were defined to be those who had previously smoked cigarettes but had stopped smoking at least 1 year prior to sampling. None of the subjects had been exposed to metallic alloys or other chemicals occupationally. Detailed clinical examination of the patients was performed. Subjects who had at least one missing tooth and also had the indication for fixed dental prostheses were included. However, subjects who had medical histories such as genetic diseases, allergies, viral infections, a history of drug use, vaccination within the previous calendar year, oral lesions, prosthetic treatment, amalgam restoration or periodontal disease, were excluded. The Local Ethical Committee of Ankara University Faculty of Dentistry, Ankara, Turkey (02.20.2012-30/7) approved this study. Prior to signing a declaration of informed consent, each patient was thoroughly informed about the purpose and the protocol of this study.

2.2.2. Characterization and application procedure of PFM-FDPs

The design and unit number of PFM-FDPs were defined according to the number and the region of the missing tooth/teeth in the jaw, the prognosis of the abutment tooth/teeth, the existence of parafunctional habits and the esthetic expectations of the patient. A minimum of 3-unit PFM-FDPs, made of Ni-Cr framework and feldspathic porcelain, were each fabricated by the same technician, using a conventional protocol. According to the treatment plan, the teeth were prepared so as to create a chamfer finish line. Impressions for interim and definitive restorations were made with irreversible hydrocolloid (Kromopan; Lascod SpA, Firenze, Italy) and condensed silicone impression material (Zetaplus, Zhermack SpA, Badia Polesine, Italy), respectively. Interim restorations were fabricated on temporary casts and temporarily cemented (Cavex Temporary Cement, Cavex, Haarlem, Netherlands) throughout the operative procedures. In the laboratory, definitive casts were prepared, mounted in a semi-adjustable articulator (Artex Type CT Articulator, Jensen Dental, North Haven, USA) and Ni-Cr (Prestige KN, Adentatec Lagerei und Warenvertriebs GmbH, Germany) metallic frameworks were fabricated from new alloy. The marginal fit of the frameworks was intra-orally evaluated and then sent to the laboratory for the fabrication of the porcelain veneer. Feldspathic porcelain (Ceramco® 3, Dentsply, New York, USA), compatible with Ni-Cr alloy, was applied on the framework and occlusal adjustments were performed intraorally. The restorations were permanently cemented with glass ionomer cement (Ketac-Cem; 3 M ESPE, Seefeld, Germany) after glazing. The composition of the materials used in this study was presented in Table 1.

2.3. Biological sampling

To evaluate the possible genotoxic and cytotoxic effects of PFM-FDPs, the BMCyt assay was used in pre- and post-treatment evaluation of the subjects. BEC samples were collected in 4 different sampling time points. The pre-treatment samples were collected immediately prior to the operative procedure (1st sampling). In the post-treatment (after cementation of the Ni-Cr based PFM-FDPs), the samples were collected a further 3 times; 1 week (2nd sampling), 1 month (3rd sampling), and 3 months (4th sampling) after the cementation of the PFM-FDPs. Each sampling simultaneously harvested a minimum volume of 3 ml