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Mutagenic Potentials of Dental Alloys Using Ames *Salmonella*/microsome Test

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Abstract: The potential mutagenicity of two Nickel-Chromium based alloys (Verabond II and Minalux) was determined using the Ames *Salmonella*/microsome test. According to ISO 6871-1 protocol, the alloys were mounted in an artificial saliva medium and incubated for 7 days at 37°C. Then aliquots were used to assess mutagenic effects of the released materials using *Salmonella typhimurium* strain TA 100 and the standard plate incorporation assay in the presence or absence of S9 fraction from rat liver. No mutagenic effects were detected for either alloys at 25, 50, 100 μL of test solutions. The S9 bioactivation also showed no mutagenic metabolite activities for these alloys. Therefore, it seems that both alloys have no mutagenic activity with or without bioactivation under the test conditions.

Key words: Dental alloys, mutagenicity, ames test, *Salmonella typhimurium*, TA100

INTRODUCTION

Concerns on the clinical performance of dental materials have led to research on their biocompatibility[1-6] but, overall, there is still a scarcity of evidence on the genotoxic and mutagenic potentials of dental materials[7,8]. At present, there are several types of commercially available dental alloys having a variety of chemical compositions. Of these alloys, Nickel-Chromium base alloys are used in combination with different elements to improve the biomechanical properties and biocompatibility of the dental materials. Because the composition of dental alloys may lead to adverse tissue reactions, the mutagenic potentials of these agents have been a topic of research interest[9-11]. Some of the dental materials may also elicit mutagenic effects in the long term[8,9]. In essence, leachable substances from dental materials may cause short-term adverse effects and even gain access to the periodontal tissue through numerous pathways[12]. The basic composition of metal base alloys is mostly Ni and in less quantity Cr, Mo, Al, Ti, Nb and Si[13]. The possibility of corrosion of dental alloys and therefore release of elements into surrounding tissue is one of the major concerns regarding the adverse effects of these alloys. Local and systemic toxicity, allergic responses and late effects such as mutagenicity and carcinogenicity of alloy components have been studied by different researchers[14-16]. The Ames *salmonella*/microsome mutagenicity test, a bacterial mutation assay, is frequently used for mutagenicity testing due to its high sensitivity (83%) and recognized validity[17]. Therefore, we decided to study the mutagenic potential of two metal base alloys, Verabond II and Minalux using Ames test.

MATERIALS AND METHODS

Chemicals and positive mutagens: D-biotin, glucose, l-histidine-HCl monohydrate, crystal violet and sodium chloride were purchased from Sigma Chemicals (UK), Ampicillin trihydrate was from Fluka (Germany), oxoid agar, oxoid nutrient broth No. 2 from Oxoid Ltd. (UK) and magnesium chloride, potassium phosphate, potassium chloride, citric acid monohydrate, sodium ammonium phosphate, sodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Merck (Germany). The positive mutagens sodium azide (NaN_3) and 2- Aminoanthracene (AA) were purchased from Sigma Chemicals (UK).

Test materials and sample preparation: According to ISO 6871-1, 3 pieces of well cleaned Verabond II, a commercially available alloy and Minalux, an Iranian made alloy, were separately mounted in the artificial saliva

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containing lactic acid (0.1 M) and sodium chloride (0.1 M) and incubated at 37°C for 7 days to allow release of elements into solution. This Emersion test was conducted as duplicate. After 7 days, the solutions were separated from alloys and kept in refrigerator until use.

Antibacterial test: This test was conducted to determine the antibacterial effect of the amounts of test materials used in the mutation assays. An overnight bacterial culture containing 1.5 x 10^8 cfu mL^{-1} was used to culture on surface of Muller-Hinton agar plates. Then wells were made on agar plates and test solutions were added to each well. Following diffusion of solutions into agar, the plates were incubated at 37°C for 24 h. The zone of inhibition of bacterial growth around the wells was examined to determine the antibacterial effects of test solutions.

Ames mutagenicity test: The *Salmonella* mutagenicity assay was carried out according to method described by Maron and Ames[18]. Oxoid nutrient broth No. 2 was used for overnight culture. For plate incorporation assays, 0.1 mL of bacterial tester strain (TA100), 0.25 mL of S9 mix if appropriate and the sample to be tested was added to 2 mL of molten top agar. The contents were mixed and poured on agar plates. After 48-72 h of incubation, revertant colonies were counted[19]. At least 3 plates were used for each dose and each experiment was repeated 2-3 times. Sodium azide was used as positive control in the absence of S9 mix and 2- Aminoanthracene was used in the presence of S9 for positive mutagen that needs bioactivation. The tester strain was checked routinely for ampicillin resistance, ultraviolet-light sensitivity, crystal-violet sensitivity, histidine requirement and spontaneous reversion rate.

Statistical analysis: The data obtained from the experiments were analyzed by SPSS 10 software. The differences between the revertant colonies of the test groups and the control group were tested with Student's t-test at 95% confidence level.

RESULTS AND DISCUSSION

Mutagenic potentials of metal base alloys: No antibacterial effect was observed for test solutions at different dilutions. The number of revertant colonies showed that Verabond II or Minalux at 25, 50 and 100 μL per plate had no mutagenic activity on TA100 tester strain (Table 1). Results of experiment in the presence of S9 bioactivation was similarly indicating that test solutions were not mutagen under the test conditions (Table 2).

Table 1: Mutagenic potential of dental alloys determined by Ames test without S9 bioactivation

Mean No. of revertant colonies (-S9 Mix)		
Dilutions	Verabond II	Minalux
25 μL $plate^{-1}$	40	44
50 μL $plate^{-1}$	50	51
100 μL $plate^{-1}$	40	44
Sodium Azide (1.5 μg $plate^{-1}$)	650	
H_2O (100 μL $plate^{-1}$)	26	

Table 2: Mutagenic potential of dental alloys determined by Ames test with S9 bioactivation

Mean No. of revertant colonies (+S9 Mix)		
Dilutions	Verabond II	Minalux
25 μL $plate^{-1}$	38	42
50 μL $plate^{-1}$	46	47
100 μL $plate^{-1}$	39	41
2-Aminoanthracene (2.5 μg $plate^{-1}$)	540	
H_2O (100 μL $plate^{-1}$)	32	

In contrast, positive controls (sodium azide and 2-aminoanthracene) showed highly mutagenic effects at corresponding conditions (Table 1 and 2).

In the present study, the mutagenic potentials of two metal base alloys, Verabond II and Minalux, were evaluated using the *Salmonella*/microsome gene mutation assay. The base metal alloys are extensively used in dentistry. The Cr-Co and Ni-Cr alloys have been used as replacement of gold alloys[20]. The possibility of corrosion of alloys over the time makes this concern about the capability of released elements in exerting toxic effects and in particular the mutagenic potentials.

Previous studies have shown that Ni-Cr alloys can undergo corrosion that leads to allergic responses[21]. This indicates the unwanted interaction of alloy elements with cells and tissues. It has been also reported that lung cancer is common among workers who are exposed to by products of Ni alloys[22]. In animal studies, development of sarcoma was observed following subcutaneous administration of Ni in rats[20]. Other studies have shown that women are 10 times more sensitive to Ni than men[21]. In addition, difference in sensitivity to Ni for immune system cells of mouth compare to skin has been previously observed[23]. *In vitro* studies have shown that released elements of Ni-based alloys can decrease the proliferation rate of mouth fibroblasts and affect the cellular metabolism[20].

The Ames *salmonella*/microsome test is well accepted as a rapid screening method that is capable of detecting 83% of the carcinogens as mutagens, when the suggested protocol is followed[18]. This implies that the Ames test is not able to detect all known carcinogens and therefore, any battery of test should include both *in vitro* and *in vivo* tests for evaluation of the mutagenic activity of chemicals[24]. Different *Salmonella* strains are available for conducting this test, among them TA100 is more

frequently used as tester strain and is also recommended by ISO ANS/ADA No. 41. In addition to the role of tester strains, the inclusion of S9 mix is important in determining the mutagenicity of compounds following metabolism. Mutagenicity tests may occasionally exhibit false-positive and false-negative results and it is not possible to draw a conclusive statement based solely on a single study. Although the results of this study showed no evidence of mutagenicity in the presence or absence of S9 bioactivation for Verabond and Minalux alloys but further *in vivo* studies are recommended to m ore elucidate this finding.

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