Cytotoxicity of electric spot welding: an in vitro study

Rogério Lacerda dos Santos*, Matheus Melo Pithon**, Leonard Euler A. G. Nascimento***, Fernanda Otaviano Martins****, Maria Teresa Villela Romanos*****, Matilde da Cunha G. Nojima******, Lincoln Issamu Nojima******, Antônio Carlos de Oliveira Ruellas******

Abstract

Objective: The welding process involves metal ions capable of causing cell lysis. In view of this fact, the aim of this study was to test the hypothesis that cytotoxicity is present in different types of alloys (CrNi, TMA, NiTi) commonly used in orthodontic practice when these alloys are subjected to electric spot welding. **Methods:** Three types of alloys were evaluated in this study. Thirty-six test specimens were fabricated, 6 for each wire combination, and divided into 6 groups: Group SS (stainless steel), Group ST (steel with TMA), Group SN (steel with NiTi), Group TT (TMA with TMA), Group TN group (TMA with NiTi) and Group NN (NiTi with NiTi). All groups were subjected to spot welding and assessed in terms of their potential cytotoxicity to oral tissues. The specimens were first cleaned with isopropyl alcohol and sterilized with ultraviolet light (UV). A cytotoxicity assay was performed using cultured cells (strain L929, mouse fibroblast cells), which were tested for viable cells in neutral red dye-uptake over 24 hours. Analysis of variance and multiple comparison (ANOVA), as well as Tukey test were employed ($p<0.05$). **Results:** The results showed no statistically significant difference between experimental groups ($P>0.05$). Cell viability was higher in the TT group, followed by groups ST, TN, SS, NS and NN. **Conclusions:** It became evident that the welding of NiTi alloy wires caused a greater amount of cell lysis. Electric spot welding was found to cause little cell lysis.

Keywords: Toxicity. Cell culture techniques. Welding in dentistry.

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* Specialist in Orthodontics, Federal University of Alfenas - UNIFAL. Master and Doctor in Orthodontics, Federal University of Rio de Janeiro - UFRJ. Adjunct Professor of Orthodontics, Federal University of Campina Grande - UFCG.
** Specialist in Orthodontics, Federal University of Alfenas - UNIFAL. Master and Doctor in Orthodontics, Federal University of Rio de Janeiro - UFRJ. Assistant Professor of Orthodontics, State University of Southwestern of Bahia - UESB.
*** Doctored Student in Orthodontics, Federal University of Rio de Janeiro - UFRJ.
**** Graduated in Microbiology and Immunology, Federal University of Rio de Janeiro. Trainee of the Microbiology Institute of Prof. Paulo de Góes - UFRJ.
***** PhD in Sciences (Microbiology and Immunology) by the Federal University of Rio de Janeiro - UFRJ. Adjunct Professor, Federal University of Rio de Janeiro - UFRJ.
****** MSc and PhD in Orthodontics, Federal University of Rio de Janeiro - UFRJ. Adjunct Professor of Orthodontics, Federal University of Rio de Janeiro - UFRJ.

INTRODUCTION

The composition of most alloys used in orthodontics is similar to that of stainless steel (18/8, i.e., 18% chromium and 8% nickel), and the manufacturing process of many devices such as facial masks, orthodontic bands and brackets involve welding of some kind. Research has shown that some ions can be released in welding[13,17,22,26,27,28] and this exposure may trigger a variety of adverse effects with direct toxic changes, be it acutely, or chronically.[1] The World Health Organization International Agency for Research on Cancer and the United States National Toxicology Program have determined that metal components in silver solder such as cadmium, copper, silver and zinc are potentially carcinogenic to humans.[1]

However, welding is widely used in orthodontic practice as an aid in moving teeth. Electric spot welding is a time saving procedure that provides ease of use, lower cost, hygiene and pleasing aesthetics.[5] However, this type of welding has been avoided due to poor mechanical strength when compared with silver solder.[14]

Type of welding machine, electrode shape and alloy wire are some of the factors that determine spot welding quality.[7] The first spot welding machine was marketed in 1934. Currently, machines have been reported that offer resistance welds by means of functions that allow proper melting of materials, reduction in the amount of oxides capable of weakening wire joining, and absence of heat around electrode contacts, which allows wires made from different types of alloys to not lose their mechanical properties.

The use of stainless steel alloy (CrNi) prevailed in orthodontics for decades but the advent of new metal alloys diversified the universe of weldable wires.

Given the proven cytotoxic activity of silver solders, other joining methods, free from the metal ions found in silver solder, have been used to reduce cytotoxic effects. The aim of this study was to test the hypothesis that cytotoxicity is present in different types of alloys (CrNi, TMA, and NiTi) subjected to electric spot welding in orthodontic practice.

MATERIAL AND METHODS

Cell culture

This study used a culture of L929 cells (mouse fibroblasts) obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), maintained in Eagle minimum essential medium (MEM-Eagle) (Cultilab, Campinas, Brazil) plus 0.03 mg/ml glutamine (Sigma, St. Louis, Missouri), 50 mg/ml Gentamicin Sulfate (Schering Plough, Kenilworth, New Jersey), 2.5 mg/ml fungizone (Bristol- Myers-Squibb, New York, USA), sodium bicarbonate solution at 0.25% (Merck, Darmstadt, Germany), 10 mM HEPES (Sigma, St. Louis, Missouri) and 10% fetal bovine serum (Cultilab, Campinas, Brazil) kept at 37°C in an environment containing 5% CO_2.

Test specimen fabrication

Three types of alloys were evaluated in this study. The test specimens were fabricated with rectangular wires (0.019x0.025-in), cut into segments of 25 mm, which were welded using combinations between stainless steel (CrNi), nickel-titanium (NiTi) and molybdenum-titanium (TMA) wires (Morelli, Sorocaba, Brazil). For the welding procedure the two wire segments were positioned one on top of the other forming an "X" and then placed in the electric spot welding machine (SMP-3000 Super Micro Point, Kernit, Indaiatuba, Brazil) and subjected to a single spot weld with power set at 30 W for all samples. After each weld, the ends of the electrodes were cleaned with 400 grit sandpaper (3M, Sumaré, São Paulo, Brazil).

Thirty-six test specimens were fabricated, 6 for each wire combination, and divided into: Group SS (steel with steel), Group ST (steel with TMA), Group SN (steel with NiTi), Group TT (TMA with TMA), Group TN (TMA with NiTi) and Group NN (NiTi with NiTi). After welding,

test specimen surfaces were cleaned with isopropyl alcohol and then sterilized by exposure to ultraviolet light (Labconco, Kansas, Missouri, USA) for 30 minutes along with the positive and negative controls. Preparation and welding of test specimens were performed by a single examiner.

Controls

To observe cellular responses to extremes, six additional groups were included, Group CC (cell control) where cells were not exposed to any material, Group C+ (positive control), consisting of a copper amalgam cylinder (Pratic NG 2, Vigodent, Rio de Janeiro, Brazil), group C- (negative control) consisting of a glass cylinder, and Group C- (steel), C- (TMA) and C- (NiTi) (negative control for each respective wire: stainless steel, TMA and NiTi) (Morelli, Sorocaba, São Paulo, Brazil), which remained in contact with the cells.

Cytotoxicity assay

After sterilization, the 6 samples of each material were placed in 24-well plates containing culture medium (MEM) (Cultilab, Campinas, São Paulo, Brazil). After 24 hours the culture medium was collected and evaluated for toxicity to L929 cells. Supernatants were placed in triplicate in a 96-well plate containing L929 confluent monolayer and incubated for 24 hours at 37°C in an environment containing 5% CO_2. After incubation, the effect on cell viability was determined using the dye-uptake technique described by Neyndorff et al[16] with minor modifications. After 24 hours of incubation, 100 µl of neutral red at 0.01% were added (Sigma, St. Louis, Missouri, USA), in a culture medium, to the microplate wells and these were incubated at 37°C for 3 hours to allow penetration of vital dye into the living cells. After this period and after disposal of the dye, 100 µl of formaldehyde solution (Reagen) at 4% were added in PBS (NaCl 130 mM; KCl 2 mM; Na_2HPO_4 $2H_2O$ 6 mM; K_2HPO_4 1mM, pH 7.2) for 5 minutes to promote cell attachment to the plates. Next, in order to extract the dye, a solution of 100 µl of acetic acid (Vetec, Rio de Janeiro, Brazil) at 1% was added along with methanol (Reagen, Rio de Janeiro, Brazil) at 50%. Twenty minutes later readings of the optical density of the experimental groups and positive and negative controls were performed in a spectrophotometer (Biotek, Winooski, Vermont, USA) at a wavelength of 492 nm (λ = 492 nm).

Statistical analyses were conducted with the aid of the SPSS 13.0 software program (SPSS Inc., Chicago, Illinois). Data were compared by analysis of variance (ANOVA) and then Tukey's test for assessment between groups, with reliability set at 5% significance level.

RESULTS

The results showed no statistically significant difference between experimental groups (SS, ST, SN, TT, TN and NN) ($P>0.05$). A statistically significant difference was found between groups CC and NN group ($P<0.05$). Cell viability was higher in the TT group, followed by groups ST, TN, SS, SN and NN (Table 1).

TMA alloy showed greater cell viability than steel and NiTi alloys. The same results were found by means of the negative controls of these respective alloys which were not welded (Table 1).

DISCUSSION

Most orthodontic materials establish some type of interaction with the environment, which may compromise their use due to deterioration of their mechanical or physical properties, or their appearance. One of these degradation processes is corrosion.[15]

The ions released by the corrosion process have the potential to interact with tissues through different mechanisms. Biological reactions occur by the interaction of the released ions with a molecule in the host, and alloy composition is of paramount importance in this process. The effects

TABLE 1 - Dye-uptake technique. Statistical description of optical density for experimental groups (n=6).

Groups	N	Time (24 h)			
		Mean	Median	SD	Viable cells (%)
CC	6	1.107[a]	0.989	0.119	100.0
C+	6	0.377	0.349	0.076	34.1
C-	6	1.098	0.991	0.129	99.2
C- (Steel)	6	1.052	0.960	0.076	95.1
C- (TMA)	6	1.092	0.946	0.139	98.8
C- (NiTi)	6	0.919	0.859	0.116	83.1
SS	6	0.927[a]	0.889	0.129	83.8
ST	6	0.994[a]	0.917	0.115	89.8
SN	6	0.897[a]	0.829	0.123	81.1
TT	6	1.039[a]	0.963	0.137	93.9
TN	6	0.943[a]	0.891	0.125	85.2
NN	6	0.787[b]	0.721	0.113	71.1

Values followed by identical letters do not show a statistically significant difference (p>0.05). SD= Standard deviation.

experienced by the body appear to be due to the influence of ions on the mechanisms of bacterial adhesion caused by toxicity, subtoxic effects or allergy to metal ions.[15]

One of the fundamental conditions for the use of metallic materials in the oral environment is that these materials resist the corrosive action of saliva and alkaline or acid foods[4,8] as well as variations in pH and temperature. Silver solders are among the materials used in orthodontics, which are very susceptible to corrosion.[10] These solders are used when one wishes to join stainless steel alloys or other alloys for the manufacture of orthodontic appliances.

Upon analysis of the biological aspects of silver solder, the results suggest that, contrary to routine orthodontic practice, silver solder should be used sparingly in the oral environment.[18,19]

Based on this premise, attempts have been made to replace it with other welding methods[27,28] — such as electric spot welding — that are free from the metal ions present in silver solder.[13,22,26,17,27,28] This study was conducted in order to investigate the behavior of CrNi, NiTi and TMA alloys subjected to spot welding, using a culture of fibroblasts.

Cell cultures have been used as part of a series of recommended tests for assessing the biological behavior of materials designed to be placed in contact with human tissue. In this study, copper amalgam was utilized as positive control, given its proven cytotoxicity,[23] and glass as negative control to validate the results.

The findings of this study showed low cell cytotoxicity in the experimental groups compared to the cell control groups and negative control group, with the sole exception of the NN group, which showed a statistically significant difference relative to the cell control group ($p<0.05$). This outcome can be explained by the considerable amount of nickel present in this alloy type compared to other types of alloys tested in this investigation.

The percentage of nickel in brackets, wires and auxiliary appliances used in orthodontic ranges from 8% (as in stainless steel) to more than 50% (as in the case of nickel-titanium).[9,20]

Nickel is notorious for its allergenic potential.[11,21,25] It is estimated that 4.5% to 28.5% of the population is hypersensitive to nickel,[3,12,21,24] with a higher prevalence in females: only one man — compared to 10 women — is allergic to nickel.[21] Given the presence of metal ions such as nickel in orthodontic appliances, this metal has been associated with hypersensitivity reactions in orthodontics.[2]

Groups NN and SN showed higher cytotoxicity compared to the groups that had titanium-molybdenum (TMA), but when negative controls were evaluated, C- (NiTi) and C- (steel), which were not welded, caused little cell lysis. All groups subjected to welding showed a larger amount of cell lysis compared to their respective controls, suggesting that metal ions — such as nickel — capable of causing cell lysis, are released during the wire melting process.

In view of the cytotoxicity observed in the groups there seems to be a relationship between the amount of nickel present in alloys and the amount of cell lysis caused by these alloys. For David and Lobner[6] and Eliades et al[9] there is clear evidence of a direct relationship between cytotoxicity and nickel. But findings by Sestini et al[27] showed that nickel and chromium caused a decrease in cell activity. Although in vitro evaluations do not simulate the oral environment, one should not assume that the in vitro environment is clinically inert.

The results of this study are consistent with those found by Sestini et al,[27] who evaluated two different alloys subjected to spot welding and concluded that both were well tolerated by different cell types, including fibroblasts and osteoblasts, which also agrees with the findings of Vande Vannet et al.[28]

Success in orthodontic practice involves not only employing corrective techniques to achieve the ideal dental occlusion, but also requires materials that are inert to the oral environment.

CONCLUSIONS

Electric spot welding was found to cause little cell lysis. Moreover, the welding of NiTi alloy wires produced the greatest amount of cytotoxicity while TMA alloy wires were the least cytotoxic.

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Contact address
Antônio Carlos de Oliveira Ruellas
Av. Professor Rodolpho Paulo Rocco, 325 - Ilha do Fundão
CEP: 21.941-617 - Rio de Janeiro / RJ, Brazil
E-mail: antonioruellas@yahoo.com.br