ISSN 1807-5274
Rev. Clín. Pesq. Odontol., Curitiba, v. 6, n. 2, p. 141-146, maio/ago. 2010

CITOTOXICITY OF ORTHODONTIC MINI-IMPLANTS

Citotoxidade de mini-implantes ortodônticos

Matheus Melo Pithon[a], Rogério Lacerda dos Santos[b], Fernanda Otaviano Martins[c], Paulo José Medeiros[d], Maria Teresa Villela Romanos[e]

[a] MsC, Orthodontist, PhD candidate, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ - Brasil, e-mail: matheuspithon@bol.com.br
[b] Graduate student, Microbiology and Immunology, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ - Brasil.
[c] PhD, Oral Maxillofacial Surgery and Traumatology, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ - Brasil.
[d] PhD, Microbiology and Immunology, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ - Brasil.
[e] Doutor em Ciências (Microbiologia) pela Universidade Federal do Rio de Janeiro (UFRJ), professor adjunto da Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, RJ - Brasil.

Abstract

OBJECTIVE: Based in the premise that Ti-6Al-4V orthodontic mini-implants can release metal ions into the body fluids, this research is aimed assess the cytotoxic effect of orthodontic mini-implant on L929 fibroblast cells. **MATERIAL AND METHODS**: Eighteen orthodontic mini-implants made of Ti-6Al-4V alloy were divided into 6 groups: 1 (golden colour, SIN), 2 (silver colour, SIN), 3 (Neodent™), 4 (INP™), 5 (Mondeal™), and 6 (Titanium Fix™). The mini-implants were immersed into Eagle's minimum essential medium for 24 hours, where supernatant removal and contact with L929 fibroblasts were performed. Cytotoxicity was evaluated in four different periods of time: 24, 48, 72, and 168 hours. After being in contact with the mini-implants immersed, the cells were incubated for further 24 hours and then 100 ml of 0.01% neutral-red staining solution were added. After this period of time, they were fixed and a spectrophotometer was used for counting the viable cells. **RESULTS**: After the 24 hours period, statistical differences were found by comparing groups 1 and 2 to groups 3,4,5, and C+ ($p < 0.05$). After the 48 hours period, groups 1 and 2 were shown to be statistically different in relation to groups 3, 4, and C+. After the 72 hours period, statistical differences were found only in group 1 compared to groups 4, 5, 6, CC, and C+ ($p < 0.05$). After 7 days, no statistical differences were found between the mini-implants. **CONCLUSION**: Although mini-implants are made of the same alloy, there are differences in their cytotoxicity because of the different concentrations of chemical elements used for manufacturing them.

Keywords: Mini-implants. Cytotoxicity. Orthodontics.

Resumo

OBJETIVO*: Baseando-se na premissa de que mini-implantes ortodônticos podem liberar íons metálicos nos fluidos corporais, pesquisou-se o efeito citotóxico de mini-implantes ortodônticos em fibroblastos L929.* ***MATERIAL E MÉTODO****: Dezoito mini-implantes ortodônticos confeccionados em liga Ti-6Al-4V foram divididos em seis grupos: 1 (dourados, SIN), 2 (prateados SIN), 3 (Neodent), 4 (INP), 5 (Mondeal) e 6 (Titanium Fix). Os mini-implantes foram imersos em meio mínimo essencial Eagle por 24 horas, onde efetuou-se remoção do supernadante e contato com fibroblastos L929. A citotoxicidade foi avaliada em 4 diferentes tempos: 24, 48, 72 e 168 horas. Após contato com os mini-implantes imersos, as células foram incubadas por mais 24 horas; então, 100 ml de solução corante neutra-vermelha foram adicionados. Após, foram fixadas e um espectrofotômetro foi usado para contar as células viáveis.* ***RESULTADOS****: Após o período de 23 horas, compararam-se os grupos 1 e 2 aos grupos 3, 4, 5 e C+. Após 72 horas, diferenças estatísticas foram encontradas somente no grupo 1, comparado aos grupos 4, 5, 6, CC e C+ ($p < 0,05$). Após 7 dias, não foram encontradas diferenças estatisticamente significantes entre os diversos mini-implantes.*

Palavras-chave*: Mini-implantes. Citotoxidade. Ortodontia.*

INTRODUCTION

The control of orthodontic anchorage has been an issue of concern to orthodontists since the early existence of this speciality. A successful orthodontic treatment, in the great majority of cases, requires that anchorage to be judiciously planned, and it would not be an exaggeration to state that this is one of the determinant factors for success or failure in many orthodontic treatments.

Mini-implants are currently being used for improving those situations in which orthodontic anchorage is needed (1-4). Their use is motivated by their positioning versatility, easy removal, and low cost (4-6). Most mini-implants are made of titanium alloy, differing in shape, design, measurements, and trademark (7). The commercially pure titanium (CP Ti) is largely utilized in the manufacture of dental and orthopaedic implants since it is considered chemically inert, in addition to have adequate mechanical properties and excellent biocompatibility (8). Despite these favourable characteristics, however, CP Ti has not been the preferred material for manufacturing orthodontic mini-implants because of its low resistance to fractures and possibility of Osseo integration (9).

Fracture resistance is one of the necessary characteristics required for insertion and removal of orthodontic mini-implants in view of their reduced size and inter-radicular placement (10). In order to overcome this problem, the material chosen for confectioning orthodontic mini-implants is the Ti-6Al-4V alloy because of its higher resistance to fracture (11).

However, the metallic alloys used in orthodontics are subject to corrosion and metal ion release into the oral cavity, which may cause adverse physiological effects such as cytotoxicity, genotoxicity, carcinogeniticity, and allergenic effects. The choice of a certain alloy depends largely on its indications. Ti-6Al-4V alloys are composed of aluminium (Al) and vanadium (V), both found to be cytotoxic elements when released in the form of ions during essays on erosion in physiological medium (12).

Ti-6Al-4V alloy is less resistant to corrosion than CP Ti (11, 13), resulting in metal ions release. As these ions can accumulate in tissues surrounding the mini-implant (14) and even in distant tissues (15) undesirable effects on human body can occur such as osteolysis, allergic reactions, renal lesions, cytotoxicity, hypersensibility, and carcinogenesis (16). In addition, metal ions are often accounted for implant failure. Because all commercially available orthodontic mini-implants are made of Ti-6Al-4V alloy, the author aims to investigate the hypothesis that there is no difference in cytotoxity between mini-implants from different manufacturers.

MATERIAL AND METHODS

Cell culture

The cell line used for this study was mouse L929 fibroblasts obtained from the American Type Culture Collection (TCC, Rockville, MD) and cultivated in Eagle's minimum essential medium

(MEM) (Cultilab™, Campinas, São Paulo, Brazil). The cell culture was supplemented with 2 mM of L-glutamine (Sigma™, St. Louis, Missouri, USA), 50 μg/ml of gentamicin (Schering Plough™, Kenilworth, New Jersey, USA), 2.5 μg/ml of fungizone (Bristol-Myers-Squib™, New York, USA), 0.25 mM of sodium bicarbonate solution (Merck™, Darmstadt, Germany), 10 mM of HEPES™ (Sigma, St. Louis, Missouri, USA), and 10% of foetal bovine serum (FBS) (Cultilab™, Campinas, São Paulo, Brazil), then being kept at 37°C in 5% CO_2 environment.

Mini-implants to be evaluated

The sample consisted of 18 Ti-6Al-4V orthodontic mini-implants from different manufacturers divided into six experimental groups, namely, Group 1 (golden colour, SIN™, São Paulo, Brazil), Group 2 (silver colour, SIN, São Paulo, Brazil), Group 3 (Neodent™, Curitiba, Brazil), Group 4 (INP™, São Paulo, Brazil), Group 5 (Mondeal™, Tuttlingen, Germany), and Group 6 (Titanium Fix™, São José dos Campos, Brazil).

Controls

To verify the cell response to extreme situations, other three groups were included in the study: Group CC (cell control), consisting of cells not exposed to any material; Group C+ (positive control), consisting of amalgam cylinder; and Group C- (negative control), consisting of a stainless steel wire (nickel free) (Morelli™, Sorocaba, São Paulo) in contact with the cells.

Assessing the cytotoxicity of the materials

The materials were previously sterilised by exposing them to ultra-violet light (Labconco™, Kansas, Missouri, USA) during 1 hour. Next, three samples of each material were placed in 24-wells plates containing Eagles' MEM (Cultilab™, Campinas, São Paulo, Brazil). The culture medium was replaced with fresh medium every 24 hours, and the supernatants were collected after 24, 48, 72, and 168 hours (7 days) for analysis of the toxicity to L929 cells. The supernatants were placed in a 96-well plate containing a single layer of L929 cells and then incubated at 37°C for 24 hours in 5% CO_2 environment. After the incubation period, cell viability was determined using the "dye-uptake" technique described by Neyndorff et al. (17) (1990), which was slightly modified. After the 24-hour incubation period, 100 μl of 0.01% neutral-red staining solution (Sigma™, St. Louis, Missouri, USA) were added to the medium within each well of the plates, and these were incubated for 3 hours at 37°C to allow the dye to penetrate into the living cells. After this period, the cells were fixed using 100 μl of 4% formaldehyde solution (Reagen™, Rio de Janeiro, Brazil) in PBS (130 mM NaCl; 2 mM KCl; 6 mM Na_2HPO_4 $2H_2O$; 1 mM K_2HPO_4, pH = 7.2) for 5 minutes. Next, 100 μl of 1% acetic acid solution (Vetec, Rio de Janeiro, Brazil) with 50% methanol (Reagen™, Rio de Janeiro, Brazil) were added to the medium to remove the dye. Absorption was measured after 20 minutes by using a spectrophotometer (BioTek™, Winooski, Vermont, USA) at a wave length of 492 nm (μ = 492 nm).

X-ray dispersion analysis

The metallic alloy of the mini-implants was characterised by X-ray dispersion using a JEOL scanning electron microscope (2000 FX™, Tokyo, Japan). For doing so, the mini-implants were cut with a precision sectioning machine (Isomet™, Buehler, Illinois, USA) and washed with an ultrasound equipment (Ultramet™ 2002, Buehler, Illinois, USA) Next, the samples were carefully dried and positioned for being submitted to SEM analysis.

Statistical analysis

Statistical analyses were performed by using a SPSS v.13.0™ software (SPSS Inc., Chicago, USA), and means and standard deviations were calculated for descriptive statistical analysis. The values for the amount of viable cells were submitted to analysis of variance (ANOVA) to determine whether statistical differences existed between the groups, and Tukey's test was applied thereafter.

RESULTS

The results regarding the 24-hour period demonstrated that statistical differences were found when Groups 1 and 2 were compared to Groups 3, 4, 5 and C+ as well as when Group C+ were compared to the other groups ($p < 0.05$). Groups 1 and 2 were shown to be statistically different compared to Groups 3, 4 and C+ at 48 hours (Table 1).

After the 72-hour period, it was observed that Group 1 was statistically different from Groups 4, 5, 6, CC, and C+ ($p < 0.05$). Statistical differences were found between Group C+ and all other groups not only at 7 hours, but also in all periods of time ($p < 0.05$).

Table 2 shows the percentage amount of chemical elements found in the mini-implants evaluated by using the X-ray dispersion analysis (EDX).

Table 1 - Statistical analysis with means and standard deviations for the groups studied

	24h		48 h		72h		7 days	
Groups	**M. Cel/DP**	**Stat.**	**M. Cel/DP**	**Stat.**	**M. Cel/DP**	**Stat.**	**M. Cel/DP**	**Stat.**
1	0,622 (±0,05)	A	0,510 (±0,12)	AC	0,855 (±0,09)	A	0,274 (±0,03)	A
2	0,636 (±0,09)	A	0,518 (±0,08)	AC	0,926 (±0,04)	AB	0,276 (±0,02)	A
3	0,492 (±0,06)	BC	0,719 (±0,10)	B	0,843 (±0,14)	AC	0,267 (±0,03)	A
4	0,495 (±0,06)	BC	0,741 (±0,04)	B	1,001 (±0,12)	BC	0,276 (±0,02)	A
5	0,498 (±0,08)	BC	0,681 (±0,06)	BC	1,034 (±0,07)	B	0,276 (±0,02)	A
6	0,561 (±0,07)	AC	0,646 (±0,05)	BC	1,049 (±0,15)	B	0,342 (±0,02)	A
C.C.	0,638 (±0,03)	AC	0,779 (±0,13)	ABC	1,051 (±0,07)	B	0,319 (±0,01)	A
C. +	0,285 (±0,06)	D	0,251 (±0,04)	D	0,648 (±0,04)	D	0,212 (±0,03)	B
C. -	0,538 (±0,00)	ABC	0,573 (±0,03)	ABC	0,952 (±0,05)	ABC	0,318 (±0,02)	A

Legend: M. Cel = mean values for the amount of viable cells;
SD = Standard Deviation;
Stat = Same letters mean no statistical difference.

Table 2 - Percentage amount of chemical elements in the mini-implants tested

Groups	Manufacturers	C	Al	Ti	V	Fe	Cu	O	N
1	SIN (golden)	2.30%	4.27%	71.91%	2.62%	-	-	16.42%	2.48%
2	SIN (silver)	2.30%	4.27%	71.91%	2.62%	-	-	16.42%	2.48%
3	Neodent	-	5.73%	91.43%	3.94%	-	-	-	-
4	INP	-	5.36%	90.33%	3.22%	-	-	-	-
5	Mondeal	1.35%	3.81%	70.39%	3.18%	0.28%	0.11%	20.87%	-
6	Titanium Fix	3.05%	5.08%	87.87%	3.58%	0.27%	0.16%	-	-

DISCUSSION

The use of cell culture has been employed as part of a series of recommend tests for evaluation of the biological behaviour of materials being put in contact with human tissues. In the present study, cytotoxicity tests were conducted in order to evaluate the biocompatibility of mini-implants for orthodontic usage.

The commercially available orthodontic mini-implants are made of Ti-6A-4V alloy. The biocompatibility of Ti ions is well described in the literature, but aluminum (Al) and vanadium (V) have not been so studied. Al ions affect proliferation, metabolic activity and differentiation of osteoblasts (18). Some of the toxic effects described in the literature are encephalopathy and Alzheimer-type senile dementia (19), and Al may be associated with osteomalacia and pulmonary granulomatosis as well (20). With regard to the level of Al ions in the orthodontic mini-implants studied, Group 3 had the highest percentage of aluminium (5.73%), followed by Group 4 (5.36%), Group 6 (5.08%), Groups 1 & 2 (4.27%), and Group 5 (3.81%).

Vanadium, whose main source is food, is an essential micro-element that is present in the majority of mammalian cells (21, 22). However, this chemical element is considered highly toxic compared to other nutritionally essential micro-elements because there is a small difference between the necessary and toxic doses (23). On the other hand, V has important pharmacological and physiological effects, playing an important role in the auxiliary treatment of diabetic patients (22).

The effects of chronic and acute V intoxication are well documented. Vanadium is cytotoxic for macrophages and fibroblasts (24), binds to certain proteins (e.g. ferritin and transferrin), affecting their distribution and accumulation throughout the body (22), stimulates local and systemic allergic reactions, inhibits cell proliferation and may cause renal lesions. Urinary excretion is the main pathway for elimination of injected vanadium in human beings (22).

Similarly to the presence of aluminum, Group 3 was found to have the lowest cell viability in all periods of time. This fact may due to the greater amount of aluminium (56%) and vanadium (3.22%) found in the samples during X-ray dispersion analysis (EDX).

These differences in cell viability between the groups may be related not only to aluminium and vanadium, but also to other components such as carbon (C), titanium (Ti), iron (Fe), copper (Cu), oxygen (O), and nitrogen (N).

Despite the differences found between several orthodontic mini-implants, in fact they showed lower cytotoxicity compared to Groups CC and C-. During the whole experiment it was observed that cell viability was higher in Group CC (mini-implant exposed to no material) and lower in Group C+, a finding that may be explained by the constant release of mercury from the amalgam – a material known to be cytotoxic (25).

After seven days, all mini-implants showed higher cell viability when compared to each other. During this period of time, no statistical differences were found between the groups studied. A drawback regarding this study was the lack of evaluation of ion content in the supernatant placed on the cells. Only ions presenting in mini-implants were evaluated. Further studies are needed to evaluate this point, which can allow us to assess any relationship between ions released by mini-implants and consequently their real cytotoxicity.

CONCLUSION

The hypothesis that no difference exists in the cytotoxicity between mini-implants made from the same alloy was not proved. Besides, small differences were observed, possibly due to the concentration of chemical elements.

CONFLICT OF INTEREST STATEMENT

The authors formally declare that there is no conflict of interest in the present manuscript.

REFERENCES

1. Melsen B. Mini-implants: Where are we? J Clin Orthod. 2005;39(9):539-47.

2. Melsen B, Costa A. Immediate loading of implants used for orthodontic anchorage. Clin Orthod Res. 2000;3(1):23-8.

3. Kanomi R. Mini-implant for orthodontic anchorage. J Clin Orthod. 1997;31(11):763-7.

4. Nojima LI, Gonçalves M, Melgaço CA, Costa LFM, Bernardes J. Dispositivos temporários de ancoragem em ortodontia estética em implantes: uma abordagem dos tecidos moles e duros. São Paulo: Santos; 2006.

5. Nascimento MHA, Araújo TM, Bezerra F. Orthodontic micro-screw: installation and peri-implant hygienic orientation. R Clin Ortod Dental Press. 2006;5(5):24-31.

6. Araújo TM, Nascimento MHA, Bezerra F, Sobral MC. Skeletal anchorage in Orthodontics with mini-implants. R Dental Press Ortod Ortop Facial. 2006;11(4):126-56.

7. Squeff LR, Simonson MBA, Elias CN, Nojima LI. Caracterização de mini-implantes utilizados na ancoragem ortodôntica. R Dental Press Ortod Ortop Facial. 2008;13(5):49-56

8. Craig RG. Restorative dental materials. 9th ed. Sl Louis: Mosby Year Book; 1993.

9. Niinomi M. Mechanical properties of biomedical titanium alloys. Materials Sci Engin. 1998;A243(1):231-6.

10. Pithon MM, Nojima LI, Nojima MGC, Ruellas ACO. Comparative study of fracture torque for orthodontic mini-implants of different trademarks. O Surg. 2008;1(2):84-7.

11. Anusavice KJ. Phillips, materiais dentários. 11a ed. Rio de Janeiro: Guanabara Koogan; 2005.

12. Khan MA, Williams RL, Williams DF. The corrosion behaviour of Ti-6Al-4V, Ti-6Al-7Nb and Ti-13Nb-13Zr in protein solutions. Biomaterials. 1999;20(3):631-7.

13. Hanawa T. Metal ion release from metal implants. Materials Sci Engin. 2004;24(4):745-52.

14. Morais LS, Serra GG, Muller CA, Andrade LR, Palermo EF, Elias CN, et al. Titanium alloy mini-implants for orthodontic anchorage: immediate loading and metal ion release. Acta Biomater. 2007;3(3):331-9.

15. Frisken KW, Dandie GW, Lugowski S, Jordan G. A study of titanium release into body organs following the insertion of single threaded screw implants into the mandibles of sheep. Aust Dent J. 2002;47(3):214-7.

16. Chassot E, Irigaray JL, Terver S, Vanneuville G. Contamination by metallic elements released from joint prostheses. Med Eng Phys. 2004;26(3):193-9.

17. Neyndorff HC, Bartel DL, Tufaro F, Levy JG. Development of a model to demonstrate photosensitizer-mediated viral inactivation in blood. Transfusion. 1990;30(2):485-90.

18. Bohrer D, do Nascimento PC, Mendonca JK, Polli VG, de Carvalho LM. Interaction of aluminium ions with some amino acids present in human blood. Amino Acids. 2004;27(1):75-83.

19. Swegert CV, Dave KR, Katyare SS. Effect of aluminium-induced Alzheimer like condition on oxidative energy metabolism in rat liver, brain and heart mitochondria. Mech Ageing Dev. 1999;112(1):27-42.

20. Schmidt R, Bohm K, Vater W, Unger E. Aluminium induced osteomalacia and encephalopathy--an aberration of the tubulin assembly into microtubules (MTs) by Al3+? Prog Histochem Cytochem. 1991;23(1):355-64.

21. Heinemann G, Braun S, Overbeck M, Page M, Michel J, Vogt W. The effect of vanadium-contaminated commercially available albumin solutions on renal tubular function. Clin Nephrol. 2000;53(6):473-8.

22. Heinemann G, Fichtl B, Vogt W. Pharmacokinetics of vanadium in humans after intravenous administration of a vanadium containing albumin solution. Br J Clin Pharmacol. 2003;55(3):241-5.

23. Woodman JL, Jacobs JJ, Galante JO, Urban RM. Metal ion release from titanium-based prosthetic segmental replacements of long bones in baboons: a long-term study. J Orthop Res. 1984;1(4):421-30.

24. Tian YS, Chen CZ, Li ST, Huo QH. Research progress on laser surface modification of titanium alloys. App Surface Science. 2005;242(1):177-84.

25. Nakajima H, Wataha JC, Rockwell LC, Okabe T. In vitro cytotoxicity of amalgams made with binary Hg-In liquid alloys. Dent Mater. 1997;13(3):168-73.

Received: 09/22/2009
Recebido: 22/09/2009

Accepted: 02/25/2010
Aceito: 25/02/2010