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AVALIAÇÃO DA CITOTOXICIDADE E DA LIBERAÇÃO IÔNICA DE MINI-IMPLANTES ORTODÔNTICOS

Porto Alegre 2010.

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Tese apresentada como parte dos requisitos obrigatórios para a obtenção do grau de Doutor na área de Materiais Dentários pelo Programa de Pós-Graduação da Faculdade de Odontologia da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Luciana Mayumi Hirakata

Co-orientadora: Renata Medina

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Dedico este trabalho:

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“Queremos saber
queremos viver
confiantes no futuro
por isso se faz necessário
prever qual o itinerário da ilusão
a ilusão do poder
pois se foi permitido ao homem
tantas coisas conhecer
é melhor que todos saibam
o que pode acontecer
queremos saber
queremos saber
todos queremos saber”
Gilberto Gil

RESUMO:

Propôs-se neste trabalho avaliar a biocompatibilidade dos mini-implantes ortodônticos novos e utilizados em tratamento ortodôntico por meio da aplicação de testes avaliativos de toxicidade celular *in vitro* e de liberação iônica *in vivo*.

O ensaio de citotoxicidade foi através da levedura *Saccharomyces Cerevisiae* que é um modelo microbiológico eucariótico. Foram realizados testes qualitativos e quantitativos, com a cepa selvagem FF18733 desta levedura. Foram executados testes de exposição direta (em meio de cultura YPD líquido) e testes de exposição indireta utilizando: MIs novos, MIs expostos ao fluoreto de sódio (NaF a 0,0125%, 0,025% e 0,05%), e MIs utilizados *in vivo*. Foram realizadas fotomicrografias com microscopia eletrônica de varredura (MEV) nos MIs utilizados *in vivo* e novos. Como resultado, a cepa selvagem FF 18733 mostrou uma diminuição de sua sobrevivência nos experimentos de exposição direta a um e a dois mini-implantes novos. No entanto, tal sensibilidade não se mostrou significativa. Os resultados de exposição à saliva artificial de novos, bem como todos os resultados referentes aos usados, não indicaram diferenças em relação aos controles. Da mesma forma, a exposição ao NaF não induziu, nos MIs, uma corrosão suficiente para causar toxicidade celular significativa nesta levedura. Apesar da corrosão leve apresentada nas fotomicrografias, não foram observadas induções de perda de viabilidade celular significativas na cepa testada de *S. cerevisiae* a partir dos componentes da liga (Ti-6Al-4V) dos MIs ortodônticos. Evidenciou-se, assim, que tais componentes não alteram de forma importante o metabolismo da levedura,

indicando que os MIs testados tendem a apresentar uma boa biocompatibilidade para uso em clínica ortodôntica.

No estudo *in situ*, o objetivo foi examinar e comparar os níveis de vários íons metálicos liberados na saliva de pacientes em tratamento ortodôntico e que iriam necessitar de tratamento envolvendo o uso de mini-implante. A amostra foi composta por 20 indivíduos que estavam entre o sexto e o oitavo mês de tratamento ortodôntico. A saliva de cada paciente foi coletada em 4 tempos diferentes: antes da inserção do MI (T1), 10 minutos (T2), 7 dias (T3) e 30 dias (T4) após a instalação do MI. As amostras salivares foram analisadas através de espectrometria (ICP- MS - inductively coupled plasma mass spectrometry; e ICP-OES - inductively coupled plasma optical emission spectrometry). A liberação de nove diferentes ions metálicos foram observados: titânio (Ti), zinco (Zn), cromo (Cr), níquel (Ni), ferro (Fe), cobre (Cu), alumínio (Al) e cobalto (Co). Os dados foram analisados através de estatística descritiva e testes de normalidade (Shapiro-Wilk). A concentração de ions metálicos na saliva nos quatro tempos diferentes de tratamento com MI foi comparado usando o teste de Wilcoxon (α=95%). No tempo T4, houve um aumento quantitativo na concentração salivar de Cu, Ti, V, Zn, bem como um decréscimo quantitativo na concentração salivar de Al, Co, Cr, Fe, Ni, quanto comparados ao grupo T1. No entanto, não houve diferenças estatisticamente significativas entre as concentrações de metais. Logo, pode ser concluído que a colocação de aparelho ortodôntico associado ao uso de mini-implante ortodôntico não leva a um aumento, estatisticamente significativo, na concentração de ions metálicos na saliva.

Palavras-chave: citotoxicidade, liberação iônica, mini-implantes, ortodontia.

ABSTRACT:

The aim of this study was to investigate the biocompatibility of new and corroded miniscrews (MSs) through the evaluation of cell toxicity *in vitro* and of ion release *in vivo*.

Saccharomyces Cerevisiae yeast was used as a eukaryotic microbiological model for the cytotoxicity assay. Qualitative and quantitative tests were carried out using the wild strain FF18733. In addition, direct exposure (in liquid YPD culture) and indirect exposure tests (using artificial saliva previously exposed to the MSs) were carried out using new MSs and MSs with sodium fluoride (NaF at 0.0125%, 0.025% and 0.05%) and used *in vivo*. Photomicrographs with scanning electron microscopy (SEM) were obtained for the new and used MSs. As a result, the wild strain FF 18733 showed a reduction in cell viability in the direct exposure to one and two new MSs. However, this reduction was not significant. The results for indirect exposure showed that there were no significant differences between new and used MSs and the control groups. Moreover, exposure to NaF did not induce corrosion of MSs sufficient to lead to significant cell toxicity in the yeast. Despite the slight corrosion revealed by the photomicrographs, there was no significant loss of cell viability in the strains of *S. cerevisiae* tested. This demonstrates that the components of MSs (Ti-6Al-4V) did not significantly alter the yeast metabolism, indicating that the MSs tested present good biocompatibility for use in orthodontic clinics.

The aim of the *in situ* study was to compare the levels of several metallic ions released in the saliva of patients undergoing orthodontic treatment

with MSs. The experimental group was made up of 20 individuals who were within the 6th and 8th months of treatment. Saliva from each patient was collected at four different timepoints: before MS placement (T1), 10 minutes (T2), 7 days (T3) and 30 days (T4) after MS placement. Saliva samples were analyzed by spectrometry (ICP- MS - inductively coupled plasma mass spectrometry; and ICP-OES - inductively coupled plasma optical emission spectrometry). The release of nine different metal ions was observed: titanium (Ti), zinc (Zn), chromium (Cr), nickel (Ni), iron (Fe), copper (Cu), aluminum (Al), vanadium (V), and cobalt (Co). Data were analyzed by descriptive statistics and tests for normality (Shapiro-Wilk). The metal ion concentrations in the saliva at the four different timepoints were compared using the Wilcoxon test (α=95%). At T4, there was a quantitative increase in the salivary concentration of Cu, Ti, V and Zn and a quantitative decrease in Al, Co, Cr, Fe and Ni, when compared to T1. However, there were no statistically significant differences in the metal concentrations. Therefore, it can be concluded that the use of orthodontic appliances with MSs does not lead to an increase, significative statistically, in salivary metal concentrations.

Key-words: citotoxicity, ionic release, miniscrews, orthodontics.

SUMÁRIO:

1. INTRODUÇÃO:

1.1 MINI-IMPLANTES E SEU USO COMO ANCORAGEM

A ancoragem ortodôntica é definida como resistência ao movimento dentário indesejável (PROFFIT e FIELDS, 1999).

A ancoragem têm sido um desafio desde a introdução dos aparelhos ortodônticos fixos. Geralmente, o movimento ortodôntico de um dente é ancorado por um grupo de dentes para que se minimize os deslocamentos indesejáveis. Uma ancoragem adequada tem se tornado difícil quando há falta de dentes posteriores. Aparelhos auxiliares intra e extra-orais tem sido usados para ajudar na movimentação, mas a efetividade desses depende do nível de cooperação do paciente (MARASSI, 2005).

Os implantes convencionais tem sido uma excelente alternativa para ancoragem, no entanto, esses só podem ser colocados em região retromolar e áreas edêntulas (ROBERTS *et al.*,1989; FAVERO *et al.*,2002). Em alguns casos, os implantes dentários convencionais são um problema para os pacientes devido à severidade da cirurgia, ao desconforto da cicatrização inicial e à dificuldade de manter a higiene oral nessa área (OHMAE *et al.*, 2001).

Assim, devido às desvantagens existentes do implante convencional, foi criado o mini-implante. Os mini-implantes foram propostos por Kanomi (1997), para ancoragem ortodôntica e apresentavam as seguintes medidas: 1.2mm de diâmetro x 6mm de comprimento. Eles são amplamente usados, pois tem poucas limitações

quanto ao local de implantação e ser um procedimento relativamente simples quanto a inserção, e de fácil controle mecânico (PARK *et al*., 2003).

A metodologia para a implementação dos mini-implantes tem sido continuamente melhorada. Algumas complicações ainda persistem e os tipos de falhas incluem a inflamação do tecido ao redor do mini-implante e a fratura do mesmo no momento de sua retirada (PARK *et al*., 2003).

1.2. BIOCOMPATIBILIDADE DOS MATERIAIS UTILIZADOS EM ORTODONTIA

A biocompatibilidade é a propriedade de um material em não causar injúrias ou efeitos tóxicos sobre os sistema biológicos, ou induzir a uma resposta adequada do hospedeiro em situações específicas (WATAHA, 2000).

A capacidade de um material ser biocompatível ou não, depende da sua composição e localização, bem como da sua interação com a cavidade oral humana (CRAIG, 1990). Um dos fatores determinantes da biocompatibilidade das ligas metálicas em Odontologia é a resistência à corrosão (WATAHA *et al.,*2002). Sabe-se que os materiais ortodônticos são confeccionados basicamente a partir de ligas de aço inoxidável, e contêm metais como o níquel e o cromo (STARKJAER; MENNÉ, 1990; BISHARA; BARRETT; SELIM, 1993; RAHILY; PRICE, 2003).

Os metais constituintes do aparelho ortodôntico sofrem um processo de corrosão estimulado, uma vez que esse meio possui elementos químicos que são capazes de alterar a estrutura do aço inoxidável (MATASA, 1995) o que pode acontecer, também, com a liga de titânio constituinte do mini-implante ortodôntico.

A corrosão das ligas metálicas utilizadas em odontologia resulta na liberação de íons metálicos que produzem efeitos deletérios aos tecidos humanos (WATAHA, 2000; GRIMAUDO, 2001; FARRONATO *et al.*, 2002; SCHMALZ e GARHAMMER, 2002; SÓRIA *et al.,* 2005 e SOUZA e MENEZES, 2008). Os principais efeitos são: alergênicos (EL AGROUDI; EL MOTAYAM; AWAD, 1986; MUNKSGAARD, 1992, GRIMAUDO, 2001), mutagênicos (FACCIONI *et al*., 2003), carcinogênicos (OLLER; COSTA; OBERDÖRSTER, 1997; BURGAZ *et al*., 2002) e citotóxicos (BOUR, 1994; NOVELLI *et al*.,1998; MORAES *et al*., 1999; FACCIONI *et al*., 2003; NIKI, *et al.* 2003).

O titânio comercialmente puro é amplamente usado como material para implante devido as suas propriedades mecânicas favoráveis e excelente biocompatilidade (APARÍCIO *et al.,* 2003). No entanto, o titânio comercialmente puro tem baixa resistência à fadiga quando comparado à uma liga de titânio. Logo, o Ti–6Al-4V foi usado para mini-implantes para melhorar essa característica (HANAWA, 2004).No entanto, a resistência à corrosão dos mini-implantes diminuiu quando essa liga foi usada, favorecendo a liberação de íons, o qual pode ser associada a falhas clínicas dos implantes, osteólise, reações cutâneas alérgicas, lesão de fígado, citotoxicidade, hipersensibilidade e carcinogênese (SEDARAT *et al*., 2001).

Para Morais *et al.* (2007) em um estudo utilizando coelhos, os resultados mostraram que, apesar da tendência da grande liberação de íons quando utilizado a liga de titânio, a quantidade de vanádio detectado não alcançou níveis tóxicos no modelo animal, em 4 semanas, quando os níveis de concentrações máximos foram medidos.

Apesar dessas evidências, não há um consenso na literatura sobre as propriedades biológicas das substâncias liberadas pelos aparelhos ortodônticos (ELIADES *et al*., 2004), bem como dos mini-implantes usados no auxílio de ancoragem ortodôntica.

2. OBJETIVOS:

2.1. OBJETIVO GERAL

Verificar a biocompatibilidade dos mini-implantes ortodônticos por meio da aplicação de testes avaliativos de toxicidade celular e de liberação iônica.

2.2. OBJETIVOS ESPECÍFICOS

- Avaliação de toxicidade celular

Análise da citotoxicidade induzida por mini-implantes ortodônticos novos e utilizados *in vivo,* utilizando como organismo-modelo a levedura *Saccharomyces cerevisiae.* Para tanto, serão realizados dois conjuntos de experimentos:

1- Sobrevivência de células de *Saccharomyces cerevisiae* após exposição direta aos mini-implantes ortodônticos, em meio de cultura líquido *in vitro*;

2- Sobrevivência de *Saccharomyces cerevisiae* frente aos químicos liberados pelos mini-implantes ortodônticos em saliva artificial comercial, *in vitro*.

3- Sobrevivência de *Saccharomyces cerevisiae* frente aos químicos liberados pelos mini-implantes ortodônticos após exposição ao fluoreto de sódio em diferentes concentrações (0,012%, 0,025% e 0,05%), *in vitro*.

- Avaliação da liberação iônica

Verificação da liberação de íons de mini-implantes ortodônticos e aparelhos fixos em diferentes grupos de pacientes, através da análise salivar em espectrometria (ICP-MS e ICP-OES), *in situ*.

3. DESENVOLVIMENTO:

3.1. Artigo 1:

Corrosion of Ti alloy miniscrews– A cytotoxicity evaluation

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Abstract:

Orthodontic miniscrews (MSs) are made of a titanium grade V alloy (Ti- 6Al-4V). This titanium alloy is highly resistant to fracture but is less biocompatible when compared to commercially pure titanium. Because of its lower resistance to intra-oral corrosion, it releases a greater amount of metallic ions. The objective of this study is to determine the cytotoxicity induced by orthodontic MSs in *Saccharomyces cerevisiae* cells *in vitro* as well as to microscopically evaluate corrosion present in MSs used intra-orally during 6 to 8 months. Direct exposure tests (in liquid YPD culture medium) were carried out using the wild *S. cerevisiae* strain FF18733 and indirect exposure tests (in artificial saliva previously exposed to the MSs) were carried out using new MSs, MSs corroded with sodium fluoride (NaF at 0.0125%, 0.025% and 0.05%) and MSs corroded *in vivo*. For the MSs corroded *in vivo*, patients who had undergone orthodontic treatment with MSs donated their used MSs after completion of mechanic molar distalization. Photomicrographs of the corroded and new MSs were obtained at scales of 1mm, 500µm and 100µm. The results show that the *S. Cerevisiae* strain utilized presented a slight, non-significant reduction in cell viability upon direct exposure to new MSs. In the indirect exposure tests using artificial saliva exposed to either new or corroded MSs, there were no differences among the experimental and control groups. Similarly, exposure to NaF did not induce of MS corrosion sufficient to cause cell toxicity in *S. cerevisiae.* Although scanning electron microscopy (SEM) revealed corrosion in the used MSs, no significant loss of cell viability was observed in the yeast. Therefore, it can be concluded that the MS titanium alloy components did not significantly alter the yeast

metabolism, indicating that the MSs tested are biocompatible for use in orthodontic clinics.

Keywords:

Cytotoxicity, miniscrews, *Saccharomyces cerevisiae*, orthodontic appliances.

Introduction:

Biomaterial is considered to be any synthetic material that substitutes or restores tissue function in the body and remains in continuous or intermittent contact with organic fluids [1]. All implanted metallic biomaterial interacts with the tissue with which it comes into contact and releases ions by dissolution, wear or corrosion [2]. Metallic ions released by metallic biomaterials can cause a number of phenomena including: the transport, metabolization and accumulation of this material in organs as well as the induction of disturbances varying from allergies to carcinomas [2].

Intra-oral corrosion is known to be a complex process that depends on the composition and thermomechanical state of the alloy, as well as on the manufacture, surface properties, mechanic aspects and the systemic state of the host [1]. The presence of chemicals in the oral cavity, such as sodium fluoride-based solutions, can also contribute to corrosion. It is known that sodium fluoride is a potent generator of corrosion in various orthodontic appliances such as brackets, wires and bands [3].

Orthodontic MSs are grade V (five) titanium screws (Ti-6Al-4V). This titanium alloy is highly resistant to fracture but is less biocompatible when compared to commercially pure titanium. Its lower resistance to intra-oral corrosion leads to the release of a greater amount of metallic ions. These ions can accumulate in the

tissues surrounding the MSs [4,5] and in distant tissues [6] thus leading to undesirable effects in the organism, such as allergic reactions, renal lesions, cytotoxicity, hypersensitivity and carcinogenesis [7].

The most noxious of the miniscrew metallic components are cobalt, in the Cr-Co alloy, nickel in stainless steel and vanadium in the Ti-6Al-4V alloy [2]. However, besides the vanadium in the Ti-6Al-4V, aluminum is also potentially toxic [7,8]. Aluminum ions affect the proliferation, metabolic activity and differentiation of osteoblasts [9]. Aluminum may also be associated with osteomalacia and pulmonary granulomatosis [7]. Vanadium is an essential micro-element present in most mammalian cells [10] and its main source is food. The difference between the essential dose and a toxic dose is small, making vanadium one of the most highly toxic micro-element among the nutritionally necessary micro-elements [11].

Despite reports that the release of titanium ions from Ti-6Al-4V is not associated with any pathological signs [5,7,12,13], particles of this metal may promote the proliferation of fibroblasts, an important factor in the development of a fibrous capsule surrounding the MSs. In addition, phagocytosis of these particles may cause peri-implant osteolysis [14]. Ti ions may induce a reduction in the number and activity of osteoblasts, macrophages and leukocytes [9], hindering osteogenesis.

In this study, *in vitro* tests, using the yeast *S. cerevisiae* as a model organism, were carried out in order to evaluate the cytotoxicity induced by metals released from orthodontic appliances. New MSs (SIN, NEODENT and INP brands) and used MSs (SIN) were analyzed in the presence or absence of sodium fluoride, through two sets

of experiments: 1- cell viability experiments after direct exposure of *S. cerevisiae* cells to the MSs in a liquid culture medium; 2- cell viability experiments after indirect exposure of *S. cerevisiae* using commercial artificial saliva previously exposed to the MSs. Metal-induced oxidative damage of the yeast respiratory metabolism was also evaluated through the *petite* colony test. In addition, screening electronic microscopy was performed in order to verify the corrosion present *in vivo* on the used MSs.

Materials and Methods:

This study was in accordance with national and international norms of research (09/04788 and 0013/09 registration number).

1- Miniscrews

Miniscrews are made of a metal alloy that contains titanium, aluminum and vanadium (Ti-6Al-4V). The MSs used in this study were obtained from three different manufacturers (Table 1): SIN (SIN- Implant Systems, São Paulo, SP, Brazil), INP (INP, São Paulo, SP, Brazil) and NEODENT (Neodent, Curitiba, PR, Brazil). The cytotoxicity evaluation was performed using brand new MS units from all of the above-mentioned manufacturers as well as used ones from SIN.

The used MSs were obtained from patients undergoing orthodontic treatment with the use of MSs anchorage for molar distalization in a private clinic.

All patients gave signed consent and were given the choice between MSs and other treatment alternatives for the same purpose. The MSs were placed between the upper first molar and the upper second premolar and had an intraoral lifetime of 6 to 8 months. The MSs were removed only after total molar distalization, with no

mechanical damage to the patients. All MSs were placed and removed by the same oral and maxillofacial surgeon (D.B.). After removal, the MSs were cleaned with distilled water for 10 seconds and sterilized in an autoclave (Cristofoli, Paraná, Brasil).

2- S. cerevisiae strain, chemicals, media and cultures

The *S. cerevisiae* strain used in this study was the wild-type FF18733 (*mat a*, *ura3-52*, *his7-3*, *leu2-1*, *trp1-289, lys1-1*). To cultivate *S. cerevisiae*, YPD medium (1% yeast extract, 2% peptone, 2% glucose) was used either in liquid or solid (with 2% agar) form. In all survival experiments, *S. cerevisiae* pre-cultures were prepared in 10 ml YPD liquid and grown overnight to the exponential phase (~ 10^{-7} cells/ ml) at 30°C. The artificial saliva was Salivan, (Apsen Farmacêutica S.A., São Paulo, Brasil). The sodium fluoride (NaF) was manipulated at a concentration of 0.012%, 0.025% and 0.05%.

3- Survival experiments for cytotoxicity analysis

The cytotoxicity analysis was performed using two types of survival experiments: 1- Direct exposure of *S. cerevisiae* cells to the MSs in YPD liquid medium; 2 – Indirect exposure to metals released by the MSs in commercial artificial saliva. Three direct and three indirect experiments were performed with each MS brand.

For the direct exposure experiments, new inocula were cultivated from the pre-culture in 5 ml YPD, each containing one or two MS units from the different brands tested. A control culture without MS was also cultivated. These cultures were

incubated at 30°C to the exponential phase (~ 10^{-7} cells/ ml). Aliquots from each culture were diluted (in 0.9% sterile saline solution) and 5µl drops from each dilution (from 10^{-2} to 10^{-5}) were plated in YPD-agar and incubated at 30°C for two days for the emergence of small colonies, which allowed a qualitative approach. For quantitative analyses, 100 µl of final dilutions were plated in YPD-agar (two plates for each dilution) for colony forming units (CFU/ml) counting after two days at 30^{o}C.

In saliva exposure experiments, two MSs from each brand were immersed in 500µl of artificial saliva for seven and twenty days. 500µl of the pre-inoculum was used for each treatment. These aliquots were centrifuged (2 min to 10.000 rpm) and resuspended at 100% of saliva exposed to the MSs. The cells were treated for 60 minutes, diluted and plated in YPD-agar as described above, for both qualitative and quantitative analyses. A control with unexposed saliva was also cultivated. Three direct and indirect experiments were performed for each MS brand tested.

To test the influence of sodium fluoride, one MS from each brand was immersed for five and fifteen days in 500µl of artificial saliva with NaF at final concentrations of 0.0125%, 0.0250% and 0.05% (only NaF). The survival analysis was performed as described for saliva exposure experiments.

4- Petite colony test

To evaluate the induction of respiratory loss in *S. cerevisiae* cells, which is an indication of oxidative stress, the colony color assay [18] was performed on plates from the quantitative analyses. In this test, the colonies were covered with a top agar (0.7%) containing 0.05% of the coloring salt triphenyltetrazolium-chloride (TTC). This

test allows the distinction, via differential coloration, of the *petite* colonies (white) among those that maintain their aerobic metabolism (red) [18].

5- Cytotoxicity and petite colony data analysis:

The average values of CFU/ml counts from the quantitative analysis of all direct and indirect experiments were compared to control values to verify the occurrence of survival differences, which is an indication of cellular toxicity. The standard deviation values among each type of experiment were used to verify the significance of observed differences. To measure the induction of significant oxidative stress, the frequencies of *petite* (white) colonies were calculated in relation to total colonies in each treatment and compared to the corresponding controls.

6- Scanning Electron Microscopy Analysis (SEM):

In order to observe topography and structure, new and clinically used MSs were mounted on appropriate aluminum bases with adhesive carbon tape and analyzed using scanning electron microscopy (Phillips XL 30). Photomicrographs (1mm, 100μm and 500μm) were obtained of the head, transmucosal portion and screw portion of the MSs.

Results and discussion:

Miniscrews have been widely used as orthodontic anchorage, mainly in situations that call for maximum anchorage, in patients that do not collaborate and in dental movements that are considered difficult or complex for traditional anchorage [15].

Most MSs are fabricated with a Ti grade V (five) alloy which is more resistant to fracture than commercially pure (cp) Ti, but which presents reduced corrosion-resistance, which favors the release of metallic ions [1]. Commercially pure Ti can spontaneously form a thin impermeable surface layer of titanium oxide (TiO_2) which confers excellent biocompatibility with the human organism [5,16]. In the Ti-6Al-4V alloy, the surface oxide is composed of TiO_2 with small quantities of Al_2O_3, hydroxide groups and water [1,2]. With the addition of aluminum and vanadium, the oxide is less stable than that of cp Ti, making it more susceptible to corrosion [16].

The experiments proposed in this study aimed to investigate the occurrence of cellular toxicity induced by orthodontic MSs in FF18733 *S. cerevisiae* cells. *S. cerevisiae* is a microorganism that has been widely used as an experimental model in biological and biomedical fields because it is easy to handle, allows for quantitative analyses in a short time, possesses unique metabolic properties, such as the formation of exclusive fermentation cells called “petite” cells, and, mainly, because it is a eukaryotic organism with biochemical and genetic characteristics similar to those of animal organisms [18,19].

In direct exposure experiments with one or two new SIN MSs, the values of CFU/ml indicated some reduction in cell viability compared to the control, although these differences were not significant (Figures 1 and 2). A similar result was observed for SIN MSs corroded *in vivo*, which indicates that the metallic corrosion in SIN MSs may not interfere in the levels of induced cytotoxicity. SIN MSs also induced some, but not a significant amount, of cytotoxicity in saliva-exposure experiments with new and corroded MSs immersed either during a period of seven or twenty days.

The results for INP and NEODENT MSs showed no differences in terms of decreased cellular survival in *S. cerevisiae* for all experiments performed, indicating that these MSs may be more resistant to corrosion than SIN MSs.

Stainless steel as well as titanium (Ti) and its alloys are commonly used as MS biomaterials [20]. In our study, we used only MSs fabricated with Ti- 6Al-4V alloy in order to evaluate this type of alloy alone.

The findings of this study corroborate with those of Morais *et al*. [1] who verified the biodegradation of orthodontic MSs (Ti6-Al4-V) and the presence of concentrations of titanium, aluminum and vanadium in rabbit organs. Small doses of all metals contained in the alloy were detected in the samples, however the quantities were only slightly significant, which supports the premise that the cytotoxicity generated by the use of these devices is not significant, as was verified in our study as well.

Some authors [21-23] have investigated the action of fluorides on titanium alloys and were unanimous in affirming that fluoride can increase the release of metallic ions in the organism, recommending that patients using titanium devices avoid toothpastes and mouthwashes containing high concentrations of fluoride. However, our findings demonstrate that the exposure of all brands of MSs to NaF (0.0125%, 0.025 and 0.05%) for five and fifteen days did not induce corrosion levels that could lead to cytotoxic effects. As was found in the other experiments in this study, the rate of *S. cerevisiae* survival after treatment of MSs with NaF solutions for five and fifteen days was not different from that of the controls.

The colony color assay was applied to verify the frequency of respiratory loss in *S. cerevisiae* cells exposed directly or indirectly to MSs. The results showed that there was apparently no mitochondrial injury induced by reactive oxygen species from MS metals, since the frequencies of *petite* colonies were not different from the controls in all experiments.

Figures 3, 4 and 5 show the structure of the corroded MSs from photomicrographs obtained by scanning electron microscopy. Discrete alterations can be seen in the head, transmucosal and screw portions of the corroded MSs when compared to the control group. The corroded MSs presented few structural defects, such as protrusions and occasional pittings located mainly in the head portion. This finding is in accordance with studies from Gioka *et al.*[24] and Schiff *et al.* [21] who found this alloy to be highly resistant to biodegradation, demonstrating good tolerance to an environment similar to that found in the oral environment.

The data presented here indicate that the MSs tested in this study did not induce significant cytotoxicity or oxidative stress in *S. cerevisiae* cells. Moreover, the corrosion observed via SEM in used MSs is probably not sufficient to generate cytotoxicity in *S. cerevisiae.* These results indicate that the MSs tested present good biocompatibility for clinical use.

Conclusion:

1- INP and NEODENT brands of MSs may be more resistant to corrosion than SIN MSs;

2- Corroded SIN MSs did not induce higher levels of cytotoxicity in *S. cerevisiae* than new SIN MSs;

3-The exposure of all MS brands to NaF (at 0.0125%, 0.025 and 0.05%) for five and fifteen days did not induce corrosion levels that could lead to cytotoxic effects;

4- Photomicrographs from scanning electron microscopy revealed discrete alterations on the surface of corroded MSs (used for 6-8 months) when compared to the control group;

5- The observed corrosion of Ti-6Al-4V MSs (SIN, Neodent, INP) is not likely to be sufficient to release metals at a concentration capable of inducing cytotoxicity in *S. cerevisiae.* This indicates that these MSs present good biocompatibility for clinical use for this period of time of 6 to 8 months.

Acknowledgements:

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Table 1- Characteristics of MSs samples.

Brand	Diameter	Length	Composition
SIN	1.6mm	10mm	Ti-6Al- 4V
Neodent	1.6mm	9mm	Ti-6Al- 4V
INP	1.6mm	10mm	Ti-6Al- 4V

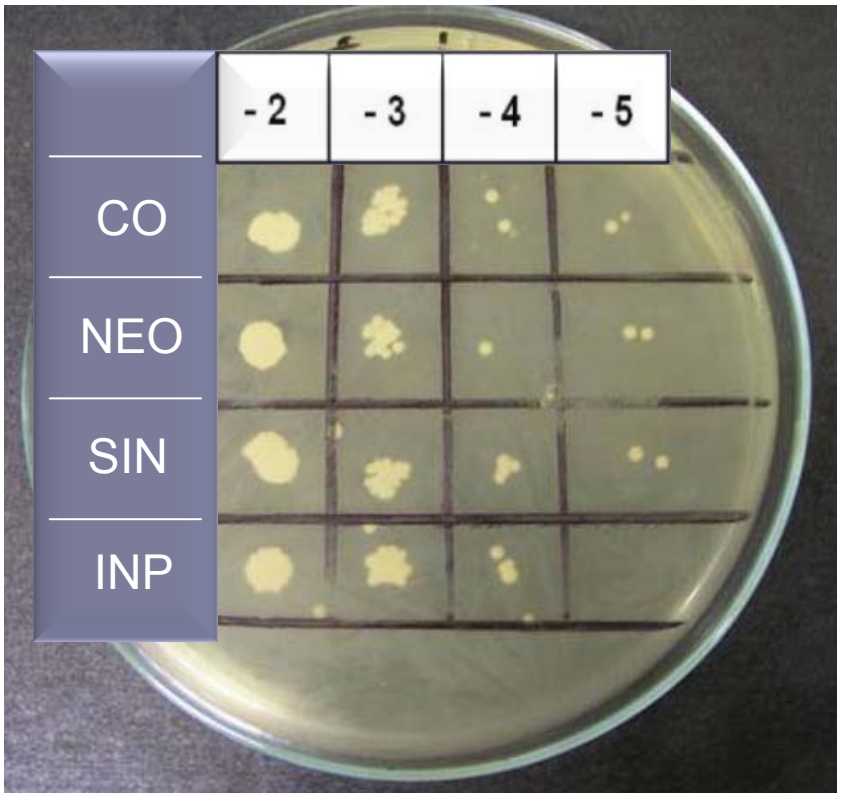


Figure 1 – One example of the survival tests (qualitative analysis) of *S. cerevisiae* after direct exposure to new MSs from Neodent (NEO), SIN and INP, showing no significant differences in relation to the control (CO) in all dilutions (10^{-2} to 10^{-5}). Similar results were observed for direct exposure to corroded SIN MSs as well as for treatments with saliva or NaF exposed to the different brands of MSs.

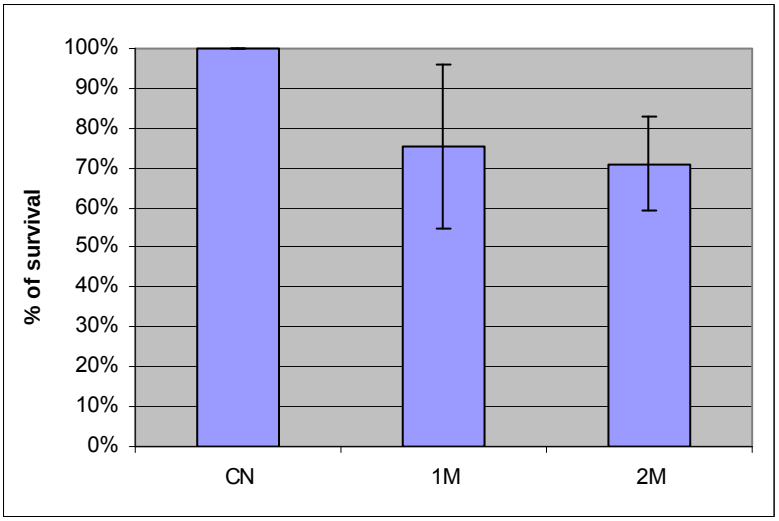
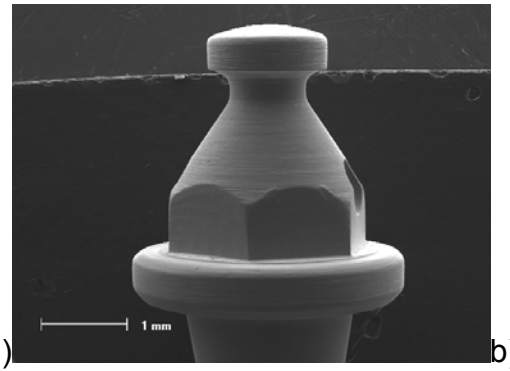


Figure 2 – Average values of thee survival tests (quantitative analysis, based on CFU/ml count) of *S. cerevisiae* after direct exposure to one and two (1M and 2M) new SIN MSs; A bar graph indicating a non significant survival decrease from the treatments in relation to the control (CN); Similar results were observed for direct exposure to corroded SIN MSs as well as for treatments with saliva or NaF exposed to the different brands of MSs.

a) 

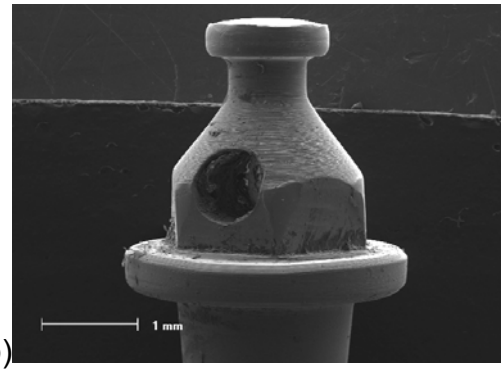
b) 

Fig.3- Microphotograph of head and transmucosal portion (sideview) of a new (a) and corroded (b) MS.

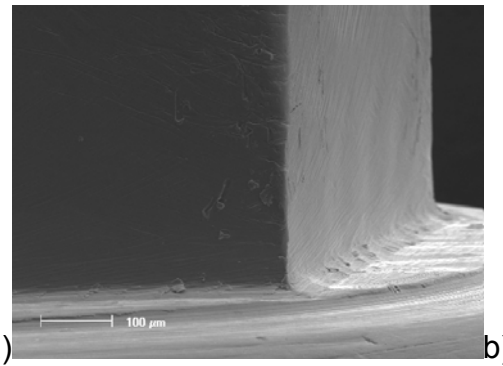
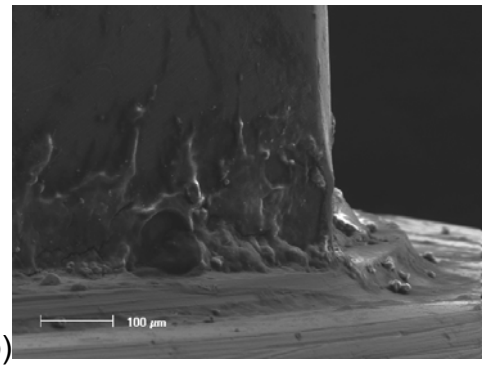
a)  b) 

Fig.4 - Microphotograph of the head of a new (a) and corroded (b) MS.

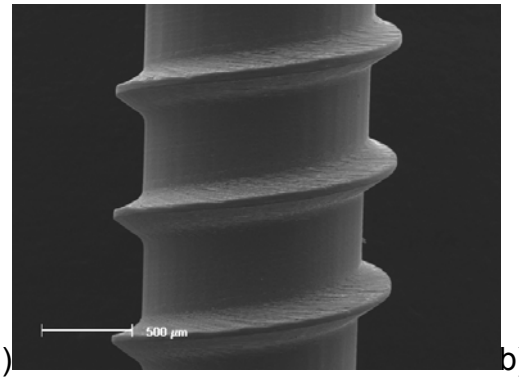
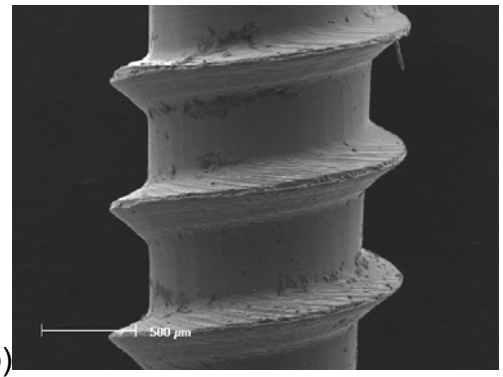
a)  b) 

Fig.5 - Microphotograph of screw portion of a new (a) and corroded (b) MS.

3.2. Artigo 2:

Titanium alloy miniscrews for orthodontic anchorage: an in vivo study of metal ion release

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ABSTRACT:

It is well known that ions of metals released from metal alloys can cause potentially toxic effects on tissues. Most orthodontic bands, brackets, and archwires are made of stainless steel, and the majority of miniscrews are made of a titanium alloy. The aim of the present study was to examine and compare levels of several metal ions released in saliva of patients with orthodontic appliances, at different time points before and after insertion of a miniscrew. The consecutively selected patients (n=20) were within the 6^{th} and 8^{th} month of orthodontic treatment. Saliva was collected at four time points: before miniscrew placement (T1), 10 minutes (T2), 7 days (T3) and 30 days (T4) after miniscrew placement. The salivary samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). The release of nine different metal ions was observed: titanium (Ti), zinc (Zn), chromium(Cr), nickel (Ni), iron (Fe), copper (Cu), aluminum (Al), Vanadium (V) and cobalt (Co). Data were analyzed by descriptive statistics and normality tests (Shapiro-Wilk). Salivary metal concentrations from different time points of miniscrew treatment were compared using Wilcoxon paired tests (α=95%). At time point T4, there was a quantitative increase in the salivary concentration of Cu, Ti, V, Zn, as well as a quantitative decrease in the salivary concentration of Al, Co, Cr, Fe, Ni, when compared with T1. However, there were no statistically significant differences in the salivary metal concentrations. Thus, it can be concluded that the placement of fixed orthodontic appliances associated with miniscrews does not lead to an increase, statistically significant, of salivary metal ion concentrations.

KEY-WORDS: Metal ions; miniscrews; ICP-MS; ICP-OES; orthodontic appliances.

INTRODUCTION

Ti -6A- 4V is the most frequently used titanium alloy for medical implants and orthodontic miniscrews because of its excellent properties [1,2]. However there have been several studies concerning the cytotoxicity and dissolution of this alloy [2-4] and its corrosion products [5-8]. In addition, the concentrations of these metals have been measured in the blood, urine and tissues of patients containing it [9].

In the alloy Ti-6Al-4V, superficial oxide is composed of TiO_2, with small amounts of Al_2O_3, hydroxylic groups and water [10]. Its superficial oxide layer is less stable than that of commercially pureTi because the Al and V, which are added to stabilize the α and β phases, respectively, desestabilize the alloy, making it more vulnerable to corrosion [11]. As V is not present in the superficial oxide layer of Ti-6Al- 4V[10], Ti and Al are the metal ions most likely to be released from the Ti-6Al-4V surface[12].

According to Hanawa (2004) [10], the most harmful components of metallic implants are Co from Cr-Co alloy, Ni from stainless steel and V from Ti-6Al-4V alloy. However, both V and Al in Ti-6Al-4V are potentially toxic [13, 14]. The Al ions affect the proliferation, metabolic activity and differentiation of osteoblasts[12]. Some toxic effects attributed to Al accumulation in the human body have been described in the literature (encephalopathia and senile dementia of the Alzheimer's type)[15]. The

element may also be associated with osteomalacia and pulmonary granulomatosis [14].

Vanadium is an essential element for the functioning of our organism [16]. However, toxic vanadium may elicit local or especially systemic reactions or inhibit cellular proliferation. Vanadium may be cytotoxic for alveolar macrophages and synovial fibroblasts, interferes with mitosis and chromosome distribution and therefore presents a real risk of carcinogenicity [16, 17].

Titanium ions may induce a decrease in the number and activity of osteoblasts, macrophages and leukocytes [12], hampering osteogenesis.

The oral environment is ideal for the biodegradation of metals due to its thermal, microbiological and enzymatic properties [18]. Thus, it is uncertain whether these alloys, which are used in miniscrews, produce corrosion debris as a result of wear and whether the debris is cytotoxic to bone [9].

It is well known that metals released from orthodontic appliances, metal restorations and metal prostheses can cause potentially toxic effects on tissues, but it is unknown whether metals released from miniscrews used as orthodontic anchorage are potentially toxic. Therefore, the aim of the present study was to examine and compare levels of several metal ions released from saliva of patients with orthodontic appliances and with miniscrews as orthodontic anchorage.

METHODS AND MATERIALS

This study was approved by The Committee of Ethics and Research of the Pontifical Catholic University of Rio Grande do Sul (PUCRS/ Brazil), in accordance with national and international norms of research using human beings (registration number = 09/04788).

Sample description:

A total of 20 patients (12 females and 8 males) were included in the study. The mean age of the sample was 21.4 years (range 16 to 32 years) and these patients were selected from a private clinic. None of the patients were smokers, had pre-existing systemic diseases or were under any pharmacological treatment.

All patients were being treated by the same orthodontist (M.B.) and were within the 6^{th} and 8^{th} month of treatment. The fixed orthodontic appliance consisted of: 8 bands and 20 bonded brackets. None of the patients had palatal or lingual appliances welded to the bands or any extraoral orthodontic appliances. None of the patients had any amalgam fillings or metallic restorations.

A preadjusted straight-wire appliance was used in all cases (10.10.971 reference, special roth brackets set with hook on the canines and premolars 0,56 x 0,76 mm- Morelli, Sorocaba/SP, Brazil). Both the permanent maxillary and mandibular molars were banded (Morelli, Sorocaba/SP, Brazil). First maxillary molar bands had triple buccal tubes with hooks, whereas first mandibular molar bands had double bucal tubes with hooks. Second maxillary and mandibular molar bands had single buccal tubes. Metallic brackets were directly bonded on incisors, canines, and premolars.

A 0.018-inch or 0.020-inch stainless steel archwire was placed on both arches (Morelli, Sorocaba/SP, Brazil) and tied with elastics (Morelli, Sorocaba/SP, Brasil).

All patients were classified as class II malocclusion with left or right subdivision, that is, they possessed malocclusion Class I on one side and class II on the counter-lateral side. These patients, therefore, needed correction for superior arch asymmetry, which was achieved through molar distalization with the use of a miniscrew as orthodontic anchorage in the superior arch, placed interdentally between the roots of the superior first and second pre-molars.

The miniscrews were placed by the same oral and maxillofacial surgeon (D.B.) with the same surgical technique.

All miniscrews remained stable as an anchorage unit for the appliance during the orthodontic treatment. Before the treatment, all patients gave signed informed consent (in accordance with bioethics norms) to the treatment plan, which consisted of implantation of one miniscrew in the superior arch. Those patients that chose not to accept the treatment with miniscrew were excluded from the study without any disruption to their treatment.

Mini-screw installation:

A surgical guide made of orthodontic wire was used in all patients to verify the receptor site. Periapical radiographs were made before miniscrew implantation, in order to verify the miniscrew site without damaging the teeth or anatomic structures.

Miniscrews were installed under local anesthesia of the soft tissues at the implant receptor site. The entire procedure was carried out under sterile conditions.

The miniscrew was inserted with a manual handpiece screwdriver (Sin Implant Systems, São Paulo,SP, Brazil) and considered immobile and stable at the moment of placement. Self-tapping miniscrews with a total length of 10mm, screw head of 3mm and 1.2 mm of diameter were obtained from Sin Implant Systems (Sin Implant Systems, São Paulo, SP, Brazil).

After installation, a periapical radiograph was taken to evaluate the position of the miniscrew. After the surgical procedure, oral hygiene with an extra-soft toothbrush and the use of a 0.12% chlorhexidine mouth rinse were prescribed. No other medications were prescribed. The miniscrews were used in the maxilla and were loaded two weeks after placement.

Collection and processing of saliva

Samples of stimulated saliva were collected by the following method: the patient thoroughly rinsed the mouth with deionized water for 1 minute (Dermapelle, Santa Maria-RS, Brazil). Next, the patient spit non-stimulated salivary secretion at different time points (Table 1). Approximately 10 ml of saliva were collected into a sterile glass tube. After collection, the samples were stored at -20°C in a freezer.

For metal determinations, saliva samples (1 ml) were digested in a hot water bath (80°C, 1 hour) with 1 ml of 14 mol l^{-1} nitric acid (Merck, Darmstadt, Germany) and 0.5 ml of 30% (v/v) hydrogen peroxide (Synth, Diadema, Brazil) in polypropylene tubes (Sarstedt, Nümbrecht, Germany). After digestion, samples were diluted to 5 ml with ultra pure water and centrifuged (Nova Técnica, Piracicaba, Brazil) at 3,000 rpm for 4 minutes prior to analysis.

Estimation of metal ions released:

Metal content in digested saliva samples was determined by inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). Co, Cr, Ni, and V were determined by ICP-MS using an inductively coupled plasma mass spectrometer (PerkinElmer-SCIEX, model Elan DRC II, Thornhill, Canada), equipped with a concentric nebulizer, a cyclonic spray chamber and a quartz torch with a quartz injector tube (2mm i.d.). Instrumental performance optimization, including nebulizer gas flow rate, ion lens voltage, and torch alignment, was carried out according to manufacturer instructions (Perkin-Elmer-Sciex, Elan version3.0, Software guide, 1006920 A, 2003, Thornhill, Canada). The operational conditions are shown in Table 2. An inductively coupled plasma optical emission spectrometer (ICP-OES- Spectro Ciros CCD, Spectro Analytical Instruments, Kleve, Germany with an axial view configuration) was used for Al, Zn, Cu, Fe and Ti determinations. Nebulization was performed through a crossflow nebulizer coupled to a Scott double pass type nebulization chamber. Plasma operating conditions and selected wavelengths used for metal determinations are listed in Table 2, and they were used as recommended by the instrument manufacturer (Spectro Ciros CCD, Software version 01/March 2003, Spectro Analytical Instruments, Kleve, Germany). For ICP-MS and ICP-OES determinations, argon 99.996% (White Martins-Praxair, Sao Paulo, Brazil) was used for plasma generation, for nebulization, and as the auxiliary gas.

Statistical analysis:

Data were analyzed with descriptive statistics and normality test (Shapiro-Wilk). Comparisons of salivary element concentrations among different times of mini-implant treatment were analyzed by Wilcoxon paired tests (α=95%). The software *Statistical Package for Social Sciences* (SPSS, Chicago, Illinois, USA) was used to perform the statistical analysis.

RESULTS:

Results from the analysis of the concentration of Al, Ti, Cr, Ni, Fe, Cu, Co, Zn and V ions released at different times of miniscrew treatment are shown in Table 3.

After 10 minutes of exposure to the treatment, it can be observed that there was an increase in the salivary concentration of the ions: Fe, Ti, V, Cr and Zn and a decrease in: Al, Co, Cu, and Ni.

At 7 days after miniscrew insertion, there was a quantitative increase of Al, Co, Cr, Cu, Ni, Ti and V.

At 30 days after insertion, there was a quantitative increase in the salivary concentration (μg/L) of: Cu, Ti, V, Zn and a quantitative decrease in the salivary concentration of: Al, Co, Cr, Fe, Ni, when compared with concentrations found before insertion.

However, there were no statistically significant differences in the salivary ion concentrations at any of the times studied.

Figure 1 display the amount of metal ions released at each time point.

DISCUSSION

This study investigates the release of metal ions from fixed orthodontic appliances, particularly with the use of miniscrews as orthodontic anchorage.

The main advantage of the present in vivo study is that the concentrations of salivary metals ions were recorded in the natural oral environment of the patient where actual adverse effects of increased metal concentrations take place. So, this study was carried out to investigate the metal ion concentrations in saliva of patients with fixed orthodontic appliances (20 brackets, 8 bands and wires) and one miniscrew.

The average number of brackets used in a study depends upon whether the patients are treated using an extraction or nonextraction approach and whether the brackets are only placed on a single arch or on both arches. In this study the patients were treated without extraction of pre-molars, brackets are placed on both arches, and the fixed orthodontic appliances were produced by the same manufacturer, in order to avoid additional variables in the study.

Thus, a systemic toxic effect from orthodontic appliances is highly unlikely. However, even such small quantities of metal ions can cause allergic reactions, especially because fixed orthodontic appliances remain in the oral cavity for a long period of time (2 to 3 years approximately). For an allergic reaction to occur in the oral mucous membrane, the antigenic potential has to be 5 to 12 times stronger than that on the skin surface. However, various clinical manifestations of hypersensitive reactions to fixed orthodontic appliances have been reported [19,20]. Moreover, it was reported that nickel ions released from dental alloys can accumulate in the cells over time, and this may have multiple harmful effects on cells [21].

A number of studies have been carried out on the biocompatibility of orthodontic materials, with the aim of determining a limit of biological tolerance and assessing whether the ions released from such materials are within these limits.

In relation to the Ti-6Al-4V alloy components from miniscrew (Ti, Al and V), it was found that Ti was increased at all time points (T1/T2/T3/T4); Al was decreased from T1 to T2, increased from T2 to T3 and decreased from T3 to T4; V increased from T1 to T2 and from T2 to T3, while from T3 to T4 it decreased, though these differences were not statistically significant. While there were no statistically significant differences, it is important to note that the quantitative increase for Ti was observed at all time points, which may be explained by the fact that it is the element with the greatest concentration in the alloy and because of the formation of a superficial titanium oxide layer.

Al and V presented a slight increase at 7 days after miniscrew insertion, which may be due to the fact that V is not present in the superficial oxide layer of Ti-6Al-4V [10] and Ti and Al are the metal íons most likely to be released from the Ti-6Al-4V surface[12].

There was a higher Ni value in T1 than T2, T3 and T4. However it cannot be affirmed that this value was due to the release of Ni from the orthodontic appliance, as it had been inside the mouth for a period of approximately 6 to 8 months. Nor could it be attributed to the orthodontic wire, as all patients had stainless steel wires.

There was a minimal difference in the V concentration at the different time points measured, which would not incur in an alarming situation, especially because the remain in the intraoral environment for a limited time. Our results are in accordance with those found by Morais *et al.,* 2007 [9], who detected varying

quantities of Ti, Al and V, proving that metal ions were released by Ti-6Al-4V orthodontic miniscrews. However, the authors observed extremely low quantities of these metal ions.

Based on the findings of this study, it can by asserted that the quantity of metal ions release is not proportional to the metal content in the alloy, which corroborates with the findings of other studies [19,20,22,23].

The method of saliva sampling, processing, and analysis also adds to the variability of the results. Several methods have been suggested to collect resting and stimulated whole saliva [24,25]. Most commonly, saliva is collected by draining or spitting into a tube [25]. This was done in our study as in previous studies [26]. Standardization of saliva collections is important when saliva is used as research material, since saliva composition varies greatly both intra- and inter-individually [18].

Although the ICP-MS and ICP-OES used in this study is a time-consuming and expensive technique, it is highly sensitive, accurate and capable of the determination of a range of metals and several non-metals at concentrations below one part in 10 [12,26].

Saliva contains acids arising from the degradation and decomposition of food, which increases the corrosion potential of stainless steel and Ti-6Al-4V. In the oral cavity, the bracket-archwire ligation induces “fretting corrosion” of metal surfaces of both the bracket and archwire, as both are moving elements. The presence of complex intraoral flora and accumulation of plaque and its byproducts also add to this variation [18]. Moreover, according to Edgar and O’ Mullane [27], hormones, drugs, and various diseases also influence saliva composition. In our study, we aimed to

exclude patients who presented any systemic disturbance in order to reduce the number of variables that could alter the results.

In summary, metals are present in the saliva of patients with metal brackets, bands and miniscrews depending on a number of variables. The metal contents differed, though not significantly, between consecutive samples per individual. No data are available for a safe limit to metal exposure in saliva. More studies are necessary, especially to investigate the amount of metal ions release when two or more miniscrews are utilized as orthodontic anchorage.

CONCLUSIONS

Based on the findings of this study, it can be concluded that:

1- Orthodontic appliances and miniscrews released metal ions, but in a quantity not proportional to the metal concentration in these materials.

2- There was no significant difference in the metal ion concentration among the different time points after miniscrew placement.

3- The placement of miniscrew leads to an increase of salivary titanium ion concentrations that is not statistically significant at all periods of saliva collection.

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Table1. Time points of saliva collection.

Group	Time of saliva collection
immediately before miniscrew insertion	Immediately before – T1
after miniscrew insertion	10 minutes- T2
	7 days- T3
	30 days- T4

Table 2. Operational Parameters metals determinations by ICP-OES and ICP-MS.

Parameter	*ICP OES*	*ICPMS*
rf power (W)	1650	1400
plasma gas flow rate (L min-1)	12.0	15.0
auxiliary gas flow rate (L min^{-1})	1.0	1.2
nebulizer gas flow rate (L min^{-1})	1.0	1.15
spray chamber	double pass, Scott type	cyclonic
nebulizer	crossflow	concentric
view	axial	
sampler and skimmer cones		Pt
ion lens (V)		7.2
dwell time (ms)		50
isotope (*m/z*)		^{109}Ag ^{59}Co ^{53}Cr ^{60}Ni ^{51}V
wavelength (nm)	396.153 Al 327.393 (Cu) 259.939 (Fe) 334.490 (Ti) 213.857 (Zn)	

Table 3 – Comparison among the metal ion concentrations in salivary samples among different time points (µg/L).

	before placement of miniscrew		10 minutes after insertion of miniscrew		7 days after insertion of miniscrew		30 days after insertion of miniscrew	
Element	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
[Al]	127.8	95.0	101.9	60.6	240.0	450.4	123.6	58.5
[Co]	0.6	0.4	0.5	0.1	2.3	5.6	0.5	0.3
[Cr]	7.1	6.2	7.2	6.2	7.4	6.3	7.0	6.2
[Cu]	78.0	67.9	54.2	33.7	87.3	142.2	100.2	73.0
[Fe]	272.5	138.3	368.3	193.7	261.5	170.1	260.3	111.1
[Ni]	7.1	2.7	6.9	2.3	6.69	2.69	6.5	3.8
[Ti]	35.3	22.8	39.9	59.6	52.6	53.8	104.3	123.0
[V]	2.2	0.7	2.4	1.1	3.4	3.7	2.5	2.3
[Zn]	168.2	101.5	220.5	172.6	154.5	102.8	209.0	137.7

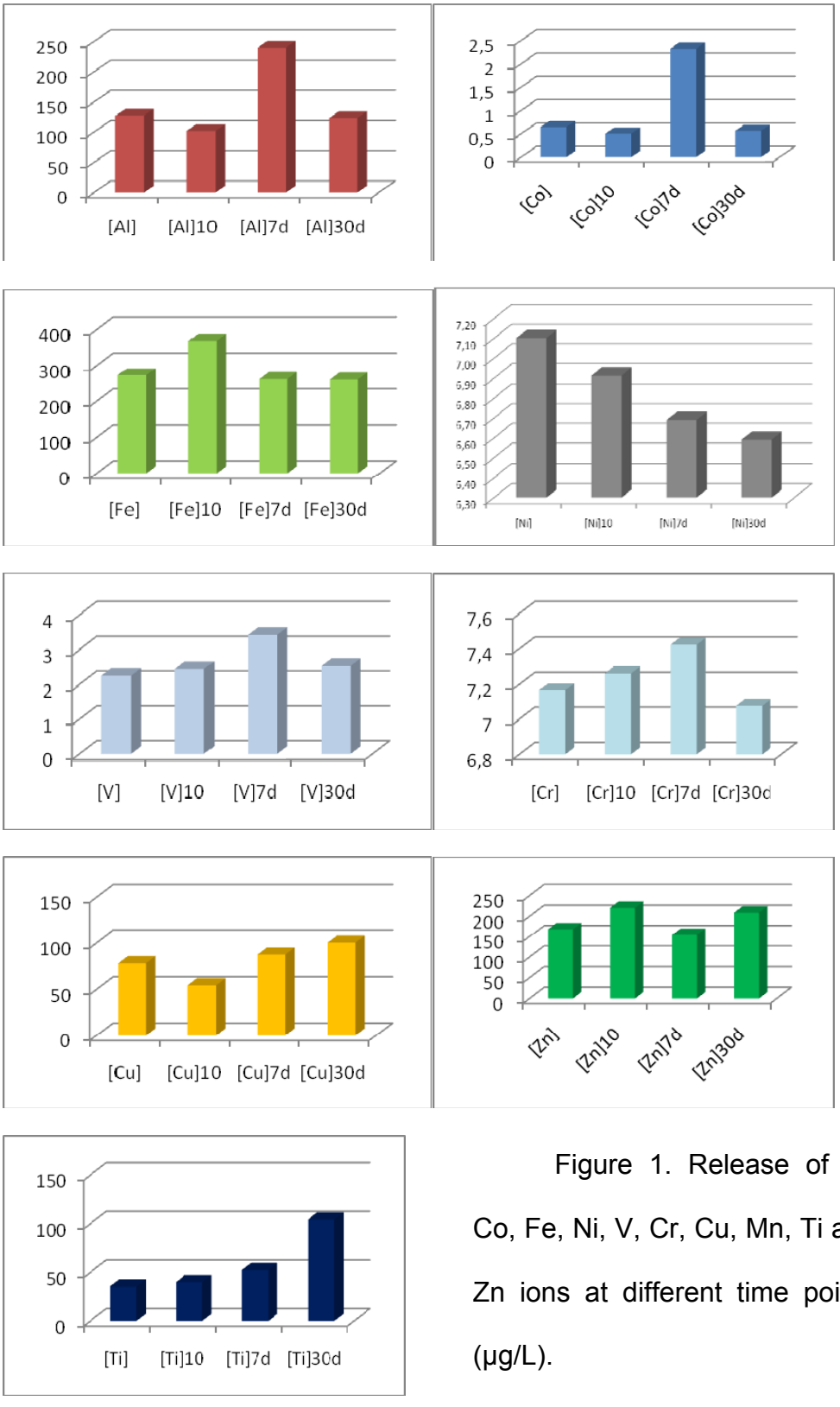

Figure 1. Release of Al, Co, Fe, Ni, V, Cr, Cu, Mn, Ti and Zn ions at different time points (µg/L).

4. DISCUSSÃO GERAL:

A Ortodontia utiliza uma variedade de dispositivos visando a movimentação dentária, e emprega, diversos materiais, entre eles as ligas metálicas. Há muito tempo têm-se estudado as propriedades dos metais e ligas, no entanto, atualmente a biocompatibilidade desses materiais têm sido alvo de estudos.

A determinação da biocompatibilidade de um determinado material representa um processo complexo que envolve testes *in vitro* e *in vivo* (WATAHA, 2000). Um dos fatores determinantes da biocompatibilidade das ligas metálicas em Odontologia é a resistência à corrosão (WATAHA *et al*., 2002).

Corrosão pode ser definida como a deterioração de um material, geralmente metálico, por ação química ou eletroquímica do meio ambiente aliada ou não a esforços mecânicos (GENTIL, 2003).

Embora as interações entre uma liga metálica e os tecidos possam ser de diversas formas, a liberação de elementos da liga na cavidade oral é o foco primário de estudo, visto que efeitos biológicos como alergias, inflamação têm sido atribuídos a esse processo (WATAHA, MESSER, 2004). Além disso, testes como citotoxicidade, bem como de liberação iônica são os mais comumente indicados (WATAHA *et al.,*1998).

A liga de titânio Ti-6Al-4V foi introduzida na fabricação de mini-implantes ortodônticos com intuito de aumentar a resistência desses dispositivos durante os procedimentos de inserção e remoção (HUANG, YEN, KAO, 2001; SERRA *et*

*al.,*2007). A resistência dessa liga foi comprovada por Gioka *et al.* (2004), porém com a adição do alumínio e do vanádio, o óxido formador da camada protetora é menos estável que o titânio comercialmente puro, apresentando menor taxa de osseointegração e maior susceptibilidade à corrosão *in vivo* (SERRA *et al*., 2007).

Logo, o titânio grau V pode apresentar citotoxicidade, reações adversas ao tecido e outras patologias associadas ao vanádio e alumínio (SEDARAT *et al.,* 2001). No entanto, através de nosso estudo a citotoxicidade não foi constatada.

A análise salivar através de ICP-MS (inductively coupled plasma mass spectrometry) pode ser considerado uma das ferramentas mais poderosas na determinação de metais devido a baixa concentração nos fluídos biológicos, o grande número de amostras requeridas para esses estudos, e o pequeno volume disponível da amostra. A capacidade multielementar, multi-isotópica e o baixo limite de detecção faz do ICP-MS uma técnica quase que ideal para análise de metais em amostras biológicas (DELVES *et al.,*1997). Entretanto, o ICP-MS tem algumas limitações como as interferências que podem afetar seriamente a habilidade analítica (SARMIENTO-GONZÁLES *et al.,* 2005). O sucesso na correção das interferências não-espectrais dependem do uso e efetividade de um padrão interno apropriado, como o utilizado em nosso estudo e demonstrado na tabela 2 do artigo 2.

Segundo Wataha (2000) um dos métodos mais relevantes para determinação da biocompatibilidade de uma liga é a quantificação dos íons liberados. Essa liberação poderia aumentar a concentração de íons no corpo, acima da concentração de ingestão ou aos metais pelo meio ambiente (BARRET, BISHARA, QUINN 1993).t

Em estudos *in vitro* é utilizado uma solução, na maioria das vezes saliva artificial, afim de verificar essa liberação metálica. No entanto, a composição da solução pode influenciar a capacidade do metal em formar uma camada protetora de óxido sobre a superfície para evitar a corrosão e a liberação iônica. Assim, a presença de um meio mais complexo com proteínas, células, sais e outras pequenas moléculas, poderia diminuir essa liberação iônica (WATAHA, NELSON, LOCKWOOD, 2001). Isso vai de encontro aos achados do presente estudo onde a liberação de íons alumínio e vanádio não foram estatisticamente significativas quando comparadas aos outros metais analisados.

Sabe-se que o processo de escovação propriamente dito pode determinar a alteração e/ou remoção da camada protetora superficial, considerada detentora do controle da liberação iônica (WATAHA, 2002). Porém nossos resultados indicam que não houve citotoxicidade relacionada a mini-implantes ortodônticos após corrosão com fluoreto de sódio (NaF a 0,0125%, 0,025% e 0,05%), e nem houve aumento da liberação iônica in vivo após escovações dentárias, ao longo dos tempos analisados.

Há que ser salientado que mais estudos devem ser realizados nesse âmbito. Logo, sugere-se a avaliação da liberação iônica salivar abrangendo um número maior de pacientes, assim como a análise de um número maior de mini-implantes inseridos durante o tratamento ortodôntico. Somando-se à isso, poderá ser realizado outras formas de análise da liberação e concentração de íons (sangue e urina), ou outras formas de análise salivar (SELDI-TOF-MS, surface–enhanced laser-desorption/ionization time of flight mass spectrometry).

Além disso, também poderá ser avaliada a citotoxicidade dos mini-implantes com cultura de fibroblastos de camundongos.

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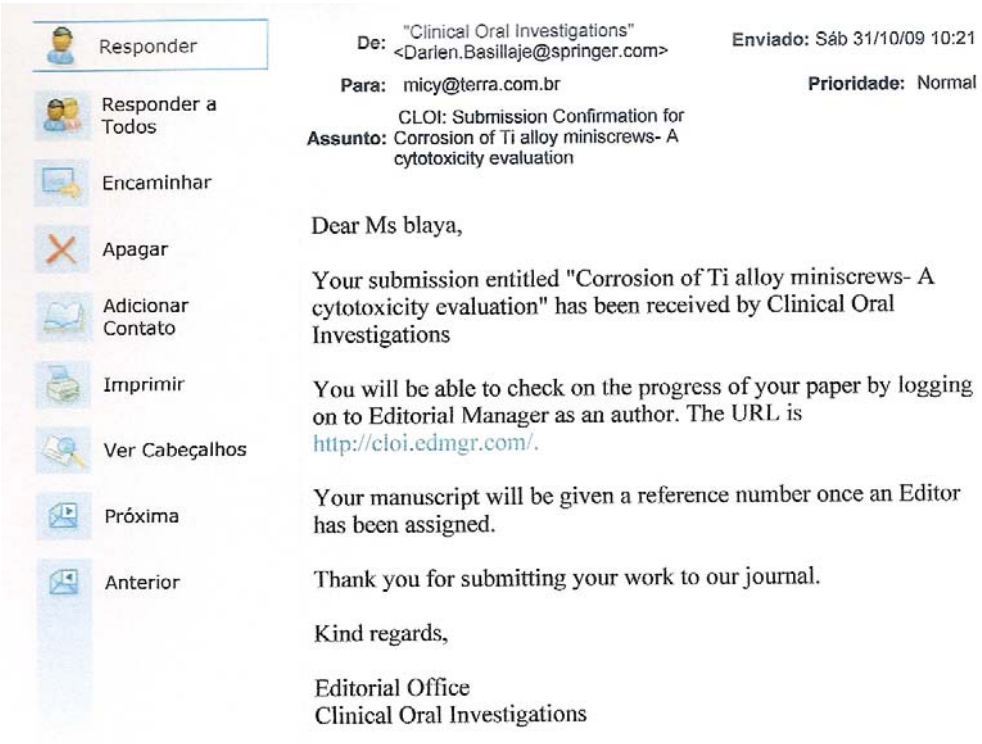
6.ANEXOS:

6.1. – E-mail de submissão artigo 1.



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Prioridade: Normal
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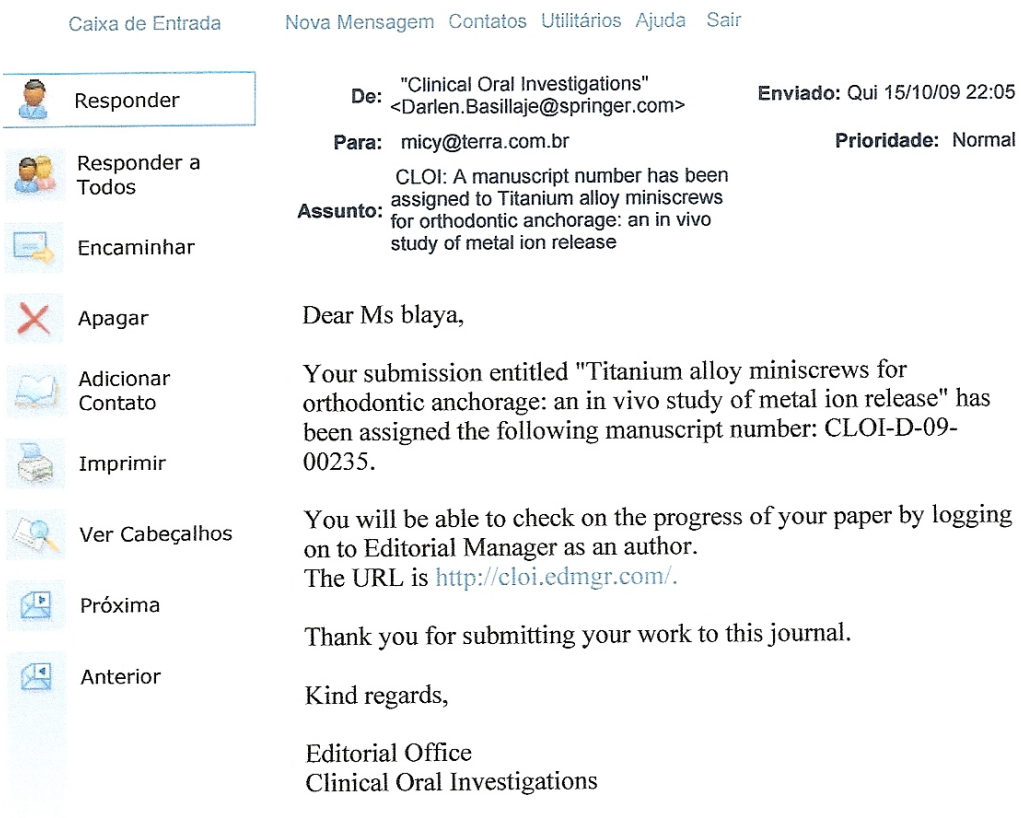
6.2.- E-mail de submissão artigo 2.



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Assunto: CLOI: A manuscript number has been assigned to Titanium alloy miniscrews for orthodontic anchorage: an in vivo study of metal ion release

Dear Ms blaya,

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