THEORETICAL EVALUATION OF NICKEL-TITANIUM MTWO® SERIES ROTARY FILES

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ABSTRACT
Nickel-Titanium rotary files are a technological development that enables dentists to prepare irregularly shaped root canals without altering them. Unfortunately, these files may fracture without any prior visible warning signs. The aim of this study was to perform a theoretical evaluation of the mechanical behaviour of Mtwo® Nickel-Titanium rotary files for endodontics, in order to determine which of the files in the basic series are most likely to fracture. Mathematical models of the Mtwo® basic file series were analyzed using the finite elements method. Bending and torsion loads were applied to the files both under normal conditions and under extreme conditions, to determine which of them had the highest Von Mises stresses. When the approximation was similar to normal use, none of the file models reached the maximum limit of failure by fracture. When used inadequately, file models 10/0.04 and 25/0.06 had the highest Von Mises stresses for bending and torsion, respectively. Thus, it is recommended that Mtwo® files10/0.04 and 25/0.06 should be used once only, to prevent fractures.

Key words: *endodontic instruments, nickel titanium alloy.*

EVALUACIÓN TEÓRICA DE LAS LIMAS ROTATORIAS DE NÍQUEL TITANIO DE LA SERIE MTWO®

RESUMEN
Las limas rotatorias de Níquel-Titanio son un avance tecnológico que permite al odontólogo llevar a cabo tratamientos en conductos con morfologías irregulares, sin alterarlas. Lamentablemente estos instrumentos pueden fracturarse sin presentar señales visibles que permitan prevenir este accidente. Por lo tanto el objetivo del presente estudio fue evaluar teóricamente el comportamiento mecánico de las limas rotatorias de Níquel-Titanio para uso en endodoncia Mtwo® para determinar cuál de los instrumentos de la serie básica es el que presenta mayor probabilidad de fractura. Con este fin se realizó un análisis por medio del método de los elementos finitos utilizando modelos matemáticos de los instrumentos de la serie básica de las limas Mtwo®. A estos instrumentos se les aplicaron cargas de flexión y torsión en condiciones normales y en condiciones extremas, para determinar cuáles presentaban los esfuerzos de Von Mises más altos. En una aproximación similar al uso normal, ninguno de los modelos de las limas alcanzó el limite máximo de falla por fractura. Ante un uso inadecuado, los modelos de las limas 10/0.04 y 25/0.06 mostraron los esfuerzos de Von Mises más altos tanto a flexión como a torsión respectivamente, por lo tanto se recomienda dar un solo uso a la lima 10/0.04 y a la lima 25/0.06 de Mtwo® para prevenir la fractura de estos instrumentos.

Palabras clave: *instrumentos endodónticos, aleación niquel titanio.*

INTRODUCTION

Biomechanical root canal preparation is the stage of endodontic treatment during which microbial pathogens – present as a result of dental pulp necrosis and decomposition – are removed, and probably the most important stage in non-surgical endodontic treatment[1,2].

Disinfection is achieved by mechanical cleaning plus the use of irrigants and medicaments. It Root canal enlargement and preparation are essential to facilitate the flow of disinfectants and help with its definitive filling[1,2] .

Small-calibre instruments, endodontic files in particular, are used for biomechanical preparation. They were originally made of carbon steel, later on of stainless steel, and currently there are also files made of Nickel-Titanium alloys[3-6].

One of the most important requirements for an ideal endodontic file is that it should not deteriorate or fracture[7].

The incidence of Nickel-Titanium rotary file fractures is reported as 5% to 21%, depending on its design[7]. The fracture of an endodontic file inside the root canal is a procedural accident which significantly reduces endodontic success[8], because the fractured fragment blocks the apical third of the canal and prevents eradication of the infection. The highest endodontic treatment failure rates occur when the initial diagnosis is necrotic pulp[7-10].

Moreover, a fractured file inside a root canal is of concern to the patient, who of course will not wish to retain a piece of metal which has been impossible to extract from his tooth, and may even sue the dentist[7].

Among of the most popular file designs in Latin America is Mtwo®, which has an italic S-shaped cross-section and widely-spaced flutes.

The memory and superelasticity properties of Nickel-Titanium mean that these files show no visible deformation during endodontic treatment. This is at the same time their greatest and most dangerous disadvantage, because the operator does not notice any deterioration until the file fractures. It has been attempted to overcome this problem by recommending that Nickel-Titanium files be used once only, but in Latin American countries their high cost necessitate a rational application to this measure, since not all files in one series are equally likely to fracture. Thus, a recommendation is needed regarding which files may be used more than once, and which should definitely be discarded after one use due to the high probability of undergoing fracture if used again.

There are two ways in which an endodontic file can fail inside a root canal: torsional failure and bending failure. Torsional failure happens when part of the instrument becomes trapped in a section of the canal and the driving motor continues to turn, causing the file to fracture. Bending failure happens when the instrument acts on canals with moderate to severe curvature. Repeated compression and tension on the instrument at each revolution may cause fracture. A study of the tendency of Nickel-Titanium files to suffer torsional or bending fracture showed that torsional facture occurs in 55.7% of fractured files, while bending fatigue occurs in 44.3%[11].

Torsional and bending fracture can be evaluated by experimental methods, as well as by theoretical systems such as the finite elements method.

The basic Mtwo® set is made up of 4 files with different tapers, 10 / 0.04, 15 / 0.05, 20 / 0,06 and 25 /0,06, and although they work on the entire length of the canal, they are used from smallest to largest diameter in order to shape the root canal gradually. It would be useful to general dentists or endodontics specialists to know which file in the series should be given special attention in order to prevent fracture. Thus, the aim of this study was to use finite elements analysis to determine which file in the Mtwo® series may theoretically have greater probability of fracture.

MATERIALS AND METHODS

Model construction

A reverse engineering process was performed at the metrology laboratory at the School of Engineering of Colombia National University. Measurements of Nickel-Titanium rotary files were taken with a Carl Zeiss Jenna profile projector, with a resolution of 0.01 mm, for geometric characterization and contour plotting of the files.

Measurements of the Mtwo® files were taken on the instrument profile at the widest and narrowest points along the entire longitudinal axis of the active part only.

Tensile stress test to determine the behaviour of the Nickel-Titanium alloy

The ASTM F2516 – 07 Standard[12] test method for evaluating of Nickel-Titanium superelastic materials, which provides a typical stress-strain curve for their behaviour (Fig. 1), was used as a reference for an experimental study using a Universal Testing Machine for tensile stress loads (Shimadzu model AG-IS), which yielded a graph of the real behaviour of the Nickel-Titanium alloy.

The test consisted of measuring tensile stress on a Nickel-Titanium (NiTiNOL) specimen 0.4 mm in diameter and 150 mm long. The specimen was subjected to traction up to 6% strain at a speed of a 0.04 mm/min. Subsequently, the wire was unloaded at 7 MPa and then subjected to traction until it fractured at a speed of 0.4 mm/min.

The experiment yielded a curve which was very similar to the one reported in standard ASTM F2516-07[12] so it was decided to apply data from the stress-strain curve obtained to a simulation by means of finite element analysis. The following data were found for mechanical properties: Young's modulus 31650 MPa, maximum limit of failure by fracture 1270.588 MPa, yielding point 352.941

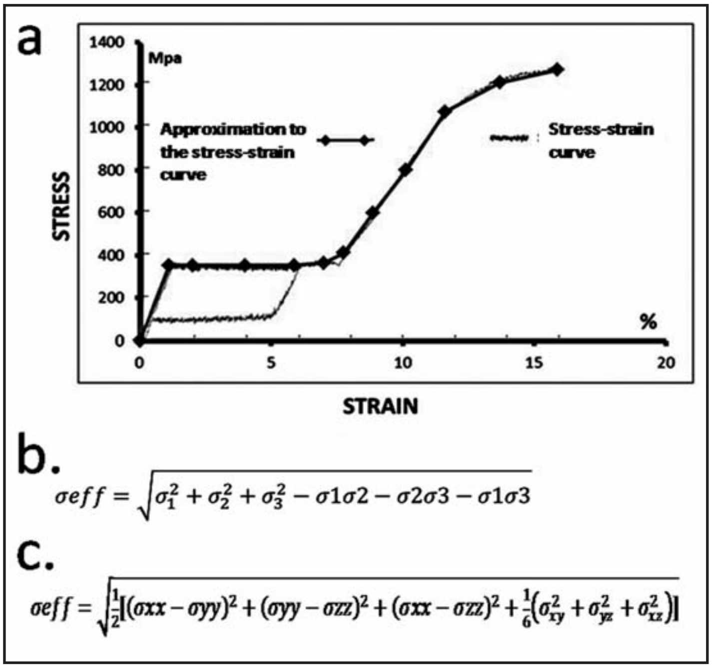

Fig. 1: a. Stress-strain curve and multi-linear approximation to the behavior of a nickel-titanium alloy. b. Equation used by the Autodesk® Simulation Multiphysics software to calculate Von Mises stresses. c. Equation used by Autodesk® Simulation Multiphysics software to calculate Von Mises stresse on Cartesian coordinates.

MPa, Poisson ratio 0.3 and density of the material 6.450 g/cm³.

The software Autodesk Inventor Professional® was used to construct each instrument based on the plots by estimating the working axis and generating the cutting helix around it, after which the characteristic Mtwo® "italic S" cross section was applied. *The Von Mises with Kinematic Hardening material curve* was selected, because it allows an elastic-plastic material to be programmed, thus approaching the real behaviour of a Nickel-Titanium alloy. Previous studies have reported the use of this material model for analyzing finite elements with other software such as Ansys® and Abaqus®[13-16]. The model also allows a behaviour curve to be programmed according to known data, which may be either experimental or previously reported in the literature. Our study was able to program the material behaviour curve according to the experimental tensile test performed on the Nickel-Titanium specimen.

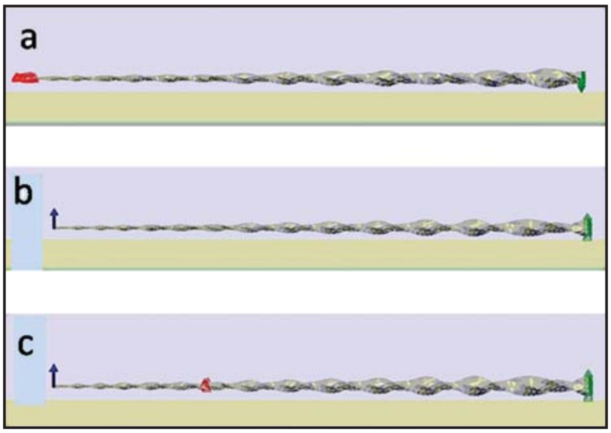

Fig. 2: a. Restriction at the tip and torque applied at file handpiece. b. Restriction in cantilever at the handpiece and force applied to the tip of the file. c. Restriction in cantilever at the handpiece and at 4 mm from the tip of the file, force applied at the tip.

Table 1: Number of elements and nodes per file.

	Elements	Nodes
10/0.04 Mtwo®	2041	881
15/0.05 Mtwo®	2045	761
20/0.06 Mtwo®	2344	934
26/0.06 Mtwo®	2228	901

Numerical analysis of the failure criterion used in the finite element analysis

In order to perform failure analysis taking into account the Von Mises criterion, the Autodesk® Simulation Multiphysics software uses equation b (Fig. 1), where *σ eff* is the Von Mises equivalent stress and *σ1, σ2* and *σ3* are the principal stresses. The software can also perform the calculation using equation c, (Fig. 1), where *σxx, σyy* and *σzz* are the principal stresses applied in each element into which the model has been discretized, expressed in terms of Cartesian coordinates.

Meshing and application of stresses and restrictions on the 3-D CAD Mtwo® Nickel-Titanium rotary files

Autodesk® Simulation Multiphysics software was used for finite element analysis for a study on three-dimensional models based on the Brick element with 8 nodes and three degrees of freedom. In some cases it was combined with the tetrahedral element with 4 or 5 nodes, and also considered a number of elements which would enable an acceptable computing time (Table 1).

To simulate the file being trapped inside the canal and the consequent torsion the file is subject to, restrictions were applied to all degrees of freedom at the end of the file which is in contact with all the walls of the canal (Fig. 2a). We used the torque recommended by the manufacturer: 1.2 N.cm for file 10/0.04, 1.3 N.cm for file 15/0.05, 2.1 N.cm for file 20/0.06 and 2.3 N.cm for file 25/ 0.06. In addition, high torque was used to evaluate the behavior of the file under extreme conditions.

To simulate bending, a restriction in cantilever was applied to all file models, as reported by Kim *et al.*[14-16] A 1N load was applied to the tip of the file models (Fig.2 b) and a restriction was placed at 4 mm from the tip, to simulate the flexion a file undergoes when working in an apical curve (Fig.2 c). Finally, a 5N load – much higher than the previous ones – was applied, to determine the bending behavior of the file in this situation.

RESULTS

Results for Bending

Under normal working conditions, no file reached the failure limit. File 10/0.04 had the highest Von Mises stress. However, under extreme conditions, files 10/0.04 and 15 /0.05 might fail and fracture, with file 10/0.04 being the most likely to fracture (Fig. 3, Table 2).

Results for Torsion

Here again, under normal working conditions, no file reached the failure limit, with file 20/0.06 having the highest Von Mises stress. However, upon applying high torque to simulate extreme working conditions, only file 25/0.06 reached the failure by fracture limit (Fig. 4, Table 3).

Figure 5 shows the result of the finite element analysis for the files which had the highest Von Mises stresses and the situations in which they occurred.

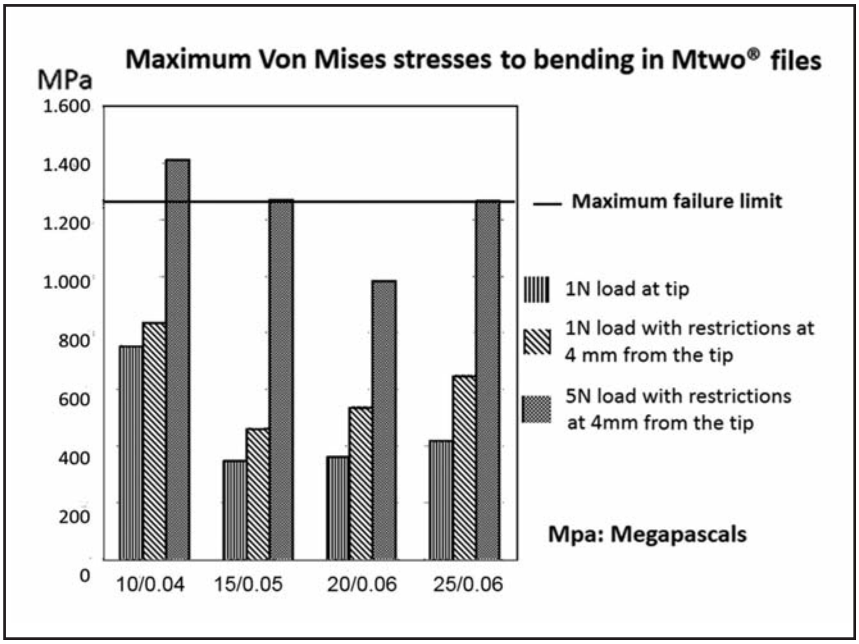

Fig. 3: Bending results for Mtwo® files.

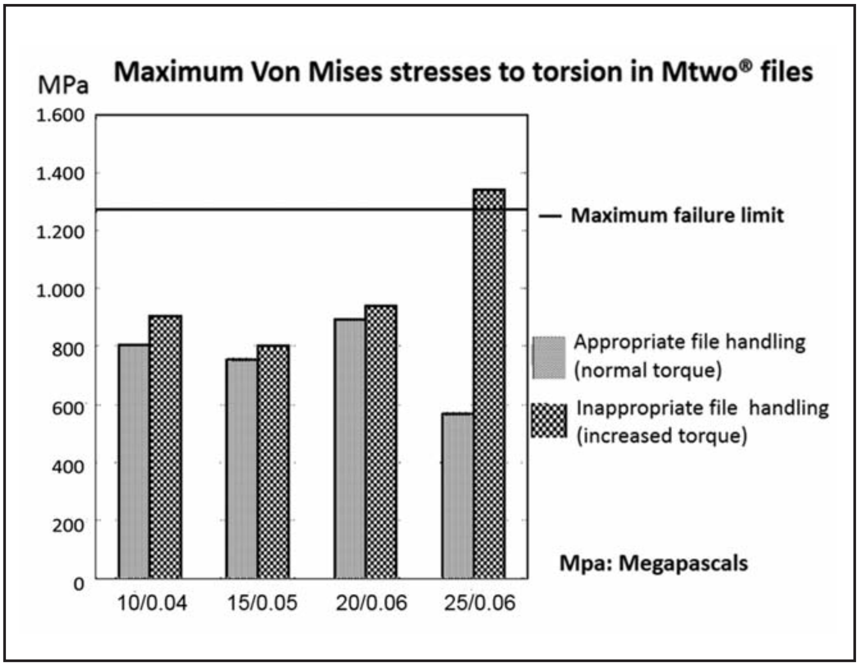

Fig. 4:Torsion results for Mtwo® files.

Table 2: Bending results for Mtwo® files.

Bending Load- File Type	10/0.04	15/0.05	20/0.06	25/0/06
1N at the tip	753.354 MPa	347.698 MPa	360.684 MPa	421.193 MPa
1N with restriction at 4mm from the tip	837.825 Mpa	460.650 MPa	536.646 MPa	648.025 MPa
5N with restriction at 4mm from the tip	1413.288 MPa	1270.930 MPa	984.267 MPa	1268.770 MPa
MPa: MegaPascals				

Table 3: Torsional results for Mtwo® files.

Torque – File type	10/0.04	15/0.05	20/0.06	25/0.06
Normal Torque	805.566 Mpa	756.870 MPa	895.147 Mpa	570.479 MPa
Increased Torque	906.705 MPa	804.179 MPa	941.204 MPa	1342.840 MPa
MPa: MegaPascals				

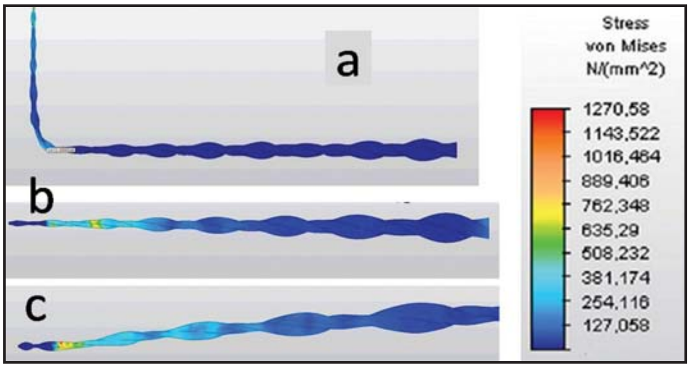

Fig. 5: Files with the highest Von Mises values: a. File 10/0.04 with restriction in cantilever and restriction at 4mm from the tip with 5N force applied to the tip. b. File 20/0.06 with restriction at the tip and 4N torque applied at the file handpiece. c. File 25/0.06 with restriction at the tip and 4N torque applied at the handpiece.

DISCUSSION

Because this is a simulation, not all clinical conditions can be replicated exactly. Nevertheless, appropriate approximations allowed the Nickel-Titanium rotary files to be evaluated mechanically.

Turpin *et al.*[17] evaluated torsion and bending by taking a segment of file and placing restrictions on one of its surfaces while applying torque on the opposite surface. They used 0.25 Nmm torque of to evaluate torsion, reporting that this value was selected because it is used by rotary instrument engine. In fact, the torque in enginesis much higher than 0.25 Nmm because they are programmed in Ncm. It should be noted that this fault in the approach appears in several of the finite element analyses reported in the literature[14-18].

In order to simulate bending behavior, a 1N load at the tip of the file has been reported[14-16]. Applying the same load to all files allows evaluation under equal conditions of the Von Mises equivalent stresses which could lead to file failure. Regarding torsion, the approximations reported in the literature place restrictions in cantilever on the file and apply torque to the tip of the file[14-16]. In reality, files do not undergo this type of stress, but rather, the restrictions should be located at the most apical part of the canal, where the file touches the walls of the canal, and torque should be on the handpiece, i.e. the point at which the file is connected to the engine. Our results show that Mtwo® file 10/0.04 had the highest Von Mises equivalent stress, thus, it may have the greatest tendency to failure by fracture due to bending stress. However, the results also seem to indicate that it is the most flexible file. Thus, the operator should be especially careful when using it. Nevertheless, being the most flexible file enables it to be used for endodontic treatment of irregularly shaped canals. It should be noted that if this file is used in a canal with moderate to severe curvature, it should not be reused for another treatment, since its mechanical properties may have been irreversibly altered, which may lead to a high possibility of fracture by bending if it is used again. Mtwo® file 25/0.06 had the lowest Von Mises stress, suggesting that it may be the most resistant to facture by bending, but at the same time, the least flexible file in the series, therefore using it for endodontic treatment of canals with abrupt curves in an inappropriate manner (e.g. not following the proper order in the series of instruments) might lead to procedural accidents such as ledges, apical transportations or perforations of the root canal. For excessive load on the file (5N) with restriction at 4mm from the tip, it was interesting to observe that all files surpassed the bending limit for failure by fracture except file 20/0.06, suggesting that it would be less likely to fail than the others when dealing with a very abrupt curve.

In this study, file 20/0.06 of the Mtwo® series showed the highest value for Von Mises stress to manufacturer-recommended torque without reaching the failure by fracture limit. With higher torque, file 25/0.06 showed the highest Von Mises stress and reached the failure by fracture limit. However, an interesting finding was that even when used inadequately, the other files of the Mtwo® series did not reach the failure by fault limit, suggesting that they may have temporary resistance to failure by fracture in the event of a procedural error.

Lee *et al.*[19], found a correlation between the number of cycles that a file is used until it fractures (cyclic fatigue) and the maximum Von Mises stress in the instrument before failure. They suggest that as the number of cycles increases prior to fracture, the value of maximum Von Mises stress value decreases, i.e. in this case, the file would be more resistant to fracture, whereas if the file withstands few cycles prior to fracturing, the value of the maximum Von Mises stress increases and the file would be less resistant.

On this basis, we will compare the results of clinical studies evaluating cyclical fatigue in Mtwo® files to the results of our study.

Plotino *et al.*[20] found that when there was severe curvature, the Mtwo® file 25/0.06 had the fewest rotations prior to fracture was (72 rotations). When the file worked under normal conditions, file 10/0.04 had the highest number of rotations prior to fracture (885 rotations). These are similar to the results of our study, which found that under difficult working conditions, file 25 /0.06 had highest Von Mises stress to torsion, and under normal working conditions, file 10 /0.04 had the lowest Von Mises stress to rotation.

Inan *et al.*[21] found that Mtwo® file 10/0.04 was the most likely to fracture by bending, in agreement with our study. File 25/0.06 came second, also in agreement with our analysis. Another study by Inan *et al.*[22] on the Mtwo® series found that file 20/0.05 was the least resistant to torsional fracture, again in agreement with our study.

To conclude, when Mtwo® series files are used in an endodontic treatment, special care should be taken in the use of files 10/0.04 and 25 /0.06. They should be used once only, otherwise there is a high risk of fracture. However, in a root canal with moderate to severe curvature, file 10/0.04 is recommended because it is highly flexible and may respect the original root canal shape, avoiding accidents such as ledges or perforations.

Similarly, caution is advised when using file 25/0.06 for which permeabilization or patency procedures are recommended to allow it to work freely in the root canal without the tip becoming trapped.

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ALVEOLAR WOUND HEALING IN RATS FED ON HIGH SUCROSE DIET

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ABSTRACT

The potential for bone repair is influenced by various biochemical, biomechanical, hormonal, and pathological mechanisms and factors such as diet and its components, all of which govern the behavior and function of the cells responsible for forming new bone. Several authors suggest that a high sucrose diet could change the calcium balance and bone composition in animals, altering hard tissue mineralization. The mechanism by which it occurs is unclear. Alveolar healing following tooth extraction has certain characteristics making this type of wound unique, in both animals and humans.

The general aim of this study was to evaluate and quantify the biological response during alveolar healing following tooth extraction in rats fed on high sucrose diets, by means of osteocyte lacunae histomorphometry, counting empty lacunae and measuring areas of bone quiescence, formation and resorption.

Forty-two Wistar rats of both sexes were divided into two groups: an experimental group fed on modified Stephan Harris diet (43% sucrose) and a control group fed on standard balanced diet. The animals were anesthetized and their left and right lower molars extracted. They were killed at 0 hours, 14, 28, 60 and 120 days. Samples were fixed, decalcified in EDTA and embedded in paraffin to prepare sections for optical microscopy which were stained with hematoxylin/eosin.

Histomorphometric analysis showed significant differences in the size of osteocyte lacunae between groups at 28 and 60 days, with the experimental group having larger lacunae. There were more empty lacunae in the experimental group at 14 days, and no significant difference in the areas of bone activity.

A high sucrose diet could modify the morphology and quality of bone tissue formed in the alveolus following tooth extraction.

Key words: *Tooth Socket, bone, dietary sucrose.*

CICATRIZACIÓN ALVEOLAR EN RATAS CON DIETA RICA EN SACAROSA

RESUMEN

El potencial de reparación ósea esta influenciado por una variedad de mecanismos bioquímicos, biomecánicos, hormonales, patológicos y factores como la dieta y sus componentes; todos rigen comportamiento y función de las células encargadas de formar nuevo hueso. Varios autores sugieren que una dieta rica en sacarosa, podría cambiar el balance del calcio y la composición ósea en animales, alterando la mineralización de tejidos duros. El mecanismo por el cual esto se produce no es claro. La cicatrización alveolar post extracción reúne características particulares que la convierten en una herida única, en animales y en humanos.

El objetivo general de este trabajo fue evaluar y cuantificar la respuesta biológica durante la cicatrización alveolar post extracción en ratas con dieta rica en sacarosa; mediante la histomorfometría de lagunas osteocíticas, recuento de lagunas vacías y medición de zonas de reposo, neoformación y resorción ósea.

Se utilizaron 42 ratas Wistar, de ambos sexos, que fueron divididas en dos grupos: grupo experimental, alimentadas con dieta modificada de Stephan Harris (43% de sacarosa) y grupo control alimentadas con dieta balanceada estándar. Se anestesiaron los animales y se extrajeron primeros molares inferiores, derecho e izquierdo, luego fueron sacrificados a las 0hs., 14, 28, 60 y 120 días. Las muestras obtenidas fueron fijadas, descalcificadas con EDTA e incluidas en parafina y se obtuvieron cortes para microscopia óptica que fueron coloreados con hematoxilina/eosina.

El análisis histomorfométrico mostró diferencias significativas de tamaño entre lagunas osteocíticas de ambos grupos a los 28 y 60 días siendo de mayor tamaño en los experimentales, se encontraron mayor cantidad de lagunas vacías en experimentales a los 14 días y no hubo diferencias significativas en las superficies de actividad ósea.

Una dieta rica en sacarosa podría producir modificaciones en la morfología y calidad del tejido óseo que se forma en el alveolo post extracción dentaria.

Palabras clave: *Alvéolo dentario, hueso, dieta rica en sacarosa*

INTRODUCTION

Following tooth extraction, a series of events takes place in the human alveolus which makes this type of wound unique in the body. The broken surface covering exposes the bone and clot to a septic cavity containing microorganisms (saprophytes or pathogens), which may upset the biological balance[1]. In addition, the entire periodontium is irreversibly damaged and healing takes place by secondary intention[2].

The potential for bone repair is influenced by a range of external factors, such as diet, which affects the behavior and function of the cells responsible for forming new bone.

Osteocytes, the most plentiful cells in bone tissue[3,4], are involved in control of extracellular concentration of calcium and phosphorus and in the process of adaptive remodeling through cell-cell interactions in response to the local environment, thus preserving bone homeostasis and ensuring bone vitality. They are found in the osteocyte lacunae and connect by means of a network of cytoplasmic processes through cylindrical canaliculi to blood vessels and other osteocytes, forming a bone microcirculation system, sending and receiving signals to and from other osteocytes, osteoblasts and bone-lining cells[5].

Several studies have shown that a high sucrose diet alters mineral metabolism in humans, inducing changes in calcium balance[6]. In experimental animals, changes in bone composition have been observed,[7] as well as variations in the mineralization of hard tissues, teeth and bones[8], taking into account that their formation processes are considerably similar[9]. Regarding dentin, it was found that there is a reduction both in its degree of mineralization[10] and in the amount formed[11]; regarding bone tissue, there is alteration of mechanical properties[12,13], with marked reductions in concentrations of calcium and phosphorous, bone density and resistance to fracture in rats of both sexes[14]. All the literature consulted shows bone alterations produced by a high sucrose diet in different bones in experimental animals; but there is no description of what happens or how this kind of diet affects the maxillaries or the alveolus following tooth extraction in humans or experimental animals.

The mechanism by which high sucrose diet negatively affects bone metabolism is still unclear. It has been reported that it might cause glucose-intolerance and hyperinsulinemia, which indirectly produce deleterious effects on the bone[15]. The consequences of this kind of diet on calcium absorption, bone calcium content and bone mechanical properties are still a matter of controversy.

The aims of this study were to assess and quantify the effects produced by a high sucrose diet on bone tissue by means of a histomorphometric study of the osteocyte lacunae in the bone tissue, surface areas of bone undergoing resorption and new formation, and quantity of osteocytes and empty lacunae per mm^2 during alveolar wound healing following tooth extraction in rats.

MATERIALS AND METHODS

Forty-two Wistar rats of both sexes were fed on the same standard feed from birth to weaning (21 days), after which they were divided into two groups of 21, a control group and an experimental group. The control group was fed a standard balanced diet (Gepsa Feeds, Grupo Pilar, Bs. As., Argentina) and the experimental group was fed a modified Stephan Harris diet[16] with high sucrose content (43%). Each group comprised four sub-groups of 5 rats (3 females and 2 males), to be killed at 14, 28, 60 and 120 days, plus one rat to be killed at 0 hours.

The food for the experimental group was prepared once a week because it did not contain any preservatives. The ingredients and quantities were strictly controlled. Food and water were provided *ad libitum*.

The rats were identified according to group. For the extractions they were given an intra-peritoneal injection of 8 mg/100g ketamine chlorhydrate (Ketalar®, Parke Davis, Morris Plains, NJ) and 1.28 mg/100g xylazine (Rompun®, Bayer, Leverkusen, Germany). The treatment area was disinfected with 0.12% chlorhexidine digluconate solution[17].

Right and left lower first molars were extracted using a dental explorer as an extraction elevator after peeling back the soft tissues, and a mosquito clamp to complete the extraction[18,19].

Rats were euthanized at 0 hours, 14, 28, 60 and 120 days by anesthetizing with ketamine chlorhydrate plus xylacine and perfusion with 10% buffered formalin.

The whole lower maxillary was extracted, a sagittal section was performed at its mid-point and the pieces were preserved in formalin at 4° C for 24 hours[20].

The right half was decalcified in EDTA at neutral pH for +/- 30 days, with radiographic control of the process. Samples were embedded in paraffin and 10 serial sections, ons 5 to 7 µm thick, were cut on a plane across the alveolus zone corresponding to two of the roots of the extracted molar – a modification of the original experimental model by Guglielmotti et al.[21] in which the section was obtained at the level of the mesial root of the extracted molar. The sections were stained with hematoxylin/eosin and the following parameters measured under conventional optical microscopy (Olympus BX 50F4): total lacuna area, lacuna perimeter, quantity of empty lacunae per mm^2 and bone surfaces.

Histomorphometry

Eight to 10 microscope images at 40X were digitalized from each case and at least 50 true lacunae were taken randomly, following a zigzag pattern[22,23]. Areas of 18861 μm^2 were evaluated using the Image Pro Plus program. The microscope-camera optical system was calibrated for each objective with a micro-rule (Carl Zeiss). The area of the complete lacunae (without canalicular projections) was measured directly using the image analyzer. Uncomplete and empty lacunae were avoided.(Fig. 1).

Empty lacunae were counted using 40X images and with relation to the number of osteocytes per mm^2.

Bone surfaces

In order to measure the percentages of bone quiescence, new formation and resorption, the alveolar area corresponding to the bisector of the angle formed between the external cortical wall and a horizontal line passing through the roof of the mandibular canal was considered.[17,18] The images were digitalized at 20X and the areas were marked manually, considering the following histological parameters:

Quiescent surface: smooth bone surface with inactive atrophic-looking osteoblasts attached to the bone wall (Fig. 2).

Area undergoing new formation: surface of bone tissue with a lining of osteoid material and hypertrophic osteoblasts attached to it (Fig. 3).

Resorption area: eroded bone surface with Howship's lacunae, with or without osteoclasts (Fig. 4).

Results were statistically evaluated by means Student's T-test for mean areas of osteocyte lacunae, Chi Square for Areas of bone remodeling and Mann Whitney's test for quantity of empty lacunae per mm^2.

RESULTS

Histomorphometric analysis

Morphological differences in the osteocyte lacunae were observed between groups at different times. The ones in the experimental group were more irregular, rounded and larger than those in the control group. Significant differences were found in mean areas at 28 and 60 days (Figs. 5, 6, 7 and 8). Table 1 shows the mean values for each group at all times. They were analyzed using Student's test, considering $p<0.05$ as significant.

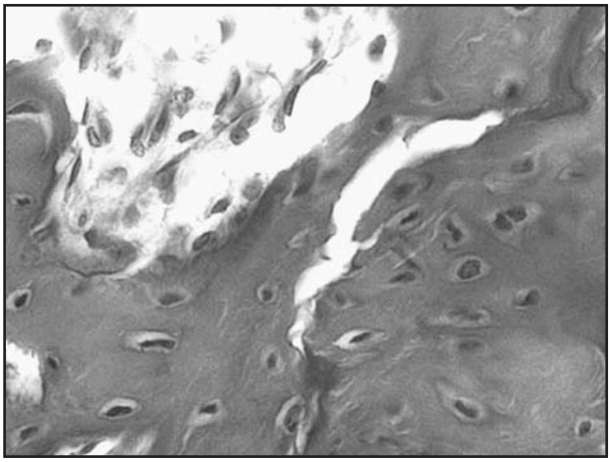

Fig. 1A: Microphotograph of osteocyte lacunae to be measured.

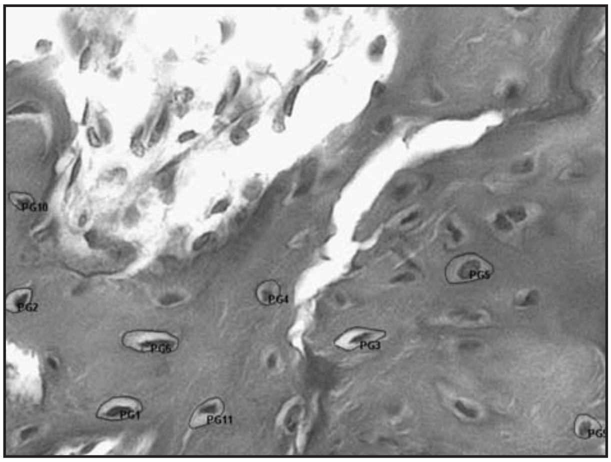

Fig. 1B: The same slide with lacunae selected semi-automatically for measurement.(hematoxylin/eosin – 40X).

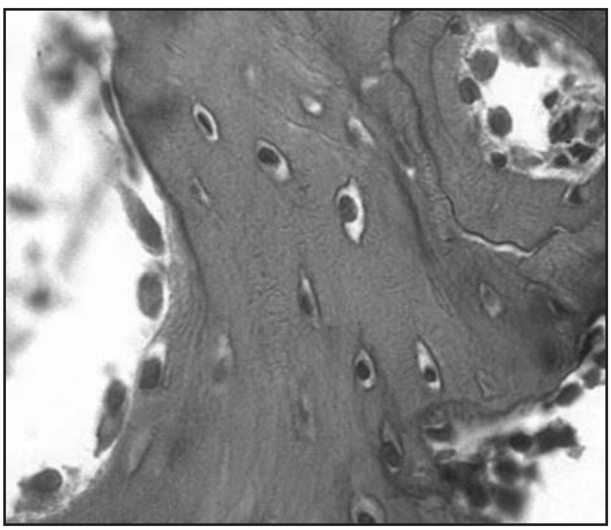

Fig. 2: Quiescent area with osteoblasts of atrophic appearance on the bone surface.(Hematoxylin/eosin – 40X).

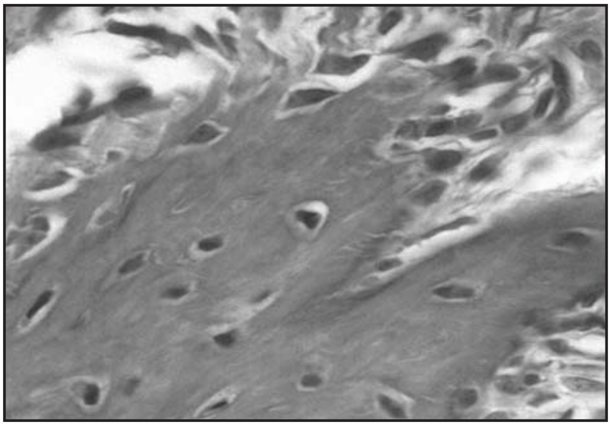

Fig. 3: Area of newly formed bone with hypertrophic osteoblasts and osteoid.(Hematoxylin/eosin – 40X).

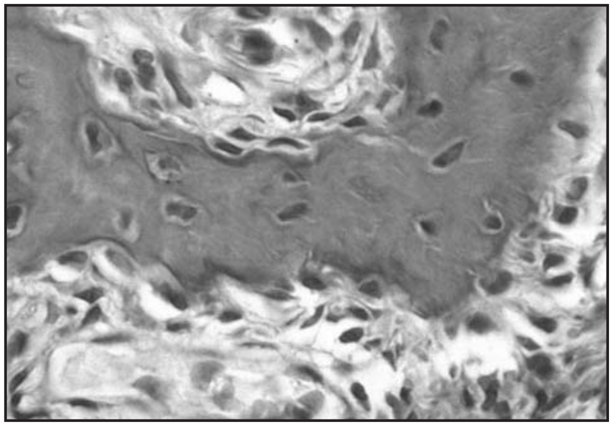

Fig. 4: Resorption area with Howship's lacunae. (Hematoxylin/eosin – 40X).

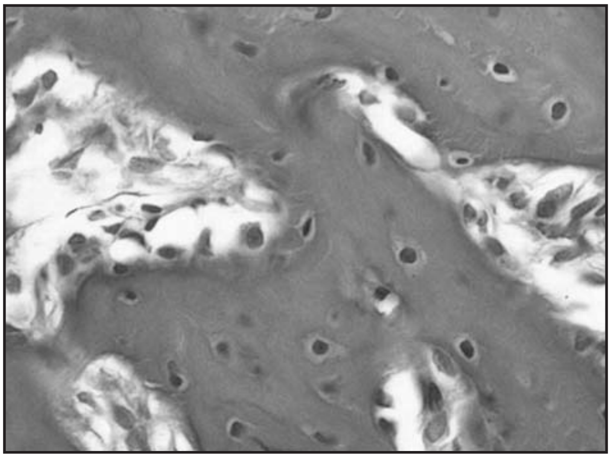

Fig. 5: Control at 28 days: the bone tissue has an orderly arrangement with clearly outlined, elliptical osteocyte lacunae of normal size containing osteocytes. (Hematoxylin/eosin – 40X).

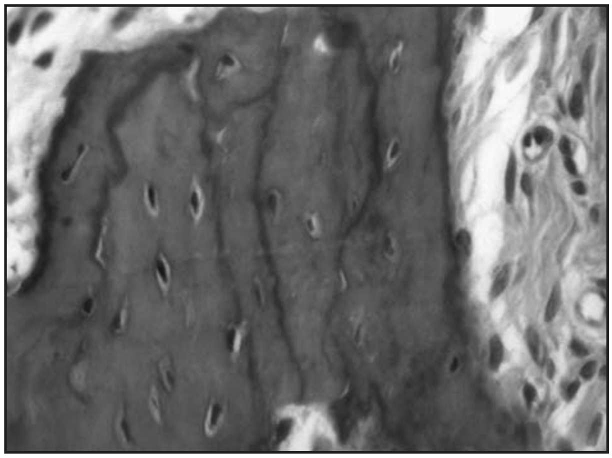

Fig. 6: Control at 60 days: osteocyte lacunae maintain their shape and are smaller than in controls at 28 days due to the change in the maturity of the bone tissue, which has basophilic lines of inversion and more compact appearance. (Hematoxylin/eosin – 40X).

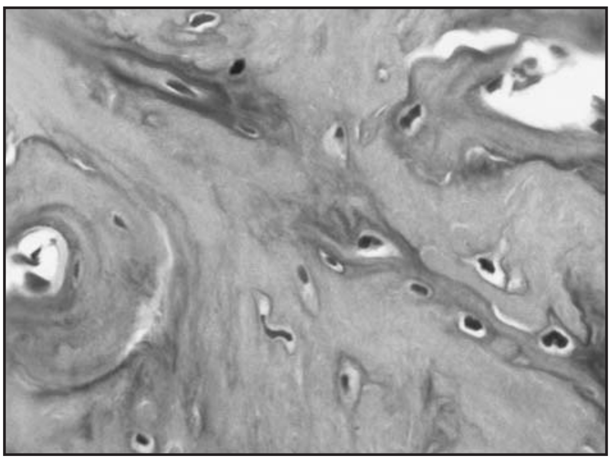

Fig. 7: Experimental at 28 days: inverse lines showing intensive bone remodeling; lacunae more irregular but clearly outlined and larger than in the controls. (Hematoxylin/eosin – 40X).

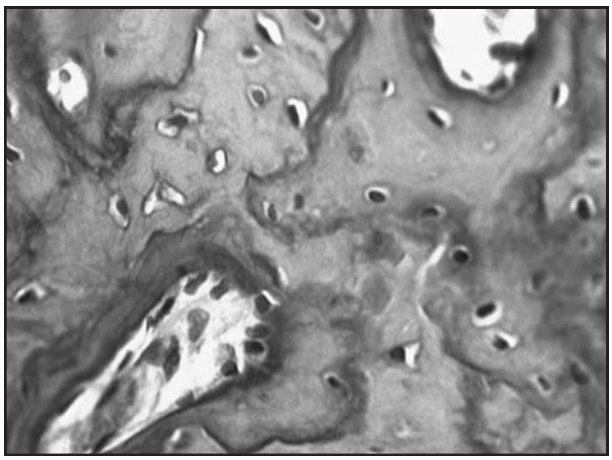

Fig. 8: Experimental at 60 days. Bone tissue with disorganized appearance, lacunae clearly larger than in controls, outlines poorly defined and morphology becomes irregular. (Hematoxylin/eosin – 40X).

Table 1: Mean areas and quantity of lacunae measured.

Time	14 days		28 days		60 days		120 days	
	Control N:5	Experimental N:5	Control N:5	Experimental N:5	Control N:5	Experimental N:5	Control N:5	Experimental N:5
Mean area (μm²)	54.0	53.3	48.4	52.6	43.3	46.6	46.0	46.7
Standard error	1.1	1.2	1.4	1.2	0.9	1.0	1.1	1.0
Mean deviation	19.97	21.09	25.31	21.06	16.20	17.75	19.53	18.08
Maximum	134.4	148.5	224.3	142.9	103.4	140.7	128.8	117.3
Minimum	16.6	18.7	13.3	16.6	13.2	14.0	11.5	9.5
Lacunae measured (n)	353	329	337	327	329	314	338	328
P value=	0.56		0.02*		0.01*		0.62	

(*) p<0.05

Analysis of number of osteocytes and empty lacunae per mm²

Empty lacunae were found at all study times in both groups (Fig. 9). The difference between experimental and control animals regarding quantity of empty lacunae compared to number of osteocytes per mm² was significant at 14 days. The values were contrasted by the Mann Whitney non-parametric test. Quantity of osteocytes per mm² was higher in the control group than in the experimental group at all times. Table 2 provides all values.

Analysis of bone surface areas

Percentages of areas with bone remodeling and different times were contrasted using the Chi-square test, but no significant difference was found.

DISCUSSION

Different studies have described the noxious effects on bone tissue of excessive sucrose intake[24]. Tjäderhane *et al.* [15] explain that all mineralized tissues are affected by sucrose intake. Our study shows negative effects on the quality of newly formed bone in the alveolus following tooth extraction in animals fed on a diet containing 43% sucrose.

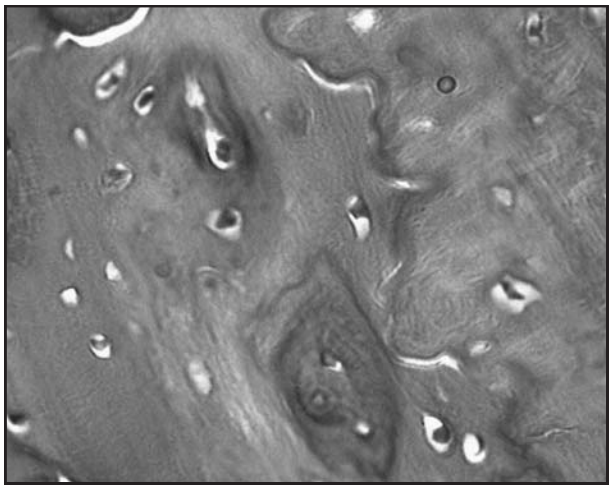

Fig. 9: Microphotograph of an experimental case showing presence of several empty lacunae. (Hematoxylin/eosin – 40X).

Table 2: Quantity of empty lacunae with relation to quantity of osteocytes per mm².

	Quantity of osteocytes per mm²			Mann Whitney's Test Empty lacunae per mm²			
Time	Mean value		Statistical contrast	Mean value		Statistical significance	
	Control N:20	Experimental N:20		Control N:20	Experimental N:20		
14 days	1643	1591	14 days	65	113	0.04	*p < 0.05
28 days	1651	1395	28 days	88	107	0.08	Non sig.
60 days	1392	1390	60 days	122	101	0.20	Non sig.
120 days	1395	1391	120 days	72	80	0.82	Non sig.

Alveolar bone wound healing provides a sustainable model for studying bone formation in rats and may be considered a sensitive indicator of bone formation in normal conditions, as reported by Guglielmotti and Cabrini [21] and Devlin[19], and under other conditions, as reported by Gorustovich (2004, 2008)[17,20] for alveolar bone histomorphometry with boron-deficient diet.
Some histological analyses suggest that at 21 days of alveolar wound healing the alveolus is occupied by a thin network of trabecular bone [25]. Elsubeihi [26] reports that the process ends in the eighth week. However, Guglielmotti et al.[27] report maximum reticular bone formation and maximum alveolar volume at 14 days after extraction, young bone formation at 30 days and mature laminar bone alveolar filling at 60 days, by quantifying the alveolar response with an image analyzer. Our study agrees with this but follows bone formation and maturation up to 120 days, when bone sclerosis is very noticeable in the experimental cases.
Studies by Hara *et al.*[28] and Lockwood [29] on high sucrose diets and by Van Schothorst [30] on a high lipids diet, showed that these kinds of diet produce hyperinsulinemia, resistance to insulin and increase in plasma glucose levels. Holl and Allen[31] reported that sucrose intake increases urinary excretion of calcium, sodium and zinc, with renal inhibition of calcium resorption. Clemens and Karsenty [32] reported that the osteoblast is an important target of insulin for controlling whole-body glucose homeostasis and regulating the osteocalcin activation mechanism to produce bone resorption.
The size of osteocyte lacunae may increase due to various factors. Ferreyra et al.[33] showed enlarged perilacunae associated to the application of orthodontic forces. Bozal *et al.*[34] reported osteocyte response to mechanical stimuli and inflammatory factors, which produce enlargement of their lacunae by osteolytic resorption, but with no change in cell volume. Krishnan and Davidovitch [35] describe osteocyte capacity to change its micro-environment in response to mechanical load. Thus, the change in size of osteocyte lacunae is related to the rigidity of the bone matrix, under normal conditions within a physiological range of size and density, for mechanical control of the forces applied on the bone tissue[36].
Quing and Bonewald [37] reported that the osteocyte has access to a very large area of its lacuna and the removal of just one Ångström (Å) of mineral per osteocyte may significantly affect circulation and systemic ion levels. In a review of the literature on osteocytic osteolysis, Tetia and Zalloneb [38] infer that it may be related to bone mineral homeostasis.
The significant differences in histomorphometry of osteocyte lacunae that we found in the alveolus following extraction may be related to the regulation of bone remodeling and to changes in the osteocyte micro-environment in response to a diet which alters mineral metabolism and the way in which bone tissue adapts in order to maintain control of homeostasis.
To conclude, excessive sucrose intake produces modifications in the morphology and quality of newly formed bone tissue in the alveolus after tooth extraction in rats, but further studies are needed to analyze and understand the molecular and cellular mechanisms that take place in it.

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