IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 8, August 2015.
www.ijiset.com
ISSN 2348 – 7968

Nanomechanical properties of dehydrated enamel surface after bleaching treatment.

Abel Hurtado-Macías[1]*, Alfredo Nevarez-Rascon[2], Santiago González-López[3], Martina M. Nevarez-Rascon[2] Víctor A. Ríos-Barrera[2] and Jesús González-Hernández[4]

[1] Metalurgia y diseño estructural, Centro de Investigación en Materiales Avanzados S.C., and Laboratorio Nacional de Nanotecnología, Miguel de Cervantes 120, Complejo Industrial Chihuahua, Chihuahua, C.P. 31109, México

[2] Facultad de Odontología, Universidad Autónoma de Chihuahua. Pascual Orozco y Avenida Universidad s/n C.P. 31000, México

[3] Department of Stomatology, University of Granada, Campus Cartuja Granada 18071, Spain

[4] Centro de Ingeniería y Desarrollo Industrial, Santiago de Querétaro 76130, Qro. México

Abstract

The abstract should summarize the content of the paper. Try to keep the abstract below 150 words. The aim of the present study was to investigate the effects of bleaching procedures of a high concentration of hydrogen peroxide (HP) on dehydrated enamel surface. Flat enamel samples embedded in epoxy resin were dehydrated and treated with 35% HP of photo-initiating bleaching system. Mechanical properties such as nanohardness (*H*), elastic modulus (*E*) and the stiffness (*S*) of the enamel were analyzed by nanoindentation method. Residual indentation of samples was recorded by the Atomic Force Microscopy (AFM) Nano Vision system attached to the nanoindenter instrument. Surfaces of treated enamel tissue, studied under scanning electron microscopy (SEM), X-ray diffraction and AFM determinate the crystallographic spectrum. The *H*, *E* and *S* as well as surface roughness of enamel surface were significantly decreased by the effects of the bleaching solution treatment (H and E< %50) the effects of demineralization observed thought changes on minerals quantities by the absence of contact with saliva.

Keywords: *Enamel, bleaching treatment, mechanical properties and nanoindentation.*

1. Introduction

By aesthetic reasons bleaching procedures are performance on human teeth surface structured by enamel rods. Enamel is a highly mineralized structure consisting of approximately 96% mineral and 4% organic material and water. The fundamental units of enamel are carbonated hydroxyapatite crystals [1]. Previous investigations have been focused on the mechanical characterization of whole teeth and larger sizes of enamel samples at microscopic scale, reporting elastic modulus within the range of 74–130 GPa; nanoindentation revealed elastic modulus of 120 GPa [2]. Nanoindentation technology has been recently introduced for the evaluation of mechanical properties of human enamel after bleaching treatments, evidencing changes of mechanical behavior of enamel at nanoscale on biomimetic materials [3]. Different studies indented the enamel with a Berkovich indenter under close loop mode, loading (loading rate 0.43 mN/s) and unloading with 20 data points and a maximum force of 15 mN to calculates the hardness and elastic modulus from the load–displacement curve based on the power-law fitting method for unloading part of the load–displacement curve [4]. Enamel in original states, contains mainly calcium phosphate salts in shapes of larges hexagonal hydroxyapatite crystals, within averages of hardness (H) and Young's modulus (*E*) of. $H > 6$GPa and $E > 115$ GPa [5]. The exact mechanism by which hydrogen peroxide affects the enamel has yet to be fully elucidated. After, bleached with 30% hydrogen peroxide on enamel surface of premolars, mean hardness decreased between 13 to 32%, whiles the mean of Young's modulus decreased by 18–32% [6], determinates the wear behavior of the enamel was by employing nano scratch test, while studied hardness and elastic modulus were by nanoindentation with Berkovich tip. Atomic force microscopy (AFM) combined with nanoindentation techniques, can also be used to observe changes on the surface roughness on enamel surface as well as mechanical properties at nanometric level [7], AFM nanoindentation in combination with electron microscopy analysis it has been use to analyzed demineralization on enamel [5], [8], also to measure the stiffness at nano scale on biological specimens [3]. Bleaching is closely correlated with variations on the amounts of Ca^{2+} extracted from the enamel, after bleaching with 30% hydrogen peroxide for 1 h, there were no changes in amounts of Ca^{2+} extracted because specimens were stored in artificial saliva [9], although enamel surface treated with 38% hydrogen peroxide for 15 minutes, did not reduce the bonding properties after orthodontic brackets position, but generate changes on the enamel surface [10]. Some authors assert that hydrogen peroxide does not cause any damages on enamel surface [3], [7]. Garrido *et al*, mention that after initial increase, the enamel exhibited a recovery of properties when it was stored in artificial saliva [11]. The mean nanohardness of the original enamel rods was calculated to be 5.31 to 7.15GPa, while the reduced elastic modulus was determined to be 86.38 to 70.96 GPa on enamel rods, affected by surface treatment [12]. Recent studios propose a reasonable doubt about bleaching therapies may have a negative effect on physical properties of the enamel [13], [14]. The present study was to investigate the effects of bleaching procedures of a high concentration of hydrogen peroxide (HP) on dehydrated enamel surface. Flat enamel samples embedded in epoxy resin were dehydrated and treated with 35% HP of photo-initiating bleaching system. Mechanical properties such as nanohardness (H), elastic modulus (E) and the stiffness (S) of the enamel were analyzed by nanoindentation method using a Berkovich tip. On the other hand, to determinate the Hertzian behavior and the yield point by the pop-ins transition through the load-penetration behavior curve were used the sphere-conical tip. Surfaces of treated enamel tissue, studied under scanning electron microscopy, X-ray diffraction and AFM determinate the crystallographic spectrum and the morphology of the structured before and after of bleaching treatment.

2. Materials and methods

2.1 Enamel samples preparation.

Four Flat enamel samples in flake shape embedded in epoxy resin were prepared from vestibular area of two human premolar teeth (specimen 1 and specimen 2) and stored in dry environment for one year. One group of enamel samples from the same specimen, were treated with 35% hydrogen peroxide and receive 30 minutes of fotocure trough halogen high intensity light exposition and finally washed with water simply alone, while the other untreated samples specimens constituted the control group. After bleaching samples were stored on dry environment.

2.2 Morphology, structural and Nanomechanical characterization.

Standard procedure Energy Dispersive Spectroscopy (EDS) by a scanning electron microscope Jeol model was used for identifying and quantifying elemental of Ca, C, O, F, Cl and P principally and morphology of the exposed tissues treated with 35% HP and the controls. The crystalline structure of the enamel before and after bleaching treatment was analyzed using a Panalytical X-Pert system. The patterns were obtained using Cu Kα radiation at 40 kV and 35 mA. The diffracted beam path included a graphite flat crystal monochromator. The grazing incidence angle was fixed at 0.5°, whereas the scanning angle 2θ was varied between 20 and 75°, with a step size of 0.02°.

On the other hand, before and 72 hrs after bleaching treatment, mechanical properties such as hardness and elastic modulus were evaluated by means of nanoindentation, employing a Nano Indenter G200 coupled with a DCM II head. The equipment was calibrated by using a standard fused silica sample. Tests parameters were the following; the constants of area function were C_0=24.05, C_1= -178.33, C_2= 6724.30, C_3= -24407.23, and C_5= 18701.80. Berkovich diamond indenter with a tip radius of 20 ±5 nm, maximum load of 35 mN, strain rate of 0.05 s^{-1}, and harmonic displacement and frequency of 1 nm and 75 Hz, passion's coefficient of ν=0.25 respectively. 90 indentations tests were evaluated in each sample. Residual indentation of samples was recorded by the AFM Nano Vision system attached to the nanoindenter system. During indentation, a curve describing the relationship between load (P) and displacement (h) is continuously monitored and recorded as illustrated in Fig. (5). On the other hand, with propose to measurement the nano-mechanical properties of the enamel before and after bleaching treatment was used the Oliver and Pharr method with controlled cycles [15]. The basic analysis of nanoindentation load-displacement curve (P-h) was established based on the elastic contact theory given by Sneddon [16] and Doerner [17]. The Sneddon equation's following was used to determinate the elastic modulus.

$$S = 2\beta\sqrt{\frac{A}{\pi}}E_r \qquad (1)$$

where β is a constant that depends on the geometry of the indenter (β = 1.034 for a Berkovich indenter), E_r is the reduced elastic modulus, which accounts for the fact that elastic deformation occurs in both the sample and the indenter and A is the contact area that is function of the penetration depth or displacement (h)[15]. The elastic modulus, E can be calculated by considering the compliance of the specimen and the indenter tip combined in series,

$$E = \frac{1-\nu^2}{\left(\frac{1}{E_r} - \frac{1-\nu_i^2}{E_i}\right)} \qquad (2)$$

where E_i, E and ν_i ,ν are elastic modulus and Poisson's ratio of diamond indenter and specimen respectively. For the diamond indenter E_i = 1140 GPa and ν_i =0.07 are used (G200 Aguilet manual, Agilent technology USA).

The hardness was calculated by the following equation

$$H=P_{max}/A(h) \qquad (3)$$

Where H is the hardness, P_{max} is the maximum load and $A(h)$ is the contact area between the sample and tip indenter, that is function of the penetration depth.
On the other hand, there is a possibility to use indentation with sphere-conical tip to probe the Hertzian behavior and yield points of a material before and after bleaching treatment, but it must be pointed out that measured indentation yield points are different from uniaxial mechanical tests due to the complex 3D-stress – distribution around the indent. A spherical indenter in contact with a specimen surface is shown in (Fig. 7 a)). The indentation area, A can be related to the indentation radius, *a*, or to the indenter radius, R=1000nm and the contact depth, h_c:

$$A = \pi a^2 = \pi(2Rh_c - h_c^2) \tag{4}$$

classical Hertzian equation for elastic contact of an elastically isotropic material denotes [18].

$$a = \left(\frac{3PR}{4E_r}\right)^{1/3} \tag{5}$$

where a contact radius, P is the applied load, R is the indenter radius and E_r is the reduced elastic modulus.

3. Results and discussion

In the Figure 1 (a) and (b) show the morphology of the specimen 1 before and after bleaching treatment respectively.

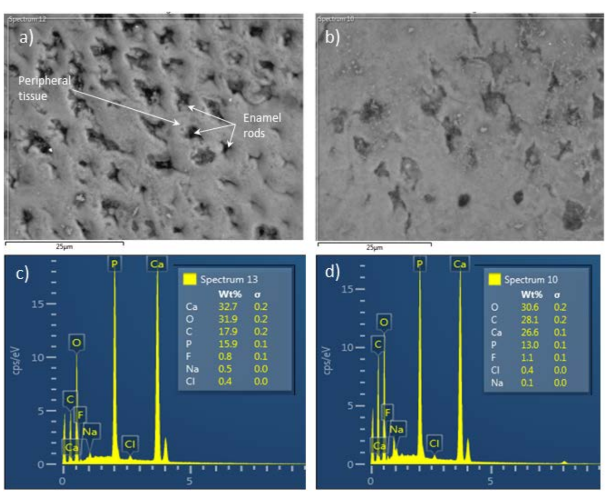

Fig. 1 Specimen1: (a), (b) Morphology and (c), (d) EDS before and after bleaching treatment respectively.

In this figure we can clearly observed the surfaces of treated enamel tissue manifest erosion. On the other hand, in the figure 1 c) and d) by the analysis EDS was possible identifying and quantifying elemental of Ca, C, O, F, Cl and P principally. In this analysis is observed that Ca decrease of the 32.7 to 26.6 Wt %. Moreover, In the Figure 2 that corresponding at the specimen 2 show the same behavior the evidence of the Ca decrease of the 33.7 to 31.2 Wt %.

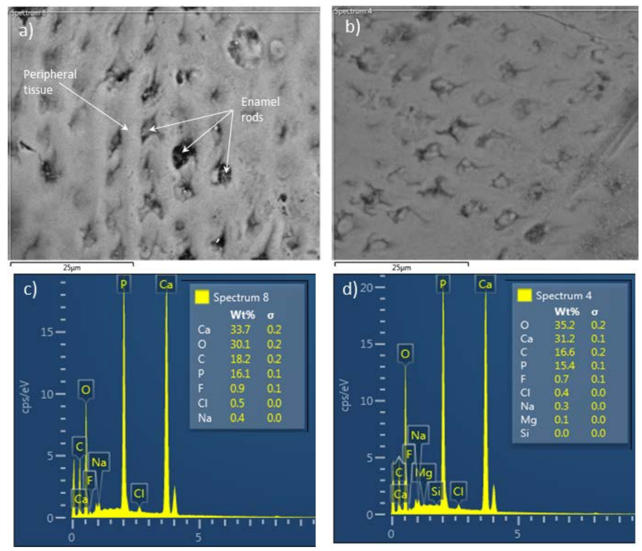

Fig. 2 Specimen 2: (a), (b) Morphology and (c), (d) EDS before and after bleaching treatment respectively.

On the other hand, the figure 3a) and 3b) show the images AFM in contact mode of the enamel (in peripheral tissue region) before and after bleaching treatment respectively. The fig. 3a) denotes small dressings pollutants of tartar material on the surface of hydroxyapatite arrangements. On the zoom images on Peritubular dentin region images it is possible to observe at high magnification, the erosive effect of the bleaching treatment, as well as the removal of contaminants from the enamel surface. Moreover, the Figure 4 shows the results of the XRD analysis of the representative specimen 1 before and after of bleaching treatment a) and b) respectively. In this figure we can clearly observed the decalcification because calcite lost after the bleaching treatment, it correspondent to lost characteristic peak (0 2 0) at ~22 of 2θ and generally intensities. Whit this results we can evidence an erosive damage of the enamel tissue.

ISSN 2348 – 7968

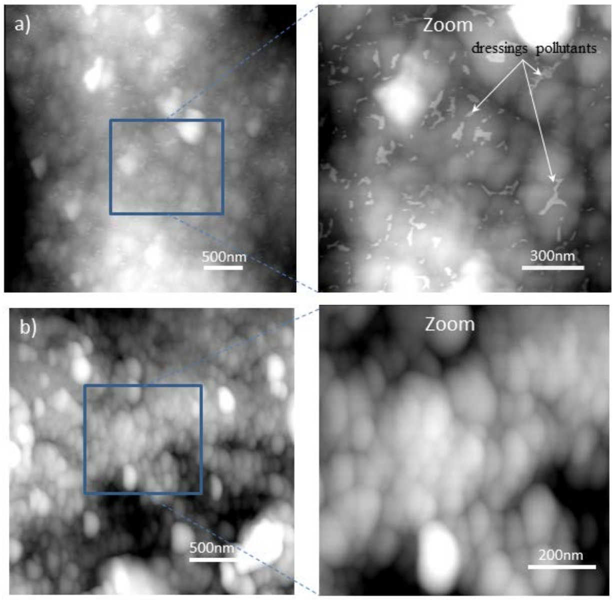

Fig. 3 a) and b) AFM imagines in contact mode of the enamel before and after bleaching treatment respectively, as well as zoom areas in Enamel tissue and interrod crystals.

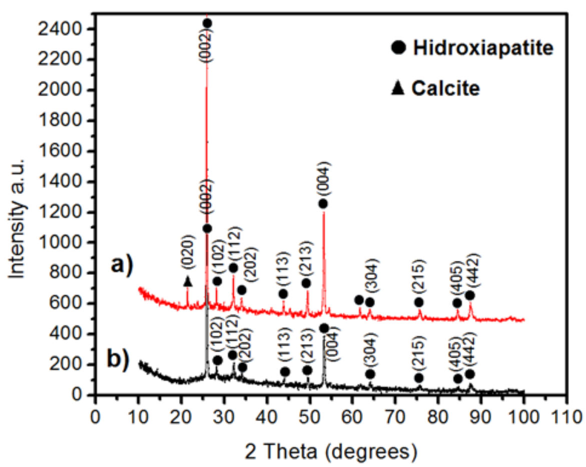

Fig. 4 XRD of representative specimen 1 before and after bleaching treatment a) and b) respectively.

On the other hand, the mechanical properties were evaluated by nanoindentation with tip Bekovich before and after treatment bleaching by the application of 35% hydrogen peroxide. Figure 5 shows the average representative's load versus penetration depth before and after bleaching treatment; a) specimen 1 and b) specimen 2. In the figure 5a) corresponding to the specimen 2 is clearly seen that mechanical behavior is different due to at the average values residual penetration or final depth ($h_f=h_r$), h_r =56 ±4nm and 200 ±5nm before and after treatment bleaching respectively.

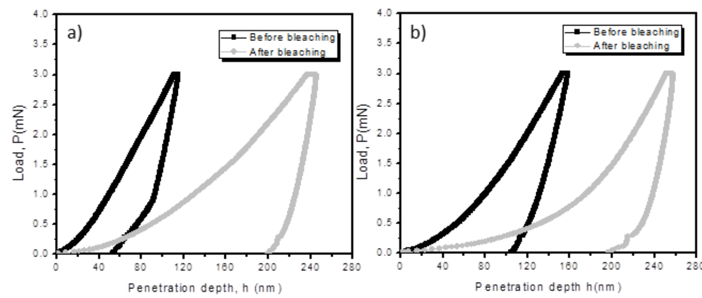

Fig. 5 Characteristic load-penetration depth curves in nanoindentation before and after bleaching treatment a) specimen 1 and b) specimen 2.

On the other hand, surface roughness observed by AFM images of the nanoindent impressions in the prisms of the surface. The figure 6 show optical image a), and b), c) AFM images in contact mode take before and after indentation test. In the figure c) show the representative Berkovich residual indentation area.

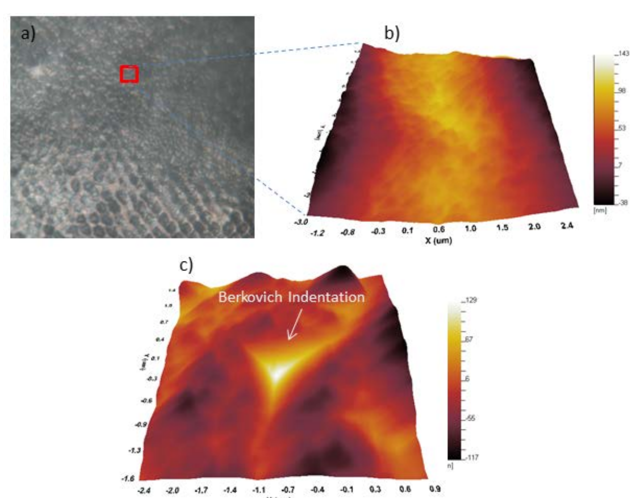

Fig. 6.- a) Optical image, b) and c) AFM images in contact mode taken before and after indentation test with Berkovich tip.

Respected the Figure 5 b) is the same behavior, the values of the residuals penetration depth are 107±2nm   199±3nm. According to the results, nanomechanical behavior of enamel was affected by the application of 35% hydrogen peroxide. For example, in the specimen 1 nanohardness

ISSN 2348 – 7968

and elastic modulus registered variations aimed at the reduction by effects of bleaching in averages values of *H*=9.2 ± 02 to *H*=3.0± 03, *E*=270±3 to *E*=105±2 respectively. There were significant decreases in the mean hardness (<60%) and Young's modulus (<50%). Mechanical properties of the two specimens before and after treatment bleaching such as elastic modulus, hardness and stiffness values showed in table 1.

Table 1.- Mechanical properties such as nanohardness (H), elastic modulus (E) and the stiffness (S) of the enamel before and after bleaching treatment evaluated by nanoindentation.

Specimen	Bleaching 35% HP	Hardness (GPa)	Elastic modules (GPa)	Stiffness (N/m)
1	Not treated	9.4±0.2	270±3	150962±234
	Treated	3.0±0.3	105±2	139622±154
2	Not treated	8.1±0.5	224±4	150281±332
	Treated	3.5±0.4	128±2	120339±247

On the other hand, with the aim to observed the elastic to plastic transition and Hertzian behavior of the enamel before and after bleaching treated was used a share-conical tip. In the figure 7a) show the SEM image of the sphere-conical tip and the zoom images show perfectly the tip radii, which is of *R*=1000 nm. The figure 7b) show the load-displacement curves before and after bleaching representative specimen 1, as well as the only elastic or Hertzian behavior to the without bleaching treatment. In this case we can see that there is not residual displacement or final depth, (h_f=h_r=0) due to the load is not enough to exceed the transition elastic to plastic. It is observed by the pop-ins evens in the load-displacement curves at 290μN and 220 μN of load to before and after bleaching treatment respectively.

Still there is some disagreement in the literature about the actual effect of bleaching agents on the mechanical properties of enamel, one of the driving forces of this work is to contribute for clarify this situation. Taking into account that this study was conducted in dehydrated enamel, our results were higher but consistent respect to others authors such as [11],[19]. Averages close to *H* >6GPa and *E* >115 GPa reported, employing similar nanoindentation techniques, also [2] reported elastic modulus within the range of 74–130 GPa on untreated enamel, while [20], reported E >114 GPa for natural enamel in white demineralized by spot lesions. Mechanical properties, chemistry and microstructure of enamel observed altered, showing a correlation with reductions of CaO wt.%, these results support the fact that the mechanical properties of enamel dependent on its degree of mineralization (CaO/P_2O_5).

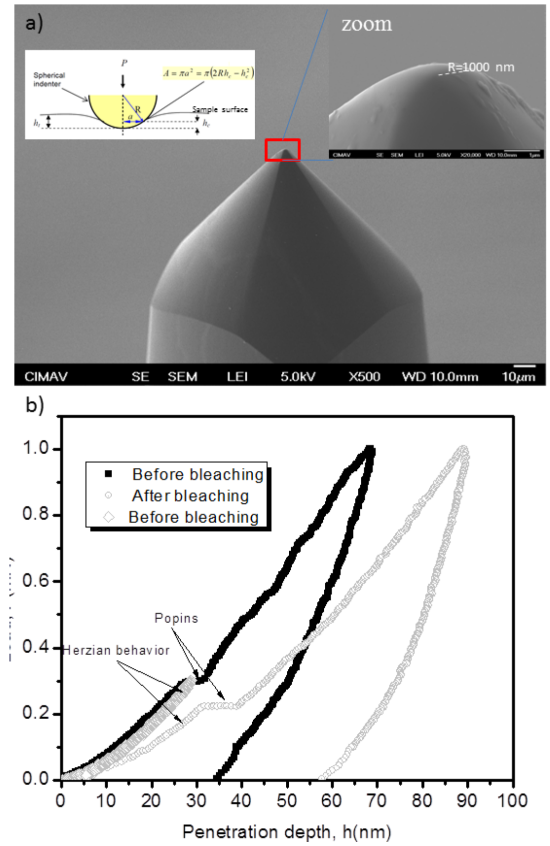

Fig. 7.- a) Optical image, b) and c) AFM images in contact mode taken before and after indentation test with Berkovich tip.

On other works, enamel exhibited a recovery of properties when it was stored in artificial saliva [6], [11], [21]. Unlike on the present work specimens' were stored at dry environment and the enamel did not receive the mineralization benefit of saliva minerals.

4. Conclusions

The hardness values were significantly decreased beyond that 60% as effects of bleaching solution. Variations on elastic modulus values observed in treated specimens' with decrement averages over the 50%. Scan electron microscopy, X-Ray diffraction and Atomic force microscopy analysis evidenced erosive roughness and levels of calcium diminished. This results as whole evidence, exhibited the nanomechanical behavior of great dearest on hardness, elastic modulus and stiffness after bleaching treatment of 35% HP on dehydrated enamel surface.

Acknowledgments

The authors wish to express their appreciation to Roberto Talamantes, Wilber Antunez and Karla Campos for SEM characterization. Assistance in X ray studies by E. Torres and Oscar Solis Canto is highly appreciated.

References

[1] L. H. He, & M.V. Swain, "Understanding the mechanical behavior of human enamel from its structural and compositional characteristics", Journal of the Mechanical Behavior of Biomedical Materials, 1(1) 2008, pp. 18-29.

[2] T. T. Huang, L. H. He, M. A. Darendeliler, M. V. Swain, "Correlation of mineral density and elastic modulus of natural enamel white spot lesions using X-ray microtomography and nanoindentation", Acta Biomater, 6, 2010, pp. 4553–4559.

[3] S. F. Ang, T. Scholz, A. Klocke, & G. A. Schneider, "Determination of the elastic/plastic transition of human enamel by nanoindentation", Dental Materials, 25(11), 2009, pp. 1403-1410.

[4] S. S. Azer., C. Machado, E. Sanchez, & R. Rashid, "Effect of home bleaching systems on enamel nanohardness and elastic modulus", Journal of dentistry, 37(3), 2009, pp. 185-190.

[5] J. Xue, W. Li, & M. V. Swain, "In vitro demineralization of human enamel natural and abraded surfaces: A micromechanical and SEM investigation", Journal of dentistry, 37(4), 2009, pp. 264-272.

[6] B. R. Hairul Nizam, C. T. Lim, H. K. Chng, & A.U. J. Yap, "Nanoindentation study of human premolars subjected to bleaching agent, Journal of biomechanics", 38(11), 2005, pp. 2204-2211.

[7] C. Poggio, M. Lombardini, A. Dagna, M. Chiesa, & S. Bianchi, "Protective effect on enamel demineralization of a CPP–ACP paste: an AFM in vitro study", Journal of dentistry, 37(12), 2009, pp. 949-954.

[8] J. C F. Z. Ge, F. Z. Cui, X. M. Wang, & H. L. Feng, "Property variations in the prism and the organic sheath within enamel by nanoindentation", Biomaterials 26(16), 2005, pp. 3333-3339.

[9] S. González-López, G. Soares de C. L. Medeiros, C. A. Defren, M. V. Bolaños-Carmona, Sanchez-Sanchez P, & Menendez-Nuñez M, "Demineralization effects of hydrogen peroxide on bovine enamel and relation to shear bond strength of brackets", Journal of Adhesive Dentistry, 11(6), 2009, pp. 461.

[10] R. J. Scougall-Vilchis, B. S. Gonzalez-Lopez, R. Contreras Bulnes, L. E. Rodriguez-Vilchis, M. W. Garcia-Niño de Rivera, Toshio Kubodera-Ito, "Influence of four systems for dental bleaching on the bond strength of orthodontic brackets", Angle Orthodontist, 81(4), 2011, pp. 700-705.

[11] M. A. Garrido, I. Giraldez, L. Ceballos, M. T. Río, & J. Rodriguez, "Nanotribological behavior of tooth enamel rod affected by bleaching treatment", Wear, 271(9), 2011, pp. 2334-2339.

[12] Y. R Jeng, T. T. Lin, & D. B. Shieh, "Nanotribological characterization of tooth enamel rod affected by surface treatment", Journal of biomechanics, 42(14) 2009, pp. 2249-2254.

[13] E. A. De Paula, S. Kossatz, D. Fernandes, A. D. Loguercio, & A. Reis, "Administration of ascorbic acid to prevent bleaching-induced tooth sensitivity: A randomized triple-blind clinical trial", Operative dentistry, 39(2), 2014, pp. 128-135.

[14] S. O. Parreiras, P. Vianna, S. Kossatz, A. D. Loguercio, & A. Reis, "Effects of light activated in-office bleaching on permeability, microhardness, and mineral content of enamel", Operative dentistry, 39(5), 2014, pp. E225-E230.

[15] W. C. Oliver, G. M. Pharr, "An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments", J Mater Res, 7, 1992, pp.1564–83.

[16] I. N. Sneddon, "The relation between load and penetration in the axisymmetric Boussinesq problem for a punch of arbitrary profile", International Journal of Engineering Science, 3 (1), 1965, pp. 47-57.

[17] M. F. Doerner, D. S. Gardner, & W. D. Nix, "Plastic properties of thin films on substrates as measured by submicron indentation hardness and substrate curvature techniques", Journal of Materials Research, 1(06), 1986, pp. 845-851.

[18] H. Hertz, Miscellaneous papers, London, Macmillan, New York, Macmillan and co., 1896.

[19] A. Joiner, "Review of the effects of peroxide on enamel and dentine properties", Journal of dentistry, 35(12), 2007, pp. 889-896.

[20] J. L. Cuy, A. B. Mann, K. J. Livi, M. F. Teaford, & T. P. Weihs, "Nanoindentation mapping of the mechanical properties of human molar tooth enamel", Archives of Oral Biology, 47(4), 2002, pp. 281-291.

[21] T. Attin, C. Hannig, A. Wiegand, & R. Attin, "Effect of bleaching on restorative materials and restorations a systematic review", Dental Materials 20(9), 2004, p852-861.

Abel Hurtado-Macías Degree in Materials Science by Morelia Institute of Technology in 2000. In 2004 he obtained a Master in Materials Science at the Center for Research and Advanced Studies (CINVESTAV), Querétaro, México. He obtained Doctorate of Science in Research and Advanced Studies of the IPN, Querétaro, México, 2008. In 2006, conducted a research stay at the Technical University of Hamburg Germany. Dr. Hurtado is currently Researcher Titular "B" at the Center for Research in Advanced Materials (CIMAV), Chihuahua. Lines of Research Dr. Abel Hurtado always been focused on nanostructured materials developed ferroelectric and ferromagnetic, evaluation of mechanical properties at micro and nano scale by nanoindentation in materials science in bulk and thin films. Dr. Hurtado is author and co-author of 27 scientific articles in international journals and four extended articles. He has directed 6 Master's Thesis, and 3 Thesis PhD focused on the mechanical properties and synthesis of ferroelectric materials.

Alfredo Nevarez-Rascon have a degree of Surgeon Dentist by the National University of Mexico UNAM in 1991, Doctorate in Stomatology by the Granada University Spain in 2007 and one postdoctoral stay of two years at the Center for Research in Advanced Materials (CIMAV), Chihuahua. Currently Dr. Nevarez is professor Titular "C" and Head of the department of scientific research at the Stomatology School of the Autonomous University of Chihuahua (UACH), Chihuahua Mexico. With main lines of Research related to development of materials for biomedical uses. Dr. Nevarez is co-author of 6 international scientific articles. He was directed 2 Master's Thesis, and proposed three patents.