ETRAN

Zbornik radova

62. Konferencija ETRAN 2018

&

5th International Conference IcETRAN 2018

Palić, 11-14.06.2018.

Društvo za ETRAN, Beograd & Akademska misao, Beograd

ETRAN Society, Belgrade & Academic Mind, Belgrade

Zbornik radova - 62. Konferencija za elektroniku, telekomunikacije, računarstvo, automatiku i nuklearnu tehniku, ETRAN 2018, Palić, 11 – 14. juna, 2018. godine

Proceedings of Papers – 5th International Conference on Electrical, Electronic
and Computing Engineering, IcETRAN 2018, Palić, Serbia, June 11 – 14, 2018

Glavni urednik / Main Editor **Dejan Popović**

Urednici / Editors **Vladimir Katić, Nikola Jorgovanović**

Izdavači / Published by **Društvo za ETRAN, Beograd i Akademska misao, Beograd / ETRAN Society, Belgrade, Academic Mind, Belgrade**

Izrada / Production **Akademska misao, Beograd / Academic Mind, Belgrade**

Mesto i godina izdanja/ Place and year of publication **Beograd, 2018./ Belgrade, 2018**

Tiraž/ Circulation **300 primeraka / 300 copies**

ISBN 978-86-7466-752-1

www.etran.rs

CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

621.3(082)(0.034.2)
534(082)
004(082)(0.034.2)
681.5(082)(0.034.2)
621.039(082)(0.034.2)
66.017(082)(0.034.2)
57+61(048)(0.034.2)
006.91(082)(0.034.2)

ДРУШТВО за електронику, телекомуникације, рачунарство, аутоматику и нуклеарну технику. Конференција (62 ; 2018 ; Палић) ETRAN [Elektronski izvor] : zbornik radova / 62. Konferencija ETRAN2018, Kladovo, 05-08. juna, 2017. godine & 5th International Conference IcETRAN 2018 Palić, 11-14.07.2018. ; urednici, editors Vladimir Katić, Nikola Jorgovanović]. - Beograd : Društvo za ETRAN : Akademska misao = Belgrade : ETRAN Society : Academic Mind, 2018 (Beograd : Akademska misao). - 1 elektronski optički disk (CD-ROM) ; 12 cm Sistemski zahtevi: Nisu navedeni. - Nasl. sa nasl. ekrana. - Tekst ćir. i lat. - Radovi na srp. i engl. jeziku. - Tiraž 300. - Bibliografija uz svaki rad. - Abstracts.

ISBN 978-86-7466-752-1 (AM)

1. International Conference on Electrical, Electronic and Computing
Engineering (5 ; 2018 ; Palić)

а) Електротехника - Зборници b) Акустика - Зборници c) Рачунарска технологија - Зборници d) Системи аутоматског управљања - Зборници e) Нуклеарна техника - Зборници f) Технички материјали - Зборници g) Биомедицина - Зборници h) Метрологија - Зборници

COBISS.SR-ID 268605452

Proceedings of Papers – 5th International Conference on Electrical, Electronic
and Computing Engineering, IcETRAN 2018, Palić, Serbia, June 11 – 14, 2018 ISBN 978-86-7466-752-1

Influence of specimen preparation and nanoindentation protocol on the mechanical properties of bovine bone

Staša Vučinić, Bojan Petrović, Sanja Kojić, *Member, IEEE*, Milica Šipovac, Sofija Stefanović, Goran Stojanović, *Senior Member, IEEE*

Abstract **The physical properties of bone tissue have been investigated at different levels, macro, micro and nano scale. The aim of this study was to assess the influence of two specimen preparation techniques and six nanoindentation protocols on modulus and hardness of bovine bone specimens. Embedding in resin do not affect the mechanical properties of bone samples. More precise and repeatable results are obtained using higher indentation forces. Larger number of measurements are required for bone indentation analysis using lower forces.**

Index Terms— **nanoindentation; bone; artefacts; specimen preparation.**

I. INTRODUCTION

During the last three decades the structure, composition and the physical properties of bone tissue have been investigated at different levels, macro, micro and nano scale [1]. Structurally, bone is composed by 3 major components: an organic matrix of collagen, a mineral substance composed by crystals of carbonate apatite and water. Biological and physical properties are substantially influenced by the interactions, characteristics and distribution of these three main components. Regarding the macro characterization of the bone, the mechanical properties most frequently reported are the elastic and shear modulus as well as Poisson's ratio. At the micro scale, the evaluations of the microstructural mechanical heterogeneity and their relations with their physico-chemical properties are usually performed and at nano scale bone anisotropic behaviour was assessed experimentally and simulated numerically [2].

Bovine bone has a laminar structure with alternating layering of parallel-fibered bone and lamellae. Two-dimensional network of blood vessels and vascular spaces lies between lamellae. Primary osteons of bovine bone are without any cement lines. Haversian bone is located near the endosteal surface, and osteonal banding at the interface between both. Haversian canals are medium in size and irregular in shape [3].

In last three decades the nanoindentation was used to test biological specimens such as bone and teeth [4]. Nanoindentation allows the quantification of the mechanical properties at the micro scale. Various experimental protocols and sample preparation techniques have been used in order to characterize the mechanical properties of bone microstructure. The most frequently used method to analyse the nanoindentation data is the Oliver and Pharr mechanical method [5,6]. It is adapted to isotropic elastoplastic materials and allows computation of a modulus of elasticity and the hardness of bone material with the assumption that it is a quasi-isotropic non-viscous material [7]. Such data may assist in predicting the pattern of behaviour of the tooth bone interface.

Despite the wide spread use of the nanoindentation for the surface characterization of the bone and other biological tissues, significant variations in the obtained data have been constantly reported regarding the same structures, and it is still not completely clear how the nanoindentation results may be influenced by the way the specimens are prepared and the nanoindentation protocol is implemented. The aim of this study was to assess the effect two specimen preparation techniques and six nanoindentation protocols, on the measurement of modulus and hardness of bovine bone specimens.

II. THE EXPERIMENTAL METHOD

A. *Sample preparation.*

1) *Bone samples*

Tests were conducted on fresh bovine metatarsal bone of a calf approximately 6 months old from a local slaughterhouse. Cortical bone from between the metatarsal metaphysis and diaphysis were cut down to 3 mm thick specimens with a water-cooled diamond-impregnated low-speed saw (Isomet Low-Speed Saw, Buehler; Lake Bluff, IL, USA). After removal of the marrow with a water jet, the sections were not dehydrated (but kept in dry environment) and half of the specimens were embedded in epoxy resin at room temperature (protocol 2). The other half of the specimens were analysed without embedding in resin (protocol 1). All 6 samples (3 per protocol) were polished before indenting using motorised silicon carbide discs of various grit sizes (200, 600 and 1200). Diamond paste of

---

Staša Vučinić is with the Faculty of Technical Science, University of Novi Sad, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia (e-mail: stasavucinic@uns.ac.rs)

Bojan Petrović is with the Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia (e-mail: bojan.petrovic@mf.uns.ac.rs)

Sanja Kojić is with the Faculty of Technical Science, University of Novi Sad, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia (e-mail: sanjakojic@uns.ac.rs)

Milica Šipovac is with the Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia (e-mail: mnsipovac@gmail.com)

Sofija Stefanović is with BioSense Institute, University of Novi Sad, Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia (e-mail: sofija.stefanovic@biosense.rs)

Goran Stojanović is with the Faculty of Technical Science, University of Novi Sad, Trg Dositeja Obradovića 6, 21000 Novi Sad, Serbia (e-mail: sgoran@uns.ac.rs)

decreasing grits (1.0 and 0.5 micron) was used for fine polishing. The final specimens had dimensions of 2 mm height × 4 mm in diameter.

2) Testing protocols

Indentations were performed distributed in the matrix 5 x 5 points with the distance of 100 µm x 100 µm in the middle region of the sample surface. These tests were performed using and Agilent Nano indenter G200, which provides adequate forces range and environment protection (heat, vibration) (Fig. 1). The prepared bone sample was mounted in the sample holder using adhesive tape (Fig. 2).

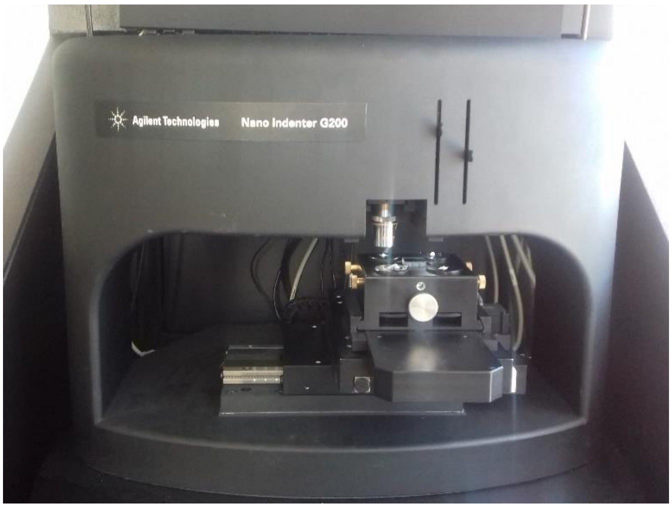

Fig. 1 Agilent Nano indenter G200 chamber

Multiple indentation (25 indentations per sample were made) tests provide measurement repeatability for the mechanical properties of analysed samples. The system has resolution of load and displacement of less than 50 nN and 0.1 nm, respectively. Nanoindentation tests were conducted with a Berkovich diamond indenter tip. The tip calibration is conducted on fused silica referent sample.

Indentation method with predefined force (G-Series, Basic Hardness, Modulus, Tip Cal, Load Control method) is used for all nanoindentation measurements. A typical load-displacement curve is depicted in Fig. 3.

Fig. 2 Sample holder with four specimens obtained after cutting and polishing (fifth sample in the center of the holder is referent fused silica sample)

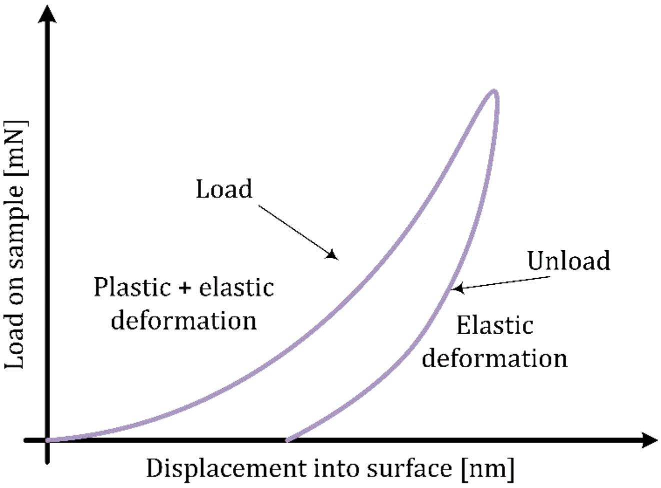

Fig. 3 Typical nanoindentation load-displacement curve

All tests are performed at 6 different max loads of 1, 5, 25, 100, 250 and 500 mN with one indentation per place. Loading segment was set on 15 s. Peak hold time segment was reduced to 1 s since there was no creep effect measurements. After that unloading segment could be observed.

3) Statistical methods

Data were analysed using standard parametric tests. Intergroup analysis was performed using t-test with the level of significance set at $p < 0.05$.

III. RESULTS AND DISCUSSION

Young's modulus for all 6 forces and both specimen preparation protocols exhibited significant variations without existence of constant decrease or increase related to the applied forces. The mean values and standard deviations of Young's modulus varied between 56.29 ± 1.18 GPa and 78.97 ± 4.24 GPa and all these values are shown in Fig. 4. Regarding the hardness, the trend of increasing values from 6.63 ± 1.86 GPa to 12.79 ± 1.29 GPa could be observed with increasing forces employed during the nanoindentation. According to results from Fig. 4, with higher forces used for both the Young's modulus and the hardness, the tendency of decrease in standard deviations was observed in samples with resin support however such tendency was not clearly found for the samples without resin.

In the study investigating human osteonal, interstitial and trabecular microstructures the reported values of elastic moduli ranged from 6.9 ± 4.3 GPa in trabecular tissue up to 25.0 ± 4.3 GPa in interstitial tissue. It has been observed that the mean elastic modulus was found to be significantly influenced by the type of lamellar structure. Similarly, according to the results presented in our investigation hardness followed a similar distribution as elastic modulus among types of lamellae, but with less significant statistical difference [4].

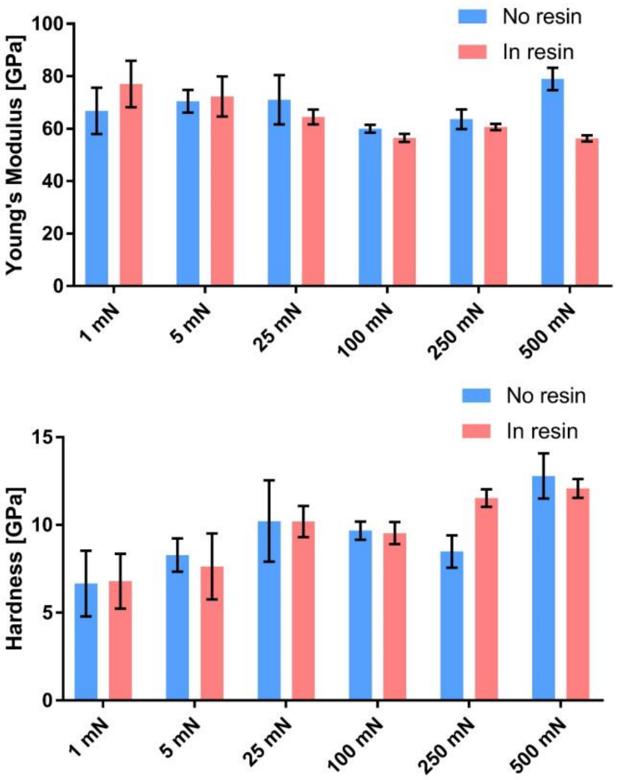

Fig. 4 Young's modulus and hardness for two different specimen preparation protocols

It can be observed that the obtained hardness data completely correspond to the majority of the previous studies, and that data regarding Young's modulus are substantially higher. It is well-known both for compact and trabecular bone, that hardness and elastic modulus are dependent on the water content, resulting in up to 40% difference in measured indentation modulus. It has been reported for wet conditions that the indentation modulus decreases [8]. In the present investigation the samples were dried and these higher results of modulus are partially attributed to desiccation and dehydration of the specimens.

The reported values for elastic modulus and hardness for both human and animal bone vary in significant wide range due to composite nature of bone microstructural organization, part of the bone, bone development and age of the donor. It has been reported that the highest values for modulus could be observed in the interstitial lamellae (30.4 ± 2.4 GPa) [4]. It has been speculated that younger bone had a lower mineral content, resulting in a lower value for elastic modulus. In contrast to that compact lamellae of the developing long bones are more elastic compared to other types or other parts of the bone. In the present investigation the long bone of rather young donor has been used and the present observation that interstitial lamellae have a higher modulus than osteons and trabecular bone is in complete agreement with the previously published report [9].

Numerous parameters should be taken into account when the results obtained from the nanoindentation experiments in anisotropic materials are compared. Following concerns could be derived from all experiment related factors- bone specimens, specimen's preparation and indentation methods and employed parameters. It has been observed and reported that even the direction of bone sections, longitudinal or transverse affect the elastic modulus of the same bone. Both elastic modulus and hardness of bone tissue is strongly related on tissue type, anatomical location, age and development.

Indentation mark presented in Fig. 5 shows middling sinking-in effect, suggesting that the differences in observed modulus variations could be attributed to this experimental feature. Roughness that can be seen in SEM micrograph can affect values of modulus and hardness as well.

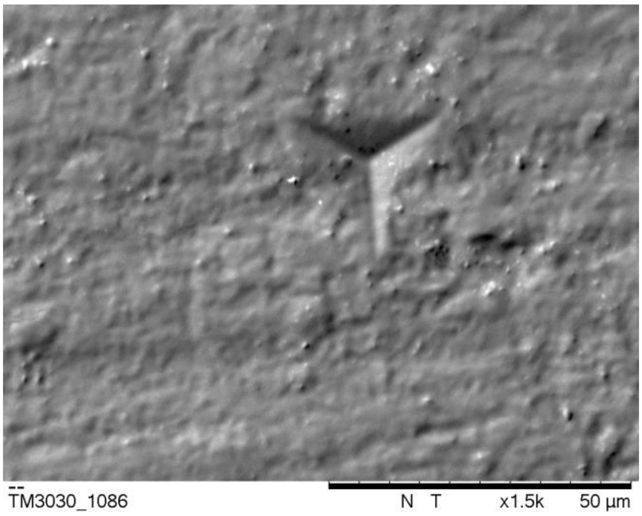

Fig. 5 SEM image of the indentation trace performed in the bovine metatarsal bone sample

Average load vs. displacement curves for two different specimen preparation techniques and six different forces are shown in Fig. 6. These curves representing load vs. displacement obtained during nanoindentation tests shows even shape and non-appearance of pop-in effect. Displacement at maximal load was similar for both protocols and was approximately 95, 210, 470, 950, 1500, 2100 nm for applied forces of 1, 5, 25, 100, 250 and 500 mN, respectively.

There are no statistical significant differences between the curves for all analysed forces employed during the experiment $p > 0.05$, t-test.

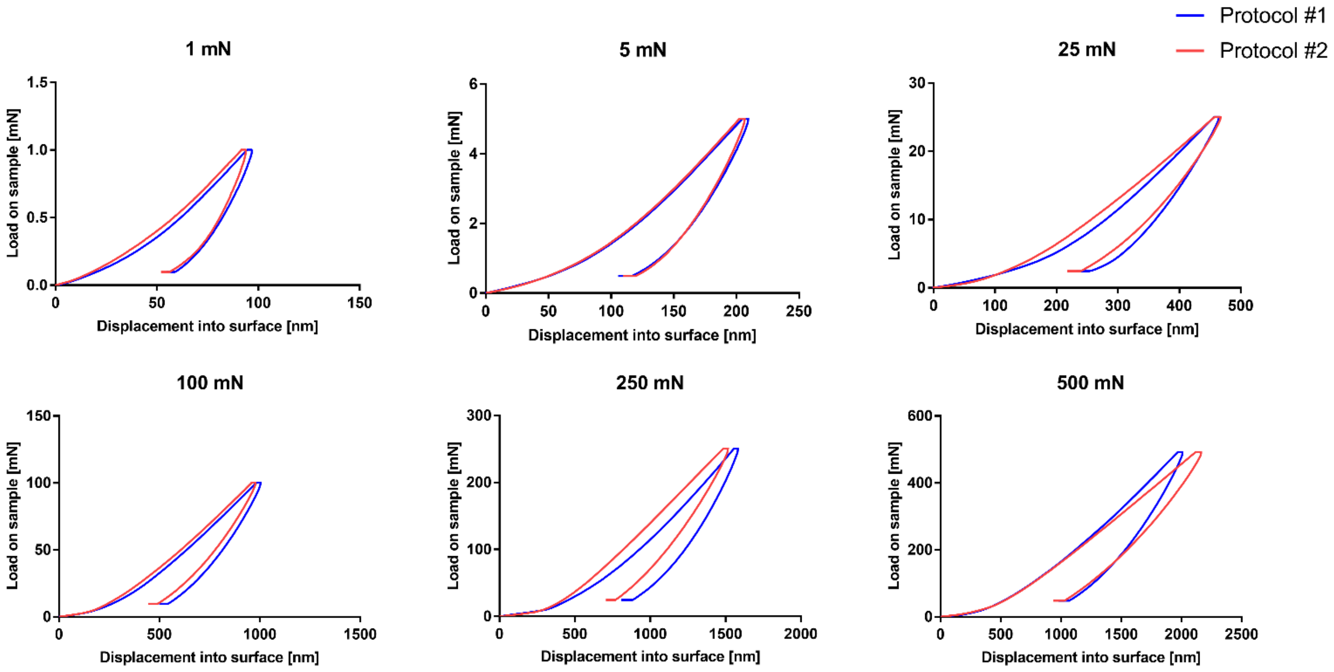

Fig. 6 Load vs. Displacement curves for two different nanoindentation protocols

IV. Conclusion

Within the limitations of the present investigation it could be concluded that regarding bone sample preparation, mounting in resin do not affect significantly the mechanical properties using forces smaller than 25 mN. On forces higher than 100 mN the effect on Young's modulus and hardness insignificantly and unevenly differ which cannot be attributed to specimen preparation protocol. More precise and repeatable results are obtained using higher indentation forces. Considering rather high values of standard deviations larger number of measurements are required for bone indentation analysis using lower forces.

V. Acknowledgment

This paper was financed through H2020 ERC project BIRTH No. 640557 and Serbian national project III45021.

References

[1] E. Zimmermann, R. Ritchie, “Bone as a Structural Material”, Adv Healthc Material, vol. 4, no. 9, pp. 1287-1304, 2015.

[2] S. Jaramillo-Isazaa, P.-E. Mazeranb, K. El-Kirata, M.-C. Ho Ba Tho, “Effects of bone density in the time-dependent mechanical properties of human cortical bone by nanoindentation”, Computer Methods in Biomechanics and Biomedical Engineering, vol 17 Suppl 1, pp. 34-35, 2014.

[3] A. Kataruka, K. Mendu, O. Okeoghene, J. Puthuvelil, AT. Akono, “Microscopic assessment of bone toughness using scratch tests”, Bone Rep., vol.6, pp. 17-25, 2016.

[4] X.J. Wang, X.B. Chen, P.D. Hodgson, C.E. Wen, “Elastic modulus and hardness of cortical and trabecular bovine bone measured by nanoindentation”, Trans. Nonferrous Met. Soc. China, vol. 16, pp. 744–748, 2006.

[5] 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., vol.7, pp. 1564–1583, 1992.

[6] J. Menčík, “Uncertainties and Errors in Nanoindentation, Nanoindentation in Materials Science", Dr. Jiri Nemecek (Ed.), InTech, 2012, DOI: 10.5772/50002.

[7] D.A. Lucca, K. Herrmann, M.J. Klopfstein, “Nanoindentation: Measuring methods and applications”, CIRP Ann. - Manuf. Technol., vol 59, pp. 803–819, 2010.

[8] O. Jiroušek, “Nanoindentation of Human Trabecular Bone – Tissue Mechanical Properties Compared to Standard Engineering Test Methods in Nanoindentation in Materials Science”, Dr. J. Nemecek (Ed.), InTech, DOI: 10.5772/50152

[9] P.K. Zysset, X.E. Guo, C.E. Hoffer, K.E. Moore, S.A. Goldstein, “Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur, Journal of Biomechanics, vol. 32, no. 10, pp. 1005–1012, 1999.

EEI

Power Engineering