Microhardness testing: can we measure the repair of demineralized lesion?

Osim što je novitet ovog broja Sonde suradnja sa studentima engleskog studija, također se i kolegica hrvatskog studija, draga nam kolegica Eva, na poticaj mentora, uvažila napisati članak na engleskom jeziku. Ovim putem potičemo daljnje pisanje stručnih članaka, kako na hrvatskom, tako i na engleskom jeziku.

Eva Filija[1]
Ivan Šalinović, dr. med. dent.[2]

[1] studentica 4. godine Stomatološkog fakulteta Sveučilišta u Zagrebu
[2] Zavod za endodonciju i restaurativnu stomatologiju, Sveučilište u Zagrebu, Stomatološki fakultet

Demineralization-remineralization balance is very fragile; consuming sugary drinks and food daily, regular snacking and inadequate oral hygiene can significantly increase the softening of the hardest tissue in human body- enamel (1). Once this outer structure is damaged, bacteria can progress deeper into the structure, thus affecting dentine and cement (2). Dental industry has provided numerous products that can prevent, slow down or even reverse the degradation of the softened tooth surfaces. However, their actual effect is hard to assess due to complex composition of hard dental tissues. Nowadays, microhardness evaluation is widely utilized to evaluate the properties of dental tissues before and after usage of the protective dental products.

Tooth mineral composition and structure

The inorganic portion of tooth hard structures composes of calcium hydroxyapatite, Ca10(PO4) 6 (OH)2, which belongs to a group of complex biological apatite. The basic cell units of biological apatite are hexagonal in shape, forming crystals of different sizes. Those units are present in enamel, dentin and cementum; however, the crystals are the biggest and most oriented in enamel (3). Tooth enamel is the most mineralized tissue in the human body with 96% of inorganic component. Dentin has 70%, and cementum 45% of the inorganic matrix, making them much challenging to repair, compared to enamel (4). High inorganic content and highly oriented crystals are the main reasons for and resilient properties of enamel (3). The composition of biological apatite is not consistent, and it depends on many variables, including the type of tissue, health, age, nutritional habits of the individual and environmental factors (5).

Demineralization and remineralization

The mineral structure of the enamel surface is partially a product of the dynamic processes of demineralization and remineralization (4). Usually, the rates of these processes on the enamel surface are balanced to keep the tooth healthy and resistant to the oral cavity environment. However, when the teeth are constantly exposed to an acidic habitat, an imbalance between the demineralization and remineralization processes shifts towards demineralization. Chemical process of detaching mineral ions from the hydroxyapatite crystals of enamel, dentin and cementum is called demineralization (2). The dental biofilm impacts this process by modulating the tooth surface pH. High sugar consumption favors the lowering of the biofilm pH by providing the substrate for the cariogenic microorganisms in the biofilm. Once pH becomes lower than 5.5, the enamel demineralization begins (6). Acidic environment causes calcium and phosphate release from the enamel, dentin and cementum. The loss of minerals leads to increased porosity, widening of the spaces between the enamel hydroxyapatite (HA) crystals and softening of the surface (7).

The demineralization process can be reversed by restoring the minerals to the HA crystals; the process is called remineralization. It occurs when calcium and phosphate are dissolved in saliva and plaque (2). A very important reactive element is the fluoride and its presence in the biofilm stimulates remineralization of HA crystals and enhances new minerals deposition. The presence of fluoride and calcium ions leads to formation of fluorapatite, FAP [Ca10(PO4) 6 F2], in which the hydroxyl molecules are replaced with fluoride in the HA crystals (6). FAP elicits stability of crystals and increases hardness, therefore defending the enamel from the aggressive acidic environment. (8)

Ion-releasing materials

A group of materials that has been increasingly examined and used in recent years are ion-releasing materials with certain degree of bioactivity. They are widely used for reconstruction and regeneration of the tissues in the orofacial region. In restorative dentistry, the term 'bioactive' can refer to the ability of a material to cause HA or FAP formation on the tooth's surface. (9) On the other hand, in implantology or oral sugary, bioactive material has the purpose to provide a direct chemical bond between the implant and the recipient bone (10).

A variety of restorative materials can induce tooth remineralization and/or inhibit demineralization. In enamel, instead of conventional treatments, early non-cavitated lesions can be remineralized by using topical remedies which contain high amounts of fluorides, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), or nano- hydroxyapatite (11). In dentine, the most commonly used restorative ion-releasing materials include glass ionomer cements, glass hybrid cements and calcium-silicate based cements (12).

Hardness

As a strong indicator of the material's ability to resist deformation, hardness, has been studied for more than a century. This ability is evaluated by a standard test where the surface resistance to indentation is measured (13). Hardness is related to other mechanical properties such as tensile strength and abrasion (14).

Many hardness testing methods developed over time and they are based on the loads that are applied to a material (macro, micro, or nano-indentation tests). Macroidentation tests are those that use larger test loads (1kgf or more) (Kgf=kilogram-force) while micro indentation tests use load from 1 to 1000 gf.(gf=gram-force). Nanoindentation tests include the nanometric level of indentation.

The most common methods that are used are Rockwell, Vickers, Knoop, and Brinell tests. Although the methods differ in details such as the shape and the size of the ident, their primary goal is to apply a specific load under controlled conditions on the desired specimen (Hardness Testing: Principles and Applications).

In dentistry, microhardness tests are widely utilized to evaluate tooth hardness and the hardness of the materials that are crucial for dental rehabilitation (15). The most commonly used microhardness methods are Vickers and Knoop tests.

Vickers microhardness test

The Vickers microhardness test uses a square-based diamond pyramid as the indenter with the angle between opposite faces of the pyramid being 136° (16). The diamond indenter is pressed into the surface of the specimen for a certain period of time. Different loads can be applied, ranging from 10 gf to 1kgf (15). Once the load is removed from the sample, the indentation area is observed and then measured by the optical microscope. The length of the diagonals of the impression is measured and the Vickers hardness number (VHN) is calculated from the following equation (17):

- VHN=0.0018544 x L/d2
- L-load in gram force(gf)
- d-mean of the two diagonals (in mm^2)

It is important to say that all the measurements are done automatically on the computer unit of the measuring advice. Figure 1 shows the Vickers microhardness tester which is in possession of the Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb.

As one of the greatest advantages of this method stands for the ability to measure the entire range of hardness, from soft to hard materials (metals, ceramics, composites) and tissues (enamel, dentin, cementum). Furthermore, it is a non-destructive method, which means that the test piece is only slightly damaged during the process.

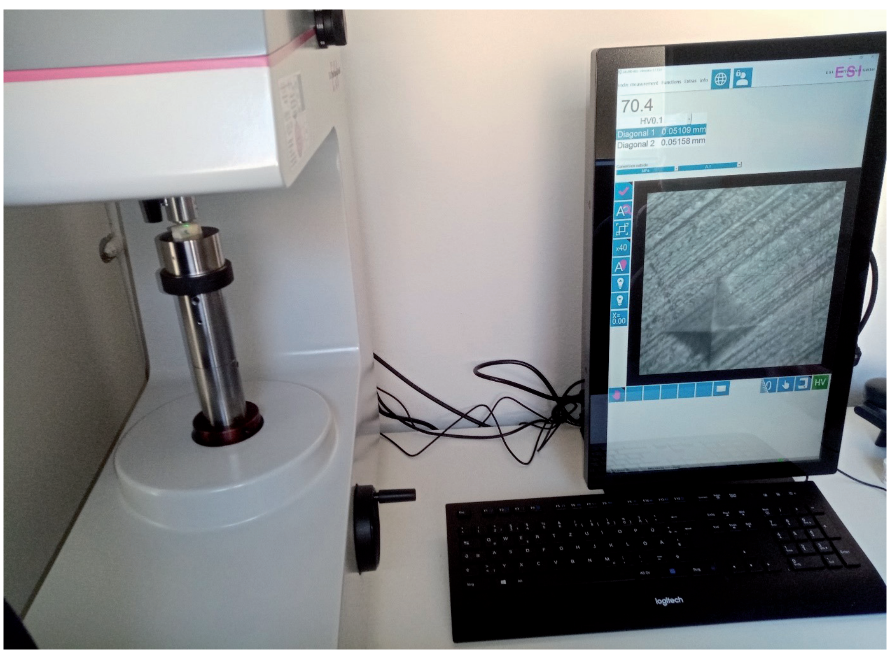

Figure 1. *Vickers Microhardness Tester (Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb)*

On the other hand, the disadvantage of the Vickers test is the amount of time which is needed for the sample preparation which is the crucial part of the procedure.

When testing the hard dental tissue and materials applied to it, researchers follow the specific protocol of the preparation. The extracted teeth are washed and disinfected using a hydrogen peroxide solution and stored in distilled water until they are tested. The extracted teeth are sectioned either transversely or longitudinally, then sectioned buccolingually and mesiodistally. The specimens' flat surface is crucial because any tilt or non-flat surface leads to greater and uneven diagonal length, thus, inaccurate results are obtained (18).

Knoop microhardness test

The principle of microhardness testing in the Knoop test is the same as in the Vickers method, but the difference is in the shape of the indenter. The Knoop Testing method uses a pyramid-shaped diamond indenter with a rhomboid base. The indenter doesn't penetrate very deep into the specimens' surface which means this method is very convenient for very thin and brittle (for example dental ceramics) materials. Figure 2 shows the difference in the shape of the indenters and the ident while using the same load.

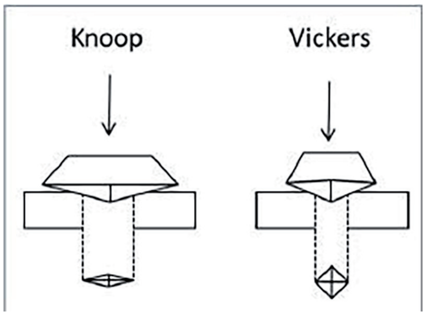

Figure 2. *Vickers and Knopp Hardness Intenter Shapes (https://niom.no/hardness-testing-of-dental-materials-and-tooth-substance/)*

Moreover, the angle between opposite faces of the pyramid is 172° in comparison to Vickers. (19)

The preparation of the specimen surface is of great importance which implies that standardized procedures need to be followed.

Another difference from the Vickers test is in the calculation which is used to determine the KHN (Knoop hardness number). The rhomboid base of the indenter leaves the rhomboid-shaped ident on the surface of the specimen. In the calculation, only the longer diagonal is used in the calculation.

Rockwell hardness

Rockwell hardness method is originally a macrohardness test but with a lower load applied, it is used in the dental material research area as well. (20)

The specificities of this method are numerous. Starting with the shape of the indenter which is either a diamond cone or a hard metal ball. This method is called the differential-depth hardness measurement method and it involves two steps.

In the first step, the indenter is pressed onto the surface of the specimen by using a minor load. The minor load is the reference point. In the second step, the load is increased. This means that the overall force is now acting on the indenter and it is held for a certain period. In the third step, the major force is removed, while the minor is still acting.

Compared with Vickers and Knoop methods which require an optical microscope to measure the diagonals, the Rockwell test only measures the depth of the penetration. The major advantages of the Rockwell method are direct hardness value measured without optical examination and no specimen preparation.

Dentistry and microhardness

Many research projects are using micro indentation tests, Knoop and Vickers equally. These tests are not only important for testing the hardness of the tooth structures-enamel and dentin, but also for a testing wide variety of materials crucial for dental rehabilitation.

However, this method has its limitations, as it merely reflects the biomechanical properties of dentin. In other words, it is possible to gain an increase in microhardness values just by releasing minerals into demineralized lesions without actually repairing them. The reason for this is the fact that dentine remineralization requires harmonious reparation of both collagen and inorganic apatite, which usually requires pulpal activity as well (21). This problem is avoided in enamel, as it is a more highly crystallized structure.

Conclusion

Microhardness testing is a valuable method for obtaining data on the efficiency of ion-releasing materials. However, the results of such examinations need to be observed within their limitations, as the method cannot fully reflect the actual hard dental tissue properties. Still, it is an easily performed method for observing the actual potential of the material and can serve as a basis for further research.

LITERATURA

1. Microhardness of tooth enamel in dentistry [Approached on October 14th 2022]. Available from: https://www.helmut-fischer.com/microhardness-of-tooth-enamel-in-dentistry.
2. Molaasadolah F, Eskandarion S, Ehsani A, Sanginan M. In Vitro Evaluation of Enamel Microhardness after Application of Two Types of Fluoride Varnish. J Clin Diagn Res. 2017 Aug;11(8):ZC64-ZC66.
3. Abou Neel EA, Aljabo A, Strange A, Ibrahim S, Coathup M, Young AM, Bozec L, Mudera V. Demineralization-remineralization dynamics in teeth and bone. Int J Nanomedicine. 2016 Sep;11:4743-4763.
4. Gutiérrez-Salazar M del P, Reyes-Gasga J. Microhardness and chemical composition of human tooth. Materials Research. 2003 June;6(3):367-373.
5. Pajor K, Pajchel L, Kolmas J. Hydroxyapatite and Fluorapatite in Conservative Dentistry and Oral Implantology—A Review. Materials (Basel)2019 Sep;12(17): 2683.
6. The Dynamic Process of Demineralization and Remineralization [Approached on October 14th 2022]. Available from: https://dimensionsoforalhygiene.com/amp/article/The-Dynamic-Process-Of-Demineralizaion-and-Remineralization/
7. Tsai M-T, Wang Y-L, Yeh T-W, Lee H-C, Chen W-J, Ke J-L, Lee Y-J. Early detection of enamel demineralization by optical coherence tomography. Sci Rep. 2019; 9: 17154.
8. Pitts NB, Zero DT, Marsh PD, Ekstrand K, Weintraub JA, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G, Ismail A. Dental caries. Nat Rev Dis Primers. 2017 May 25;3:17030.
9. Spagnuolo G. Bioactive Dental Materials: The Current Status. Materials (Basel). 2022 Mar; 15(6):2016.
10. Sonarkar S, Purba R. Bioactive materials in conservative dentistry. Int J Contemp Dent Med Rev. 2015;2015:1-4.
11. Salinovic I, Schauperl Z, Marcius M, Miletic I. The Effects of Three Remineralizing Agents on the Microhardness and Chemical Composition of Demineralized Enamel. Materials (Basel). 2021 Oct 13;14(20):6051.
12. Chen L, Shen H, Suh BI. Bioactive dental restorative material,review. American Journal of Dentistry. 2013 Aug 01; 26(4):219-227.
13. Chuenarrom C, Benjakul P, Daosodsai P. Effect of indentation load and time on knoop and vickers microhardness tests for enamel and dentin. Materials Research. 2009;12(4):473–76.
14. Mehulić, K i suradnici. Dentalni Materijali : Uvod u materijale. Prvo izdanje. Zagreb :Medicinska naklada, 2017.
15. Şakar-Deliormanli A, Güden M. Microhardness and fracture toughness of dental materials by indentation method. J Biomed Mater Res B Appl Biomater. 2006 Feb;76(2):257-64.
16. Vickers Hardness Testing. [Approached on October 14th 2022]. Available from: https://www.hardnesstesters.com/test-types/vickers-hardness-testing
17. Identation hardness. [Approached on October 14th 2022]. Available from: https://en.wikipedia.org/wiki/Identation_hardness#:~:text=Indentation%20hardness%20From%20Wikipedia%2C%20the%20free%20encyclopedia%20Indentation,be%20performed%20on%20a%20macroscopic%20or%20microscopic%20scale.
18. Keeble M. Vickers vs. Knoop Hardness Testers for Measuring Case Depth in Carburized Parts. Geartechnology. 2020 Jan/Feb.
19. Broitman, E. Indentation Hardness Measurements at Macro-, Micro-, and Nanoscale: A Critical Overview. Tribol Lett 65, 23 (2017).
20. Ayyıldız S, Soylu EH, Ide S, Kılıç S, Sipahi C, Pişkin B, Gökçe HS. Annealing of Co-Cr dental alloy: effects on nanostructure and Rockwell hardness. J Adv Prosthodont. 2013 Nov;5(4):471-8.
21. Bertassoni L E, Habelitz S, Kinney J H, Marshall S J, Marshall-Jr G W. Biomechanical perspective on the remineralization of dentin. Caries Res 2009; 43: 70–77.