Acta of Bioengineering and Biomechanics
Vol. 15, No. 3, 2013

Original paper
DOI: 10.5277/abb130308

Dentinogenesis imperfecta – hardness and Young's modulus of teeth

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Dentinogenesis imperfecta type II (DI-II) is the most common dental genetic disease with reported incidence 1 in 8000. Elasticity and hardness of the enamel of teeth are important values which are connected with their resistance to attrition. It is hypothesized that values of physical properties for healthy teeth and teeth with DI-II are different. The aim of the study was to investigate some physical properties of teeth extracted from patients with DI-II in comparison with normal teeth. The material of the study was six teeth: three lower molars, with clinical signs of DI-II, which were extracted due to complications of pulp inflammation and three other lower molars which were extracted for orthodontic reasons – well formed, without any signs of pathology. The surfaces of DI-II and normal teeth were tested on the CSM Instruments Scratch Tester machine (producer CSEM Switzerland) by Oliver & Pharr method. The indenter used was Vicker's VG-73 diamond indenter. Additionally, the Scanning Electron Microscopy (SEM) analysis of the surface of the teeth with DI-II was made. Vickers hardness of the teeth with dental pathology (DI-II) was seven times smaller, and Young's modulus six times smaller than those of healthy teeth. The parameters of hardness and elasticity of enamel of teeth with clinical diagnosis of DI-II were very much smaller than in normal teeth and because of that can be responsible for attrition.

Key words: *nanoindentation, enamel, dentinogenesis imperfecta*

1. Introduction

Dentinogenesis imperfecta is the most common dental genetic disease with reported incidence 1 in 8000. Clinically teeth are discolored with color scale from brown to blue, and sometimes described as amber or grey color. Teeth show structural hypoplastic or hypocalcified defects, such as bulbous crowns, short roots and small pulp chambers. Underlying defects of mineralization often result in the shearing off of the overlying enamel, leaving exposed, weakened dentine which is prone to wear. Dentinogenesis imperfecta was probably first time recognized by Barret in 1882. The first published report describing the disorder as "enamel defected", was by Talbot, as quoted by Witkop [1]. The term "hereditary opalescent dentin" was first used by Skillen [2], Finn [3] and Hodges [4] to describe brown translucent teeth that have an opalescent sheen and lack a pulp chamber. The name dentinogenesis imperfecta hereditaria was first used in 1939 by Robert and Schor. There are three forms of this disease. Dentinogenesis imperfecta type I – osteogenesis imperfecta, in which teeth of both dentitions have typically amber color and are translucent, also show significant attrition. In dentinogenesis imperfecta type II teeth have bulbous crowns with marked cervical constriction. In a tri-racial population in southern Maryland so-called type III Brandywine was found. This character is recognized only in the dentition [1], [5]. These changes should be distinguished from amelogenesis imperfecta which relate only to the enamel. In these abnormalities the hypomineralization of enamel occurs and the teeth surface is covered with characteristic holes and depressions [6].

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Received: July 12th, 2012
Accepted for publication: March 18th, 2013

Dentinogenesis is a process in which the organic predentine matrix is progressively mineralized by ectomesenchymally-derived cells called odontoblasts. The odontoblasts differentiate at the bell stage of tooth development forming a layer of cells lining the pulp cavity where they secrete the organic predentine matrix into the underlying space. The predentine (10–40 μm in thickness) is an unmineralised region containing type I collagen which separates the odontoblast cell bodies from the mineralization front. The collagenous component of the matrix is thought to provide the correct three-dimensional structure into which the mineral component of dentine is deposited while dentine phosphoprotein is also thought to act as a nucleator of hydroxyapatite crystals during the mineralization process. As dentinogenesis continues, the odontoblasts migrate deeper into the pulp cavity, extending their processes as they go, while secreting new dentine matrix. The first-formed, or mantle, dentine of the tooth crown is approximately 15–20 μm thick and is built upon a dentine matrix containing thick collagen type III fibrils arranged at right angles to the dentine-enamel junction. As the odontoblasts migrate further, the matrix they secrete becomes dominated by finely textured collagen type I fibrils orientated parallel to the dentine-enamel junction, resulting in a denser mineralised dentine known as primary, or circumpulpal, dentine [7]–[9]. Dentinogenesis is the process of formation by odontoblasts extracellular collagen fibres, which is the scaffolding for hydroxyapatite. Dentin has in its composition two proteins: dentin phosphoprotein (DPP) and dentin sialoprotein (DPS). Disturbances in the secretion of DPS and DPP, and thus in the proper shape and placement of dentin matrix crystals of apatite, manifested clinically as dentinogenesis imperfecta hereditaria [10].

In histological view the dentine of the crown is relatively normal, but the dentine in pulpal on third showed numerous interglobular areas. Radiculary dentine, on the other hand, is atubular and amorphous and even showed occasionally empty lacunae. The root canals are reduced in dimension and contained a thin strip of pulp tissue [11].

The indentation testing methodologies are rapid. During an indentation test, an indenter, typically diamond, is pressed into the specimen. From the specimen's deformation in response to the indentation load, various mechanical properties of the specimen can be deduced. It is a popular method for determining the hardness of a wide range because it is inexpensive. It can also be effectively used in small volumes of materials. In the development of depth-sensing indentation methodology, which involves the continuous tracking of applied load and indenter's displacement, the elastic properties of the material can also be deduced. In dentistry, the indentation test method has been employed to determine the mechanical properties of dental hard tissues, investment materials, and composites [12].

Currently available restorative materials for the reconstruction of lost tooth tissue have been developed based on the (mechanical) properties of normal formed hard tissues of the tooth. However, the same materials used for the reconstruction of lost tissues in patients with dentinogenesis imperfecta in longitudinal studies do not meet clinical requirements [5], [13], [14]. It therefore seems that in these cases there is a difference in the mechanical properties of dental structures. The adhesion of restorative materials to tooth tissue is most vulnerable in conditions of occlusal load when micromovement is likely to occur. Adequate elastic properties of enamel are relevant to the behavior of this tissue under masticatory load which is why it was considered necessary to examine the elasticity of dental tissue in cases of dentinogenesis imperfecta.

The aim of the study was to investigate some physical properties of teeth extracted from patients with clinical diagnosis of dentinogenesis imperfecta type II (DI-II) in comparison with normal teeth.

2. Materials and methods

The material of the study was six teeth: three lower molars with clinical signs of DI-II, which were extracted due to complications of pulp inflammation and three other lower molars which were extracted for orthodontic reasons – well formed, without any signs of pathology. All human teeth, any available from other patients, were collected after patients had signed an informed consent, in accordance with the ethics committee of Jagiellonian University KBET/174/B/2011.

The study was required to satisfy the following inclusion criteria: tooth without fillings.

All experiments were done at AGH University of Science and Technology, Faculty of Mechanical Engineering and Robotics, Cracow, Poland, in the same conditions. Teeth were stored in physiological saline in separate tubes at 4 °C. Immediately before the test, each tooth was sectioned transversally (relative to the tooth) to create the surface for indentation, then again at the base to create a flat surface for mounting. Test-

ing surfaces were polished with 400 grit sandpaper for 4 min and finally with Pikal polishing paste (Nihon Maryo-Kogyo Co., Japan) for 20 min to ensure a smooth surface. The polished samples were then briefly washed in a water to remove any debris [15]. After this the teeth were laterally stabilized in resin in the research sleeves with enamel surface on the top. The micromechanical parameters of the structures of enamel were studied using the method of nanoindentation. Poison's ratio of the enamel was hypothetic and assumed to be 0.3. The indentation test was done on wet samples. This method makes it possible to measure the hardness and also Young's modulus in the structure tested. The method contrasts with traditional methods of hardness testing, based on measuring the imprint formed by the penetration of the indenter; nanoindentation enabled the characteristics of load/deformation during loading and unloading cycles to be recorded. The resulting characterization was used to calculate the microhardness and Young's modulus. The enamel surfaces of DI-II and normal teeth were tested on the CSM Instruments Scratch Tester machine (producer CSEM Switzerland) and the resultant force-displacement data analyzed by the Oliver & Pharr method [16]. The indenter used was Vicker's VG-73 diamond indenter. Successive loading and unloading cycles in randomly selected areas on the buccal surfaces of enamel of DI-II and normal teeth are shown in Fig. 1A, B and Fig. 2A, B, respectively.

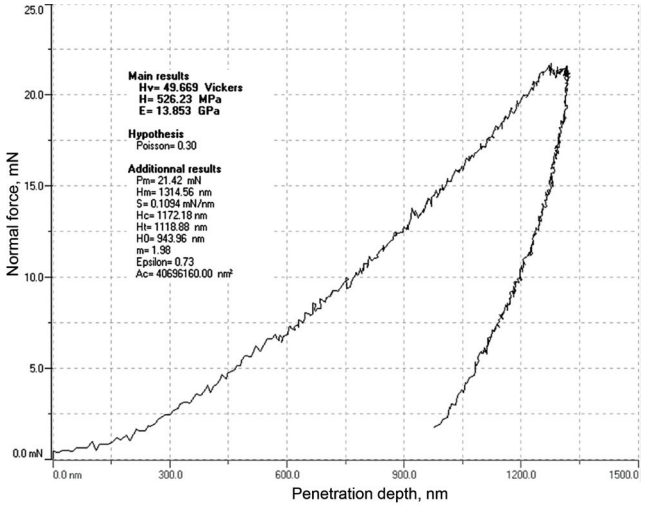

Fig. 1A. Scheme of loading and unloading cycles in randomly selected areas on the buccal surfaces of DI-II at max load 20 mN

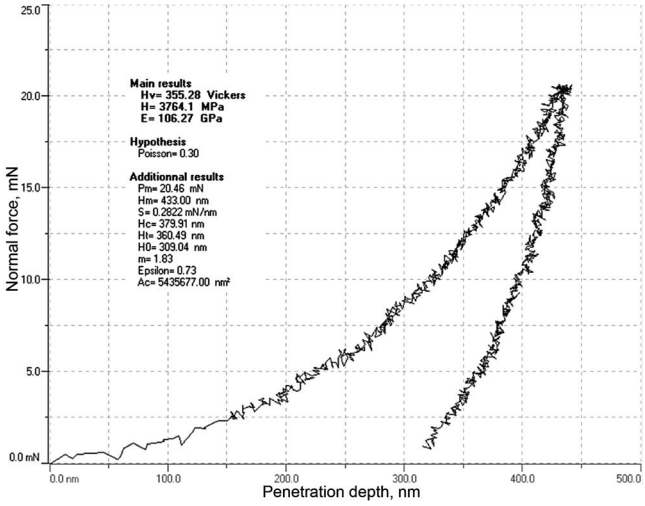

Fig. 2A. Scheme of loading and unloading cycles in randomly selected areas on the buccal surfaces of normal teeth at max load 20 mN

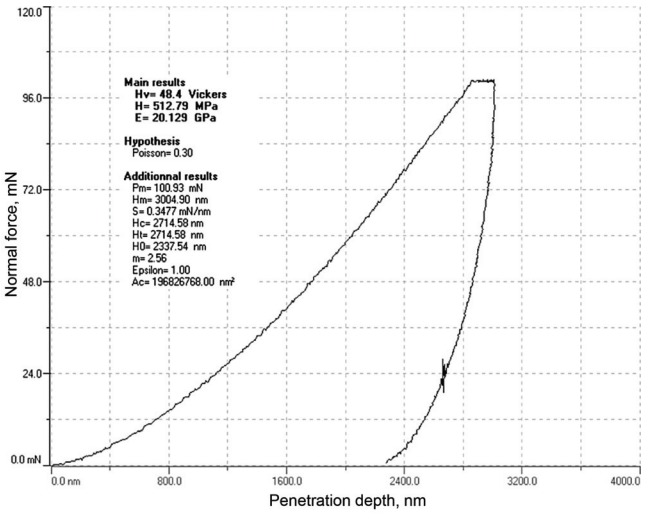

Fig. 1B. Scheme of loading and unloading cycles in randomly selected areas on the buccal surfaces of DI-II at max load 100 mN

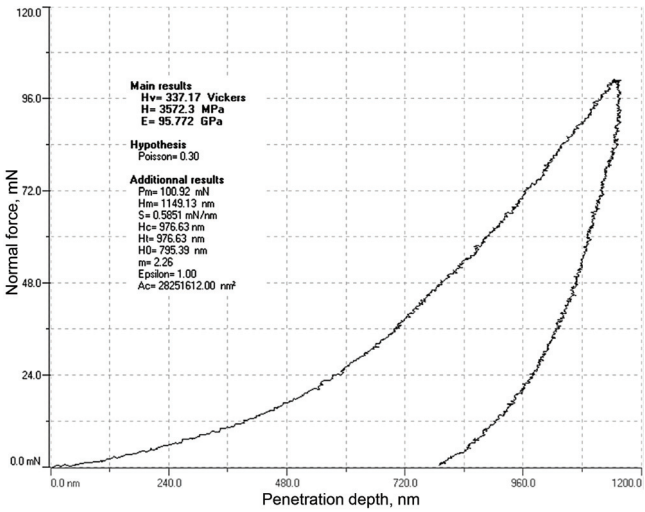

Fig. 2B. Scheme of loading and unloading cycles in randomly selected areas on the buccal surfaces of normal teeth at max load 100 mN

They were made under designated loading and unloading cycles. Microhardness determined during the test was equivalent to the pressure occurring between the test tooth surface of enamel and the indenter diamond, at maximum load. The maximum indentation loads used were 20 mN and 100 mN, and the rates of change of load were 20 mN/min, 40 mN/min, 100 mN/min, 200 mN/min. The investigation was repeated 10 to 15 times on the surface of each sample in a manner similar to previous studies [17]–[20].

Additionally, analysis of the surface of one randomly chosen tooth with DI-II in Scanning Electron Microscopy (SEM) was made. The tooth was rinsed in distilled water, fixed, buffered and stored in 4% glutaraldehyde. Next, they were dehydrated in ethyl alcohol in serial strength up to absolute, and finally dried in vacuum for 24 hours in preparation for the next stage. The surface was coated with gold in vacuum and next examined in the scanning electron microscope Jeol JSM 35CF (Jeol, Tokyo, Japan).

3. Results

Based on the analysis of the imprint indenter results and deformation curves obtained, the microhardness and modulus of elasticity of the surface layer of pathological (DI-II) and normal enamel on the buccal surface were determined. For teeth with DI-II the hardness HV was in the range 48.4–49.7 Vickers (0.47 Gpa–0.49 GPa), and for normal enamel in the range 337.2–355.3 Vickers (3.31 Gpa–3.8 GPa). Young's modulus of elasticity depends on the properties of both the indenter and the structure of the test tooth. With known parameters of the indenter the elastic properties of dental tissues can be assessed. This parameter is used to characterize the tissue in terms of elastic response, the effect of which is determined by contact stiffness. Results for the determination of Young's modulus for teeth with DI-II ranged from 13.9 GPa to 20.1 GPa, and for normal enamel from 95.8 GPa to 106.3 GPa. These results showed very substantial differences in the mechanical properties of dental tissues.

The result of SEM analysis of the surface of the teeth is shown in Fig. 3. The deep enamel cracks are visible. The slots of enamel were divided into smaller fragments as a result of significantly reduced mechanical resistance and chipping of the enamel.

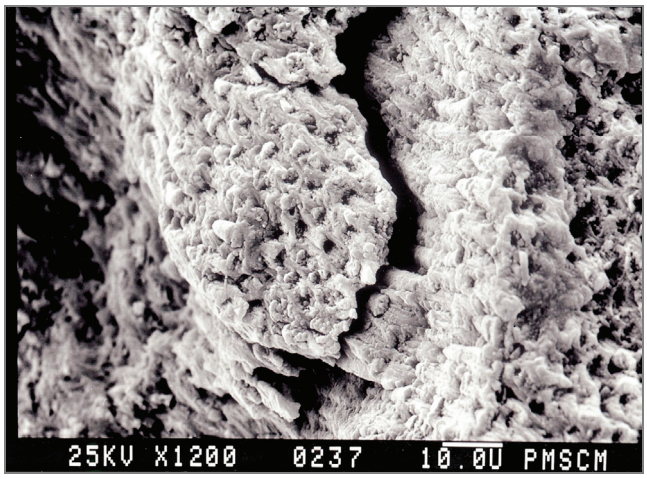

Fig. 3. SEM. Surface of the DI-II tooth – enamel with the visible deep cracks

4. Discussion

Vickers hardness of the enamel of teeth with dental pathology (DI-II) was seven times smaller, and Young's modulus six times smaller than in healthy teeth. In the graphs showing the ratio of force to penetration of the indenter at the tooth surface (Young's modulus = stress/strain), in the final stage there was observed a flattening of the characteristic graph indicating further penetration of the nanoindenter for a smaller than usual increase in load. This combination of hard tissue properties that occurs in DI-II cases can create difficulties in the placement of clinical dental fillings, and classic restorations, the most commonly used biomaterials. Substantially reduced hardness, elasticity and stiffness of dental tissues in DI-II allow the phenomenon of micromovement and potential loss of retention for tooth restorations. At the same time it is necessary to regard the strength parameters of pathological and normal tissue; while the strength parameters of biomaterials have much higher hardness and much higher resistance to stress [21].

Using indentation, Willems et al. reported Young's modulus of elasticity for enamel $E = 90.59 \pm 16.13$ GPa and Mahoney et al. reported hardness $H = 4.88 \pm 0.35$ GPa (497.6 ± 35.69 Vickers) [22], [23]. The values depend on the particular tooth surface on which the study was done. Our data shows similar results in healthy teeth. In teeth with clinical diagnosis DI-II the values for hardness and elasticity from our study are more similar to those from Kinney et al. for intertubular dentine near dentine-enamel junction: hardness 2.23–2.54 GPa and elastic modulus 17.7–21.1 GPa [24]. It can be assumed that the explanation for the

results of the test is abnormal tooth structure, which was established during its development.

In our previous study we analyzed the mineral composition of permanent teeth in dentinogenesis imperfecta type II. The results of tests performed on dental mineral elements show many irregularities. A substantial decrease in calcium ion values in all measurements and an increase in phosphorus content in the enamel on the surface were observed. The calcium to phosphorus ratio in every site tested was significantly reduced. The magnesium values were found to be reversed on the tooth surface. The values of other elements determined, such as sodium, potassium, fluorine, sulphur, chlorine, iron, strontium and cadmium were not significant [25]. The results of the above examinations can explain the differences in the value of the modulus of elasticity and hardness.

The parameters of hardness and elasticity of enamel of teeth with clinical diagnosis DI-II were very much smaller than in normal teeth and because of that can be responsible for attrition.

Acknowledgements

This study was supported by Grant K/ZDS/002455 which is founded by Ministry of Education and Research for Jagiellonian University, Collegium Medicum.

Research was done in accordance with the requirements of the ethics committee of Jagiellonian University KBET/174/B/2011.

Our sincere thanks are due to Peter Likemann PhD, DDS (UK) for helping us in correction of the manuscript in English.

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

[1] WITKOP C.J. Jr.. *Manifestations of genetic diseases in the human pulp*, Oral Surg. Oral Med. Oral Pathol., 1971, Vol. 32, 278–316.

[2] SKILLEN W.G., *Histologic and clinical study of hereditary opalescent dentin*, J. Am. Dent. Assoc., 1937, Vol. 24, 1426–1433.

[3] FINN S.B., *Hereditary opalescent dentin: I, An analysis of the literature on hereditary anomalies of tooh colour*, J. Am. Dent. Assoc., 1933, Vol. 25, 1240–1249.

[4] HODGE H.C., FINN S.B., *Hereditary opalescent: A dominant hereditary teeth anomaly in man*, J. Heredit., 1938, Vol. 29, 359–364.

[5] SUBRAMANIAM P., MATHEW S., SUGNANI S.N., *Dentinogenesis imperfecta: a case report*, J. Indian Soc. Pedod. Prev. Dent., 2008, Vol. 26, 85–87.

[6] GIBEK J., PAWŁOWSKA E., KLIMEK L., SZCZEPAŃSKA J., *Studies of microhardness of hard dental tissues in the course of amelogenesis imperfecta based on the chosen model of transgenic mice*, J. Stoma., 2013, Vol. 66, 10–23.

[7] BARRON M.J., MCDONNELL S.T., MACKIE I., DIXON M.J., *Hereditary dentine disorders: dentinogenesis imperfecta and dentine dysplasia*, Orphanet J. Rare Dis., 2008, Vol. 3, 31.

[8] YAVUZ I., ZULKUF AKDAG M., DASDAG S., ZELAL ULKU S., ZEKI AKKUS Z., *Influences of extremely low frequency magnetic fields on mineral and trace elements content of rat teeth*, African Journal of Biotechnology, 2008, Vol. 7, 3811–3816.

[9] YIN J., ZHANG Y., BAI Y., WANG B., YANG K., *Expression patterns of homeobox genes in different teeth of the minipig embryo*, African Journal of Biotechnology, 2011, Vol. 10, 5831–5837.

[10] de COSTER P.J., CORNELISSEN M., de PAEPE A., MARTENS L.C., VRAL A., *Abnormal dentin structure in two novel gene mutations [COL1A1. Arg134Cys] and [ADAMTS2, Trp795-to-ter] causing rare type I collagen disorders*, Arch. Oral Biol., 2007, Vol. 52, 101–109.

[11] SHIELDS E.D., BIXLER D., EL-KAFRAWY A.M., *A proposed classification for heritable human dentine defects with a description of a new entity*, Arch. Oral Biol., 1973, Vol. 18, 843–853.

[12] CHUNG S.M., YAP A.U., TSAI K.T., YAP F.L., *Elastic modulus of resin-based dental restorative materials: a microindentation approach*. J. Biomed. Mater. Res. B Appl. Biomater., 2005, Vol. 72, 246–253.

[13] CHLADEK W.T., KARASINSKI A., *Experimental evaluation of occlusal forces*, Acta Bioeng. Biomech., 2001, Vol. 3, 25–38.

[14] PIENIAK D., NIEWCZAS A.M., *Phenomenological evaluation of fatigue cracking of dental restorations under conditions of cyclic mechanical loads*, Acta Bioeng. Biomech., 2012, Vol. 14, 9–17.

[15] WHITENACK L.B., SIMKINS D.C. JR., MOTTA P.J., HIRAI M., KUMAR A., *Young's modulus and hardness of shark tooth biomaterials*, Arch. Oral Biol., 2010, Vol. 55, 203–209.

[16] OLIVER W.C., PHARR G.M., *A new improved technique for determining hardness and elastic modulus using load and sensing indentation experiments*, J. Mater. Res., 1992, Vol. 7, 1564–1582.

[17] ANJUM A., OTSUKI M., MATIN K., TAGAMI J., *Preservation in the liquid media produces alterations in enamel surface properties*, J. Dent., 2009, Vol. 37, 884–890.

[18] CUY J.L., MANN A.B., LIVI K.J., TEAFORD M.F., WEIHS T.P., *Nanoindentation mapping of mechanical properties of human molar teeth enamel*, Arch. Oral Biol., 2002, Vol. 47, 281–291.

[19] HE L.H., SWAIN M.V., *Nanoindentation derived stress-strain properties of dental materials*, Dental. Materials, 2007, Vol. 23, 814–821.

[20] MEREDITH N., SHERRIFF M., SETCHELL D.J., SWANSON S.A., *Measurement of the microhardness and Young's modulus of human enamel and dentine using an indentation technique*, Arch. Oral Biol., 1996, Vol. 41, 539–545.

[21] STAINES M., ROBINSON W.H., HOOD J.A.A., *Spherical indentation of tooth enamel*, J. Mater. Sci., 1981, Vol. 16, 2551–2556.

[22] MAHONEY E., HOLT A., SWAIN M., KILPATRICK N., *The hardness and modulus of elasticity of primary molar teeth: an ultra-micro-indentation study*, J. Dent., 2000, Vol. 28, 589–594.

[23] WILLEMS G., CELIS J.P., LAMBRECHTS P., BRAEM M., VANHERLE G., *Hardness and Young's modulus determined by nanoindetation techniquie of filler particles of dental restorative materials compared with human enamel*, J. Biomed. Mater. Res. B Appl. Biomater., 1993, Vol. 27, 747–755.

[24] KINNEY J.H., BALOOCH M., *Hardness and Young's modulus of human peritubular and intertubular dentine*, Arch. Oral Biol., 1996, Vol. 41, 9–13.

[25] WIECZOREK A., LOSTER J., *Analysis of the mineral composition of permanent teeth in dentinogenesis imperfecta type II*, J. Stoma., 2012, Vol. 65, 404–410.[