Preliminary study of distribution of mechanical properties and mineral density by depth of liquid saturated carious dentine

E V Sadyrin1,2*, B I Mitrin1, D V Yogina2 and M V Swain1,3

1 Research and Education Center “Materials”, Don State Technical University, Rostov-on-Don, Russia
2 Rostov State Medical University, Rostov-on-Don, Russia
3 The University of Sydney, Camperdown NSW 2006, Australia

* email: e.sadyrin@sci.donstu.ru

Abstract. Fissure caries in a form of brown spot lesion is a serious problem for patients. Having passed the dentine-enamel junction, cariogenic bacteria cause demineralization of dentine in its vicinity. Over time, the area of demineralization spreads deeper towards the pulp of the tooth, creating a gradient of strength characteristics in dentine. The aim of this work was to determine the mechanical properties and mineral density in the direction from the dentine-enamel junction to the dental pulp using nanoindentation and X-ray computed microtomography. The results can be used as control ones for the further research of remineralization techniques used in clinical practice.

1. Introduction

The biofilm formed by bacteria attached to the pellicle on the tooth enamel surface, together with their waste products, form dental plaque [1]. A by-product of the metabolism of plaque bacteria (primarily of the Streptococcus mutans and Lactobacillus groups) in the presence of carbohydrates in the oral cavity is organic acid [2-6], mainly lactic acid. It locally lowers Ph in the area of plaque and penetrates into the tooth enamel, dissolving its main structural components - hydroxyapatite crystallites [7-10]. This phenomenon is called demineralization. Demineralization can begin in two ways: either the centers of the crystallites dissolve, or areas on their periphery [11], thus caries begins. In case of fissure caries, bacteria not only inhabit plaque on the occlusal surface, but are also able to penetrate the enamel, reaching the dentine-enamel junction and then penetrating into the dentinal tubules [12], propelling themselves down them [13]. The odontoblast inside the tubule, sensing the appearance of a bacterium, gives a signal about the invasion of an alien microorganism into the pulp, which in turn releases growth factors that allow the odontoblasts, as in the case of proximal caries, to pump more minerals from the pulp. However, in the case of fissure caries, minerals are used to build secondary dentine that attempts to block the tubules (creating a dead path in the dentine, although tubules are not blocked at the full extent, thereby slowing down the bacterial ingress into the pulp from an external threat). Simultaneously with the blockade of the tubules, the possibility of odontoblasts to deliver mineral content to the enamel crystallites disappears, which causes the risk of the final destruction of the crystals in the body of the lesion and, as a consequence, the formation of a cavity inside the tooth [14]. When the caries develops into the dentine below a fissure the biofilm is now very active and results in both substantial acid...
development and also an increase in the bacteria causing the acid plus difficulty in terms of being exposed to the saliva or cleaned by the tongue or tooth brush. This then causes both acid demineralization of the dentine but also proteases that break up the collagen.

In this case, a density gradient arises in dentine (from the dentine-enamel junction, the density increases towards the pulp). The lower the density, the more severely damaged hydroxyapatite crystallites. The stronger the crystal is damaged, the greater part of it is replaced by water. Also, demineralization of dentine is accompanied with the breakdown of organic component.

Understanding the process of dentine demineralization in case of fissure caries demands obtaining of complex of physical and mechanical characteristics of the carious area. This work aims to study three such characteristics in vitro: mineral density, reduced Young’s modulus and indentation hardness by the depth of carious dentine. The results of the study are crucial for further linkage of the properties mentioned above with the microstructural features of carious tissue, and can serve as reference data for researches of efficacy of minimally invasive methods for clinical treatment.

2. Materials and methods

2.1. Sample

An extracted permanent human molar was collected for orthodontic purposes from an individual in the dental department of Rostov State Medical University clinic. Local independent ethics committee of Rostov State Medical University approved the study (statement № 13/20 from 10.09.2020), the patient provided informed consent. The fissure caries in the stage of brown spot lesion (BSL) was assessed by a clinician. Following extraction, the sample was kept in 1 wt. % NaClO solution for 10 min. Then the sample was stored in Hanks Balanced Salt Solution (HBSS) at 4 ºC with thymol granules (Unifarm, Russia), added to prevent fungal growth and disinfection. The ratio of thymol to HBSS was 1:1000.

2.2. X-Ray computed microtomography (micro-CT)

The non-destructive determination of mineral density of the sample was conducted using a micro-CT device (Xradia Versa 520, Carl Zeiss X-ray Microscopy, USA). The tooth was placed inside the chamber of the device in the container filled with distilled water with the calibration sample (phantom), placed above the tooth. A calibration sample was made from four phantoms - parallelepipeds 15 mm high, base 50 x 50 mm with measured density: PET (ρ = 1.35 g/cm³), magnesium alloy Ma2-1M (ρ = 1.78 g/cm³), aluminium alloy SAS1-400 (ρ = 2.69 g/cm³) and fluorite (ρ = 3.09 g/cm³).

The phantoms were used for constructing a linear relationship between grey level and mineral density, that allowed conversion of the measured grey value of the tooth tissues to an estimated mineral density [15]. Thus, in VG Studio MAX 3.5 software (Volume Graphics GmbH, Heidelberg, Germany), we took the region of interest as maximum cylinder in width, length and height, which could be inserted into each phantom (the same for all phantoms). Inside it the grey value was averaged.

Acquisition parameters were as follows: magnification objective 0.4x, voltage 60 kV, power 4.5 W, source filter HE6, exposure time 16 s, voxel size 19.1 µm. Totally 1601 X-ray projections were acquired, which were afterwards reconstructed using XRMReconstructor 12.0.8086.19558 software (Carl Zeiss AG, Germany) with automatically adjusted center shift values, σ = 0.5 Gauss blurring filter. Adaptive motion compensation correction of sample drift was enabled. Beam hardening correction 0.19 was chosen in a such way to minimise beam hardening on the calibration phantom.

For each scan, the sample was placed at the closest possible distance to the X-ray source in a such way, that the whole sample could be acquired. The 2048x2048 pixels CCD camera was maintained at −59°C. The acquisition was performed with camera binning factor = 2, which resulted in up to 1024x1024 pixels sized projection images. The X-ray source filters were selected based on the observed transmittance values according to the recommendations of the Xradia Versa 520 manufacturer. The exposure time was selected to maintain count (intensity) values greater than 5000 with the selected source parameters and filter. The Dynamic Ring Removal option, which enables small random motions of the sample during acquisition, was enabled for all the projections for each sample. During each tomography procedure, 10 reference X-ray images were acquired with equal time intervals between
them (air was used as reference). The average of these references was applied to each projection. Prior each acquisition, up to one-hour warm-up scan was performed with the same source parameters.

After data acquisition, the images were exported in DICOM format for volume rendering in VG Studio MAX 3.5 software.

2.3. Sample preparation
Following micro-CT study the tooth was prepared for nanoindentation test. Thus, a tooth was placed into 1-inch diameter cylinder filled with epoxy resin. Then, the longitudinal section through the region containing a BSL was cut using a precision saw (Isomet 4000, Buehler, USA) with an abrasive SiC disc (MetAbrase, Buehler, USA). The cut surface was carefully ground using the SiC-based abrasive paper P1200 (Siwat 1913, Sia Abrasives, Switzerland) with distilled water used as lubricant. Then, polishing was conducted with diamond oil-based suspensions (Allied, USA) with particles of 15, 9, 3, and 1 μm diameters. Next, the final polishing was made using de-agglomerated alumina 0.05 μm diameter (Allied, USA) suspension. The lubricant hexilene glycole GreenLube (Allied, USA) was applied during polishing steps. All suspensions were applied on no nap cloth according to manufacturer’s recommendations: 15 and 9 μm diameter suspensions were placed on Plan B cloth (Allied, USA), 3 μm on Final P cloth (Allied, USA), 1 and 0.05 μm on FinalPrep cloth (Allied, USA). Each grinding and polishing step was followed by ultrasonic cleaning of the sample in distilled water (Sonorex RK 31, Bandelin, Germany) for 10 min. The similar sample preparation procedure was previously demonstrated excellent surface quality for nanoindentation of enamel and dentine [16].

2.4. Optical microscopy
An optical Greenough stereomicroscope (Stemi 305, Zeiss, PRC) with a colour video camera (Axiocam 105, Zeiss, Germany) was used for visual observation of BSL are of prepared tooth in reflected light.

2.5. Nanoindentation
The research of distribution of mechanical properties by depth of the BSL was carried out using nanoindentation device (NanoTest 600 Platform 3, Micro Materials, UK). The experiments were carried out at a constant temperature of 27.0 ± 0.1 °C in a closed chamber, a calibrated diamond Berkovich indenter was used. The maximum load was 5 mN. The loading and unloading rates were 1 mN/s, dwell period at maximum load was 30 s. The thermal drift was recorded and corrected using the software of the device. To prevent shrinkage of dentine the sample was maintained wet with saline droplets using a syringe pump (Terufusion TE-332, Terumo, Belgium). The droplets were applied on the sample in the time between indentations in order to prevent the influence of the droplet impact on the indenter. Values of the reduced Young’s modulus $E_r$ and indentation hardness $H$ were calculated using the Oliver – Pharr method [17].

3. Results and discussion
Four segments were marked on the image obtained by optical microscopy. The same plane (as a virtual cross-section) was found on the non-destructive map of mineral density obtained using micro-CT. Across these segments the values of reduced Young’s modulus, indentation hardness and mineral density were measured, the graphs of which are presented on the figure 1, whereas the segments themselves can be seen on the figure 2. The curves, showing dependence of indentation depth $h$ on indentation load $P$ are depicted on figure 3.

Segment 1 demonstrates the control results, obtained from the sound dentine part. We observed, that the values of the mechanical properties decreased from the pulp to the dentine-enamel junction, although in general they are illustratory of dentine [18-23]. It is possible that the differences in values are related to anisotropy (the exit of dentinal tubules at different angles at the current cross-section). At the same time results for dentine were consistent with minimal fluctuations.

Segment 2 shows a gradual decrease in the values of both mechanical properties and density from the pulp to the dentine-enamel junction. In general, this is predictable (since in the vicinity of the pulp dentine was least affected by demineralization). Interestingly, in the image from an optical microscope, two points are clearly visible, where the segment touches a strongly brown area near the pulp, and at
these points on the graph one can see the dips in the modulus of elasticity and hardness (and the subsidence of density).

Figure 1. Results of measurements of mechanical properties and mineral density along the segments: 1 (a,b); 2 (c,d); 3 (e,f); 4 (g,h)
Figure 2. Lines across which the mechanical properties and mineral density were measured on the optical image after sample preparation (a) and on the non-destructive virtual cross section of mineral density map (b).

Figure 3. $P-h$ curves for the segments 1 (a), 2 (b), 3 (c), 4 (d).
Segment 3 reveals a sharp drop and rise in the values of mechanical properties, followed by a decrease (also quite rapid) in the same direction. The decrease in density values is also obvious, although it is more gradual. As in the case of segment 1, there is a peak in the density values approximately in the same place as the peak in the values of mechanical properties inside the segment (and, as in the case of segment 1, it is shifted to the right).

Segment 4 demonstrates a very sharp drop in the values of the modulus of elasticity and hardness also from the pulp to the dentine-enamel junction. At the same time, the drop in density is also strong, but not so sharp. Judging by the density of dentine in the first half of the segment, it is only slightly affected by carious process, at the same time the load resistance mechanism is already severely impaired.

4. Conclusions
The mechanical properties and mineral density in the direction from the dentine-enamel junction to the dental pulp was studied in vitro across four segments using nanoindentation and micro-CT in the presence of liquid. The features connected to the abnormality of the indentation response were recorded. The results are planned to be extended in order to include the results for dehydrated dentine on the same tooth and on some other samples to collect more statistical data. It is also planned to include prediction of behaviour of carious tissues using the approaches in mathematical modelling that are able to describe indentation into inhomogeneous media. Such an approach based on bilateral asymptotic method was suggested in our previous papers [24-29].

One of the possible ways to explain the changes of the values of the characteristics observed for the sound dentine can be further examination of the sample using atomic-force microscopy by a protocol [30] proved to be reliable for the research of teeth tissues. The results can serve can serve as reference data for researches of efficacy of remineralizing methods for clinical treatment [31-34].

Acknowledgments
This research was funded by the grant of the Government of the Russian Federation, grant number 14.Z50.31.0046. The experiments were carried out in the Research and Education Center “Materials” of Don State Technical University (https://nano.donstu.ru/).

References
[1] Marsh P D 2004 Caries Res. 38 204–211
[2] Geddes D A 1975 Caries Res. 9 98–109
[3] Loesche W J 1986 Microbiol. Rev. 50 353
[4] Loesche W J, Syed S A 1973 Caries Res. 7 201–216
[5] Leverett D H, Proskin H M, Featherstone J D B, Adair S M, Eisenberg A D, Mundorff-Shrestha S A, Shields C P, Shaffer C L, Billings R J 1993 J. Dent. Res. 72 538–543
[6] Leverett D H, Featherstone J D B, Proskin H M, Adair S M, Eisenberg A D, Mundorff-Shrestha S A, Shields C P, Shaffer C L, Billings R J 1993 J. Dent. Res. 72 529–537
[7] Featherstone J D 2000 J. Am. Dent. Assoc. 131 887–899
[8] Hannig M, Hannig C 2010 Nat. Nanotechnol. 5 565
[9] Takahashi N, Nyvad B 2011 J. Dent. Res. 90 294–303
[10] Takahashi N, Nyvad B 2016 Caries Res. 50 422–431
[11] Yanagisawa T, Mlake Y 2003 Microsc. 52 605–613
[12] Bjorndal L 2002 Endodontic Topics 2 10–23
[13] Koutsi V, Noonan R G, Horner J A, Simpson M D, Matthews W G, Pashley D H 1994 Pediatr. Dent. 16 29–29
[14] Oliveira E F, Carminatti G, Fontanella V, Maltz M 2006 Clin. Oral Investig. 10 134–139
[15] Alyahya A, Alqareer A, Swain M 2019 Med. Princ. Pract. 28 247–255
[16] Kislyakov E A, Karotiyan R V, Sadyrin E V, Mitrin B I, Yogina D V, Kheygetyan A V, Maksyukov S Y 2020 Modeling, Synthesis and Fracture of Advanced Materials for Industrial and Medical Applications ed S M Aizikovich, H Altenbach et al (Cham: Springer) pp. 75–83
[17] Oliver W C, Pharr G M 1992 J. Mater. Res. 7 1564–83
[18] Kinney J H, Balooch M, Marshall S J, Marshall Jr G W, Weihs T P 1996 Arch. Oral Biol. 41 9–13
[19] Chng H K, Ramli H N, Yap A U J, Lim C T 2005 J. Dent. 33, 363–369
[20] Elfersi S, Grégoire G, Sharrock P 2002 Dent. Mater. 18 529–534
[21] Marangos O, Misra A, Spencer P, Bohaty B, Katz J L 2009 Acta biomater. 5 1338–48
[22] Jíra A, Němeček J 2014 Key Engineering Materials (vol. 606) ed P Haušild (Kapellweg: Trans Tech Publications Ltd) pp. 133–136
[23] Zimmerman B, Datko L, Cupelli M, Alapati S, Dean D, Kennedy M 2010 J. Mech. Behav. Biomed. 3 339–346
[24] Volkov S S, Vasiliev A S, Aizikovich S M, Sadyrin E V 2018 AIP Conf. Proc. (vol. 1959) ed E Kustova, G Leonov et al (New York: AIP Publishing LLC) p 070037
[25] Volkov S S, Vasiliev A S, Sadyrin E V 2018 MATEC Web of Conf. (vol. 226) ed H-G Gross, H Khaled et al (Les Ulis: EDP Sciences) p 03018
[26] Zelentsov V B, Sadyrin E V, Sukiyazov A G, Shubchinskaya N Y 2018 MATEC Web of Conf. (vol. 226) ed H-G Gross, H Khaled et al (Les Ulis: EDP Sciences) p 03027
[27] Aizikovich S M, Vasiliev A S 2013 J. Appl. Math. Mech. 77 91–97
[28] Vasiliev A S, Sadyrin E V, Mitrin B I, Aizikovich S M, Nikolaev A L 2018 Russ. Eng. Res. 38 735–737
[29] Vasiliev A S, Swain M V, Aizikovich S M, Sadyrin E V 2016 Arch. Appl. Mech. 86 1247–54
[30] Sadyrin E V, Kislyakov E A, Karotkiyan R V, Yogina D V, Drogan E G, Swain M V, Maksyukov S Yu, Nikolaev A L, Aizikovich S M 2020 Plasticity, Damage and Fracture in Advanced Materials ed H Altenbach, M. Brüning et al (Cham: Springer) chapter 8 pp. 135–150
[31] Lavigne O, Vu A M, Richards L, Xie Z 2018 J. Oral Sci. 60 121–128
[32] Tschoppe P, Zandim D L, Martus P, Kielbassa A M 2011 J. Dent. 39 430–437
[33] Zhang X, Neoh K G, Lin C C, Kishen A 2012 J. Mater. Sci.: Mater. Med. 23 733–742
[34] Zhong B, Peng C, Wang G, Tian L, Cai Q, Cui, F 2015 J. Tissue Eng. Regen. M 9 1004–16