Original Article

ASSESSMENT OF MORPHO-STRUCTURAL CHANGES AT INCREASED ERASABILITY OF TEETH WITH ATOMIC FORCE MICROSCOPY

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ABSTRACT

The purpose of this work was to study the possibility of using atomic force microscopy in the assessment of morphostructural changes at increased erasability of teeth. For the experiment, 100 teeth samples from patients of various age groups living in the North Caucasus Federal District of Russia were obtained. Atomic force microscopy made it possible to establish that in teeth with manifestations of increased erasability, the diameter of the tubes and their number are smaller both on the surface (3.8 times) and in the middle layer (2.2 times) than in the control group. The orientation of the tubes was indistinctly expressed, the surface has a flattened, smoothed relief. The uniformity of the dentin structure was most likely associated with inclusions of foreign substances, pigments penetrating from the oral cavity through the exposed surface of dentin in the abrasion facet, as well as with the protective function of the pulp and activation of the synthesis of substitutive (irregular) dentin. The lumen of the surface tubules was reduced or even completely obliterated. Hypermineralization zones around dentine tubules were expanding, associated with a dense arrangement of mineral crystals and globules, and the microhardness of the surface layer was increasing. In deeper layers, pathological changes were less pronounced. The structure of dentin resembles the structure of the 2nd and 3rd layers of healthy dentin. However, the lumen of the dentine tubules was still smaller (up to 0.5 µm).

Key words: Morpho-structural, Teeth, Atomic force microscopy, Erasability.

Introduction

Changes in the morphological structure of the tooth during pathological processes significantly affect the resistance of the enamel to adverse factors [1, 2]. This affects the durability of the seal, marginal adaptation, and compliance of the restoration work with clinical and aesthetic evaluation criteria [3, 4]. Notably, quantitative and qualitative indicators of the micro- and nanostructure of hard dental tissues, as well as the processes occurring in enamel and dentin with increased erasability, have not been practically studied to date. Moreover, there are no studies in the available literature justifying the choice of filling materials for the restoration of teeth subject to increased abrasion.

It can be assumed that the appearance of nanofilled adhesive systems and composite materials capable of interacting with tooth tissues and embedding into their structure at the nanoscale will solve this problem [5-8]. The functional monomers of the adhesive system react with hydroxyapatite and form a nanointeractive hybrid zone [9-11]. In addition, nanofilled materials, having sufficient strength to occlusal load, make it possible to preserve the polishing luster of restoration for a long time, and reproduce anatomical features, color nuances, and transparency of hard tooth tissues [12-15].

In this aspect, an integral assessment of the morphological features of the structure of the hard tissues of teeth with increased erasability using modern experimental microscopic research methods becomes relevant [16]. Scanning electron microscopy is one of the most often used methods to study the structure of biosystems [17]. Transmission electron microscopy uses the wave properties of moving electrons to obtain high-resolution images of the object under study [18]. Using this method, the features of teeth microstructure in representatives of various age groups have been studied [19-21]. Scanning electron microscopy makes it possible to study biomolecules in subnanometer resolution since the surface of the sample is examined using a very thin beam with a diameter of only a few angstroms [22]. The process of forming a hybrid zone during sealing with various composite materials and adhesive systems has been studied through the use of this particular microscopy technique [23].

The most promising microscopy method that allows us to obtain images on an atomic and nanometer scale is atomic force microscopy (AFM). AFM generates high-resolution images of a sample by scanning it using microscopic mechanical, electrical, optical, thermal, and other probes [24, 25]. The probe is located at the free end of a miniature cantilever [26]. It measures the weak interaction forces arising between the tip and the surface of the sample, determining changes in the reflection of the laser beam [27, 28]. The image of the surface relief is recorded using a movable piezoelectric slide table, which moves either the sample over the tip or the tip over the surface of the sample [29]. In biomedical applications, AFM is used to study the structure and physical properties of proteins, erythrocytes, DNA, etc. [30-32]. However, there is very limited information in the available literature on the use of this method to study the morphological structure of hard tooth tissues in pathological processes [33].

Thus, the purpose of this work was to study the possibility of using AFM in the assessment of morphostructural changes at increased erasability of teeth.

Materials and Methods

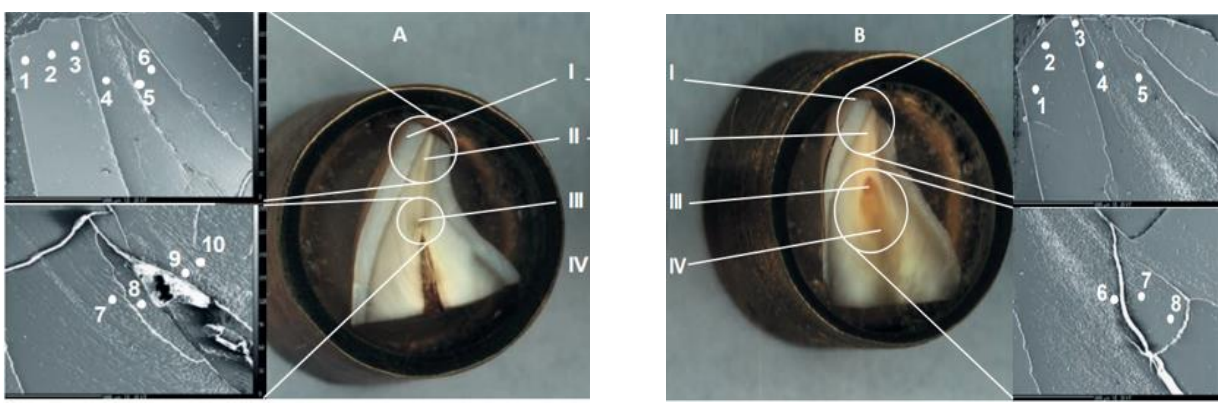
For the experiment, 100 teeth samples from patients of various age groups living in the North Caucasus Federal District of Russia were obtained. The experimental group was formed from teeth with manifestations of increased tooth abrasion. The control group consisted of samples of intact teeth removed for orthopedic and orthodontic indications.

The study of the microstructure and surface properties of enamel and dentin was carried out using Explorer AFM (Thermo Microscopes, USA). The surfaces of longitudinal sections of teeth with a thickness of 1-1.5 mm prepared using a low-speed drill and a diamond separation disc were studied [34]. Flexible abrasive discs were used for sample grinding.

Optical electron microscopy was performed on an electron probe microanalyzer SX 100 (Cameca, USA). To carry out the analysis, special checkers with polished longitudinal sections of teeth fixed in epoxy resin were sprayed with carbon [35]. The analysis was performed at an accelerating voltage of 15 kV and a current strength of 40 nA. Natural minerals such as fluorapatite, chlorapatite, dolomite, albite and anhydrite were used as standard samples [36, 37]. Micrographs of various sections of dental tissues were obtained in the mode of secondary electrons with a voltage of 20 kV.

Results and Discussion

In the samples of intact incisors and incisors with increased erasability, four main zones were studied, which stand out visually well and are of the greatest interest in connection with the changes occurring in them during the development of the sclerosing process: I – enamel, II – surface (cloak) dentin, III – deep (periculpar) dentin, IV – newly formed sclerosed dentin, which forms in the tooth cavity with increased erasability. Detailed images of these zones in two teeth obtained in the mode of secondary electron microscopy are shown in **Figure 1**. It can be seen that a tooth with increased erasability is characterized by an inhomogeneous granular structure of the mantle dentin **(Figure 1b)**, (sections 3-5). The zone of overgrowth with highly mineralized sclerosed dentin is clearly distinguished **(Figure 1b)**, (sections 7, 8). Differences in the direction of growth of crystals of intact periculpar dentin are visible in the zones directly adjacent to the pulp chamber **(Figure 1a)**, (sections 8, 9).

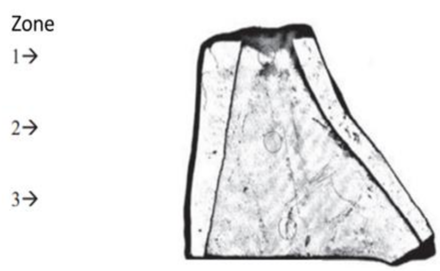


a) b)

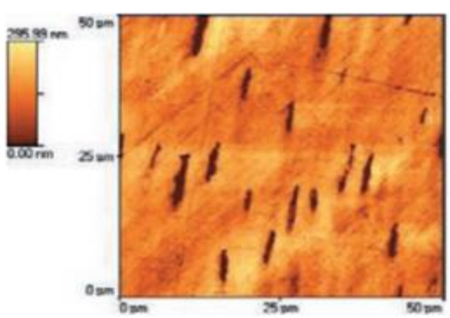
Figure 1. The point of the tested hard tissues of an intact tooth (a) and a tooth with the manifestation of erasability (b) and micrographs of individual zones of teeth obtained in the mode of secondary electron microscopy.

Figure 2 shows photographs demonstrating the features of the topology (relief) of the surface of intact teeth and teeth with increased erasability, obtained using AFM. As can be seen, dentin is constructed from a basic substance (collagen fibrils, mineral phases – hydroxyapatites, phosphates, carbonates, calcium fluorides, an adhesive substance – hyaluronic acid, glycosaminoglycans), permeated by tubes in which the processes of odontoblasts and the endings of nerve fibers penetrating from the pulp are located, which is in the line with the report of Salman and Hussein [38]. The interchannel substance is the most highly mineralized, and has a high density and significant hardness [39]. Dentine tubes start from the inner surface of the dentine and reach the enamel-dentine border. The tubes are unevenly distributed in the dentin substance [40]. It is worth noting, that in all the teeth studied, the largest number of tubules was located in the area adjacent to the pulp (periculpar dentin), and as they moved away from the pulp, their number decreased. All this is quite clearly shown in AFM scans **(Figures 2 and 3)**.

a)



b)



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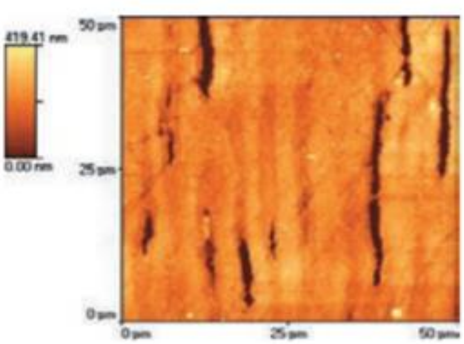
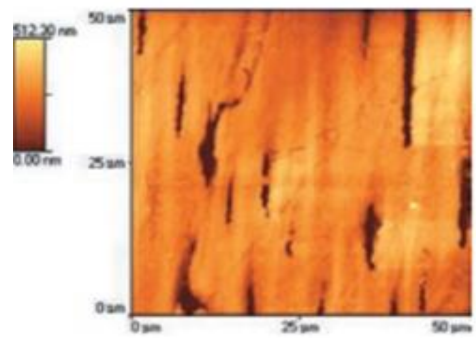
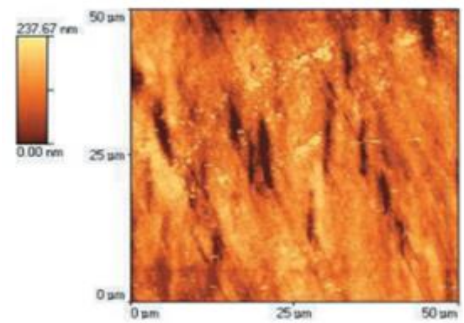


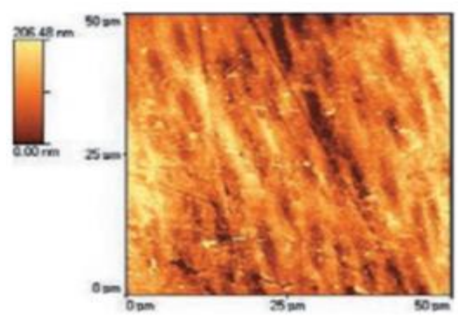
d)



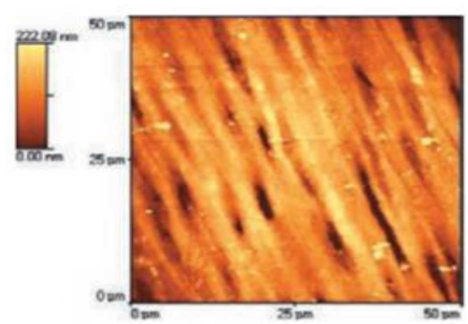
e)



f)

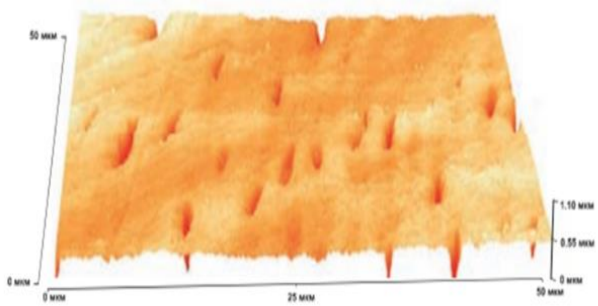


g)

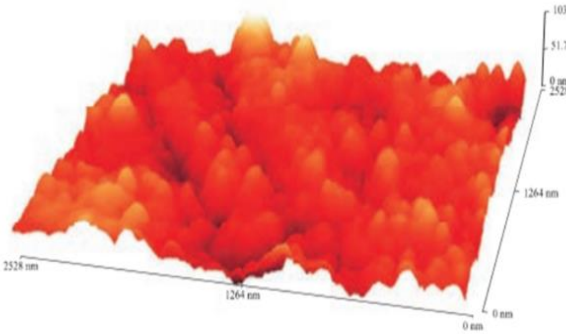


h)

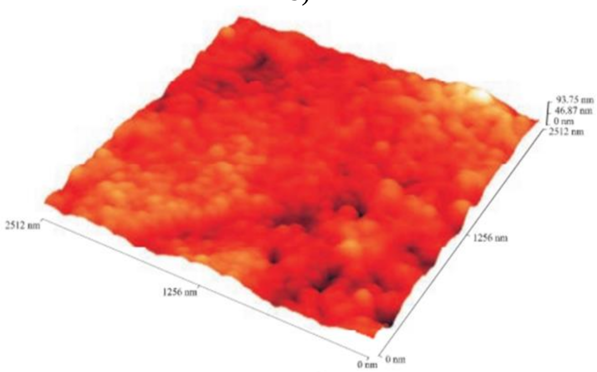
Figure 2. Surface topography was obtained using optical (a, b) and atomic force (c-h) microscopes for an intact incisor (a, c (Zone 1), d (Zone 2), e (zone 3)) and incisor with a manifestation of increased erasability (b, f (Zone 1), g (Zone 2), h (Zone 3)).



a)



b)



c)

Figure 3. Three-dimensional image of the surface obtained with an atomic force microscope for an intact incisor (a, b (Zone 3)) and incisor with manifestations of increased erasability (c (Zone 3)).

According to the results obtained, in intact teeth, the diameter of the tubules varied from 0.5 to 2.5 microns. The width of the interchannel zone was from 4 to 8 microns. The maximum diameter of the tubules and the maximum number of tubules themselves were found in the periculpar dentin. The minimum values were found in the surface layers of dentin. In the intermediate layer of the mantle dentin, the diameter of the tubes was wider than in the surface zone, but their number was less than in the deep dentin. The dentine surface has uneven outlines due to bulges and crater-like depressions. The mouths of the tubules are usually located in the center of these depressions [41]. The walls of dentine tubes were uneven, which is due to the protrusion of individual mineral nanocrystals into their lumen, as well as micropores in the walls that serve for exchange between the tube and the intertubular zone [42]. Along the course of the tube, there were branches of a smaller diameter **(Figures 2c-2e)**.

In teeth with manifestations of increased erasability, the diameter of the tubules and their number were smaller both on the surface (3.8 times) and in the middle layer (2.2 times) than in the control group. The orientation of the tubes was indistinctly expressed, the surface had a flattened and smoothed relief. The uniformity of the dentin structure was most likely associated with inclusions of foreign substances, pigments penetrating from the oral cavity through the exposed surface of dentin in the abrasion facet, as well as with the protective function of the pulp and activation of the synthesis of substitutive (irregular) dentin [43, 44]. The lumen of the superficial tubules was reduced or even completely obliterated. Hypermineralization zones around dentine tubules were expanding, associated with a dense arrangement of mineral crystals and globules, and the microhardness of the surface layer was increasing. In deeper layers, pathological changes were less pronounced [45]. The structure of dentin resembles that of the 2nd and 3rd layers of healthy dentin. However, the lumen of the dentine tubules was still smaller up to 0.5 μm **(Figures 2f-2h)**.

Conclusion

Within the framework of this study, it was found that atomic force microscopy has great potential for application in the assessment of morphostructural changes in teeth with increased erasability. Notably, the experimentally revealed morphostructural changes in the hard tissues of teeth indicate the need for a differentiated approach to aesthetic and functional restoration with increased erasability and require a reasonable choice of restoration materials.

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Conflict of interest: None

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Ethics statement: All patients and their attending physicians have given voluntary, informed consent to the use of extracted teeth as a research object.

References

1. Beniash E, Stifler CA, Sun CY, Jung GS, Qin Z, Buehler MJ, et al. The hidden structure of human enamel. Nat Commun. 2019;10(1):4383. doi:10.1038/s41467-019-12185-7
2. Willershausen I, Krautkremer F, Hilbert T, Seidel CL, Geppert CI, Ghanaati S, et al. The comparison of the morphology of the mid-palatal suture between edentulous individuals and dentate jaws shows morphological differences. Ann Anat. 2022;243:151948. doi:10.1016/j.aanat.2022.151948

3. Gil-Bona A, Bidlack FB. Tooth enamel and its dynamic protein matrix. Int J Mol Sci. 2020;21(12):4458. doi:10.3390/ijms21124458
4. Al-Zahawi AR, Ibrahim RO, Talabani RM, Dawood SN, Garib DSH, Abdalla AO. Age and sex-related change in tooth enamel thickness of maxillary incisors measured by cone beam computed tomography. BMC Oral Health. 2023;23(1):971. doi:10.1186/s12903-023-03639-y
5. Sadyrin E, Swain M, Mitrin B, Rzhepakovsky I, Nikolaev A, Irkha V, et al. Characterization of enamel and dentine about a white spot lesion: Mechanical properties, mineral density, microstructure and molecular composition. Nanomaterials (Basel). 2020;10(9):1889. doi:10.3390/nano10091889
6. Gutnova TS, Kompantsev DV, Gvozdenko AA, Kramarenko VN, Blinov AV. Vitamin D Nanocapsulation. Izv Vyssh Uchebn Zaved Khim Khim Tekhnol. 2021;64(5):98-105. doi:10.6060/ivkkt.20216405.6399
7. Akalın TT, Bozkurt FO, Tuncer AK, Bağ HG, Özcan M. Clinical performance of nanofilled and microhybrid direct composite restorations on endodontically treated teeth. Eur J Prosthodont Restor Dent. 2019;27(1):39-47. doi:10.1922/EJPRD_01638Akalin09
8. Maran BM, de Geus JL, Gutiérrez MF, Heintze S, Tardem C, Barceleiro MO, et al. Nanofilled/nanohybrid and hybrid resin-based composite in patients with direct restorations in posterior teeth: A systematic review and meta-analysis. J Dent. 2020;99:103407. doi:10.1016/j.jdent.2020.103407
9. Antoniou I, Mourouzis P, Dionysopoulos D, Pandoleon P, Tolidis K. Influence of immediate dentin sealing on bond strength of resin-based CAD/CAM restoratives to dentin: A systematic review of in vitro studies. Biomimetics (Basel). 2024;9(5):267. doi:10.3390/biomimetics9050267
10. Omar N, Salem HN, Abdou A, Moharam LM. Effect of various irrigation protocols and antioxidant application on bonding performance of two adhesive systems to coronal dentin. J Clin Exp Dent. 2024;16(4):e406-15. doi:10.4317/jced.61303
11. Shabestari Khiabani S, Zivari-Ghader T, Aghazadeh M, Keyhanvar P, Shabestari Khiabani Z, Reyhanifar F, et al. Loading of VEGF and BMP2 to PNIPAAm-based hydrogel as a scaffold for bone regeneration. J Med Pharm Chem Res. 2024;6(9):1301-11. doi:10.48309/jmpcr.2024.450418.1165
12. Nikkerdar N, Golshah A, Salmani Mobarakeh M, Fallahnia N, Azizi B, Shoohanizad E, et al. Recent progress in the application of zirconium oxide in dentistry. J Med Pharm Chem Res. 2024;6(8):1042-71. doi:10.48309/jmpcr.2024.432254.1069
13. Gpv S, Shukla V, Raut AA, Ghosh M, Mustafa M, Suleiman Alofi R. In-Vitro evaluation of dental adhesive bond strength with diode laser irradiation before photopolymerization. Cureus. 2024;16(3):e56935. doi:10.7759/cureus.56935
14. García-Varela S, Ramos JC, Ginzo-Villamayor MJ, Castelo-Baz P, Méndez-Díaz R, Anache-D'Abate MA, et al. Effect of dentin irrigants on push-out bond strength in resin cementation protocols for fiber posts in endodontically treated teeth: An in vitro study. Materials (Basel). 2024;17(6):1432. doi:10.3390/ma17061432
15. Sheikhzadeh M, Nakhostin A, Sarlak H, Tahmasebi V. Effect of different bleaching procedures on hardness and surface roughness of nanohybrid composite restorations. J Med Pharm Chem Res. 2024;6(5):548-57. doi:10.48309/jmpcr.2024.420594.1019
16. Kornsombut N, Takenaka S, Sotozono M, Nagata R, Ida T, Manuschai J, et al. Antibiofilm properties and demineralization suppression in early enamel lesions using dental coating materials. Antibiotics (Basel). 2024;13(1):106. doi:10.3390/antibiotics13010106
17. Fischer ER, Hansen BT, Nair V, Hoyt FH, Dorward DW. Scanning electron microscopy. Curr Protoc Microbiol. 2012;25:2B. doi:10.1002/9780471729259.mc02b02s25
18. Leth Larsen MH, Dahl F, Hansen LP, Barton B, Kisielowski C, Helveg S, et al. Reconstructing the exit wave of 2D materials in high-resolution transmission electron microscopy using machine learning. Ultramicroscopy. 2023;243:113641. doi:10.1016/j.ultramic.2022.113641
19. Marchand E, Bertrand B, Hedouin V, Demondion X, Becart A. Study of root transparency in different postmortem intervals using scanning electron microscopy. Diagnostics (Basel). 2023;13(17):2808. doi:10.3390/diagnostics13172808
20. Baruwa AO, Martins JNR, Maravic T, Mazzitelli C, Mazzoni A, Ginjeira A. Effect of endodontic irrigating solutions on radicular dentine structure and matrix metalloproteinases-A comprehensive review. Dent J (Basel). 2022;10(12):219. doi:10.3390/dj10120219
21. Weerakoon AT, Cooper C, Sokolowski KA, Meyers IA, Thomson D, Ford PJ, et al. Effect of dentine site on resin and cement adaptation tested using X-ray and electron microscopy to evaluate bond durability and adhesive interfaces. Eur J Oral Sci. 2022;130(5):e12890. doi:10.1111/eos.12890
22. Witzke K, Frank M, Specht O, Schulz U, Oehlschläger C, Behrend D, et al. Comparative sample preparation using focused ion beam and ultramicrotomy of human dental enamel and dentine for multimicroscopic imaging at micro- and nanoscale. Materials (Basel). 2022;15(9):3084. doi:10.3390/ma15093084
23. Akabane C, Pabisch S, Wagermaier W, Roschger A, Tobori N, Okano T, et al. The effect of aging on the nanostructure of murine alveolar bone and dentin. J Bone Miner Metab. 2021;39(5):757-68. doi:10.1007/s00774-021-01227-0
24. Xia F, Youcef-Toumi K. Review: Advanced atomic force microscopy modes for biomedical research.

Biosensors (Basel). 2022;12(12):1116. doi:10.3390/bios12121116
25. Ho TM, Abik F, Mikkonen KS. An overview of nanoemulsion characterization via atomic force microscopy. Crit Rev Food Sci Nutr. 2022;62(18):4908-28. doi:10.1080/10408398.2021.1879727
26. Feng Y, Roos WH. Atomic force microscopy: An introduction. Methods Mol Biol. 2024;2694:295-316. doi:10.1007/978-1-0716-3377-9_14
27. Hu J, Gao M, Wang Z, Chen Y. Review on the applications of atomic force microscopy imaging in proteins. Micron. 2022;159:103293. doi:10.1016/j.micron.2022.103293
28. An Y, Manuguri SS, Malmström J. Atomic force microscopy of proteins. Methods Mol Biol. 2020;2073:247-85. doi:10.1007/978-1-4939-9869-2_14
29. Eaton P, Batziou K. Artifacts and practical issues in atomic force microscopy. Methods Mol Biol. 2019;1886:3-28. doi:10.1007/978-1-4939-8894-5_1
30. Kodera N, Ando T. Visualization of intrinsically disordered proteins by high-speed atomic force microscopy. Curr Opin Struct Biol. 2022;72:260-6. doi: 10.1016/j.sbi.2021.11.014
31. Demchenkov EL, Nagdalian AA, Budkevich RO, Oboturova NP, Okolelova AI. Usage of atomic force microscopy for detection of the damaging effect of CdCl2 on red blood cell membranes. Ecotoxicol Environ Saf. 2021;208:111683. doi:10.1016/j.ecoenv.2020.111683
32. Haynes PJ, Main KHS, Akpinar B, Pyne ALB. Atomic force microscopy of DNA and DNA-protein interactions. Methods Mol Biol. 2022;2476:43-62. doi:10.1007/978-1-0716-2221-6_5
33. Nezafat NB, Ghoranneviss M, Elahi SM, Shafiekhani A, Ghorannevis Z, Solaymani S. Microstructure, micromorphology, and fractal geometry of hard dental tissues: Evaluation of atomic force microscopy images. Microsc Res Tech. 2019;82(11):1884-90. doi:10.1002/jemt.23356
34. Doss BL, Konkol JA, Liu Y, Brinzari TV, Pan L. Correlative atomic force microscopy and raman spectroscopy in acid erosion of dentin. Microsc Microanal. 2023;29(5):1755-63. doi:10.1093/micmic/ozad094
35. Tamilselvi S, Nagate RR, Al-Ahmari MMM, Kokila G, Tikare S, Chaturvedi S. Comparison of the effect of sodium bicarbonate and glycine air polishing systems on tooth surface roughness: An atomic force microscopic analysis. Technol Health Care. 2021;29(3):489-98. doi:10.3233/THC-202210
36. Reier S, Turyanskaya A, Heimel P, Frischauf N, Meusburger D, Heuser T, et al. Cross-modality imaging of bisphosphonate-treated murine jawbones. Analyst. 2021;146(14):4683-99. doi:10.1039/d0an02373f
37. Cai J, Moradian-Oldak J. Triple function of amelogenin peptide-chitosan hydrogel for dentin repair. J Dent Res. 2023;102(13):1434-43. doi:10.1177/00220345231198228
38. Salman SQ, Hussein BMA. Topographical analysis of human enamel after phosphoric acid etching and Er, Cr: YSGG laser irradiation. J Lasers Med Sci. 2023;14:e68. doi:10.34172/jlms.2023.68
39. Solaymani S, Ghoranneviss M, Elahi SM, Shafiekhani A, Kulesza S, Ţălu Ş, et al. The relation between structural, rugometric, and fractal characteristics of hard dental tissues at micro and nano levels. Microsc Res Tech. 2019;82(4):421-8. doi:10.1002/jemt.23183
40. Steinbauer P, Rohatschek A, Andriotis O, Bouropoulos N, Liska R, Thurner PJ, et al. Single-molecule force spectroscopy reveals adhesion-by-demand in statherin at the protein-hydroxyapatite interface. Langmuir. 2020;36(44):13292-300. doi:10.1021/acs.langmuir.0c02325
41. Ibrahim S, Strange AP, Aguayo S, Shinawi A, Harith N, Mohamed-Ibrahim N, et al. Phenotypic properties of collagen in dentinogenesis imperfecta associated with osteogenesis imperfecta. Int J Nanomedicine. 2019;14:9423-35. doi:10.2147/IJN.S217420
42. Bakan F, Sezen M, Gecgin M, Goncu Y, Ay N. Structural and chemical analysis of hydroxyapatite (HA)-Boron Nitride (BN) nanocomposites sintered under different atmospheric conditions. Microsc Microanal. 2017;23(5):891-9. doi:10.1017/S1431927617012405
43. Mester A, Piciu A, Piciu D, Petean I, Lucaciu PO, Apostu D, et al. Disorders of dental hard tissues induced by radioiodine-131 (I-131) therapy used in differentiated thyroid cancer: An in vitro study. Biomedicines. 2020;8(11):475. doi:10.3390/biomedicines8110475
44. Chen Z, Nadeau B, Yu K, Shao X, He X, Goh MC, et al. Whole-field macro- and micro-deformation characteristic of unbound water-loss in dentin hard tissue. J Biophotonics. 2018;11(9):e201700368. doi:10.1002/jbio.201700368
45. Ahmadi S, Torab A, Negahdari R. Effect of zinc phosphate and non-eugenol temporary dental cement on different modifications of abutment in implant restoration. J Med Pharm Chem Res. 2020;2(12):1172-82.