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Dental Calculus – Nanocharacterization

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SUMMARY

Emerging technologies and new nanoscale information have potential to transform dental practice by improving all aspects of diagnostics and therapy. Nanocharacterization allows understanding of oral diseases at molecular and cellular levels which eventually can increase the success of prevention and treatment. Opto-magnetic spectroscopy (OMS) is a promising new technique based on light-matter interaction which allows insight into the quantum state of matter. Since biomolecules and tissues are usually paramagnetic or diamagnetic materials it is possible to determine the dynamics of para- and diamagnetism at different teeth structures using that method. The topography of the surface of a sample can be obtained with a very high resolution using atomic force microscopy (AFM), which allows observation of minimal changes up to 10 nm, while magnetic force microscopy (MFM) is used to record the magnetic field gradient and its distribution over the surface of a sample. The aim of this study was to determine the possibility of AFM and MFM for the characterization of dental calculus, and a potential application of OMS for the detection of subgingival dental calculus.

Keywords: dental calculus; nano-characterization; atomic force microscopy (AFM); magnetic force microscopy (MFM); opto-magnetic spectroscopy (OMS)

INTRODUCTION

According to the World Health Organization report, periodontal disease is one of leading worldwide diseases with high prevalence rate. The main cause of periodontal disease, dental calculus, contains irritating substances such as endotoxins and bacterial antibodies. Its complete removal is of great importance for the maintenance of periodontal health. The past few years have witnessed the development of several calculus detection techniques based on different technologies, such as fiberoptic endoscopy, spectro-optical technology, and autofluorescence. The main problem is the penetration of light through the gingival soft tissue, and masked intensity of fluorescence when calculus is covered with soft tissue [1].

The following methods for the characterization of dental calculus have been described in the literature: light microscopy, polarized light microscopy, scanning electron microscopy (SEM), high resolution electron microscopy (HREM) [2-6]. With the introduction of new microscopic and molecular methods, researchers have revealed that biofilms are not simple bacterial layers on different surfaces but rather biological systems highly organized, where bacteria develop coordinated and functional communities [7].

Atomic force microscopy (AFM) is a novel, nondestructive method for providing insight into the critical properties associated with bacterial cells and their related surface proteins. AFM has proved to be a powerful tool not only for imaging bacterial ultra-structure under *in situ* conditions, but also for determining mechanical properties and intermolecular forces [8].

The aim of this study was to determine the possibility of using atomic force microscopy (AFM) and magnetic force microscopy (MFM) for the characterization of dental calculus, and the potential application of innovative technique, opto-magnetic spectroscopy (OMS), for the detection of subgingival dental calculus.

MATERIAL AND METHODS

Sample preparation

Extracted human teeth with deposits of subgingival and supragingival dental calculus were used in this study. Teeth were extracted during regular student practical sessions at the Clinic of Oral Surgery, School of Dentistry, University of Belgrade. Teeth were cleaned and immersed in saline, and then “embedded” in resin. After the polymerization, the samples were prepared by sectioning parallel to the long axis of teeth using a microtome (Isomet 4000, Buehler).

Structural characteristics of the tooth surface with dental calculus were analyzed using AFM (JEOL, Japan), MFM (JEOL, Japan) and OMS (NanoLab, Belgrade).

Opto-magnetic spectroscopy (OMS)

Opto-magnetic spectroscopy (OMS) is a novel method based on interaction of electromagnetic radiation with electrons of the tested material [9]. In this method light is used as a “measuring probe” to test the material properties.

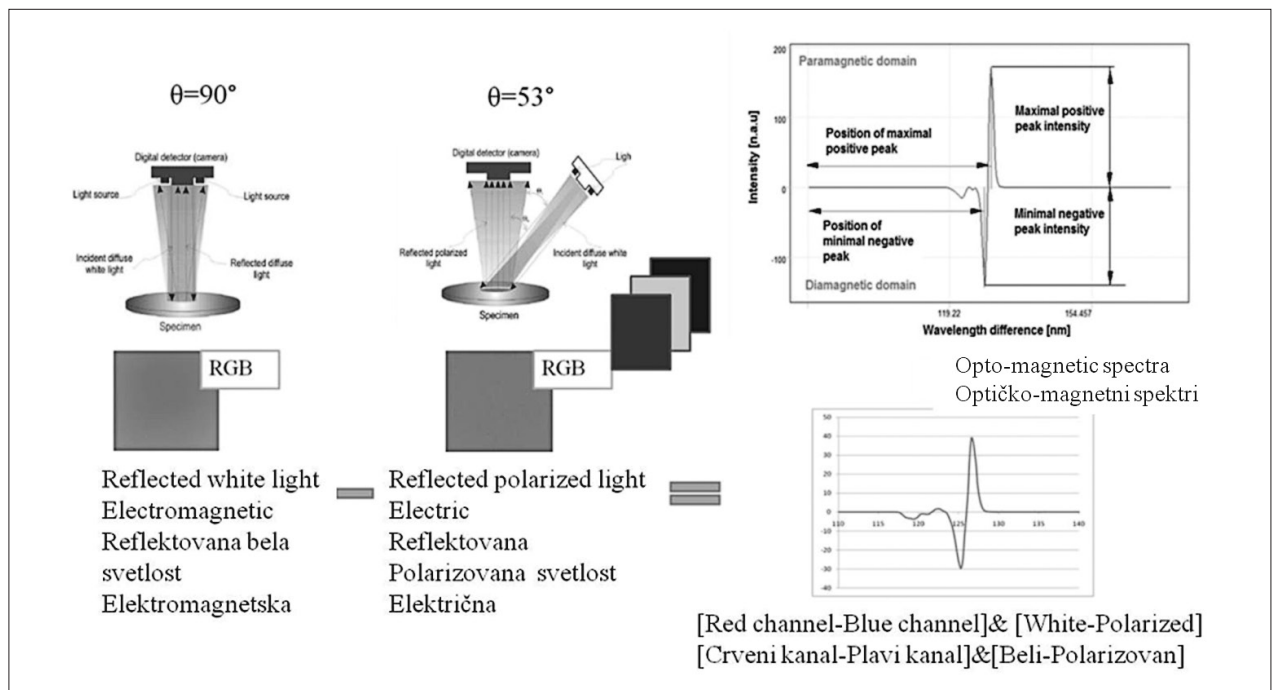


Figure 1. Schematic representation of the working principle for OMS device
Slika 1. Princip rada uređaja za optomagnetnu spektroskopiju

Only a light photon is small enough sensory element, created by nature, which guarantees high sensitivity and noninvasive interaction with matter [10, 11]. White diffuse light, with wavelengths in the range $\lambda \approx 400-800$ nm, is used in this method. It enables gathering information about higher levels of organization of biological macromolecules (tertiary and quaternary structure). An image obtained by classical optical microscopy is based on electromagnetic properties of both light source and matter, while OMS is based on subtraction of diffuse reflected white light and polarized reflected white light. Polarized reflected light is obtained when incident light in interaction with a sample acquires characteristics of polarized light. Polarization occurs due to the dependence of the direction of reflected light beam on the angle of incidence. In the case of sample illumination under certain angle the most of the incident light beam is weakened and the reflection of the smaller part of light which becomes plane polarized is achieved. The special value of the angle of incidence at which this effect is achieved is named Brewster's angle (Figure 1).

Atomic Force Microscopy (AFM)

The main advantage of AFM is that it enables simultaneous visualization and material characterization by:

- Measuring morphological characteristics on micro- and nano- dimensional level. Unlike other microscopy techniques (SEM, TEM) AFM spatial 3D topographic measuring gives quantitative information regarding third dimension, height, in every measuring point of the sample surface. This specific information and the nanometer level of precision gives basics for positional sensor control used in physico-chemical characterization of the sample.

- Characterization of physical quantities (mechanical, electrical, magnetic) in conjunction with advanced measuring techniques such as phase-contrast imaging which provides complex information about the sample. Interpretation of such information leads to new insights regarding material properties.

Mentioned advantages give an excellent basis for digital processing of recorded signals giving substantially facilitated way for quantitative analysis, not possible with other methods.

In this study the system JSPM-5200, JEOL (Japan) was used. Nano-console sensors were manufactured by Micro-Masch (Estony) i Nanosensors (Switzerland).

RESULTS AND DISCUSSION

OMS is a method for characterization of biological materials which enables insights into quantum states of matter due to the magnetic component of light waves. The change in quantum states of a sample surface has a unique bilateral correspondence with conformational changes of molecules on the sample surface (to the depth of few nanometers, or even few millimeters, depending on the translucency of the sample) therefore; OMS is a complementary method with AFM and MFM. While NanoProbe methods enable getting conformational states of individual molecules from scanned surfaces of a sample in range of 10 nm – 100 μ m, with OMS it is possible to get total (statistically relevant) information regarding conformational changes of surface molecules in the measured sample. It is important to have in mind that OMS is qualitative and not quantitative method used for the characterization of surface structure of the material. So far, in the research of surface structure of teeth and tooth deposits, conducted

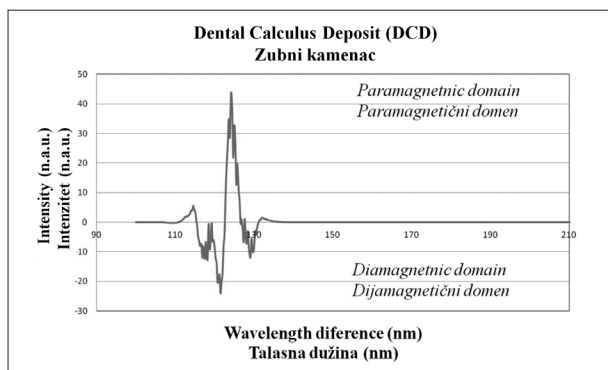


Figure 2. OMS diagram for dental calculus deposit
Slika 2. OMS dijagram za naslage zubnog kamenca

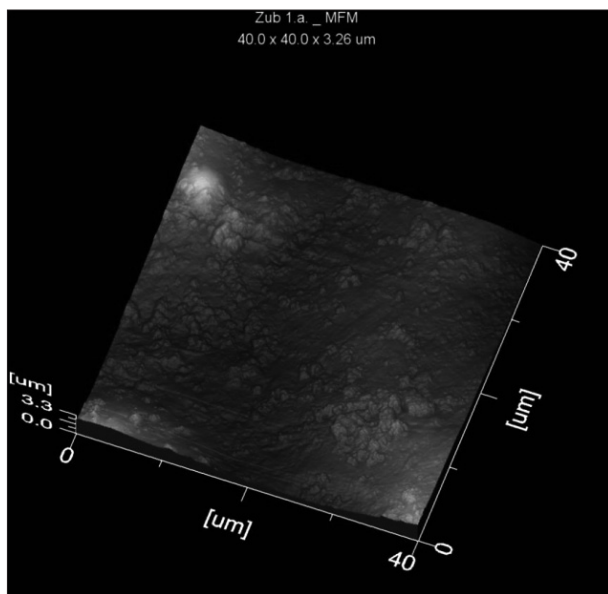


Figure 4. 3D topography of DCD on a tooth surface
Slika 4. 3D topografija naslage kamenca na površini zuba

in the NanoLab laboratory of the Faculty of Mechanical Engineering in Belgrade, OMS has shown high specificity. Figure 2 shows an OMS diagram for dental calculus deposits (DCD). Diagrams like this enable comparative characterization of DCD on different teeth regarding chemical composition, micro and nano topography of the surface and magnetic properties of the material. With the application of this method on different tooth samples it is possible to determine dynamics of para- and diamagnetism depending on different factors such as teeth age, the existence of carries or changes in enamel structure (e.g. hypomineralization). Based on Figure 2 it is possible to draw a conclusion that the DCD on a given tooth shows dominantly paramagnetic properties, peak in the paramagnetic domain for $\Delta\lambda=124.16$ nm with the intensity of $I=43.68$ n.a.u. and peak in the diamagnetic domain for $\Delta\lambda=120.71$ nm with the intensity of $I=020.35$ n.a.u.

The analysis of the topography of DCD in a few spots, which was routinely implemented in associated software for image processing, was also conducted. Figure 3 shows a generic report as an illustration of quantitative analysis regarding surface roughness and morphometric characteristics of DCD, which can readily be performed with the use of AFM.

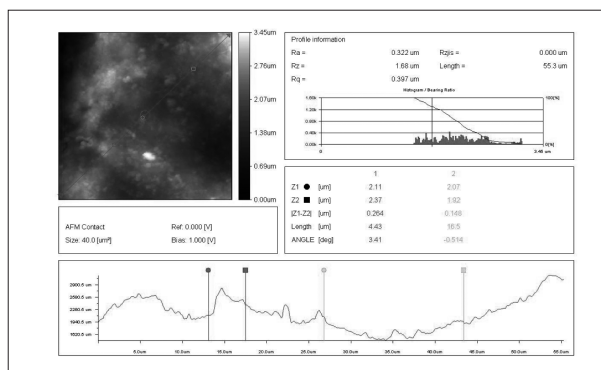


Figure 3. Generic report as an illustration of quantitative analysis done by associated software, which can readily be performed in samples scanned with AFM

Slika 3. Generički izveštaj pripadajućeg softvera kao ilustracija kvantitativne analize uzorka koju je moguće rutinski uraditi kod uzoraka snimljenih metodom AFM

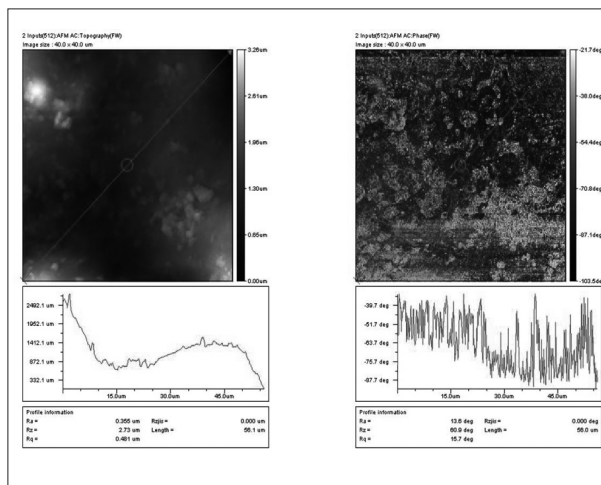


Figure 5. MFM of DCD
Slika 5. Mikroskopija magnetnih sila kamenca recentnog zuba

The actual report refers to the measurement at a particular direction. The detailed image of the analyzed region is given in the top left corner. The top right corner gives information for marked direction regarding average roughness (R_a , R_z , R_q), marker distances (red and green markers) as well as roughness histogram and bearing ratio. In the middle of the right side the height bar is given and in the bottom of the image the diagram with the height profile for marked direction. Marker placement (red and green markers) enables getting the information about structure dimensions for the chosen direction. It is possible to perform analysis for both linear and surface elements of the image. It is also possible to perform multi profile analysis. Similar analysis can also be conducted for physical quantities (magnetic and electric properties, mechanical material characteristics). The software allows various methods of digital image processing and analysis, enabling e.g. extraction of grains of a given size, and associated statistical analysis [12].

Figure 4 shows 3D topography of DCD on the surface of an extracted tooth. This allows studying the spatial structure of a given surface morphology with nanometer precision, potentially giving the possibility to detect different morfo-biological formations.

Magnetic force microscopy (Figure 5) allows the comparison of results related to magnetism with the results obtained using OMS. Top left picture of Figure 5 shows recorded surface topography, in the bottom is the diagram with the profile in selected direction, in the top right part is the magnetic field gradient of the same area and the lower right is the profile of the phase shift of the probe due to the magnetic field gradient in chosen direction.

CONCLUSION

Nanocharacterization of tooth surface, supra and subgingival deposits, may lead to a better understanding of the structural and functional physiological and biophysical characteristics of these structures. OMS, as a non-invasive method that has high specificity in the comparative characterization of tooth surfaces, potentially has the ability for accurate diagnostics of dental diseases in early stages. Opto-magnetic method can detect very small difference between normal and pathological tissue which is the most important advantage in comparison with classical methods. Using AFM technique, which is complementary to OMS method, diseases such as dental caries, periodontitis and oral cancer can be quantified based on morphological, biophysical and biochemical nanoscale properties of oral cavity surfaces.

ACKNOWLEDGEMENTS

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Zubni kamenac – karakterizacija na nanonivou

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KRATAK SADRŽAJ

Inovativne tehnologije i nove informacije na nanonivou imaju potencijal da poboljšaju stomatološku praksu, unapređujući sve aspekte dijagnostike i terapije. Nanokarakterizacija omogućava razumevanje razvoja oboljenja na ćelijskom i molekularnom nivou, što za krajnji rezultat može imati poboljšanje prevencije i uspeha lečenja. Optomagnetna spektroskopija (engl. *opto-magnetic spectroscopy* – OMS) je nova tehnika koja obećava, a zasnovana je na interakciji svetlosti i materije koja omogućava uvid u kvantno stanje materije. S obzirom na to da biomolekuli i tkiva mogu ispoljavati osobine paramagnetičnosti, odnosno dijamagnetičnosti, ovom metodom moguće je utvrditi dinamiku paramagnetizma i dijamagnetizma na različitim strukturama zuba. Pomoću mikroskopije atomskih sila (engl. *atomic force microscopy* – AFM) moguće je dobiti topografiju površine uzorka vrlo visoke rezolucije, čime se uočavaju i najmanje promene sve do 10 nm, dok se mikroskopija magnetnih sila (engl. *magnetic force microscopy* – MFM) koristi za snimanje gradijenta magnetnog polja i njegove raspodele po površini uzorka. Cilj ovog rada bio je da prikaže mogućnosti primene AFM i MFM za karakterizaciju zubnog kamenca, kao i primene OMS za otkrivanje subgingivalnog kalkulusa.

Glavne reči: zubni kamenac; nanokarakterizacija; mikroskopija atomskih sila (AFM); mikroskopija magnetnih sila (MFM); optomagnetna spektroskopija (OMS)

UVOD

Prema izveštaju Svetske zdravstvene organizacije, oboljenje parodontijuma je veoma često oboljenje kod ljudi širom sveta s visokom stopom prevalencije. Glavni uzročnik parodontopatije je zubni kamenac, koji sadrži iritirajuće supstance, kao što su endotoksini i bakterijska antitela. Njegovo potpuno uklanjanje je izuzetno važno za očuvanje zdravlja parodontijuma. U poslednjih nekoliko godina za otkrivanje subgingivalnog kamenca primenjuju se fiberoptička endoskopija, spektrooptička tehnologija i autofluorescencija. Glavni problem predstavlja prodiranje svetlosnog zraka kroz meko tkivo gingive, a intenzitet fluorescencije je maskiran kada je kalkulus prekriven mekim tkivom [1].

Dosad su u literaturi opisane sledeće metode za karakterizaciju zubnog kamenca: svetlosna mikroskopija, mikroskopija s polarizovanom svetlošću, skening-elektronska mikroskopija (engl. *scanning electron microscopy* – SEM), elektronska mikroskopija visoke rezolucije (engl. *high resolution electron microscopy* – HREM) [2-6]. Primenom novih mikroskopskih i molekularnih metoda istraživači su pokazali da biofilmovi nisu proste bakterijske naslage na različitim površinama, već da su u pitanju biološki sistemi s visokim stepenom organizovanosti, u kojima mikroorganizmi grade koordinisane i funkcionalne zajednice [7].

Mikroskopija atomskih sila (engl. *atomic force microscopy* – AFM) je nova, nedestruktivna metoda za sticanje uvida u međusobnu povezanost bakterijskih ćelija i njihove veze s površinskim proteinima. AFM bi mogao biti moćno sredstvo ne samo za snimanje bakterijskih ultrastrukturnih površina u uslovima *in situ*, već i za određivanje mehaničkih osobina i međumolekularnih snaga [8].

Cilj ovog rada bio je da se prikažu mogućnosti primene AFM, mikroskopije magnetnih sila (engl. *magnetic force microscopy* – MFM) za karakterizaciju zubnog kamenca, kao i primene inovativne tehnike, optomagnetne spektroskopije (engl. *opto-magnetic spectroscopy* – OMS) za otkrivanje subgingivalnog kalkulusa.

MATERIJAL I METODE RADA

Priprema uzoraka

U ovoj studiji su korišćeni humani ekstrahovani zubi s naslagama subgingivalnog i supragingivalnog zubnog kamenca. Zubi su izvađeni uobičajenim postupkom tokom redovnih studentskih vežbi na Klinici za oralnu hirurgiju Stomatološkog fakulteta Univerziteta u Beogradu. Uzorci su očišćeni od krvi i potopljeni u fiziološki rastvor, a potom „uronjeni“ u akrilat. Nakon polimerizacije, sečeni su na mikrotomu (*IsoMet 4000*, *Buehler*) šajbnom, paralelno s uzdužnom osom zuba.

Strukturna obeležja površine zuba s kamencem analizirana su metodama AFM (*JEOL*, Japan), MFM (*JEOL*, Japan) i OMS (*NanoLab*, Beograd).

Optomagnetna spektroskopija (OMS)

OMS je tehnika zasnovana na interakciji elektromagnetnog zračenja s valentnim elektronima materije [9]. Kod ove metode svetlost se koristi kao merno sredstvo (sonda) kojim se ispituju osobine materijala zbog toga što je samo foton dovoljno mali senzorni modalitet koji je stvorila priroda kojim se može ostvariti garantovano dovoljna osetljivost i neinvazivnost interakcije s materijom [10, 11]. Kod ove metode se koristi bela difuzna svetlost, čije su talasne dužine u opsegu od ≈400 do 800 nm, što omogućava dobijanje informacija o višim nivoima organizacije bioloških makromolekula (tercijarna i kvarтерна struktura). Slika dobijena klasičnim optičkim mikroskopom zasnovana je na elektromagnetnim osobinama izvora i materije, dok je OMS zasnovana na različitim difuzno reflektovane bele svetlosti i polarizovano reflektovane bele svetlosti. Polarizovano reflektovana svetlost nastaje kada upadna svetlost u interakciji s uzorkom poprimi osobine polarizovane svetlosti. Do polarizacije dolazi usled pravca prostiranja reflektovane svetlosti od upadnog ugla. U slučaju osvetljavanja površine uzorka pod određenim uglom postiže se slabljenje većeg dela upadne svetlosti i odbijanje samo njenog manjeg dela, koji

postaje planarno polarizovan. Posebna vrednost upadnog ugla pri kojoj se postiže ovaj efekat naziva se Brusterovim uglom (Slika 1).

Mikroskopija atomskih sila (AFM)

Glavna prednost metode AFM sastoji se od mogućnosti istovremene vizuelizacije i ispitivanja osobina materijala kroz:

- merenja morfoloških osobina na nivou mikro i nano dimenzione skale. Informacija dobijena topografskim merenjima odnosi se na trodimenzionalan prikaz uzorka u svakoj tački na osnovu kojeg je moguće steći kvantitativnu informaciju o trećoj, visinskoj, dimenziji uzorka, što je osobina koja se ne sreće kod drugih mikroskopskih metoda (SEM i TEM). Upravo ta informacija, kao i nivo njene tačnosti (nanometarska), jesu osnova za upravljanje pozicijom senzora koji se koristi u fizičko-hemijskoj karakterizaciji uzoraka;
- karakterizaciju fizičkih veličina (mehaničkih, električnih, magnetnih) na osnovu kojih se uz napredne režime, poput fazno-kontrastnog snimanja, dobijaju kompleksne informacije o uzorku čijom interpretacijom se stižu novi uvidi u osobine materijala.

Pomenute prednosti su odlična osnova za digitalnu obradu i manipulaciju snimljenih signala kojom se na znatno olakšan način mogu vršiti kvantitativne analize koje nisu moguće drugim metodama.

U ovom istraživanju je korišćen sistem *JSPM-5200*, *JEOL* (Japan). Nanokonzolni senzori su proizvodi firme *MicroMasch* (Estonija) i *Nanosensors* (Švajcarska).

REZULTATI I DISKUSIJA

OMS, kao metoda karakterizacije bioloških materijala, zahvaljujući magnetnoj komponenti svetlosti, omogućava uvid u kvantno stanje materije. Kako je promena kvantnih stanja površine uzorka obostrano jednoznačno korespondentna s konformacionim promenama molekula na površini uzorka (dubine od nekoliko nanometara do nekoliko milimetara, u zavisnosti od prozirnosti uzorka), to je OMS komplementarna metoda AFM i MFM. Dok se tehnikom *NanoProbe* sa skenirane površine od 10 nm do 100 μ m mogu dobiti konformaciona stanja pojedinačnih molekula, OMS tehnikom se mogu dobiti ukupne (statistički relevantne) informacije o konformacionim promenama molekula snimanog uzorka. Bitno je naglasiti da je OMS kvalitativna, a ne kvantitativna metoda, koja se koristi za karakterizaciju površinskih struktura materijala. OMS je u dosadašnjim istraživanjima u laboratoriji Mašinskog fakulteta u Beogradu pokazala visoku specifičnost pri ispitivanju površinskih struktura zuba i zubnih naslaga. Na slici 2 je prikazan OMS dijagram za kamenac zuba. Ovakvi dijagrami nam omogućavaju komparativnu karakterizaciju naslaga kamenca na različitim zubima, u pogledu hemijskog sastava, mikrotopografije i nanotopografije površine, te magnetnih svojstava materijala. Upotrebom ove metode na različitim uzorcima zuba moguće je utvrditi dinamiku paramagnetizma i dijamagnetizma u zavisnosti od različitih faktora, kao što su starost zuba, postojanje karijesa i promene strukture gleđi (npr. hipomineralizacija). Može se uočiti da kamenac na datom zubu pokazuje dominantno para-

magnetna svojstva, da je vrh krive u paramagnetnom domenu na različitoj talasnoj dužini 124,16 i inteziteta od 43,68 jedinica normalizovane dodeljene vrednosti, a vrh krive u dijamagnetnom domenu na različitoj talasnoj dužini 120,71 i inteziteta od -20,35 jedinica normalizovane dodeljene vrednosti.

Takođe je urađena analiza topografija zubnog kamenca u nekoliko tačaka koja se rutinski vrši u programu za obradu snimaka. Na slici 3 je prikazan generički izveštaj pripadajućeg softvera kao ilustracija kvantitativne analize (hrapavosti i morfometrije) uzorka zubnog kamenca koju je moguće rutinski uraditi kod uzoraka snimljenih metodom AFM. Konkretni izveštaj se odnosi na merenje parametara na datom pravcu. U gornjem levom uglu prikazan je snimak detalja analizirane regije. U gornjem desnom delu su date informacije o pravcu označenom na slici u pogledu proračunate srednje hrapavosti (R_a , R_z , R_q), iznosa označenog rastojanja, kao i raspodele hrapavosti. Srednji deo s desne strane i donji deo slike daju informaciju o visini svih tačaka na izabranom pravcu. Postavljanjem markera moguće je dobiti i informaciju o dimenzijama strukture u datom pravcu (crveni i zeleni markeri). Analizu je moguće izvesti kako na linijskim, tako i na površinskim elementima snimka. Takođe se može uraditi uporedna analiza nekoliko snimaka odjednom. Sličnu analizu moguće je obaviti i za merne podatke fizičkih veličina (magnetna i električna svojstva, mehaničke osobine materijala). Softver dozvoljava i različite metode digitalne obrade i analize slike, čime je omogućeno npr. izdvajanje zrna zadatih dimenzija, te pripadajuća statistička analiza [12].

Na slici 4 je prikazana trodimenzionalna topografija naslaga kamenca na površini ekstrahovanog zuba. Ovakav prikaz omogućava proučavanje prostorne morfologije površine date strukture s nanometarskom preciznošću, što pruža mogućnost otkrivanja različitih morfobioloških formacija.

Korišćenje metode MFM (Slika 5) omogućava i upoređivanje rezultata koji se odnose na magnetizam s rezultatima dobijenim metodom OMS. Gore levo prikazana je topografija snimane površine, dole levo profil na izabranom pravcu, gore desno snimak gradijenta magnetnog polja za istu površinu, a dole desno profil faznih pomeranja sonde usled gradijenta magnetnog polja na izabranom pravcu.

ZAKLJUČAK

Proučavanje površina zuba i zubnih naslaga na nanometarskom nivou može dovesti do boljeg razumevanja strukturnih, funkcionalnih, fizioloških i biofizičkih odlika ovih struktura. OMS, kao neinvazivna metoda koja pokazuje visoku specifičnost pri komparativnoj karakterizaciji zubnih površina, pruža mogućnost precizne dijagnoze bolesti zuba u ranom stadijumu. Mogućnost optomagnetne metode da otkrije vrlo malu razliku između normalnih i patoloških tkiva je njena glavna prednost u odnosu na klasične metode. Pomoću tehnike AFM, komplementarne OMS, bolesti kao što su karijes, parodontopatije i oralni karcinom mogu se kvantifikovati na osnovu morfoloških, biofizičkih i biohemijskih nanosvojstava površina tkiva u unutrašnjosti usne duplje.

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