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Mapiranje bioimpedancijskog spektra zdrave oralne sluznice: eksperimentalna studija

Mapping Electrical Impedance Spectra of the Healthy Oral Mucosa: a Pilot Study

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Sažetak

Svrha ispitivanja: Bioimpedancija je otpor prolasku električne struje kroz tkivo i ovisi o njegovoj strukturi i kemijskom sastavu. Svrha istraživanja bila je mapirati bioimpedancijske spektre za svaku regiju usne šupljine. **Ispitanici i postupci:** U mjerenju bioimpedancije sudjelovalo je tridesetero ispitanika s urednim nalazom oralne sluznice, a obavljalo se na 14 lokalizacija u usnoj šupljini s lijeve i desne strane te je ponovljeno nakon sedam i četrnaest dana. **Rezultati:** Najniže vrijednosti izmjerene su na dorzumu jezika, a najviše na tvrdom nepcu. Nije ustanovljena statistički značajna razlika između mjerenja na lijevoj i desnoj strani. Statistički značajno više vrijednosti izmjerene su ženama na sluznici gornje usne, dorzumu jezika i na ventralnom dijelu jezika. Utvrđena je statistički značajna razlika između nepušača i pušača na sluznici donje usne i sluznici dna usne šupljine. Vrijednosti bioimpedancije bile su u negativnoj korelaciji s količinom sline na sluznici gornje usne, na tvrdom nepcu, dorzumu jezika i na podjezičnoj sluznici. Mjerenja pri niskim frekvencijama imala su veću varijabilnost. **Zaključak:** Vrijednosti bioimpedancije najčešće ovise o stupnju keratinizacije sluznice. Na njihove vrijednosti vjerojatno utječu demografski i klinički čimbenici, što je potrebno razjasniti u daljnjim studijama s većim brojem ispitanika.

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Ključne riječi
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Uvod

Električna impedancija (EI) ili električna otpornost mjera je kojom se izražava otpor prolasku električne struje kroz tkivo. EI je obilježje svih živih tkiva i ovisi o njegovoj strukturi i kemijskom sastavu. Promjene u strukturi i/ili kemijskom sastavu rezultirat će promjenom električne otpornosti tkiva. Na tom principu zasniva se primjena EI-ja u dijagnostičke svrhe u medicini i stomatologiji (1, 2). Dijagnostičkim metodama koje se temelje na mjerenju EI-ja koristimo se u različitim područjima medicine. Najčešće, i povijesno najdulje (Fere i Tarhanov 1888. godine), bioimpedancijska spektrografija upotrebljava se u slučaju promjena na koži (3 – 6). EI se primjenjuje i u određivanju veličine ishemijske bolesti mišića (2), a metode bioimpedancijske tomografije pomažu u procjeni veličine ishemičnih srčanih zastoja i plućnih edema (7, 8). U sportskoj medicini i u medicinskoj rehabilitaciji mjerenjima EI-ja određuje se povećanje tjelesne mase zbog nagomilavanja masti i vode (9). Posljednjih godina ove se metode sve češće primjenjuju u detekciji tumorskih promjena u različitim tkivima. Tako se impedancijskom spektrografijom mogu procjenjivati različite tumorske tvorbe na koži, u dojci i na sluznici ženskih reproduktivnih organa (3, 4, 10, 11). Bioimpedacijska mjerenja već su ustaljena u stomatološkoj praksi korištenjem različitih apeksnih lokatora za određi-

Introduction

Electrical impedance (EI) or electrical resistance is a measure which represents the resistance to electric current flow through a tissue. EI is a property of all living tissues and depends on the structure and chemical composition of the tissue. Structural and/or chemical changes result in changes in the electrical resistance of the tissue. The application of EI in medical and dental diagnostics is based on this principle (1, 2). Diagnostic methods based on EI measurement are used in different fields of medicine. EI spectroscopy, which is the most widely used method, has a long history (Fere and Tarhanov 1888.g), and has been mostly used for the assessment of skin lesions (3-6). EI is also applied in the assessment of the severity of muscle ischaemia (2). Methods of EI tomography assist in the evaluation of ischemic heart disease and pulmonary edema (7, 8). In sports medicine and rehabilitation, EI measurements determine the increase of body mass due to accumulation of fat and water (9). Lately, these methods have been increasingly used in the detection of tumors in different tissues. Thus, EI spectroscopy can be used to assess different tumor lesions on the skin, breast and female reproductive organs (3, 4, 10, 11). The application of EI measurements has already been established in dentistry through the use of different apex locators for the root canal length de-

vanje dužine korijenskih kanala (12, 13). Primjena EI-ja za dijagnostiku patoloških promjena na oralnoj sluznici dosad nije dovoljno evaluirana. Nicader i suradnici (14) su, mjereći EI oralne sluznice zdravih ispitanika, ustanovili da postoje razlike između keratinizirane, nekeratinizirane i specijalizirane oralne sluznice. Isti autori (15) utvrdili su statistički značajne razlike na EI-ju zdrave oralne sluznice i sluznice tretirane kemijskim iritansom (natrijev lauril-sulfat). Ching i suradnici (16) te Sun i njegovi kolege (17) utvrdili su statistički značajne razlike na EI-ju zdrave oralne sluznice i ranoga karcinoma jezika. Murdoch i suradnici (18) izvijestili su o značajno nižim vrijednostima EI-ja u slučaju oralnih karcinoma i lezija s teškom displazijom, u usporedbi s umjerenom displazijom i zdravom oralnom sluznicom. Zbog neinvazivnosti, metode temeljene na EI-ju imaju određenu prednost nad invazivnim metodama – jednostavnije se obavljaju, ugodnije su pacijentu i manji su problem kad je riječ o dezinfekciji, sterilizaciji i kontroli infekcije (1, 16, 19). Da bismo se EI-jom mogli koristiti u dijagnostici patoloških promjena oralne sluznice, potrebne su polazišne osnove za komparaciju tj. treba se odrediti raspon referentnih vrijednosti EI-ja na zdravoj oralnoj sluznici. Svrha ovog eksperimentalnog istraživanja bila je:

- utvrditi raspone vrijednosti EI-ja na zdravoj oralnoj sluznici zdravih mladih ispitanika,
- mapirati EI spektre različitih regija usne šupljine,
- ustanoviti na kojoj su frekvenciji mjerenja najkonzistentnija,
- utvrditi utječu li na vrijednosti EI-ja demografski i klinički parametri, tj. čimbenici poput dobi, spola, količine sline ili navike poput pušenja.

Ispitanici i postupci

Ispitanici

U istraživanju je sudjelovalo 30 zdravih ispitanika u dobi od 20 do 40 godina. Svima je objašnjena svrha studije, pa su dragovoljno pristali sudjelovati o čemu svjedoče potpisani informirani pristanci. Kriterij za uključivanje bio je klinički uredan nalaz oralne sluznice, odsutnost sistemskih bolesti i razumijevanje teksta informiranog pristanka. Za potrebe istraživanja zabilježeni su sljedeći podatci: dob, spol, pušenje i količina nestimulirane sline. Količina sline mjerila se prije svakog mjerenja, između 8 i 10 sati Wu Wangovom metodom (20). Jedan sat prije mjerenja ispitanici nisu jeli, pili, prali zube niti žvakali žvakaću gumu. Tijekom pet minuta ispitanici su slinu skupljenu u ustima izbacivali u graduiranu sterilnu epruvetu. Ukupni volumen sline podijeljen je s pet kako bi se dobila količina izražena u ml/min.

Postupci

Mjerenje EI-ja obavljalo se s pomoću posebnog uređaja izrađenog za tu namjenu (21), a sastojao se od intraoralnog senzora, mjernog instrumenta i prijenosnog računala (slika 1.). Intraoralni senzor imao je ugrađene tri koncentrične elektrode izrađene od sinterirane aluminijske legure visoke provodljivosti, ukupnog promjera 8 milimetara obložene

termination (12, 13). However, the application of EI in the diagnostics of oral mucosal lesions has not been adequately evaluated so far. Nicader et al. (14) performed EI measurements of the oral mucosa in healthy subjects and established differences between keratinized, non-keratinized epithelium and specialized oral mucosa. The same authors (15) found statistically significant EI variations between the healthy oral mucosa and mucosa treated with a chemical irritant (sodium lauryl sulphate). Ching et al. (16) and Sun et al. (17) found statistically significant differences in EI between the healthy oral mucosa and early tongue cancer. Murdoch et al. (18) reported significantly lower values of EI in oral cancer and severely dysplastic lesions compared to mild dysplasia and healthy oral mucosa. Being non-invasive, methods based on EI have certain advantages over invasive methods: they are simpler to perform, more comfortable for the patient and pose a lesser problem regarding disinfection, sterilization and infection control (1, 16, 19). In order to use EI spectroscopy in the diagnostics of oral mucosal lesions, initial values for the comparison are needed i.e. it is necessary to determine the range of EI reference values on the healthy oral mucosa. The aims of this pilot study were: to determine the ranges of EI values on the healthy mucosa in healthy young subjects, to map EI spectra of different regions of the oral cavity, to establish frequencies which provide the most consistent measurements, to evaluate the effect of demographic and clinical parameters such as age, gender, the amount of saliva, or habits such as smoking on the EI measurement.

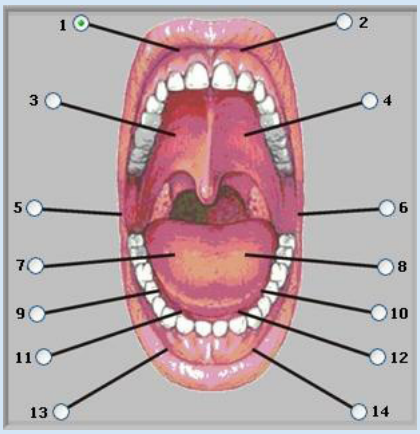
Materials and methods

Subjects

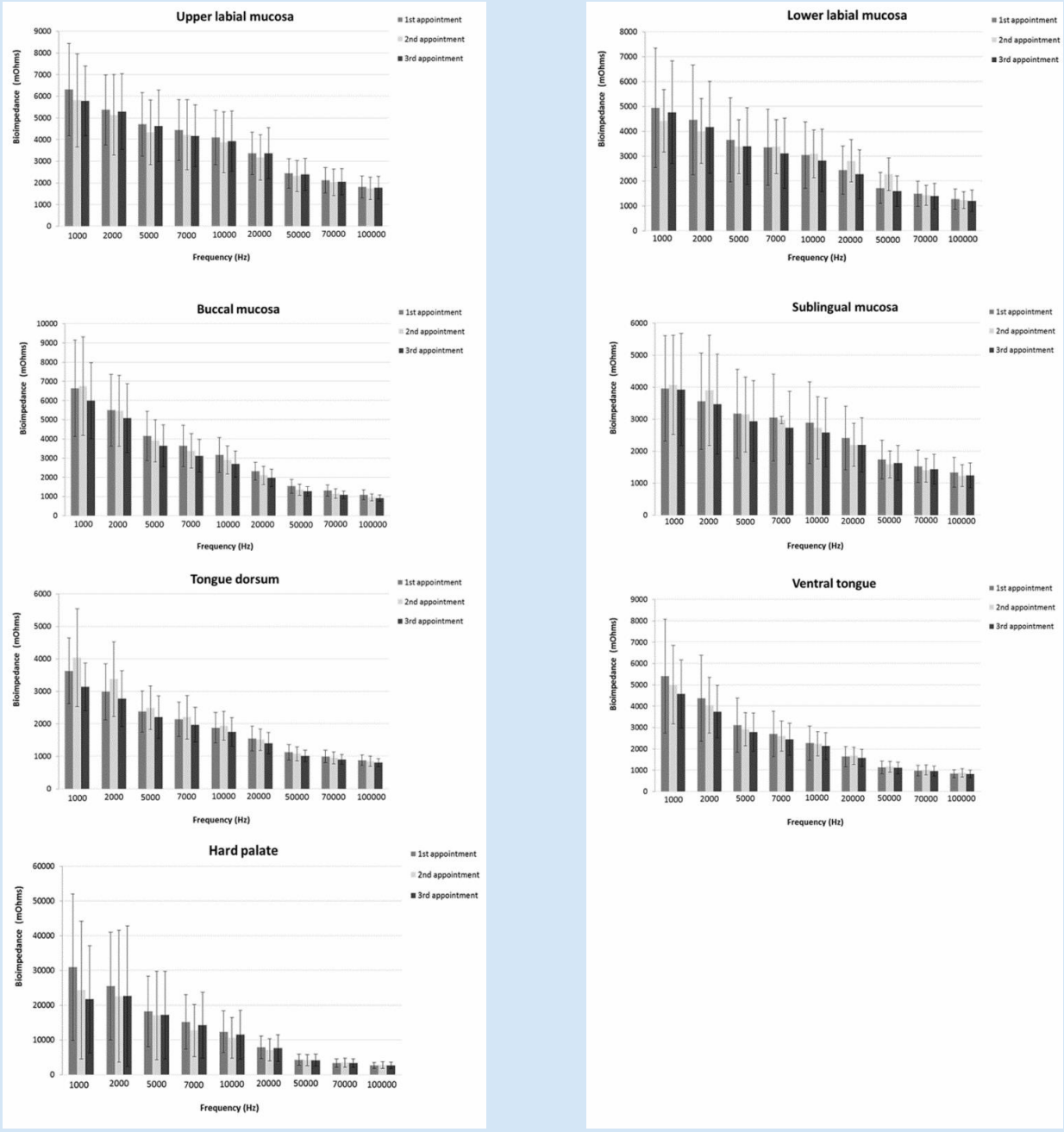
The study included 30 healthy subjects aged between 20 – 40 years. All subjects were informed about the aims of the study and signed the informed consent according to the declaration of Helsinki. The inclusion criteria were clinically normal oral mucosa, absence of systemic diseases and understanding the text of informed consent. The following patient data were recorded: age, gender, smoking and unstimulated salivary flow rate. Salivary flow was measured prior to each impedance measurement, between 8–10 AM, using the method by Wu Wang (20). One hour before the measurement, the subjects did not eat, drink, brush their teeth, or chew a chewing gum. During 5 min, participants expectorated saliva accumulated in the mouth into a sterile graded test tube. The total volume of saliva was divided by 5 to obtain the quantity in ml/min.

Methods

EI measurement was performed using a measuring set designed specifically for this purpose (21). The measuring set included an intraoral sensor, a measuring device and a laptop (Figure 1). The intraoral sensor consisted of three concentric rings made of high conductivity aluminum alloy, with a total diameter of 8 mm coated with an insolation lay-



Slika 1 Mjerni uređaj.
Figure 1 Set for the electrical impedance measurement.
Slika 2. Anatomske regije s mjernim točkama.
Figure 2 Anatomical regions with selected measuring points.



Slika 3. Intraindividualne varijacije mjerenja
Figure 3 Intra-individual variation (values of individual measurements).

izolacijskim slojem od teflona. Da bi se ujednačio pritisak elektrode i kontakt sa sluznicom, senzor je bio spojen na stomatološku sisaljku koja je proizvodila konstantni podtlak od 250 mB čime se osiguravala stabilnost senzora tijekom mjerenja. Preko električnih vodiča intraoralni senzor bio je spojen na mjerni uređaj NI USB-6251(National Instruments®, Austin, SAD) koji je USB-konekcijom bio povezan s prijenosnim računalom. Mjerni program, izrađen na osnovi programskog paketa LabWiev 8.5.1.(National Instruments®, Austin SAD), pretvarao je električne impulse iz uređaja u digitalne zapise i pohranjivao ih u bazu podataka. Provjera i kalibracija mjernog uređaja obavljena je u Zavodu za automatiku i elektroniku Tehničkog fakulteta u Rijeci korištenjem konstantnoga električnog otpora u nadomjesnoj shemi. EI se mjerio na sedam referentnih lokalizacija u usnoj šupljini kako slijedi:

1. sluznica gornje usne – u projekciji između vrhova korjenova gornjih drugih sjekutića i očnjaka;
2. sluznica donje usne – u projekciji između vrhova korjenova drugog donjeg sjekutića i očnjaka;
3. sluznica tvrdog nepca – u razini vrha palatinalnog korijena prvog gornjeg kutnjaka na obraznoj sluznici;
4. obrazna sluznica – ispod prvoga gornjeg kutnjaka u razini kuta usana;
5. dorzum jezika – 1 centimetar prema medijalnoj liniji, na zamišljenoj crti koja spaja meziobukalne kvržice prvih donjih kutnjaka;
6. rub jezika – u projekciji prvoga donjeg kutnjaka;
7. podjezična sluznica – 1 centimetar od grebena prema medijalnoj liniji u razini prvog donjeg kutnjaka.

Mjerenje se obavljalo na lijevoj i desnoj strani, pa je ukupno bilo 14 mjernih točaka (slika 2.). Mjerilo se tako da je ispitivač prislonio senzor na odabranu točku na sluznici. U trenutku kad se postigao podtlak od 250 mB, što je potvrđeno mjernim instrumentom (Yuyao Yadong Plastic©, Huangzhou, Kina), asistent je pokrenuo računalni program koji bi zabilježio EI za svaku mjernu točku na temelju devet zadanih frekvencija (1, 2, 5, 7, 10, 20, 70 i 100 kHz). Mjerenje se na isti način ponovilo još dva puta u razmaku od po sedam dana da bi se dobio uvid u intraindividualna odstupanja.

Za statističku obradu podataka korišten je program SPSS (IBM Inc, SAD). Normalnost distribucije testirana je Kolmogorov-Smirnovljevim testom. Za ispitivanje razlika među skupinama korišteni su Hi-kvadrat test, Studentov t-test i analiza varijance. Za procjenu razlika između pojedinog mjerenja za svakog ispitanika korištena je analiza varijance za ponovljena mjerenja. Korelacije između pojedinih varijabli izražene su Pearsonovim koeficijentom korelacije. Vrijednosti p manje od 0,05 ($p < 0,05$) smatrale su se statistički značajnima.

Rezultati

U istraživanju je sudjelovalo 20 žena i 10 muškaraca prosječne dobi 27,4 ± 5,9 godina. Demografske i kliničke karakteristike ispitanika prikazane su u tablici 1. Nisu utvrđene statistički značajne razlike u dobi, pušenju i količini sline

er of Teflon. In order to assure uniform pressure and contact with oral mucosa, the sensor was attached to a dental aspirator which produced a constant sub-pressure of 250 mbar to ensure stability of the sensor during measurements. The intraoral sensor was connected to the measuring device NI USB-6251 (National Instruments®, Austin SAD) by electric conductors. The device was attached to the laptop through a USB connection. The measurement program, based on LabView 8.5.1. software package (National Instruments®, Austin SAD), converted electrical impulses from the device into digital records and stored them in a database. Validation and calibration of the measuring device and the program were performed at the Department of Automation and Electronics of the Faculty of Engineering in Rijeka, using established resistance values in the standard replacement scheme. EI was measured at 7 reference locations in the oral cavity as follows:

1. Upper labial mucosa - in the projection between the root apex of the maxillary second incisor and canine
2. Lower labial mucosa - in the projection between the root apex of the mandibular second incisor and canine
3. Hard palate mucosa - at the level of apex of the palatal root of the maxillary first molar
4. Buccal mucosa – at the projection of the first maxillary molar
5. Dorsum of the tongue – 1 cm towards the midline, on the imaginary line connecting the mesiobuccal cusps of the mandibular first molars
6. Ventral tongue - in the projection of the mandibular first molar
7. Sub-lingual mucosa - 1 cm from the mandibular ridge to the midline at the level of the mandibular first molar

The measurements were performed on the right and left side, making the total of 14 measuring points (Figure 2). Measurements were performed in such a way that the investigator placed the sensor on the selected point on the mucosa. When sub-pressure of 250 mbar was obtained, as verified by the manometer (Yuyao Yadong Plastic©, Huangzhou, China), the assistant started a computer program which recorded EI for each measuring point at nine frequencies (1, 2, 5, 7, 10, 20, 70 and 100 kHz). The same measuring procedure was repeated two more times after 7 and 14 days respectively, in order to observe intraindividual EI variations. Data analysis was performed using SPSS® software (IBM Inc, SAD). Normality of distribution was assessed by the Kolmogorov Smirnof test. To evaluate differences between groups, chi-square test, the Student's test and analysis of variance were used where appropriate. To assess differences between individual measurements for each subject, repeated measures analysis of variance was used. Correlations between variables were expressed by the Pearson correlation coefficient. P values with $p<0.05$ were considered statistically significant.

Results

The study included 20 females and 10 males with the average age of 27.4 ± 5.9 years. Demographic and clinical characteristics of the subjects are shown in Table 1. No statistically significant differences in age, smoking and the salivary

Tablica 1. Demografske i kliničke karakteristike ispitanika.
Table 1 Demographic and clinical characteristics of the participants.

Gender N (%)		**p**
Females	20	
Males	10	
Age (Mean ± SD)	27.4 ± 5.9	0.628
Smoking N (%)		
Yes	9 (0.3)	0.095
No	21 (0.7)	
Salivary flow rate ml/min (Mean ± SD)		
1. visit	0.6 ± 0.3	0.075
2. visit	0.5 ± 0.3	0.496
3. visit	0.6 ± 0.3	0.821
Between-visit salivary flow difference p	0.391	

Tablica 2. Sumarni rezultati mjerenja električne impendancije za 7 regija oralne sluznice.
Table 2 Summary of electrical impedance values for the seven regions of the oral mucosa.

	1 kHz	2 kHz	5 kHz	7 kHz	10 kHz	20 kHz	50 kHz	70 kHz	100 kHz
Sluznica gornje usnice (u kΩ) • Upper labial mucosa (in kΩ)									
Sred. vrijednost • Mean value	5.98	5.27	4.56	4.28	3.97	3.28	2.39	2.07	1.78
Stand. dev. • Standard deviation	1.99	1.73	1.53	1.48	1.35	1.05	0.71	0.60	0.51
Minimum	1.85	1.53	1.35	1.24	1.07	0.83	0.62	0.56	0.51
Maksimum • Maximum	11.90	10.24	8.57	8.99	7.92	6.11	3.94	3.50	3.10
Sluznica tvrdog nepca • Hard palate mucosa									
Sred. vrijednost • Mean value	25.97	23.64	17.51	14.10	11.56	7.60	4.24	3.39	2.70
Stand. dev. • Standard deviation	19.33	18.18	11.78	8.34	6.29	3.43	1.61	1.23	0.92
Minimum	2.42	2.07	1.79	1.71	1.65	1.52	1.33	1.22	1.09
Maksimum • Maximum	96.90	94.69	54.07	39.30	29.46	16.05	9.29	7.00	5.21
Bukalna sluznica • Buccal mucosa									
Sred. vrijednost • Mean value	6.47	5.35	3.90	3.38	2.92	2.13	1.39	1.18	0.99
Stand. dev. • Standard deviation	2.38	1.83	1.17	0.96	0.80	0.48	0.32	0.26	0.21
Minimum	1.89	1.58	1.33	1.24	1.16	0.96	0.71	0.63	0.54
Maksimum • Maximum	13.63	10.72	7.40	6.34	5.77	3.31	2.48	2.04	1.64
Sluznica dorzuma jezika • Dorsum of the tongue mucosa									
Sred. vrijednost • Mean value	3.62	3.05	2.36	2.11	1.86	1.49	1.07	0.95	0.85
Stand. dev. • Standard deviation	1.19	0.99	0.66	0.54	0.45	0.35	0.21	0.18	0.16
Minimum	1.53	1.37	1.20	1.12	0.93	0.80	0.66	0.60	0.55
Maksimum • Maximum	8.90	6.55	4.15	3.51	2.94	2.52	1.75	1.51	1.29
Sluznica ventralne površine jezika • Ventral surface of the tongue mucosa									
Sred. vrijednost • Mean value	4.99	4.05	2.94	2.58	2.22	1.63	1.13	0.99	0.85
Stand. dev. • Standard deviation	2.09	1.56	0.99	0.85	0.67	0.43	0.43	0.23	0.18
Minimum	1.16	0.98	0.82	0.77	0.71	0.62	0.62	0.47	0.44
Maksimum • Maximum	11.15	9.16	6.29	5.32	4.45	2.78	2.78	1.61	1.33
Sublingvalna sluznica • Sub-lingual mucosa									
Sred. vrijednost • Mean value	3.99	3.65	3.09	2.92	2.74	2.27	1.66	1.46	1.27
Stand. dev. • Standard deviation	1.64	1.60	1.27	1.20	1.12	0.85	0.53	0.45	0.40
Minimum	1.19	1.07	0.96	0.92	0.89	0.84	0.72	0.64	0.57
Maksimum • Maximum	8.58	8.20	6.60	6.53	5.99	4.89	2.97	2.63	2.57
Sluznica donje usnice • Lower labial mucosa									
Sred. vrijednost • Mean value	4.72	4.35	3.69	3.29	2.99	2.52	1.88	1.51	1.30
Stand. dev. • Standard deviation	1.97	1.81	1.53	1.34	1.19	0.96	0.69	0.50	0.42
Minimum	1.78	1.58	1.28	1.14	1.03	0.85	0.65	0.58	0.52
Maksimum • Maximum	10.55	9.83	7.67	6.96	6.33	5.05	3.97	2.84	2.47

između žena i muškaraca. Sumarni podatci mjerenja (minimum, maksimum, srednja vrijednost i standardna devijacija) za svaku regiju nalaze se u tablici 2. Najniže vrijednosti EI-ja na svim frekvencijama izmjerene su na dorzumu jezika (3,62 ± 1,19kΩ pri frekvenciji od 1 kHz), a najviša vrijednost EI-ja na svim frekvencijama zabilježena je na tvrdom nepcu (26 ± 19,3 kΩ pri frekvenciji od 1kHz). Nije utvrđena statistički značajna razlika između mjerenja na lijevoj i desnoj strani ni u jednoj ispitivanoj regiji. Statistički značajno više vrijednosti EI-ja izmjerene su ženama na sluznici gornje usne (pri frekvencijama od 1 kHz, 2 kHz i 5 kHz), dorzumu jezika (pri svim frekvencijama) i na ventralnom dijelu jezika (pri frekvenciji od 1 kHz). Statistički značajno više vrijednosti EI-ja izmjerene su pušačima na podjezičnoj sluznici (pri frekvencijama od 70 kHz i 100 kHz) i na sluznici donje usne (pri frekvencijama od 50 kHz, 70 kHz i 100 kHz). Utvrđena je negativna korelacija EI-ja s količinom sline na sluznici gornje usne (pri frekvencijama od 5 kHz i 7 kHz), na tvrdom nepcu (pri svim frekvencijama, osim od 5 kHz), dorzumu jezika (pri frekvencijama od 1 kHz, 2 kHz, 5kHz i 7 kHz) i na podjezičnoj sluznici (na 1 kHz, 2kHz, 7kHz).

Vrijednosti pojedinačnih mjerenja prikazane su na slici 3. Statistički značajne razlike u vrijednostima EI-ja između pojedinačnih mjerenja utvrđene su na tvrdom nepcu (pri frekvencijama od 1 i 2 kHz), obraznoj sluznici (pri frekvencijama od 7, 10, 50, 70 i 100 KHz) i na dorzumu jezika (na 1, 2 i 20 kHz). Mjerenja na niskim frekvencijama imala su veću varijabilnost. Koeficijenti unutar klasne korelacije za različite regije izračunati su kako slijedi: sluznica gornje usne: 0,23 (1 kHz) -0,30 (20 kHz); sluznica donje usne: 0,2 (1 KHz) -0,42 (20 kHz); tvrdo nepce: 0,22 (1 KHz) -0,57 (100 KHz); sluznica obraza: 0,09 (5 kHz) -0,31 (100 kHz); dorzum jezika: 0,1 (1 kHz) -0.40 (7 kHz); sluznica ventralne strane jezika: 0,16 (20 kHz) -0.24 (7 kHz) i podjezična sluznica: 0,23 (2k HZ) -0.41 (100 kHz).

Rasprava

Svrha ove studije bila je odrediti referentne vrijednosti EI-ja na oralnoj sluznici. Slično istraživanje proveo je i Nicader sa suradnicima (14, 15), a ispitivali su osnovne vrijednosti EI-a na šest različitih anatomskih lokacija u ustima. Zaključili su da su razlike u usnoj šupljini veće negoli na različitim područjima kože, te da se metodom mjerenja EI-a mogu preciznije detektirati eksperimentalno uzrokovane blage alergijske reakcije u odnosu prema drugim metodama pretraga, kao što su inspekcija sluznice i histološke pretrage (22). Za potrebe ovog eksperimentalnog istraživanja izabrana je skupina ispitanika u dobi od 20 do 40 godina. Iako je među njima bilo 66,6 posto žena, nisu utvrđene statistički značajne razlike u dobi, količini sline i pušenju između žena i muškaraca, pa se može zaključiti da je ispitna skupina bila homogena. Najniže vrijednosti EI-ja izmjerene su na sluznici dorzuma jezika, a najviše na tvrdom nepcu. Ovakav nalaz povezuje se s različitom građom sluznice na pojedinim područjima. Čimbenici kao što su slabija prokrvljenost,

flow rate between females and males were found. Measurement summary data (minimum, maximum, mean value and standard deviation) for each region of the oral cavity are presented in Table 2. Measurements for the left and the right side of each region were unified. The lowest EI vales at all frequencies were measured on the dorsum of the tongue (3.62 ± 1.19kΩ at the frequency of 1 kHz). The highest EI values were recorded on the hard palate at all frequencies (26 ± 19.3 kΩ at the frequency of 1 kHz). Statistically significant differences between measurements on the left and right side of any region were not found. Significantly higher EI values were measured in females on the upper labial mucosa (at frequencies 1kHz, 2kHz and 5 kHz), tongue dorsum (at all frequencies) and on the ventral surface of the tongue (at the frequency of 1kHz). Significantly higher EI values were determined in smokers in the sub-lingual mucosa (at frequencies 70 kHz and 100 kHz) and on the lower labial mucosa (at frequencies 50 kHz, 70 kHz and 100 kHz). A negative correlation between EI and the amount of saliva measured by sialometry was found on the upper labial mucosa (at frequencies 5 kHz and 7 kHz), hard palate (at all frequencies except 5 kHz), tongue dorsum (at frequencies 1 kHz, 2 kHz, 5 kHz and 7 kHz) and on the floor of the mouth (at 1 kHz, 2kHz, 7kHz). Individual measurements are shown in Figure 3. Statistically significant differences in EI between individual measurements were found on the hard palate (at frequencies 1 and 2 kHz), buccal mucosa (at frequencies 7, 10, 50, 70 and 100 kHz) and on the tongue dorsum (at 1, 2 and 20 kHz). Variability of measurements was higher at lower frequencies. Intraclass correlation coefficients for different regions were determined as follows: upper labial mucosa: 0.23 (1kHz)-0.30 (20 kHz), lower labial mucosa: 0.2 (1KHz)-0.42(20kHz), hard palate: 0.22 (1kHz)-0.57 (100kHz), buccal mucosa: 0.09(5kHz)-0.31(100kHz), dorsum of the tongue: 0.1 (1kHz)-0.40 (7 kHz), mucosa of the ventral surface of the tongue: 0.16 (20kHz)-0.24(7kHz), and sublingual mucosa: 0.23 (2kHZ)-0.41(100kHz).

Discussion

The aim of this study was to determine EI reference values on the healthy oral mucosa. A similar investigation was conducted by Nicader et al. (14, 15) who analyzed EI measures on six different anatomical locations in the mouth of healthy subjects. The authors concluded that differences in EI values in the oral cavity among individuals were higher than EI values on the skin. Moreover, the authors claimed that the method of EI measurement allowed a more precise detection of experimentally caused mild allergic reaction than other methods such as inspection of the mucosa and histological tests (22). For the purpose of this pilot study, a group of subjects aged 20-40 years were selected. Although 66.6% of subjects were females, no statistically significant differences in age, the amount of saliva and smoking were found between females and males, thus suggesting that the study group was homogenous. The lowest EI measures were found on the tongue dorsum and the highest on the hard palate. This finding can be linked to a different structure of

vlažnost, količina vezivnog tkiva i debljina stratum korneuma mogu utjecati na prolazak električne struje i posljedični otpor. Dorzum jezika izrazito je dobro prokrvljen, sadržava znatno manju količinu veziva i ima znatno tanji stratum korneum od tvrdog nepca, te se može očekivati da će vrijednosti električnog otpora biti niže. Slina koja se zadržava između filiformnih papila također može smanjiti otpor između polova elektroda i tako pridonijeti smanjenju ukupne vrijednosti otpora. Ovaj nalaz u skladu je s rezultatima naših prijašnjih istraživanja (23), te s onim Nicadera i suradnika (14, 15) koji su također pronašli najveće razlike u EI-ju između sluznice tvrdog nepca i sluznice dorzuma jezika. Nisu utvrđene statistički značajne razlike u vrijednostima EI-ja između lijeve i desne strane ni na jednoj od ispitnih točaka. Ovaj nalaz potvrđuje da bioimpedancija ovisi o histološkim i anatomskim svojstvima određene regije usne šupljine, bez obzira na lijevu ili desnu stranu. Nepostojanje razlika između lijeve i desne strane u istim regijama zabilježili su u svojim radovima Rantanen te Nicader sa suradnicima (15, 19, 22). Time se otvara mogućnost da se kontralateralna područja sluznice koriste kao referentna točka za mjerenje EI-ja u slučaju unilateralnih oralnih promjena. Spol je jedan od čimbenika čiju ulogu u određivanju referentnih vrijednosti EI-ja na oralnoj sluznici još treba razjasniti. U ovom istraživanju utvrđene su statistički značajno više vrijednosti EI-ja kod žena na sluznici gornje usne i na ventralnoj strani jezika pri niskim frekvencijama (1, 2 i 5 kHZ) te na dorzumu jezika pri svim frekvencijama. Takav nalaz može biti posljedica stvarne razlike između spolova, ali i posljedica malog broja ispitanika i/ili većeg broja ženskih ispitanika u odnosu na muške. Nalaz je potrebno potvrditi ili odbaciti u studijama s većim brojem ispitanika. Podataka u literaturi za usporedbu nema, a mjerenjem EI-ja na koži nisu utvrđene statistički značajne razlike između muškaraca i žena (24). Pušači su u pravilu imali više vrijednosti EI-ja u odnosu prema nepušačima, ali su razlike bile statistički značajne samo na sluznici donje usne i sluznici dna usne šupljine. Poznato je da duhanski dim i povišena temperatura uzrokuju jaču keratinizaciju sluznice (25, 26), što može rezultirati povećanim otporom pri prolasku električne struje. Je li ovakav nalaz doista posljedica pušenja ili posljedica malog broja ispitanika, ustanovit će se budućim studijama. Zanimljivo je ipak istaknuti da su i donja usna i dno usne šupljine lokalizacije koje su pri pušenju u najduljem kontaktu s cigaretom i komponentama duhanskoga dima. Vrijednosti EI-ja na tvrdom nepcu (na području koje je također u neposrednom dodiru s duhanskim dimom) nisu se razlikovale između pušača i nepušača. Vrijednosti EI-ja na tvrdom nepcu bile su visoko varijabilne i možda su zato teško uočljive razlike između pušača i nepušača. Količina sline također bi mogla utjecati na vrijednosti EI-ja na oralnoj sluznici. U ovoj studiji utvrđena je negativna korelacija s količinom sline na sluznici tvrdog nepca gotovo pri svim frekvencijama, osim na onoj od 5 kHZ. Osim toga, utvrđena je i negativna korelacija s količinom sline na sluznici gornje usne, dorzuma jezika i na sublingvalnoj sluznici, uglavnom pri nižim frekvencijama (od 1 kHZ do 7 kHz). Niži otpor vjerojatno je posljedica veće količine sline i bolje provodljivosti. U literaturi nema studija s kojima bi se ovaj nalaz mogao usporediti. Iz istraživanja pro-

the mucosa at these locations. Factors such as vascular flow, humidity, amount of the underlying connective tissue and the thickness of the *stratum corneum* may affect the electric flow and its consequent resistance. The tongue dorsum has a rich vasculature, contains prominently less connective tissue and has thinner *stratum corneum* than the hard palate, so it is more likely to have lower electrical resistance. Saliva retained between filiform papillae can also lower resistance between poles of electrode and lead to decreased resistance. This finding complies with the results of our previous studies (23), and with results reported by Nicader et al. (14, 15) who also established the greatest differences in impedance between the hard palate and tongue dorsum. There were no statistically significant variations in EI values between the left and right side at any of the measuring points. This finding suggests that EI depends on histological and anatomical features of a specific region of the oral cavity regardless of the side. Absence of the difference between the left and right side in the same regions was also reported by Rantanen al. and Nicader et al. (15, 19, 22). Thus, there is a possibility that contralateral regions may be used as reference points for EI measurement of unilateral oral lesions. Gender is one of the factors the role of which should be clarified in determining EI values of oral mucosa. This study established significantly higher EI values in the females on the upper labial mucosa and on the ventral surface of the tongue at low frequencies (1, 2 and 5 kHz), and also on the dorsum of the tongue at all frequencies. This finding may result from the actual difference between genders, but it may occur due to a small number of subjects and/or a larger number of female subjects than male ones. These results need to be confirmed or rejected in studies with greater number of participants. There are no literature data available for comparison, while EI measurement on the skin did not show statistically significant differences between males and females (24). Smokers had somewhat higher EI values compared to non-smokers, but the differences were statistically significant only on the lower labial mucosa and on the floor of the mouth. It is known that tobacco smoke and elevated temperature can increase mucosal keratinization (25, 26), which may lead to increased resistance to the electric current flow. Whether this finding is actually an effect of smoking or it results from a small number of participants, remains to be determined by further studies. However, it is interesting to note that both the lower lip and the floor of the mouth are the sites which have the longest contact with a cigarette and ingredients of tobacco smoke. The EI values on the hard palate mucosa, (a localization which is also in intimate contact with tobacco smoke) did not differ between smokers and non-smokers. EI values on the hard palate were highly variable, and possible differences between smokers and non-smokers could therefore be undetected. Salivary flow may also affect the EI values. A negative correlation between impedance values and salivary flow was observed on the hard palate at all frequencies except at 5 kHz. Moreover, a negative correlation was also found between salivary flow and EI values on the upper labial mucosa, tongue dorsum and floor of the mouth at lower frequencies (1 kHZ to 7 kHz). Lower resistance is likely to be an effect of higher

vedenih na koži i oralnoj sluznici može se zaključiti da količina tekućine u tkivu utječe na vrijednosti impedancije jer upalne promjene i alergijske reakcije kože i sluznice (karakterizirane većom količinom ekstracelularne tekućine) imaju niži iznos impedancije u odnosu prema zdravoj sluznici/koži (1, 19, 22, 27, 29). Rezultati mjerenja EI-ja u ovoj studiji pokazuju intraindividualnu varijabilnost koja se očitovala u niskim koeficijentima unutarklasne korelacije. Varijabilnost je bila izraženija na nižim frekvencijama. Ovaj nalaz pokazuje da vrijednosti EI-ja svakog pojedinca nisu konstantne, nego se kreću unutar određenog raspona. Taj raspon intraindividualnih vrijednosti nije prevelik, jer u većini slučajeva vrijednosti pojedinačnih mjerenja nisu bile statistički značajno različite. Dostupnih podataka o intraindividualnim razlikama impedancije na oralnoj sluznici u literaturi nema. Ove razlike mogu nastati zbog neujednačenog pritiska oralnih senzora na sluznicu (što smo se trudili izbjeći upotrebom ujednačenoga podtlaka iz aspiratora) te zbog količine i kvalitete sline u sklopu mjerenja. Promjene krvne cirkulacije pod utjecajem vegetativnoga živčanog sustava također mogu utjecati na varijabilnost rezultata mjerenja jer je poznato da vrijednosti bioimpedancije mogu ovisiti o količini tkivne tekućine (1, 7, 19, 23, 27). Najveća varijabilnost zabilježena je na tvrdom nepcu u kojem je standardna devijacija bila veća od 70 posto srednje vrijednosti (0,74 na 1 kHz i 0,77 na 2 kHz), a na drugim se ispitnim točkama kretala u rasponu od 32 do 42 posto. Ova varijabilnost najvjerojatnije je uzrokovana slabim prianjanjem elektrode na palatinalnu sluznicu. Sve navedeno govori u prilog potrebi da se odrede referentne vrijednosti EI-ja na zdravoj oralnoj sluznici kojima bismo se koristili kao polazišnom osnovom za usporedbu s patološkim promjenama. U ovoj studiji ima nekih ograničenja/nedostataka koje je potrebno spomenuti. U prvom redu to je mali broj ispitanika. Iako dobiveni rezultati pokazuju određeni uzorak – utjecaj spola, pušenja i količine sline na vrijednosti impedancije, to je potrebno potvrditi ili odbaciti u studijama s većim brojem ispitanika. Nadalje, potrebno je standardizirati postupak mjerenja kako bi se nehomogenost rezultata svela na najmanju moguću mjeru. Tijekom istraživanja uočeno je da povećanje podtlaka kojim sustav elektroda prianja uz sluznicu pridonosi smanjenju intra- i interindividualnih razlika. Ovo istraživanje provedeno je uz podtlak od 250 mBara, što je ostvarilo solidan kontakt sa sluznicom i pacijentima je bio ugodan. Upotrebom viših iznosa podtlaka moglo bi se postići stabilnije prianjanje elektrode na sluznicu, što bi eventualno rezultiralo stabilnijim rezultatima mjerenja.

Na temelju rezultata ove eksperimentalne studije može se zaključiti da je primijenjenom metodom moguće utvrditi raspone vrijednosti EI-ja oralne sluznice zdravih ispitanika. Vrijednosti EI-ja najviše ovise o stupnju keratinizacije sluznice. Demografski i klinički čimbenici kao što su spol, pušenje i količina sline vjerojatno utječu na njihove vrijednosti, što je potrebno razjasniti u daljnjim studijama s većim brojem ispitanika. Ova metoda potencijalno se može razviti u dijagnostičko pomagalo koje može biti upotrijebljeno za određivanje intenziteta i proširenost lezija u oralnoj sluznici. Studije u kojima je procjenjivana bioimpedancijska spektroskopija u detekciji malignih i potencijalno malignih lezija u ustima,

amount of saliva and better conductivity. There are no literature data to compare these findings with. Studies performed on the skin and oral mucosa suggest that the amount of tissue fluid has an impact on EI since inflammatory and allergic reactions of the skin and mucosa (characterized by increased extracellular fluid) have lower impedance values compared to the healthy mucosa/skin (1, 19, 22, 27, 29). The results of EI measurement obtained in this study show intraindividual variability manifested by low intraclass correlation coefficients. Variability was more pronounced at lower frequencies. This finding showed that the impedance measures in each individual were not constant, but varied within a specific range. This range of intraindividual values is not too wide since in most cases the values of individual measurements did not differ significantly. No literature data on intraindividual variations in oral mucosal EI are available for comparison. Intraindividual variations may result from non-uniform pressure of the sensor on the mucosa (which we tried to eliminate with aspirator system) as well as the amount and quality of saliva during measurements. Circulatory changes caused by the vegetative nervous system may also lead to measurement variability since it is known that impedance values may depend on the amount of fluid in the tissue (1, 7, 19, 23, 27). The highest variability was noted on the hard palate where standard deviation was over 70% of the mean (0.74 at 1 kHz and 0.77 at 2 kHz), unlike other locations where standard deviations were 32-42%. This variability is most likely the effect of poor attachment of the sensor to the palatal mucosa. All of the abovementioned confirms the need for the determination of EI reference values on the healthy oral mucosa which could be used as the starting point for comparison with different mucosal lesions. This study has certain limitations which need to be addressed. First of all, there was a small number of participants. Although the obtained results suggest a specific pattern of influence of gender, smoking and salivary flow on the EI values, these results should be confirmed in studies with greater number of participants. Furthermore, it is necessary to standardize the measuring procedure in order to avoid artifacts and minimize non-homogeneity of the results. The standardization of the measuring procedure refers primarily to ensuring uniform pressure of the sensor on the mucosa. During the investigation, it was observed that the increase of subpressure exerted by the sensor contributed to lowering the intraindividual and interindividual differences. This investigation was conducted using sub-pressure of 250 mbar which created an adequate contact with the mucosa and was comfortable for the patients. Using higher sub-pressure, a more stable attachment of the sensor to the mucosa may be achieved and lead to more stable measurements. Based on the results of this pilot study, it can be concluded that the applied method may be used for determining the EI values of the oral mucosa in healthy subjects. EI values mostly depend on the degree of keratinization of the oral mucosa. Demographic and clinical factors such as gender, smoking and salivary flow probably affect their values, but they need to be clarified in further studies on a larger number of participants. The method shows a potential for developing into a diagnos-

govore u prilog spomenutoj tvrdnji (15 –18). Prije nego što to bude moguće bit će potrebno ukloniti čimbenike koji pridonose pogreškama u mjerenju, primarno otkloniti smetnje pri prianjanju oralnoga senzora i kontrolirati količinu sline u mjernom području.

tic tool which may be used to determine the intensity and spread of oral mucosal lesions. Studies which assessed EI spectroscopy for the detection of malignant and potentially malignant lesions of the oral cavity speak in favor of the above claim (15-18). Before this is accomplished, factors that contribute to measurement deviations need to be eliminated, primarily the attachment of the sensor to oral mucosa and the amount of saliva in the measurement area.

Abstract

Objective: Electrical impedance is the resistance to the electric current flow through a tissue and depends on the tissue's structure and chemical composition. The aim of this study was to map electrical impedance spectra for each region of the healthy oral mucosa. **Materials and Methods:** Electrical impedance was measured in 30 participants with healthy oral mucosa. Measurements were performed in 14 points on the right and the left side of the oral cavity, and repeated after 7 and 14 days respectively. **Results:** The lowest values were measured on the tongue dorsum and the highest values were measured on the hard palate. No significant differences were found between the right and the left side. Significantly higher values were found in females on the upper labial mucosa, tongue dorsum and the ventral tongue. Significant difference between smokers and non-smokers on the lower labial mucosa and floor of the mouth was found. Electrical impedance was negatively correlated with salivary flow on the upper labial mucosa, hard palate, tongue dorsum and sublingual mucosa. Higher variability of measurements was found at low frequencies. **Conclusions:** Electrical impedance mostly depends on the degree of mucosal keratinization. Demographic and clinical factors probably affect its values. Further studies with bigger number of participants are required.

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