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## Nalaz anaerobnih bakterija oko implantata i homolognog zuba od 2 do 14 godina nakon ugradnje

### *Anaerobic Bacteria in Implants and Homologous Teeth 2-14 Years after Implantation*

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#### Sažetak

**Svrha:** Svrha studije bila je utvrditi ima li razlike u prisutnosti potencijalno patogenih anaerobnih mikroorganizama oko implantata i homolognog zuba kod pacijenata koji su nakon postavljanja dentalnih implantata bili upućeni u individualni pristup u održavanju oralne higijene. **Materijal i postupci:** U istraživanju je sudjelovalo 30 ispitanika (10 muškaraca i 20 žena) prosječne dobi 49,6 godina (22 – 78 godina). Implantati su bili protetički opskrbljeni metalceramičkim krunicama prosječne starosti 5,26 godina (2 – 14 godina). Na kontrolnom pregledu parodontnom sondom zabilježeni su sljedeći indeksi i mjere: aproksimalni indeks plaka (API), indeks krvareće papile (PBI), dubina sondiranja parodontnih džepova (PD) i recesija gingive. Vestibularno se uzorkovala tekućina oko implantata i gingivalna sulkusna tekućina oko homolognog zuba na kontralateralnoj strani. **Rezultati:** Rezultati naše studije pokazali su pozitivan API na 30 % implantata, a na 70 % bio je negativan. Vrijednosti PBI-ja bile su identične vrijednostima API-ja. Izmjerena je prosječna retrakcija mukoze oko implantata od 0,15 mm i prosječna vrijednost dubine sondiranja oko implantata od 2,25 mm. Na homolognim zubima API je bio pozitivan na 78,3 % zuba, kao i PBI. Izmjerena je prosječna retrakcija gingive od 1,06 mm i prosječna vrijednost dubine sondiranja od 1,85 mm. U skupini od 30 ispitanika, anaerobne bakterije pronađene su kod njih 12 (40 %). Kod sedam ispitanika anaerobne bakterije izolirane su samo na implantatu, kod tri samo na homolognom zubu, a kod dva i na implantatu i na homolognom zubu. **Zaključak:** Zapaženo je više anaerobnih bakterija na implantatu u odnosu prema homolognom zubu.

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#### Ključne riječi

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#### Uvod

Prošlo je gotovo pet godina otkako su Albrektsson i suradnici (1) zaključili da je periimplantitis *infekcija sa supuracijom povezana s klinički značajnim i progresivnim krestalnim gubitkom kosti nakon faze adaptacije*, no čini se da je broj pacijenata s periimplantatnom infekcijom neprestano u porastu (2). Etiologija periimplantitisa je kompleksna, a niz rizičnih čimbenika koji utječu na njegov nastanak i progresiju može se objasniti jedino multikauzalnim modelom. Ipak, organizacija i rast biofilma na dentalnim implantatima potiče odgovor domaćina koji izaziva razvoj periimplantatnog mukozitisa, a ubrzo i periimplantitisa (3). Biofilm se može definirati kao agregacija jedne ili više različitih skupina mikroorganizama uloženi u matriks koji sami proizvode i pričvršćen je na neku čvrstu površinu (4).

#### Introduction

It has been almost five years since Albrektsson et al. (1) concluded that peri-implantitis is “an infection with suppuration associated with clinically significant progressive crestal bone loss after the adaptive phase”, but it appears that the number of patients with peri-implant infections is constantly rising (2). The etiology of peri-implantitis is complex, and the series of risk factors that influence its emergence and progression can only be explained by the multicausality model. Nevertheless, the organization and formation of biofilm on dental implants foster the host's reaction, which leads to the development of peri-implant mucositis, and soon also to peri-implantitis (3). Biofilm can be defined as an aggregation of one or more groups of different microorganisms, embedded in a self-produced matrix and adhering to a firm surface (4).

Može se reći da su inicijalne faze razvoja biofilma na zubima i implantatima identične. Pelikula na površini implanta-ta/suprastrukture veoma je slična pelikuli na prirodnim zubima. U prvoj fazi nastanka biofilma *Streptococcus mutans* čini od 60 do 80 % svih ranih kolonizatora s različitim bakterijskim adhezivima odgovornima za prijanjanje na pelikulu. Rast i diverzifikacija biofilma na implantatima donekle se razlikuju od onih na prirodnim zubima, no neki elementi su identični. Na primjer, kolektivna svijest koju bakterije razvijaju pospješena je stimulirajućim peptidima koji se otpuštaju nakon izloženosti niskom pH (5). Površina implantata poželjno je nepravilna, no upravo to pogoduje rastu biofilma, organizacije koju danas smatramo primitivnim višestaničnim mikroorganizmom (6). Četiri su elementa koja pogoduju rastu i razvoju biofilma na površini dentalnih implantata: (a) slučajni dolazak bakterija nošenih slinom na površinu implantata, (b) inicijalna (reverzibilna) adhezija, (c) kolonizacija površine te (d) snažna adhezija na površinu (7).

Kontrola biofilma jedan je od glavnih preduvjeta za održavanje zdravlja periimplantantnog tkiva, baš kao i za održavanje zdravlja parodonta. Ipak, zbog morfoloških i anatomskih razlika periimplantantna tkiva podložnija su razvoju inflamacije od parodontalnih, a i oko dentalnih implantata, čini se, upala brže napreduje (8). Niz je čimbenika, ponajprije na bakterijskoj razini, koji utječu na to u kojoj će mjeri biofilm biti izazov za domaćina. Nastanak upale izravno potiče značajne promjene u sastavu biofilma (9) – uglavnom se povećava ili smanjuje udjel određenih vrsta (10). Te su razlike posebno izražene u slučaju gingivitisa (11), no nepoznate su uloge određenih vrsta unutar biofilma pri nastanku i progresiji periimplantantnog mukozitisa i periimplantitisa. Kako je periimplantitis teška parodontna bolest koja može završiti progresivnom difuznom destrukcijom potporne kosti i okolnih tkiva, sigurno je vrlo važno na vrijeme identificirati lokalne parametre koji u većoj mjeri utječu na inicijaciju, odnosno na progresiju bolesti (12).

Upravo zbog toga, ovim smo istraživanjem pokušali utvrditi ima li razlike u prisutnosti tzv. potencijalno parodontopatogenih bakterija (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*) oko implantata i homolognog zuba kod pacijenata koji su nakon postavljanja dentalnih implantata bili upućeni u individualni pristup u održavanju oralne higijene.

## Materijali i metode

### Ispitanici

U istraživanju je sudjelovalo 30 ispitanika (10 muškaraca i 20 žena) prosječne dobi 49,6 godina (22 – 78 godina). Svi su morali zadovoljiti kriterij da na kontralateralnoj strani od implantata imaju prirodan zub bez protetičkog nadomjestka. Implantati su bili protetički opskrbljeni metalkera-

The initial phases of biofilm formation on teeth and on implants can be considered identical. The pellicle on the surface of implants/suprastructures is very similar to the pellicle on natural teeth. In the initial phase of biofilm formation, *Streptococcus mutans* makes up for 60%-80% of all early colonizers, with different bacterial adhesives responsible for the adhesion to the pellicle. Even though the growth and diversification of biofilm are somewhat different on implants than on natural teeth, certain elements remain identical. For example, the collective consciousness that the bacteria develop is enhanced by the stimulating peptides that are released after the exposure to low pH (5).

The surface of implants is preferably uneven, but that is precisely what favors the formation of biofilm, the organization that is nowadays considered to be a primitive multicellular organism (6). There are four elements that are favorable for the growth and formation of biofilm on the surface of dental implants: (a) random transport of bacteria to the surface of the implant through saliva, (b) initial (reversible) adhesion, (c) colonization of the surface and (d) strong adhesion to the surface (7).

Controlling the biofilm is one of the main prerequisites for keeping the peri-implant tissue, as well as the periodontal tissue, healthy. However, due to morphological and anatomical differences, peri-implant tissue is more prone to developing inflammation than periodontal tissue, and it appears that the inflammation around dental implants progresses faster (8). There is a series of factors, primarily bacteria-related, that affects the extent to which the biofilm will constitute a challenge for the host. The emergence of inflammation leads directly to significant changes in the composition of biofilm (9), primarily in terms of increasing and decreasing the proportion of certain species (10). These differences are especially noticeable when it comes to gingivitis (11), but the roles of certain species within biofilm in developing peri-implant mucositis and peri-implantitis are unknown. Since periimplantitis is a serious condition that can lead to progressive destruction of the supporting alveolar bone and adjacent tissues, it is of general interest to identify the local parameters which significantly influence the initiation and progression of this disease (12).

Precisely in view of that, in this study we have attempted to establish whether there is a difference in the presence of potentially so-called periodontopathogenic bacteria (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*) around the implant and homologous tooth in patients who, after having the dental implants placed, received information about an individual approach to maintaining oral hygiene.

## Materials and methods

### Subjects

The study included 30 subjects (10 males and 20 females), whose average age was 49.6 years (ranging from 22 to 78 years). Aside from having implant-prosthetic interventions done, the chosen participants were required to have natural teeth as well. On top of the participant's implants,

mičkim kronicama čija je prosječna starost prije pregleda bila 5,26 godina (2 – 14 godina). Četiri implantata bila su ugrađena u području prednjih zuba (sjekutići), a 26 u stražnjem dijelu (pretkutnjaci i kutnjaci). Ispitanici su bili zdravi i nisu imali kliničkih znakova parodontne bolesti. Svi su potpisali informirani pristanak za sudjelovanje u znanstvenom istraživanju koje je odobrilo Etičko povjerenstvo Stomatološkog fakulteta Sveučilišta u Zagrebu.

## Metode

Ispitanicima se na kontrolnom pregledu ustanovilo stanje potpornog aparata zuba i stanje tkiva oko implantata. Parodontnom sondom (Tekno-Medical Optik-Chirurgie, Tuttlingen, Njemačka) zabilježeni su sljedeći indeksi i mjere: aproksimalni indeks plaka (API), indeks krvareće papile (PBI), dubina sondiranja parodontnih džepova (PD) te recesija gingive.

Nakon dezinfekcije 3-postotnim vodikovim peroksidom ( $H_2O_2$ ), ispuhivanjem komprimiranim zrakom te postavljanjem vaterolica, papirnatim štapićem (25) (Absorbent Paper Point Pearl Endopia, Pearl Dent, Kyunggi-Do, Koreja) vestibularno se uzorkovala tekućina oko implantata i gingivalna sulkusna tekućina oko homolognog zuba na kontralateralnoj strani. Štapić je 90 sekunda držan u subgingivnom području implantata i homolognog zuba nakon čega je uronjen u anaerobni transportni medij (Thioglycollate Medium G, Biolab, Budimpešta, Mađarska) (slika 1.) do prijenosa u mikrobiološki laboratorij gdje je odmah nakon dostave inkubiran u istom mediju tri dana na temperaturi od 37 °C. Zatim su uzorci kultivirani na anaerobnom krvnom agaru Columbia s dodatkom 5 % konjske krvi (Columbia Agar Base, Biolife, Milano, Italija) te su bakterije identificirane metodom masene spektrometrije proteina (engl. *matrix-assisted laser desorption/ionization time-of-flight mass spectrometer* – MAL-I-TOF-MS).

dental prostheses in the form of metal-ceramic crowns had been fixed, whose average age before the examination was 5.26 years (ranging from 2 to 14 years). Four implants were placed in the area of front teeth (incisors), and 26 were placed in the back (premolars and molars). The subjects were healthy and did not show clinical signs of periodontal disease. All the participants had signed an informed consent form for participating in a scientific study, approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb.

## Methods

The condition of the participant's tooth-supporting apparatus and of the tissue surrounding the implant was established during an examination. A periodontal probe (Tekno-medical Optik-Chirurgie, Tuttlingen Germany) was used to record the following indexes and measurements: the approximal plaque index (API), the papilla bleeding index (PBI), the periodontal pocket probing depth (PD) and the gingival recession.

After disinfecting with the 3% hydrogen peroxide ( $H_2O_2$ ), drying with compressed air and placing dental cotton rolls, paper points (25) (Absorbent Paper Point Pearl Endopia, Pearl Dent, Kyunggi-Do, Korea) were used to vestibularly sample the fluid around the implant and the gingival sulcus fluid around the homologous tooth on the contralateral side. The paper points were kept in the sub gingival area of the implant for 90 seconds, after which they were stored in the anaerobic transport medium (Thioglycollate Medium G, Biolab, Budapest, Hungary) (Figure 1) until they were transported to the microbiological laboratory, where they were immediately incubated in the same medium at 37°C for three days. The samples were then cultivated on the Columbia anaerobically agar base with 5% horse blood (Columbia Agar Base, Biolife, Milano, Italy) and the bacteria were identified using the protein mass spectrometry method (matrix-assisted laser desorption/ionization time-of-flight mass spectrometer, MALDI-TOF-MS).



**Slika 1.** Papirnatı štapić (25) (Absorbent Paper Point Pearl Endopia, Pearl Dent, Kyunggi-Do, Koreja), anaerobni transportni medij (Thioglycollate Medium G, Biolab, Budimpešta, Mađarska)

**Figure 1** Paper Point (25) (Absorbent Paper Point Pearl Endopia, Pearl Dent, Kyunggi-Do, Korea), anaerobic transport medium (Thioglycollate Medium G, Biolab, Budapest, Hungary)

## Statistička analiza

Dobiveni rezultati analizirani su statističkim programom SPSS 21 (IBM, Armonk, SAD). Vjerojatnost povezanosti nalaza anaerobnih bakterija s dubinom sondiranja određena je s pomoću modela višestruke linearne regresije ( $p < 0,05$ ), a procjene statistički značajnih razlika pri usporedbi homolognog zuba i implantata bile su analizirane t-testom o razlici sredina dviju populacija. Preostali rezultati kojima se opisuju karakteristike uzorka obrađeni su deskriptivnom statistikom, odnosno mjerama centralne tendencije, mjerama varijabilnosti i mjerama asimetrije (tablica 1 – 7).

## Statistical Analysis

The results obtained were analyzed by the statistical program SPSS 21 (IBM, Armonk, USA). The probability of the correlation of anaerobic bacterial findings with the depth of probing was determined by multiple linear regression model ( $p < 0.05$ ), while estimates of statistically significant differences when comparing the homologous tooth and implant were analyzed by t-test on the difference between the two populations. The remaining results describing the characteristics of the sample were processed with the help of descriptive statistics, regarding measures of central tendency, measures of variability and measures of asymmetry (Table 1-7).

**Tablica 1.** Apsolutne i relativne frekvencije API-ja i PBI-ja izražene u broju pozitivnih i negativnih nalaza  
**Table 1** Absolute and relative frequencies of APIs and PBIs expressed in the number of positive and negative findings

	Apsolutne frekvencije • Absolute frequencies	Postotne frekvencije • Percent frequencies	Kumulativni postotak • Cumulative Percentage
<b>PLAK VESTIBULARNO (H) • PLAQUE VESTIBULAR (H)</b>			
Negativan • Negative	6	20,0	20,0
Pozitivan • Positive	24	80,0	100,0
<b>PLAK ORALNO (H) • PLAQUE ORAL (H)</b>			
Negativan • Negative	7	23,3	23,3
Pozitivan • Positive	23	76,7	100,0
<b>UPALA VESTIBULARNO (H) • BLEEDING VESTIBULAR (H)</b>			
Negativan • Negative	6	20,0	20,0
Pozitivan • Positive	24	80,0	100,0
<b>UPALA ORALNO (H) • BLEEDING ORAL (H)</b>			
Negativan • Negative	7	23,3	23,3
Pozitivan • Positive	23	76,7	100,0
<b>PLAK VESTIBULARNO (I) • PLAQUE VESTIBULAR (I)</b>			
Negativan • Negative	21	70,0	70,0
Pozitivan • Positive	9	30,0	100,0
<b>PLAK ORALNO (I) • PLAQUE ORAL (I)</b>			
Negativan • Negative	21	70,0	70,0
Pozitivan • Positive	9	30,0	100,0
<b>UPALA VESTIBULARNO (I) • BLEEDING VESTIBULAR (I)</b>			
Negativan • Negative	21	70,0	70,0
Pozitivan • Positive	9	30,0	100,0
<b>UPALA ORALNO (I) • BLEEDING ORAL (I)</b>			
Negativan • Negative	21	70,0	70,0
Pozitivan • Positive	9	30,0	100,0

**Tablica 2.** T-test o procjeni razlike dubine sondiranja oko implantata i homolognog zuba (mm)

**Table 2** T-test for estimating the depth of probe depth around the implant and the homologous tooth (mm).

Var/pokazatelj • Var/indices	N	SD	AS	MD	t	p	Donja • Lower 95%	Gornja • Upper 95%
Dubina sondiranja (H) • Probing depth (H)	30	2,43	7,50	-1,47	-2,74	<0,05	-2,56	-0,37
Dubina sondiranja (I) • Probing depth (I)	30	3,29	8,97					

**Tablica 3.** T-test o procjeni razlike retrakcije mukoze oko implantata i gingive oko homolognog zuba (mm)

**Table 3** T-test for estimating the difference of mucosa recession around the implant and gingiva around the homologous tooth (mm).

Var/pokazatelj • Var/indices	N	SD	AS	MD	t	p	Donja • Lower 95%	Gornja • Upper 95%
Retrakcija (H) • Recession (H)	30	2,76	2,13	1,83	4,38	<0,05	0,98	2,69
Retrakcija (I) • Recession (I)	30	1,06	0,30					

**Tablica 4.** Deskriptivna statistika dubine sondiranja oko homolognog zuba prema API-ju  
**Table 4** Descriptive statistics of depth probes around the homologous tooth by API

API (H)		Dubina džepa distalno • Depth probes distal (mm)	Dubina džepa mezijalno • Depth probes mesial (mm)	Dubina džepa vestibularno • Depth probes vestibular (mm)	Dubina džepa oralno • Depth probes oral (mm)
Negativan • Negative	N	6	6	6	6
	A. sredina • A. mean	1,83	1,83	1,67	1,33
	Medijan • Median	2,00	2,00	2,00	1,00
	Std. devijacija • Std. Deviation	0,75	0,75	0,52	0,52
	Koeficijent asimetrije • Asymmetry coefficient	0,31	0,31	-0,97	0,97
	Koeficijent zaobljenosti • Curvature coefficient	-0,10	-0,10	-1,88	-1,88
	Raspon podataka • Data range	2	2	1	1
	Minimum	1	1	1	1
	Maksimum • Maximum	3	3	2	2
Pozitivan • Positive	N	24	24	24	24
	A. sredina • A. mean	2,38	2,33	1,21	1,79
	Medijan • Median	2,00	2,00	1,00	2,00
	Std. devijacija • Std. Deviation	0,92	1,17	0,41	0,66
	Koeficijent asimetrije • Asymmetry coefficient	2,75	1,60	1,53	0,24
	Koeficijent zaobljenosti • Curvature coefficient	10,33	3,17	0,38	-0,55
	Raspon podataka • Data range	5	5	1	2
	Minimum	1	1	1	1
	Maksimum • Maximum	6	6	2	3

**Tablica 5.** Deskriptivna statistika retrakcije gingive prema API-ju  
**Table 5** Descriptive gingival recession statistics according to the API

API (H)		Retrakcija vestibularno • Recession vestibular (mm)	Retrakcija oralno • Recession oral (mm)
Negativan • Negative	N	6	6
	A. sredina • A. mean	1,50	0,83
	Medijan • Median	0,50	0,00
	Std. devijacija • Std. Deviation	2,07	2,04
	Koeficijent asimetrije • Asymmetry coefficient	1,21	2,45
	Koeficijent zaobljenosti • Curvature coefficient	0,20	6,00
	Raspon podataka • Data range	5	5
	Minimum	0	0
	Maksimum • Maximum	5	5
Pozitivan • Positive	N	24	24
	A. sredina • A. mean	1,42	0,67
	Medijan • Median	1,00	0,00
	Std. devijacija • Std. Deviation	1,64	1,17
	Koeficijent asimetrije • Asymmetry coefficient	1,32	2,51
	Koeficijent zaobljenosti • Curvature coefficient	1,72	7,67
	Raspon podataka • Data range	6	5
	Minimum	0	0
	Maksimum • Maximum	6	5

## Rezultati

Rezultati naše studije pokazali su pozitivan API na 30 % implantata, a na 70 % bio je negativan. Vrijednosti PBI-ja bile su identične vrijednostima API-ja. Izmjerena je prosječna retrakcija mukoze oko implantata od 0,15 mm (0 – 5 mm) i prosječna vrijednost dubine sondiranja oko implantata od 2,25 mm (0 – 6 mm). Na homolognim zubima API je bio pozitivan na 78,3 % zuba, kao i PBI. Izmjerena je prosječna

## Results

The results of our study have shown a positive API on 30% of the implants and a negative one on 70% of the implants. The PBI values were identical to the API values. The average mucosal retraction measured around the implants was 0.15 mm (ranging from 0 to 5 mm), and the average probing depth was 2.25 mm (ranging from 0 to 6 mm). As regards the homologous teeth, the API and PBI were positive

**Tablica 6.** Deskriptivna statistika dubine sondiranja oko implantata prema API-ju  
**Table 6** Descriptive statistics of probe depth around the implant to the API

API (H)		Dubina džepa distalno • Depth probes distal (mm)	Dubina džepa mezijalno • Depth probes mesial (mm)	Dubina džepa vestibularno • Depth probes vestibular (mm)	Dubina džepa oralno • Depth probes oral (mm)
Negativan • Negative	N	21	21	21	21
	A. sredina • A. mean	2,52	2,71	1,57	2,29
	Medijan • Median	2,00	3,00	1,00	2,00
	Std. devijacija • Std. Deviation	1,03	1,42	1,03	0,96
	Koeficijent asimetrije • Asymmetry coefficient	0,54	0,56	1,92	0,50
	Koeficijent zaobljenosti • Curvature coefficient	0,34	-0,19	5,55	-0,44
	Raspon podataka • Data range	4	5	5	3
	Minimum	1	1	0	1
	Maksimum • Maximum	5	6	5	4
Pozitivan • Positive	N	9	9	9	9
	A. sredina • A. mean	2,11	2,11	2,22	2,22
	Medijan • Median	2,00	2,00	2,00	2,00
	Std. devijacija • Std. Deviation	0,78	0,78	1,20	0,83
	Koeficijent asimetrije • Asymmetry coefficient	-0,22	-0,22	1,68	-0,50
	Koeficijent zaobljenosti • Curvature coefficient	-1,04	-1,04	3,69	-1,28
	Raspon podataka • Data range	2	2	4	2
	Minimum	1	1	1	1
	Maksimum • Maximum	3	3	5	3

**Tablica 7.** Deskriptivna statistika retrakcije mukoze oko implantata prema API-ju  
**Table 7** Descriptive recession of mucose around the implant to the API

API (H)		Retrakcija vestibularno • Recession vestibular (mm)	Retrakcija oralno • Recession oral (mm)
Negativan • Negative	N	21	21
	A. sredina • A. mean	,24	,00
	Medijan • Median	,00	,00
	Std. devijacija • Std. Deviation	1,09	,00
	Koeficijent asimetrije • Asymmetry coefficient	4,58	
	Koeficijent zaobljenosti • Curvature coefficient	21,00	
	Raspon podataka • Data range	5	0
	Minimum	0	0
	Maksimum • Maximum	5	0
Pozitivan • Positive	N	9	9
	A. sredina • A. mean	,33	,11
	Medijan • Median	,00	,00
	Std. devijacija • Std. Deviation	1,00	,33
	Koeficijent asimetrije • Asymmetry coefficient	3,00	3,00
	Koeficijent zaobljenosti • Curvature coefficient	9,00	9,00
	Raspon podataka • Data range	3	1
	Minimum	0	0
	Maksimum • Maximum	3	1

retrakcija gingive od 1,06 mm (0 – 6 mm) i prosječna vrijednost dubine sondiranja od 1,85 mm (1 – 6 mm).

Nadalje, rezultati analize upućuju na statistički značajnu razliku između dubine sondiranja oko implantata i oko homolognog zuba (MD = -1,47; t = -2,74; p < 0,05). U vezi s

on 78.3% of the teeth. The average gingival retraction measured was 1.06 mm (ranging from 0 to 6 mm), and the average probing depth was 1.85 mm (ranging from 1 to 6 mm).

Furthermore, the results of the analysis indicate the presence of a statistically significant difference between the probe

tim, tijekom analize rezultata retrakcije uočena je statistički značajna razlika, pri čemu je na homolognom zubu izmjerena veća retrakcija negoli na implantatu (MD = 1,83;  $t = 4,38$ ;  $p < 0,05$ ) (tablica 3.).

Od ukupno 30 ispitanika, anaerobne bakterije pronađene su kod njih 12 (40 %), a kod 18 ispitanika (60 %) nisu pronađene potencijalno patogene anaerobne bakterije. Od toga je sedam ispitanika imalo anaerobne bakterije samo na implantatu, tri samo na homolognom zubu, a kod dva ispitanika dokazane su anaerobne bakterije i na implantatu i na homolognom zubu. Kod osoba s pozitivnim nalazom pronađeno je 13 anaerobnih bakterija, među kojima su *Streptococcus anginosus* na dva implantata, *Propionibacterium acnes* na jednom implantatu, *Lactobacillus fermentum* na dva implantata i dva homologna zuba, *Lactobacillus* spp. na jednom implantatu i jednom homolognom zubu, *Bifidobacterium dentium* na jednom implantatu, *Veilonella* spp. na jednom homolognom zubu i *Veilonella parvula* na jednom homolognom zubu. U nalazu jednog ispitanika na implantatu su identificirane *Veilonella parvula* i *Prevotella denticola*. Jednom ispitaniku je na implantatu izolirano pet anaerobnih bakterija (*Prevotella nigrescens*, *Fusobacterium nucleatum*, *Selenomonas infelix*, *Capnocytophagia* spp., *Parvimonas micra*). Nije pronađena povezanost između vrste bakterije i vremena ugradnje implantata.

Modelom višestruke linearne regresije željela se ispitati statistički značajna povezanost između dubine sondiranja i nalaza anaerobnih bakterija. Rezultati analize homolognog zuba upućuju na nepostojanje statistički značajne povezanosti, pri čemu je modelom protumačeno 11,40 % ukupne varijance. Ni jedna dubina sondiranja na homolognom zubu ne ostvaruje statistički značajnu povezanost s postojanjem anaerobnih bakterija. Analizirani su i rezultati oko implantata, te je dokazana parcijalna statistički značajna povezanost između dubine sondiranja i nalaza anaerobnih bakterija. Konkretno, modelom je protumačeno 31,1 % ukupne varijance, a na jednom od četiriju mjesta mjerenja dubine sondiranja dokazano je da su statistički značajno povezani s nalazom anaerobnih bakterija ( $b_2 = 0,637$ ;  $t = 2,82$ ;  $p < 0,05$ ). Dakle, s povećanjem dubine sondiranja, povećava se vjerojatnost nalaza anaerobnih bakterija.

## Rasprava

Rezultati ovog istraživanja pokazuju da, unatoč očekivanim potencijalno patogenim bakterijama tzv. parodontnim patogenima (13) (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*), ni kod jednog ispitanika, od ukupno njih 30, nisu izolirane potencijalno patogene anaerobne bakterije iz crvenoga kompleksa bakterija (*Porphyromonas gingivalis*, *Treponema denticola* i *Tannerella forsythia*) ni na implantatu ni na prirodnom homolognom zubu.

Kod 40 % (14) ispitanika izolirane su druge anaerobne bakterije, uključujući i one iz narančastog kompleksa. Cortelli i suradnici (14) u svojem su istraživanju dokazali trend

depth around the implant and about the homologous tooth (MD = -1.47;  $t = -2.74$ ;  $p < 0.05$ ) (Table 2). In the previous study, a statistically significant difference was observed when retraction results were measured, with a higher retraction in the homologous tooth compared to the implant (MD = 1.83;  $t = 4.38$ ;  $p < 0.05$ ) (Table 3).

Anaerobic bacteria were found in 12 out of 30 participants (40%), while no potentially pathogenic anaerobic bacteria were found in the remaining 18 participants (60%). Out of 12 participants, in 7 of them the anaerobic bacteria were present only on the implant, in 3 of them only they were present on the homologous tooth, while in 2 participants the anaerobic bacteria were present on both the implant and the homologous tooth. In those subjects, 13 anaerobic bacteria were found, including *Streptococcus anginosus* on 2 implants, *Propionibacterium acnes* on 1 implant, *Lactobacillus fermentum* on 2 implants and 2 homologous teeth, *Lactobacillus* spp. on 1 implant and 1 homologous tooth, *Bifidobacterium dentium* on 1 implant, *Veilonella* spp. on 1 homologous tooth, and *Veilonella parvula* on 1 homologous tooth. In one subject, *Veilonella parvula* and *Prevotella denticola* were identified on the implant, while in one subject 5 anaerobic bacteria were isolated on the implant (*Prevotella nigrescens*, *Fusobacterium nucleatum*, *Selenomonas infelix*, *Capnocytophagia* spp., *Parvimonas micra*). A connection between the type of bacteria and the time of placing the implant has not been found.

The model of multiple linear regressions was used to investigate the existence of statistically significant correlation between probing depth and anaerobic bacterium findings. The results of the homologous tooth analysis indicate the absence of statistically significant correlation, with the model comparing 11.40% of the total variance. No depth of probing in the homologous tooth has a statistically significant association with the presence of anaerobic bacteria. The results of the implants were also analyzed, showing a partial statistically significant correlation between the depth of the probe and the findings of anaerobic bacteria. Specifically, 31.1% of the total variance was interpreted in the model, and in one of the four sites for the measurement of the depth of the probes, they have statistically significant correlation with anaerobic bacterial findings ( $b_2 = 0.637$ ,  $t = 2.82$ ,  $p < 0.05$ ). Additionally, with the increasing depth of probe, the probability of finding anaerobic bacteria increases.

## Discussion

The results of the study have shown that despite the expected potentially pathogenic bacteria, the so-called periodontal pathogens (13) (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*), potentially pathogenic anaerobic bacteria from the red complex (*Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*) have not been isolated in any of the 30 subjects, neither on the implant nor on the homologous tooth.

In 40% of the subjects (14), other anaerobic bacteria have been isolated, including the bacteria from the orange complex. Cortelli et al. (14) demonstrated the trend of a more

češćeg pojavljivanja više anaerobnih bakterija na prirodnim zubima negoli na implantatima, a rezultati našeg istraživanja pokazali su da je više anaerobnih bakterija izolirano na implantatima negoli na homolognim zubima.

Prema dostupnoj literaturi, s obzirom na formiranje biofilma, očekuje se izolacija većeg broja anaerobnih bakterija na prirodnim zubima, odnosno da će ispitanicima kojima su izolirane anaerobne bakterije na prirodnom zubu biti izolirane i na implantatu (15, 16). Neki autori smatraju da su bakterije prirodnih zuba primarni izvor patogena te da izravno djeluju na ishod novopostavljenih implantata (17). No, Schierano i suradnici (18) obavili su analizu biofilma, vezanu za parodontne patogene oko klinički zdravih zuba i oko implantata, te nisu ustanovili značajne razlike u broju i vrsti bakterija s obzirom na dva mjesta uzorkovanja. Botero i suradnici (19) navode da je postojala značajna povezanost između subgingivnih bakterija na implantatima i susjednim zubima. Na temelju rezultata u našem istraživanju, uočena je prisutnost subgingivnih bakterija na implantatu i susjednom zubu kod dvoje ispitanika.

Koyanagi i suradnici (20) ustanovili su da je mikrobnna flora raznolikija u slučaju periimplantitisa u odnosu prema parodontitisu, te da su *Fusobacterium* spp. i *Streptococcus* spp. dominantni patogeni u oba stanja. No istaknuli su da je samo kod oboljelih od periimplantitisa izolirana *Parvimonas micra*, što je u skladu s rezultatima našeg istraživanja.

Neki autori (21) smatraju da je parodontna bolest povezana s periimplantitisom te da su *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia* i *Treponema denticola* bile izolirane u zdravom tkivu, ali i kod osoba s periimplantatnim mukozitisom i periimplantitisom.

Sumida i suradnici (22) ustanovili su da postoji prijenos *Porphyromonas gingivalis* i *Prevotella intermedia* iz parodontnih džepova na područje oko implantata. Stingu i suradnici (23) izolirali su *Prevotella intermedia* i *Prevotella nigrescens* kod zdravih osoba i onih s parodontnom bolešću. *Prevotella intermedia* ubraja se u narančasti kompleks bakterija, zajedno s *Fusobacterium nucleatum*, *Fusobacterium periodonticum*, *Parvimonas micra*, *Streptococcus constellatus*, *Eubacterium nodatum* i *Campylobacter rectus* (24, 25). *Prevotella intermedia* teško se može razlikovati od *Prevotella nigrescens* uobičajenim laboratorijskim metodama, uključujući i plinsku kromatografiju (26, 27), no može se identificirati i razlikovati od *Prevotella nigrescens* s pomoću metode masene spektrometrije proteina (MALDI-TOF-MS metode). Posljednjih je godina *Prevotella nigrescens* prihvaćena kao mogući parodontni patogen. Smatra se da potiče produkciju medijatora upale i da s pomoću lipopolisaharida može uzrokovati resorpciju alveolarne kosti (28). U nekim novijim studijama *Prevotella nigrescens* izolirana je u značajno većem omjeru na mjestima klinički jače izražene upale pridružene dubljim parodontnim džepovima, što je sukladno našoj studiji, a to je dokazano kod jednog ispitanika kojemu je dubina sondiranja vestibularno iznosila 5 mm. (25). Također je detektirana u većem postotku kod pacijenata s izraženim lokaliziranim i generaliziranim oblikom parodontitisa te generaliziranim agresivnim i kroničnim parodontitisom (29, 30). U skladu s ovom studijom,

frequent presence of anaerobic bacteria on natural teeth than on implants, while the results of our study have shown that more anaerobic bacteria have been isolated on implants compared to homologous teeth.

According to the available literature on the subject, in view of the biofilm formation, it is expected that a larger number of anaerobic bacteria will be isolated on natural teeth or that in the subjects in which anaerobic bacteria have been isolated on natural teeth, they will be isolated on the implant as well (15, 16). Some authors believe that bacteria on natural teeth are the primary source of pathogens and that they directly affect the outcome of the newly-placed implants (17). However, Schierano et al. (18) analyzed the biofilm in relation to periodontal pathogens around clinically healthy teeth and around implants and they have not found any substantial differences in terms of the number and type of bacteria considering the two sampling locations. Botero et al. (19) stated that there was a significant connection between the subgingival bacteria in implants and in the neighboring teeth. The results of our study show the presence of subgingival bacteria on the implant and on the neighboring tooth in two subjects.

Koyanagi et al. (20) found that the microbial flora in periimplantitis is more varied than in periodontitis and that *Fusobacterium* spp. and *Streptococcus* spp. are the dominant pathogens in both conditions. However, they found that *Parvimonas micra* had been isolated only in the patients with periimplantitis, which is consistent with the results of our study.

Some authors (21) believe that periodontal disease is related to periimplantitis and that *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia* and *Treponema denticola* were isolated in healthy tissue, but also in individuals with peri-implant mucositis and peri-implantitis.

Sumida et al. (22) found that *Porphyromonas gingivalis* and *Prevotella intermedia* are transported from periodontal pockets of healthy teeth onto the area around the implant. Stingu et al. (23) isolated *Prevotella intermedia* and *Prevotella nigrescens* both in healthy individuals and in patients with the periodontal disease. *Prevotella intermedia* species belongs to the orange complex of bacteria, together with *Fusobacterium nucleatum*, *Fusobacterium periodonticum*, *Parvimonas micra*, *Streptococcus constellatus*, *Eubacterium nodatum* and *Campylobacter rectus* (24, 25). It is difficult to identify *Prevotella intermedia* and distinguish it from *Prevotella nigrescens* using ordinary laboratory methods, including gas chromatography (26, 27), but that can be achieved using the protein mass spectrometry method (MALDI-TOF-MS method). *Prevotella nigrescens* has recently been accepted as a possible periodontal pathogen. It is believed that it fosters the production of mediators of inflammation and that its lipopolysaccharide may cause alveolar bone resorption (28). In some more recent studies *Prevotella nigrescens* was isolated in a substantially larger proportion from the places with a clinically more prominent inflammation associated with deeper periodontal pockets, which was demonstrated in one of the subjects of our study, whose probing depth was 5 mm vestibularly (25). A higher percentage of *Prevotella nigrescens* was also detected in patients with localized and generalized forms of peri-



rezultati našeg istraživanja također su pokazali da je, uz klinički izraženu upalu oko implantata s vestibularnom dubinom sondiranja od 5 mm, *Prevotella nigrescens* izolirana kod jednog ispitanika.

Lisa Heitz-Mayfield (31) uočila je povezanost između loše oralne higijene, anamnestičkih podataka o parodontitisu i pušenja kao najznačajnijih rizičnih čimbenika za nastanak periimplantitisa.

Poznato je da hrapavost površine implantata utječe na kolonizaciju biofilma. Titanijska površina s hrapavošću koja je prosječno  $Ra < 0,088$ , inhibira kolonizaciju i sazrijevanje biofilma (32). Suprotno tomu, hrapavost površine  $Ra > 0,2 \mu\text{m}$  povećava stvaranje biofilma te pogoduje nastanku periimplantitisa (33).  $Ra < 0,2 \mu\text{m}$  nema utjecaja na stvaranje supragingivnog i subgingivnog plaka (34) te su zato neki istraživači (35) zaključili da  $Ra < 0,2 \mu\text{m}$  nema učinka na mikrofloru.

Istraživanje provedeno 2016. godine dokazalo je da su mikroorganizmi u periimplantantnim lezijama slični onima u parodontnim lezijama, ali da je korelacija između dosadašnjih studija dosta teška zbog drukčijih načina uzorkovanja te da bi nove metagenomske tehnike uzorkovanja trebale biti metoda izbora za buduća istraživanja (36).

## Zaključak

U skupini od ukupno 30 ispitanika nisu izolirane potencijalno patogene anaerobne bakterije iz crvenoga kompleksa (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*).

Iz rezultata ovog istraživanja jasno je da su od 30 ispitanika kod njih 12 (40 %) izolirane druge vrste anaerobnih bakterija, uključujući i one iz narančastog kompleksa, bilo na zubu bilo na implantatu.

Zapaženo je više anaerobnih bakterija na implantatu negoli na homolognom zubu. Anaerobne bakterije koje su bile istodobno i na implantatu i na homolognom zubu nađene su u manjem broju uzoraka.

S obzirom na formiranje biofilma, moglo se očekivati da će se više anaerobnih bakterija izolirati na prirodnom zubu, ili da će ispitanicima kojima su izolirane anaerobne bakterije na prirodnom zubu biti izolirane i na implantatu (15, 16).

Neki autori smatraju da se mikroorganizmi povezani s nastankom parodontne bolesti razlikuju u raznim dijelovima svijeta i mogu varirati zbog niza čimbenika. Upravo ta spoznaja trebala bi potaknuti svaku zemlju da uspostavi vlastiti dentalni mikrobiološki profil kako bi se pripremile smjernice za provedbu odgovarajućih preventivnih mjera te u skladu s tim poduzele ciljane terapijske mjere (23).

U svrhu dobivanja što relevantnijih rezultata, potrebna su daljnja istraživanja na što većem broju ispitanika.

## Sukob interesa

Nije bilo sukoba interesa.

odontitis, as well as in patients with generalized aggressive and chronic periodontitis (29, 30). In accordance with this study, we have also isolated *Prevotella nigrescens* in one subject with a clinically prominent inflammation around the implant, with the probing depth of 5 mm.

Using meta-analysis, Heitz-Mayfield (31) determined that poor oral hygiene, history of periodontitis and smoking are the most important risk factors for the development of periimplantitis.

It is known that the rough surface of implants has an effect on the colonization of biofilm. A titanium surface with the average roughness of  $Ra < 0.088 \mu\text{m}$  inhibits the colonization and maturation of biofilm (32). Conversely, the surface roughness of  $Ra > 0.2 \mu\text{m}$  leads to increased biofilm formation and favors the emergence of peri-implantitis (33).  $Ra < 0.2 \mu\text{m}$  does not have an influence on the formation of supragingival and subgingival plaque (34); consequently, some scholars have concluded (35) that  $Ra < 0.2 \mu\text{m}$  does not affect micro flora. A study carried out in 2016 demonstrated that the microorganisms present in peri-implant lesions are similar to those in periodontal lesions, but that correlation between the conducted studies is quite unlikely because of different sampling methods; it was concluded that new metagenomic sampling techniques should be the method of choice for future studies (36).

## Conclusion

In a group of 30 subjects no potentially pathogenic anaerobic bacteria from the red complex have been isolated (*Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola*).

The study has shown that in 12 out of 30 subjects (40%) other types of anaerobic bacteria have been isolated, including bacteria from the orange complex, either on a tooth or on an implant.

Anaerobic bacteria were more abundantly present on implants than on homologous teeth. The presence of anaerobic bacteria both on a tooth and on an implant was found in few samples.

In view of the biofilm formation, it was expected that a larger number of anaerobic bacteria would be isolated on natural teeth or that in the subjects in which anaerobic bacteria were isolated on natural teeth, they would be isolated on the implant as well (15, 16).

Some authors believe that the microorganisms related to the development of periodontal diseases differ in different parts of the world and that they can vary due to a series of factors. In light of that presumption, every country should establish its own dental microbiological profile in order to develop guidelines for the implementation of the corresponding preventive measures and in order to take targeted therapeutic measures accordingly (23).

In order to obtain more relevant results, further studies with larger samples are needed.

## Conflict of interest

None declared

**Abstract**

**Objective:** The objective of the study was to establish whether there is a difference in the presence of potentially pathogenic anaerobic microorganisms around the implant and the homologous tooth in implant-prosthetic patients who received individual information about maintaining their oral hygiene. **Material and methods:** The study included 30 subjects with dental implants and metal-ceramic crowns. A periodontal probe was used to record the approximal plaque index (API), the papilla bleeding index (PBI), the periodontal pocket probing depth (PD) and the gingival recession. The fluid around the implant and the gingival sulcus fluid around the homologous tooth on the opposite lateral side were sampled. **Results:** The results have shown a positive API and PBI on 30% of the implants and a negative one on 70% of the implants. The average mucosal retraction measured around the implants was 0.15 mm, and the average probing depth was 2.25 mm. The API and PBI were positive on 78.3% of the homologous teeth. The average gingival retraction measured was 1.06 mm, and the average probing depth was 1.85 mm. Anaerobic bacteria were found in 12 out of 30 subjects (40%). Anaerobic bacteria were isolated only on the implant in 7 subjects, only on the homologous tooth in 3 subjects and both on the implant and the homologous tooth in 2 subjects. **Conclusions:** Anaerobic bacteria were more abundantly present on implants than on homologous teeth.

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**Key words**

Anaerobic Bacteria, Dental Implants; Peri-Implantitis; Dental Plaque Index; Periodontal Pocket; Gingival Recession

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