ISSN 2306-4269. Lviv Clinical Bulletin. 2022, 3(39) - 4(40): 40-44 https://orcid.org/10.25040/lkv2022.03-04.040

УДК 616.314–089.23:616.31–022–07

V. Vovk[1, 2], M. Panas[1], M. Sobchyshyn[3], P. Kriukov[2]
[1] Danylo Halytsky Lviv National Medical University
[2] Medical dentistry centre Bioimplantservice, Lviv
[3] Aesthetic dentistry clinic PERFECTDENT, Lviv

Colonization of New and Reused Dental Implant Healing Abutments by Oral Microbiota during Implantation Period

Introduction. Dental implants placement procedure has become an important option in dentistry to replace missing teeth and restore their function and has been commonly used in recent years [21]. Implant healing abutment (IHA) placement is one of the stages of implantation. However, peri-implant diseases such as mucositis and peri-implantitis are still big problems facing implantologists [6].

An exposed dental implant surface is prone to microbial colonization and biofilm formation [1]. Such biofilms are the main source of pathogens for peri-implant disease, they may trigger infection and cause inflammatory destruction of the peri-implant tissue [11].

Oral bacteria are the main components of the oral microbiota and naturally form biofilm communities with each other on the surface under almost any environmental condition or hygienic status of the oral cavity as a natural biotope [5]. Such communities have much more virulent characteristics compared to bacteria in planktonic state, since they are less penetrable by antibodies, neutrophils or antimicrobial factors of the host [14]. Many bacterial species in biofilms exhibit greater tolerance to different environmental factors, such as pH, oxygen, UV radiation, drying etc [7].

Oral cavity is a constantly changing dynamic ecosystem continuously colonized by microorganisms. Development of the oral microbial community involves competition as well as cooperation among colonizers of the hard surface. Changes in the local microenvironment can cause changes of the biofilm microflora, enabling certain species to overgrow, enhance their virulence and eventually become opportunistic pathogens. Dysbiotic biofilms may elevate community virulence, and the resulting community targets specific aspects of host immunity to further disable immune surveillance while promoting an overall inflammatory response [10]. Inflammation and dysbiosis reinforce each other and stimulate the inflammatory tissue destruction, as in the case of bone loss in peri-implantitis [17]. Therefore careful oral hygiene, prevention of the presence of bacteria in the region alongside with the use of sterile instruments and components to avoid cross-infection between patients are important goals of implantology and essential for long-term implant success [2]. Nevertheless, the reuse of implant healing abutments (IHA) is common in dental practice. It is considered that effective elimination of bacteria, fungus and viruses is accomplished by conventional cleaning and sterilization. But multiple cycles of sterilization could affect the biocompatibility of IHAs surface and could result in microfractures of the temporary components [9]. Therefore, the aim of our study is to analyze and compare the colonization by microbial symbionts of the surface of new and reused dental implant healing abutments in patients undergoing implantation.

The aim of the study. To analyze and compare the colonization of new and reused dental implant healing abutments by oral microbiota in patients during implantation.

Materials and methods. The study began with the selection of patients who gave informed consent to participate in the study according to the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, relevant laws of Ukraine and international acts (Art. 43 of the Law of Ukraine "Basics of the Legislation of Ukraine on Health Care", Order of the Ministry of Health of Ukraine dated 14.02.2012 No. 110, Order of the Ministry of Health of Ukraine dated 23.11.2004 No. 566, Orders of the Ministry of Health of Ukraine No. 751 and No. 1422).

4 groups of 5 randomly selected patients in each were enrolled in this study, 12 women and 8 men, with partial secondary adentia, without significant medical anamnesis, all non smokers and having good oral hygiene. A total of 36 submerged dental implants (implantSwiss) were placed using two stage surgery protocol and were completely buried under mucosa, with an appropriate healing time (3-6 months).

The second stage surgery was considered as a baseline, all 36 implants were surgically exposed and 36 different

 © Vovk V., Panas M., Sobchyshyn M., Kriukov P., 2022

healing abutments (3 and 5 mm in height) from the same implant system were placed. Group I included 12 IHAs 3 mm in height reused after proper cleaning and sterilization, Group II – 10 new IHAs 3 mm in height, Group III included 8 reused 5 mm IHAs, Group IV – 6 new 5 mm IHAs used for the first time. After 10 to 14 days sutures were removed and patients were instructed not to brush the surgical area. The only preventative treatment prescribed for patients was mouth rinsing with CHX (0.12 %) 2 times a day for 7 days.

The examination of the oral cavity was performed 2 to 3 weeks after total healing of the mucosa, without any signs of inflammation in the mouth, because the presence of sutures and swelling could determine an uncontrolled deposition of plaque and, therefore, led to biased results. We used Mira2Tone tablets to color formed on healing abutments biofilm. Each tablet was ground and dissolved in 0.9% NaCl, then established suspension was injected using sterile syringes into the oral cavity of each patient until all healing abutments were stained enough.

At this stage we evaluated the level of plaque formation on the IHAs surface: only cervical part of healing abutment covered with biofilm (fig. 1,*a*); ⅓ of the surface covered with biofilm (fig.1,*b*); more than ½ of the surface covered with biofilm (fig.1,*c*).

a *b* *c*

Fig. 1. a – Cervical part of healing abutment is covered with biofilm; *b* – ⅓ of the healing abutment surface is covered with biofilm; *c* – more than ½ of the healing abutment surface is covered with biofilm.

Microbiological examination was performed using the classical cultural method, which allows to analyze quantitative indicators of colonization by microorganisms - symbionts of the oral cavity. To prevent material contamination by environmental microflora a sterile excavator was used aseptically. 1.0 ml sample of the biological substrate from the IHA placed in transport media was streaked during one hour after the material sampling on the following growth media: 5 % sheep blood agar, meat peptone agar, selective salt egg agar, Mitis-Salivarius agar and placed in 37° C incubator for 24 hours, after what cultured bacterial colonies were counted (fig. 2, 3).

Aerobic bacteria belonging to a precise genus were determined on the basis of morphological characteristics, culture properties, and due to establishment of biochemical properties [8]. The colonization rate was estimated at the colony level.

Statistical calculation of the results was performed using personal computer and software package for statistical data analysis for biomedical research "Instat" (GraphPad Software Inc.). The results were obtained in the form of the average value of the studied parameter (M), the standard error (deviation) of the studied parameter (m) and the reliability index (*p*).

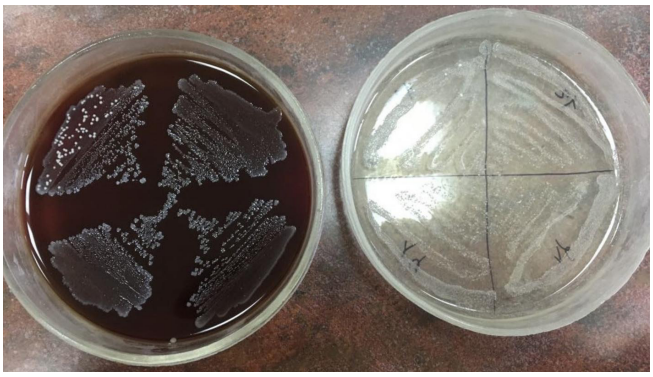
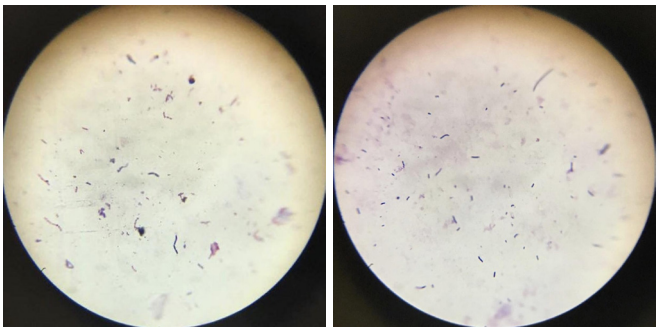


Fig. 2. Microorganisms growing on 5 % sheep blood agar, meat peptone agar



a *b*

Fig. 3. Streptobacillus (*a*), Streptococcus, Gram+ monococcus (*b*), light microscope view, magnification x630.

Results and discussion. All 36 dental implant healing abutments were contaminated with biofilm. 38.9 % of all IHAs were covered with plaque only in cervical area, 30.6 % - ⅓ of the surface and more than ½ of the surface each. However, some differences were observed in groups under investigation. In particular, group I included 25 % of IHAs covered with biofilm in the cervical area, 41.7 % - ⅓ of the surface and 33.4 % IHAs were covered with biofilm more than ½ of the surface. Group II included 50 %, 30 % and 20 % respectively. 25 % of IHAs from group III patients exposed plaque only in the cervical area and on ⅓ of the surface, while in 50 % of abutments ½ or more of the surface was covered with plaque. In group IV patients 66.7 % of the abutments were contaminated only in the cervical region, 16.7 % – on ⅓ of the surface, and 16.7 % – on half or more of the IHAs surface.

Dental implant healing abutments with formed dental plaque on which corresponded to the condition of the oral cavity treated as "satisfactory hygiene" (the plaque was found on ⅓ of the abutment surface after the staining) were enrolled in the microbiological study.

The obtained results confirmed the oral hygiene of our patients as satisfactory, since no aerobic gram-negative microbiota (in particular, enterobacteria) was detected in the material under investigation, and the existing Staphylococci did not show lecithinase activity or other signs of virulence.

Obviously, the greater formation of dental plaque on the higher healing abutments led to the formation of more significant microbiological indicators.

While studying the microbial spectrum of biofilm formed on IHAs from different groups, the following features were established (table): *Streptococcus spp* were the leading factors of contamination in all groups, in group III - 98.13 ± 0.32 CFU/ml, in group I - 97.30 ± 0.32 CFU/ml. Group II showed the lowest level of detected in biofilm microorganisms: *Staphylococcus epidermidis* - 25.60 ± 0.42 CFU/ml, in Group IV (5mm IHAs). Reuse of healing abutments led to an increase of staphylococcus population level in spite of its height - 57.80 ± 0.56 and 65.60 ± 0.64 CFU/ml ($p < 0.05$). *Streptobacillus spp* in Group I were detected 44.20 ± 0.61 CFU/ml, Group III - 53.30 ± 0.69 CFU/ml compared to Group II and IV - 26.70 ± 0.69 and 35.50 ± 0.79 CFU/ml - a significant increase of the indicated parameters (by 1.65 and 1.5 times).

Quantitative evaluation of gram+ monococci showed a more intensive formation of dental plaque on the reused abutments, although these differences were not reliably significant (table).

Population levels of groups of microorganisms found in dental plaque from new and reused healing abutments

Genus of microorganism	I group (research) n = 12 10^3 CFU/ml	II group (control) n = 10 10^3 CFU/ml	III group (research) n = 8 10^3 CFU/ml	IV group (control) n = 6 10^3 CFU/ml
Streptococcus spp	97.30 ± 0.32	94.40 ± 0.69	98.13 ± 0.32	95.80 ± 0.52
Staphylococcus epidermidis	57.80 ± 0.56*	25.60 ± 0.42	65.60 ± 0.64*	46.00 ± 0.80
Streptobacillus spp	44.20 ± 0.61*	26.70 ± 0.69	53.30 ± 0.69*	35.50 ± 0.79
Gram+ monococcus	19.50 ± 0.58	17.20 ± 0.41	20.30 ± 0.48	17.50 ± 0.24

Note.* – indicators of groups significantly different from each other ($p < 0.05$).

The implant healing abutments are important temporary components of implantology systems: they are essential for soft tissue conditioning since they provide a scaffold for tissue growth [20] and are used to improve aesthetic result of implantation. IHA is exposed to a unique combination of conditions, with one part supragingival and exposed to the oral cavity microbiota, and the other part – subgingival, being in contact with soft tissue. Reuse of dental implant healing abutments is common in clinical practice, primarily for economic reasons [19]. The purpose of this study was to compare the features of biofilm formation on new and reused IHAs. We hypothesize that reusable IHAs would have lower corrosion resistance and higher level of surface degradation compared to new IHAs, and that these changes could potentially affect the colonization of the surface by microorganisms [12, 15], as it is known that biofilm formation on implant surface is controlled not only by growth conditions, but also, by the nature of the colonized surface. In addition, several studies have indicated that a combination of mechanical and chemical cleansing is ineffective in complete removal of biological debris and biofilm from abutments [4, 18], the other retrieval study showed the presence of viable bacteria [3] and organic carbon [13] attached to IHA surface post-sterilization. Moreover, procedures used for routine sterilization cannot inactivate prions as they can survive autoclaving even at high temperatures [16].

Further studies are needed to compare the aspects of biofilm formation on single-use and reused implant healing abutments.

Conclusions. The obtained data demonstrated that the plaque formation was statistically higher on the reused IHAs compared to the new ones despite no differences in prescribed oral hygiene. Microbiological analysis showed the highest level of contamination in group III (reused 5mm IHAs), and the lowest in group II (new 3mm IHAs). Group I (reused 3mm IHAs) was less contaminated than Group IV (reused 5mm IHAs), but more contaminated than group II (new 5mm IHAs). Retention of oral cavity microorganisms to hard surface, in particular of healing abutment, depends on the characteristics of this surface. Repeated cycles of cleaning, sterilization and use of IHA changes their surface characteristics, which could affect the initiation of biofilm formation, primary colonization and adhesion of microorganisms. Streptococci – the most important components of the oral microbiota – were detected in higher population levels compared to other microorganisms of the oral microbiota, but the differences in their colonization of new and reused healing abutments were unsignificant, unlike Staphylococci, which do not belong to the specific microbiota of oral cavity, but demonstrate much stronger effect on colonization and adhesion to the artificial material. Filamentous bacteria and streptobacilli are more actively involved in biofilm formation on the changed surface due to their specific morphology. Excessive colonization leads to co-aggregation of pathogenic microorganisms, which can cause mucositis or peri-implantitis and, as a result, loss of the implant. Therefore the practice of reusing healing abutments between patients should be reconsidered.

References

1. Belibasakis GN, Charalampakis G, Bostanci N, Stadlinger B. Peri-implant infections of oral Adv Exp Med Biol. 2015;830:69-84. https://doi.org/10.1007/978-3-319-11038-7_4
2. Bressan E, Tessarolo F, Sbricoli L, Caola I, Nollo G, DiFiore A. Effect of chlorhexidine in preventing plaque biofilm on healing abutment: a crossover controlled study. Implant 2014;23(1):64-68. https://doi.org/10.1097/ID.0000000000000018

3. Cakan U, Delilbasi C, Er S, Kivanc M. Is it safe to reuse dental implant healing abutments sterilized and serviced by dealers of dental implant manufacturers ? An in vitro sterility Implant Dent. 2015;24(2):174-179. https://doi.org/10.1097/ID.0000000000000198
4. Charalampakis G, Ramberg P, Dahlén G, Berglundh T, Abrahamsson I. Effect of cleansing of biofilm formed on titanium discs. Clin Oral Implants 2015;26(8):931-936. https://doi.org/10.1111/clr.12397
5. Etxeberria M, López-Jiménez L, Merlos A, Escuín T, Viñas M. Bacterial adhesion efficiency on implant abutments: A comparative study. Int 2013;16:235-242.
6. Grössner-Schreiber B, Griepentrog M, Haustein I, Müller WD, Lange KP, Briedigkeit H et al. Plaque formation on surface modified dental implants. Aninvitrostudy. Clin Oral Implants 2001;12(6):543-551. https://doi.org/10.1034/j.1600-0501.2001.120601.x
7. Hojo K, Nagaoka S, Ohshima T, Maeda N. Bacterial interactions in dental biofilm bevelopment. J Dent 2009;88(11):982-990. https://doi.org/10.1177/0022034509346811
8. Holt JG, Krieg NR, Sneath PHA et al. Bergey's manual of determinative bacteriology. 9thed. Baltimore (MD): Williams&Wilkins; 1994:1599.
9. Jain SS, Schramm STJ, Siddiqui DA, Huo W, Palmer KL, Wilson TG Jr et al. Effects of multiple implantations of titanium healing abutments: Surface characteristics and microbial colonization. Dent 2020;36(9):e279-e291. https://doi.org/10.1016/j.dental.2020.05.016
10. Lamont RJ, Hajishengallis G. Polymicrobial synergy and dysbiosis in inflammatory disease. Trends Mol 2015;21(3):172-183. https://doi.org/10.1016/j.molmed.2014.11.004
11. Paquette DW, Brodala N, Williams RC. Risk factors for endosseous dental implant failure. Dent Clin North 2006;50(3):361-374. https://doi.org/10.1016/j.cden.2006.05.002
12. Rodrigues DC, Valderrama P, Wilson TG, Palmer K, Thomas A, Sridhar S et al. Titanium corrosion mechanisms in the oral environment: a retrieval study. Materials (Basel). 2013;6(11):5258-5274. https://doi.org/10.3390/ma6115258
13. Sánchez-Garcés MA, Jorba M, Ciurana J, Vinas M, Vinuesa MT. Is the re-use of sterilized implant abutments safe enough? (Implant abutment safety). Med Oral Pathol Oral Cir Bucal. 2019;24(5):e583-e587. https://doi.org/10.4317/medoral.22967
14. Schaudinn C, Gorur A, Keller D, Sedghizadeh PP, Costerton JW. Periodontitis: an archetypical biofilm disease. J Am Dent 2009;140(8):978-986. https://doi.org/10.14219/jada.archive.2009.0307
15. Sridhar S, Wilson TG Jr, Palmer KL, Valderrama P, Mathew MT, Prasad S et al. In vitro investigation of the effect of oral bacteria in the surface oxidation of dental implants. Clin Implant Dent Relat Res. 2015;17 Suppl 2:e562-575.
16. Sushma B, Gugwad S, Pavaskar R, Malik SA. Prions in dentistry: A need to be concerned and known. J Oral Maxillofac 2016;20:111-114. https://doi.org/10.4103/0973-029X.180961
17. Toncheva KD. Biofilm in dentistry. Actual problems of modern medicine: Bulletin of the Ukrainian Medical Stomatological Academy. 2015;616.31-74:615.46
18. Vezeau PJ, Keller JC, Wightman JP. Reuse of healing abutments: an in vitro model of plasma cleaning and common sterilization techniques. Implant 2000;9(3):236-246. https://doi.org/10.1097/00008505-200009030-00009
19. Wadhwani C, Schonnenbaum TR, Audia F, Chung KH. In-vitro study of the contamination remaining on used healing abutments after cleaning and sterilizing in dental practice. Clin Implant Dent Relat 2016;18(6):1069-1074. https://doi.org/10.1111/cid.12385
20. Wheelis SE, Wilson TG Jr, Valderrama P, Rodrigues DC. Surface characterization of titanium implant healing abutments before and after placement. Clin Implant Dent Relat 2018;20(2):180-190. https://doi.org/10.1111/cid.12566
21. Wifaq M.Ali Al-Wattar, Warkaa M.Al-Wattar, Afya Sahib Diab Al-Radha. Microbiological and cytological response to dental implant healing abutment. J Int Den Med Res. 2017;10(3):891-898.

The article was received by the journal on 27.09.2022

Conflict of interest
The authors of this article argue that there is no conflict of interest.

Colonization of New and Reused Dental Implant Healing Abutments by Oral Microbiota during Implantation Period

V. Vovk, M. Panas, M. Sobchyshyn, P. Kriukov

Introduction. Reuse of implant healing abutments is common in dental practice, mainly due to economical reasons.

The aim of the study. To analyze and compare the colonization of new and reused dental implant healing abutments by oral microbiota in patients subjected to dental implantation.

Materials and methods. 4 groups, 20 patients, 36 healing abutments were examined using clinical and microbiological methods.

Results. Clinical and microbiological analysis showed that biofilm formation was statistically higher on the reused IHAs compared to the new ones.

Conclusions. The practice of administration of the reused healing abutments between patients should be reconsidered.

Keywords: dental implant, healing abutment, biofilm, bacteria, peri-implantitis, mucositis, reuse.

Колонізація представниками мікробіоти порожнини рота нових і використовуваних повторно формувачів ясен після проведення дентальної імплантації

В. Ю. Вовк, М. А. Панас, М. Р. Собчишин, П. С. Крюков

Вступ. Процедура дентальної імплантації останнім часом стала дуже популярною і щораз частіше застосовується у практиці лікаря-стоматолога. Проте асоційовані з нею патологічні стани, такі як перимукозит і періімплантит, досі становлять велику проблему, з якою стикаються імплантологи. Повторне використання формувачів ясен є поширеним явищем у стоматологічній практиці, переважно через економічні причини, проте це може бути небезпечно.

Мета. З'ясувати й порівняти колонізацію представниками мікробіоти порожнини рота нових і повторно використовуваних формувачів ясен після проведення дентальної імплантації.

Матеріали й методи. У дослідженні брали участь чотири групи по п'ять випадково відібраних пацієнтів у кожній, усього 36 формувачів ясен 3 і 5 мм заввишки. Аналіз утворення нальоту на формувачах проведено клінічно, за допомогою таблеток для забарвлення нальоту. Матеріалом для мікробіологічного дослідження слугували формувачі ясен і сформована на них біоплівка. Мікроорганізми були ідентифіковані відповідно до класифікаційних даних, запропонованих у дев'ятому виданні посібника Bergey. Статистичну обробку результатів проводили за допомогою програмного забезпечення статистичного аналізу даних для біомедичних досліджень Instat (GraphPad Software Inc.).

Результати. Клінічний і мікробіологічний аналіз показав, що біоплівка утворювалася інтенсивніше на формувачах ясен пацієнтів, які використано повторно, ніж на нових.

Висновки. Варто переглянути повторне використання формувачів ясен у пацієнтів під час імплантації у практиці лікаря-стоматолога.

Ключові слова: дентальний імплантат, формувач ясен, біофільм, бактерії, періімплантит, перімукозит, повторне використання.

Information about the authors

1. Vovk Volodymyr; Danylo Halytsky Lviv National Medical University, Department of surgical dentistry and maxillofacial surgery (69 Pekarska str., Lviv, 79010; +38 (032) 276-97-63); Doctor of Phylosophy (PhD), Associate Professor; Medical dentistry centre Bioimplantservice, (8 Dontsova str., Lviv, 79010; +38 (032) 275 09 91); Doctor surgeon and orthopedist ; 148/5 Ivana Franka str., Lviv, 79011; + 38 067 100 30 87; dr. vovk1987@gmail.com; https://orcid.org/0000-0003-0568-3966
2. Panas Marta; Danylo Halytsky Lviv National Medical University, Microbiology Department (69 Pekarska str., Lviv, 79010); Associate Professor of the Microbiology Department; 12 Zelena str., Lviv, 79005, +380989438783 ; panas.marta@gmail.com; https://orcid.org/0000-0002-3302-6974
3. Sobchyshyn Mariya; Aesthetic dentistry clinic (1B Terchakovtsiv str., Lviv, 79005); General dentist; 60 Shevchenka str., Lviv, 79039, +380991399429; marysobchyshyn@gmail.com ; https://orcid.org/0000-0003-1044-0360
4. Kriukov Petro; Medical dentistry centre Bioimplantservice (8 Dontsova Street, Lviv, 79010; +38 (032) 275 09 91); Doctor orthopedist ; 30/14 Malogoloskivska str., Lviv, 79066; petrokryukov@gmail.com ; https://orcid.org/0000-0002-0304-5402