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ORIGINAL PAPER

Microbiological assay of soft acrylic and silicone-based materials used in overdentures on mini dental implants

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Abstract

The aim of this study was to assess species composition of microbiota developed on soft acrylic and silicone based relining materials, used as soft matrices, in overdentures retained by mini dental implants. Four different soft materials, two acrylic (Tissue Conditioner; Visco-gel) and two silicone based (Mollosil; Elite H-D) were simultaneously applied in the mandibular overdenture base, at the housing site. After seven days, these materials were removed from the overdenture base, and microbiologically assayed. Microbiological profile of soft materials adjacent to mini dental implants was diverse, consisting mainly of Gram negative bacilli even in healthy conditions. Some of the species identified were the same in the materials applied simultaneous, and others were different. Different commercial products of soft materials showed different resistance degrees to microbial colonization, the highest number of microbial strains being identified in case of Tissue Conditioner, and the lowest for Elite H-D and Visco-gel. Further studies are recommended to be conducted in order to identify the best materials for specific clinical purposes.

Keywords: microbial strains, dental prosthesis, peri-implantitis, dentures, relining

1. Introduction

Overdenture retained by mini dental implants is a treatment alternatives for completely edentulous patients, indicated especially in the elderly, due to increasing prostheses retention by a relatively minimally invasive surgical intervention. This treatment alternative registers an increased use nowadays, maybe in correlation to the aging population and due to the better treatment outcome when compared to still most use conventional denture (PREOTEASA & al [1]; PREOTEASA & al [2]). One of the main complications of this treatment, which can have a negative effect on the outcome, are implant mucositis and peri-implantitis. These are strongly linked to accuracy of oral hygiene maintenance, which can be a problem in the aged due to relatively frequent decreased dexterity and age related visual impairment. Considering in implant mucositis gum inflammation is found, and peri-implantitis is characterized by inflammatory bone loss, which is suspected to be trigger by infection, knowledge of microbiologic profile associated with healthy and diseased implant site are important aspects to be clarified (RAKIC & al [3]). The state of the art is constantly advancing on the issue of microbiota adjacent to dental implants, even so it isn't still well clarified, being necessary to differentiate between different clinical situations e.g., fixed or removable dental prostheses on

dental implants. The evidence of microbiota found in mini dental implant overdenture patients, in different normal and pathological situations is relatively scarce, it being needed to be clarified in order to have an important information that can contribute significantly to a better treatment outcome. During overdenture treatment several materials are used, corresponding to different treatment steps. Soft materials, acrylic or silicone based are sometimes used for different purposes e.g., as soft matrices, tissue conditioning, relining. Considering their usage, sometimes for an extended period, sometimes during healing period, sometimes during the critical phase of osseointegration, an optimal biocompatibility is a necessary condition, this meaning low cytotoxicity and low microbial loading (PREOTEASA & al [4]; POLL & al [5]; CINTEZA & al [6]; KOSANIĆ & al [7]). In this regard is necessary to identify which is the best soft material in terms of low colonization potential, in order to ensure proper condition for a good outcome of implant overdenture treatment.

The aim of this study was to assess species composition of microbiota developed on soft acrylic and silicone based relining materials, used as soft matrices, in overdentures retained by mini dental implants, with O-ring as attachment system.

2. Materials and Methods

Two patients, treated by overdentures retained by mini dental implants were included in this study. They were both completely edentulous in both jaws. The first patient had both maxillary and mandibular overdenture retained by mini dental implants. The second patient had a maxillary conventional denture in the maxilla and on overdenture retained by mini dental implants in the mandible. The overdentures were manufactured like conventionally complete dentures, with complete coverage of the support area, until the anatomical and functional borders, with a lingualized occlusion. The mini implants used were mini1SKY (Bredent), one piece narrow dental implants, with O-ring as attachment system.

In these patients, 4 different soft tissue materials were used as soft matrices, all being applied in the mandibular overdenture base, at the housing site. There were used both soft acrylic materials (Tissue Conditioner, GC Corporation; Visco-gel, Dentsply) and silicone base materials, (i.e. Mollosil, Detax; Elite H-D, Zhermack). All materials corresponding to the four commercial products previously named were applied in both patients. In the first patient all four materials were applied simultaneous, as having an overdenture retained by four mini-dental implants, in the second patient the materials were applied in a sequence of two pairs, as having an overdenture retained by two mini dental implants. After seven days of wearing, these materials were removed from overdenture base, sampled, coded and microbiologically assayed.

The samples of soft materials were send to the microbiology laboratory in vials with sterile thioglycolate broth and processed within 24 hours. For microbiological analysis of the mixed-species biofilms, the inoculated thioglycolate media were plated on Columbia blood agar supplemented with 5% sheep blood plates, for incubation at 37 °C in aerobic and anaerobic atmosphere). The isolated colonies were identified based on culture, colony and biochemical characteristics.

3. Results and discussion

Eight samples, two from each soft material, were collected and analyzed, all yielding between one and three microbial strains. A total of nine different strains were identified in all samples (Table 1).

Table 1. Microbial strains identified, in respect to soft material used and patient to which was applied

Material	Patient 1	Patient 2
Tissue Conditioner	*Bifidobacterium (n=1)* *Pseudomonas aeruginosa (n=2)*	*Aeromonas caviae (n=1)* *Enterobacter sakazakii (n=1)* *Pseudomonas aeruginosa (n=2)*
Visco-gel	*Ewingella americana (n=1)*	*Enterobacter cloacae (n=3)*
Elite H-D	*Ewingella americana (n=1)*	*Lactobacillus acidophilus (n=1)*
Mollosil	*Bifidobacterium spp. (n=1)* *Ewingella americana (n=1)*	*Pseudomonas putida (n=1)* *Staphylococcus aureus (n=1)*

The microbiota was diverse, with Gram negative bacilli and Gram positive rods in both patients, and also Gram positive cocci only in one of the patients. Yeasts were found in none of them. In both patients, the majority of microbial strains were Gram negative bacilli. The only microbial strain found in both patients was *Pseudomonas aeruginosa*. Analyzing the microbiota found in the four materials used, we observed that some microbial strains were found repeatedly in some different materials, at the same patient. In the first patient *Ewingella americana*, a Gram negative bacillus, was found in 3 out of 4 soft materials recollected, and *Bifidobacterium s*pp., a Gram positive rod, in 2 different type of materials (i.e, one acrylic, and one silicone based material) out of 4 soft materials tested. In the 2nd patient Enterobacter spp. and *Pseudomonas s*pp., both gram negative bacilli, were found in 2 out of 4 soft materials.

The soft materials sampled yielded various number of microbial strains, the highest being identified in Tissue Conditioner, and the lowest in Elite and Visco-gel. These results suggest that the microbiological profile of soft materials adjacent to mini dental implants is diverse, consisting mainly of Gram negative bacilli. It was observed a tendency of soft materials, regardless of type or commercial products, to be colonized by same species when applied simultaneous. Different commercial products of soft materials showed different colonization potential, the highest number of microbial strains were identified in Tissue Conditioner, a soft acrylic material, and lowest in Elite H-D, a silicone based material, and Visco-gel, a soft acrylic material.

Oral microbiota is known to be very diverse, including polymicrobial communities with complex interactions, with strains that are etiological agents of human opportunistic infection, that are particularly dangerous for some population categories, as the aged (ZAWADZKI & al [8]; CRISTEA & al [9]). Some dental treatments, as overdentures treatments retained by mini-dental implants, are mostly used in the category of elderly, a segment well represented in the population, characterized by need to apply often difficult dental treatment to persons with altered general state (MURARIU-MAGUREANU & al [10]). Therefore better knowledge of these materials' characteristics and other dental materials, as microbial colonization, should be better known, considering there is a strong link between oral health and general health (SUCIU & al [11]; PERLEA & al [12]).

The microbial strains that colonized the soft materials used as soft matrices in overdenture retained by mini dental implants, identified in this study, were mostly Gram-negative bacteria.

According to the current evidence, a predominantly gram-negative flora is found in disease cases, with peri-implantitis, in healthy cases usually being found a predominantly gram-positive flora (DHIR [13]). The cases presented showed a positive outcome at 9 years, none of the implants exhibiting signs of implant mucositis or peri-implantitis during sample recollection or soon after, and having a good long-term outcome. These findings may be explained by the great diversity of oral microbiota, or to the dental treatment particularities,

implant overdenture being less researched compared to other treatment alternative as are the fixed prosthesis on dental implants.

From the Gram-negative bacteria identified some were reported to be encountered in implants with peri-implantitis. *Enterobacter* is one of the most common enteric bacteria found in peri-implantitis (RAKIC & al [3]). *Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen, that can be responsible for severe infections in immunocompromised patients (MIHALACHE (RADU) & al [14]; CHIFIRIUC & al [15]; POLL & al [16]). It was also found in diseased implants in complete edentulous patient, being assessed as a non-periodontal species that are more frequently found in this clinical situation, being among factors that could influence treatment outcome (VALENTE & ANDREANA [17]).

From gram positive cocci, Staphylococcus aureus was also found more frequently in patients with peri-implantitis (PERSSON & RENVERT [18]). It is known to exhibit a large spectrum of virulence factors, and generally a pathogen that can complicate some medical conditions (GHEORGHE & al [19]). Staphylococcus is believed to be responsible for infections associated with metallic biomaterials, being demonstrated to adhere also to titanium surfaces, therefore playing an important role in colonization of dental implants and subsequent infection (PYE & al [20]).

Rods were reported as being frequently found in completely edentulous patients (DHIR [13]), this being supported by this study results. Regarding the ones identified in these patients, *Lactobacillus* and *Bifidobacterium* spp, these are seen as gastrointestinal bacteria that raise interest, as playing a role in controlling the growth of oral microorganisms (ALLAKER & DOUGLAS [21]). A recent randomized controlled trial even reported that administration of a daily lozenge of Lactobacillus Reuteri Prodentis improved the clinical parameters of implants with mucositis or peri-implantitis (GALOFRE & al [22]). Their presence in the oral microbiota may play a positive role for a good outcome.

Candida or other yeasts were not identified in the cases reported. These are known pathogens found in implant mucositis or peri-implantitis, and even considered to rapidly colonize the soft materials. It is especially important in denture and overdenture wearers, as known to be present with an increased frequency (IOSIF & al [23]).

The materials used are different commercial products, two of them belonging to the category of soft acrylics, and two of them to silicone based materials. These four materials most probably have different properties that are linked to different behavior in oral condition, and may be related to different microbial loading properties. For example, Tissue Conditioner, the material with the highest surface wettability yielded the highest number of microbial strains, while Elite, the material with the lowest surface wettability, yielded the lowest number of microbial strains in both patients (PREOTEASA & al [24]). Therefore, future studies should address microbial loading patterns in conjunction with other material properties, considering clinical indication, in order to identify the one which is most recommended in a specific treatment step. This is especially indicated for opportunistic pathogens, as *Pseudomonas aeruginosa* and *Candida albicans*, frequently found in the oral environment and responsible for a wide range of infections (TELCIAN & al [25])

Soft denture materials were analyzed regarding their microbial adhesion especially by in vitro research. A combined in vitro and in vivo research brought evidence that Candida adhere more on soft materials compared to conventional acrylics but failed to identify difference between the soft materials tested (OKITA & al [26]). Our study and others contradict this research. An in vitro study of Candida albicans adherence to silicone-based soft materials identified significant difference between the six commercial products tested (GEDIK & al [27]). In the study mentioned, one of the commercial products tested is Mollosil, which was also

analyzed in this study, it showing medium susceptibility to Candida adherence. In our research Candida was not identified to colonize any material, it maybe explicable by factors related to duration of wearing the soft relining material, of only one week, in conjunction with the particularity of *in vivo* observation, different from in vitro ones. It was suggested that soft denture relining materials showed a lower rate of deterioration and a decreased microbial colonization in vivo, compared to expected knowledge derived from in vitro studies (TAYLOR & al [28]). Even so, soft relining materials are usually indicated to be used for a relatively short period of time, with time length increasing the surface roughness, therefore promoting increased susceptibility to microbial colonization. *In vitro* research brings valuable data, but especially on the issue of treatment conduct it is necessary to check it *in vivo* when possible. Among the latter, gold standard is randomized controlled trials, which can be conducted in different alternatives, e.g. parallel group design, split-mouth design. Comparative analysis of soft reliners, when used as soft matrices in implant overdenture study, is one problem that can be researched accurately by a split mouth design (simultaneous application of two or more soft liners, in randomized blocks). This research can be considered a pilot study confirming that that study design is appropriate, and a more extensive research, on a bigger sample, is recommended.

4. Conclusion

Microbiota of soft materials used as soft matrices in implant overdentures is diverse, consisting mainly of gram negative bacilli, even in healthy situations. The soft materials, regardless of type (soft acrylic or silicone based) or commercial products, have a tendency to be colonized by same species when used simultaneously. Different commercial products of soft materials showed different colonization potential, further studies being recommended to be conducted in order to identify best material in this regard, in the context of the usage for this specific clinical purpose.

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In this article, all authors have an equal contributions as the first author.

References

1. E. PREOTEASA, M. IMRE, C.T. PREOTEASA. A 3-year follow-up study of overdentures retained by mini-dental implants. *Int. J. Oral. Maxillofac. Implants*, 29(5):1170-1176 (2014).
2. E. PREOTEASA, L.I. FLORICA, F. OBADAN, M. IMRE, C.T. PREOTEASA, Minimally invasive implant treatment alternatives for the edentulous patient-fast & fixed and implant overdentures, In: I. TURKYILMAZ, ed., Current concepts in dental implantology, InTech, 2015, pp: 77-103.
3. M. RAKIC, M.G. GRUSOVIN, L.CANULLO. The microbiologic profile associated with peri-implantitis in humans: a systematic review. *Int. J. Oral. Maxillofac. Implants*, 31(2):359-368 (2016).
4. C.T. PREOTEASA, A. NABIL SULTAN, L. POPA, E. IONESCU, L. IOSIF, M.V. GHICA, E. PREOTEASA. Wettability of some dental materials. *Optoelectron. Adv. Mat.,* 5(8):874-878 (2011).
5. A. POLL,V.R. NIMIGEAN, D. BĂDIŢĂ, R.A. BĂLĂCEANU, S.C. CISMAS, P. PERLEA, S.A. MORARU, V. NIMIGEAN. In vivo experimental model for the evaluation of dental implant integration. *Rom. Biotechnol. Lett.*, 23(2):13505-13510 (2018).
6. L.O. CINTEZA, S.N. VOICU, M. POPA, L. MARUTESCU, S. NITU, R. SOMOGHI, C.L. NISTOR, C. PETCU. Rational design of silver nanoparticles with reduced toxicity and enhanced antimicrobial activity. *Rom. Biotechnol. Lett.*, 23(4):13878-13886 (2018).
7. M. KOSANIĆ, B. RANKOVIĆ, T. STANOJKOVIĆ. Evaluation of antioxidant, antimicrobial and anticancer effects of three selected marine macroalgae. *Rom. Biotechnol. Lett.*, 23(4):13804-13813 (2018).
8. P.J. ZAWADZKI, K. PERKOWSKI, M. PADZIK, E. MIERZWINSKA-NASTALSKA, J.P. SZAFLIK, D.B. CONN, L. CHOMICZET. Examination of oral microbiota diversity in adults and older adults as an approach to prevent spread of risk factors for human infections. *BioMed. Research International*, 2017:ID 8106491 (2017).

9. A.D. CRISTEA, C.T. PREOTEASA, M. POPA, L. MARUTESCU, M.C. CHIFIRIUC, I. GHEORGHE, V. LAZAR, A.A. ILIESCU, P. PERLEA, G.F. MOLDOVEANU, I. SUCIU. In vitro testing of susceptibility to endodontic irrigants and disinfectants of bacterial strains isolated from chronic apical periodontitis. *Rom. Biotechnol. Lett.*, 21(1):11217-11224 (2016).
10. C. MURARIU-MĂGUREANU, C.T. PREOTEASA, L. IOSIF, M. IMRE, M. CUCULESCU, E. PREOTEASA. Anatomical features and prosthetic considerations of edentulous patients with mandibular reconstruction with autograft performed more than 40 years ago. *Rom. J. Morphol. Embryol.,* 58(1):231-234 (2017).
11. I. SUCIU, V. TARMURE, E. IONESCU, I. SUCIU, M. CHIRILA, I. GHEORGHE, M. POPA, A. DUMITRIU, H. URSU. Possible interaction between carious lesions, chronic marginal periodontitis, periapical pathology and salivary iodine level –preliminary results. *Rom. Biotechnol. Lett.*, 23(1):13297-13300 (2018).
12. P. PERLEA, I. SUCIU, M. MELESCANU IMRE, M. CIOCARDEL, R.I. BARTOK, D. CRISTEA, A.A. ILIESCU, S. MILICESCU. Evaluation of apical filling using different obturation techniques. *Rom. Biotechnol. Lett.*, 23(2):13375-13382 (2018).
13. S. DHIR. Biofilm and dental implant: The microbial link. *J. Indian Soc. Periodontol.,* 17(1):5-11 (2013).
14. M.R. MIHALACHE (RADU), A.C. RATIU, A. NEAGU, V. LAZAR, M.C. CHIFIRIUC, A.A. ECOVOIU. Expression profiles of genes involved in mannose metabolism are modulated during experimental infection of Drosophila melanogaster males with Pseudomonas aeruginosa. *Rom. Biotechnol. Lett.*, 23(1):13225-13234 (2018).
15. M.C. CHIFIRIUC, G. MIHAESCU, V. LAZAR, Microbiology and medical virology. University of Bucharest Press, Bucharest, 2011.
16. A. POLL, C.A. MINCULESCU, V.R. NIMIGEAN, D. BĂDIŢĂ, R.A. BĂLĂCEANU, D.L. PĂUN, S.A. MORARU, V. NIMIGEAN. Experimental model for the study of autogenous mandibular bone grafts integration. *Rom. Biotechnol. Lett.*, 23(3):13681-13689 (2018).
17. N.A. VALENTE, S. ANDREANA. Peri-implant disease: what we know and what we need to know. *J. Periodontal. Implant. Sci.,* 46(3):136-151 (2016).
18. G.R. PERSSON, S. RENVERT. Cluster of bacteria associated with peri-implantitis. *Clin. Implant. Dent. Relat. Res.*, 16(6):783-793 (2014).
19. I. GHEORGHE, A.L. TATU, I. LUPU, O. THAMER, A.I. COTAR, G.G. PIRCALABIORU, M. POPA, V.C. CRISTEA, V. LAZAR, M.C. CHIFIRIUC. Molecular characterization of virulence and resistance features in Staphylococcus aureus clinical strains isolated from cutaneous lesions in patients with drug adverse reactions. *Rom. Biotechnol. Lett.*, 22(1):12321-12327 (2017).
20. A.D. PYE, D.E. LOCKHART, M.P. DAWSON, C.A. MURRAY, A.J. SMITH. A review of dental implants and infection. *J. Hosp. Infect.,* 72(2):104-110 (2009).
21. R.P. ALLAKER, C.W.I. DOUGLAS. Non-conventional therapeutics for oral infections.*Virulence,* 6(3):196-207 (2015).
22. M. GALOFRÉ, D. PALAO, M. VICARIO, J. NART, D. VIOLANT. Clinical and microbiological evaluation of the effect of Lactobacillus reuteri in the treatment of mucositis and peri-implantitis: A triple-blind randomized clinical trial. *J. Periodontal Res.,* 53(3):378-390 (2018).
23. L. IOSIF, C.T. PREOTEASA, C. MURARIU-MĂGUREANU, E. PREOTEASA. Clinical study on thermography, as modern investigation method for Candida-associated denture stomatitis. *Rom. J. Morphol. Embryol.,* 57(1):191-195 (2016).
24. C.T. PREOTEASA, A.N. SULTAN, L. POPA, M.V. GHICA, E. IONESCU, A.M.C. TANCU, E. PREOTEASA. Studies regarding the wettability of acrylic and silicone dental materials. *Farmacia,* 59:871-878 (2011).
25. A. TELCIAN, D.H. MOHAMMED, M.C. CHIFIRIUC, C. BLEOTU, A.M. HOLBAN, C. CURUTIU, E. GROSU, A. FICAI, G. MIHAESCU, R. GRIGORE, L.M. DITU. Assessment of the anti-biofilm activity and biocompatibility of novel PE and PVC polymers. *Rom. Biotechnol. Lett.*, 22(5):12997-13004 (2017).
26. N. OKITA, D. ORSTAVIK, J. ORSTAVIK, K. OSTBY. In vivo and in vitro studies on soft denture materials: microbial adhesion and tests for antibacterial activity. *Dent. Mater.,* 7(3):155-160 (1991).
27. H. GEDIK, Y.K. ÖZKAN. In vitro evaluation of candida albicans adherence to silicone-based soft lining materials. *Balk. J. Stom.,* 13:91-95 (2009).
28. R.L. TAYLOR, K. BULAD, J. VERRAN, J.F. MCCORD. Colonization and deterioration of soft denture lining materials in vivo. *Eur. J. Prosthodont Restor. Dent.*, 16(2):50-55 (2008).