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Original Article

Functional denture soft liner with antimicrobial and antibiofilm properties

Tuba Baygar [a*], Aysel Ugur [b], Nurdan Sarac [c], Uydu Balci [c], Gulfem Ergun [d]

[a] *Material Research Laboratory, Research Laboratories Center, Mugla Sitki Kocman University, Mugla, Turkey*
[b] *Section of Medical Microbiology, Department of Basic Sciences, Faculty of Dentistry, Gazi University, Ankara, Turkey*
[c] *Department of Biology, Faculty of Science, Mugla Sitki Kocman University, Mugla, Turkey*
[d] *Department of Prosthodontics, Faculty of Dentistry, Gazi University, Ankara, Turkey*

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Abstract *Background/purpose:* Denture soft liners, especially used for elders who have poor disinfection habits, provide a favourable environment for accumulation and colonization of microorganisms. This *in vitro* study is aimed to investigate the effectiveness of natural carvacrol incorporation into soft lining material on the inhibition of oral pathogens.
Materials and methods: Antimicrobial susceptibility of carvacrol was primarily determined by disc diffusion method. Soft lining material was prepared as recommended by the manufacturer and 10 μL carvacrol was added aseptically to the soft liner discs. Inhibition zones for the control discs without carvacrol (C) and carvacrol-incorporated discs (CL) were determined by disc diffusion method. The biofilm inhibition percentages of carvacrol on soft liner was determined by MTT assay and also observed by Scanning Electron Microscopy (SEM).
Results: Carvacrol displayed great antimicrobial activity for yeast, Gram-negative and Gram-positive strains. The highest inhibition zone of carvacrol (41.33 ± 1.53 mm) was measured for *Bacillus subtilis* strain which is followed by *Candida albicans* and *Streptococcus sanguis* (34.00 ± 1.73 mm and 32.33 ± 0.58 mm, respectively). The inhibition zones were also similar for soft liner discs with carvacrol, with the highest inhibition zones against *B. subtilis*, *Streptococcus mutans* and *C. albicans* (43.67 ± 0.58 mm, 40.33 ± 0.58 mm and 38.33 ± 1.15 mm, respectively). Incorporation of carvacrol into the soft liner decreased (98.03 ± 0.2%) of the biofilm formation for *C. albicans*.

* Corresponding author. Research Laboratories Center, Mugla Sitki Kocman University, Mugla TR-48000, Turkey. Fax: +90 252 211 18 87.
E-mail address: tbay48@hotmail.com (T. Baygar).

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Conclusion: Carvacrol-incorporation obviously decreased the colonization and plaque formation of oral pathogens, especially *C. albicans* accumulation. Carvacrol may be useful as a promising agent for antibacterial and antifungal management for denture soft lining materials.

Introduction

The oral cavity contains numerous species of microorganisms which include bacteria, viruses, and fungi organized into dental plaque. These microorganisms are able to form biofilms, which are resistant to mechanical stress or antibiotic treatment.[1] Dental plaque is a film of microorganisms on the tooth surface that plays an important part in the development of caries and periodontal diseases.[2] Patients who use removable prosthetic devices may have oral health problems if they do not apply adequate hygiene rules. The most common lession is denture stomatitis which is characterized as inflammation and erythema of the oral mucosal areas covered by the denture.[3–5] There are many reports suggest that most of the patients who wear removable dentures can suffer from denture stomatitis.[5–8] Denture relining materials are permanently used for sensitive patients to limit the traumatic effect of dentures by providing cushioning effect and also for denture relining to evenly distribute the loads transferred onto soft tissues during motion.[9,10] They are primarily used in patients with thin atrophic mucosa, with normal mucosa with an atrophied ridge, with a sharp alveolar ridge and when the mucosa exhibits a low tolerance to the load applied by the dentures.[11,12]

Soft lining materials are easily contaminated in the oral environment and it is not possible to clean or brush effectively.[13] For creating an efficient denture disinfection, the hygiene of the denture soft lining materials or tissue conditioners play significant role.[10] Denture disinfectant agents such as chlorhexidine gluconate, sodium hypochlorite, hydrogen peroxide and more were reported to cause unfavourable changes to the physical and chemical properties of the soft liner.[14,15] Studies have reported that the fungi and bacterial species can enter porous spaces within the denture liner and that their colonization may reduce the intra-oral life of the material.[9,16] Antibiofilm activity of the soft liner is also an efficient feature to prevent the patients from infections with resident oral microorganisms.[17] It is essential to apply the best infection management strategy for the risk group of denture wearers.[13] Douglas and Walker[18] had the idea of enhancing the therapeutic effects of a tissue conditioner with an antifungal agent to prolong the action of drug and treat the tissue trauma with lower cost. An antimicrobial soft liner will help to patients who can not perform routine denture care. Medicinal herbs are nowadays using as an alternative treatment method as a naturopathic remedy.[19]

Carvacrol (2-methyl-5-(1-methylethyl) phenol) ($C_{10}H_{14}O$) is an essential oil component of numerous aromatic plants such as oregano and thyme (*Thymus* and *Origanum* sp.).[20–22] It has been granted Generally Recognized as Safe (GRAS) status by the FDA in 21 CFR (Code of Federal Regulation) part 172.515.[23] Antimicrobial activity of carvacrol has been demonstrated against bacteria, mold and yeast.[22,24] The inhibitory effect of its volatile phase against bacteria was also studied.[25] Recently some antimicrobial drugs, such as eugenol, have the most attention for dental cleaning purpose. As far as we know, there is no study about the use of carvacrol for denture soft lining applications. With this current study, it is aimed to investigate the antimicrobial properties of a soft liner incorporated with a natural agent which posses great biocidal activity against several microorganisms. For this purpose, antimicrobial and antibiofilm activities of carvacrol and carvacrol-incorporated soft liner have been studied.

Material and methods

Materials

Carvacrol was purchased from Sigma–Aldrich. Sabouraud Dextrose Broth/Agar, Brain Heart Infusion Broth/Agar, Mueller Hinton Agar, Tryptic Soya Broth and D-Glucose were purchased from Merck. Autopolymerized soft denture liner material (UFI Gel) is based on A-silicone (addition silicone) and consists from mixture of different polyalkylsiloxanes, fumed silica, catalysts, butanone and additives. UFI Gel P (Voco GMBH, Cuxhaven, Germany) were purchased from a local dental market. Test microorganisms were all provided from Mugla Sitki Kocman University Culture Collection.

Microbial strains and culture conditions

The antimicrobial activity of the carvacrol was individually tested to a group of microorganisms including; *Candida albicans* ATCC 10239 (yeast) which is directly associated with the pathogenesis of denture stomatitis; *Staphylococcus aureus* ATCC 25923 (Gram/+), *Streptococcus mutans* ATCC 25175 (Gram/+) and *Streptococcus sanguis* ATCC 10556 (Gram/+) which are known to be oral pathogens; *Bacillus subtilis* ATCC 6633 (Gram/+), *Escherichia coli* ATCC 25922 (Gram/-) and *Pseudomonas aeruginosa* ATCC 27853 (Gram/−) which are standard pathogenic strains for antimicrobial studies and represent the clinically important strains of Gram (+) and Gram (−) bacteria. All strains were provided from Culture Collection of Mugla Sitki Kocman University (MUKK). *B. subtilis*, *E. coli* and *S. aureus* strains were incubated at 37 ± 0.1 °C for 24–48 h, *C. albicans* and *P. aeruginosa* were incubated at 30 ± 0.1 °C for 24–48 h and *Streptococcus* strains were incubated anaerobically at