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Antimicrobial activity of silver and gold in toothpastes: A comparative analysis

Jonas Junevičius, Juozas Žilinskas, Kęstutis Česaitis, Gabrielė Česaitienė, Darius Gleiznys, Žaneta Maželienė

SUMMARY

Objective. In this study, we compared the antimicrobial activity of identical toothpastes differing only in silver or gold nanoparticles against the activity of one of the common toothpastes containing a chemical active ingredient. We also compared the active concentrations of the toothpastes.

Methods. For this study, we selected "Royal Denta" toothpastes containing silver and gold particles, and the "Blend-A-Med Complete" toothpaste containing zinc citrate as the active ingredient. We used 8 standard microorganism cultures on the basis of their individual mechanisms of protection. The antimicrobial activity of each studied preparation was evaluated at 9 concentrations.

Results. Most effective against gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) was the *"Silver Technology"* – MIC was 0.004–0.0015 g/mL. Neither *"Silver Technology"* nor *"Orange and Gold Technology"* had any effect on *Escherichia coli* or *Proteus mirabilis*. Antimicrobial activity against the motile bacterium *Proteus mirabilis* was observed in *"Silver Technology", "Orange and Gold Technology",* and *"Blend-A-Med Complete"* – the MIC was 0.015 g/mL or lower. No antimicrobial activity against *Candida albicans* fungus at the studied concentrations was observed in the *"Orange and Gold Technology"*. The toothpaste *"Blend-A-Med" demonstrated the* most effective antimicrobial activity – the MIC of 0.0015 g/mL and 0.015 g/mL inhibited *Staphylococcus aureus* and *Enterococcus faecalis*, respectively, and the MIC of 0.15 g/mL inhibited the growth of the bacteria *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and fungus *Candida albicans*.

Conclusions. Silver in toothpaste has a greater antimicrobial effect than gold, but its effect is still inferior to that of a chemical antimicrobial agent.

Key words: toothpaste, antimicrobial activity.

INTRODUCTION

Silver has long been known as an antimicrobial substance (1, 2). It was used for water purification, wound care, bone prostheses, restorative orthopedic surgery, catheters, and surgical instruments. The development of biotechnologies allowed for the incorporation of ionized silver into fabrics used in hospitals for the prevention of nosocomial infections as well as for the improvement of personal hygiene (3). The use of this naturally found metal has been gaining popularity, and there are even those who believe that colloidal silver solution may cure over 650 diseases (4). Gold is also seen as the symbol of health and well-being. It has long been used in cosmetics and medications as the vehicle of active substances (5). The growing interest in nanoparticles of precious metals prompted an increase in studies and possibilities for the use of such substances. Literature contains data on the effectiveness of nanoparticles against gram-positive, gram-negative,

[1]*Clinical Department of Dental and Maxillofacial Orthopedics, Faculty of Odontology, Medical Academy, Lithuanian University of Health Sciences*
[2]*Private practice in odontology*
[3]*Faculty of Odontology, Medical Academy, Lithuanian University of Health Sciences*
[4]*Institute of Microbiology and Virology, Faculty of Veterinary Medicine, Veterinary Academy, Lithuanian University of Health Sciences*

***Jonas Junevičius**[1] – D.D.S., PhD, lect.*
***Juozas Žilinskas**[1] – D.D.S., PhD, lect.*
***Kęstutis Česaitis**[2] – D.D.S.*
***Gabrielė Česaitienė**[3] – student*
***Darius Gleiznys**[1] – D.D.S.*
***Žaneta Maželienė**[4] – M.D., assist. prof.*

Address correspondence to: Kęstutis Česaitis, Ukmergės g. 32-18, Kaunas, Lithuania.
E-mail address: kcesaitis@gmail.com

and even antibiotic-resistant bacterial cultures such as MRSA or MRSE (6,7,8).

Mouth is one of the most common zones of human interaction with the environment. With time, the composition of its microflora changes, the variety of the species of microorganisms increases, and opportunistic or infection-causing microorganisms emerge (9, 10).

According to odontologists' recommendations, oral care should be ensured by using a toothbrush and toothpaste. Thus, microorganisms are affected by mechanical brushing and the active substances of the toothpaste dissolved in saliva (11). For these reasons, the use of oral hygiene items containing gold and silver particles is becoming increasingly common.

In this study, we wanted to compare the antimicrobial activity of identical toothpastes differing only in silver or gold nanoparticles against the activity of one of the widely available common toothpastes. We also wanted to compare the active concentrations of the toothpastes.

MATERIALS AND METHODS

This microbiological study was conducted in aseptic conditions. The following standard microorganism cultures were used: *Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 33499, Proteus mirabilis* ATCC 12459*, Pseudomonas aeruginosa ATCC 27853, Bacillus cereus ATCC 8035,* and *Candida albicans ATCC 60193*. These bacteria and fungi were selected as representatives of their classes because of their specific characteristics (thick walls, spore formation, etc.). The standard microorganism cultures were grown in commercial standardized BBL (Becton Dickinson and Company) growth media – tryptic soy agar (bacteria) and Sabouraud agar (*Candida albicans*). Bacterial cultures were cultivated in the tryptic soy agar for 24 hours at the temperature of 37 ºC, and *Candida albicans* – in Sabouraud agar for 24 hours at the temperature of 25 ºC. The microorganism cultures were then washed with a sterile 0.9% sodium chloride solution, and their suspensions were prepared [10^5 CFU (colony-forming units)/1 mL].

The microbiological study assessed the antimicrobial activity of the preparations *"Royal Denta (R.D.) Silver", "R. D. Orange and Gold",* and *"Blend-A-Med Complete 7 Extra fresh"*) in a solid growth medium - Mueller-Hinton agar. The antimicrobial activity of each studied preparation was evaluated at 9 different concentrations: 0.21 g/mL; 0.18 g/mL; 0.15 g/mL; 0.118 g/mL; 0.058 g/mL; 0.029 g/mL; 0.015 g/mL; 0.004 g/mL, and 0.0015 g/mL. These concentrations were obtained by mixing a certain amount of the preparation in a Petri dish with 2 mL of sterile saline and 15 mL of Mueller-Hinton agar at 50 ºC temperature. After the agar solidified, the prepared suspensions of the standard microorganisms were introduced into the marked segments. The cultures were incubated for 24 hours in a thermostat at the temperature of 37 ºC, and then microorganism growth at various concentrations of the preparations was evaluated. The testing series were repeated 5 times.

RESULTS

No statistically significant difference was found between the 5 test series ($p<0.01$). The data of the study showed that in case of the toothpaste *"R. D. Silver",* the MIC (minimal inhibitory concentration) affecting the growth of gram-positive bacteria *S. aureus* was 0.004 g/mL, and the MIC affecting the growth of fungus *C. Albicans* – 0.18 g/mL. The MIC affecting the growth of gram-negative bacteria *K. pneumoniae* and *P. aeruginosa* was 0.15 g/mL, whereas no inhibition of gram-negative bacteria *E. coli* or *P. mirabilis* was achieved at the studied concentrations. The toothpaste *"R. D. Silver"* was most effective against the bacteria *E. faecalis* and *B. cereus* because no growth of these bacteria was observed even at the lowest tested concentration of the toothpaste.

In case of the toothpaste *"R. D. Orange and Gold",* the MIC affecting the growth of gram-positive bacteria *S. aureus, E. faecalis,* and *B. cereus* was 0.015 g/mL, and the MIC affecting the growth of gram-negative bacteria *K. pneumoniae* and *P. aeruginosa* – 0.15 g/mL. No inhibition of the growth of gram-negative bacteria *E. coli* and *P. mirabilis* or fungus *C. albicans* was observed at the studied concentrations.

In case of *"Blend-A-Med Complete 7 Extra Fresh"* toothpaste, the MIC affecting the growth of gram-positive bacteria *S. aureus* and *B. cereus* was 0.0015 g/mL, the MIC affecting the growth of *E. faecalis* – 0.015 g/mL, and the MIC affecting the growth of gram-negative bacteria *K. pneumoniae* and *P. aeruginosa* was 0.15 g/mL. The MIC affecting the growth of gram-negative bacteria *E. coli* and *P. mirabilis,* and the fungus *C. albicans* was 0.15 g/mL.

The comparison of the quantitative and qualitative antimicrobial effectiveness of the studied toothpastes against gram-positive bacteria, gram-negative bacteria, and fungi is presented in Figures 1-3.

DISCUSSION

In this study, we evaluated the antimicrobial activity of the tested preparations against bacteria with a prokaryotic cell structure and fungus *Candida albicans*, which has a eukaryote cell structure. In addition to that, we selected standard bacterial cultures differing in specific, unique properties. For instance, only *Staphylococcus aureus* and *Bacillus cereus* have a thick cellular membrane composed of peptidoglycan, which is characteristic only of gram-positive bacteria. Meanwhile, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Proteus mirabilis* have a dual-layered membrane characteristic of gram-negative bacteria. A specific biological feature of *Bacillus cereus* is the ability to produce spores that protect these bacteria against unfavorable environmental factors. *Escherichia coli* are gram-negative bacteria that have a characteristic cell membrane and are highly heterogeneous in their biological characteristics. *Klebsiella pneumoniae* creates a capsule – a thick layer of mucus protecting it from phagocytosis and other unfavorable environmental factors. *Pseudomonas aeruginosa* have saprophytic, potentially pathogenic, and pathogenic properties, which facilitates their spread in healthcare institutions. *Proteus mirabilis* is highly motile and causes genitourinary infections (12,13).

The comparison of the exact compositions of "Royal denta" toothpastes showed that their compositions were nearly identical, the only difference being in a few components: *"R.D. Silver"* contained silver particles, whereas *"R.D. Orange and Gold"* contained gold particles as well as tangerine and orange oils. Therefore, these two toothpastes were selected for the study in order to avoid the influence of differing compositions of toothpastes on their antimicrobial activity. The widely available classical *"Blend-A-Med Complete 7 Extra Fresh"* was selected as control toothpaste. In this toothpaste, zinc nitrate is the substance that has an antimicrobial effect.

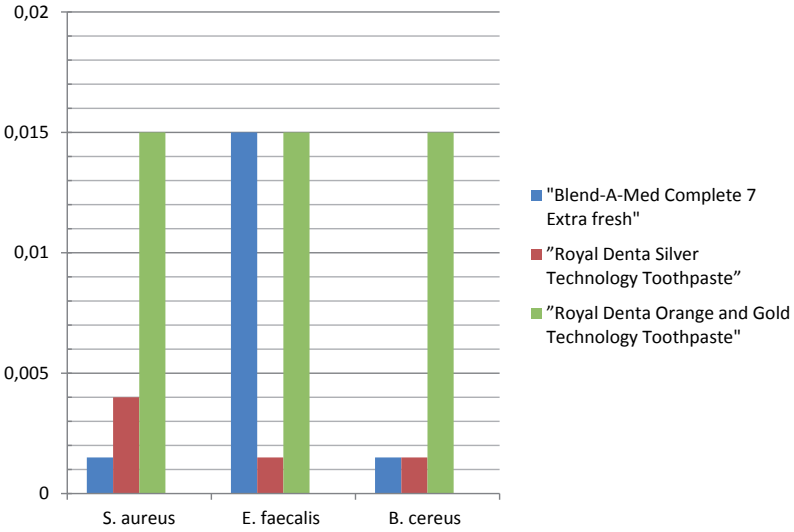

Fig. 1. Sensitivity of gram-positive standard microorganism cultures to different toothpaste concentrations used in the study

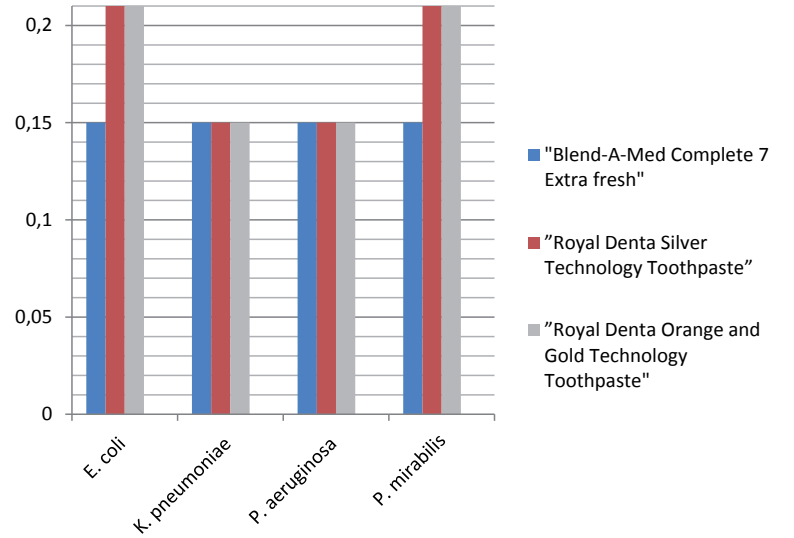

Fig. 2. Sensitivity of gram-negative standard microorganism cultures to different toothpaste concentrations used in the study

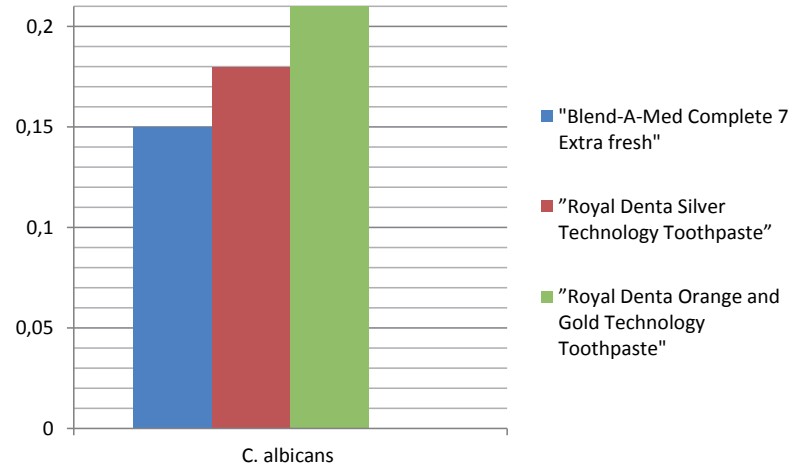

Fig. 3. Sensitivity of C. albicans standard microorganism culture to different toothpaste concentrations used in the study

The evaluation of the obtained results clearly showed that the studied toothpastes were more effective against gram-positive than against gram-negative bacteria. Natural botanical components of

all *"Royal Denta"* toothpastes were more effective against gram-positive bacteria. Due to the stronger effect of silver ions on gram-negative bacteria, silver-containing toothpastes had a broader antimicrobial effect, compared to toothpastes containing gold particles. The data of the study showed that zinc nitrate in the *"Blend-A-Med Complete 7 Extra fresh"* toothpaste provided a more efficient antimicrobial effect compared to toothpastes containing silver or gold particles.

Scientific literature does not provide any data on gold as an antimicrobial substance. The toothpaste containing gold particles also showed the poorest antimicrobial effect. This suggests that gold in the toothpaste was used purposes other than its antimicrobial effect. The antimicrobial effectiveness of gold particle-containing toothpastes is mainly due to the oils in the toothpaste – which explains the efficacy of this toothpaste against gram-positive bacteria.

Odontologists recommend using a pea-sized amount of toothpaste for oral hygiene. Such amount of toothpaste applied on the gums weighs ca. 1.5-2 g, which approximately corresponds to the 0.118 g/mL concentration of the solution used in the study. We expanded the scale of solution concentrations in order to identify the minimal amount of toothpaste that would have an antimicrobial effect. However, in some studied microbes, we failed to determine the effective concentration of the toothpaste because the amount required was significantly higher than the amount recommended for optimal oral hygiene.

When evaluating any toothpaste, their possible adverse effects have to be taken into consideration. Sylvie Gaillet et al. indicated that silver nanoparticles in toothpaste and other products may be associated with the development of inflammations of the gastrointestinal tract (14). However, since the toothpaste is indicated for topical rather than systemic use, adverse reactions are unlikely.

CONCLUSIONS

All the evaluated toothpastes had antimicrobial effects, yet none of them inhibited all the investigated microorganisms. Silver or gold particles in the *"Royal Denta"* toothpastes were more effective against gram-positive bacteria than against gram-negative bacteria. Silver nanoparticle-containing *"Royal Denta"* toothpastes were more effective than gold nanoparticle-containing "Royal Denta" toothpastes. The traditional *"Blend-A-Med Complete 7 Extra fresh"* toothpaste with a chemical active ingredient demonstrated better antimicrobial activity compared to silver- or gold-containing toothpastes.

The amount of the toothpaste applied on the toothbrush (ca. 1.5-2 g) is sufficient for inhibiting the growth of a part of microorganisms found in the oral cavity.

Statement of conflict of interest

The authors declare that there are no conflicts of interest.

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