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The influence of silver nanoparticles introduced into RTV-silicone matrix on the activity against *Streptococcus mutans*

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ABSTRACT

Purpose: The silicone based room temperature vulcanized (RTV) polymers are commonly used materials for medicine, especially for dentures and maxillofacial prostheses. Unfortunately, the colonization of those materials by pathogenic microorganisms is well-known problem related with their applications. The aim of presented study was to examine antibacterial properties of RTV silicone for dentistry modified with silver nanoparticles.

Design/methodology/approach: The silver nanoparticles were introduced into two-component system silicone based materials. The presence of silver nanoparticles was investigated with scanning electron microscope (SEM). The antibacterial activity against *Streptococcus mutans* was determined. The result were statistically analysed with a Statistica 12.5 software and non-parametric Kruskal-Wallis test ($\alpha = 0.05$).

Findings: The silver nanoparticles introduction into RTV - silicone allowed to enhance the antimicrobial resistance against standard strain of *Streptococcus mutans*.

Research limitations/implications: In this research only *Streptococcus mutans* bacterium strain was used. In future activity of presented materials against other pathogenic bacteria living in oral cavity should be determined. Additionally long term investigation should be prepared.

Practical implications: The colonization of dental materials with pathogenic bacteria and fungus is one of the most important and still unresolved problems related to exposition on oral environment. The low microbiological resistance of RTV-silicones and antimicrobial potential of silver were reported in numerous studies. The gram-positive *Streptococcus mutans* is commonly found in the human oral cavity and it is an important factor to tooth decay.

Originality/value: The resistance against *Streptococcus mutans* of modified material was enhanced. The investigated materials could be a potential factor a potential conducive to reducing the risk of oral cavity infections.

Keywords: Dental materials; RTV-silicone; Nanosilver; Streptococcus mutans; Denture; Rehabilitation

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MATERIALS

1. Introduction

Silicone elastomers are a group of materials widely used for dentistry. These polymers are used as impression materials (additional silicones high temperature vulcanized silicone and condensation silicones), long-term soft denture lining materials (used in dentures to mitigate difficulties related to unfavourable alveolar ridge shape, sore spots under the dentures, poor retention and/or painful mucosa are present), for relining in implantology as well as to perform postoperative obturators and maxillofacial prosthesis [1-5]. The most frequently are used the room temperature vulcanized (RTV) additional silicones. These materials are valued due to their dimensional stability during polymerisation and after it, relatively good stability of mechanical properties during longer exhibition periods on oral environment, low sorption and solubility, favourable modulus of elasticity and possibility of tailoring viscoelastic properties [5,6]. During polymerization reaction the addition of silyl hydride groups (-SiH) to vinyl groups (CH2=CH-) attached to the silicone is involved with the aid of a platinum catalyst [5,7]. For some applications as the disadvantages are reported their hydrophobic properties, selective adhesive property or the need to use bonding systems when connections with other materials is required [5,6]. The other important and unresolved problem is the low microbiological resistance against pathogenic microorganisms when silicones are exposed for longer period to the destructive impact of the oral environment [8]. The high humidity, elevated temperature and the presence of proteins, sugars, and other ingredients creates ideal conditions for microbial growth [9,10]. Colonies of microbes in the surface of such materials appears farthest after a few months [8]. In addition, the study of Bulard et al. [1] undoubtedly indicates that microorganisms penetrate the interior of materials. Some researchers suggest that the use of cleaning agents can promote the formation of grooves and fissures on material surface and increase difficulties with the removing of microorganisms [11]. Other work suggests storing of materials in some denture cleaners can conduce to a greater ability for forming biofilms than after storing in distilled water [12].

Important way to increase the properties of dental materials is introduction of nanofillers [13,14]. Microbiological resistance of silicone materials can be also modified with additional fillers [15]. The efficacy inter alia of nanosilver against the yeast – like fungus *Candida albicans* was demonstrated [16]. Anyway it should be also considered if those materials can enhance antibacterial properties against some pathogenic bacteria in oral cavity. One of the representative microorganisms is *Streptococcus mutans*, a facultative anaerobic, gram-positive bacterium recognized as an important contributor to tooth decay.

Therefore, the zero hypothesis of this study was that the use of filler in the form of silver nanoparticles can increase the resistance of RTV-silicone based materials against typical in the oral cavity bacteria represented by *Streptococcus mutans* strain. The aim of this study was to investigate the effect of introducing the silver particles into RTV silicone on the activity of investigated materials against *Streptococcus mutans* strain.

2. Materials and methodology

2.1. Materials

Room temperature vulcanized silicone soft liner material Ufi Gel SC (VOCO GmbH, Cuxhaven, Germany) was used as a base for all studies. The composition of the material is presented in Table 1. The methodology of material preparation were reported previously [15]. The silver nanoprarticles colloid in hexane were diluted in n-hexane down to a concentration of 30 ppm. The silicone material components were dissolved separately in hexane, achieving a concentration of 7% (w/w) in 300 mL Erlenmeyer flasks with a magnetic stirrer at room temperature for 2h. The silver nanoparticles suspension masses necessary for a composite with a particular concentrations were calculated according to the equation:

$$m_{AgNP} = \frac{c_{AgNP} \times m_{SC} \times 10^6}{c_{AgNP-sol} \times (10^6 - c_{AgNP})} \quad (1)$$

where m_{AgNP} was the silver nanoparticles suspension mass, g; c_{AgNP} was suspected silver nanoparticles concentration in a manufactured composite component, ppm; m_{sc} was the silicone component mass in g, and $c_{AgNP-sol}$ was the silver nanoparticles concentration in the solvent.

Then, the mass of suspension was calculated according to equation (1) and added to the solution of silicone based material. The mixture was next stirred with a magnetic stirrer for 15 min.

Table 1.
The starting compositions of materials used in the studies

Materials type	Ingredients	Manufacturer
The silicone "base" component	Vinyl terminated polydimethylsiloxane, methylhydrosiloxane-dimethylsiloxane copolymer, functionalized silica filler, pigments.	VOCO GmbH, Cuxhaven, Germany
The silicone "catalyst" component	Vinyl terminated polydimethylsiloxane, functionalized silica filler, platinum-complex catalyst	VOCO GmbH, Cuxhaven, Germany
Silver nanoparticles solution	The silver nanoparticles 0.1 % (w/w), n-hexane – the rest	AMEPOX Co. Ltd., Łódź, Poland
95% n-hexane	Min. 95% n-hexane	Avantor, Gliwice, Poland

The hexane was then preliminarily evaporated from the flask with a rotary evaporator (IKA RV-10) for 15 min under reduced pressure (100 mbar) and the preliminarily condensed material was placed into a Petri dish and next finally warmed in a dryer for 24 h at 50°C.

Following the previously described procedure, both components of RTV-silicone based material were modified. Samples were prepared with the silver nanoparticles concentrations: 0.001; 0.002; 0.004; 0.008; 0.01 and 0.02% (w/w).

The modified materials components ("base" and "catalyst") samples were mixed together in a mass ratio of 1:1 and polymerised with standard flasking method used in dental prosthetic.

To confirm presence of silver nanoparticles measurements were performed on a scanning electron microscope Quanta 250 ESEM FEG (FEI Company) operating at 30 kV in environmental mode, using the wet-STEM detector to detect STEM images in SEM and the gaseous secondary electron detector (GSED). The chamber pressure was 10 mmHg. Also TEM investigations on the TECNAI F20 TWIN microscope were performed.

2.2. Antibacterial test

The in vitro antibacterial activities of materials were examined according to Melaiye et al. [17] and Chladek et al. [16] with some modifications. Probes were exposed to a standard strain of *Streptococcus mutans* ATCC 33535. The polymerised material samples were cut from the larger plates. Specimens were square 10 mm × 10 mm and 2.3 mm thick. Before testing, the specimens were plasma sterilised. Each sample was introduced into 4 mL of bacterial suspension in tryptone water, containing approximately 1.5×10^5 colony forming units (CFUs) of *Streptococcus mutans* in 1 mL. A suspension of 1.5×10^5 CFU/ml of *Streptococcus mutans* in tryptone water was tested as a positive control (blank). Samples of pure tryptone water without bacterial was tested as negative control. All mixtures were next incubated at 37°C in static conditions for 17 h under microaerophile conditions. After incubation, 20 µL of each mixture was seeded onto a Columbia agar with 5% sheep blood plates. These plates were finally incubated at 37°C for an additional 48 h under microaerophile conditions. Then, the number of *Streptococcus mutans* colonies was counted. The antibacterial effect of introduced nanoparticles was calculated according to the following equation:

$$AE = \frac{V_0 - V_n}{V_0} \times 100\ \% \quad (2)$$

where V_0 was the average number of viable bacterial colonies of the unmodified material and V_n was the number of viable bacterial colonies of the specimen with nanosilver.

2.3. Statistical analysis

The results were subjected to a statistical analysis. The Statistica software (version 12.5, StatSoft, Tulsa, OK, USA) was used. The non-parametric Kruskal-Wallis test ($\alpha = 0.05$) was also used.

3. Results

Electron microscopy investigations confirmed that nanoparticles were successively introduced into materials. The STEM and GSED detectors used during SEM investigations showed numerous aggregations characterized by a different number of mass in relation to the PDMS-based matrix (Fig. 1). Measurements showed the presence of both large nanoparticles aggregations (Figs. 2a, b and c) but TEM investigations also have shown particular nanoparticle in the composites (Fig. 2d). Silver particles ranged frequently from 10 to 30 nm. The higher the silver nanoparticles concentration, the greater the number and the larger the sizes of NP aggregations. Aggregations were observed in all composites, but frequently they were registered starting with a concentration 0.008% ppm. They usually measured from 100 to 300 nm, but also larger aggregations exceeded 1 μm have been occurred.

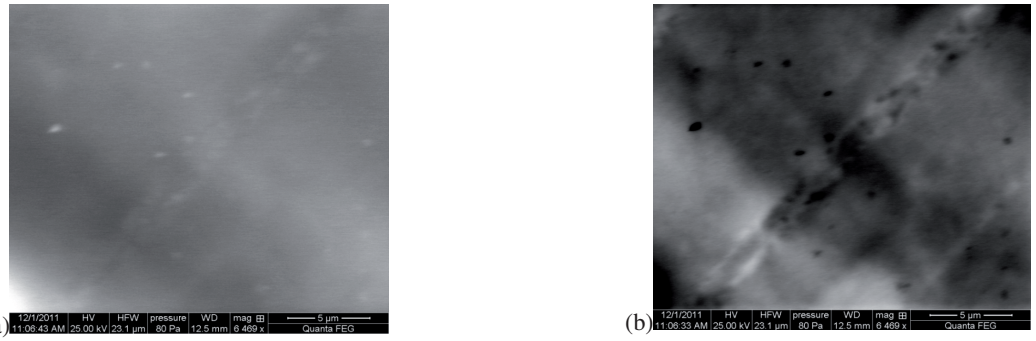

Fig. 1. Micrographs presenting nanoparticles and their aggregations in composite with 0.012 % of introduced silver; scanning transmission electron image using the gaseous secondary electron detector (GSED) (a) and the wet-STEM detector (b)

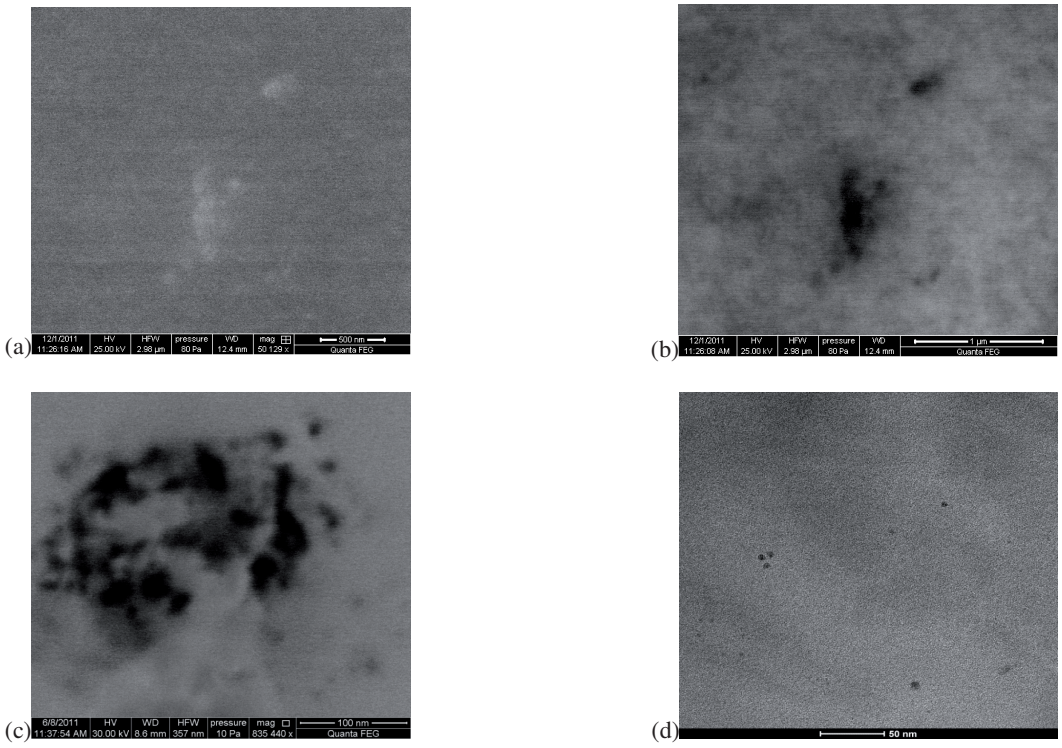

Fig. 2. Nanoparticles and their aggregations in composite with 0.012% of introduced silver; scanning transmission electron image of larger aggregations using the gaseous secondary electron detector (GSED) (a) and wet-STEM detector (b), (c) and TEM image showing particular nanoparticles (d)

The antibacterial test results were presented in Fig. 3. Positive and negative controls showed expected results. The antibacterial effectiveness significantly increased (p=0.0005) with increasing of silver nanoparticles concentrations. For the lowest concentration generally there wasn't registered antimicrobial effect and additionally for one sample AE had a negative value so the number of viable bacterial colonies slightly increased. The introduction of 0.002% of silver nanoparticles into the polymer led to reduction of bacterial units of 13.9%, so it was still relatively low. The median value for samples with 0.004% was much higher and reached 35.6%. An additional increase in the silver nanoparticles concentration resulted in clearly visible AE increase. For the highest examined concentration the median AE was 66%, the minimum values was 63.9% and the maximum value was 68%.

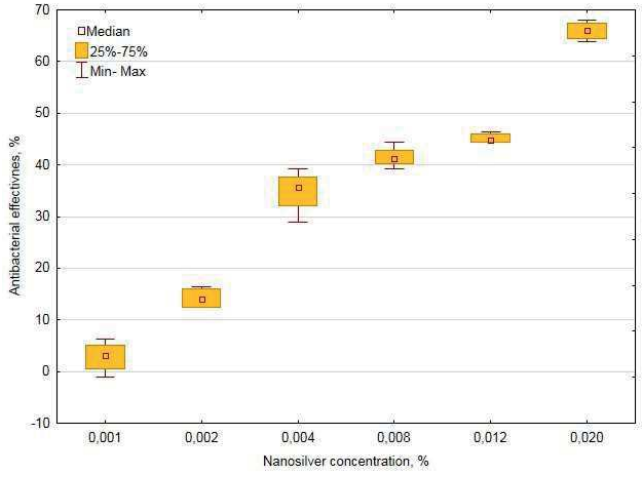

Fig. 3. The medians, interquartile ranges, minimum and maximum values of antibacterial effect of introduced nanoparticles

4. Discussion

The bacterial colonization of soft lining materials used in dentistry is a less important problem than fungal infections. It can be a key feature only if those materials are used when natural teeth are still present in the oral cavity, e.g. in case of partial dentures, overdentures, obturators and maxillofacial prostheses. The colonization of soft linings by pathogenic bacteria can play some role in dental caries or periodontal diseases. First, it is recognized as tooth decay affected by acidogenic bacteria like *Streptococcus mutans, Lactobacillus* and *Actinomyces*. The clinical symptoms are the damage of the mineralized tissues, which is a cause pain and in some cases the loss of the tooth [18]. The periodontal diseases are also associated with microorganisms species, which they are connected with the infection, inflammation and pathological changes in the tissues that support the teeth [19,20]. For this reasons many studies presented a new antimicrobials materials intended for dentistry. Also the antibacterial activity of nanosilver or materials against *Streptococcus mutans* was studied in other works. Nakamura et al. [21] show antibacterial effect of calcium phosphate submicrospheres with dispersed silver nanoparticles. Emmanuel et al. [22] also reported efficacy of green synthesized drug blended silver nanoparticles against different pathogenic microorganisms living in oral cavity. Kasraei et al. [23] confirmed antibacterial properties of dental composite resins. Studies [24] show *in vivo* positive effect of silver nanoparticle against different bacteria during orthodontic treatment. However for polydimethylsiloxane - based materials investigations were not yet reported. Antibacterial effect achieved in presented work is generally comparable with reported for other materials. Also it should be in mind that numerous important factors may affect antimicrobial properties of silver nanoparticles. The important factor which can determine antimicrobial effect is the tendency to aggregation of nanoparticles. It reduces effective surface of particles which may contact with bacteria or fungi, hence antimicrobial effect may decreased [25]. In presented work numerous aggregations were observed and better dispersion of silver particles could enhance an effectiveness against *Streptococcus mutans*. Due to the silver ion emission into the environment noted for different composites [26], the cytotoxic test of presented materials is needed. Some *in vitro* studies reported cytotoxicity of silver nanoparticles to different cell lines [27]. This toxicity is not satisfactory recognized [28]. However, some results reported toxicity only in the cases of exposure at high concentrations [29,30]. It may suggest that nanosilver bears no risk in considered applications. Anyway, this questions should be extensively studied in future, because the potentially adverse impact of nanosilver and other nanomaterials on the human health especially in longer periods, is still under debate [31,32].

5. Conclusions

In this study, antibacterial effect of silicone based materials modified with silver nanoparticles was achieved. Antibacterial effect is needed in preventing colonisation of denture linings and maxillofacial prostheses with *Streptococcus mutans*. The zero hypothesis that the use of

filler in the form of silver nanoparticles can increase the resistance of RTV-silicone based materials against typical in the oral cavity bacteria represented by *Streptococcus mutans* strain was confirmed.

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