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Original paper

Developing the procedure of modifying the denture soft liner by silver nanoparticles

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Colonization of denture soft lining materials by fungi and denture plaque leads to infections of mucosa. Microorganisms such as *Candida albicans* colonize not only the surface of the soft liners, but they also penetrate inside those materials. Therefore the use of common disinfectants, e.g., surface active cleaners, is not a perfect solution for keeping a proper hygiene of soft linings. Modifying soft lining by silver nanoparticles (AgNP) seems to be a right way to overcome those problems. The procedure of modifying two-component silicone material by silver nanoparticles (AgNP) is presented in the article. The solubility tests for both material components have been carried out in the first stage of examinations. On the basis of test results, a solvent has been selected, being a dispersion medium for AgNPs and both soft liner components. The effective method for evaporating a solvent from the composition has been developed. Material components with various AgNP concentrations (10, 20, 40, 80, 120 and 200 ppm) have been obtained. Cured samples of the composites have been examined by SEM to confirm the effectiveness of the procedure.

Key words: *silver nanoparticles, dental materials, soft liner, modification, antimicrobial effectiveness*

1. Introduction

Soft lining materials are usually used for lining dentures in order to distribute the forces which are applied to soft tissues under the denture during chawing. They are mainly used for patients with a sharp alveolar ridge, thin atrophic mucosa, normal mucosa with a resorbed ridge and when mucosa shows a low tolerance to the load applied by denture [1]–[2]. It has been revealed that the use of soft lining materials can intensify the process of the growth of microorganisms [3]–[4]. Fungal and bacterial growth is supported by environmental conditions under the denture (high humidity, elevated temperature) as well as the structure of materials [5]–[7]. Microorganisms first adhere to the surface of the lining and then they penetrate inside the material [3]–[10]. This phenomenon is particularly disadvantageous, because it shows restricted possibilities of conventional cleaners commonly used by patients.

Modification of soft linings by AgNPs can be an improvement in that respect. Fungicidal and bactericidal properties of silver have been known for centuries and they have been scientifically proved. Additionally few reports confirm the effectiveness of AgNPs in their use for dentistry [11]–[15].

Modification procedure of two-component silicone denture soft lining material for long-term use by AgNP has been presented in this paper. Both material components have been modified to develop better dispersion of AgNP in cured material.

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2. Materials and methods

The silicone material, i.e., Ufi Gel SC (UG), (Voco GmbH, Germany), used for making long-term denture soft lining has been examined. The chemical composition of Ufi Gel SC Base (UGB) and Ufi Gel SC Catalyst (UGC) has been confirmed by ^{1}H NMR spectroscopy. ^{1}H NMR spectra have been registered by the UNITY/NOVA (VARIAN, FT, 300 MHz) spectrometer. Deuterated chloroform ($CDCl_3$) has been used as a solvent. Chemical shifts have been measured against tetramethylsilane.

The purpose of solubility tests for two components of UG was to find a solvent which would be a dispersion medium for AgNP and the solvent base for UG components as well. The examinations have been carried out at room temperature. The samples of UG components (0.25 ± 0.01 g) were put into flasks with 50 g of solvent. The components have been mixed intensively with a magnetic stirrer for 24 hours. The following solvents have been used: *n*-methylpyrrolidone, benzyl benzoate, ethyl benzoate, glyceryl triacetate, dimethyl sulfoxide, acetone, dichloromethane, chloroform, toluene, xylene, ethylene glycol monoethyl ether, 1-butanol, methanol, tetrahydrofuran, 1,4-dioxane, diethyl ether, *n*-hexane, *n*-heptane, ethyl lactate, 1,3-dioxolane and dimethylformamide. Effective solvents have been classified according to the following criteria: polarity (preferably non-polar solvents have been selected) and boiling point (the lowest temperature is essential in the process of evaporating the solvent). Then a bigger quantity of UG has been dissolved in selected solvent up to the point where an organoleptic increase in viscosity of the solution is observed. The aim of this experiment was to choose a suitable concentration which would allow us to mix the solution of material component with a colloid of AgNP in a solvent.

AgNP colloid of 1000 ppm (w/w) concentration in *n*-hexane (Amepox Ltd. Łódź) has been used for modification. In order to increase the accuracy of dosing the colloid and to improve the uniformity of the mixing process of the components, the colloid has been diluted with 95% *n*-hexane (POCH, Gliwice, Poland) to make the final solution of 30 ppm concentration. The AgNP sizes in the colloidal solutions were then determined using dynamic light scattering (DLS) spectroscopy (Brookhaven). The particle hydrodynamic dimensions in the solution were assessed by performing measurements at a single angle of θ = 90°. Measurements showed an average NP size of 22.8 nm. The size distribution of AgNPs is presented in figure 1.

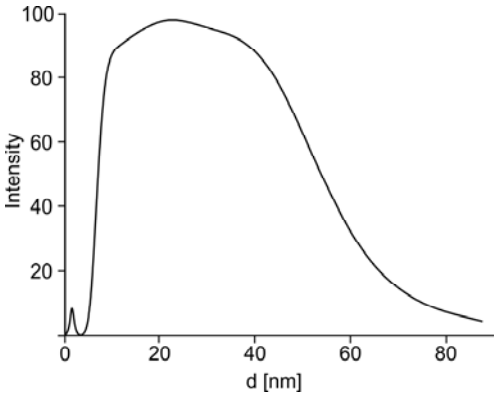

Fig. 1. AgNP size distribution in 30 ppm colloid

The procedure of the solvent evaporation from the solvent-UG component-AgNP compositions has been established for UGB. The mass of AgNP colloid in *n*-hexane necessary for the composite preparation of the particular AgNP concentration was calculated according to the following equation:

$$m_{\mathrm{Aghex}} = \frac{c_{\mathrm{AgT}} \times m_T \times 10^6}{30 \times (10^6 - c_{\mathrm{AgT}})}, \tag{1}$$

where:

m_{Aghex} – AgNP colloid mass (g),

c_{AgT} – AgNP concentration in the modified material (ppm),

m_T – mass of the modified component (g),

30 – Ag concentration in the *n*-hexane colloid (ppm).

Before adding AgNPs colloid to the material, 7% (w/w) solution of 2.5 g UGB in 95% *n*-hexane (POCH SA) has been made by mixing them with a magnetic stirrer at room temperature for 2 h.

The amount of AgNP colloid calculated according to equation (1) has been mixed with a magnetic stirrer and added to the solution of modified material component. This mixture was stirred with a magnetic stirrer for 15 min. In the next step, the effectiveness of four procedures of solvent evaporation from the mixture has been checked:

- evaporating was carried out for 6 h on rotary evaporator at room temperature, a water pump was used for decreasing the pressure to 100 mbar,
- evaporating of *n*-hexane was carried out on rotary evaporator at the temperature of 50 °C, under vacuum (10 mbar) using oil pump,
- evaporating was carried out for 6 h on rotary evaporator at the temperature of 50 °C and water pump was used for decreasing the pressure to 100 mbar,
- evaporating was carried out in a drying oven at 50 °C for 2 h and optionally for 24 h.

The process of evaporation of the solvent at lowered pressure has been performed on IKA RV-10 rotary evaporator equipped with Vacuubrand DVR 2 vacuum meter.

The effectiveness of each procedure has been assessed based on the analysis of ^{1}H NMR spectra of modified UG components. Spectra have been created in order to exclude or to detect the presence of signals registered for the *n*-hexane which was used in the process of modification (signals coming from CH_3 groups at $\delta = 0.89$ and from CH_2 group at $\delta = 1.28$).

The conclusions drawn from the examinations allowed us to established a final procedure for modifying the contents of one cartouche containing UGB or UGC (27±0.5 g). The next step was to modify both components of the material with the following AgNP concentrations: 10, 20, 40, 80, 120 and 200 ppm. The components (base and catalyst) of UG and composites have been mixed at 1:1 weight ratio, next they were placed between two glasses separated by 2.3 mm thick dividers and cured at temperature of 45 °C. From cured plates square specimens (10 mm × 10 mm) were cut.

A solid triangular samples of silicone were frozen in liquid nitrogen and mounted directly onto a specimen holder in the cryo-chamber of an ultramicrotome (Leica, EM UC7) which was then cooled down to –120 °C. Trimming with a glass knife helped to prepare the plane from which the sample was cut out. Sections were cut out at −120 °C with a speed of 5 mm/sec (the section thickness set at 200 nm). The leafs of material were manipulated on the Cu grid using an eyelash probe, and the sample was squeezed between ceramic surfaces and directly transferred using the high-vacuum technique to a glass holder adapted for drying. The samples have been examined with Scanning Electron Microscope (SEM) in order to confirm the presence of AgNP in the modified materials. SEM measurements have been performed with a Quanta 250 ESEM FEG scanning electron microscope (FEI Company) operating at 30 kV in environmental mode, using wet-STEM detector to detect STEM images in SEM and the gaseous secondary electron detector (GSED). The chamber pressure was 10 mmHg.

3. Results

3.1. Chemical composition

^{1}H NMR spectra for UGB and UGC have been presented in figure 2. A strong signal (A) coming from methyl groups which were in the neighbourhood of silicone atoms could be noticed on spectra representing UGB and UGC at $\delta = 0.072$ ppm. That clearly indicates the poly(dimethylsiloxane) structure of the two material components. There were also three multiplets within the range of $\delta = 5.69$–6.19 ppm, coming from double bonds of a vinylic protons (E, D, C). (B) signal at $\delta = 4.70$ ppm corresponds to the proton of neighbouring silicon atom. Introducing Si–H units into poly(dimethylsiloxane) chain makes cross-linking possible due to the reaction of –CH=CH_2 group with Si–H group. A catalyst of cross-linking reaction contained in UGC has not been detected by ^{1}H NMR. The signal at $\delta = 1.23$ ppm comes from water. A signal * at $\delta = 7.26$ ppm comes from the $CDCI_3$ solvent in which the measurement has been carried out.

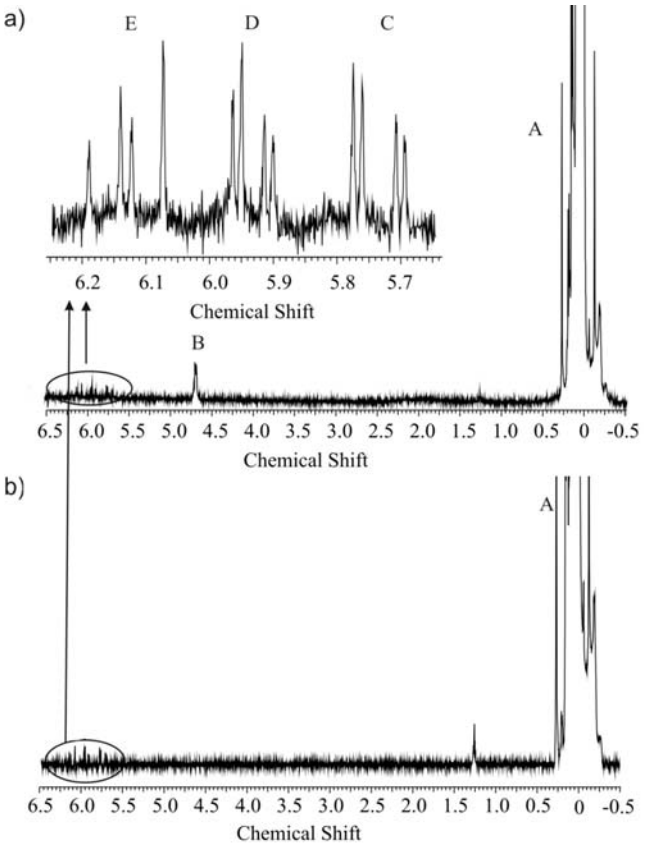

Fig. 2. ^{1}H NMR spectra representing UGB (a) and UGC (b)

3.2. Solubility tests

Both components of the material dissolved in the same type of solvent; however the UGB dissolved faster. The tests have been completed successfully for three solvents of moderate polar properties: methylene chloride, chloroform, tetrahydrofurane and for four non-polar ones: toluene, xylene, hexane, heptane. The

process of fast sedimentation was not observed in any of the above mentioned cases. The process of partial separation of the solvent fractions was slow and lasted about twenty-four hours. On the basis of the examinations carried out, *n*-hexane has been chosen for further tests. The boiling point of *n*-hexane, i.e. 68 °C, is lower that those of the other three solvents [16] which makes the evaporation process easier. The tests revealed that the solution became more condensed when 8–10% of material was added to *n*-hexane so 7% concentration was chosen to further tests. The time needed for obtaining a 7% homogeneous solution was about 80–90 min for a base and 110–120 for a catalyst.

Table. The times of solvent evaporation on rotary evaporator at 100 mbar pressure in the process of modifying 27 g of both material components

c_{AgT} (ppm)	Evaporation time (min)	
	UGB	UGC
10	19–20	17–18
20	19–20	17–18
40	19–20	17–18
80	21–22	19–20
120	23–24	21–22
200	27–28	25–26

3.3. Developing the effective method of removing *n*-hexane from the solution

Evaporating *n*-hexane on rotary evaporator at 100 mbar pressure and at room temperature was not satisfactory. There were peaks on ^{1}H NMR spectrum which indicated that after 6 h the residual amount of solvent was still present in the mixture obtained (figure 3a).

While evaporating *n*-hexane on rotational evaporator at the temperature of 50 °C under 10 mbar vacuum, the distillate became light yellow. This phenomenon excludes the use of such low values of pressure (vacuum pump) for removing *n*-hexane because this means that AgNP passes into the distillate. Increasing the pressure up to 100 mbar made it possible to obtain a composite without *n*-hexane after 6 h of the process of evaporation which has been confirmed by the analysis of ^{1}H NMR spectrum (figure 3b).

Heating up the solution in a dryer at 50 °C for 2 h did not give satisfactory results (figure 3c); however, prolonging the time of heating up to 24 h enabled us to get rid of the solvent in the process of evaporation (figure 3d).

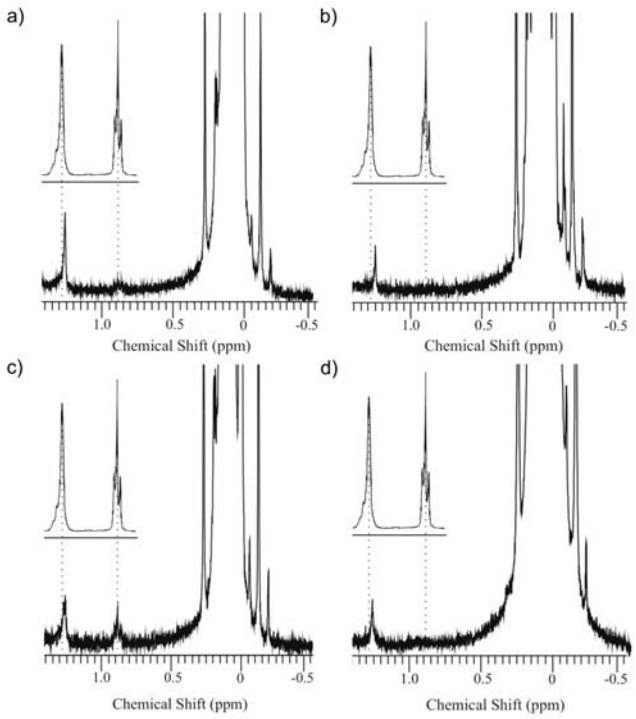

Fig. 3. Fragments of ^{1}H NMR spectra prepared for evaluating the effectiveness of evaporation of solvent from UGB–AgNPs after: evaporating on rotary evaporator at 100 mbar and at room temperature (a) and at 50 °C (b), heating up in a dryer at 50 °C for 2 h (c) and for 24 h (d)

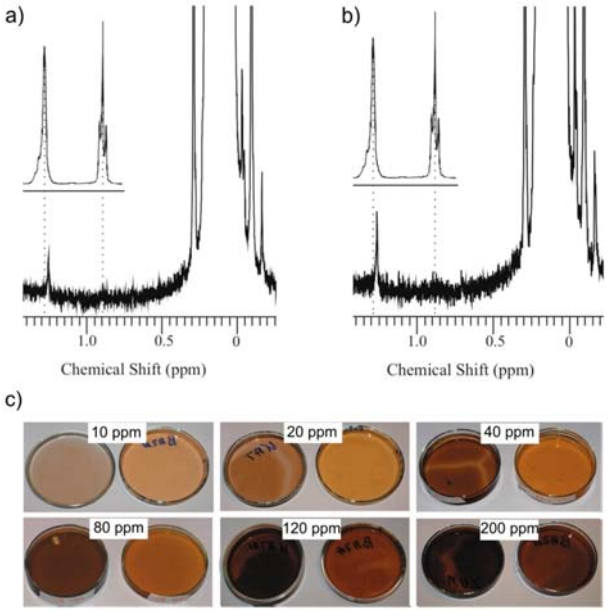

Fig. 4. Example fragments of ^{1}H NMR spectra which confirm the effectiveness of the final evaporation of a solvent from UGB containing 40 ppm of AgNP (a) and UGC containing 80 ppm of AgNPs (b) and modified components of the materials on Petri dishes (c)

Based on the results presented above, the final procedure has been developed which allowed us to

produce 27 g of UG component of 10, 20, 40, 80, 120 or 200 ppm concentration of AgNP. Initial evaporation of the solvent from a round-bottom flask on a rotary evaporator at 100 mbar pressure was the first stage of the process. The evaporation times at decreased pressure are given in the table. The same evaporation time was used for solvents on the evaporator for composites of AgNP concentration from 10 to 40 ppm because the mass of the *n*-hexane used in the solution with AgNPs only slightly increased compared to the mass of *n*-hexane used for dissolution of the material. In the case of the "catalyst", 2 min shorter evaporation times were used because of high viscosity of the solution. After initial evaporation, the solution had a consistence of a moderate-viscosity syrup. Next it was poured into a Petri dish of 200 mm diameter and it was held in a drying oven at the temperature of 50 °C for 24 h. Example ^{1}H NMR spectra which confirm the effectiveness of the procedure established are shown in figures 4a and 4b. Modified components of the material of different AgNP concentrations in a non-cured form are presented in figure 4c.

3.4. Scanning electron microscopy investigations

Scanning transmission electron microscopy investigations confirmed the success of modification process. Measurements have shown the presence of both single particles and large nanoparticles aggregations (figure 5) in the composites. Single particles from all specimens ranged usually from 10 to 30 nm. The higher the AgNP concentration, the greater the number and the larger the sizes of NP aggregations. Starting with a concentration of 80 ppm, numerous large aggregations, mostly ranging from 100 to 300 nm, have been observed, but simultaneously some of them exceeded 1 μm (figure 5d).

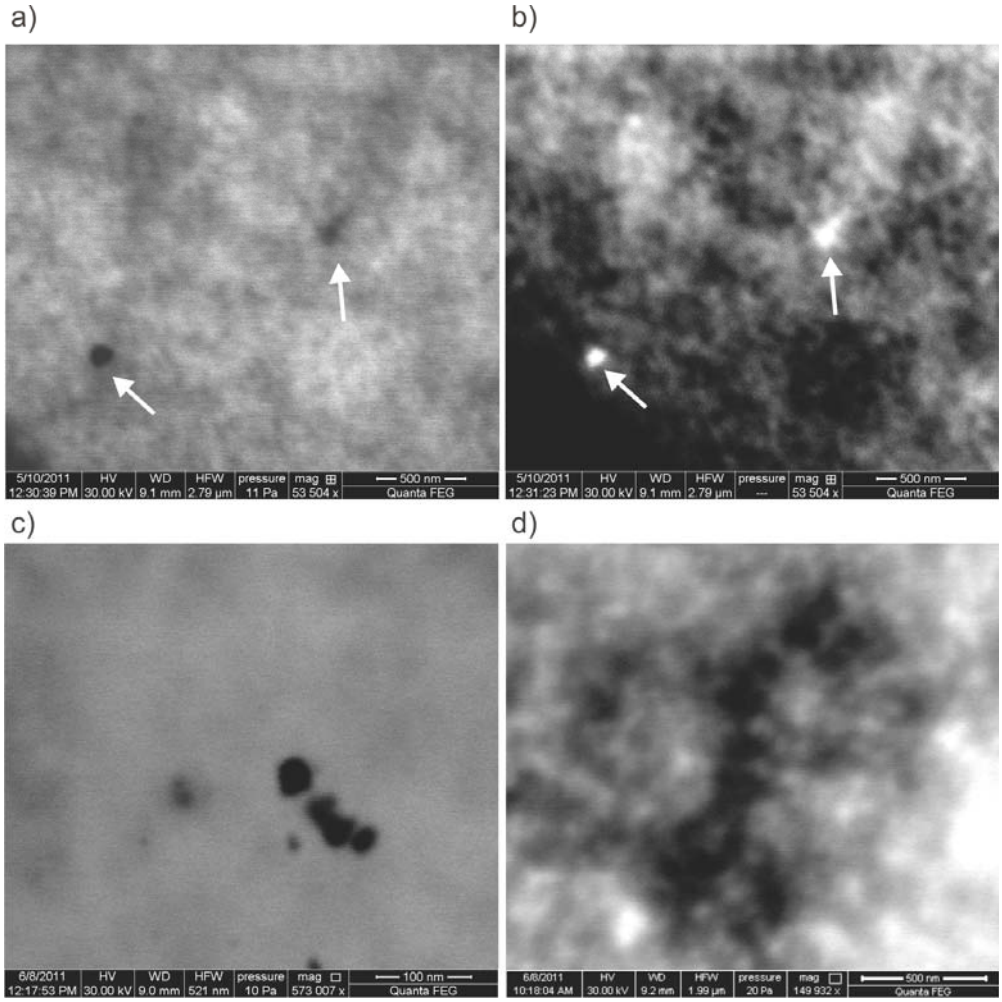

Fig. 5. Micrographs showing AgNPs in composites. Scanning transmission electron image using the wet-STEM detector (a) and the gaseous secondary electron detector (GSED) (b) in a composite with 120 ppm of AgNP (arrows indicates NPs), SEM images using the wet-STEM detector showing a smaller aggregation (c) and huge aggregation in a composite with 200 ppm of AgNPs (d)

4. Discussion

A soft lining material of antimicrobial properties has not been designed so far, even though the materials such as UG are commonly colonized and penetrated by microorganisms [1]–[17]. Simultaneously numerous reports confirm an adverse effect of AgNP on the basic pathological microflora in oral cavity such as a *Candida albicans* and *Streptococcus mutans* [15], [18]–[20]. Additionally KVITEK et al. [21] report that AgNPs do not generate any danger if its concentration is limited in order to inhibit the growth of microorganisms.

The method of modifying soft lining materials by solvents has been presented in this investigation. In the first stage of the examinations, *n*-hexane solvent has been selected as a dispersion medium for AgNPs and the components of the UG. The tests of evaporating the solvent from *n*-hexane–UG–AgNP mixture confirm that at pressures lower than 10 mbar it is not possible to carry out the modification process because AgNPs pass into the distillate. It has also been proved that at temperature up to 50 °C the process of evaporation is much more effective. On the basis of the tests the final two-stage procedure has been developed which allowed *n*-hexane to be removed from the mixture. To accelerate the whole evaporation process, the solution was initially compensated on rotary evaporator at 100 mbar pressure. Continuing the process in Petri dish reduced losses while transporting the material, e.g., during the process of curing (taking out the ready material component from the flask after a completed process of evaporation of the solvent is quite difficult and time-consuming). Cured material samples of different AgNP concentration have been subjected to SEM examinations which confirmed the presence of AgNP in the modified material. The observed tendency of AgNP to form large aggregations of more than several hundred nm in the material, particularly noticeable at concentrations of AgNP exceeding 80 ppm, can result in a decrease in antimicrobial effectiveness of the NP introduced into the material by the reduction of effective surface of AgNP which can contact with microorganisms [21]. Antifungal and antibacterial properties and the impact of modification process upon the basic properties of the composites obtained, defined by European Standards [22], need to be confirmed by further examinations.

Acknowledgements

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