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Bactericidal properties of experimental dental composites based on dimethacrylate resins reinforced by nanoparticles

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ABSTRACT

The aim of this study was to examine the possibility of replacing a part of the filler in experimental dental composites by nanoparticles with antibacterial potential. Experimental dental composites containing silver nanoparticles deposited on titanium and silica dioxide of different size were analyzed in terms of antibacterial properties. The depth of cure and mechanical properties of composite surface layer were examined. Bactericidal properties were tested according to the LIVE/DEAD cell viability assay. The mechanical properties were determined with a NanoTest 600 instrument. Composites containing silver nanoparticles deposited on titanium dioxide and nanosilica carrier exhibit the strongest antibacterial properties. High content of TiO_2 causes strong absorption of light irradiation, which makes their curing process more difficult. Hardness of surface layer increases but the deeper layers of the samples remain uncured. Composites containing nanosilver on titanium dioxide and nanosilica carrier exhibit the highest antibacterial activity. High content of TiO_2 causes strong absorption of light irradiation impairing their curing process. The presence of nanosilver changes composite color causing limited light penetration and lower surface hardness.

1. Introduction

The interest in "nanotechnology" and the need for this rapidly expanding area of research with huge potential is enormous nowadays. The transition from microparticles to nanoparticles causes a significant increase in specific surface area of materials. This, in turn, dramatically changes the output characteristics, which refers to their physical, chemical and optical properties. Studies on the improvement of mechanical strength by the introduction of nanoparticles into the resin matrix have been extensively carried out. However, the development of new dental composite materials is a very complex matter and requires the analysis of several key aspects. The crucial issue is the process of durable interactions formation between dimethacrylate matrix and inorganic filler particles, which requires filler surface modification by silane coupling agent [1-4].

Silver, in the form of nanoparticles, has made a remarkable comeback. The possibility of using silver nanoparticles in dental materials has been studied over the past several years [5-15]. Due to the expanded, porous structure, and thus excellent sorption properties, SiO_2 as the most popular filler of dental composites, is an excellent base to various modifications [16]. Yamamoto *et al.* [5], Kawashita *et al.* [17] and Jeon *et al.* [18] have investigated various modification possibilities of silica by silver compounds. In fact, the mechanism of antibacterial action of Ag-modified fillers is similar to the action of antibiotics introduced into the matrix and depends on the releasing of Ag^+ ions in contact with moist oral environment. Silver blocks metabolic processes occurring in bacterial cells by reacting with -SH groups of enzymes, deactivating their catalytic action and distorting the metabolic processes. However, the antibacterial activity of Ag-modified silica, similarly to chlorhexidine, is short-lasting.

The use of zeolites as carriers of silver seems to be more promising. According to the theory, Ag^+ is released from the zeolite (exchanged) at a rate controlled by the concentration of cations present in the environment. As the ionic strength of oral environment is not high, the modification of zeolites with silver would probably cause the desirable, slow release of silver ions. This, in turn, potentially creates an opportunity to extend the time of effective antibacterial action. Nevertheless, the application of silver zeolite in dental composites to evaluate its bactericidal efficacy in oral environment requires further studies [19].

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Table 1. Antibacterial additives.

Additive	Manufacturer	Information
$NanoZrO_2$	Aldrich Chemical Co., Milwaukee, USA	d<100 nm
SiO_2+nanoAg	Insitute of Industrial Chemistry, Warsaw, Poland	concentration of nanosilver on silica ~ 32000 ppm
$NanoTiO_2$	Aldrich Chemical Co., Milwaukee, USA	d~21 nm
TiO_2+nanoAg	Amepox Sp. z o. o., Lodz, Poland	1000 ppm of nanosilver on titanium dioxide (Aldrich)
$NanoTiO_2$+nanoAg	Amepox Sp. z o. o., Lodz, Poland	1000 ppm of nanosilver on nanotitanium dioxide (Aldrich)
Arsil+nanoAg	Amepox Sp. z o. o., Lodz, Poland	1000 ppm of nanosilver on microparticulate silica (Rudniki)
Aerosil+nanoAg	Amepox Sp. z o. o., Lodz, Poland	1000 ppm of nanosilver on nanosilica Aerosil 380 (Degussa)

The aim of this study was to examine the possibility of replacing a part of the filler in experimental dental composites by nanoparticles with antibacterial potential. We examined whether a small addition of such additives changed the viability of *S. mutans* in contact with the sample and whether the addition of nanoparticles influenced the mechanical properties and the curing depth of composite.

2. Experimental

2.1. Materials

2.1.1. Preparation of a standard matrix

2,2-*Bis*-[4,4-(2'-hydroxy-3'-methacryloxypropoxy)phenyl] propane (Bis-GMA, Aldrich Chemical Co., Milwaukee, USA) was poured into the crystallizer, placed on a hot plate. After a few minutes, lower viscosity monomer-triethylene glycol dimethacrylate (TEGDMA, Aldrich Chemical Co., Milwaukee, USA) was added in weight ratio of 6:4 and the mixture was stirred for 30 minutes using a magnetic stirrer. After that, appropriate quantities of camphorquinone (CQ, Aldrich Chemical Co., Milwaukee, USA), 2,6-di-*tert*-butyl-4-methyl phenol (BHT, Fluka Chemie AG, Buchs, Switzerland) and 2-(dimethylamino) ethyl methacrylate (DMAEMA, Merck KGaA, Darmstadt, Germany) were added and mixed for another 3 hours until all ingredients were completely dissolved. Additional components were added to dimethacrylate resins in the amount not exceeding 1.6 wt%.

2.1.2. Modification of filler surface

The surface of Arsil precipitated silica powder (Rudniki S.A., Poland) was modified using the 3-metacryloxypropyl-trimethoxysilane (U-511, Unisil, Tarnow, Poland). The quantity of silane coupling agent, necessary to produce a silane monolayer on the surface of silica, was calculated according to the formula (1):

$$[wt.\,\%\,] = \frac{\text{Surface area of filler } [\frac{m^2}{g}]}{\text{Coverage area of silane } [\frac{m^2}{g}]} \times 100\,\% \qquad (1)$$

The mixture containing acetone, water, appropriate quantity of amine and silane (4 wt%. in relation to the total amount of solvents) was prepared. The solution was then left for the next 5 min. allowing for pre-hydrolysis of silane to take place. Then, the silane solution was gradually stirred and silica was incorporated. Mixing was continued for another 1 hour. After filtration, the modified silica was washed twice with acetone. After this treatment, the silica was transferred into an oven, where it remained for 10 hours at temperature of 110 °C.

2.1.3. Preparation of dental composites

All composites were made using 2 g of resin mixture. Modified Arsil filler and different potentially antibacterial additives (Table 1) were added in seven portions and carefully ground in an agate mortar for 35 minutes. Special attention was paid to achieve the desired consistency.

Experimental resin composites studied are shown in Table 2. The materials were extruded directly into the silicon mold and their surface was secured with a microscope cover glass to minimize the contact with air oxygen. Then, the top surface was photo-polymerized for 60 s using a light-curing unit (SmartLite PS, Dentsply DeTrey, Konstanz, Germany) operating in a standard mode and emitting the radiation of 950 mW/cm^2.

2.2. Methods

2.2.1. Depth of curing

The study composite materials were placed in a silicone mold (cylinder of length l = 5 mm and diameter d = 3 mm) and covered with a microscope slide. The samples were cured for 60 s. Then, the samples were pulled out from the mold, the uncured portion of the material was removed and the cross-linked layer depth was measured. Three tests were performed for each sample group. The Kruskal-Wallis equality-of-populations rank test was performed. The significance level was set at 0.05.

2.2.2. Nanoindentation

Mechanical properties of the surface layer of light-cured dental composites were determined with a NanoTest 600 instrument (Micro Materials Ltd., Wrexham, UK) [24]. A Berkovich diamond penetrated the surface layer of material with the loading/unloading rate of dP/dt = 0.1 mN/s up to the maximum force of 5 mN. All the experiments were conducted under controlled temperature (T = 20±2 °C) and relative humidity (60±2 %). Ten tests were performed for each sample group. The data registered were analyzed according to Oliver and Pharr [20]. An analysis-of-variance (ANOVA) model for balanced designs was employed. The significance level was set at 0.05.

2.2.3. Bactericidal tests

Bactericidal tests were conducted according to the following procedure: a sterile area (5×5 mm) of cured dental composites was covered with suspension of *S. mutans*. After 24-hour incubation at 37 °C, bacteria on the sample surface were stained using 5 µL *bis*-benzidine solution (100 mg/mL) in 0.1 M phosphate buffer (pH = 7.4). Subsequently, the sample surface was covered with 2 µL propidine iodide solution (500 mg/mL in 70 % ethanol). The samples were incubated for another 2 hours and observed under the fluorescent microscope (Olympus GX71, Japan) equipped with a digital camera (DP70, Japan). This procedure allowed distinguishing live (blue-white color) and dead cells (purple-red color). Ten tests were performed for each sample group. An analysis-of-variance (ANOVA) model for balanced designs was employed. The significance level was set at 0.05.

3. Results

3.1. Bactericidal tests

The examined samples contained precipitated silica (Arsil) as basic filler and different additives with potential bactericidal activity.

Table 2. Formulation of the composite studied.

Sample	Matrix	Basic filler - Arsil [wt.%]	Nano-additive [wt.%]	Summary filler content [wt.%]
"0"		50.1	-	50.1
A		53.1	$NanoZrO_2$ [4.4]	57.5
B		56.5	SiO_2 + nanoAg [0.4]	56.9
C	Standard	36.0	Arsil + nanoAg [14.1]	50.1
D	*Bis*-GMA:TEGDMA (3:2)	26.8	Aerosil + nanoAg [14.6]	41.4
E	and CQ, BHT, DMAEMA	43.6	$NanoTiO_2$ [12.2]	55.8
F		41.4	TiO_2+nanoAg [11.,7]	53.1
G		41.4	$NanoTiO_2$ + nanoAg [11.7]	53.1
H		53.0	$NanoTiO_2$ + nanoAg [1.1]	54.1

Table 3. The depth of curing.

Sample	Depth of cure [mm]
"0"	4.7
A	2.0
B	0.5
C	2.0
D	1.6
E	0.8
F	0.4
G	0.8
H	1.8

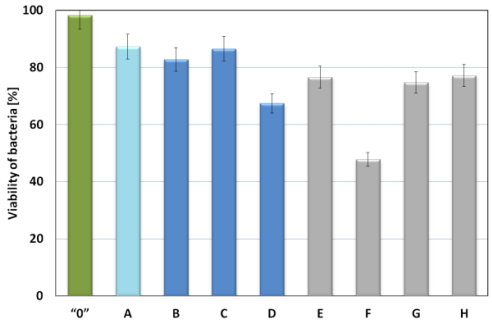

Figure 1. Viability of *S. mutans* on the surface of samples studied.

In all cases, a decrease in the number of *S. mutans* in contact with the surface of samples was observed (Figure 1). The viability of bacteria on the surface of the reference sample "0" was similar to the control sample, which indicated no antibacterial activity of the precipitated silica.

It is worth emphasizing, that pure nanozirconium dioxide exhibits some antibacterial potential. The addition of only 4.4 wt% of $nanoZrO_2$ (samples A) caused significant decrease in number of living cells (87.3 %). Similar bacteria mortality was observed in the case of samples B (SiO_2+nanoAg) and C (Arsil+nanoAg). However, silica dioxide and silver (composite D) resulted in 32.6 % inhibition of bacteria growth that differed significantly to other composites. In the case of sample F (containing nanoparticles of silver deposited on the surface of titanium dioxide) over 52 % population of bacteria *S. mutans* did not survive in contact with the surface of the cured composite; the level of living cells was significantly lower than for other composites. The addition of nanosilver on nanotitanium dioxide (sample G) resulted in only a slight improvement in bactericidal activity in comparison to sample E. Tenfold reduction $nanoTiO_2$+nanoAg in composite H (in relation to sample G), caused no significant difference in the average viability of bacteria. The addition of pure $nanoTiO_2$ (composite E) and the introduction of a similar amount of $nanoTiO_2$ with nanosilver (composite G) showed no significant difference in the number of living cells.

3.2. The depth of curing

The curing depth of composites B and E-G is in the range of 0.4-0.8 mm and there is no significant difference between these values (Table 3). The tenfold reduction in the amount of nanosilver on nanotitanium dioxide (composite H) significantly increased the depth of cure from 0.8 mm up to 1.8 mm. The highest depth of cure was observed in the case of experimental composites A, C and D. These values (composite A, C, D and H) of curing depth were significantly higher than values of other experimental samples.

3.3. Nanoindentation experiments

The surface layer hardness of composite E, containing $nanoTiO_2$, amounted to 230 MPa (Table 4). The tenfold reduction in the amount of $nanoTiO_2$ in composite H (150 MPa), lowered the hardness of the surface layer of about 10 % only. The absence of sample F was due to the failure to prepare the specimens, which was caused by very low depth of cure. A significant difference was found only in case of composite H and the reference sample. Moreover, the addition of nanosilver changed the sample color from cream to brown.

4. Discussion

Biphasic composite restorative materials for filling tooth cavities are undoubtedly one of the most important achievements in modern dentistry. However, to provide better durability of restorations dentistry has been looking for new ways of matrix reinforcement. As satisfactory mechanical properties of restorative materials are important, composites should also exhibit low polymerization shrinkage, good bridgeability and should be easy to handle.

Table 4. Nanohardness and Young modulus of the composite studies.

Sample	Depth of indentation, h [nm]	Nanohardness, H [MPa]	Young modulus, E [GPa]
"0"	1160	210	4.0
A	1150	170	3.4
B	1060	185	3.4
C	1190	195	4.0
D	1205	200	3.5
E	1245	230	2.5
G	1275	170	3.5
H	1375	155	2.7

In addition, dental materials providing bactericidal properties may allow prevention of severe complications associated with the presence of harmful bacteria in the oral cavity.

Bactericidal tests conducted in our study showed that silver nanoparticles deposited on the titanium dioxide surface exhibited the strongest activity. Over 52% population of bacteria *S. mutans* did not survive in contact with the surface of the cured composite. The addition of nanosilver on nanotitanium dioxide resulted only in a slight improvement in bactericidal activity, and effectiveness was not specifically dependent on the additive amount. Tenfold reduction of nanoTiO_2 + nanoAg in the composite H (in relation to sample G), caused only a slight increase in average viability of bacteria. We hypothesize that in the case of silver deposited on nanotitanium dioxide, it is not nanosilver but rather the carrier itself, which exhibits antibacterial properties. The comparison of the silica fillers containing nanosilver shows that the combination of two types of nanoparticles: silica dioxide and silver (composite D) exhibit the most effective antibacterial action. This system is definitely more effective than nanosilver deposited on microparticulate silica filler. Preliminary studies demonstrated that the viability of bacteria in contact with the material containing 53 wt% of silanized Aerosil 380 only, achieved the level of 82 %. The addition of nanosilver (about 100 ppm to the composite) caused the reduction in *S. mutans* viability of approximately 15 %.

Kawashita *et al*. reported similar activity of silver-containing filler prepared using the sol-gel method, where *S. mutans* was completely killed due to the contact with the experimental composite containing 70% Ag-filler for 12 hours [17].

Antimicrobial activity of silver nanoparticles can be explained by various hypotheses. This process can evolve in two parallel streams: silver is either incorporated in the cell membrane or it penetrates into the cell, which eventually, leads to cell death [14,21-23]. Bactericidal effect is reported to be dependent on the shape of particles (truncated triangular shaped particles have greater action as compared with spherical and rod-shaped particles), and the type of microorganism- Gram positive or Gram negative, where the former are more resistant [23,24]. This can be attributed to the difference in cell wall structure between gram negative and gram positive microorganisms. *S. mutans*, gram positive bacteria, may be, therefore, less susceptible to silver nanoparticles than for example *E. coli* [24].

Yoshida *et al*. [25] have found that the antibacterial action of silver is not always caused by the release of Ag^+ ions to the environment. This situation was observed for example in the case of silver immobilized on silica gel, or zirconium phosphate-$Zr_2(HPO_4)_3$. It was suggested that, as a result of the catalytic action of silver, oxygen was changed into active oxygen (including hydroxyl radicals) by the action of light energy and/or H_2O in the air or water only at polar surfaces, and that this active oxygen caused structural damage in bacteria.

The satisfactory results of antibacterial properties of composites encouraged us to verify their mechanical properties and usefulness in dental office. And indeed, the depth of cure of composites E-G occurred to be too small (Table 3) to use these composites (especially F) in clinical conditions. Tenfold reduction in the amount of nanosilver on nanotitanium dioxide (composite H), increased the depth of cure from 0.8 mm to an acceptable level. Smaller amount of titanium oxide with nanosilver in composite F, may act in the same way, without losing very good bactericidal properties. This, however, has not been confirmed. The highest depth of cure was observed in case of composites A, C and D. It means that the addition on nanosilver on silica carriers (concentration of about 1000 ppm) does not change significantly the color of samples and it does not deteriorate the absorbance of light irradiation. The small depth of cure of composite B can be explained by the high concentration of silver (about 32000 ppm in basic filler) and the change in the sample color.

Incorporation of 20% or more silver-zeolite, and 10% or more of silver-apatite has been reported to reduce significantly mechanical properties such as the tensile strength, compressive strength, and elastic modulus [26-28]. Although the majority of these values for these experimental composites can be clinically acceptable, further studies remain to be performed.

The next important issue is that specific optical properties of TiO_2, which is capable of absorbing light radiation, might cause some problems with crosslinking. The surface layer of the composite containing nanoTiO_2 was the hardest one. Titanium dioxide strongly absorbs light radiation. This phenomenon results in the effective cross-linking of the surface layer of these samples, producing the material of a very hard surface skin. Lower depth of curing of this composite can be explained by blocking the penetration of radiation quanta into deeper layers of the material, which was caused nanoTiO_2 particles. We have also observed a similar effect in samples with nanoTiO_2+nanoAg (G, H). The reduction in crosslinking density and the depth of cure was also reflected by lower (compared to the sample "0") hardness of the surface layer of the experimental composites with the exception of samples containing TiO_2+nanoAg.

Color stability is one of the most important properties of esthetic materials, therefore, it should be investigated which should be investigated thoroughly in every newly developed composite. Unfortunately, this study shows that adding nanosilver to experimental composite causes its brownish appearance. The higher amount of nanosilver in the material made the color darker and the curing process incomplete.

Poor color stability of silver-containing materials, dental composites especially, was also reported in other studies [27,28]. Composites containing silver-zeolite became heavily discolored after only one day of immersion in artificial saliva [27]. However, the discoloration of the experimental composite incorporating 10% silver-apatite was much smaller than silver-zeolite composites [28].

Zinc oxide (ZnO) has been incorporated into resin composites as opaque reinforcing filler. However, ZnO powders may exhibit also antimicrobial properties [29]. It is claimed, that smaller particles of zinc oxide (ZnO nanoparticles; ZnO-NPs) are more effective than larger particles against both gram negative and gram positive bacteria [30,31]. However, the antimicrobial behavior ZnO-NPs incorporated into dental composites has not been widely reported yet. Antimicrobial properties of ZnO-NPs may be due to the generation of active

oxygen, which inhibits growth of microbes. Another potential mechanism of ZnO-NPs antibacterial activity may result from the leaching of Zn^{2+} ions into the growth media, reducing acid production by *S. mutans* and *S. sorbinus* and additionally disrupting enzyme systems of dental biofilms by displacing Mg^{2+} essential for enzymatic activity of the dental plaque [32].

According to Sevinc and Hanley [29], the incorporation of small amount of ZnO-NPs filler into dimethacrylate resin do not significantly inhibit the bacteria growth in contact with experimental composites. Some inhibitory effect was observed in the case of higher amount of ZnO-NPs (10 wt%) [29]. Our experiments revealed that addition of 4.4 wt% of ZnO change the bacteria viability in small extent only (11% compared to the reference sample). Such promising results prompt us to undertake more detailed study. Experimental composites with higher content of ZnO-NPs will be further investigated.

Both ZnO-NPs and TiO_2-NPs can exhibit photocatalytic activity under ultraviolet light leading to the production of antimicrobial active oxygen species. The antimicrobial properties of TiO_2 in the presence of UV light have been also attributed to their production of active oxygen species i.e. H_2O_2. However, TiO_2-NPs are apparently unable to inhibit bacterial growth significantly in the absence of light. The addition of TiO_2 is associated with low optical absorption [33]. Several strategies have been adopted to overcome that drawback including e.g. coupling with SiO_2 or some other oxide carriers (titania-silica). Titania-silica nanocomposites exhibit high thermal stability, surface area and higher photocatalytic activity than pure TiO_2 [34]. The experimental antibacterial composites generated in this study proved to exhibit adequate antibacterial activity against cariogenic microorganisms. However, mechanical and optical properties need improvement. Moreover, further experiments simulating clinical situations should clarify whether the materials are effective in inhibiting bacterial growth or bacterial attachment under in vivo conditions.

5. Conclusion

Composites containing nanosilver on titanium dioxide and nanosilica carrier exhibit the highest antibacterial activity. High content of TiO_2 causes strong absorption of light irradiation impairing their curing process. The presence of nanosilver changes composite color causing limited light penetration and lower surface hardness.

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