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Preparation and characterization of ZnO-PMMA resin nanocomposites for denture bases

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Purpose: The aim of the paper was to investigate the antifungal activity of zinc oxide nanoparticles (ZnONPs) against *Candida albicans*. Some attempts have been made to find out the best way to introduce ZnONPs into polymethyl methacrylate (PMMA) resin material and to determine some parameters of a newly formed composite. *Material and methods*: Zinc oxide nanoparticles were manufactured and their basic physical parameters were determined (average particle size, density, specific surface area). Minimal inhibitory concentration (MIC) of ZnONPs was determined for the *Candida albicans* standard strain. The average size of ZnO conglomerates in the monomer solution of PMMA resin was measured using a dynamic light scattering instrument. PMMA resin samples with incorporated ZnONPs were produced. The morphology of nanopowder and the newly formed composite was examined under a scanning electron microscope (SEM). In addition, the roughness parameter of PMMA resin material was investigated before and after ZnONPs modification. *Results*: Nanopowder with the average particle size of 30 nm, density of 5.24 g/cm^3 and surface area of 39 m^2/g was obtained. MIC was determined at the level of 0.75 mg/mL. The average size of ZnO conglomerates in the monomer solution of acrylic resin dropped by 11 times after ultrasound activation. SEM examination of a newly formed composite showed a successful introduction of ZnONPs confirmed by the energy dispersive X-ray spectroscopy (EDS) analysis. There were no statistically significant differences in the biomaterial roughness before and after the modification of ZnONPs. *Conclusion*: Zinc oxide nanoparticles were successfully incorporated into acrylic resin used for the production of denture bases. The presence of nanoparticles with sizes below 100 nm was confirmed. Nevertheless a newly created composite needs to be further investigated to improve its homogeneity, and to check its microbiological properties, strength and biocompatibility prior to its possible clinical use.

Key words: *ZnO nanoparticles, Candida albicans, acrylic resins*

1. Introduction

Denture stomatitis is defined as all types of inflammatory lesions in oral cavity, directly caused by prosthetic appliances. The main causes of its development comprise mechanical injury, denture plaque related to poor hygiene and fungal infection (24). The etiology of this phenomenon is therefore multifaceted and epidemiological studies indicate its occurrence even in 70% of denture wearers (11). Polymethyl methacrylate (PMMA) resin is the most often used material in dental prosthodontics, especially in prosthetic rehabilitation of edentate or nearly edentate patients. In such cases the reduced efficiency of the immune system must be taken into consideration,

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primarily due to advanced age, systemic diseases and related medication. The denture base then becomes a significant risk factor for the development of denture stomatitis complicated by fungal infection. *Candida albicans* is the microorganism most frequently isolated in these cases. It occurs as a saprophytic organism in a healthy oral cavity, but due to environmental changes and microbial imbalances under a denture base, it may lead to the formation of inflammatory lesions (1). Complete denture creates specific conditions in oral cavity by impairing natural flow of saliva, exerting mechanical compression, increasing the temperature or decreasing the light access, especially when the denture, against dentist's advice, is worn without night-breaks (24). In these cases, *Candida* strains are present not only on the mucosa, but they also form a biofilm structure on the acrylic denture. This structure is difficult to remove and increases the resistance to antifungal treatment (7). Using antifungal agents designed to eradicate *Candida* it is necessary to consider their toxicity and possible development of multiresistant strains. Besides, it is impossible to eradicate *Candida* from the previously infected denture, which results in oral mucosa recontamination and disease recurrence (7). It is, therefore, essential to conduct research aimed at modifying PMMA resin to impede or prevent the process of forming a bacterial and fungal biofilm. Currently, the research is aimed to modify the surface of the polymerized PMMA resin by forming a layer impeding biofilm formation. This layer is designed inter alia to smooth all the microroughness and change free surface energy by exerting effect on the hydrophilic/hydrophobic properties of the material. For this purpose, photopolymerized coatings (14), titanium dioxide (3), mannan or mannose (23) are designed to disrupt non-specific and specific interactions between the microbes and the denture base, thus preventing their adhesion to the biomaterial. This is a simple and fast method in clinical practice, however the biocompatibility of the applied coating gives rise to doubts, as well as its durability owing to mechanical and chemical agents used for cleaning dentures. This provides the rationale for making an attempt to modify the entire chemical composition of PMMA resin before the polymerization process. Due to the constant progress in the field of nanotechnology, some attempts have been made to add nanoparticles to PMMA resin, i.e. silver (15) or platinum (20) to make use of their microbiological properties. The best method known to date is the modification of the material by silver nanoparticles with a proven antibacterial activity for both Gram positive and Gram negative or strains resistant to conventional antibiotics (13). Silver nanoparticles show a lower cytotoxicity and genotoxicity compared with substances containing silver in the scale of the micrometer and are less prone to the formation of strains resistant to treatment compared with antibiotics (8). Li et al. (15) evidenced a reduction of bioactivity and biomass of biofilm with increasing concentrations of nano-Ag solution. Anti-adhesive properties against *Candida* showed PMMA resin modified by a 5% content of nanoparticles. Microscopic analysis revealed that the 2% silver content samples already showed disorder in the formation of biofilm, while at 5% the analyzed average thickness of the biofilm and the number of live cells showed only a few cells of *Candida*. It is worth mentioning that the authors of this study are not familiar with reports on the clinical use of biomaterials containing silver nanoparticles for denture bases.

The aim of the paper was to investigate antifungal activity of zinc oxide nanoparticles (ZnONPs) against standardized strain of *Candida albicans.* Some attempts have been made to find out the best way to introduce ZnONPs to PMMA resin material and to determine some parameters of newly formed nanocomposites.

2. Material and methods

Preparation of ZnO nanopowder

The materials used for the ZnONPs synthesis were: zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, pure for analysis, CHEMPUR, Poland) and ethylene glycol ($C_2H_4(OH)_2$, pure for analysis, CHEMPUR, Poland). Zinc oxide was obtained by dissolving zinc acetate in ethylene glycol. For ZnONPs synthesis the microwave solvothermal synthesis (MSS) technology was used (17). After 45 min of additional stirring, the reaction solution was transferred into a capped teflon vessel and heated using microwave radiation. The microwave reactor MSS2 (IHPP, ITeE – PIB, ERTEC, Poland) (16) was operated at 2.45 GHz and at a power density adjusted to approximately 10 W/ml. The duration of the reactions was 12 min under a constant pressure of 3 bars at a microwave power of 3 kW. After the synthesis, the obtained powder was sedimented, washed three times with deionized water (1 class, HLP 20 UV, Hydrolab, Poland) centrifuged (MPW-350, MPW Med Instruments, Poland) and dried in freeze dryer (Lyovac GT-2, SRK Systemtechnik GmbH, Germany).

ZnONPs characteristics

1. Powder X-ray diffraction

The X-ray diffraction (XRD) patterns were collected in the 2Θ range of 10°–100° at room temperature, with a step of 0.02° using an XRD (X'Pert PRO diffractometer, Cu $K_{\alpha 1}$ radiation, PANalytical BV, Almelo, The Netherlands). The average crystallite size was determined using the Scherrer formula. XRD peak profile analysis was performed using analytical formula for polydispersive powders [X, Y]. While Scherrer method provides a single size parameter, this technique provides four parameters: (a) average crystallite size, (b) error of the average crystallite size, (c) dispersion of size and (d) error of dispersion of sizes. Hence, one obtains full crystallite size distribution curve and estimation of "thickness" of this curve (error bars). On-line tool science24.com/xrd is a webpage, where diffraction files can be directly dropped. Files are processed on server to extract crystallite size distribution for XRD peaks. Unlike standard fitting, the tool does not act in reciprocal space at all, but solves sets of equations in few auxiliary spaces simultaneously. This allows us to analyze XRD data with heavily convoluted reciprocal space peaks.

2. Density and specific surface area (SSA) measurements

Density measurements were carried out using a helium pycnometer (AccuPyc II 1340, Micromeritics, USA) using an in-house procedure (25). This method enabled the density of ZnONPs to be measured with an accuracy of 0.01 g/cm^3. The specific surface area of the powder was measured by the Brunauer–Emmett –Teller (BET) method (Gemini 2360, Micromeritics, USA). The powder was subjected to desorption at 150 °C for 2 h prior to the measurement. The average diameter of the particles was determined on the basis of SSA and density; identity and sphericity of the particles were assumed (25).

3. Morphology analysis

The morphology of the nanopowder was investigated with a scanning electron microscope (SEM) (Ultra Plus; Carl Zeiss Meditec AG, Jena, Germany) and ZnONPs thin layer of carbon using a sputter coater (SCD 005/CEA 035, BAL-TEC, Switzerland). Imaging was performed using an InLens detector. With InLens detector superficial contaminations become visible. It also allows the quality and purity of nanoparticles to be specified.

4. The transmission electron microscope (TEM) and high-resolution TEM (HRTEM) investigations

The specimens for the TEM observations were prepared by dropping the methanol particle dispersion, created by an ultrasonic technique, on a carbon film supported on a 300 mesh copper grid. In addition, TEM studies were used to determine the nanoparticles size distribution. The particle size histograms were obtained by considering a region of a sample consisting of ca. 200 spherically-shaped nanocrystals. The obtained histograms were fitted to either normal or log-normal distributions (Chi^2 test and Person's coefficient).

5. Determination of minimal inhibitory concentration (MIC)

The overnight cultures of *Candida albicans* 14053 (obtained from the collection of the Department of Dental Microbiology, Medical University of Warsaw, Poland) were adjusted to 10^4 viable cells per mL. Prior to adjustment, they were incubated for 24 h in Sabouraud medium (DifcoTM, USA) containing various amounts of ZnONPs. The density of inoculums was standardized to obtain 10^4 colony forming units (CFU) per spot on the agar. The lowest ZnONPs concentration resulting in the invisibility of turbidity was taken as the minimal inhibitory concentration (MIC). Sabouraud medium plates were prepared as recommended by the manufacturer. A dilution series of antimicrobial agents ZnONPs (from 31.2 μg/ml to 2 mg/ml) was produced, including a drug-free control. These were followed by adding 19 ml of Sabouraud agar to each container, mixed thoroughly, and placed on prelabeled sterile Petri dishes at a level surface. Plates were incubated at 35–37 °C for 18 h (9, 10).

Preparation of denture base PMMA resin samples containing ZnONPs

1. Average size of ZnONPs in the suspension of PMMA resin monomer

Average size measurements in suspension were carried out using a dynamic light scattering (DLS) instrument (Zetasizer Nano-ZS, Malvern Instruments, Ltd., England) according to the ISO 22412:2008 standard. Measurements were made for 0.01wt% solution of ZnONPs in PMMA resin monomer (Superacryl Plus, Spofa Dental, Czech Republic). The mixture was shaken for 10 min in a Vortex shaker VX-200 and additionally sonicated for 60, 120 or 240 s in an Elmasonic S 10/(H) (30W, Elma Schmidbauer GmbH, Germany).

2. Nanocomposite preparation

A thermally polymerized PMMA resin Superacryl Plus was used to manufacture the denture bases. The recommended mixing ratio was 22 g of powder polymer and 10 ml of liquid monomer, which represents a volume ratio of 3:1. Zinc oxide nanopowder was

suspended in liquid monomer of PMMA resin. The mixture was shaken in a Vortex VX-200 shaker (Labnet, USA) for 10 min. Then an appropriate amount of PMMA resin powder was added, so that the final 2% mass concentration of ZnONPs could be obtained. This was preceded by calculating the weight loss of PMMA-nanocomposite after polymerization. The experimental evaluation involved weighing each of the mixture components before a 10-min stirring and sonication process. Weight loss resulted from the release of free monomer as well as evaporation of a volatile liquid monomer while stirring and sonication of solution (based on the experiment it was designated as 4.5%). Furthermore, ZnONPs in the solution of liquid monomer after the evaporation process increases its weight – 1 g of ZnONPs absorbs into its interior 0.1 g of monomer, which must also be taken into account, however, it is of less importance compared with weight loss during the nanocomposite preparation. Finally, the mixture consisted of 0.61 g ZnONPs, 9.5 g liquid monomer and 22 g of PMMA powder. PMMA resin without adding ZnONPs was used as the control group. Originally, samples were prepared with a model wax Vertex Regular (Vertex-Dental BV, The Netherlands), of size 10 × 10 × 2 mm, applying a standard procedure for conversion of wax to PMMA resin with hard gypsum class III (Stodent, Zhermack, Italy). The material was conventional heat-polymerized in the polymerization integral machine (PS-2, P.E.M., Poland) according to the manufacturer's instructions. After cooling and removal of the samples from a polymerization can, only necessary treatments were applied to remove residual gypsum with silicon rubber, without subjecting the samples to the polishing process. This procedure was designed to get the texture of the surface as similar as possible to the mucosal surface of the denture base. Thirty samples were made for both study and control groups. The samples were used in the roughness test and SEM examination. Due to the results obtained in the DLS examination the procedure was additionally modified. ZnONPs solution in liquid monomer of PMMA was additionally sonicated for 240 s using an Elmasonic S 10/(H) (30 W, Elma Schmidbauer GmbH, Germany). The rest of the procedure was the same as described previously. The obtained nanocomposite was used in the SEM examination.

3. Roughness test of nanocomposite

The surface roughness was examined using a stylus profiler Dektak XT. Linear and aerial measurements were made. The former measures a single line on a sample surface, the latter measures an area of the surface. Linear scans, 5 mm long, were taken step by step every 1 mm for both reference and modified samples. Moreover, a 10 micron resolution 3D map of the representative modified surface was recorded for an area of 4 mm^2. In statistical analysis of the roughness test results Student's *t*-test for independent samples was used. Data were evaluated for normal distribution using Kolmogorov –Smirnov test and homogeneity of variance was found using two independent assays, Brown–Forsythe and Levene tests. The level of significance was established at $p = 0.05$. All data were computed using the Statistica 10.0 program (StatSoft, Inc. Tulsa, OK, USA).

4. SEM examination and elemental composition of nanocomposites

The morphology of the obtained nanocomposites was investigated with a scanning electron microscope (SEM) (Ultra Plus; Carl Zeiss Meditec AG, Jena, Germany). An Energy Dispersive X-ray (EDS) (Quantax 400, Bruker, Billerica, Massachusetts, USA) detection system built in the SEM instrument was used to record the X-ray emission spectra of the samples interacting with the electron beam. For the SEM and EDS analyses breakthroughs of nanocomposites made in liquid nitrogen were used. Breakthroughs were sprayed with a thin layer of carbon using sputter coater. The aim of the study was to obtain a qualitative microscopic characteristics of the obtained polymer nanocomposites. For imaging two detectors, ESB and SE2, were used. The energy selective backscattered (ESB) detector is suitable for clear compositional contrast. The ability to detect back-scattered electrons (BSE) makes the subsurface information and nano-scale composition visible. The SE2 image contrast reveals both topographical and compositional information due to the greater sample interaction depth of SE2 electrons. Pores and fractures appear dark in the SE2 image, therefore, porosity information can be readily characterized with the SE2 electrons. In this paper, we present the results of the nanocomposite surface.

3.Results

ZnONPs characteristics

The obtained ZnONPs had a density equal to 5.24 g/cm^3 (Table 1), which is 6.6% less than the value given in the literature, 5.61 g/cm^3 (5). It was observed that the presence of nanoparticles caused the material density reduction, probably due to the significant contribution of the surface layers, which are less densely packed than the bulk. The material specific surface area was 39 m^2/g, and the average parti-

cle diameter was 30 nm; the calculation was based on SSA. The equation to calculate the particle diameter is as follows

$$D = \frac{N}{SSA \cdot \rho}$$

where D – is a particle size, N – factor dependent on the shape of the particles for sphere equal to 6, SSA – specific surface area, and ρ – density.

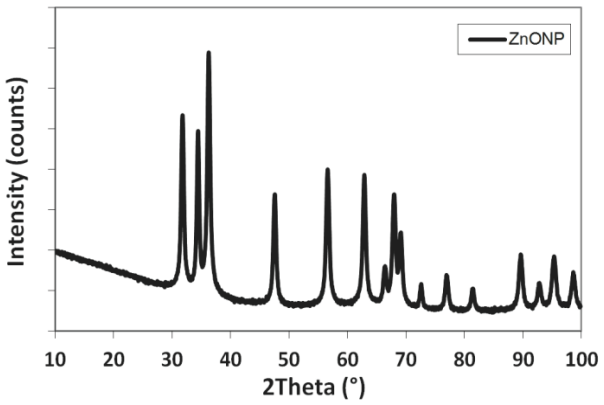

Fig. 1. XRD pattern of ZnONPs synthesized using microwave radiation

The XRD analysis for ZnONPs is presented in Fig. 1. The results confirmed that ZnONPs is a pure well crystallized hexagonal wurtzite structure with nano-sized particles ca. 25 nm in diameter (Table 1). No indication of the presence of other crystalline phases or any amorphous component was found.

Figure 2 presents the results of the SEM and TEM investigations. As can be seen ZnONPs exhibit strong homogeneity. The average particle size of the synthesized ZnONPs was estimated to be 25–30 nm. Figure 3 shows an image of the spherical particles taken in the dark field. According to TEM investigations, the average particle size was ≈26 nm (Fig. 4). According to XRD investigations, the average crystalline size was 26 nm (Fig. 5). Determination of minimal inhibitory concentration (MIC) is widely used in the comparative testing of new agents. The lowest concentration of antimicrobial agent inhibiting the visible growth of a microorganism is known as the MIC. For the standardized strain of *Candida albicans14053* MIC of ZnONPs was established at 0.75 mg/mL level.

Nanocomposite characteristics

The analysis of ZnONPs (Cp = 0.01 wt%) suspension in a liquid monomer of PMMA resin showed the average ZnO conglomerates of size 1608 nm with polydispersion index (Pdl) 0.597. After activating the solution with ultrasounds, an eleven-fold decrease in the average size of particles (down to 141 nm) with Pdl = 0.173 was observed (Table 2).

Table 1. Density, specific surface4 area and average particle size of ZnONPs

Sample	SSA, [$a_s \pm \sigma.m^2/g$]	Density, [$\rho \pm \sigma.g/cm^3$]	Average particle size from SSA BET, [$d \pm \sigma.nm$]	Average crystallite size, Scherer's formula, based on XRD, [$d_c.nm$]	Average crystallite size, Nanopowder XRD Processor Demo, based on XRD, [$d \pm \sigma.nm$]	Average particle size from TEM, [$d \pm \sigma.nm$]
ZnONPs	39 ± 1	5.24 ± 0.05	30 ± 2	25	25 ± 7	26 ± 1

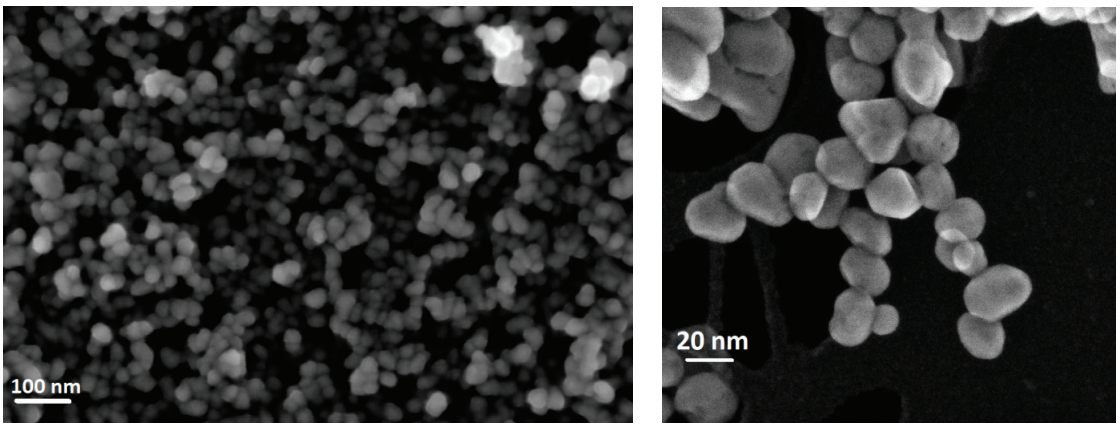

Fig. 2. Images of ZnONPs: left image – SEM, right image – TEM

Table 2. Average diameter of ZnONPs in methyl methacrylate suspension

Sample ZnONPs/MMA, Cp = 0.01 wt%	Size by DLS, Z-Average [d ± σ.nm]	Polydispersion index, Pdl
Mixing for 10 min	1608 ± 145	0.597 ± 0.054
Mixing for 10 min and ultrasonic mixing for 60 s	447 ± 23	0.471 ± 0.024
Mixing for 10 min and ultrasonic mixing for 120 s	144 ± 3	0.201 ± 0,022
Mixing for 10 min and ultrasonic mixing for 240 s	141 ± 2	0.173 ± 0,016

Cp = 0.01 wt%, DLS – dynamic light scattering; ZnONPs – zinc oxide nanoparticles.

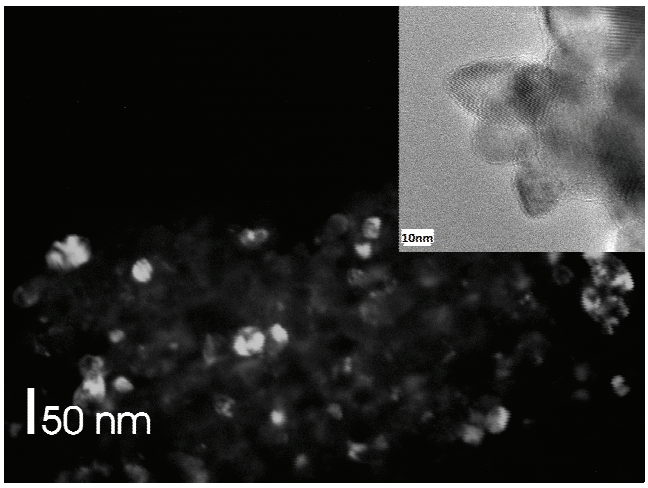

Fig. 3. The dark fieldTEM image of ZnONPs

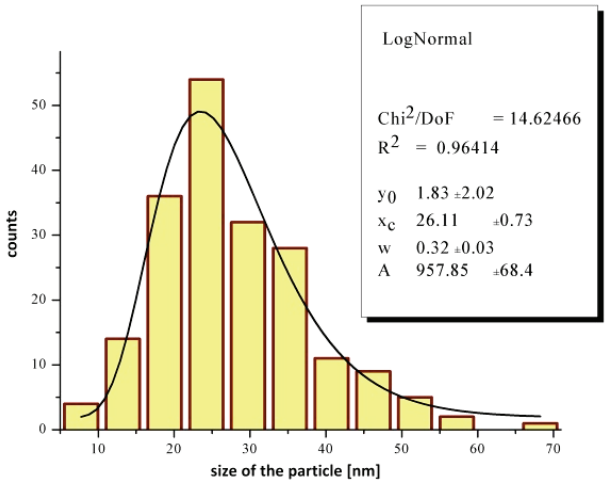

Fig. 4. Histogram of the ZnO particle size distribution

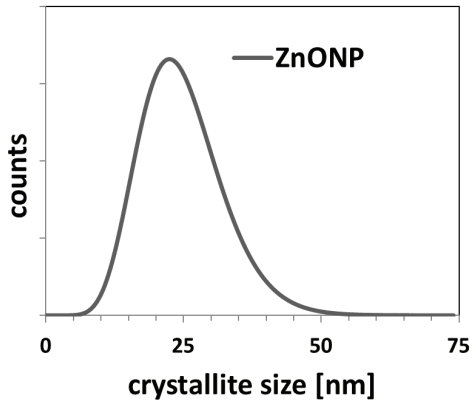

Fig. 5. Crystallize size distribution of ZnONPs, obtained in the web program Nanopowder XRD Processor Demo pre α ver.0.0.8, © Pielaszek Research, http://science24.com/xrd

Mapping of zinc content in the nanocomposite without sonication process (Fig. 6) showed the presence of ZnO clusters in the polymer, which indicated some deficiencies in the nanocomposite preparation method. Clusters of even a few micrometers in diameter were visible in the whole volume of the sample. Nevertheless, large areas of well dispersed nanoparticles were visible. After 240 s of the sonication process it was still possible to find areas with large clusters (Fig. 7), but in the whole volume of the sample ZnO was better dispersed in polymer matrix (Fig. 8). The SEM taken scans at a magnification of 50k× for both nanocomposites without (Fig. 9) and with (Fig. 10) the sonication process confirmed that the material also contained particles smaller than 100 nm.

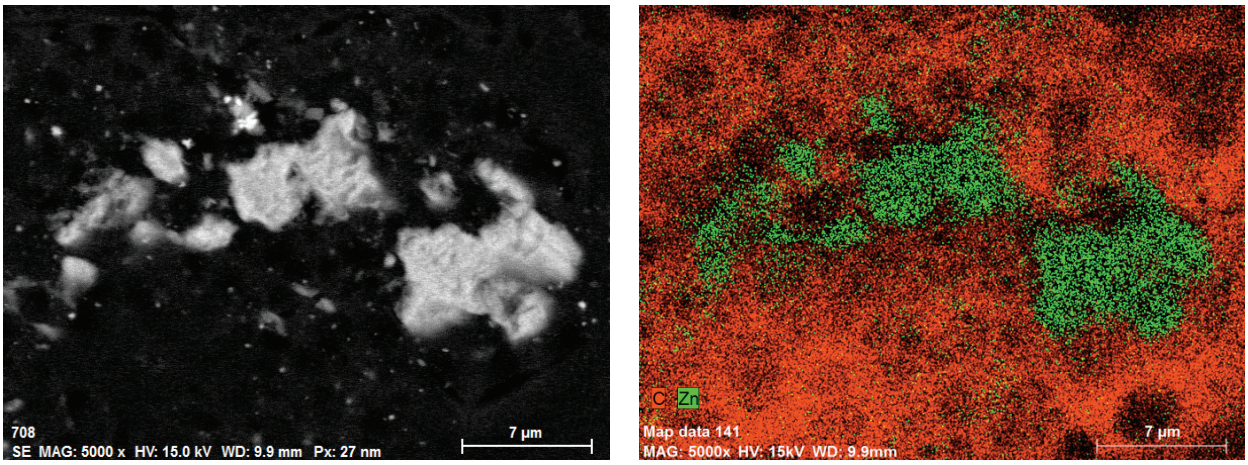

Fig. 6. Representative element map of PMMA resin speciments with ZnONPs, Cp = 2 wt% for C and Zn using EDS on SEM (nanocomposite manufactured without sonication process)

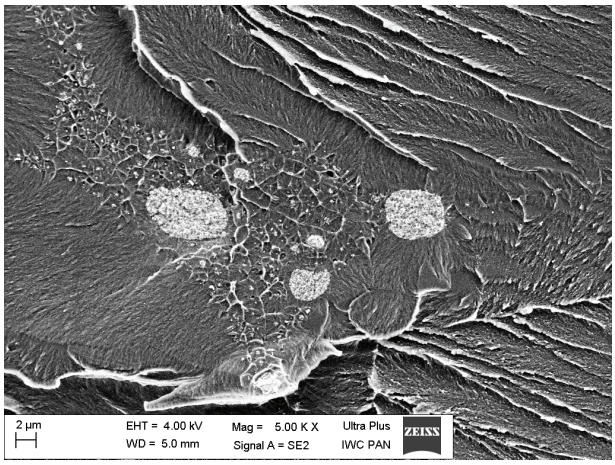

Fig. 7. Representative SEM image of nanocomposite (ZnONPs Cp = 2 wt%) manufactured using the sonication method

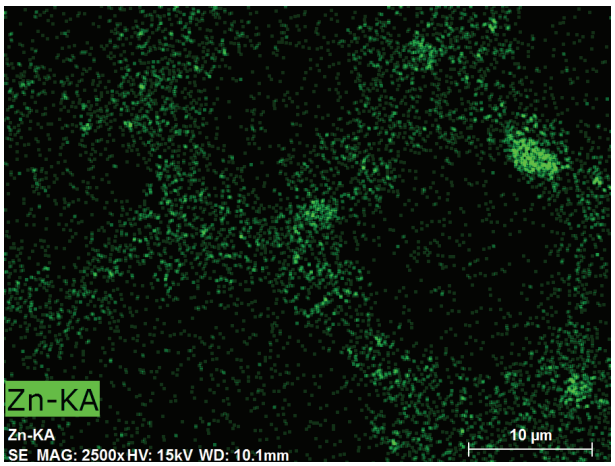

Fig. 8. Element map of nanocomposite (sonication method) for Zn (ZnONPs Cp = 2 wt%) using EDS on SEM

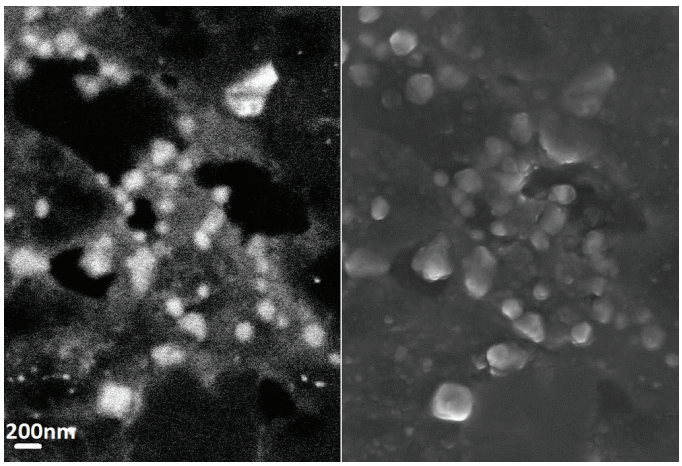

Fig. 9. SEM images of cross section area of acrylic resin material modified with ZnONPs, Cp = 2 wt% (nanocomposites manufactured without sonication process)

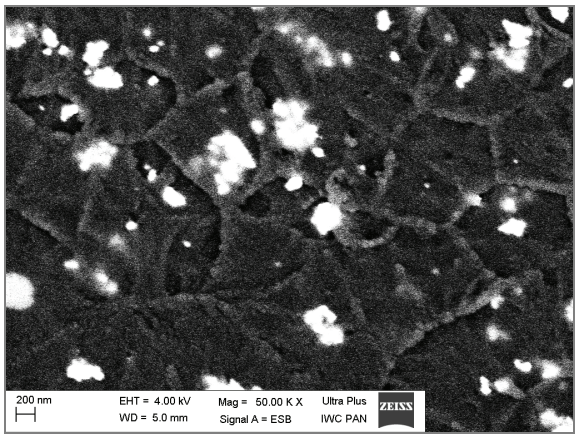

Fig. 10. SEM image of cross section area of nanocomposite (ZnONPs, Cp = 2wt%) manufactured using the sonication method

Roughness test

The study of the roughness of acrylic samples modified by the addition of 2% ZnONPs showed the Ra parameter of 3.26 microns (SD = 0.96 microns), while for non-modified materials, Ra was 3.38 microns (SD = 1.06 microns). Normal distributions, confirmed by the Kolmogorov–Smirnov test, were obtained for both the study and control groups (d = 0.12021, $p > 0.2$ and d = 0.20844, $p < 0.15$, respectively) and homogeneity of variance was found using two independent assays, Brown–Forsythe (F = 0.146664, p = 0.702975) and Levene (F = 0.761664, p = 0.385972). Thus the conditions for the use of parametric Student's t-test for independent samples (t = –0.503941, p = 0.615981) were met. The survey results revealed no statistically significant differences between the study and control groups (Fig. 11). All the analysis was performed for the significance level of p = 0.05. 3D mapping of the representative sample modified with ZnONPs (Ra = 3.05 microns) is shown in Fig. 12a and 12b).

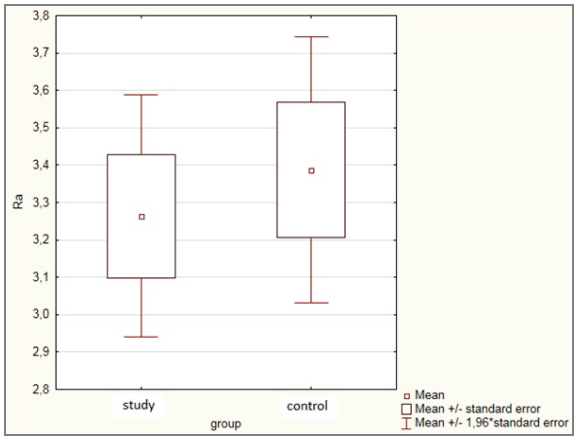

Fig. 11. The roughness parameter (Ra) of acrylic resin material before and after modification of ZnONPs, Cp = 2 wt%

Discussion

Nanomaterials are defined as materials with at least one dimension of the structural elements in the range of 1–100 nm (18). The antibacterial properties of zinc oxide have been used in dentistry over decades. With the development of nanotechnology the opportunity to produce not only a specific size of ZnO grains, but also to modify their shape has appeared. Potent microbial action of nanoparticles, compared to their micrometer counterparts, is made possible due to a very large surface area relative to the volume of the particle [18]. This was confirmed by the study carried out by Gondal et al. [12], who determined minimal inhibitory concentration (MIC) for *Aspergillus niger* (5.0 mg/mL for ZnO, and 2.5 mg/mL for nano-ZnO) and for *Candida* (10 mg/mL and 5 mg/mL, respectively). Thus, in both cases, MIC for the nanoparticles

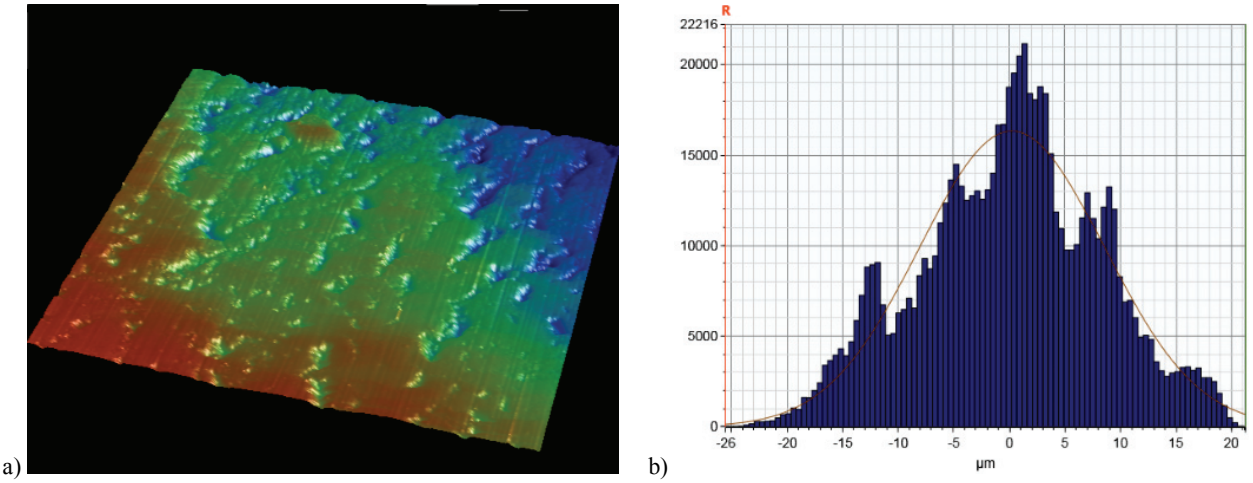

Fig. 12. The surface topography of the sample modified with ZnONPs, Cp = 2 wt%: #d map obtained using a stylus profiler (a) and its corresponding height histogram (b)

used was reduced twice. Khan et al. [13] studied the effect of ZnONPs on Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria and fungi (*Candida albicans*) using the disk diffusion method. It was observed that the inhibition of growth was evident at both concentrations of 0.50 mg/mL and 0.25 mg/mL and was proportional to the concentration of nanoparticles. Furthermore, the fungicidal activity for *Candida albicans* at a concentration of 0.50 mg/mL was comparable to that of nystatine, in the absence of the adverse effects, which may occur during the antibiotic treatment. Raghupathi et al. [22] have shown that microbial activity is directly proportional to the concentration of ZnONPs, and inversely proportional to their diameter. The use of inorganic nanooxides has many advantages over organic oxides, namely stability, long life and durability. Comparing the nano zinc oxide with other nanooxides, such as nano CuO or nano Fe_2O_3, a much stronger antibacterial activity was found [4]. In addition, ZnONPs are regarded as a non-toxic and biocompatible compound, therefore, it has found application as a carrier of drugs, cosmetics, or as a component dressing for wound healing [2].

Numerous studies have attempted to determine the mechanism of the antibacterial action of zinc oxide nanoparticles. One hypothesis is about activation of reactive oxygen species (ROS), reactive molecules containing oxygen, such as H_2O_2, superoxide anion O_2^-, OH^- or singlet oxygen O_2, which are responsible for the destruction of DNA, lipids or bacterial proteins [26]. According to another theory ZnO joins bacteria via the electrostatic forces and disorganization of their wall or cell membrane, which results in direct killing of bacteria [21]. Association of ZnO can dissociate into ions Zn^{2+} while changing the pH and potential bactericidal reaction. However, the studies carried out by Raghupathi et al. [22] demonstrated first, that microbial activity cannot depend only on the solution pH, and second, that the antibacterial properties of nanoparticles are much superior to that of Zn^{2+}. Brayner et al. [6] observed the deposition of ZnONPs on the surface of the bacteria and their accumulation in the cytoplasm, which may damage their functions. Moreover, Raghupathi et al. [22] showed that ZnO particles larger than 100 nm exhibit bacteriostatic properties only, while smaller ones exhibit both bacteriostatic and bactericidal properties. Therefore, it seems justified to make an attempt to introduce ZnONPs to PMMA resin material and to make use of their antibacterial properties for the prevention and treatment of denture stomatitis complicated by fungal infection.

In this study, nanoparticles of zinc oxide were successfully incorporated into PMMA resin serving as a material for denture bases. The only visible difference between the control and the study groups after the polymerization process was a slight whitening of the material, which is fully acceptable from both clinical and esthetic points of view. This work shows the way of the zinc oxide powder incorporation into PMMA resin. In the environment of liquid PMMA resin monomer ZnO shows the grains form. Upon stirring in a shaker the conglomerates with an average size of up to 1600 nm are created, which is a reason for very rapid sedimentation of particles. An eleven-time decrease in the average size of conglomerates after a 240-second sonication was observed, which increased the stability of the solution (Table 2). Both the SEM and EDS studies confirmed the presence of zinc compounds in polymerized PMMA resin material (Fig. 13). The examination of relevant sections (Figs. 9 and 10) also confirmed the presence of ZnO particles in the whole volume of the sample. This has important clinical implications, as the machining removes the outer layer of the biomaterial. By modifying the material prior to the polymerization process a constant supply of nanoparticles is provided, even after the destruction of the outer layer of the denture. The SEM taken scans at a magnification of 50k × (Figs. 9 and 10) confirmed that the material also contains particles smaller than 100 nm meeting the conditions required for nanomaterials. In Figure 6, it can be seen that the molecules form clumps uniformly distributed throughout PMMA resin. The clustering could be the result of the material porosity and tendency for occupying the natural niches by ZnO particles or due to water on the high surface of ZnONPs. Accumulated water on the surface can form large clusters of ZnONPs. Prior to polymerization the sonication process decreases ZnONPs conglomerates providing more favorable conditions for better dispersion of nanoparticles in polymer matrix. However, in some parts of nanocomposite huge conglomerates are still visible contributing to further improvement of the nanocomposite manufacturing technology.

The roughness testing of the samples performed before and after modification revealed no statistically significant differences between the two groups. This is in agreement with the results obtained by Li et al. [8], who also revealed no significant differences between PMMA and PMMA with nano Ag composite. The texture of the denture mucosal surface varied due to the anatomical structure of palate, as well as to the mode of denture preparation. Surface roughness was of the order of a few microns (Fig. 12b). This creates

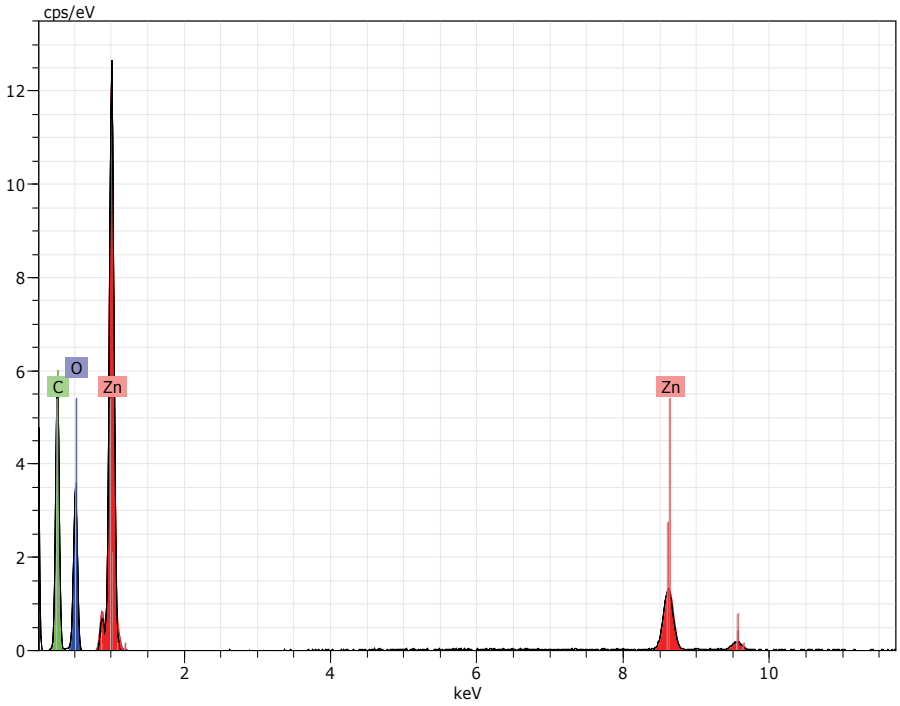

Fig. 13. EDS analysis of the acrylic resin material modified by ZnONPs, Cp = 2 wt%

favorable conditions for the development of microorganisms and makes an effective denture cleaning more difficult. Therefore, it seems unlikely that the particles in the nano-scale could have a significant impact on the above-mentioned parameter. It merely confirms the fact that the effect of nanoparticles does not rely on the roughness modification, one of the main causes of denture stomatitis [24].

The study confirmed the biological activity of zinc oxide nanoparticles against *Candida albicans,* the most common pathogen of denture stomatitis. In this study minimal inhibitory concentration value was established at 0.75 mg/mL while Gondal et al. [12] determined MIC at a level of 5 mg/ml. The differences could be due to other characteristics of ZnONPs, as well as to the use of another strain typing.

5. Conclusions

In this study, we successfully managed to incorporate ZnONPs to PMMA resin, confirming its presence in the whole volume of the sample. To the best of our knowledge, this is the first successful attempt to produce PMMA resin for bases of dentures modified with nanoparticles of zinc oxide. Nevertheless, a newly created nanocomposite needs to be further investigated to improve its homogeneity, and to check its microbiological properties, strength and biocompatibility prior to its possible clinical use. The development of the material able to display fungistatic or fungicidal activity over the lifetime creates an opportunity to reduce significant clinical problems related to the prevention and treatment of denture stomatitis complicated by fungal infection.

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