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Modification of Zinc Selenium nanoparticles with fish oil and their effect on bacteria

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Abstract: The aim of this study was develop suitable nanomaterial modified by fish oil using microwave-assisted synthesis. Dietary omega-6 and omega-3 polyunsaturated fatty acids can influence an inflammatory process, as well as they can modulate the inflammatory responses to stress situations. Thus, these fatty acids can be beneficial for wound healing. Also, the NPs are important components for many scientific fields, including nanomedicine and development of new and better wound dressing. This work deals with synthesis of ZnSe NPs with fish oil, and their subsequent *in vitro* testing on different bacterial strains. The synthesized nanomaterial was characterized by scanning electron microscopy, which showed the spherical NPs covered by fish oil. *Staphylococcus aureus*, methicillin resistant *S. aureus*, and *Escherichia coli* were used for the monitoring of NPs effect on bacteria. Wound care is inevitably important clinical challenge, which definitely requires fast growing.

Key Words: nanoparticles, zinc, selenium, microwave synthesis, fish oil

INTRODUCTION

Nanotechnology is a part of technology, which manipulates with nanoscale matter (Rajendar et al. 2018). Nanomaterials are significant for many scientific fields, because they have really important applications in a large variety of areas. For instance, they are utilized in many commercial products (sunscreens, cosmetics, pharmaceuticals, bandages for wound healing, etc.). Some nanoparticles (NPs) are characterized by their excellent biological properties, which make them applicable in medical field (Pivodova et al. 2015). For example, nanotechnology is used for novel drugs development, for better and effective drug delivery systems, or also for nanoelectronic biosensors or *in vivo* imaging (Rajendar et al. 2018). Nanotechnology is definitely prominent discipline of this millennium (Ovais et al. 2018).

Fish oil is rich mainly for omega-3 unsaturated fatty acids, such as docosahexaenoic (DHA) and eicosapentaenoic acid (EPA), naturally found in fish oil (Azizi et al. 2019). These unsaturated fatty acids are important for their well-known physiological functions or potential disease prevention abilities. They can help to reduce blood lipids, activate brain cells and enhance memory. They are important for regulation of physiological, biochemical reactions, and as well they help to increase anti-inflammatory abilities (Ding et al. 2019). Many studies have proved the fact that omega-3 unsaturated fatty acids have substantial effect on the disease prevention. Besides, it is assumed that, they have important role in cardiovascular disease prevention, treatment of inflammation, and autoimmune diseases (Ding et al. 2019).

Wound healing is a regulated sequence of a well-orchestrated cellular and biochemical processes, which restore the integrity of the skin (Hajialyani et al. 2018). It is sequential process, which exists in three related phases: inflammation, cell proliferation, and tissue remodeling (Komprda et al. 2018).

Omega-3 can generate bioactive lipid mediators, which reduce the inflammation. PUFA (poly-unsaturated fatty acids) change the proinflammatory cytokine production, however this is not clearly understood, yet (McDaniel et al. 2008). Limited evidences suggest that omega-3 PUFA can enhance the local inflammatory responses at wound healing (Kiecolt-Glaser et al. 2014).

MATERIAL AND METHODS

Microwave assisted synthesis of ZnSe NPs with fish oil

The synthesis was inspired by Moulick et al. (2017). One milliliter of zinc acetate dehydrate (52.51 mg/ml) was mixed with MilliQ water (85 ml) and 1 ml of mercaptosuccinic acid (60 mg/ml). The pH of solution was adjusted by 1 M ammonia to 7.5. Further it was added 1.5 ml of sodium selenite (5.26 mg/ml) and 10 ml fish oil (Fagron a.s.). Sodium borohydride (40 mg) was added as a reducing agent. The suspension was stirred for 2 hours (400 RPM). After 2 hours the suspension was filled up to 100 ml by MilliQ water. From this solution was taken two milliliters to small vessel and then it was heated under microwave irradiation (Multiwave3000, Anton-Paar, GmbH, Graz, Austria). The solution was heated at 80 °C, 90 °C, 100 °C or 110 °C for 10 minutes by 300 W (ramping time was 10 minutes) by using microwave oven. Prepared ZnSe NPs were finally stored at 4 °C in dark. Using these temperatures should be not a problem. Fournier et al. (2006) claims that temperature when the fish oil start degradation is 180 °C and higher.

Characterization of NPs by scanning electron microscopy

Scanning electron microscopy (SEM; Tescan, Brno, Czech Republic) was used to determination of the morphology of ZnSe NPs with fish oil. It was set to working distance 3 mm and 5 kV voltage. In this case, a MIRA 3 LMU for documentation of the nanomaterial morphology was utilized.

In vitro antibacterial testing of ZnSe NPs

The effect of NPs on bacteria was detected by agar plating technique. For the measurements, the bacterial cultures of *Escherichia coli* NCTC 13216, *Staphylococcus aureus* NCTC 8511 and methillicin-resistant *S. aureus* (MRSA) CCM 7110 were used. The bacterial strains were purchased from Czech Collection of Microorganisms (Brno, Czech Republic). The cultures were cultivated on MH (Muller-Hinton) agar (Oxoid, Hampshire, UK) at 37 °C. The colonies from overnight cultures were resuspended in MH broth and diluted to 0.1 optical density at 600 nm (~1 × 10^8 CFU/ml). Bacterial suspensions were then diluted hundred times more. Five hundred µ of bacterial cultures were mixed with 500 µl of ZnSe NPs. The control sample was prepared by mixing of 500 µl of bacteria suspension with 500 µl MiliQ water. After 2 hours of incubation at 37 °C, 100 µl from each inoculum was spread on MH agar. The rest of inoculum was incubated overnight at 37 °C and then applied on MH agar. After 24 hours cultivation at 37 °C the colonies were observed.

RESULTS AND DISCUSSION

Characterization of composites by SEM

ZnSe NPs were successfully synthesized using the microwave irradiation and different temperatures during synthesis. The SEM micrographs (Figure 1 and Figure 2) confirmed the formation of NPs with fish oil. From SEM pictures is obvious that higher treating temperature helps to create NPs. In all cases, NPs were correctly formed together with fish oil. Fish oil was bound to the surface of the NPs. The detection of NPs can be proved thanks to Figure 2 (B), where was used backscattered electrons, because on this figure can be seen NPs. The reason is that NPs are composed of heavier elements (Zn, Se) than fatty acids (C, H, O). There is a difference in comparison with picture from SEM. Therefore, it is assumed that the difference is made up of an envelope of NPs from fatty acids. All pictures that are included in Figure 1 and Figure 2 were selected in scale 2 µm.

Figure 1 Characterization of ZnSe NPs composites with fish oil by SEM by using 80 °C, 100 °C and 110 °C

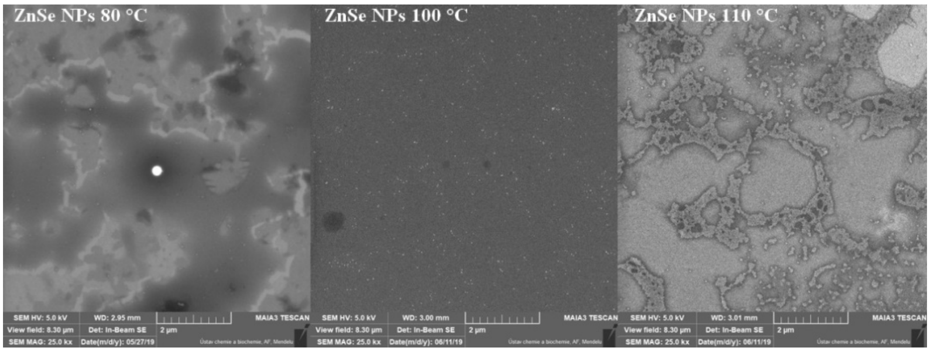
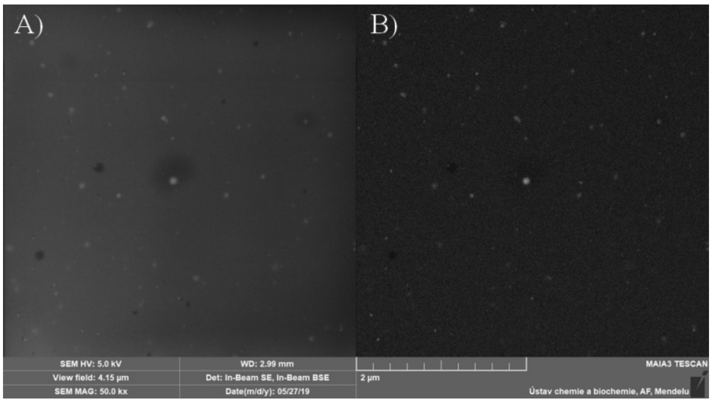


Figure 2 Characterization of ZnSe NPs composites prepared at 90 °C with fish oil A) by SEM B) by SEM with using backscattered electrons



The influence of ZnSe NPs with fish oil on bacteria

The effect of ZnSe NPs with fish oil on bacteria was determined by agar plating method after incubation of bacteria with NPs. The samples were cultivated with bacteria for 2 and 24 hours. As tested microorganisms, the Gram-positive, *S. aureus* and MRSA, and Gram-negative, *E. coli*, strains were utilized. On the Figure 3, the effect of ZnSe NPs with fish oil after 2 hours incubation is shown. The highest inhibitory effect (and only one) was recorded on *S. aureus* by using NPs synthesized at 80 °C. The rest of samples prepared at different temperatures showed no antimicrobial effect.

The effect of ZnSe NPs with fish oil after 24 hours cultivation is presented in Figure 4. In this case, none inhibitory effect has been observed.

Figure 3 The effect of Znse NPs with fish oil after 2 hours of incubation

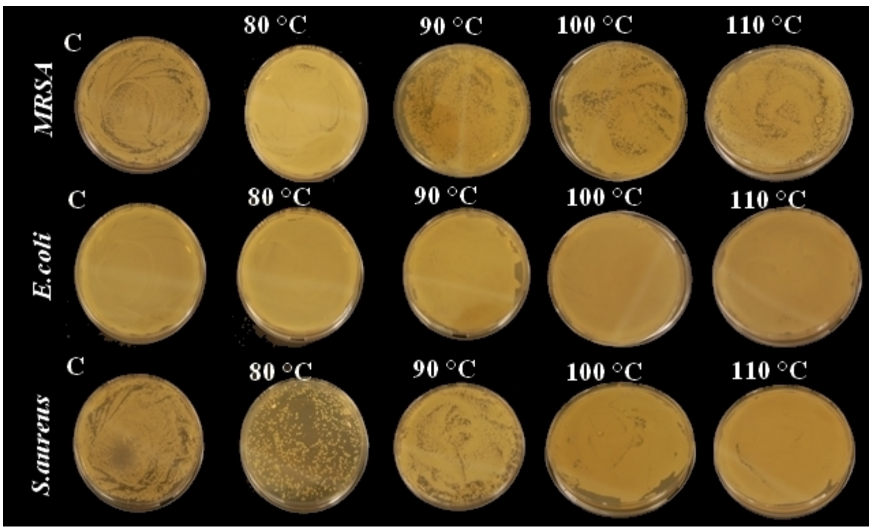
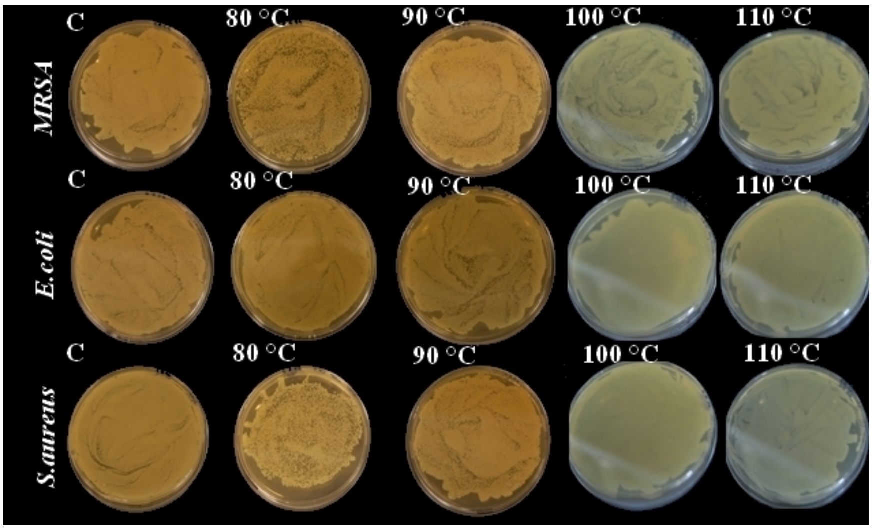


Figure 4 The effect of ZnSe NPs with fish oil after 24 hour of incubation



CONCLUSION

This study dealt with synthesis of ZnSe NPs with fish oil and determination their antimicrobial activity potential. The results showed that the binding of NPs with fish oil by using different temperatures was successful (fish oil was bound to surface of nanocomposites). The antibacterial activity of these NPs with fish oils was observed only in one case (*S. aureus* by using 80 °C). The results of antibacterial activity could be influenced by the fish oil presence. It is possible to assume that the fish oil could block antibacterial effect of unmodified NPs. So, this testing is a subject of further exploring. Nanotechnology offers interesting and new strategies for regenerative medicine.

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