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Synthesis of zinc selenium-based nanoparticles modified by algal oil and their effect on bacterial growth

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Abstract: The aim of this study was to develop suitable zinc selenium-based nanoparticles modified by algal oil containing mainly polyunsaturated fatty acids. Dietary omega-6 and omega-3 polyunsaturated fatty acids can affect inflammation reaction of the human body. Polyunsaturated fatty acids include eicosapentaenoic fatty acid and docosahexaenoic fatty acid, which could favourably enhance the correct wound healing. The algal oil would be a great complementary element to accelerate wound healing in combination with nanoparticles. Nowadays, the nanomaterials are beneficial part in different branches, including medicine (e.g. development of new cover materials containing nanoparticles to accelerate the healing effect, targeted drug delivery), cosmetics, textiles, protective equipment, and agriculture. This study includes synthesis of ZnSe nanoparticles modified by algal oil and their characterization by scanning electron microscopy. For the determination of *in vitro* effect of these nanoparticles on microorganisms, *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant *S. aureus* were employed. No antibacterial activity was found at these nanoparticles, therefore they could be used as nanocarrier for polyunsaturated fatty acids.

Key Words: nanomaterial, microwave synthesis, algal oil, scanning electron microscopy

INTRODUCTION

Many recent studies have shown positive correlation between consumption of docosahexaenoic unsaturated fatty acid (DHA) and various health benefits, such as protection against cardiovascular diseases, anxiety, depression, and inflammatory processes in the body (Waghmare et al. 2018, Bernstein et al. 2012). DHA is predominantly found in fatty fish, meat and eggs (Key et al. 2006). Another source of mentioned long chain polyunsaturated fatty acid may be microalgae. These microalgae use photosynthesis for the synthesis of sugar and producing of algal oil, which contains DHA. Algal oil is kind of vegetable oil. It has a similar chemical composition as vegetable oil with many unsaturated fatty acids content. The recommended daily dose of DHA is 300 mg per day (Simopoulos et al. 1999).

Nanotechnology has recently been a very frequent research topic. Nanotechnology deals with sub-microscopic particles with at least one dimension less than 100 nm (Khurana et al. 2019). At this size scale are differences in many material properties that are normally not seen at larger scales in the same materials. For example, differences in chemical, biochemical and physicochemical properties mainly due to their ratio of surface to volume (Khanna et al. 2019). It leads to differences in catalytic and biological activity, mechanical properties, optical absorption, thermal and electrical conductivity, and melting point (Shah et al. 2015). Nanoparticles (NPs) should work as a carrier of some substances, too (El-Bayoumy and Sinha 2004). They are used in many fields, like medicine, cosmetics, agriculture. Nanomaterial plays a key role particularly in the formulation and delivery of drugs or other substances at pathological sites with increased success. They may therefore influence drug's circulation, the half-life period, sustained release, and longer duration of action in the body. This could help heal faster in the patient's postoperative condition (Pavitra et al. 2019).

The main purpose of this work was to find a suitable method for the synthesis of zinc and selenium-based nanoparticles modified by algal oil. Microwave synthesis at temperatures 80 °C, 90 °C, 100 °C, 110 °C was chosen. These nanoparticles were expected to have an antimicrobial effect which was further tested on three microbial strains *in vitro* - *E.coli*, methicillin-resistant *Staphylococcus aureus* and *S. aureus*.

MATERIAL AND METHODS

Chemicals

Mercaptosuccinic acid (MSA), Ammonium salt, Sodium selenite, Sodium borohydride, and Zinc acetate dihydrate, were obtained from Sigma–Aldrich (St Louis, MO, USA). Algal vegetable oil was obtained from DSM Nutritional Products (South Carolina, USA). Brain Heart Infusion Broth and Müeller-Hinton (MH) broth and agar were purchased from Oxoid (Hampshire, UK). The deionized water was prepared using reverse osmosis equipment Aqual 25 (Tišnov, Czech Republic). The deionized water was further purified by using the apparatus MilliQ Direct QUV, equipped with the UV lamp (Aqua osmotic, Tišnov, Czech Republic). The resistance was 18.2 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany).

Microwave assisted synthesis of ZnSe-based NPs modified by algal oil

Synthesis of ZnSe nanoparticles were performed by microwave irradiation inspired by Moulick et. al. 2015. The solution was formed by 1 ml aqueous solution of zinc acetate dihydrate (52.51 mg/ml) and 1 ml of MSA (60 mg/ml) dissolved in 85 ml of Milli-Q water. The pH of the solution was adjusted to value 7.5 with solution of ammonia (1M). Then 1.5 ml of sodium selenite (5.26 mg/ml) and 10 ml of algal oil were added. Finally, 40 mg of sodium borohydride was added as a reducing agent. This final solution was stirred at 400 RPM on a magnetic stirrer for two hours at room temperature. Subsequently, the Milli-Q water was added to reach 100 ml and the solution was placed into microwave oven (Multiwave 3000, Anton-Paar, GmbH, Graz, Austria). The mixture was heated under microwave irradiation (300 W) with a different temperature treatment: 80 °C, 90 °C, 100 °C, and 110 °C for 10 minutes retention (10 minutes ramping time).

Determination of ZnSe NPs effect on bacterial cultures *in vitro*

The effect of ZnSe NPs on microorganisms was tested on three bacteria cultures: *Staphylococcus aureus* NCTC 8511, *Escherichia coli* NCTC 13216 and methicillin-resistant *S. aureus* (MRSA) CCM 7110 (Czech Collection of Microorganisms, Brno, Czech Republic). The mentioned cultures were cultivated on Müeller-Hinton agar (Oxoid, Hampshire, UK) at 37 °C overnight.

The cultures were resuspended in MH broth to achieve an approximate value of 1×10^8 CFU/ml. The value was determined by optical density at 600 nm. This solution was subsequently diluted 1 : 100 with MH broth to a volume of 10 ml of suspension. Then, 0.5 ml of bacterial solution with 0.5 ml ZnSe NPs was mixed. As a control solution, 0.5 ml of bacteria suspension with 0.5 ml Milli-Q water was prepared.

All samples were shaken in incubator at 37 °C for 2 hours. After that, 100 µl of solution was applied onto MH agar and the remaining inoculum was cultivated at 37 °C for overnight. The bacterial growth on MH agar was evaluated after 24 h incubation at 37 °C.

Scanning electron microscopy (SEM)

The structure of ZnSe NPs modified by algal oil, were observed by scanning electron microscopy (Tescan, Brno, Czech Republic). It was used working distance (WD) 3 mm and voltage of 5 kV. MIRA 3 LMU was used for nanostructure documentation.

RESULTS AND DISCUSSION

ZnSe nanoparticles with algal oil characterization

The experiment was focused on developing suitable NPs that contain zinc and selenium, because these elements are assumed to have good antioxidant and anticarcinogenic effects (Abu-El-Zahab et al. 2019). The aim of this work was to bind algal oil to ZnSe NPs. Individual pictures of ZnSe NPs, modified by algal oil are shown in Figure 1. All pictures are on the same magnification (2 μm).

In the pictures, we can clearly see created NPs at 90 °C, 100 °C and 110 °C. It can be observed, that NPs has corona, which is made up of the mentioned algal oil. In mentioned temperatures we can see the bound algal oil directly on the nanoparticles.

At 80 °C are not visible precisely formed nanoparticles. The nanoparticles are partially recognizable, but arranged in clusters.

Figure 1 The influence of different temperatures on synthesis of ZnSe MPs modified by algal oil

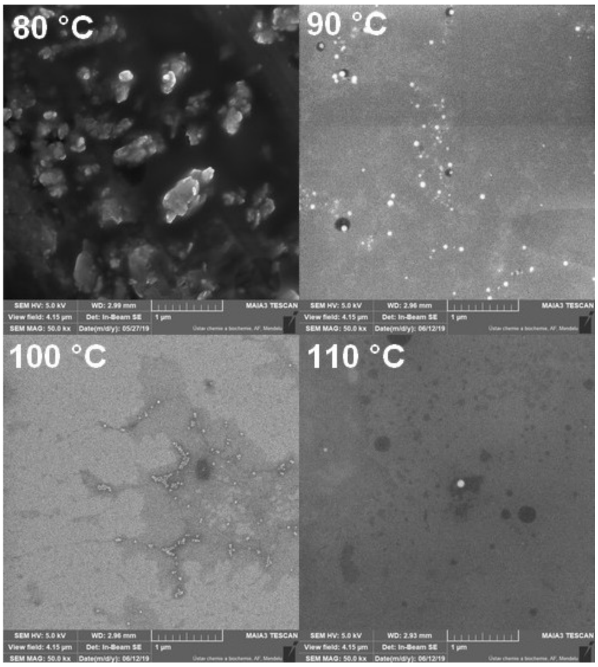
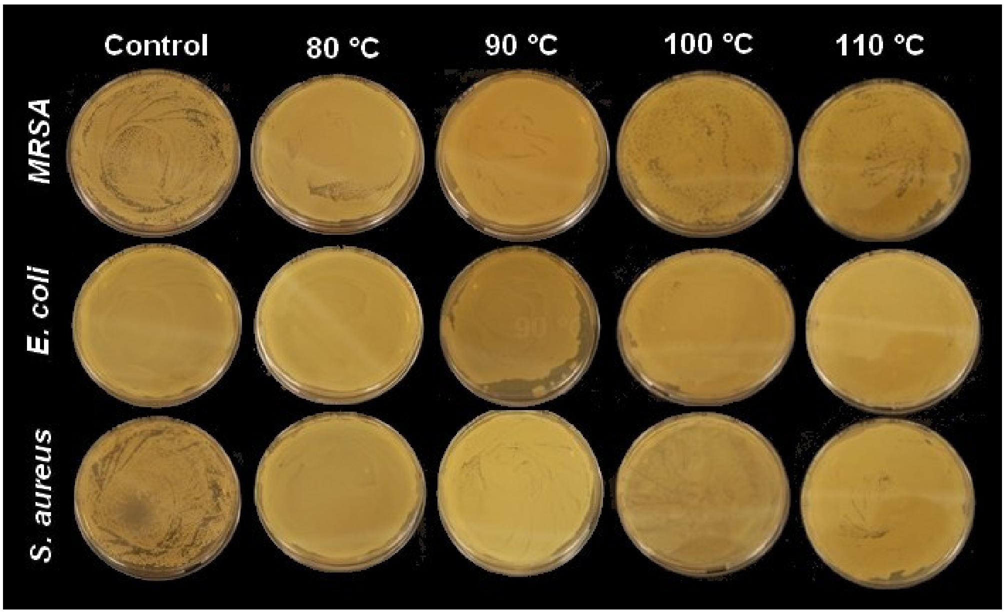


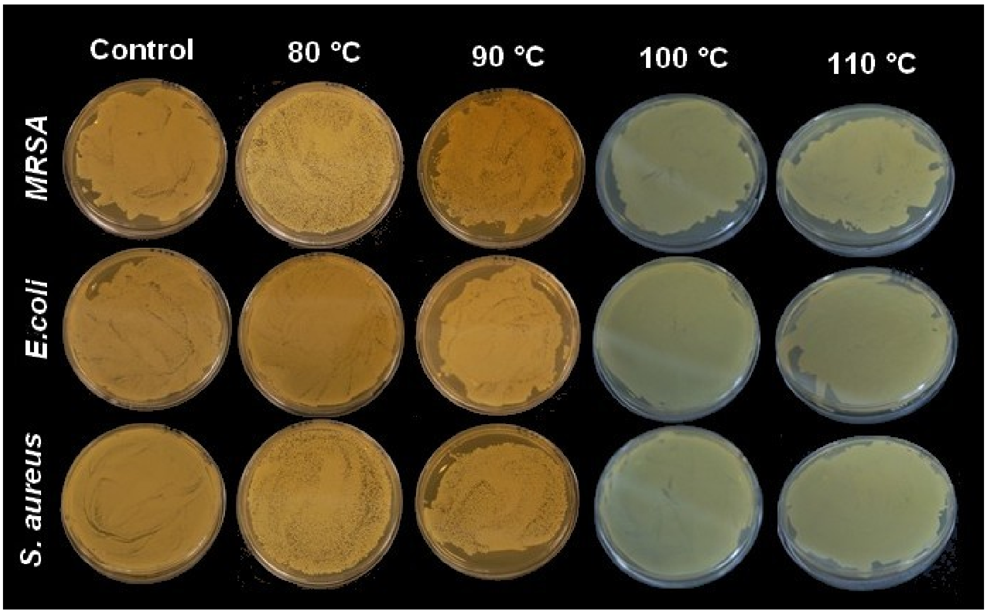
Figure 2 The bacterial growth after 2 hours incubation with ZnSe NPs with algal oil



The effect of ZnSe NPs modified by algal oil on bacterial growth

For culture method, *E. coli*, *S. aureus* and MRSA, were used. ZnSe NPs were incubated with bacteria cultures for different time periods, 2 and 24 hours. The synthesized ZnSe NPs showed no influence on mentioned bacteria growth (Figure 2 and Figure 3). The incubation time had no effect on the microbial inhibition of bacteria cultures.

Figure 3 The bacterial growth after 24 hours incubation with ZnSe NPs with algal oil



CONCLUSION

The formation of ZnSe NPs modified by algal oil differed in individual temperatures of synthesis. The results showed that successful microwave synthesis was at temperatures 90 °C, 100 °C and 110 °C. On the contrary, synthesis of ZnSe NPs with algal oil at 80 °C was successful but ZnSe NPs are arranged in the clusters.

According to the results of our experiment, ZnSe NPs modified by algal oil showed no effect on bacterial growth. Therefore, ZnSe NPs could be considered as a suitable nanocarrier for polyunsaturated fatty acids, like DHA. The nanocomposite with the appropriate oil would have a beneficial effect on wound healing, mainly because fatty acids such as DHA and EPA have an anti-inflammatory effect and accelerate the healing process after topical application (Hidalgo-Lucas et al. 2014). The advantages of using a nanomaterial as a carrier for mentioned oil are, in particular, improved efficiency, protection against oil degradation and controlled oil release from the composite. As a result, algal oil can act on the wound for several days. This could help in the second phase of the healing process, where an inflammatory reaction occurs which could be alleviated by the oil and thus contribute to better healing of the wound. Not only the nanoparticles, but also fatty acids with anti-inflammatory effects would contribute to the healing process. These results could be subject to further investigation, especially in terms of cytotoxicity of ZnSe NPs to tissue cells, so that they can be safely applied to wound healing in postoperative conditions of the patient.

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