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Characterization and applications of a new composite material obtained by green synthesis, through deposition of zinc oxide onto calcium carbonate precipitated in green seaweeds extract

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ABSTRACT

Zinc oxide was deposited onto calcium carbonate precipitated using a template of polysaccharides from *Ulva lactuca* green seaweeds (Black Sea). The resulted composite material was characterized by X-ray diffraction, scanning electron microscopy, energy-dispersive X-ray spectroscopy, UV–vis and FTIR spectroscopy. The therapeutic effect of prepared composite material was assessed *in vivo* as a topical application for the burns treatment and compared with that of ZnO. An increased antioxidant activity by combining zinc oxide with calcium carbonate capped with polysaccharides from green seaweeds extract in a composite material was demonstrated.

1. Introduction

Zinc is an essential component of more than 300 metalloenzymes and over 2000 transcription factors, and besides other physiological roles it exhibits antioxidant properties, being useful in preventing UV induced damage and in reducing the incidence of malignancy [1–4]. Also, zinc plays an important role in all skin functions (*i.e.* morphogenesis, repair, maintenance, protection and defense) [5]. Zinc oxide is currently investigated as antibacterial agent in both microscale and nanoscale formulations. In dermatology, zinc oxide has used as photoprotector, soothing agent, active ingredient of antidandruff shampoos, and in the treatment of infections (warts, leishmaniasis), inflammatory dermatoses (acne vulgaris, rosacea), pigmentary disorders (melasma), and neoplasias (basal cell carcinoma). Topically applied zinc oxide improves the healing of leg ulcers and increases the rate of re-epithelialization. The sparingly soluble zinc oxide was found to be more effective and several studies showed that topically absorbed zinc into the wounds promotes their faster healing because it stimulates the cleaning of wounds. Sparingly soluble forms may offer benefit of a long-lasting, slow-release of zinc ions, maximizing their bioavailability [3,6–9]. The various factors, which affect the antibacterial activity of ZnO nanoparticles against pathogens, were investigated [10]. It was reported that the crystalline structure and particle shape had little influence on the antimicrobial activity [11], but it is inversely proportional to the particle size [12]. Zn(II) ions can form varied complexes in solution/biological systems, resulting in the solubilization of zinc oxide, which also influences its antimicrobial activity [13].

The seaweeds are important source of polysaccharides, which are widely used as biopolymers for the synthesis of inorganic materials by biotechnological methods. In last years, many polysaccharides are also studied as antibacterial agents, mainly due to their biocompatibility. Furthermore, the natural polysaccharides have not drawbacks like bacterial resistance, high toxicity to humans, short shelf life, high production costs, *etc.* [14]. The researchers demonstrated that algal polysaccharides exhibit radical scavenger properties preventing the oxidative damage in living organisms [15]. The polysaccharides extracted from *Ulva* green algae are a group of hetero-polysaccharides, mainly consisted in sulfated rhamnose and glucuronic acid, iduronic acid, xylose, glucose, with lower amounts of mannoses, arabinose, and galactose, which are generally referred as ulvan [16,17]. The antioxidant activity of polysaccharides depends on several parameters like the content and position of sulfate groups, the molecular weight, sugar type, and glycosidic branching [18]. Other biological effects of sulfated polysaccharides from seaweed like anticoagulant, antithrombotic, anti-inflammatory, antitumoral, contraceptive, and antiviral were also revealed [19]. Particularly, *U. lactuca* was evaluated as a source for controlling the human pathogenic microorganisms. Thus, *U. lactuca* extract was tested against *Salmonella paratyphi*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Staphylococcus aureus*, *Shigella dysentriae* and *Klebsiella pneumonia* strains [17].

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The polysaccharides bioactivity could be enhanced by chemical modification or through the incorporation of metal compounds. The natural hydrogels obtained from polysaccharides (like sodium alginate) were used as template in the synthesis of inorganic and composite materials [20,21]. For the green synthesis of ZnO [22–25] and calcium carbonate [26,27] various methods and vegetal extracts were used.

Herein, we reported the obtaining of a new material by deposition of zinc oxide onto calcium carbonate precipitated in a matrix of polysaccharides from green seaweeds. The expected synergy between the bioactivity of zinc ions (involved in wounds healing being active against various pathogens) and anti-inflammatory effect of polysaccharides is a good premise for a new composite material for pharmaceutical and cosmetics industries [14]. Calcium salts of polysaccharides (*e.g.* calcium alginate) are usually used in dermatology. For instance, because calcium alginate exhibits hemostatic and gel-forming properties it is used in the treatment of burns and to control bleeding after dental interventions. On contact with blood, calcium alginate fibers release calcium ions due to ionic exchange with sodium ions, resulting in a greatly increased local calcium concentration, which promotes rapid coagulation [28]. The new developed composite material was tested *in vivo* in the treatment of burns and its antioxidant properties were evaluated based on the tissue level of malondialdehyde.

2. Experimental

2.1. Materials

The high purity reagents were obtained commercially from Loba Chemie (zinc oxide, ZnO), Sigma-Aldrich (calcium chloride, $CaCl_2$; sodium carbonate, Na_2CO_3; calcium carbonate, $CaCO_3$; glycerol, $C_3H_8O_3$; trichloroacetic acid, Cl_3CCOOH; 2-thiobarbituric acid, $C_4H_4N_2O_2S$), Prima (talcum powder) and Merck (1.0 M Tris–HCl solution).

2.2. Preparation of dry seaweed powder

The green seaweeds of *Ulva lactuca* used in our experiments were collected from the Black Sea coastline, in Constanta city. The seaweeds were washed with water, dried in an oven at 70 °C to constant mass and stored as fine powder (SW) in a refrigerator.

2.3. Synthesis of composite material (ZnO/PCC)

The soluble polysaccharides sodium salt were extracted by adapting a method for calcium alginate obtaining from red algae (calcium alginate method) [29].

2 g of SW were mixed with 100 mL H_2O and magnetically stirred over 30 min for the hydration of seaweeds. A solution of 1 M Na_2CO_3 (10 mL) was added and the mixture was further stirred for 1 h, for solubilization of polysaccharides as sodium salts. The solid residue was filtered off and 1.20 g of ZnO powder was added in the filtrate. After 30 min of magnetic stirring of the resulted suspension, a 1 M $CaCl_2$ solution (10 mL) was added. A white powder was obtained immediately. The suspension was further stirred for 1 h. The precipitate was filtered off, washed with water and dried.

2.4. Characterization of composite material

The powder was investigated by X-ray diffraction (XRD) performed on a Rigaku Miniflex 2 diffractometer with Ni filtered CuKα radiation, in the range of 2θ, 10–70°, scan rate of 2°/min and a step of 0.02°. The morphology of powder was analyzed using a Tescan Vega 3LMH scanning electron microscope (SEM), equipped with a back-scattered electron (BSE) detector. In order to identify the material composition, energy dispersive X-ray spectroscopy (EDX) was performed on the scanning electron microscope equipped with a Bruker X-ray energy dispersive detector. The UV–Visible diffuse reflectance spectra of powders were recorded in the range of 220 – 850 nm, on a Jasco V 550 spectrophotometer, with an integrating sphere, using MgO as the reference sample. The UV–Visible spectra for solutions were recorded on a Cecil 2125 spectrophotometer. The FTIR spectrum was recorded on Bruker Tensor 27 spectrometer using KBr pellets technique, in the wave number range of 400–4000 cm^{-1}.

2.5. Topical application

2.5.1. Preparation of ZnO based pastes

The mixture denoted M1 was prepared from 3 g of ZnO powder and 3 g of talcum (1:1 w/w), to which was added a solution of 9 g glycerol in 9 g H_2O (1:1 w/w) (12.5% ZnO). For mixture denoted M2, ZnO powder was replaced with ZnO/PCC powder (12.5% ZnO/PCC). The mixtures were triturated to homogeneity.

2.5.2. In vivo assessment of antioxidant activity

Eighteen 180–200 g female Wistar rats with age of 12 weeks, acquired from the Animal Facility of Ovidius University of Constanta, Romania, were randomly divided into 3 groups of 6 animals each. The groups were denoted A (control), (A + ZnO) and (A + ZnO/PCC). The rats from all groups were thermal burn, on a circular tegument area with a radius of 1.2 cm. The burns were further treated for 3 days with paste M1 - group (A + ZnO), respective M2 - group (A + ZnO/PCC). The tegument samples were taken after 3 days, under anesthesia, from the area of treated skin. The rats were deeply anaesthetized by a single dose injection with a cocktail between ketamine (3 mg/kg) and xylazine (0.75 mg/kg) in sterile normal saline solution, administered subcutaneous. All procedures for *in vivo* experiments were approved by the Ethics Committee on Animal Welfare of Ovidius University of Constanta, in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Council of Europe No. 123, Strasbourg 1986. The antioxidant activity was estimated *in vivo* by the determination of malondialdehyde (MDA) tissue level, through spectrophotometric thiobarbituric acid reactive species assay [30], which considers that MDA is the main advanced lipid oxidation product able to react with thiobarbituric acid (TBA), and the condensation product MDA-TBA_2 can be measured by UV–vis spectrophotometry [31]. MDA values were expressed as nmol/mg protein.

2.5.3. Statistical analysis

The data were analyzed by Student's *t*-test and the statistical significance was $p < 0.05$ (p = the thresold of significance established on the basis of t value).

3. Results and discussion

The green seaweeds extract is a rich source of polysaccharides, which can be employed as matrix for the synthesis and surface modification of inorganic materials. It was investigated the antioxidant activity of ZnO in the case of topical application, namely in the treatment of burns, by deposition of ZnO particles onto calcium carbonate obtained through the precipitation method in a green seaweeds extract.

3.1. Characterization of ZnO/PCC

Calcium carbonate (precipitated calcium carbonate, PCC [32]) was obtained in the green seaweeds extract using a biosynthesis approach and during its synthesis ZnO was deposited onto its surface (one-pot synthesis).

The XRD pattern (Fig. 1) demonstrated the presence in the composite sample of ZnO as wurtzite, crystallized in the hexagonal symmetry ($P6_3mc$ space group [33]) (JCPDS No. 36-1451), and $CaCO_3$ as calcite with hexagonal symmetry ($R\bar{3}c$ space group) (JCPDS No. 88-1807).