

DECORATION OF ELECTROSPUN FIBERS WITH CHITOSAN NANOPARTICLES LOADED WITH ESSENTIAL OILS FOR BACTERICIDAL AND ANTI-INFLAMMATORY APPLICATION

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Introduction

Infections are the most common postsurgical complications, which present serious threat to patients life [1]. In addition, more and more bacteria strains are becoming resistant to antibiotics [2]. Therefore, attention of researchers has been recently attracted by essential oils (EO) – a wide group of substances, from which some oils exhibit very good antibacterial and anti-inflammatory properties. Especially carvacrol (CRV) and thymol (TM) possess high activity against *E.coli*, *S. aureus* and *P. aureginosa* [3] – strains, which inhabit our skin and are mostly responsible for causing infections [1]. What is more, traditional bandages accumulate blood and other liquids from wound, which are potential source of nutrients for bacteria. This may result in development of infection and cause serious problems for human health. In this study, we focused on designing of an active wound dressing made of electrospun polymeric fibers, decorated with chitosan nanoparticles loaded with a bioactive agent (essential oil: CRV or TM).

Materials and Methods

Preparation of oil-loaded chitosan particles

All chemicals were purchased from Sigma Aldrich and used as received. CRV or TM (150 mg, pure grade) was mixed with Tween 80 (106 μ l, HLB 15), then 15 ml of 0.5% (w/v) low average molecular weight chitosan (50-190 kDa) in 1% (v/v) acetic acid was added. Mixture was vortexed for ~30 s and 5 ml of 0.3% (v/v) tripolyphosphate (TPP) solution was added dropwise and stirring was carried out for 30 min in 25°C with stirring speed 700 rpm. Particles were collected by centrifugation at 13 000 rpm (10 min) in ambient temperature, washed with 20 ml of 0.5% Tween80 solution and twice with distilled water. Supernatants were collected to determine encapsulation efficiency. Obtained particles were dispersed in 1 ml of distilled water and kept in 4°C.

Characterisation of loaded chitosan particles

Dynamic light scattering was used to measure hydrodynamic diameter and Zeta potential of obtained spheres. SEM imaging was carried out to determine morphology and size of particles. UV-vis absorption spectra of SN were measured (abs. max.: 247 nm CRV and TM). Concentrations of EO in supernatants were calculated from calibration curve. IR spectrophotometry was carried out to confirm successful loading of EO into polymer matrix. Also, thermogravimetry (TGA) was performed in temperature range of 25-600°C with heating rate of 10°C/min and with N₂ flow rate of 50 ml/min.

In vitro release of oils from chitosan nanoparticles

Essential oils were released using dialysis membranes in PBS with 0.1% (v/v) Tween80, pH = 7.4 in 37°C. After each period of time, samples in dialysis bag were moved into new container with fresh PBS. Samples of PBS were measured using UV-vis.

Electrospinning and decoration of fibers with NPs

Polycaprolactone (PCL) was dissolved in mixture of dichloromethane and dimethylformamide obtaining 10% solution. Solution of polymer and suspension of chitosan nanoparticles loaded with EO in ethanol were electrospun simultaneously on rotating collector (100 rpm), with pumping rates of 1 ml/h (PCL) and 2 ml/h (NPs). Nozzles were swiping above the collector (3 cm/s) to prevent separation of polymer and particles.

Results and Discussion

Obtained particles exhibited hydrodynamic diameter of 290 nm (DLS) and incipient stability with zeta potential around 20 mV. SEM imaging has shown that dry NPs possessed spherical shape, regular size distribution and diameter ca. 90 nm (FIG. 1, left). Mean encapsulation efficiency of EO was 25% and 28% for CRV and TM respectively. *In vitro* release studies showed release efficiency around 31% and 22% respectively for CRV and TM. Encapsulated EO were released in initial burst effect lasting 6-8 hours (FIG. 2, right). SEM imaging confirmed successful decoration of electrospun PCL fibers with chitosan nanoparticles loaded with EO (FIG. 1, right).

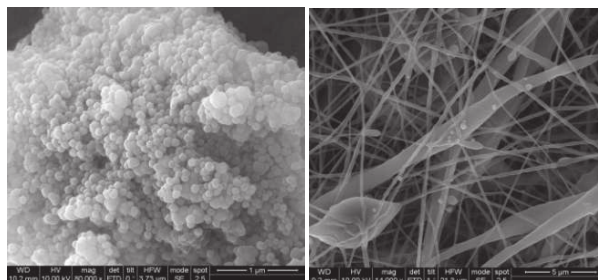


FIG. 1. SEM images of chitosan NPs loaded with OE (left) and decorated with them electrospun PCL fibers (right).

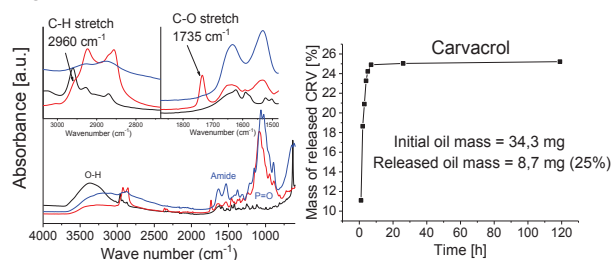


FIG. 2. IR spectra (left) and exemplary release profile (right).

Conclusions

Chitosan nanoparticles loaded with EO were obtained by ionic gelation technique. Loading of EO into chitosan NPs was confirmed by UV-vis, IR (FIG. 1, left) and TGA. Encapsulation efficiency was ca. 25%. Release rate can be considered as effective, as all of released drug would be released during time of using single wound dressing. Simultaneous electrospinning of PCL fibers and chitosan particles loaded with EO is an effective method to functionalise fibers with polymeric NPs.

References

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