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Featured Letter

Encapsulation of indocyanine green in poly(lactic acid) nanofibers for using as a nanoprobe in biomedical diagnostics

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ABSTRACT

Some chronic illnesses do not show any symptoms at an early stage, which causes a difficult to treating the situation. Especially cardiovascular diseases, liver functions and cancer could be prevented at early stages of diseases by advanced biomedical imaging techniques. In this study, indocyanine green (ICG), which must be stabilized in aqueous media for the development of its use in biomedical applications, was stabilized by encapsulating into the biodegradable polymer of poly(lactic acid) (PLA) using a coaxial electrospinning (CES) method to produce ICG near infrared (NIR) fluorophore nanoprobe with the one-step process.

1. 1.Introduction

Indocyanine green (ICG) is a water-soluble, non-toxic, and non-ionizing tricarbocyanine dye, which has been approved by the United States Food and Drug Administration (FDA) in 1956 for clinical usage such as the evaluation of cardiac output, visualization of the retinal and choroidal vasculatures [1,2]. But, it has some limitations in diagnostic imaging for detection of early stage tumours [3]. Easy binding of ICG to plasma proteins in the blood, make it eliminates rapidly from the body (2–4 min of plasmatic half-life) and therefore it is almost impossible to reach the targeted tissues [2]. Instability in aqueous solutions, susceptibility to aggregation and photo-thermal degradation, are other main disadvantages of ICG [3,4]. In this study, to overcome these limitations, ICG was encapsulated into biodegradable PLA by using CES method. The previous study has shown that CES, brings different materials together in CNFs [5]. In addition, it has been indicated that the shell of CNFs prevents premature (burst) release of the encapsulated water-soluble drug [6]. Sustained drug release profile of PLA/ICG CNFs also studied over 21 days [7].

2. Materials and methods

Poly (L-lactic acid) (PLA) (2003D (good biocompatibility for cells [8]) was obtained from NatureWorks LLC, Minnetonka, MN) was dissolved in mixed solvents of (4:1, v/v) dichloromethane: dimethylformamide (DCM: DMF) (Fisher Scientific, Atlanta, GA) at the concentration of 8% (w/v) at the room temperature (RT). After mixing, Tween80 (Sigma Aldrich) were added to the PLA solution at the ratio of 3% (w/v) and the solution was gently stirred for a further 600 s at RT. ICG (IR-125, Acros Organics) (1 mg/ml) was dissolved in methanol (Sigma-Aldrich) as a concentration of 100 μL at the RT. The electrospinning solutions were characterized for viscosity (DV-E, Brookfield AMETEK, USA), density (10 mL specific density bottle), surface tension (Sigma 703D, Attension, Germany), and electrical conductivity (Cond 3110 SET 1, WTW, Germany). The morphology {Scanning Electron Microscope (SEM) (EVO LS 10, ZEISS)} and molecular contents {Fourier-transformed infrared (FT-IR) (PerkinElmer, Waltham, Mass., USA)} of nanofibers were examined. Encapsulated ICG was viewed with a confocal microscope (Zeiss,

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LSM 700) from the PLA/ICG CNFs. ICG releasing was carried out at different pH medium. The PLA/ICG mats (10 mg, 3 pieces) were immersed in 1 mL of pH 7 and pH 4 buffer solutions (PBS) and were shaken with the thermal shaker (BIOSAN TS-100) at 37 °C. At the defined time intervals, the two PBS sets were removed from each sample and 1 mL of fresh PBS was added again to continue releasing over 21 days. UV spectroscopy (Shimadzu UV-3600) was used for monitoring of ICG releasing profile at 780 nm [9].

3. Results and discussion

The PLA polymer solution {outer nozzle (OD: 3 mm, ID: 2.87 mm)} and the ICG solution {inner nozzle, (OD: 2.13 mm, ID: 1.88 mm)} were placed in a 10-mL plastic syringe and connected to the multi nozzle spinneret indicated in Fig. 1. Solutions were infused to the multi-nozzle end with syringe pumps (New Era Pump Systems, Inc., USA) separately. While syringe pumps were infused the polymer solution into the spinneret a high voltage (0–40 kV) was given to the between nozzle and grounded collector drum at the electrospinning device (NS24, Inovenso Co., Turkey). After forming of Taylorcone, PLA/ICG CNFs were collected onto collector drum at the distance of 15 cm. Pure PLA nanofibers were collected with same procedure by using a single nozzle. All the experiments were performed at the ambient pressure and RT. Optimized electrospinning conditions and the physiochemical properties of solutions were stated in Table 1. The high electrical conductivity and low viscosity of the spinning jet have strong effects on the produced fiber diameters [10]. It is seen clearly, that the electrical conductivity of ICG is significantly higher whereas the viscosity of ICG is significantly lower than the PLA solution. As a result, PLA/ICG CNFs (ϕ = 816.08 ± 166.83 nm) exhibited more uniform fiber distributions and nanoscale diameters, when compared with PLA nanofibers (ϕ = 1473.83 ± 493.88 nm), as shown in Fig. 2a–d. This result suggests that the physical properties of ICG including the higher conductivity and the lower viscosity are effective in reducing fiber diameters, when encapsulated in PLA polymer matrix, as seen in Fig. 2c and d. The confocal fluorescence images were taken to prove that ICG is successfully encapsulated within the CNFs, as indicated in Fig. 2e. It is clearly demonstrated that ICG is localized within the electrospun CNFs. FT-IR spectra of PLA, PLA/ICG nanofibers and ICG are given in Fig. 2f. Spectrum confirmed that the ICG is entrapped (embedded) in the PLA matrix of CNFs. In details, the characteristic peaks of PLA (O—H stretch bands between 3600 and 2800 cm^{-1}, C=O vibration peak at 1756 cm^{-1}, CH_3 asymmetrical scissoring at 1454 cm^{-1}, C—O asymmetrical stretching and CH_3 twisting at 1180 cm^{-1}, C—CH_3 stretching at 1045 cm^{-1} and C—COO stretching at 868 cm^{-1}) are clearly visible in the PLA/ICG spectra [11]. The characteristic peaks of ICG (N—H bonds at 3430 cm^{-1}, C—H bond at 2910 cm^{-1}, sulfone group at 1300 cm^{-1} and sulfonic group at 1345 cm^{-1}) are presented in the ICG spectrum [12]. Band intensities of ICG-related peaks increased in the PLA/ICG spectrum confirm that ICG was successfully encapsulated into the PLA nanofibers.

The in vitro ICG release patterns of PLA/ICG CNFs with PBS at different pH are shown in Fig. 3. The hydrophilic drugs encapsulated in the hydrophobic polymer matrix exhibit tendency to migrate from nanofiber surface through opened channels [7]. Thus, ICG got out of through the PLA outer layer as a sustained and the leakage process is schematically presented in Fig. 3a. The UV spectra obtained with the different concentrations of ICG (0.2–1 mg/ml) and the calibration curve plotted from the ICG absorption values (r^2 = 0.9753) obtained from these spectra are given in Fig. 3b and c, respectively. In addition, in order to investigate the effect of pH on the release of ICG, the release experiments were conducted in pH 4 and pH 7, respectively. As can be seen in Fig. 3d, ICG exhibit more sustained release at pH 4 (59. 88%), whereas it exhibits the burst release properties at pH 7 (78. 56%). Thus, it has been demonstrated that ICG release amount was decrease in the low pH environment. These results are consistent with some study. As previous was reported by Gorman and co-workers, both pH and temperature had a strong effect on the rate of degradation. Their results suggested that intramolecular transesterification (backbiting) was the pathway for the degradation of the PLA brush in basic, aqueous solution, similar to what has been observed for the depolymerization of PLA oligomers in basic solution [13]. Also, as previously was reported by Brzeziński, when the PLA-based microcapsules were exposed to acidic medium, their diameters didn't change and therefore no active ingredient release were occurred. Conversely, in the basic medium, the structure of microcapsules was degraded and the active ingredient was released [14]. In a study by Mehnath et al., pH dependent release profile of hydrophobic paclitaxel (PTX) anticancer drug was investigated in the hybrid structure of hydrophilic (PCPP) and hydrophobic (PLA) polymers. It was demonstrated that ICG integrated hybrid poly-

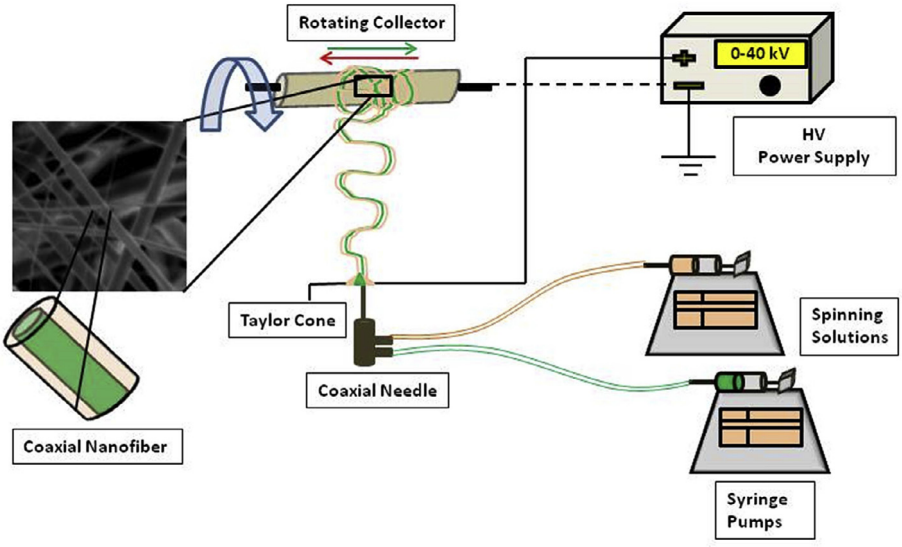

Fig. 1. Schematic diagram of CES apparatus and brief mechanism for encapsulation process.