

Fabrication of Poly(Ethylene Oxide)/Chitosan/Indocyanine Green Nanoprobe by Co-Axial Electrospinning Method for Early Detection

Zeynep R. Ege, Aydin Akan, Faik N. Oktar, Betul Karademir, Oguzhan Gunduz

Abstract—Early detection of cancer could save human life and quality in insidious cases by advanced biomedical imaging techniques. Designing targeted detection system is necessary in order to protect of healthy cells. Electrospun nanofibers are efficient and targetable nanocarriers which have important properties such as nanometric diameter, mechanical properties, elasticity, porosity and surface area to volume ratio. In the present study, indocyanine green (ICG) organic dye was stabilized and encapsulated in polymer matrix which polyethylene oxide (PEO) and chitosan (CHI) multilayer nanofibers via co-axial electrospinning method at one step. The co-axial electrospun nanofibers were characterized as morphological (SEM), molecular (FT-IR), and entrapment efficiency of Indocyanine Green (ICG) (confocal imaging). Controlled release profile of PEO/CHI/ICG nanofiber was also evaluated up to 40 hours.

Keywords—Chitosan, coaxial electrospinning, controlled releasing, indocyanine green, nanoprobe, polyethylene oxide.

I. INTRODUCTION

LETHAL tumors can be diagnosed at early stages by advanced biomedical imaging techniques. Because near infrared (NIR) probes exhibit higher tissue penetration and low absorption; they are promising materials for early detection [1]. In the NIR window (650-750 nm), NIR dyes are effective and sensitive detection probes due to low beam losing through the tissues [2]. ICG NIR organic dye which is approved by the U.S. Food and Drug Administration (FDA) has been used for the imaging of retinal-choroidal vasculature and evaluating of cardiac output since 1970 [3]. However there are some disadvantages that spectacular *in vivo* imaging agent possesses, limit its using in targeted biomedical imaging

techniques. These limitations are mainly optical decay dependent of photo-thermal conditions, aggregation-degradation in aqueous solution and elimination rapidly from the body (2-4 min of plasmatic half-life) [4]. Electrospinning technique was used to enhance optical, thermal and bioavailability properties of ICG in biological environment by encapsulating in polymeric nanofibers.

CHI is non-toxic, non-antigenic, biodegradable, biocompatible, cost effective, and antibacterial natural polymer which make it remarkable for drug delivery, controlled releasing and biomedical imaging systems [5]. However, CHI has poor electro spinnability due to its low solubility, stability and mechanical properties [6]. In the present study, CHI was incorporated by electro spinnable synthetic polymer of poly(ethylene oxide) (PEO) via co-axial electrospinning method at one step without blending before spinning application.

II. EXPERIMENTAL

A. Materials and Methods

PEO (average Mv: 600,000 powder) and CHI (low molecular weight) were purchased from Sigma-Aldrich. The solvent of acetic acid (CH₃COOH, 99–100% for synthesis), was purchased from Merck. Tween80 (viscous liquid) was purchased from Sigma Aldrich. ICG (IR-125) was purchased from Acros Organics. Co-axial electrospinning process was performed with dual electrospinning apparatus which was built by our research group in Turkey. 3-ends nozzle system was also made in Turkey ((outer nozzle (OD: 3 mm, ID: 2.87 mm) inter nozzle, (OD: 2.13 mm, ID: 1.88 mm) inner nozzle, (OD: 1.30 mm, ID: 1.20)). In order to infuse solutions as a controlled amount (ml/h), each syringe was connected to the controllable syringe pumps (NE-300, New Era Pump Systems, Inc., USA) separately. A high voltage (0-40 kV) was given to the between 3-ends nozzle and collector drum. Morphological investigation of nanofibers was made by Scanning Electron Microscope (SEM) (EVO LS 10, ZEISS) and molecular contents of nanofibers were investigated by Fourier Transform Infrared Spectroscopy (FT-IR) (PerkinElmer, Waltham, Mass., USA). Encapsulated ICG was viewed with a confocal microscope (Zeiss, LSM 700). UV spectroscopy (Shimadzu UV-3600) was used for monitoring of ICG releasing profile at 780 nm from multilayer nanofiber up to 48 hours [6].

B. Preparation of Electrospinning Solutions

5% PEO (w/v) and 1, 2 and 3% of CHI (w/v) solutions were

Zeynep Ruya Ege is PhD candidate with the Biomedical Engineering Department, University of Istanbul, Turkey and she studied as a researcher at Advanced Nanomaterials Research Laboratory, Marmara University, Turkey (phone: +905301473301; e-mail: zruyadeveli@gmail.com).

Aydin Akan is with the Biomedical Engineering Department, Faculty of Engineering and Architectural, University of Izmir Katip Celebi, Turkey (e-mail: aydin.akan@ikc.edu.tr).

Faik Nuzhet Oktar is with Bioengineering Department, Faculty of Engineering, University of Marmara, Turkey (e-mail: foktar.biomaterials@gmail.com).

Serap Durdane Kuruca is with Physiology Department of Faculty of Medicine, University of Istanbul, Turkey (e-mail: sererdem@yahoo.com).

Betul Karademir is with the Biochemistry Department, Faculty of Medicine, University of Marmara, Turkey (e-mail: betulkarademir@marmara.edu.tr).

Oguzhan Gunduz is with Metallurgical and Materials Engineering Department, Faculty of Technology, University of Marmara and he is head of the Advanced Nanomaterials Research Laboratory at Marmara University Turkey (e-mail: ucemogu@gmail.com).

prepared separately in 50% acetic acid. Then 1% Tween 80 was added to PEO and CHI solutions. PEO solution was stirred for 24 h, CHI solutions was stirred for 12 h on the magnetic stirrer at 250 rpm at room temperature. ICG (1 mg/ml) was dissolved in methanol as a concentration of 200 μ L at the room temperature by vortex.

C. Co-Axial Electrospinning System

Prepared PEO polymer solution, CHI solution and ICG solution were filled into separate 10 ml plastic syringes. All syringes were connected to the multi-nozzle system. Solutions were infused as a controlled amount (ml/h) by the controllable syringe pumps (NE-300, New Era Pump Systems, Inc., USA) separately. A high voltage (0-40 kV) was given to the between nozzle and collector drum. Electrospinning parameters (applied voltage, distance of needle and collector, flow rates of solutions) were optimized. All the experiments were carried out at room temperature.

D. SEM

The morphology of multilayer nanofibers was observed by SEM. In order to measure of fiber diameters, 100 fibers were randomly selected from SEM images and their diameters were measured by using analysis software (SmartSEM, Zeiss).

E. FT-IR

The molecular contents of the multilayer nanofiber were determined by FT-IR spectrum for each nanofiber.

F. Confocal Microscopy

In order to demonstrate that ICG was encapsulated in multilayered nanofibers, confocal images were taken by Zeiss LSM 700.

G. Controlled ICG Release Study

PEO/CHI/ICG nanofiber mats (10 mg) with 1, 2 and 3% CHI ratios were cut into small pieces were immersed in 1 mL PBS with pH 7. All samples were shaken with thermal shaker (BIOSAN TS-100) at 37 °C horizontally. At defined time intervals, supernatant of samples were removed for UV analysis and 1 mL of fresh PBS was added again to continue releasing. The samples at all-time points were run in triplicate. The ICG release ratio was calculated by (1);

$$ICG \text{ release } (\%) = \frac{Q_t}{Q_s} \times 100 \% \quad [7] \quad (1)$$

where Q_t is the Quantity of ICG released at time t , Q_s is the total amount of released ICG.

III. RESULTS AND DISCUSSION

A. Fabrication of PEO/CHI/ICG Nanofibers

The PEO and CHI polymer solutions and the ICG solution were connected with 3-ends nozzle system as shown Fig. 1. This electrospinning setup was repeated for different ratios of CHI concentrations. Electrospinning parameters were optimized. All optimized electrospinning parameters for different PEO/CHI/ICG nanofibers were given in Table I.



Fig. 1 (a) Co-axial Electrospinning setup (b) and (c) 3-ends nozzle

TABLE I
OPTIMIZED CO-AXIAL ELECTROSPINNING PARAMETERS

Sample	Voltage (kV)	Flow Rate (ml.h ⁻¹)	Distance (mm)
Pure PEO	19.5	1.8	15
PEO/1%CHI/ICG	23.2	1.4/0.3/0.5	15
PEO/2%CHI/ICG	23.1	1.3/0.3/0.5	15
PEO/3%CHI/ICG	23.0	0.8/0.4/0.4	15

No Optimized co-axial electrospinning parameters for multilayered nanofibers with different CHI concentrations.

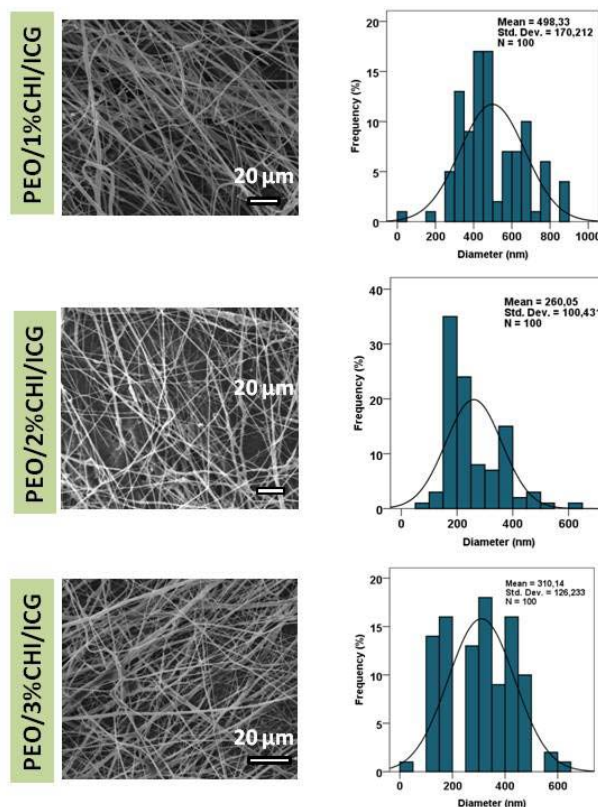


Fig. 2 SEM images and Fiber diameters of PEO/CHI/ICG coaxial nanofibers with different ratio of CHI

B. SEM and Fiber Size Analysis

The SEM images and fiber diameters distribution of the PEO/CHI/ICG nanofibers were presented in Fig. 2. As can be seen in Fig. 2, ICG encapsulated PEO/CHI/ICG nanofibers diameters are quite fine. At 2% CHI concentration, PEO/CHI/ICG diameter ($\Phi = 260.05 \pm 0.10$ nm) has ultrafine fiber

structure. As shown in Fig. 2, no beaded PEO/CHI/ICG nanofibers were successfully fabricated. In addition, ICG also has a considerable effect on fiber formation. As seen in our previous study, ICG has high electrical conductivity and low viscosity [8]. Thus, with the effect of high electrical conductivity and low viscosity of ICG, ultrafine fiber structures were successfully obtained. This is because of the spinning jet properties have strong effects on the produced fiber diameters.

C. FT-IR

FT-IR analysis was used to demonstrate that ICG was encapsulated in PEO and CHI polymer multilayered

nanofibers. Molecular interactions of the electrospun nanofibers were given in Fig. 3. The characteristic infrared bands of PEO, CHI and ICG were also shown on the spectrum at Fig. 3. At 3558 cm^{-1} , there are main characteristic peaks of the pure CHI which are N-H and -OH stretching vibrations and hydrogen bonding [9]. This broad band which is assigned to stretching mode of CHI and ICG at around 3500 cm^{-1} was clearly seen at the spectrum of PEO/CHI/ICG nanofiber mat. Moreover, stretching mode of the C=O band and bending mode of the N-H band were also seen at spectrum of PEO/CHI/ICG nanofiber mat at 1730 cm^{-1} and 1620 cm^{-1} respectively.

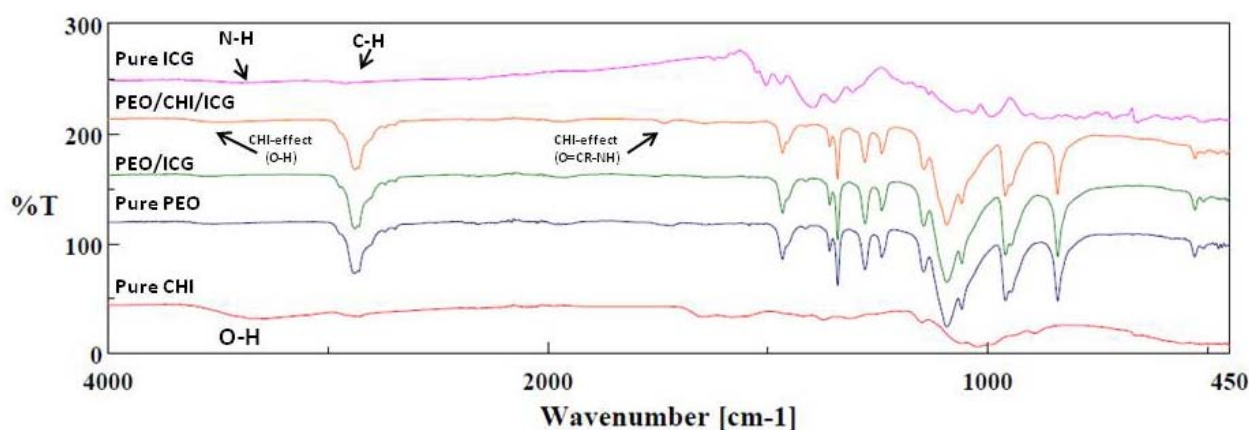


Fig. 3 FT-IR of the Pure CHI, pure PEO, PEO/ICG, /PEO/CHI/ICG and Pure ICG from bottom to up

D. Confocal Microscopy

Confocal images of PEO/CHI/ICG nanofiber mats with 2% CHI concentration were given in Fig. 4. In this study, enhancing ICG stabilization was aimed in the biological environment. As can be seen in Fig. 4, ICG was successfully encapsulated inner layer of PEO and CHI polymer nanofiber structures.

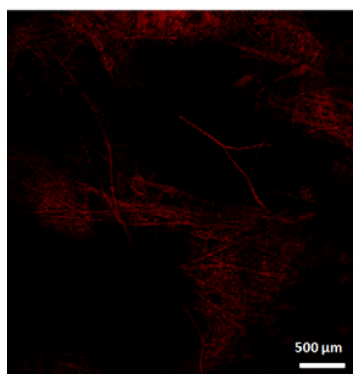


Fig. 4 Confocal images of PEO/CHI/ICG coaxial nanofiber with 2% ratio of CHI

E. Controlled ICG Release Study

In vitro release profile of ICG from PEO/(2%)CHI/ICG electrospun nanofiber was examined in PBS at pH 7 via UV spektrophotometer at defined time intervals. The released

amount of ICG was calculated by calibration curve which is plotted with the different concentrations of ICG ($0.2\text{--}1\text{ mg.ml}^{-1}$) absorption values at 780 nm ($r^2 = 0.9753$) [8].

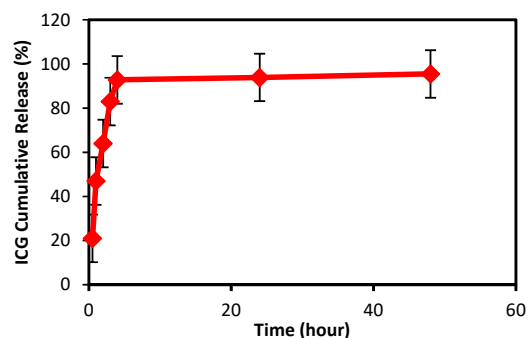


Fig. 5 Releasing profile of ICG from PEO/CHI/ICG with the ratio of 2% CHI

As can be seen in Fig. 5, there was no burst release of ICG. This result can be explained with ultrafine fiber which has porous structure. This porous nanofiber structure served as a sieve, which is allowing the ICG dye to be released in a sustainable manner.

IV. CONCLUSION

In the present work, unspinnable CHI was electrospun

with spinnable PEO and ICG, which have high electrical conductivity, at one step by co-axial electrospinning. At the same time, by encapsulating ICG in the PEO/CHI nanofibers, stability and bioavailability of ICG increased. The sustained release of the ICG dye allows the early detection of tumor tissues without destroying any healthy tissues by targeting it to the desired site via nanofiber carriers. As a result, the ICG nanoprobe can be used in biomedical imaging applications and patient welfare can be increased by early diagnosis.

ACKNOWLEDGMENT

This study was financially supported by TUBITAK (Project number: 217M028).

REFERENCES

- [1] C. Zheng, "Indocyanine green-loaded biodegradable tumor targeting nanoprobe for in vitro and in vivo imaging," in *Biomaterials*, vol. 44, April 2012, pp. 5603–5609.
- [2] C. Yue, "IR-780 dye loaded tumor targeting theranostic nanoparticles for NIR imaging and photothermal therapy," in *Biomaterials*, vol. 34, May 2013, pp. 6853–6861.
- [3] T. Desmettre, "Fluorescence Properties and Metabolic Features of Indocyanine Green (ICG) as Related to Angiography" in *Survey of Ophthalmology*, vol. 45 July 2000, pp. 15-27.
- [4] S. Vishal, "Polymeric nanoparticulate delivery system for Indocyanine green: biodistribution in healthy mice" in *Int. J. Pharm.*, vol. 308, February 2006, pp. 200-204.
- [5] M. Pakravan, "A fundamental study of chitosan/PEO electrospinning," in *Polymer*, vol. 52, August 2011, pp. 4813–4824.
- [6] M. Sadri, "New Chitosan/Poly(ethylene oxide)/Thyme Nanofiber Prepared by Electrospinning Method for Antimicrobial Wound Dressing," in *Nanostruct.*, vol. 4, September 2016, pp. 322-328.
- [7] Z. C. Yao, C. Zhang, Z. Ahmad, J. Huang, J. S. Li, M. W. Chang, "Designer fibers from 2D to 3D Simultaneous and controlled engineering of morphology, shape and size," *Chemical Engineering journal.*, vol. 2, Oct. 2017, pp. 89–98.
- [8] Z. R. Ege, A. Akan, F. N. Oktar, C. C. Lin, B. Karademir, O. Gunduz, "Encapsulation of indocyanine green in poly(lactic acid) nanofibers for using as a nanoprobe in biomedical diagnostics," *Materials Letters*, vol. 228, June 2018, pp.148–151.
- [9] F. Cheng, J. Gao, L. Wang, X. Hu, "Composite chitosan/poly(ethylene oxide) electrospun nanofibrous mats as novel wound dressing matrixes for the controlled release of drugs," *J. APPL. POLYM. SCI.*, January 2015, pp. 42060-42068.

Zeynep R. Ege (M'2018) was born in Turkey/Bayburt in 1986. Her BC is Physics (2009), MSc (2013) is Physics and Ph.D. (continue) is Biomedical Engineering in Istanbul University.

She is doctorate student in Istanbul University with Prof. Dr. Aydin Akan and she has been studying in the Advanced Nanomaterial Research Lab since 2016 spring with TUBITAK project in Marmara University with Assoc. Prof. Dr. Oguzhan Gunduz.

Her interest areas are biomaterials, cancer diagnosis and treatment and biomedical devices.