ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ B- TEORİK BİLİMLER

Eskişehir Technical University Journal of Science and Technology B- Theoretical Sciences

2020, 8(2), pp. 293 - 302, DOI: 10.20290/estubtdb.658998

EFFECTS OF GENTAMICIN LOADED PCL NANOFIBERS TO CELL VIABILITY AND RELEASE RATE OF PLASMID DNA

Muhammet CEYLAN [1,*] Shang-you YANG [2], Ramazan ASMATULU [3]

[1] Mechatronics Engineering, Engineering Faculty, Istanbul Commerce University, Istanbul, Turkey
[2] Via Christi Hospital, Wichita, Kansas, USA
[3] Mechanical Engineering, Engineering Faculty, Wichita State University 1845 Fairmount, Wichita, Kansas, USA

ABSTRACT

Polycaprolactone (PCL) nanofiber with constant plasmid DNA addition and with different concentration of gentamicin designed and studied for their effect on cell viability and release rate. PCL nanofibers were fabricated using electrospinning method with plasmid DNA and gentamicin addition. The plasmid DNA used in PCL nanofiber were extracted from E. coli. The scanning electron microscopy (SEM) images show that the nano-scale fiber structures have an average diameter of 113.9 nm. UV microplate reader confirmed the existence of plasmid DNA in the PCL nanofibers. Elisa reader study showed the addition of gentamicin in the fibers. Cell viability tests indicated that PCL nanofibers with 10% gentamicin on a fibroblast cell showed high cell viability, which is related to surface areas and pore size of the electrospun fibers besides to the interaction among gentamicin, plasmid DNA and electrospun fiber matrix.

Keywords: Electrospinning, PCL nanofibers, Cell viability, Wound Dressing

1. INTRODUCTION

Electrospinning process has improved popularity in the most recent decade due to larger fibers could able to go from micron size to nanometer size. At the point when the diameters of the polymeric fibers diminished from micrometers to nanometers, it is offered a few astounding aspects, for instance, high surface range to volume ratio, high porosity, adaptability in surface functionalities, and predominant mechanical properties. These exceptional properties of electrospun nanofibers make them to be the ideal applicants for a wide area of significant use in broadly different areas, for example, drug delivery, tissue engineering, scaffolding, wound dressing, sensors and filters [1].

Electrospinning is the methodology of expelling a fine fiber from a charged polymer solution towards to grounded collector. The fiber is constantly elongate by electric static forces and solvent evaporate while traveling through air to collector [2]. The history of electrospinning has started with electrospraying about 100 years ago. Instead of fibers, polymer droplets were formed by electrostatic forces. In 1934, Formhals patented the device that effectively electrospun fibers [3]. Formhals kept on improving upon his work with extra patents throughout the following few years with minor, yet compelling progressions, to the electrospinning setup [4, 5]. In 1969, Taylor created a mathematical model to show the form of a liquid droplet under an electric field. This shape is known as the Taylor cone. In order to achieve successful jet formation, the droplet cone angle was 98.6 degrees [6]. In 1971, Baumgarten studied solution parameters and their effects on fiber diameter [7]. Baumgarten study showed that increasing viscosity of solution cause increased fiber diameters therefore electrospun fibers could be in nanometer range. Publications about electrospinning has increased since 2000 [2]. Production of fibers in nano size has turned into promising feature for biomedical applications such as wound dressings, drug delivery, tissue engineering and also filtration [8, 9].

*Corresponding Author: mceylan@ticaret.edu.tr
Received: 20.12.2019 Published: 31.08.2020

Diameter of electrospun fibers form have been attained from microns to nanometers size [10]. Electrospun fibers have high surface area to volume ratio and this allow for improve cell reproduction. Electrospinning have promising applications including vasculature [11, 12], neural [13] bone tissue [14], antibacterial, filtration, scaffolding [15]. Natural and synthetic polymers, such as collagen [16], gelatin [17], and fibrinogen [18], have been electrospun. There are several inclusions such as drug, antibiotics, graphene, and TiO_2 nanoparticles [19]. Using these additions can be added to polymer solution as a result electrospun fiber property can be increased. Electrospinning technology opens up new chances for the generation of fiber based biomaterials, including antimicrobial fibers [19, 20].

Poly (ε-caprolactone) (PCL) is a biodegradable, hydrophobic and food and drug administration (FDA) approved polymer that has extensive uses in biomedical applications and it shows biocompatibility [21]. In the present study, we examined the PCL nanofibers and developed antibiotic and also plasmid DNA loaded fibril structures that display various release characteristics for wound healing applications. Gentamicin was chosen as a drug to have gentamicin loaded PCL nanofibers. The reason to have gentamicin as a drug is the ability of antibiotic function of inhibiting or killing germ and bacteria that are common after surgical infection. It has been examined that the choice of polymer, distribution of plasmid DNA as well as antibiotic within the nanofibers affect the morphology and the release rate of the PCL nanofibers. Preliminary antibiotic effectiveness against fibroblast cell (L929) and neuroblast cell (B104) have also been tested to set up the suitability of these nanofibers to be applied for decreasing frequency and severity of post-surgery infections.

2. MATERIALS AND METHODS

2.1. Materials

Polycaprolactone (PCL) was bought from Scientific Polymer Products and acetonitrile obtained from Sigma Aldrich. pCMVb-GFP DNA were acquired from Addgene. Plasmid DNA was amplified with E. coli. This is the way plasmid DNA handled. These items were straightforwardly utilized within the electrospinning procedure without further purification. Fibroblast cell (L-929), neuroblast cell (B104) were bought from American Type Culture Collection (ATCC).

2.2. Methods

2.2.1. Electrospinning of Nanofibers

Electrospinning process is a complex system since there are multiple parameters and using these parameters various electrospun fibers can be produce for different applications. Electrospinning set up includes syringe, charged polymer solution, a pump, a grounded collector, and high power supply as it can be seen in Figure 1.

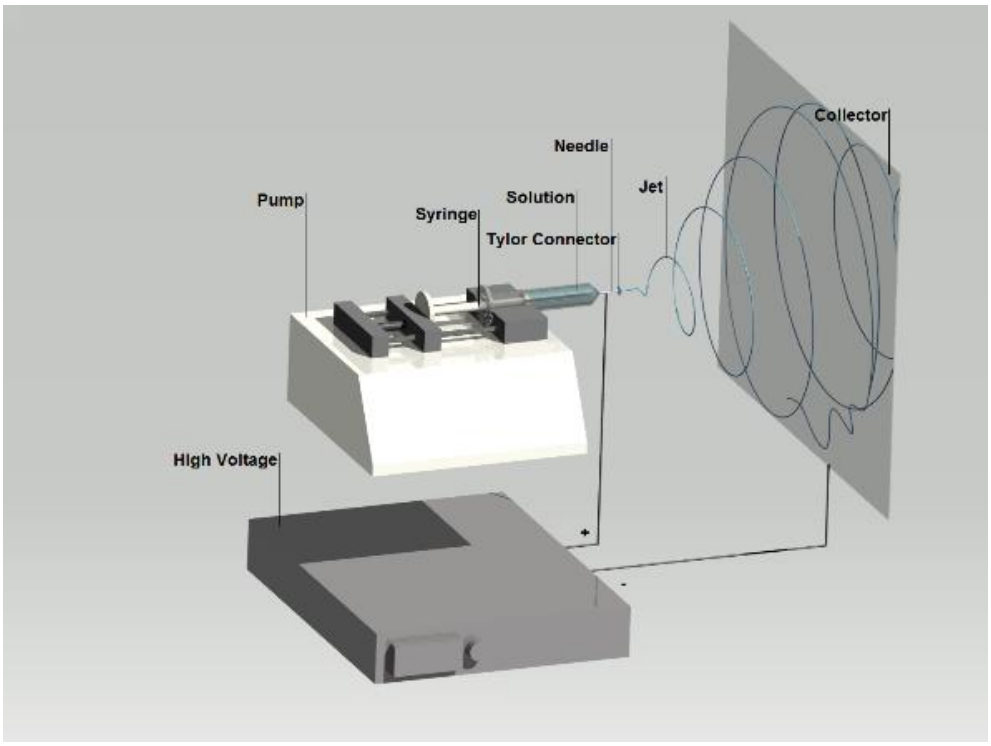

Figure 1. A schematic illustration of an electrospinning process

Polymer solution is composed of melted polymer and a solvent. The solvent has a role here to bring the polymer resin into solution and then when solution expose to air solvent is going to evaporate. In order to have a solid fiber evaporation of the solvent is necessary. The pump holds the syringe containing polymer solution also flow rate of the solution can be adjusted using the pump. High power supply range can be change 10-30 kV. An electrode from high power supply is applied to solution for the most of the time through syringe needle or conductive material which is attached to syringe and it is in contact with polymeric solution. There are various of collectors depending on the application such as for scaffolding to have different shape of fibers it can be use wire mesh, a fluid bath, a flat plate or a rotating drum which the polymer wraps itself [22]. The distance between tip of the needle to collector called the gap distance. Grounded collector and the charged polymer solution creates a static electric field. When voltage is applied to needle, the solution droplet at the tip experiences columbic forces exerted by the electric field and electrostatic repulsion between surface charges [2]. As the applied charges increases, the solution droplet changes from round shape to the Taylor cone. When the charge overcomes the surface tension of the solution droplet, a thin polymer jet goes from Taylor cone towards to collector. As the jet travels through the air a whipping instability occurs and that causes jet to progress in a conical or zigzag pattern. Before arrival to collector this will further thins the fiber diameter.

In our fabrication, 15wt% of PCL was dissolved in an acetonitrile. Then 0, 2.5, 5, 10 wt % of gentamicin and 0.55µg plasmid DNA were added into PCL solutions. The final solution was placed on a hot plate (stir plate) for 24 hours, which was heated up to 540C at a speed of 500rpm. Mixed solution was transferred into 10 mL syringe and placed in a KD scientific syringe pump at a flow rate of 2 mL/hr. Electrospinning process was completed in an ambient condition. Fibers collected after they were dried for at least 2 days.

2.2.2. Materials characterization

Scanning electron microscopy (SEM) (ZEISS) was used to analyze the morphology of the PCL electrospun fibers. Elisa Reader used for Cytotoxicity reading plate at OD 590nm. UV Microplate reader (CytoFlour Series 4000, Perceptive Biosystems) used for release rate study.

2.2.3 Isolation of pCMVb-GFP plasmid DNA

Plasmid DNA encoding EGFP with CMV promoter was amplified with E. coli which grew on a shaker at 37oC overnight. Micro-centrifuge tubes were filled with saturated bacterial culture grown in lysogeny broth (LB) containing ampicillin and were centrifuged at a speed of 4000 rpm at 4oC for 3 min, followed by the addition of 0.2ml ice-cold solution I (50mM glucose, 25mM Tris-Hcl pH 8.0, 10mM EDTA Ph 8.0), 0.4 ml solution II (1% sodium dodecyl sulfate(SDS), 0.2 N NaOH), and 0.3 ml ice-cold solution III (3 M K+ 5 M Acetate). The micro-centrifuge tubes were next put on ice for 10 min. Then micro-centrifuge tubes were centrifuged at 12000 rpm at 4oC for 5 min and supernatant was transferred to a fresh micro-centrifuge tube, respectively. Then isopropanol was added to fill remainder of centrifuge tubes and tubes were put on ice for 10min, followed by centrifuged by 12000 rpm at 4oC for 5 min. 1ml ice-cold 70% ethanol was added to each of the micro-centrifuge tubes, which were then centrifuged at a speed of 7500 rpm at 4oC for 2 min. Finally, 50µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was added to each tube. Using Elisa Reader the concentration of plasmid DNA was detected from the UV absorbance at a wavelength of 260 nm (A260).

2.2.4 Biocompatibility and cytotoxicity assay of PCL nanofibers

Sterile nanofibers in 2ml Eppendorf tube were submerged with Dulbecco's Modified Eagle's Medium (DMEM) (ATCC) containing 5% fetal bovine serum (FBS), 2mM glutamine, 100µ/ml penicillin and 0.1mg/ml streptomycin. We collected the medium on first, fourth, seventh day, and refilled these tubes with fresh medium. Cells were seeded in a 96-well plate at the density of 5x104 cells/well and cultured in a standard incubator (37oC, 5%CO2) for 3 days. Then 20 µl 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was added to each well. After six hours, cells were cracked by 10% sodium dodecyl sulfonate (SDS) solution, followed by reading plate at OD 590nm. Elisa Reader used for this process.

2.2.5 DNA release rates

The electrospun nanofiber were cut into quarter size and each one of them was immersed in 1 ml TE buffer in Eppendorf tubes at 37oC. The amount of DNA released into solution was quantified by using PicoGreen assay, according to the manufacture's protocol. Briefly, the elution TE buffers were collected at 1h, 4h, 1 day, 4 days and 7 days, and labeled with PicoGreen to detect DNA content. For each time point, there were six samples. DNA standard solution (100µg/ml in TE(10 mM Tris-HCl, 1 mM EDTA, pH 7.5) buffer) was diluted into 2µg/ml stock solution, which was a dilution of 1:2. According to the concentration of DNA in the standard solution, one can calculate how much double-strand DNA was released into the TE buffer. PicoGreen reagent was made 200-fold dilution in TE buffer. The elution solutions were measured at 520 nm (with excitation at 480nm) in a UV Microplate Reader (CytoFlour Series 4000, Perceptive Biosystems). According to the standard curve, the plasmid DNA concentration in each sample can be calculated.

3. RESULTS AND DISCUSSION

PCL nanofibers with addition of gentamicin and plasmid DNA were produced using electrospinning. From the SEM images, it can be seen that the fiber diameter was around 113.9 ± 36.60 nm. Figure 2 shows SEM images for the following combinations of Plasmid DNA and Gentamicin: 0% plasmid DNA

and 0% gentamicin, plasmid DNA, 2.5% gentamicin and plasmid DNA, 5% gentamicin and plasmid DNA, and 10% gentamicin and plasmid DNA.

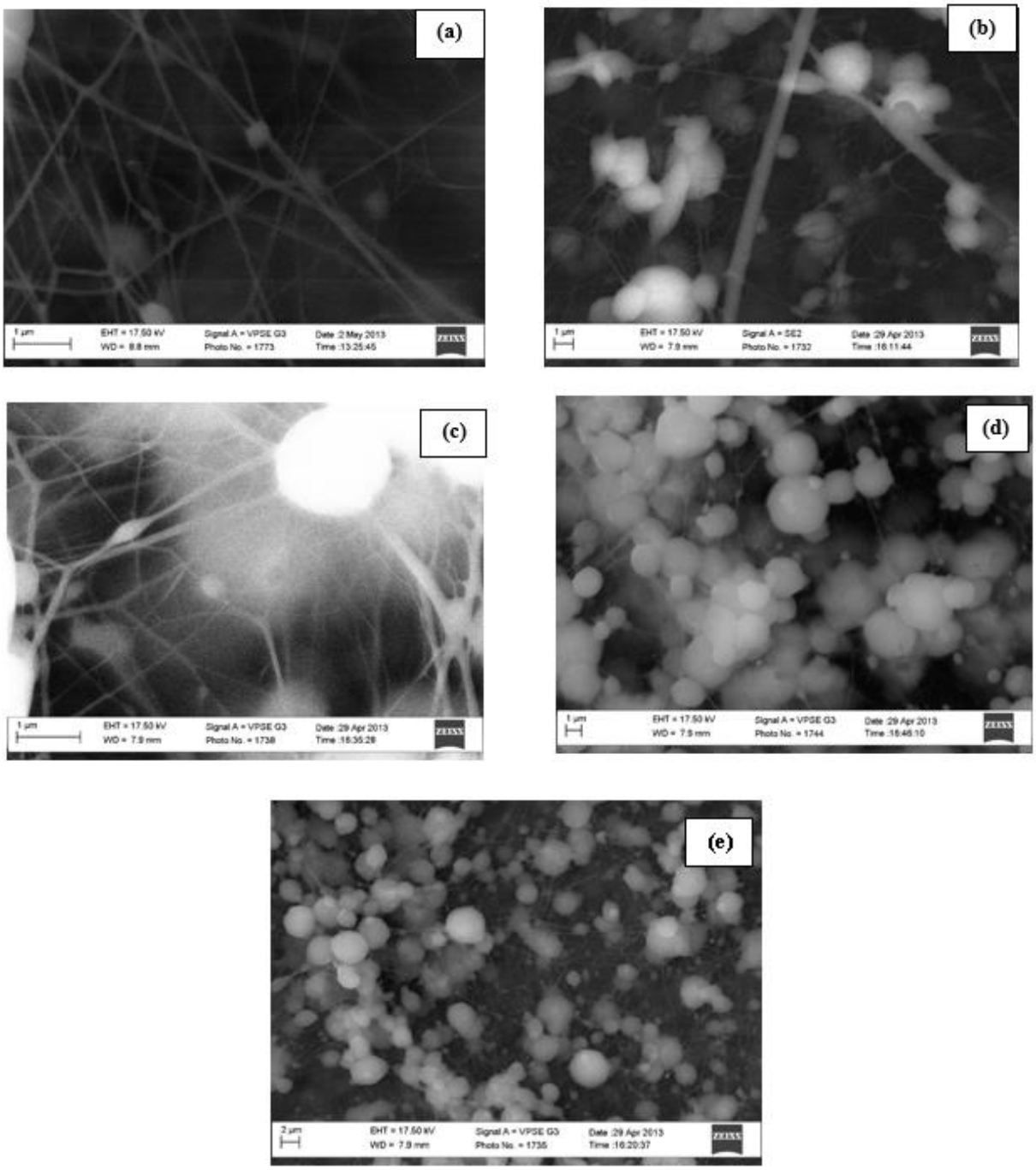

Figure 2. SEM images of PCL nanofiber composed of the following materials:(a) 0%gentamicin and 0% plasmid DNA (b) 0%gentamicin and plasmid DNA, (c) 2.5% gentamicin and plasmid DNA, (d) 5% gentamicin and plasmid DNA, and (e) 10% gentamicin and plasmid DNA.

The diameter of nanofibers calculated using 2 SEM images from each nanofiber sample and figure 3 is generated. It can be seen that 10% gentamicin and plasmid DNA addition has the smallest diameter.

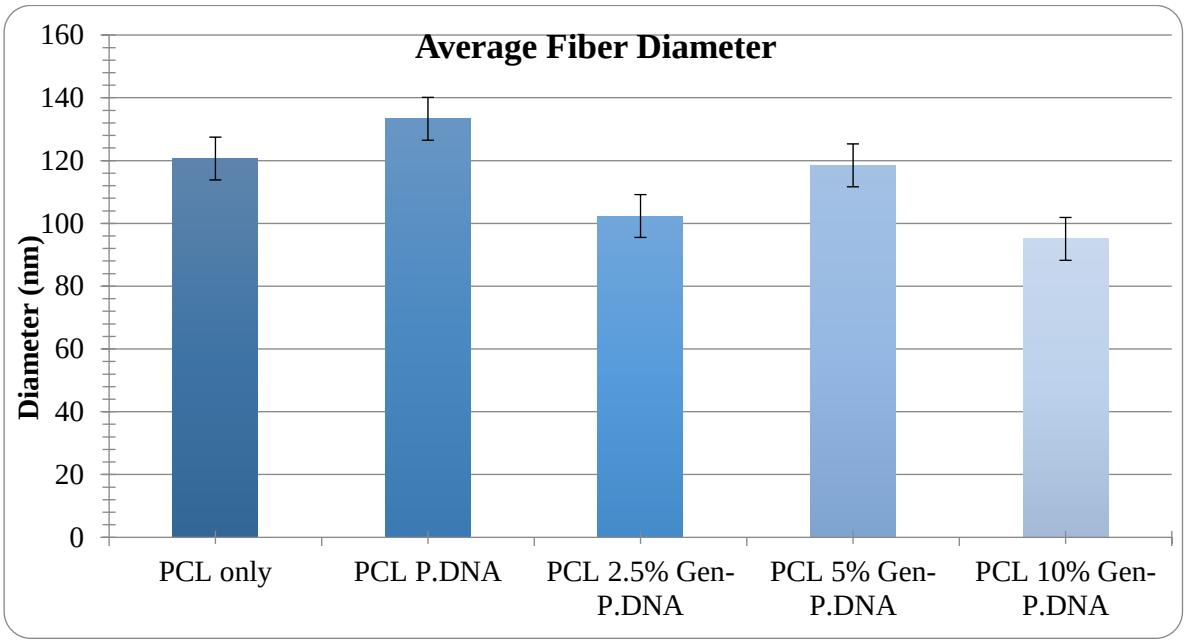

Figure 3. Diagram shows average fiber diameter of PCL nanofibers with different percentage of gentamicin and plasmid DNA.

PCL nanofiber investigated using UV Elisa reader. Figure 4 shows DNA release as a function of time for PCL nanofibers composed of gentamicin and plasmid DNA. As it can be seen from figure 4 2.5% gentamicin loaded PCL nanofiber had good amount of release.

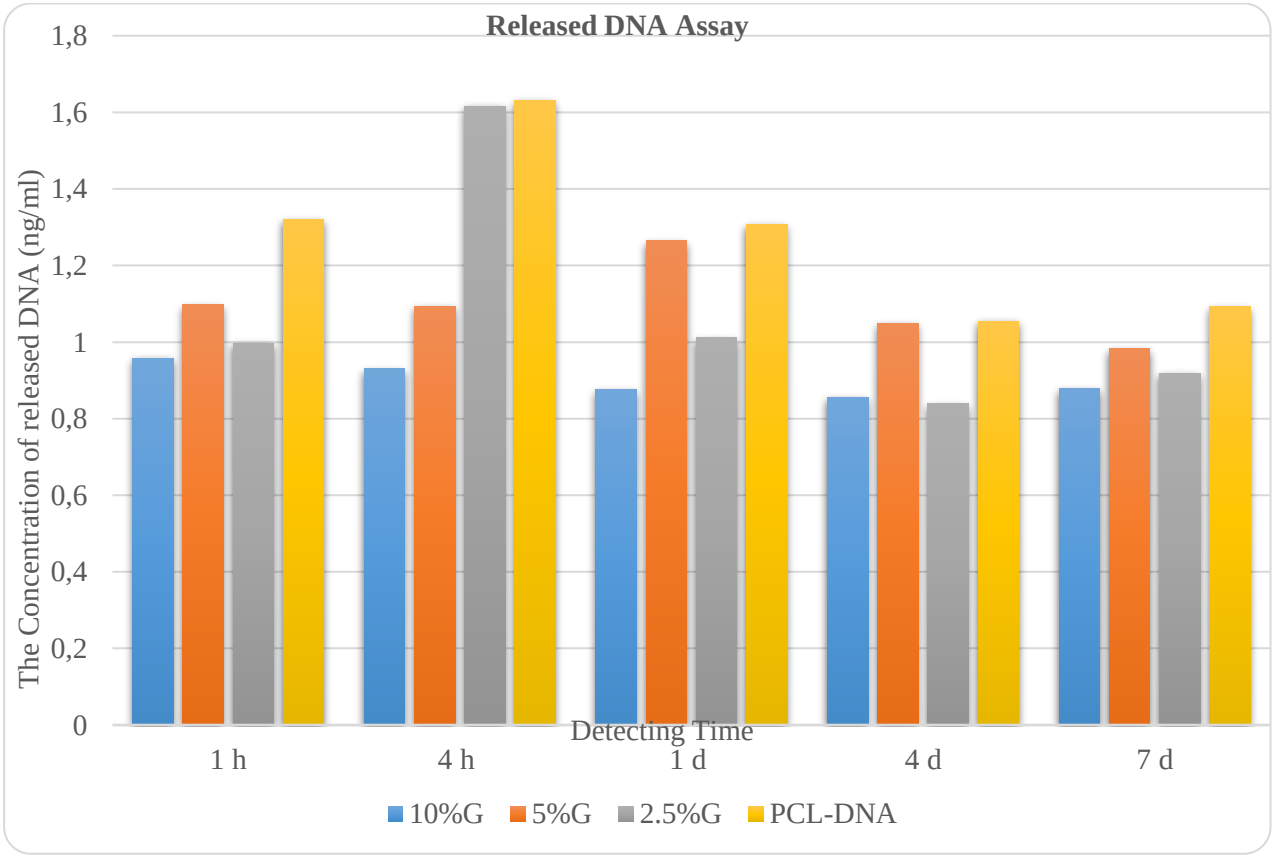

Figure 4. DNA release rates as a function of time for different composed of PCL nanofiber

For the evaluation of the antimicrobial properties of the PCL nanofibers with various concentration of gentamicin and plasmid DNA nanofibers evaluated through cell viability. B104 and L929 cells were used for both cases cell viability above 70%. Figure 5 and Figure 6 were generated using Elisa reader. Figure 5 shows the cell viability of neuroblast cells and figure 6 is exhibits fibroblast cell viability. As it can be seen from figure 5 there is not significant effect of gentamicin on neuroblast cells. On the other hand, gentamicin shows significant effect on fibroblast cell viability. 5% gentamicin addition shows the highest cell viability among other gentamicin addition.

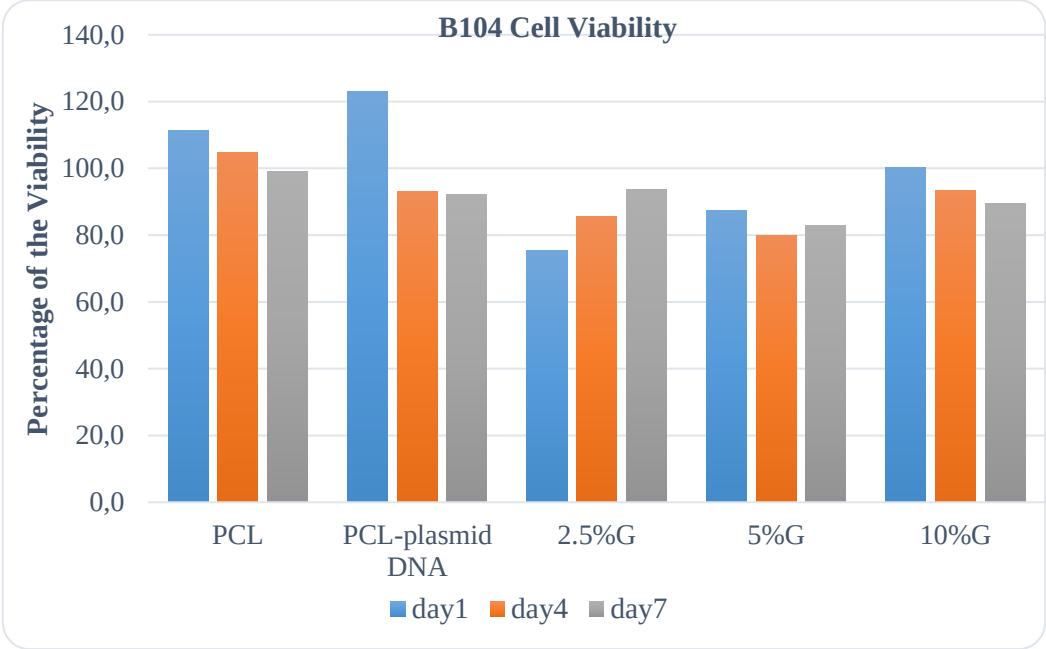

Figure 5. Cell viability of B104 neuroblast cells

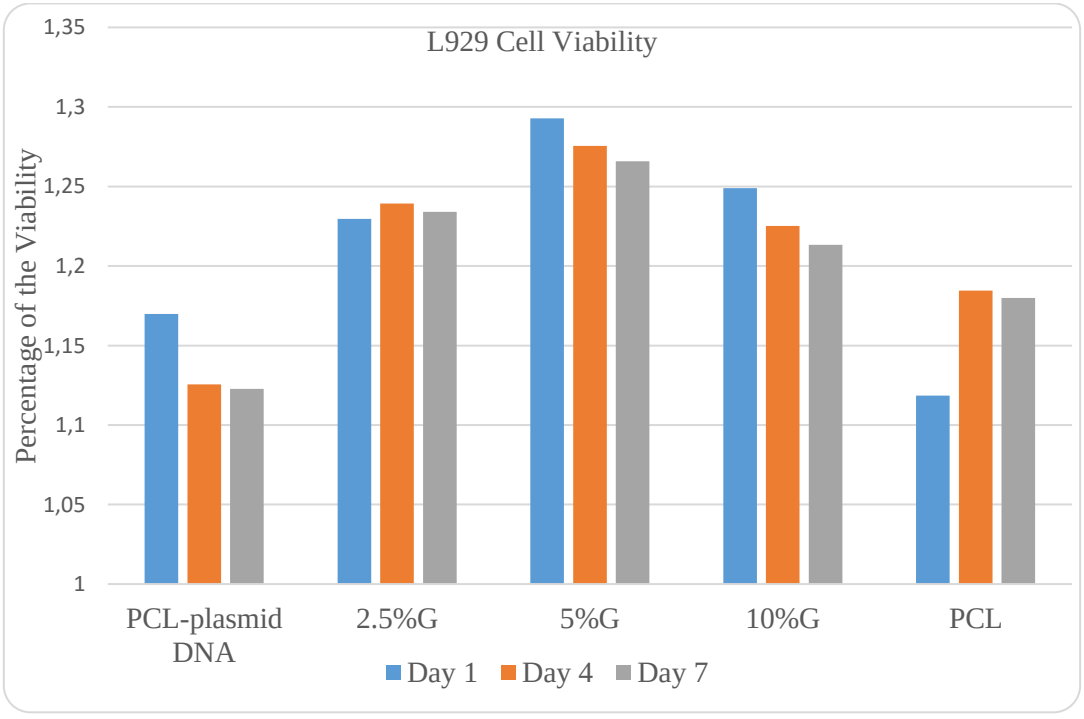

Figure 6. Cell viability of L929 fibroblast cells

Our results show that L929 cells is more susceptible to gentamicin than B104 cells. This result is comparable to that demonstrated by Ozdemir et al., although they demonstrated the Cytotoxicity of soft lining material with L929 cells [23]. Furthermore, a study by Lanbeck et al., involved with four antibiotics tests with endothelial cells. The antibiotics used for their testing were erythromycin, dicloxacillin, cefuroxime, and benzylpenicillin. Their results show that time related manner, erythromycin and dicloxacillin reduced DNA synthesis in all types of cells however, cefuroxime and benzylpenicillin did not show any effects [24]. Our results show that the PCL nanofibers in fibroblast cells without gentamicin did not show high cell viability as compared to with antibiotic addition. The release of gentamicin depends on a various of factors including the nature of the polymer matrix such as chemical composition of the polymer and water solubility. Also the distribution of nanofiber, matrix design, capacity of loading, interaction between drug and matrix, diameter of fibers, surface area and pores structure of fibers is important factor on gentamicin release. From figure 6, it can be seen that gentamicin addition in PCL nanofibers affect cell viability. When the gentamicin reaches 10 percent in the nanofibers, the nanofibers did not show the higher cell viability, which may be because of strong interactions among PCL, plasmid DNA, and gentamicin, low surface areas and volumes of the PCL nanofibers. However, as gentamicin in the PCL nanofiber reaches 5%, a higher cell viability show is displayed. From figure 5, it can be seen that there is not much affect on cell viability with inclusion of gentamicin. It may be because of the cell type or strong interactions between PCL, plasmid DNA and gentamicin and also low surface are and volume size.

These results confirm that PCL nanofibers are potential candidate for the wound dressing application. These findings further encourage more examination in the future work correlating mechanical properties and porosity of nanofibers.

4. CONCLUSION

PCL nanofibers were successfully produced using electrospinning with the addition of gentamicin and plasmid DNA. SEM images showed that the electrospun fibers are truly on a nano-scale and have the plasmid DNA and gentamicin with in the fiber. As a result, it was concluded that there was no significant change by adding gentamicin and plasmid DNA to PCL. UV Elisa reader confirmed that plasmid DNA in the PCL nanofiber. However, gentamicin was added to the PCL nanofibre and no significant change was observed on DNA release. Elisa reader confirmed that inclusion of gentamicin in the PCL nanofiber. Biological test showed that the gentamicin successfully remained active and was effective with fibroblast cells as an antimicrobial agent. Fibroblast cells showed that increase of gentamicin concentration in the fibers shows better cell viability. PCL nanofibers addition of gentamicin showed significant cell viability in fibroblast cells compare to without gentamicin inclusion. These results further confirmed that PCL nanofibers are potential candidate for wound dressing antimicrobial applications.

ACKNOWLEDGMENT

The authors wish to thank Ms. Zheng Song, Li Yongchao, Li Yao, Jimmy Nguyen and Jianhoa Jiang for their excellent technical assistance.

REFERENCES

[1] Ramakrishna S, Fujihara K., Teo W-E., Lim T-C., Ma Z. An introduction to electrospinning and nanofibers. World Scientific Publishing Co.; 2005:398.

[2] Doshi, J. D. Reneker. Electrospinning process and applications of electrospun fibers. Proceeding of IEEE-Industry Applications Society Annual Meeting 1993, Toronto, Canada :1698.

[3] Formhals A. Method and apparatus for the production of fibers. US2116942 A, Patents, 1938.

[4] Formhals A. Artificial thread and method of producing same. US2187306 A, Patents, 1940.

[5] Formhals A. Method and apparatus for the production of artificial fibers. US2158416 A, Patents, 1939.

[6] Taylor G. Electrically Driven Jets. Proceedings of the Royal Society of London. Series A, Mathematical and Physical Sciences (1934-1990) 1969; 313: 453-475.

[7] Baumgarten P. Electrostatic spinning of acrylic microfibers. Journal of Colloid and Interface Science 1971; 36(1): 71-79.

[8] Sill TJ, Von Recum HA.Electrospinning: Applications in drug delivery and tissueengineering. Biomaterials 2008; 29(13):1989-2006.

[9] Dietzel JM, Kleinmejer J, Harris D, Beck Tan NC. The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 2001;42: 261-272.

[10] Huang C, Chen S, Lai C, Reneker Darrell H, Qiu H, Ye Y, Hou H. Electrospun polymer nanofibres with small diameters. Nanotechnology 2006; 17(6):1558–1563.

[11] Heydarkhan-Hagvall S, Schenke-Layland K, Dhanasopon AP, Rofail F, Smith H, Wu BM, Shemin R, Beygui RE, MacLellan WR. Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. Biomaterials 2008; 29(19): 2907-2914.

[12] Balguid A, Mol A, Marion MH, Bank RA, Bouten CVC, Baaijens FPT. Tailoring fiber diameter in electrospun poly (e-caprolactone) scaffolds for optimal cellular infiltration in cardiovascular tissue engineering. Tissue Engineering; Part A 2008;14.

[13] Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/micro scale poly (L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials 2005; 26:2603-2610.

[14] Yoshimoto H, Shin YM, Terai Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials 2003; 24(12): 2077-2082.

[15] Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS. Electrospun antibacterial polyurethane–cellulose acetate–zein composite mats for wound dressing. Carbohydrate Polymers 2014; 102: 884-892.

[16] Matthews JA, Wnek GE. Simpson DG, Bowlin G L. Electrospinning of collagen nanofibers, Biomacromolecules 2002; 3(2): 232–238.

[17] Li Y, Ceylan M, Shrestha B, Wang H, Lu RQ, Asmatulu R.,Yao L. Nanofibers Support Oligodendrocyte Precursor Cell Growth and Function as a Neuron-Free Model for Myelination Study. Biomacromolecules 2014; 15(1):319–326.

[18] McManus MC, Boland ED, Simpson DG, Barnes CP, Bowlin GL. Electrospun fibrinogen: feasibility as a tissue engineering scaffold in a rat cell culture model. Journal of Biomedical Materials Research Part A 2007;81(2):299-309.

[19] Asmatulu R, Ceylan M, Nuraje N. Study of Superhydrophobic Electrospun Nanocomposite Fibers for Energy Systems. Langmuir 2011; 27(2): 504–507

[20] Nuraje N, Khan WS, Lei Y, Ceylan M, Asmatulu R. Superhydrophobic Electrospun Nanofibers. Journal of Materials Chemistry A 2012; 1:1929–1946.

[21] Orozco-Castellanos LM, Marcos-Fernandez A, Martínez-Richa A. Hydrolytic degradation of poly(ε-caprolactone) with different end groups and poly(ε-caprolactone-co-γ-butyrolactone). Characterization and kinetics of hydrocortisone delivery. Polymer for Advanced Technologies 2009 DOI:10.1002/pat.1531

[22] Boland ED, Matthews JA, Pawlowski KJ, Simpson DG, Wnek GE, Bowlin GL. Electrospinning collagen and elastin: preliminary vascular tissue engineering. Frontiers in Bioscience 2004; 9:1422-1432.

[23] Ozdemir KG, Yilmaz H, Yilmaz S. In Vitro Evaluation of Cytotoxicity of Soft Lining Materials on L929 Cells by MTT Assay. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2009; 90 (1):82-86.

[24] Lanbeck P, Paulsen O. Short-term Effects of four Antibiotics on DNA Synthesis in Endothelial Cells. Pharmacology & Toxicology 2001; 88:204-208.