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Poly(lactic acid) nanofibers containing phosphorylcholine grafts for transdermal drug delivery systems

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ABSTRACT

The continuous and prolonged releases of chemotherapeutic drugs are required for successful treatment in cancer treatment. The project focused on a new material design to meet this requirement. We developed a constant and sustained release system and investigated the release profiles of Paclitaxel (PTX). Polylactic acid (PLA) nanofiber surface was grafted with poly (methacryloyloxyethyl phosphorylcholine) (PMPC) by the UV-induced grafting method. The morphological structure of the PLA nanofibers did not change with an increase in the MPC content. PMPC blocks contribute to the solubility of PTX, which shows low resolution. When the amount of MPC is 5%, the PTX loading efficiency increased two times compared with PLA nanofiber. The nanofiber mats exhibited an initial fast release during the first 3 h. Endothelial cells were cultured on nanofiber mats to investigate whether this material was toxic or not. The mats showed good biocompatibility with HUVEC. Thus, it was confirmed that nanofiber mats would not be toxic when releasing drugs during in vivo use. We think that PMPC facilitates the pass of drugs through the lipid-rich biological membrane and so anticancer drugs can be delivered to direct tumor sites.

1. Introduction

Cancer is a primary public health problem. Population aging and rapid population growth will lead to an increase in cancer cases [1]. Chemotherapy is a high effect treatment method for primary and metastatic cancer. However, the nanocarrier polymer systems that have been developed in recent years have been very useful for the effective use of drugs and their long-term release in optimal doses [2]. Paclitaxel (PTX) as a chemotherapeutic agent exhibits strong and rapid inhibitory against a variety of tumor cells including breast, ovarian, pancreatic, and brain cancers [3]. PTX provides the formation of stable microtubules by disassembly of microtubules [4]. However, the very low water solubility of PTX leads to a decrease in its superior anti-tumor activity [5]. Furthermore, a low solubility profile causes side effects. To minimize side effects, one of the best methods is the incorporation of PTX by phospholipids derivatives. The lipid derivatives are generally well-tolerated in the physiological environment and their residues are non-toxic to the human body [6]. In addition, phospholipids enhance the hydrophilicity of the hydrophobic drugs due to their good emulsifying property [7].

Nanofibers have a high surface-to-volume ratio, tunable porosity, and high flexibility [8]. Besides, they can mimic both the form and functionality of the native extracellular matrix (ECM) [9]. Nanofibers combined with certain nanocarriers and vesicles have received a great deal of attention in recent years in the biomedical field for preparing drug delivery systems [10]. The large surface area of the nanofibers allows surface grafting [11]. UV-induced surface treatment can be used for the functionalization of a solid surface due to its low cost, high efficiency, and simple equipment requirement [12]. The surface radicals are formed using the photoinitiator such as benzophenone and react with the monomer [13].

Polylactic acid (PLA) is a biodegradable and biocompatible polymer, which can be produced from 100% bio-resources [14]. Several drugs have successfully been loaded into PLA nanofibers by using various techniques. Transdermal delivery of Plai oil from PLA nanofibers showed no skin irritation, indicating them as promising prototypes for medical applications [15]. Zhao et al. investigated the release profile and antimicrobial activity of chlortetracycline hydrochloride and amphotericin B from the

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polycaprolactone (PCL)-PLA fibers [16]. The adsorption and stability of the drug are the two parameters for efficient drug delivery. However, the polymers like PLA, poly (lactic acid-*co*-glycolic acid) (PLGA), PCL could not avoid plasma protein adsorption and fibrosis formation due to their hydrophobic character [17]. Amphiphilic block copolymers can be used to enhance drug stability. For example, Xu et al. studied the drug delivery of poly (ethylene glycol) (PEG) -PLA nanofibers with dual drugs [18]. The reported loading efficiency of the drug into the amphiphilic block copolymer is low (below 30%) [19].

In our work, a new PLA/phospholipid-derivate nanofiber mat was designed to increase the adsorption and stability of the drug. The amphiphilic structure was created with a core-sheath by using UV-photo grafting of methacryloyloxyethyl phosphorylcholine (MPC) onto PLA nanofiber. As a phospholipid polymer, MPC-based polymers have been used for drug delivery systems [20–22]. A therapeutic drug PTX was loaded into the nanofiber mats. Among several types of PTX loaded PLA drug delivery, PLA decorated with PEG [23], poly (γ-glutamic acid) [24], and poly (amine-ester) [25] can be mentioned. The polymer-lipid-based systems so-called "next-generation drug delivery systems" will make a new contribution to this area. These hybrid drug delivery systems can be prepared by encapsulation. However, optimization of formulation parameters is necessary [26]. For this study, a new polymer-based nanofiber has been developed containing a phosphorylcholine group in the side chain that is the most suitable monomer to mimic the phospholipid polar groups contained with the cell membrane. The cytotoxicity of the prepared nanofibers was evaluated with HUVEC umbilical vein endothelial cells as normal cell line and MDA-MB-231 breast cancer cell line. After MPC grafting, the nanofibers showed better biocompatibility. In addition, a higher PTX loading efficiency was achieved.

2. Materials and methods

2.1. Materials

PLA 4043D (D-isomer 4.5–5 wt. %, 110.000 g/mol) was kindly supplied by Pelsan Tekstil. 2-Methacryloyloxyethyl phosphorylcholine (MPC) (97%), benzophenone (99%), methanol (anhydrous, 99.8%), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (≥99%) were obtained from Sigma Chem. Co (St Louis, MO).

2.2. PLA nanofiber

The PLA nanofiber was prepared by using electrospinning. First, 0.5 g of PLA was dissolved in 10 mL HFIP to achieve a concentration of 5% w/v. 10 mL PLA solution was loaded into a stainless steel syringe and the flow rate was fixed at 0.5 mL/h. The electric field of 17.9 kV was applied between the syringe and the collector plate. The collector plate was placed at a distance of 16 cm from the tip of a syringe.

2.3. Surface functionalization of the PLA nanofiber by UV-induced grafting

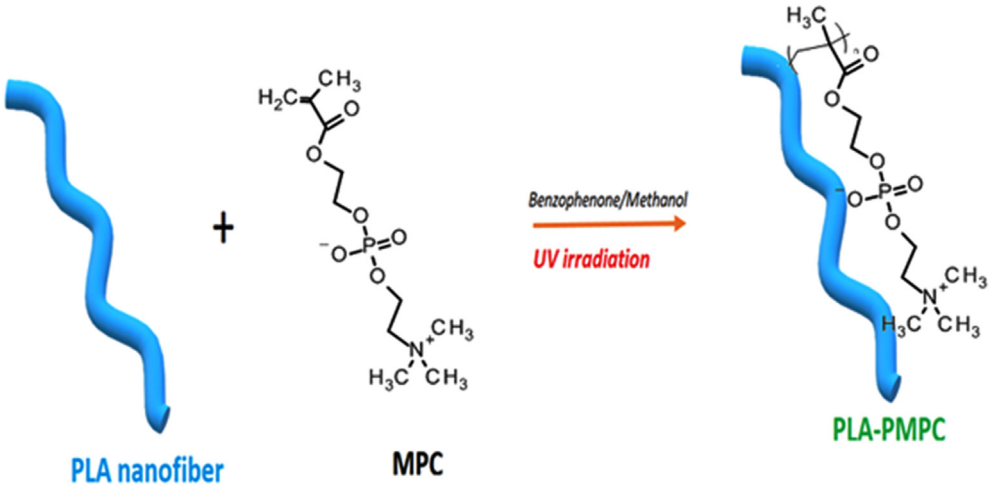
4 cm × 4 cm samples were cut from the PLA nanofiber mat. The samples were washed with methanol and dried under a vacuum. MPC monomer solutions (1%, 3% and 5% (w/v) monomer concentrations in 1 mL methanol) were prepared. Benzophenone was used as a photoinitiator and added to the monomer solutions. The PLA nanofiber mats were dipped in the prepared MPC monomer solutions for 60 min. The MPC monomer soaked nanofibers were irradiated by a UV lamp for 15 min. Then all nanofiber samples were washed with fresh methanol three times to remove the residual unreacted monomer and photoinitiator. The reaction pathway is shown in Scheme 1.

2.4. Nanofiber characterization

The structural analysis of the nanofibers after grafting was performed by Fourier transform infrared spectroscopy (FTIR) (on Perkin Elmer, ATR-FTIR). The spectra were collected at the range from 4000 to 380 cm^{-1}. The morphology of the nanofibers and diameter was performed by scanning electron microscopy (SEM) (on Phillips XL 30 ESEM-FEG). Thermal transitions (Tg and Tm) of samples were evaluated by differential scanning calorimeter (DSC) (on Perkin Elmer, Diamond DSC).

2.5. Drug loading and in-vitro release

PTX was used as a model drug. The drug loading was performed in acetonitrile. PTX (50 μg) was dissolved in 50 μL of acetonitrile and then vortexed. The nanofiber was immersed in the drug solution for 24 h. Afterward, they were taken out and washed with fresh acetonitrile. The drug release of the nanofibers was evaluated at pH 7.4 and pH 5. The release studies were performed as stated in our



Scheme 1. The preparation of PLA-PMPC.

previous study [27]. Briefly, the PTX-loaded nanofibers were loaded in 2 mL of 10 mM phosphate in a 37 °C water bath with constant shaking. At various time intervals, 1 mL of sample was drawn from the medium and an equal volume of phosphate buffer was added to the dissolution medium to maintain a constant volume. The release of PTX was followed by using Shimadzu UV-spectrophotometer.

2.6. Cell culture

HUVEC umbilical vein endothelial cell line and MDA-MB-231 breast cancer cell line were used for cell culture studies. Cell culture was performed as stated in our previous study [28]. Cytotoxic effects of the compounds were evaluated by (3-[4,5-dimethylthiazol-2-yl]- 2,5 diphenyltetrazolium bromide) (MTT) assay, which is reduced by living cells to yield a soluble formazan product by using the method of Mossman modified by our laboratory [29].

3. Results and discussion

3.1. Structural characterization

Fig. 1 shows FTIR spectra of PLA and PLA-PMPCs. In the spectrum, the characteristic peaks between 3000 and 2950 cm^{-1}

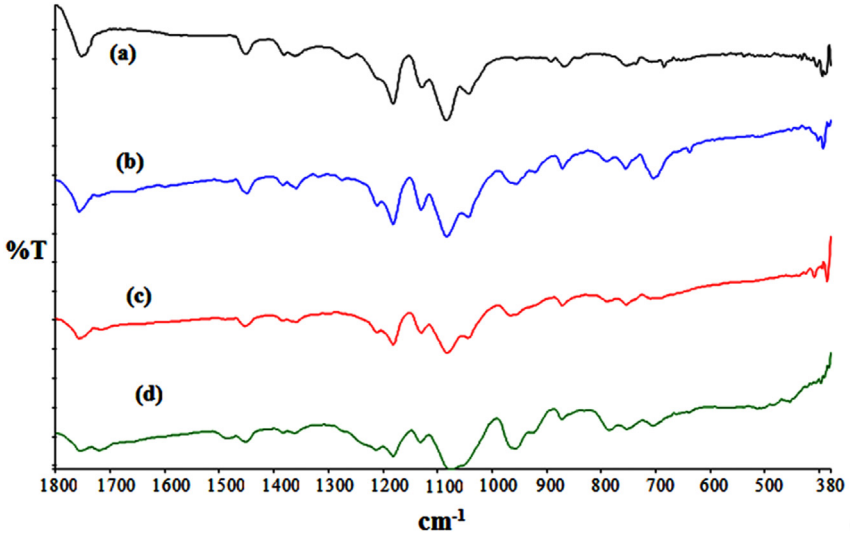


Fig. 1. FT-IR spectra of PLA and PLA-PMPCs.

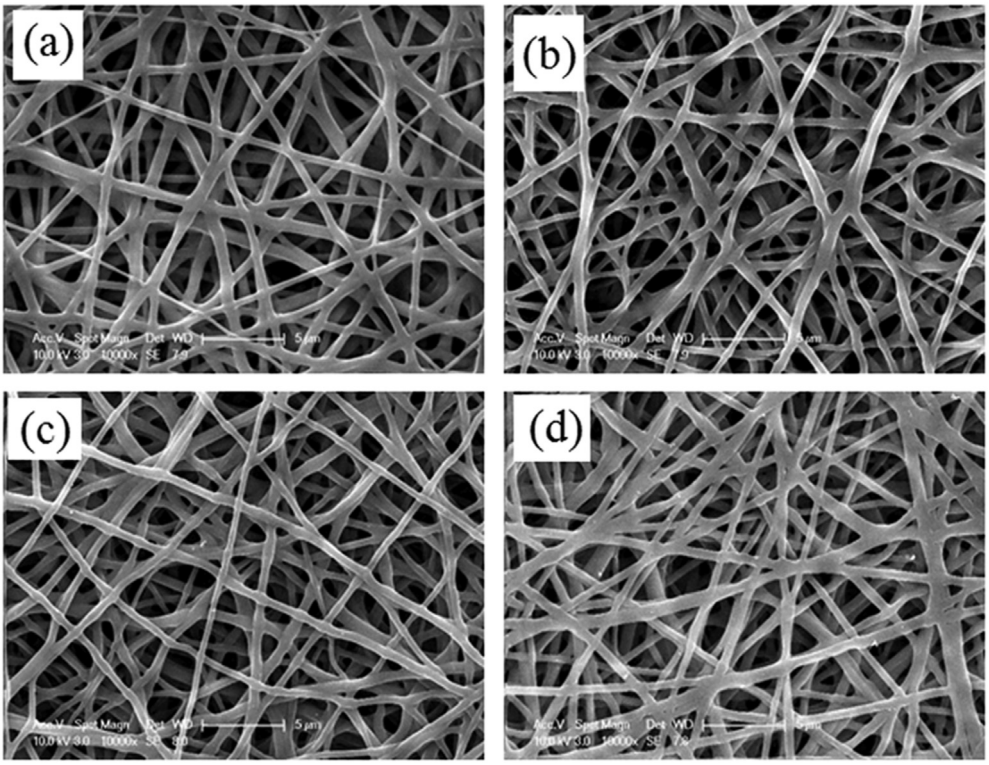


Fig. 2. SEM images of nanofibers at 1000× magnification (a) PLA, (b) PLA-PMPC-1, (c) PLA-PMPC-2 and (d) PLA-PMPC-5.

attributed to aliphatic C-H groups. The carbonyl (C═O) bond stretching was detected at 1752 cm^{-1}. The C-O absorption of the CH-O group was observed at 1183 cm^{-1}. Furthermore, the peaks at 1082 and 1043 cm^{-1} are C-O stretchings of the O-C═O group [30]. After MPC polymer grafting, the new peak appeared at around 1720 cm^{-1} due to the carbonyl group. The two characteristic peaks at 1083 and 1044 cm^{-1} can be attributed to $POCH_2$- bending vibration. However, these peaks overlapped with the C-O stretching of PLA. As can be seen, the intensity of the peaks increased after the reaction with MPC. The peak at 1210 cm^{-1} is asymmetric stretching of $POCH_2$- and the peak at 956 cm^{-1} is indicative of -$N(CH_3)_3$ [31,32].

3.2. DSC results

Tg and Tm of PLA and PLA-PMPCs were measured by DSC. PLA is a semi-crystalline polymer. Thus it exhibits two thermal transitions due to its amorphous and crystalline regions [33]. The glass transition temperature (Tg) of PLA was found as 64 °C. In addition, the melting temperature (Tm) of PLA was determined as 130 °C. After grafting with MPC, Tg of the PLA nanofiber mats decreased from 64 °C to 59 °C for PLA-PMCP-1. By further increase in grafting level of PLA, Tg value reduces drastically to 45 °C. Grafting makes two main effects: The first one is the local motions are decreased due to reduction of linear segmental length and the second one is the increase in the number of chain ends which enhances segmental mobility. Since the grafted PLA polymer has more chain ends it will have a larger free volume, which reduces Tg [34]. In addition, the Tm of PLA-PCM1 was found as 95 °C. The reduction of Tm could be attributed to the imperfect crystallinity of PLA due to its branching [35]. However, Tm was not observed for PLA-PCM2 and PLA PCM5.

Table 1
The drug loading capacities of nanofibers.

b	pH 7.4	pH 5
	PTX uptake (mg drug/g nanofiber)	PTX uptake (mg drug/g nanofiber)
PLA	72,17	127,87
PLA-PMPC-1	80,34	128,55
PLA-PMPC-2	119,72	155,4
PLA-PMPC-5	156,67	168,36

3.3. SEM images of after and before modification by UV-grafting

Fig. 2 shows SEM images of after and before modification of PLA nanofiber mat by UV-photo grafting. As can be seen in SEM images, PLA nanofibers have narrow size distribution and bead-less morphology. After grafting of PMPC the diameter of the fibers increased. But, the characteristic nano-structure is apparent. The diameter of the PLA and PLA-grafted PMPC nanofibers was in the range of 300−500 nm.

3.4. In-vitro drug release of PTX from the nanofiber mats

PTX was selected as a model anticancer drug. The loading percent of PLA, PLA-PMCP-1, PLA-PMCP-2, and PLA-PMCP-5 nanofiber mats were shown in Table 1. The high surface area of nanofibers is allowed to interact with drugs. One can see from Table 1 that the drug uptake of PLA-PMCP mats is higher than the PLA nanofiber mat. It is known that the hydrogen bond formation or hydrophobic interactions between the drug and the carrier enhance the drug uptake [36]. Previously reported that the dendritic phospholipid nanocarriers showed nearly 10% anticancer drug (DOX) loading capacity [37]. The percent of drug loading decreased as the molar percent of lipid derivatives such as saturated phospholipids in drug delivery by liposomes due to the high rigidity of their lipid bilayers [38−40]. In this study, it was observed that the loading efficiency increased with the grafting ratio of PMPC compare to other phospholipid-based drug nanocarriers. PMCP fringes enable both cell compatibility and high drug loading efficiency with their phospholipid-type structure. The reason for increased loading capacity may be the less entangled PMPC chains with higher mobility on the large nanofiber surface leading to higher molecular interaction with the drug. In addition, the PTX loading capacity of the nanofibers was higher at pH 5 compared with pH 7.4.

The release of PTX from the nanofiber mats was performed at both physiological pH (pH 7.4) and lysosomal pH (pH 5.0) at 37 °C. The *in-vitro* release profile of PTX from the nanofiber mats shows biphasic patterns (Fig. 3 and Fig. 4). All drug-loaded samples showed a fast burst release of the drug. When compare to aqueous drug solution, transdermal drug systems exhibit rapid burst release within the first 5 min. Almost all of the drugs are released after

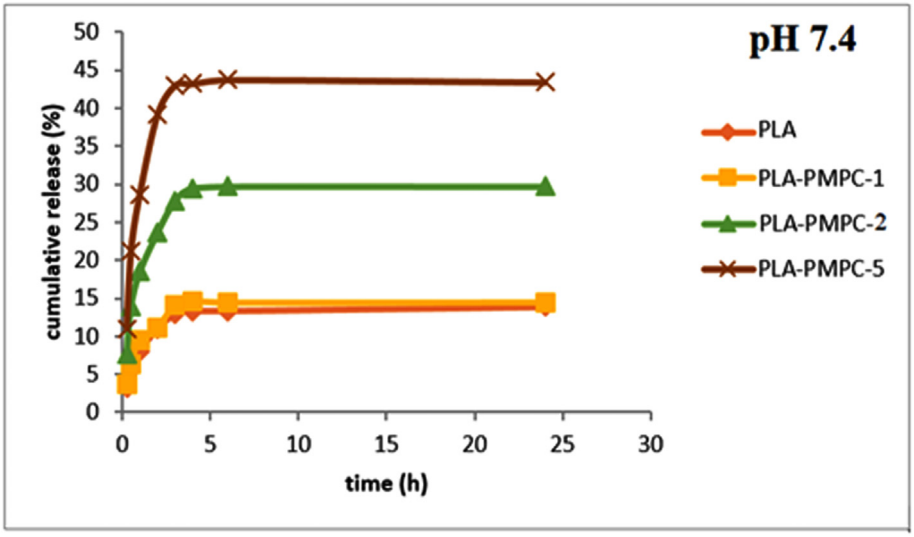


Fig. 3. PTX release profile from the nanofibers at pH 7.4.

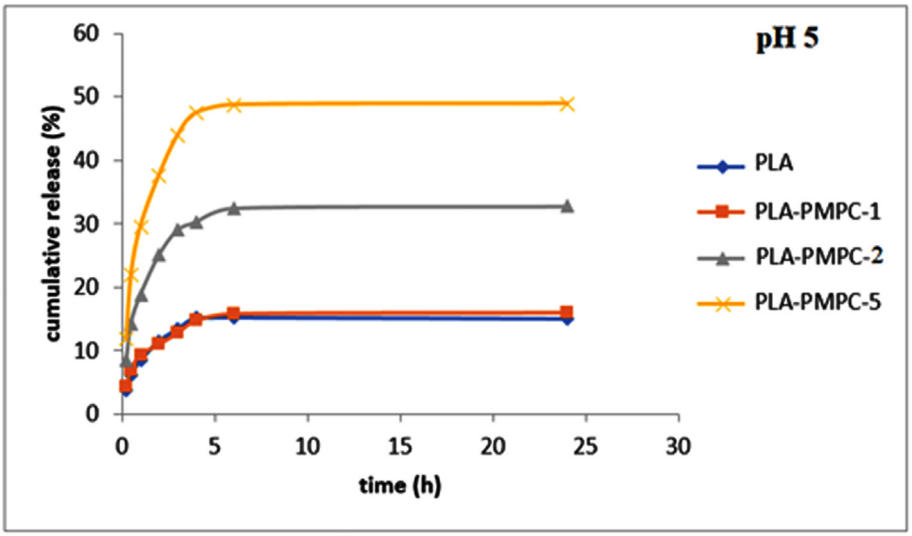


Fig. 4. PTX release profile from the nanofibers at pH 5.

60 min [41,42]. As can be seen, the release of PTX from the nanofiber materials within 5 h was completed.

After 24 h, the cumulative drug release of PLA was 13% at pH7 and 15% at pH5. For both pHs, the cumulative release of the drug was increased drastically (>43%) by grafting. Previous reports in the literature show that the cumulative release of PTX is usually between 5% and 35% [43]. PTX release from the nanofiber mats was more rapid at pH 5 than at pH 7.4. At pH 5, a partial positive surface charge on grafted nanofiber mats was generated from the protonation of phosphonate and trimethylammonium groups improved

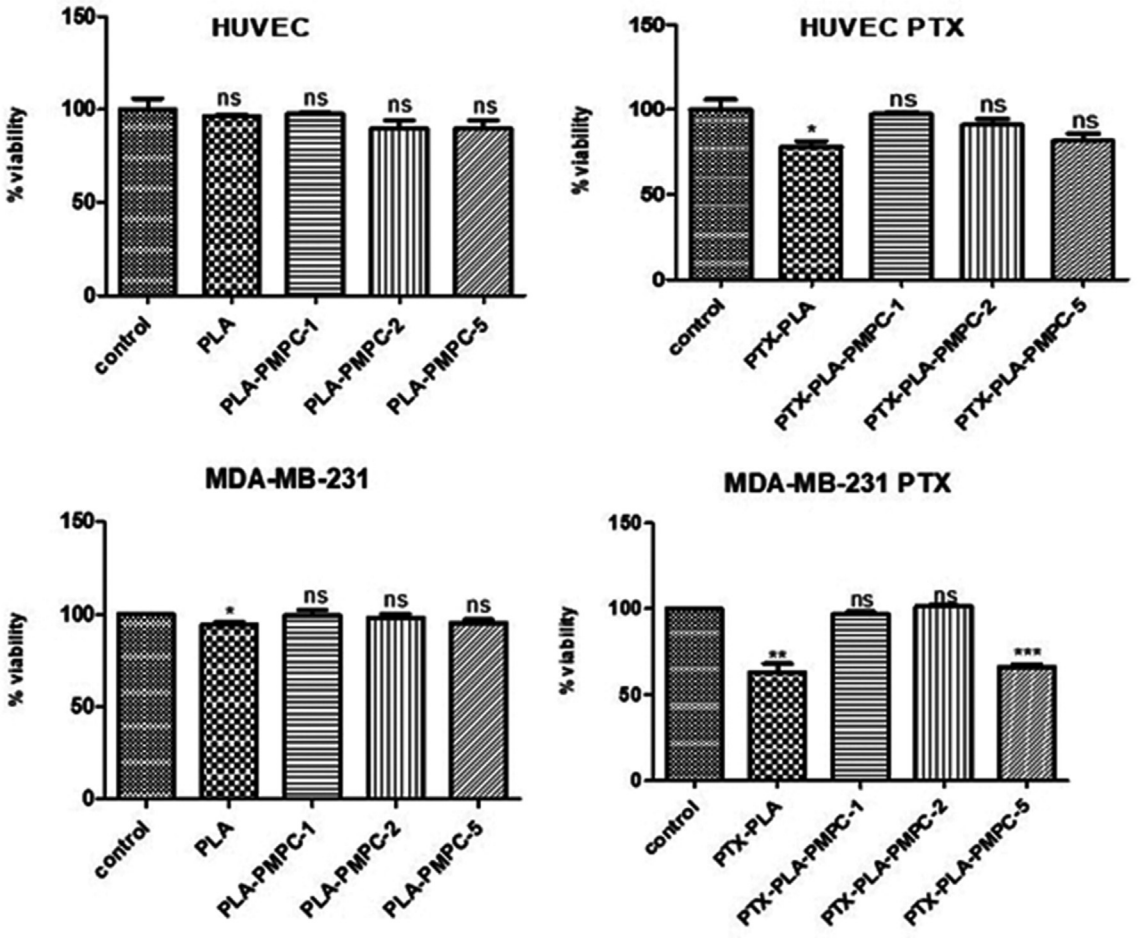


Fig. 5. Cell viability of HUVEC and MDA-MB-231 cells on the loaded with PTX and PTX-free nanofibers.

the electrostatic repulsion, and leads to faster release of loaded drug molecules. In addition, the solubility of PTX may be higher under pH7.4. Therefore, a faster PTX release was observed at low pH [44]. As can be seen, the PTX release from PLA-PMPC-1 to PLA-PMPC-5 increased. The hydrophilicity of PLA nanofiber surface increases with grafting, leading to higher drug release [45]. The PTX release was affected by loaded-drug density. The higher drug loaded-nanofiber exhibited a faster release due to the diffusion process since the release becomes easier for a high drug-loading. However, the release slowed down later due to reduced drug density [46]. We can say, lower doses could be needed as compared with conventional cancer therapy because of its constant and sustained release [47].

3.5. Cytotoxicity of PTX loaded PLA and PLA-PMPC nanofiber mats

The cytotoxicity of all the nanofibers was evaluated by MTT colorimetric assay. After 72 h, the MTT assay is shown in Fig. 5. For this study, the prepared PLA nanofiber mat exhibited high cell compatibility. After grafting of MPC monomer, the nanofiber mats showed higher biocompatibility. The viabilities of the nanofiber mats not containing PTX drug were found over 100% in both cells.

In the nanofiber mats loaded with PTX; the cytotoxic effect was observed in PTX loaded PLA polymer and PTX loaded PLA-PMPC-5 polymer in both cell lines. Cytotoxic effects were detected in HUVEC cells by 21,76% ratio in PTX-loaded PLA polymer and 18,54% ratio in PTX-loaded PLA-PMPC-5 polymer. The decrease in cell viability in MDA-MB-231 cells was detected 36,93% ratio in PTX-loaded PLA polymer and 33,92% ratio in PTX-loaded PLA-PMPC-5 polymer. When we compare it to normal cells and cancer cells, PTX loaded polymers showed more cytotoxic effect in MDA-MB-231 breast cancer cells.

The effective PTX doses on the cells are detailed in Table 2. By applying PTX alone to MDA-MB-231 and HUVEC cells, 50% viability was achieved with doses of 42.80 μM and 46.76 μM, respectively. On the other hand, with a dose of 2.17 μM, which is calculated according to the release rate, a decrease in viability of 33.92% in MDA-MB-231 cells and 18.54% in HUVEC cells was detected (Table 2). Based on these data, our polymer PTX loaded PLA-PMPC-5 gave an effective result despite the low dosage. It is also an important finding that the polymer has a more cytotoxic effect in cancer cells compared to normal cells.

In-vitro attachment and growth of HUVEC cells were also investigated by SEM. Fig. 6 shows SEM images of cells after 72 h

Table 2
Comparison of effective PTX concentrations treated to cells according to release rates.

Cell lines	PTX release rate from nanofibers (%)	The amount of PTX released from nanofibers μM (pH = 7.4)	PTX-loaded nanofibers PLA, PMPC-5 (% viability)	PTX alone (IC_{50}) μM	PTX alone (%viability)
MDA-MB-231	43,41	2,17	33,92	42,80	50
HUVEC	43,41	2,17	18,54	46,76	50

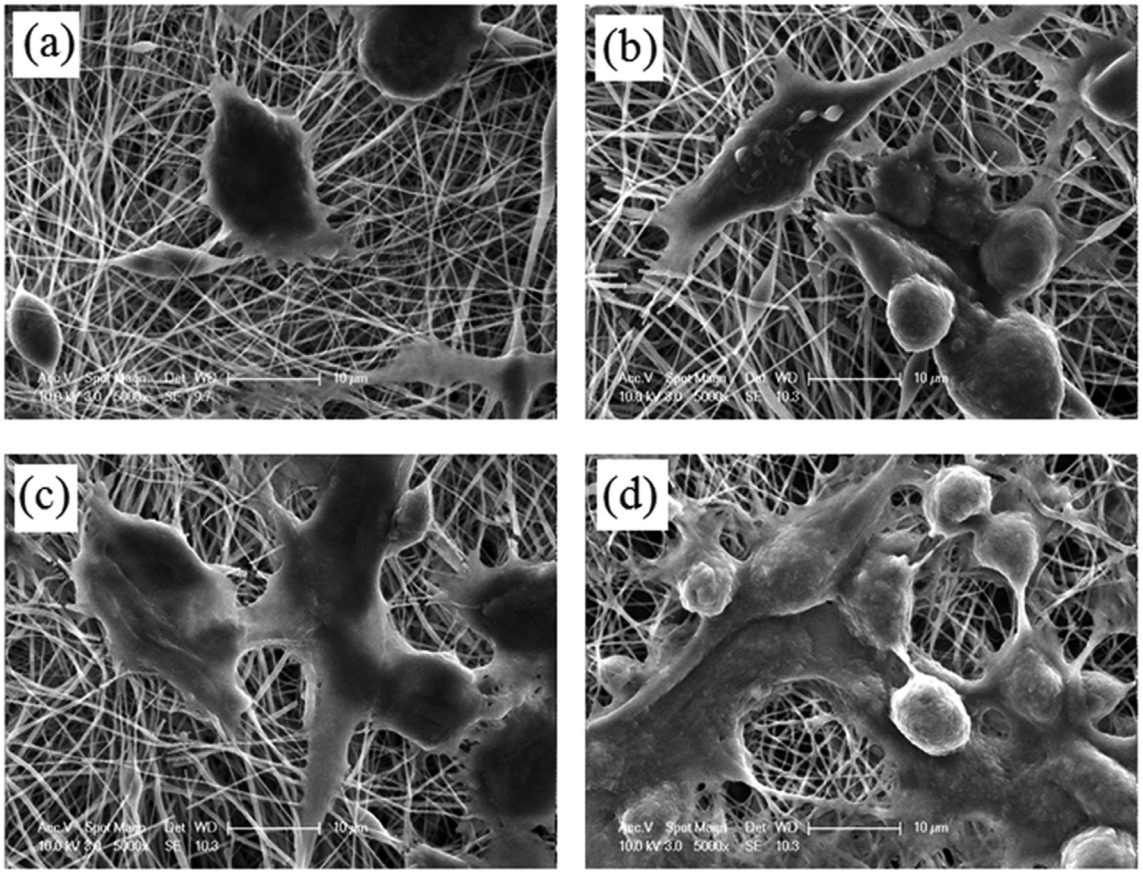


Fig. 6. SEM micrographs of HUVEC on nanofibers after 72h of culture at 5000× magnification (a) PLA, (b) PLA-PMPC-1, (c) PLA-PMPC-2 and (d) PLA-PMPC-5.

incubation. HUVEC cells well adhered and spread onto the fiber surface. The cells showed better propagation with MPC. Zwitterionic phosphatidylcholine groups of cell membrane exhibit excellent non-thrombogenic and anti-fouling properties. The structure of PMPC mimics the structure of phosphatidylcholine in the cell membrane. In addition, PMPC has resistance to the adsorption of various proteins and so generates a non-fouling interface [48].

4. Conclusion

In this study, a new polymer/phospholipid hybrid nanofiber was designed for drug delivery systems. PLA nanofiber mats were prepared by electrospinning. The PLA fibers were covered with a phospholipid polymer by UV-induced grafting. The nanofiber had more PTX loading efficiency with the increasing MPC ratio. The nanofibers exhibited a biphasic release profile. Furthermore, *in-vitro* cellular experiments demonstrated the PLA/PMPC nanofibers were biocompatible. This study demonstrated that a quite low drug level in PTX loaded PLA-PMPC polymer compared to free PTX is highly effective in the treatment of cancer cells. This selective effect may constitute an option suitable for clinical use. For future works, a wide variety of diagnostic nanoparticles with anticancer drugs could be loaded to the prepared nanofiber-based drug delivery system. Effective drug carriers can be developed for cancer treatment.

Credit author statement

Burcu Oktay: Investigation, Writing - review & editing. **Güneş Özen Eroğlu:** Investigation, Writing - original draft**. Serap Demir:** Investigation, Writing – original draft. **Serap Erdem Kuruca:** Investigation, Writing - original draft**. Nilhan Kayaman Apohan:** Supervision, Writing - review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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