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Electrospinning of ampicillin trihydrate loaded PLA nanofibers: effect of drug concentration and PLGA addition on its morphology, drug delivery and mechanical properties

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Abstract

The aim of the study is to produce ampicillin trihydrate loaded PLA and PLA/PLGA polymeric nanofibers using HFIP as solvent via electrospinning. The effect of ampicillin trihydrate concentration (4-12%), the addition of PLGA and the amount of added PLGA (20-80%) on the spinnability of the solutions and morphology, average nanofiber diameter, encapsulation efficiency, in vitro drug release and mechanical properties of PLA and PLA/PLGA nanofibers were examined. All nanofibers have shown to have favorable encapsulation efficiency and mechanical properties. As the amount of ampicillin trihydrate increased and PLGA was added, nanofiber diameter increased while mechanical properties decreased. However, as the amount of added PLGA increased, a decrease in nanofiber diameter was observed. The increase in the drug amount caused an increase in the burst effect. The ideal drug concentration was determined to be 8% (F2), as it allows the prolonged and controlled drug release for up to 10 days. While in vitro drug release decreased with the addition of PLGA to PLA, it increased with the increasement of added PLGA to PLA. As a result of the study, it was concluded that the amount of the drug and the added PLGA concentration may affect the average nanofiber diameter, morphology, in vitro drug release and mechanical properties of the obtained electrospun PLA nanofibers.

Keywords

ampicillin trihydrate; electrospinning; nanofiber; PLA nanofiber; PLA/PLGA nanofiber

Introduction

Polymeric nanofibers have been widely used in many fields such as tissue engineering and drug delivery systems. Electrospinning is the most commonly used polymeric nanofiber preparation method. Because it is a single-step, low cost, reproducible method. It allows the production of extracellular matrix-like nanofibers that provides easy scale-up and have different properties with many polymers and solvents [1-4]. Drug loaded electrospun polymeric nanofibers have many unique properties such as accelerating healing and providing controlled drug release, stimulation of cell growth and proliferation due to their similarity to extracellular matrix, large surface area, high encapsulation efficiency, high porosity and superior mechanical properties [5-7].

In our study, FDA-approved polylactic acid (PLA) and poly lactic-co-glycolic acid (PLGA), which are frequently preferred polymers in the production of polymeric nanofibers, were used. Because they are biodegradable, biocompatible, non-toxic and provide high mechanical strength [1,8]. In this study, ampicillin trihydrate, an FDA approved β-lactam antibiotics, a broad-spectrum semi-synthetic derivative of aminopenicillin, was used. Ampicillin trihydrate acts by inhibiting the synthesis of peptidoglycan, a critical component of the bacterial cell wall [9]. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was used as solvent in the study. It is preferred due to its sufficiently low surface tension and sufficiently high dielectric constant and volatility [10].

In our previous study, we produced ampicillin trihydrate loaded electrospun PLA and PLA/PCL nanofibers and the effect of PLA concentration and added PCL amount on the nanofibers properties were investigated [11]. In this research, ampicillin trihydrate loaded PLA and PLA/PLGA nanofibers with controllable morphology, nanofiber diameter, mechanical properties, encapsulation efficiency and in vitro drug release were prepared via electrospinning. The spinnability and properties of the PLA

nanofibers associated with drug concentration (4-12%) and PLGA addition and the amount of added PLGA (20-80%) were also investigated. The aim of this study was to produce and characterize ampicillin trihydrate loaded implantable PLA and PLA/PLGA electrospun polymeric nanofibers for controlled drug release with favorable properties for use in tissue engineering. Although there are studies on electrospun PLA/PLGA nanofibers, there are few studies on the effect of PLA:PLGA ratios on nanofiber morphology, nanofiber diameter, in vitro drug release and mechanical properties.

Results and Discussion

Preparation and characterization of ampicillin trihydrate loaded electrospun nanofibers

Stable jet and continuous nanofiber formation was observed in all PLA nanofibers containing different amount of drug and in PLA/PLGA nanofibers with different ratios of PLGA (Figure 1 and 2). All PLA and PLA/PLGA nanofibers were randomly aligned, smooth and bead-free (Figure 1 and 2).

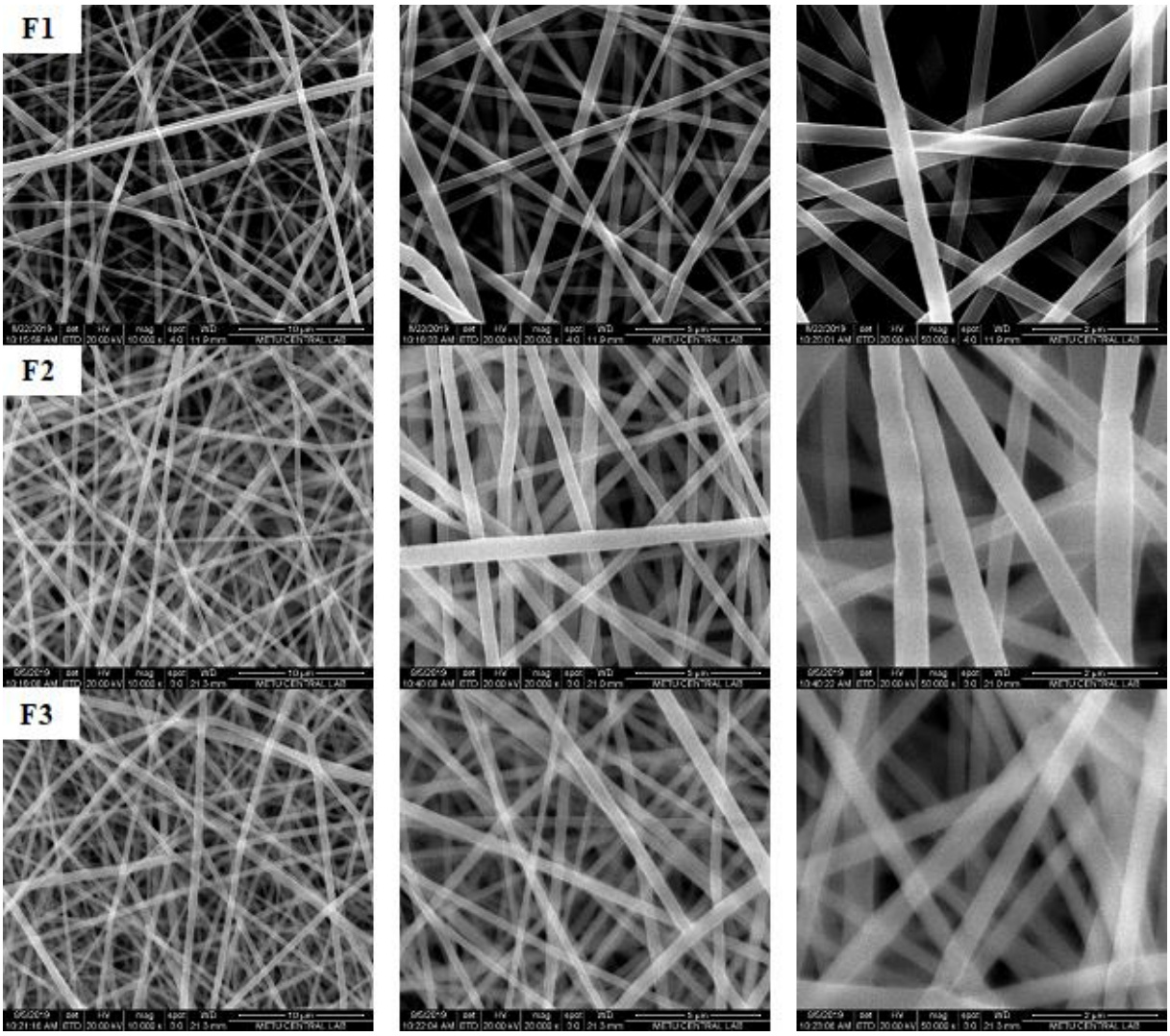

Figure 1: SEM images of nanofibers produced by change in ampicillin trihydrate concentration (F1:%4, F2:%8 and F3:%12) (A: 10.000 x, B: 20.000 x, C: 50.000 x)

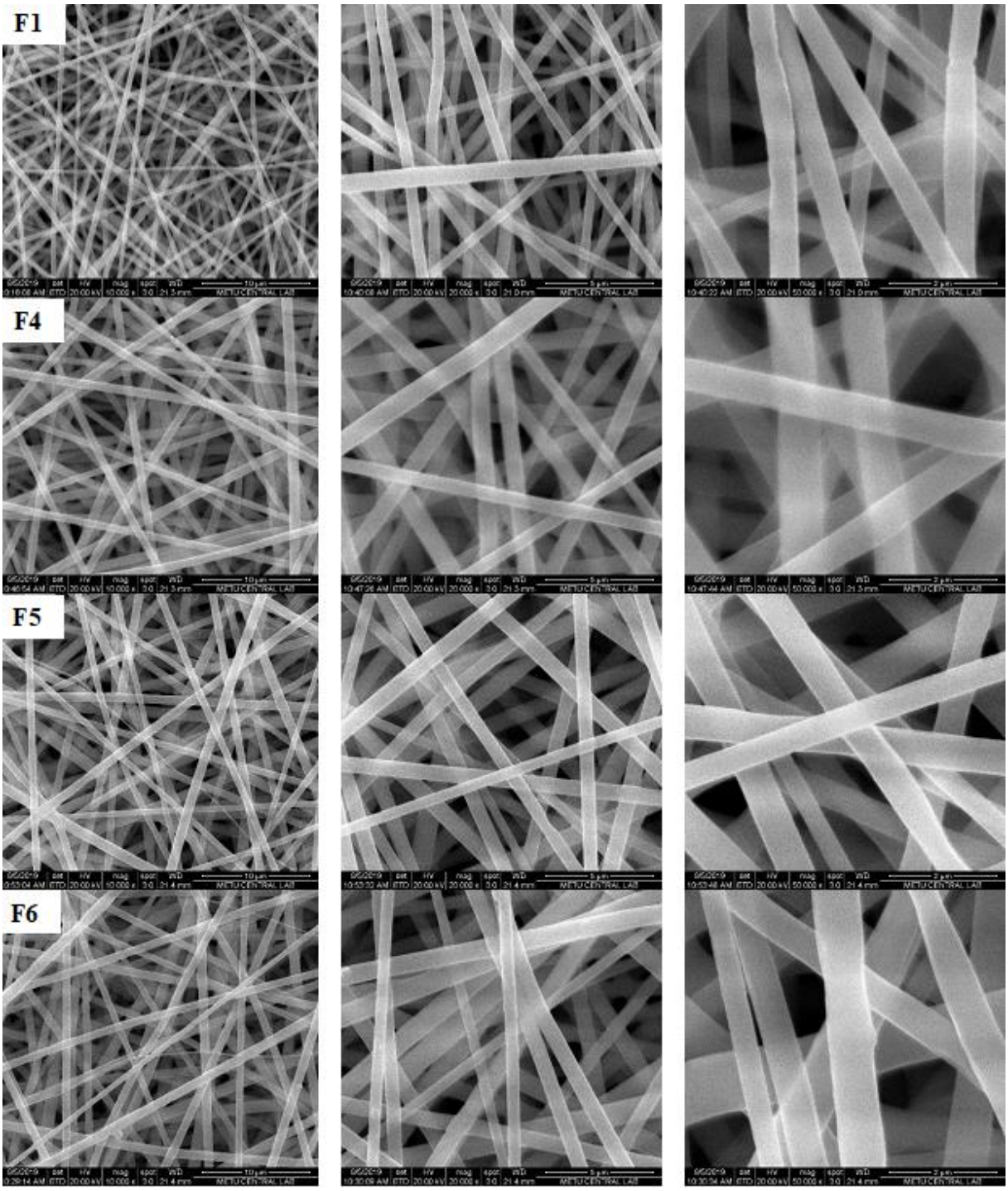

Figure 2: SEM images of PLA/PLGA [F2: PLA(100:0); F4: PLA/PLGA(80:20); F5: PLA/PLGA(60:40); F6: PLA/PLGA(20:80)]

The average nanofiber diameters calculated using SEM images of nanofibers in ImageJ were given in Table 1 and Table 2.

Table 1: PLA nanofibers prepared in the study

Formulation	Polymer	Polymer Concentration (%)	Polymer Ratio(%)	Ampicillin trihydrate (%)	Voltage (kV)	Capilary-collector distance (cm)	Flow rate (ml/h)	Diameter±SD (nm)	Encapsulation efficiency (%)
F1	PLA	10	100:0	4	11.5	10	0.8	416.5±8.4	91.3
F2	PLA	10	100:0	8	11.5	10	0.8	432.7±11.4	90.0
F3	PLA	10	100:0	12	11.5	10	0.8	476.7±9.8	64.5

Table 2: PLA/PLGA nanofibers prepared in the study

Formulation	Polymer	Polymer Concentration (%)	Polymer Ratio(%)	Ampicillin trihydrate (%)	Voltage (kV)	Capilary-collector distance (cm)	Flow rate (ml/h)	Diameter±SD (nm)	Encapsulation efficiency (%)
F2	PLA:	10	100:0	8	11.5	10	0.8	432.7±11.4	90.0
F4	PLA: PLGA	10	80:20	8	11.5	10	0.8	820.0±10.4	89.4
F5	PLA: PLGA	10	60:40	8	11.5	10	0.8	747.9±14.7	89.9
F6	PLA: PLGA	10	20:80	8	11.5	10	0.8	447.1±6.6	91.2

The diameters of the PLA nanofibers ranged from 417 to 477 nm (Table 1). As the amount of drug in the nanofiber increased, the nanofiber diameter increased (Table 1, Figure 1). While the diameter of the nanofiber containing 4% drug was 417 nm, when the amount of drug was increased to 8% and 12%, the nanofiber diameter increased

to 433 and 477 nm, respectively ($p<0.05$). This could be attributed to the increase in the amount of drug resulting in surface loading [12].

In order to examine the effects of PLGA addition on PLA nanofibers, PLA/PLGA nanofibers were produced by replacing 20% to 80% of PLA with PLGA in the F2 coded formulation containing 8% drug (Figure 2). As can be seen in Table 2, the diameters of the PLA/PLGA nanofibers ranged from 447 to 820 nm. The addition of PLGA to PLA led to an increase in nanofiber diameter. The highest increase in nanofiber diameter was in F4 coded nanofiber, which's PLGA ratio was 20%. The nanofiber diameter increased from 433 nm to 820 nm with the replacement of 20% PLGA ($p<0.05$). However, as the amount of added PLGA increased, a decrease in nanofiber diameter was observed. When the PLGA ratio was increased to 40% and 80%, the nanofiber diameter was 745 nm and 447 nm, respectively. The increase in nanofiber diameter with the addition of PLGA can be explained by the higher molecular weight of PLGA than that of PLA. Because the increase in polymer molecular weight increases the viscosity, which leads to the increase of nanofiber diameter [13-15]. Another reason for this was that the average diameter of nanofibers changes with the change of polymer type [16-17]. In a study conducted by Liu et al. (2012), unlike our results, the diameter of PLGA/PLA nanofibers increased with a decrease in the amount of PLGA [8].

In a study, it was found that by increasing the amount of PCL in PLGA/PCL nanofibers from 10% to 20%, the fiber diameter decreased from 1000 nm to 500 nm, but as the amount of PCL increased to 30%, the diameter increased to 2000 nm [1]. Similarly, in our previous study, the increase in the amount of PCL initially caused an increase in fiber diameter, while it decreased as the amount of added PCL increased. In our previous study, fiber diameter increased from 1168 nm to 1334 nm when 10% of PLGA was replaced by PCL. However, the fiber diameter decreased to 1128 nm by adding

25% PCL, and to 770 nm by adding 50% PCL to the formulation [15]. These demonstrated that the effect of PCL addition on the diameters of PLGA fibers was not linear. In the present study, it was proven that adding PLGA to PLA caused an increase in nanofiber diameters independent of the increasement in the amount of added PLGA.

Encapsulation Efficiency of Nanofibers

The encapsulation efficiency of PLA nanofibers containing up to %8 drug was quite high. As the amount of ampicillin trihydrate increased, the encapsulation efficiency decreased. A significant decrease in encapsulation efficiency was observed with increasing the drug content to 12% (Table 1) ($p<0.05$). While the encapsulation efficiency of nanofibers containing 4% and 8% ampicillin trihydrate was about 90%, it decreased to 65% when the amount of ampicillin trihydrate was increased to 12%. It is thought that the encapsulation efficiency is reduced because of the excess drug loading leading to undissolved drug in solution [18]. In addition, F3 coded nanofiber containing 12% ampicillin trihydrate may have formed a heterogeneous matrix instead of a homogeneous matrix.

The addition of PLGA and the amount of added PLGA to PLA did not cause a change in encapsulation efficiency. The encapsulation efficiency of PLA/PLGA nanofibers was also quite high (about 90%).

Dissolution Studies

In vitro drug release from PLA and PLA/PLGA nanofibers was examined. The *in vitro* drug release of PLA electrospun nanofibers produced by varying the amount of ampicillin trihydrate in Table 1, was shown in Figure 3.

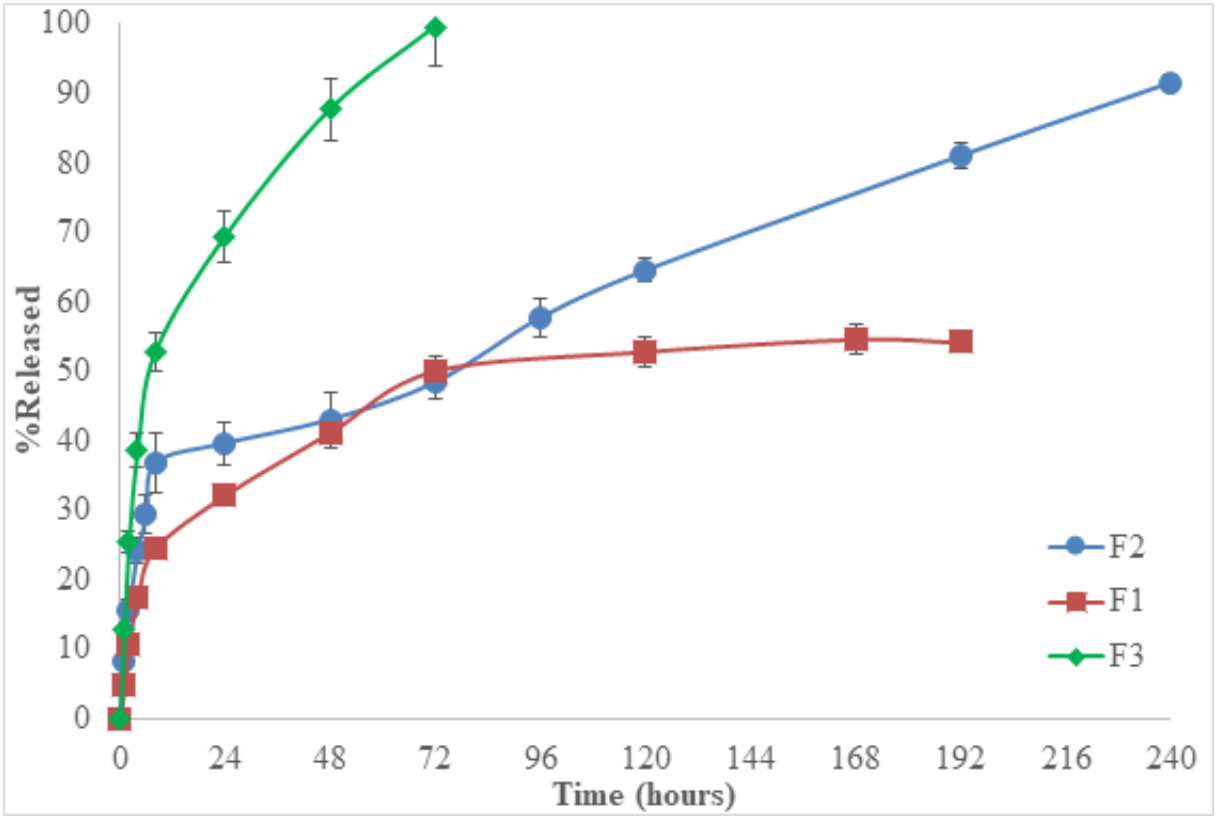

Figure 3: Effect of drug amount on in vitro release from PLA nanofibers

As can be seen in Figure 3, increasing the amount of drug increased the burst effect. Cumulative drug release in 24 hours was 32.1%, 39.6% and 69.4% for those containing 4% (F1), 8% (F2) and 12% (F3) ampicillin trihydrate, respectively. Drug release ended within 3 days in the F3 coded formulation containing 12% ampicillin trihydrate, while the drug release ended on the 7th day in the F1 coded formulation containing 4% ampicillin trihydrate. In the F2 coded formulation containing 8% drug, drug release continued for up to 10 days (Figure 3). It was concluded that the optimum ampicillin trihydrate concentration in PLA nanofibers was 8% due to the prolonged and most controlled *in vitro* drug release. In other studies conducted on different polymer and polymer blends and drugs, it has been shown that the increase in the amount of drug caused a higher burst effect and faster drug release [12,19-20].

In vitro drug release of PLA/PLGA electrospun nanofibers produced by replacing 20% to 80% of PLA with PLGA in the F2 coded formulation containing 8% drug was shown in Figure 4.

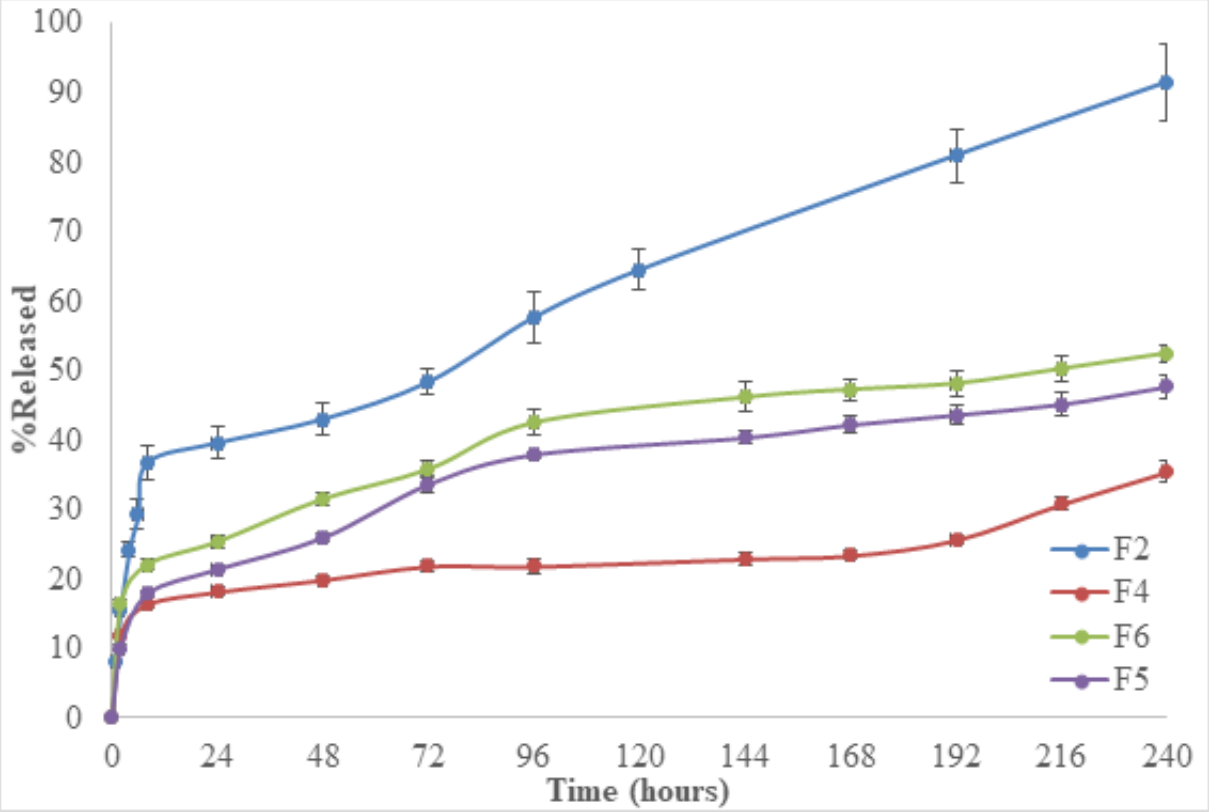

Figure 4: Effect of PLGA amount on in vitro release from PLA/PLGA nanofibers

As can be seen in Figure 4, *in vitro* drug release decreased with the addition of PLGA to PLA, and increased with the increasement of added PLGA used in the production of PLA/PLGA electrospun nanofibers. The addition of PLGA and the increase in the amount of PLGA also caused a decrease in the burst effect (Figure 4). As can be seen in Table 2 and Figure 4, the *in vitro* drug release decreased in association with the reduction in nanofiber diameters with the addition of PLGA. Drug release was slower in large diameter nanofibers due to the greater distance required for the drug to diffuse and lower specific surface areas relative to fine diameter fibers [15,21-22].

Another reason why drug release was slower in PLA/PLGA nanofibers compared to PLA nanofiber may be that the PLGA molecular weight was higher than the PLA molecular weight. Srikar et al. (2008) showed that the change in polymer content and molecular weight also affects the drug release rate as they affect the nanoporosity and desorption enthalpy of nanofibers [23]. Because the increase in the molecular weight of the polymer led to a decrease in nanoporosity and an increase in viscosity of polymer solution and diameter of nanofibers [13,23], drug release from PLA/PLGA nanofibers with a smaller surface area was slower than PLA nanofibers with a larger surface area.

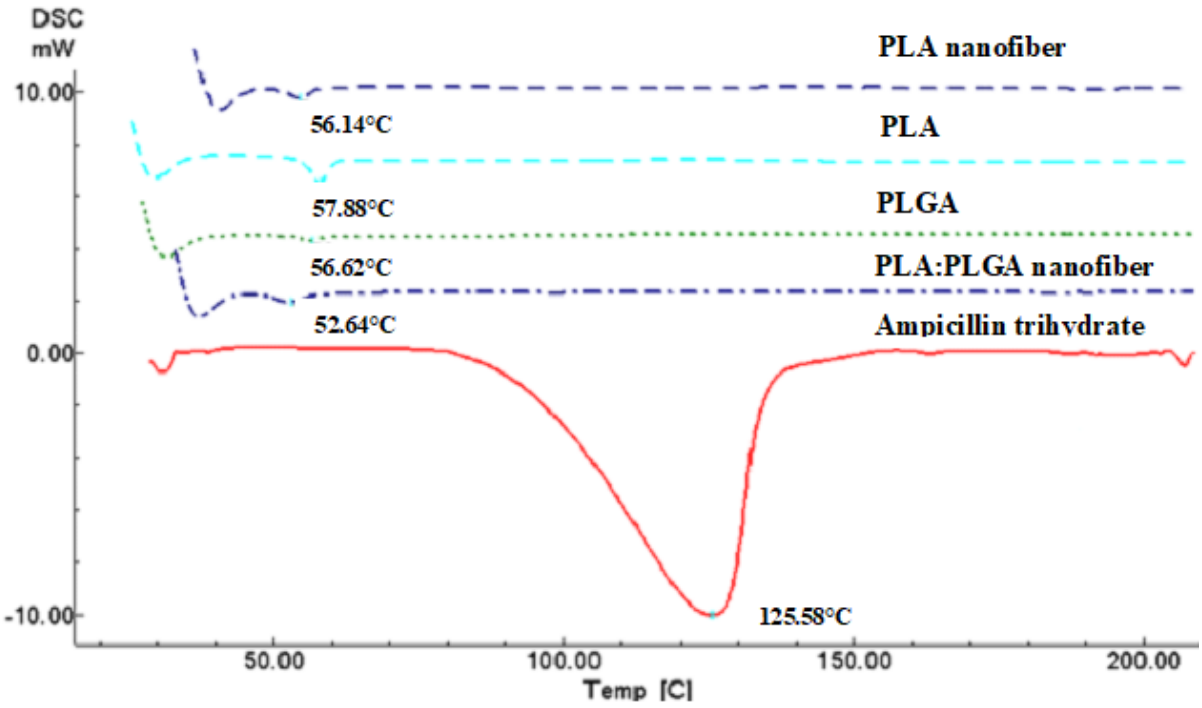

Figure 5: DSC thermograms of ampicillin trihydrate, PLA, PLGA, PLA nanofiber and PLA/PLGA nanofiber

As shown in Figure 5, the absence of the melting endotherm peak at 125.58°C specific to pure ampicillin trihydrate in the DSC thermograms of the PLA, PLGA, PLA nanofibers and PLA/PLGA nanofibers proved that ampicillin trihydrate was loaded in the nanofibers in amorphous form.

Mechanical Properties of Nanofibers

Mechanical properties of nanofibers depend on their composition, porosity, average size and distribution, individual nanofiber orientation, interaction between nanofibers, and arrangement and entanglement of the nanofibers [24-26].

Mechanical properties of PLA nanofibers containing different amounts of drug were shown in Table 3. Due to the increase in the amount of drug causing an increase in nanofiber size, both the tensile strength and the tensile modulus of the nanofibers decreased ($p<0.05$) (Table 1 and Table 3).

Table 3: Mechanical properties of PLA nanofibers

Formulation	Tensile strength±SD (mPa)	Elongation at break±SD (%)
F1	2.62±0.46	21.59±7.51
F2	2.06±0.34	11.64±0.95
F3	1.77±0.24	9.52±1.26

The tensile strength and elongation at break value of PLA nanofibers containing 4% drug were 2.62 mPa and 21.59%, respectively. When the amount of drug increased from 4% to 8%, they decreased to 2.06 mPa and 11.64%, while the nanofiber diameter increased from 417 nm to 433 nm ($p<0.05$). Similarly, increasing the drug amount to 12% caused an increase in nanofiber diameter and a decrease in mechanical properties ($p<0.05$). As the increase in the amount of drug caused an increase in nanofiber size, both tensile strength and tensile modulus of the nanofibers decreased ($p<0.05$) (Table 1 and Table 3).

The size of the nanofiber affects the deformation behavior. This is because larger diameter of fibers tend to display bulk-like properties [27]. The effect of nanofiber diameter on the mechanical properties observed in this study was similar to our previous studies with linezolid loaded PLGA and PCL/PLGA nanofibers [15,28]. Chew

et al. (2006) also showed that the increase in the amount of bovine serum albumin caused an increase in nanofiber diameter and a decrease in the mechanical properties of poly (caprolactone-co-ethyl ethylene phosphate) nanofibers [29].

The tensile strength of the PLA nanofiber was 2.06 mPa and the elongation at break value was 11.64%. As PLGA was added to PLA nanofiber, the mechanical properties of PLA/PLGA nanofibers increased and the nanofibers had a harder structure. When the PLGA concentration was 20% (F4), 40% (F5) and 80% (F6), the tensile strength increased to 2.58 mPa, 2.66 mPa and 2.15 mPa, respectively (Table 4).

Table 4: Mechanical properties of PLA/PLGA nanofibers

Formulation	**Tensile strength±SD (mPa)**	**Elongation at break±SD (%)**
F2	2.06±0.34	11.64±0.95
F4	2.58±0.27	12.46±1.04
F5	2.66±0.20	11.49±0.40
F6	2.15±0.17	11.94±0.85

The difference in mechanical properties could be explained by the increase in nanofiber diameter. As can be seen in Table 2 and 4, the increase in mechanical properties was directly proportional to the increase in nanofiber diameter. While the increase in both nanofiber diameter and mechanical properties in F4 and F5 coded PLA/PLGA nanofibers was statistically significant compared to F1 coded PLA nanofibers ($p<0.05$), these increases were not statistically significant in F6 coded PLA/PLGA nanofibers ($p>0.05$). The reason that the increase in diameter with the increase of PLGA led to an increase in mechanical properties was that nanofibers had a compact arrangement and a stable structure. This may also be due to the increase in diameter causing reduced porosity [8].

Conclusion

Ampicillin trihydrate loaded smooth, bead-free PLA and PLA/PLGA electrospun nanofibers have been successfully developed as an implantable system. They can be effectively use in tissue engineering and controlled drug delivery due to their structure features a morphological similarity to the extracellular matrix. Characterization of the nanofibers was also performed.

As a result of this study, F2 coded PLA nanofiber with the ideal drug concentration (8%) was chosen as the ideal PLA nanofiber, since it allows the best controlled drug release with its favorable encapsulation efficiency, nanofiber diameter, morphology and mechanical properties. In vitro drug release decreased with the addition of PLGA to PLA, while it increased with the increasement of PLGA used in the production of PLA/PLGA electrospun nanofibers.

From our study, it may be concluded that the average nanofiber diameter, mechanical properties and in vitro drug release of PLA nanofibers are dependent on drug concentration and the amount of added PLGA to PLA. It has been also concluded that the systemic side effects of the drug can be reduced by local application of drug loaded nanofiber with increased patient compliance and treatment efficacy.

Experimental

Materials

Ampicillin trihydrate was obtained from Atabay (Istanbul, Turkey) as a gift. PLA (MW of 103000 g/mol), ester terminated PLGA (MW of 190000–240000 g/mol, a lactide/glycolide ratio of 85:15) and HFIP were obtained from Sigma-Aldrich. All the other chemicals used were analytical grade.

Electrospinning

PLA nanofibers and PLA/PLGA nanofibers prepared in the study were given in Table 1 and Table 2, respectively. 10% (w/v) polymer solution was prepared by dissolving polymer in HFIP as solvent. Then 4-12% w/w ampicillin, based on dry weight of polymer, was dissolved in polymer solutions. For electrospinning, the solutions were poured in a plastic syringe (5 ml) fitted with a 21 G needle. The syringe was then placed in a syringe pump and a high voltage was applied between the needle and grounded stationary rectangular metal collector. The process parameters used in the current study were shown in Table 1 and Table 2 (Electrospinning machine Ne-200, Inovenso, Turkey). The collector covered by a piece of aluminum foil was used for the fiber deposition. The deposited fiber mats were dried for 72 hours at room temperature and stored in a desiccant until the analysis.

Characterization of nanofibers

Differential scanning calorimetry analysis (DSC)

DSC thermograms of drug, polymer and nanofibers were obtained using DSC (Shimadzu DSC-60, Kyoto, Japan). The samples were heated from 25 to 200 °C at a rate of 10 °C/min under a nitrogen atmosphere.

Encapsulation efficiency of electrospun nanofibers

Nanofibers (1x1 cm) were weighed and 1 mL of dichloromethane was added to dissolve fiber mats and an hour later 7 mL of pH 7.4 phosphate buffer was added to dissolve drug released from the nanofibers (n=3) . After removing the DCM, the volume of each solution was made up to 10 mL with buffer and the amount of ampicillin trihydrate was analyzed with a UV spectrophotometer (Thermo Scientific Evolution

201). The encapsulation efficiency of the nanofibers was calculated using the following equation.

Encapsulation efficiency = (The amount of drug loaded/Theorical drug amount in the nanofiber)×100

In vitro drug release

Static method was used to evaluate the in vitro drug release of the nanofiber mats. Nanofiber mats (2×2 cm) were weighed and incubated in 5 mL of pH 7.4 phosphate buffer solution at 37ºC (n=3). All of the release medium was removed and 5 mL of fresh solution was added at predetermined time intervals. The amount of drug released was assayed with the UV spectrophotometer.

For calibration and validation of ampicillin trihydrate, solutions were prepared at 2.5-30 µg/mL concentrations by dilution from 200 µg/mL ampicillin trihydrate stock. Absorbances were measured at a wavelength of 213 nm (y= 0.0309x+0.0466, r^2=0.9984).

Morphologies of electrospun nanofibers

Nanofibers were firstly gold-coated and morphologies of the electrospun nanofibers were observed by scanning electron microscope (SEM) (QUANTA 400F Field Emission SEM, Holland). The average diameters of resulting nanofibers were calculated by the measurement of 100 single nanofibers from SEM images using analysis software (Image J, USA).

Mechanical properties

The mechanical properties such as tensile strength and elongation at break values of the electrospun nanofibers (2×1 cm) were evaluated on Texture Analyzer (TAXT Plus, Stable Micro Systems, United Kingdom) with an extension rate of 10 mm/sec (n=3).

Tensile strength (mPa) and elongation at break (%) values of the nanofibers were calculated from the strain-stress curves.

Statistical analyses

All data were expressed as mean ± SD. Statistical analyses were performed using SPSS 20.0 for Windows (SPSS, Chicago, IL). The significance was evaluated with one-way ANOVA followed by Tukey's post hoc test (SPSS 20.0). The data were considered to be significant at $p < 0.05$.

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