Electrospinning of doxorubicin loaded silica/poly(ε-caprolactone) hybrid fiber mats for sustained drug release

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Abstract
Loading of anticancer drugs into electrospun fiber matrices is a portentous approach for clinical treatment of diseased tissues or organs. In this study, doxorubicin hydrochloride (DOX) is added to silica nanoparticles (SiO2) during the formation of SiO2 via the sol-gel approach. The obtained DOX@SiO2 nanoparticles are then added to poly(ε-caprolactone) (PCL) and poly(ethylene oxide) (PEO) blend before electrospinning process via different methods. The effects of DOX addition as a free form or as DOX@SiO2 nanoparticles on physical and chemical properties of obtained PCL-PEO fibers, as well as release profiles are evaluated to give a continual DOX release for several days. The morphology observed with scanning electron microscope (FESEM) revealed significant changes in the average diameter of obtained fibers ranging from 2164 nm to 659 nm and distribution of drug-loaded nanoparticles in the final mats according to the mode of additions. With the same manner, the releasing performances of obtained mats are quite different. Therefore, fabrication of drug loaded mats would offer a powerful approach to minimize serious side effects for clinical patients and allows us to control the drug concentration in the bloodstream.

Keywords: electrospun fibers, poly(ε-caprolactone), doxorubicin hydrochloride, release profiles, silica nanoparticles, different methods
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micro- or nanofibers have been applied as a drug carrier, because of the wide scope for direct incorporation of hydrophobic and hydrophilic drugs also biomacromolecules like proteins or RNA into the electrospun fibers mats [6]. The interconnected porous feature and high surface area of electrospun fibers help us to decrease the drug dose within the implant, leading to a less systemic toxicity and reduce not desired side effects. Moreover, the release profile of drug from the electrospun webs can be modified by adjusting the fiber morphology, porosity and texture [7]. Therefore, electrospun polymer fibers would provide an ersatz approach for long-term antitumor activity via incorporation of the target therapeutic agent into electrospun fibers [4]. Several anticancer drugs have been loaded into various electrospun fibers for postsurgical cancer therapy [8, 9]. Doxorubicin hydrochloride (DOX) is one of the most widely employed chemotherapeutic drugs in cancer treatment. It is a class of anthracycline antibiotic which acts on the S-phase of the cell cycle and causes cell death by damaging DNA and its synthesis through the intercalation between nucleotides, generation of oxygen-free radicals and inhibition of topoisomerase II [10]. When the drug loaded into the surface of the nanofibers an undesirable burst release is constantly inevitable in account of their high ionic strength in solution and the solvent quick evaporation through electrosprining [11]. To overcome this issue, several inorganic nanocarriers have been entrapped into electrospun nanofibers so as to prevent initially burst release of drug and provide a slow sustainable release [12]. Silica nanoparticles are chosen as the drug carrier as: silica nanoparticles (SiO$_2$), have raised much interest in the preparation of drug delivery systems because of their high surface areas and porous interiors which permit them to be used as reservoirs for loading both hydrophilic and hydrophobic drugs. In addition, SiO$_2$ was reported to be able to promote the dissolution of the indigent water-soluble indications and enhance their bioavailability [2, 13, 14].

Among the famous synthetic polymers used in electrospinning are hydrophobic polycaprolactone (PCL) and hydrophilic polyethylene oxide (PEO). PCL is a semi-crystalline biodegradable thermoplastic polymer and has been confirmed by the Food and Drug Administration (FDA) in the USA as a biodegradable material for biomedical purposes as drug delivery and tissue engineering applications [15]. PEO is a water-soluble amphiphilic polymer with good biological features, including biocompatibility and soft toxicity [16]. This work aimed to load DOX (as an anticancer drug) into the silica nanoparticles and find out the right set of conditions to incorporate drug loaded nanoparticles into PCL-PEO nanofiber mats. The chemical also physical characteristics of the fabricated fiber mats and nanoparticles are characterized. In addition, the suitability of the prepared polymeric mats, as a substrate for drug release application, was investigated by studying the drug releasing profile.

2. Materials and methods

2.1. Materials

Poly($\varepsilon$-caprolactone) (PCL with average molecular weight 80,000 g mol$^{-1}$) and polyethylene oxide (PEO) with molecular weight 900,000 g mol$^{-1}$) were bought from Sigma Aldrich. Doxorubicin hydrochloride (DOX, $M_w$ = 580) was secured from the Beijing Huafeng United Technology Co, Ltd. Chloroform purchased and tetraethyl orthosilicate (TEOS) were get from Sigma-Aldrich. Methanol, absolute ethanol and ammonia solution (25%) were obtained from El-Nasr Company.

2.2. Methods

2.2.1. Preparation of SiO$_2$ and DOX@SiO$_2$. Silica nanoparticles are prepared via sol-gel process based on the Stöber method using tetraethyl orthosilicate (TEOS, $\text{Si(OCH}_3\text{H}_2)$$_3$) as a precursor [17]. In brief, 110 ml ethanol and 5 ml ammonia solution (25%) is stirred together for about 30 min in a closed polyethylene bottle. Then, 8 ml TEOS is appended to the above solution and is further stirred at room temperature for 24 h. The particles in the sol are afterward gathered by centrifugation and washed three times with distilled water. Finally, the obtained wet particles are dried at 40 $^\circ$C for about 24 h. For the drug loaded silica nanoparticles, DOX is incorporated during the formation of silica nanoparticles. At first, different amounts of DOX with respect to weight of silica (0.5, 2, 4%wt of SiO$_2$) are dissolved in ethanol/ammonia solution before TEOS addition. The obtained DOX@SiO$_2$ nanoparticles are named $D_{0.5}$, $D_2$ and $D_4$, respectively.

2.2.2. Preparation of the electrospinning solution. PCL with an optimized concentration (10%) and PEO with an optimized concentration (3% relative to PCL) are dissolved in a blend of chloroform:methanol (3:1 v/v) for all the samples. The PCL-PEO solution is loaded into a 1 ml plastic syringe to which a metal blunt ended needle (G18) is attached. The injection velocity through the syringe pump into the nozzle is adjusted with a flow rate at 10 ml h$^{-1}$. The high voltage supply positive output lead is attached to the needle on the syringe, while the working platform is connected with the negative pole. For drug contained fiber, five methods are applied to incorporate DOX and DOX@SiO$_2$ nanoparticles into the polymer solution as follow: (i) Method (named $M_1$), a definite amount of DOX are added to PCL-PEO solution. After dissolution, the prepared solution is transferred to a 1 ml plastic syringe for electrospinning. (ii) Method (named $M_2$), DOX loaded to SiO$_2$ nanoparticles are mixed with the polymer solution instead of free DOX. (iii) Method (named $M_3$), the PCL-PEO and DOX@SiO$_2$ mixture is sonicated for about 30 min. (iv) Method (named $M_4$), the DOX@SiO$_2$ nanoparticles are separately sonicated for about 30 min before the addition to PCL-PEO solution. The obtained mixture are again stirred for 1 h and sonicated for 30 min. (v) Method (named $M_5$) (core shell), DOX@SiO$_2$ nanoparticles are dispersed in chloroform/methanol mixture via ultrasonic and then loaded into 1 ml plastic syringe. The PCL-PEO solution is transferred into another syringe fitted with a needle having a tip diameter larger than the tip diameter of the syringe used for DOX@SiO$_2$ nanoparticles solution. Finally, the formed electrospun mats are dried at room temperature for 24 h to permit the total solvent removal.
2.3. Characterization

2.3.1. Microstructure characterizations. The morphology of the electrospun fibers is examined under FESEM that processed using FESEM model (Quanta FEG 250 FEI, Holanda). The diameter of the fiber and their distribution is estimated from image j software. The as prepared silica and DOX@SiO₂ nanoparticles are examined by TEM, JeolJem-1230. Fourier transform infrared spectroscopy (FTIR) of all mats is performed by Jasco, FTIR-6100 type A. The FTIR spectra are obtained by using a high-resolution spectrophotometer Bruker D8 advance CuK target with secondary monochromator at 40 kV and 40 mA. The thermal properties and weight changes are analyzed as a function of temperature by thermal gravimetric analysis (TGA), NETZSCH STA 409 instrument. The test is performed at a heating rate of 10 °C min⁻¹ under nitrogen atmosphere from RT up to 1000 °C. Differential scanning calorimetry (DSC) measurements are carried out using Mettler DSC 822/400 thermal analyzer instrument having sub-ambient capability.

2.3.2. In vitro drug release. All the electrospun mats are cut into 1.0 × 1.0 cm² square pieces. The sample weight is recorded before dipping into a centrifuge tube filled with 10 ml of phosphate buffered saline solution (PBS). At time periods of 1 h, 2 h, 3 h, 2 d, 6 d, 10 d, 14 d, 21 d and 33 d the amount of DOX released in the PBS is measured using UV-Vis spectrophotometer by measuring the absorbance at 480 nm. The experiments are processed in triplicate per sample. The percentage of released drug is calculated using the following equation:

\[
\text{Released drug (\%)} = \frac{M_t}{M_{tot}} \times 100,
\]

where \(M_t\) is the mass of DOX released at time \(t\) and \(M_{tot}\) is the total amount of DOX present on the fibrous mat [4].

3. Results and discussion

3.1. Morphological observations of electrospun PCL/PEO fibers

Figure 1 shows the FESEM images of the electrospun PCL-PEO fibers loaded with DOX and DOX@SiO₂ via different methods at different magnifications. As can be seen in figures 1(A), (A1) and (A2), \(M_1\) method leads to agglomeration of DOX particles on the surface of the fibers. This could be due to the diameter of PCL-PEO fiber which is smaller than DOX aggregates. The average diameter of PCL-PEO fiber is 1109.91 nm (figure 2(\(M_1\))). The same agglomeration is also seen for DOX@SiO₂ nanoparticles loaded on the fibers by the \(M_2\) method as shown in figures 1(B), (B1) and (B2)) with an average diameter of about 659.217 nm (figure 2(\(M_2\))). On the other hand, the obtained \(M_3\) method fiber shows less agglomeration of DOX@SiO₂. The dumbbell-shaped features of PCL-PEO suggest the encapsulation of drug particles inside the nanofiber shown in figures 1(C), (C1) and (C2)). This could be due to ultrasonication of the samples before electrospinning. The average diameter of the obtained fibers is 1492.124 nm (figure 2(\(M_3\))). However, the loading of DOX@SiO₂ by the \(M_4\) and \(M_5\) method resulted to the insertion of the all particles inside the electrospun PCL-PEO nanofibers as shown in figures 1(D), (D1), (D2), (E), (E2) and (E3)). The average diameter of obtained fibers is increased to 1880.713 nm for \(M_4\) fibers and 2163.978 nm for \(M_5\) method (figures 2(\(M_4\)) and (\(M_5\))). Some of the DOX@SiO₂ nanoparticles find in agglomeration inside the fibers caused the formation of the swollen beads on the nanofibers as shown by the dashed green circles. The above results focus on the effect of ultrasonication on morphology and nanoparticles distribution within the fiber mats, in which the loaded nanoparticles are highly agglomerate in absence of ultrasonic (\(M_2\)) and less agglomeration distribution, is obtained with \(M_5\) due to the sonication that separate agglomerated particles from each other. In this consequence, uniform distribution is achieved via sonication of DOX@SiO₂ nanoparticles before and after the addition to the polymer solution, \(M_5\) method. Finally, \(M_5\) method provides an alternative approach for high drug encapsulation capability as presented by FESEM micrographs.

3.2. The microstructure analysis of PCL-PEO fibers and DOX@SiO₂ nanoparticles

The chemical structure analyses of the obtained fiber mats and DOX@SiO₂ nanoparticles are characterized by FTIR (figure 3). The FTIR spectrum of pure PCL-PEO nanofiber is shown in curve A of figure 3(a) through this spectrum the band at about, 1723.7 cm⁻¹ is associated with CO – stretching vibration of ester in PCL, the band occurred at 1237.7 cm⁻¹ is related to the O–C–O asymmetric stretching vibrations of PCL, and that of symmetric stretching occur at 1178.03 cm⁻¹, the bands at about 1104 cm⁻¹ and 1043.1 cm⁻¹ may be associated with the vibration of C–O present on PCL and the vibrational band at about 726.06 cm⁻¹ is related to CH₂ of PCL [18].

The intense doublet band at about 2944.09 cm⁻¹ and 2868.8 cm⁻¹ assigned to asymmetric and symmetric vibration of CH₃ of PEO, respectively. The two bands at 1462.2 cm⁻¹ and 1290.2 cm⁻¹ are concerned with scissoring and twisting vibration of CH₂, respectively [19]. The band at 1365.4 cm⁻¹ is associated with wagging vibration of CH₃ belongs to PCL and PEO. Also the band at about 960.7 cm⁻¹ is assigned to rocking vibration of CH₂ found on PCL and PEO. On the other hand, there are some changes occur on the FTIR spectra of PCL-PEO fibers loaded with DOX or DOX@SiO₂. As illustrated in curves B, C and D of figure 3(a) for \(M_1\), \(M_2\) and \(M_3\) methods, respectively, we can see that the bands at about 1462.2 cm⁻¹, 1365.4 cm⁻¹, 1237.7 cm⁻¹, 1137.03 cm⁻¹, 1043.1 cm⁻¹ do not exist in the spectra. Also the intensity of the band at about 1723.7 cm⁻¹ and intensity of doublet bands at about 2944.09 cm⁻¹ and 2868.8 cm⁻¹ decrease in these spectra as compared to the spectrum of pure PCL-PEO. All these alterations could be related to the presence or adsorption of loaded particles on their surface. For the other methods \(M_4\) and \(M_5\), the spectra are resembled to the spectrum of pure
PCL-PEO which may be because of DOX@SiO₂ particles that are encapsulated by PCL-PEO fibers.

Figure 3(b) shows the FTIR spectra of pure SiO₂ and samples of DOX loaded on silica nanoparticles at different ratios 0.5, 2, 4% named D₀.₅, D₂, D₄, respectively. The spectrum of pure SiO₂ nanoparticles illustrates the presence of absorption bands arising from asymmetric vibration of Si–O on the range of (1046 – 1232 cm⁻¹), asymmetric vibration of Si–OH at (945.3 cm⁻¹), and symmetric vibration of Si–O appeared at (797.1 cm⁻¹). The intense characteristic absorption band at the range (3095.7 – 3640 cm⁻¹) ascribed to O–H of stretching in H-bonded water. Also the band assigned at (1629.2 cm⁻¹) is due to scissor bending vibration of molecular water. The loading of DOX into the silica nanoparticles led to obvious changes on the vibrational bands. The broadening of asymmetric vibration band of Si–O increased with the loading of drug into the silica nanoparticles. The intensity of vibration band of Si–OH decreases with the incorporation of DOX into the silica nanoparticles and shifts to higher wavenumbers. This may be due to: presence of drug into the silica nanoparticles increases their diameters which in turn decrease the length of the bond between Si–OH which cause its shift to higher wavenumbers.

TEM images of pure SiO₂ and DOX@SiO₂ nanoparticles are indicated in figures 4(A), (B), (B1) and (B2). As can be seen, the as prepared pure silica nanoparticles are spherical in shape that agglomerated to form clusters. The loading of DOX into the silica nanoparticles leads to formation of uniform spheres larger than pure silica. The diameter of DOX@SiO₂ nanoparticles are ranged from 30 – 50 nm. In addition, figure 4(C) shows the XRD spectra of the synthesized pure silica nanoparticles and DOX loaded silica nanoparticles. The XRD pattern of pure SiO₂ exhibits a broad peak at 2θ = 22°, which reveals the amorphous nature of the silica nanoparticles. The broadening of this peak is obvious owing to the smaller grain size effect. The absence of other peaks indicates that there are no impurities in the prepared silica nanoparticles [20]. For DOX@SiO₂ sample, there is a small shift of 2θ to 22.5°, as indicated from the XRD pattern in figure 4(C). In addition, the broadening of the peak decreases
slightly with the loading of DOX into the silica nanoparticles. These changes reflect the incorporation of DOX within the structure of silica nanoparticles.

3.3. Thermal analysis of SiO$_2$ and DOX@SiO$_2$

For investigating the amount of encapsulated DOX into the SiO$_2$ nanoparticles excluding the interference of the drug content changes into the measurement, the TGA thermal analysis of pure silica nanoparticles and loaded with doxorubicin is investigated. Figures 5(A)–(C) illustrate the stages of weight loss. The TGA analysis of pure silica nanoparticles exhibited four stages of weight loss which could be indicated as follow: first stage of weight loss occurred at around 30°C – 249.1°C, in which the mass loss during this stage is about 0.38%. This is related to the vaporization of physically adsorbed water on
the silica surface. Second stage of weight loss happens during the temperature range about 249 \( ^\circ \)C – 508.2 \( ^\circ \)C which contributes to a weight loss of about 3.87%. The loss in weight for this stage is related to the escape of entrapped water and apportion of residual solvent (ethanol) in the porous structure of silica matrix. The third stage of weight loss covers the range of temperature (508.66 \( ^\circ \)C – 688.56 \( ^\circ \)C). During this stage the weight loss (2.3%) may be due to the dehydroxylation of the silanol group and the degradation of residual reactant (TEOS). The fourth stage 688.56 \( ^\circ \)C – 990.02 \( ^\circ \)C is suggested to be due to the degradation of residual TEOS and dehydroxylation of silanol groups. The weight loss at this stage is about 1.44% [21]. Therefore, the total weight loss of silica nanoparticles along all range of temperature 10\( ^\circ \)C – 1000\( ^\circ \)C is about 7.986% of the total mass. This means that the prepared silica nanoparticles are thermally stable up to 1000 \( ^\circ \)C. The observation of TGA thermal analysis of DOX@SiO\(_2\) shows that there are apparently two stages of weight loss: (i) first one occurred over the temperature range of 10.0 \( ^\circ \)C – 579.7 \( ^\circ \)C, the weight loss in this case is about 53.22%, which may due to loss of water, large portion of solvents and also the decomposition of all present DOX; (ii) second stage appears to cover the temperature range of 579.7 \( ^\circ \)C – 987.7 \( ^\circ \)C, the weight loss is about 8.69%, this may be due to the escape of the residual solvents. The overall amount of loaded DOX is estimated by TGA. In the TGA measurement, the drug desorbs after decomposing, which is detected as a temperature dependent weight reduction. However quantification of the loaded-drug fraction in the pores of material is not as simple as just quantifying the total drug content of the sample [22].

Figure 5(D) shows the DSC thermal analysis of the pure silica nanoparticles and DOX@SiO\(_2\). For pure silica nanoparticles, there is an endothermic peak centered at 88.1 \( ^\circ \)C. This is attributed to removal of physically adsorbed water molecules, ethanol and ammonia at the surface of silica. The endothermic peak at about 695 \( ^\circ \)C may be due to removal of residual water present on pores and vaporization of other solvents present due to the dehydroxylation process (release of water molecules due to condensation of silanol groups and formation of siloxane...
linkage) [21–23]. For DOX@SiO$_2$, the first endothermic peak centered for the pure silica and DOX@SiO$_2$ at the same temperature is due to dehydration. Doxorubicin has a melting point ($T_{m}$) at about 230$^\circ$C as illustrated in literature [24], resulting in an endothermic peak in the DSC curve giving the evidence for crystal structure of the drug. The absence of native doxorubicin $T_{m}$ peak on DSC curve of DOX@SiO$_2$ nanoparticles shows that the drug in nanoparticles is in an amorphous phase. In other words, encapsulation process disrupts the native doxorubicin crystals, indicating that the encapsulation process is appreciable. However on the DSC curve of DOX@SiO$_2$, there is an exothermic peak centered at about 350$^\circ$C. This may refer to the process of condensation of silanol (−Si–OH) groups that occurred at the surface of the silica particles and accompanied with the elimination of water molecule. This process supports the gathering of silica particles that cause the formation of new siloxane (−Si–O–Si−) bond at the interface. The presence of siloxane permits the more ordering of the product which demonstrate the appearance of the exothermic and peak negative enthalpy change [25]. This means that loading of DOX into silica nanoparticles lead to occurrence of dehydroxylation process at a lower temperature. The endothermic peak at about 695$^\circ$C may be due to removal of residual water molecules and other solvents present in pores.

3.4. Release of DOX using different mats

The in vitro release of DOX using the electrospun mats is studied by the immersion of the fibrous mat in PBS solution ($pH = 7.4$) at 37$^\circ$C. For comparison, the release of DOX by PLC-PEO/DOX and PLC-PEO/DOX@SiO$_2$ in which DOX@SiO$_2$ is loaded by different methods; $M_2$, $M_3$, $M_4$ and $M_5$ are also investigated. As indicated in figure 6, all the electrospun mats show gradual increase of DOX release with time. The sample contains DOX loaded directly into the nanofiber. Curve in figure 6 indicates three stages of release profile when compared to other samples which exhibit two stages of release profile. The release profile using PLC-PEO/DOX indicates at first an initial rapid release within the first period of incubation, followed by a moderate increase in release with time and then almost constant release during the last periods of incubation. However the total amount of DOX release at the last period of incubation is 91.77%. This fast initial release could be attributed to the presence of DOX agglomerated on the surface of PCL-PEO nanofiber which leads to the direct exposure of DOX to the PBS that result in initial rapid release of DOX. For the other samples in which DOX@SiO$_2$ is loaded into the fiber by different methods, the release profile exhibits two stages of release profile; initial, slightly fast release, and secondly sustained release profile. The inhibition of the proliferation of the tumor cells through the early periods of implantation of fibrous mats loaded with anticancer drug is an important demand. This can be satisfied via the rapid initial drug release from the implanted mat. Also the sustained release of drug which follows initial rapid release provides an adequate concentration of anticancer drug over an extended period, this beneficial to avoid repeated administration of drug [3]. For the delivered samples, the DOX release rate is slower
and the maximum amount of DOX release is less than that in case of using PLC-PEO-DOX [26].

The release profile of DOX@SiO2 loaded into the nanofiber affected by the method of loading. As shown in curves B, C, D and E of figure 6, the release profile is higher (for both initial and sustained stages) for sample in which DOX@SiO2 is loaded by M2 method as compared with M1, M3, M4 methods of loading. For example, the release of DOX at 33rd day (last day of incubation) is about 68.64% for this method and about 58.02%, 59.71%, 37.03% for the other three used methods respectively. M2 method results in the presence of DOX@SiO2 agglomerated on the surface of the nanofiber (as illustrated in FESEM micrographs) while the other methods of loading incorporate DOX@SiO2 inside the nanofiber. This may be due to, DOX is released from SiO2 to the medium when present on the surface while when incorporated into the fiber, tends to be first released from the SiO2 matrix. Subsequently release is occurred from the polymer matrix to the medium [4]. While both M4 and M5 methods load DOX@SiO2 into the nanofiber, the release profile of DOX from core shell is slower. This may be due to the preparation by core shell which results in the formation of core which contains dispersed DOX@SiO2 and also thick sheath formed of polymer which means that DOX undergoes more difficulties in steps when released to the medium [27].

4. Conclusion

PCL-PEO fiber mats are prepared via electrospinning method as a carrier for DOX and sol gel derived DOX@SiO2 nanoparticles. Morphological observations (FESEM micrographs) reveal that the incorporation method is greatly affects the distribution of DOX@SiO2 nanoparticles within the fibrous structure of the polymeric mats. Moreover, in-vitro drug release results in reflectance of the capability of minimal side effects of DOX using encapsulation of drug loading nanoparticles by electrospin fibers. The results reported here provide useful information for the fabrication of DOX releasing implants for clinical treatment of cancer because of high potential of control and localization of a drug dose.

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