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## Formulation of polycaprolactone meshes by melt electrospinning for controlled release of daunorubicin in tumour therapy

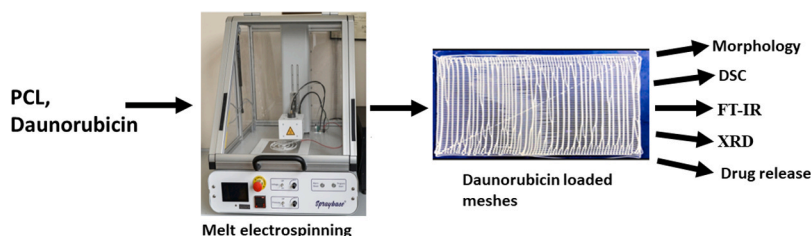
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### GRAPHICAL ABSTRACT



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### ABSTRACT

Several types of chemotherapeutic agents are used in cancer treatment. Among these agents, daunorubicin hydrochloride which is a cell-cycle non-specific antitumor agent is commonly used for treating various types of cancers. This work aims to design daunorubicin loaded polymeric fibre meshes with melt electrospinning using poly ( $\epsilon$ -caprolactone) (PCL) polymer for potential localized antitumor application. The prepared meshes had smooth surface with uniform distribution of daunorubicin as indicated by fluorescent microscope. The meshes thickness increased by increasing the daunorubicin concentration loaded into the PCL fibres. The process of melt electrospinning did not result in any chemical interactions between PCL and daunorubicin neither changed the crystalline structure of these components. Concentration dependent slow-release profile of daunorubicin from the melt electrospun fibres was achieved. Cytotoxicity of the released daunorubicin was assessed on melanoma and ovarian cancer cells and revealed that the cytotoxicity was increased by increasing the time of meshes incubation due to the slow-release profile of daunorubicin. These results prove that PCL-based fibre meshes loaded with daunorubicin are a suitable therapeutic option for local application of antitumour agents. This can enhance the therapeutic outcomes and reduce the unwanted toxicities of these anticancer molecules.

### 1. Introduction

The global increase in the incidence of various cancer cases and the emerging of treatment failure resulted in a considerable effort for

improving the therapeutic options and the formation of new drug delivery systems for chemotherapeutic agents. Radiotherapy, surgery, and chemotherapy are the main clinical options for cancer treatment [1]. Despite being very effective in many cases, chemotherapeutics has high

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toxicity resulting in organ damage, hepato- and nephrotoxicity, along with lowering the overall quality of life for the patients. This is primarily due to the undesired distribution of these chemotherapeutic agents to normal tissues following oral or intravenous administration. Effective anticancer formulation can ensure the accumulation of anticancer agent at the tumour site and prevent the tumour progression or the tumour recurrence following tumour removal by surgery as long as improving the patients' quality of life [2]. One of the proposed methods for reducing the anticancer toxicities against normal tissues is through the local administration by placing the anticancer drug directly at the tumour site. This local administration of the anticancer agents requires the use of drug delivery system that can achieve prolonged drug release that will eventually result in promoting the tumour tissue penetration.

Several proposed drug delivery systems have been investigated for this purpose including micelles, liposomes and other nanoparticles, hydrogels, and many others [3,4]. However, issues related to burst drug release from these formulations along with the low anticancer drugs encapsulation of these formulations remain among the major challenges against the wide use of these drug delivery systems [5,6].

Electrospinning is a polymer fabrication method used to generate polymeric fibres with micro to nanometre diameter. These fabricated polymers have several applications in the field of drug delivery when loaded with various types of medications for slow and controlled release purposes and in tissue engineering scaffolds [7,8]. The used polymers in these purposes are biocompatible with various properties that favours drugs loading and controlled release of these drugs [9,10]. This will result in the evaluation of the use of these electrospun fibres for controlled drug delivery as well as for tissue regeneration. The intended application of the melt electrospun fibre polymers depends on the loading of drugs within these fibres along with the polymer types, compositions, and solubilities [11]. Moreover, several comprehensive reviews are available on the feasibility of scale-up processes for electrospinning along with their applications [12–15].

Drug delivery application is among the highly investigated biomedical applications of these electrospun polymers prepared as polymeric meshes. This is related to the possibility of achieving high drug loading and encapsulation into these fibres, the diversity of polymers that can be used to prepare fibres with high compatibility, the possibility to modify the drug release from the polymeric fibres, and the simplicity and cost-effectiveness of the electrospinning process [16,17].

Due to these features of electrospinning, several efforts have been reported to understand the melt electrospinning technique and the application of the generated polymeric fibre meshes in overcoming some of the clinical gaps related to cancer treatment. Long-acting drug delivery systems with controlled release profile is being developed and investigated in many anticancer formulations to overcome challenges in cancer treatment such as those related to chemotherapeutics toxicities, overcoming treatment failure, and improving patient adherence. In this study, Poly ( $\epsilon$ -caprolactone) (PCL) based fibre meshes were prepared by solvent free melt electrospinning and loaded with daunorubicin. This was an attempt to develop a local drug delivery system for daunorubicin to be applied locally in certain types of cancer treatment such as vaginal and skin cancers. PCL has been widely employed in the preparation of fibre meshes using electrospinning as it has low melting point around 60 °C which facilitates the process of melt electrospinning and enhance the encapsulation of several drugs. PCL polymer characterised by its extended degradation time, enhancing its suitability to achieve slow and extended release of the loaded drug inside the polymeric fibres [18,19].

Daunorubicin hydrochloride was chosen in these formulations as its widely used anticancer molecules used in various types of tumours like vaginal and skin cancers. PCL meshes loaded with daunorubicin were prepared with melt electrospinning and characterised for their physicochemical properties such as morphology, thickness, thermal behaviours, and crystallinity. The fluorescence properties of daunorubicin enable the evaluation of its distribution within the fibre meshes through fluorescent microscope imaging. Moreover, the drug release profile was

assessed through evaluating the cytotoxicity of daunorubicin on two different cancer cells models.

## 2. Materials and methods

### 2.1. Materials

Polycaprolactone (Mn 80000), tablets of phosphate-buffered saline (PBS, pH 7.4), [(3- (4, 5-dimethylthiazole-2-yl) -2) 5- Diphenyl-tetrazolium bromide (MTT) powder, Serum-free and antibiotic-free medium Roswell Park Memorial Institute medium (RPMI 1640), foetal bovine serum (FBS), and penicillin–streptomycin were purchased from Sigma-Aldrich (UK). Daunorubicin hydrochloride was purchased from TCI chemicals (Japan). The human melanoma cells (A375) and the ovarian cancer cells (A2780) were purchased from American Type Culture Collection (ATCC®).

### 2.2. Melt electrospinning

Polymeric fibre meshes made of PCL and loaded with daunorubicin were fabricated using Spray base melt electrospinner (Spraybase® Avectas Ltd., Ireland), melt electrospinning involves the use of high-voltage power source, controlled temperature, and a head containing the melted polymer. The melted polymer was released through the nozzle in the form of fine fibre using air compressor to aid in the emission of the melted polymer. The melted and casted polymer was collected on a movable collector during the polymer writing process. The required quantity of PCL with daunorubicin were mixed together and then placed in the electrospinner the holder. The polymer and the drug were kept at 90 °C for at least 15 minutes so the PCL polymer is fully melted. Rectangular single-layer PCL fibre meshes loaded with daunorubicin were prepared using nozzle with size 0.25 mm and deposited on the movable metal stage. The fibre meshes were prepared using the following melt electrospinner parameters: pressure: 2.75 bar, voltage: 3.75 mV, and temperature: 90 °C.

Two PCL fibre meshes were prepared with different daunorubicin percentages, D1 with 0.05 % daunorubicin (prepared from 2 mg daunorubicin with 4 g PCL) and D2 with 0.025 % daunorubicin (prepared from 1 mg daunorubicin with 4 g PCL). Blank PCL meshes were prepared under the same conditions mention above without the use of daunorubicin.

### 2.3. Morphology of meshes

Images for the prepared PCL meshes with and without daunorubicin were taken using scanning electron microscope (SEM) (Quanta 250, FEI, USA) which was an FEI Quanta 250 field emission gun. To prevent charging, low vacuum chamber conditions were deployed (0.5 mbar) and secondary electrons collected using a gaseous secondary electron detector.

### 2.4. Fluorescence of daunorubicin loaded meshes

The fluorescence of meshes loaded with daunorubicin and thus the drug distribution throughout the meshes was evaluated using fluorescence microscope (OPTIKA, Japan). Images were taken from different spots at different magnifications to evaluate the distribution of daunorubicin hydrochloride through the electrospun fibres.

### 2.5. Meshes thickness

The thickness of the prepared meshes was measured using electric micrometre calliper with a 0.01 mm precision. Measurements were taken from three different places for three triplicates and the results were presented as the average  $\pm$  SD.

## 2.6. Differential scanning calorimetry

Thermal behaviour of the raw materials and the prepared meshes by melt electrospinning was evaluated using differential scanning calorimeter (DSC) (a Mettler Toledo DSC analyzer) using nitrogen gas for cooling. The thermal behaviour of free PCL, free daunorubicin, blank PCL meshes and daunorubicin loaded meshes were evaluated. Five mg from each sample were placed in aluminium pans and the thermal analysis was carried out by heating each sample with 10 °C/min rate to 320 °C using a 20 ml/min nitrogen flow. DSC analysis was done in triplicate for each sample and the results were reported as an average  $\pm$  SD.

## 2.7. Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FT-IR) spectra were evaluated using SHIMADZU FT-IR spectrophotometer with ATR probe within the range from 4000 – 400  $\text{cm}^{-1}$ . Measurements were taken for the polymers alone, daunorubicin alone, empty and loaded meshes.

## 2.8. X-ray diffraction (XRD)

To identify the crystalline of the meshes components, a small piece (10–50 mg) of each mesh was analysed using transmission XRD data collected on a Bruker D8 Discover Multi-well transmission diffractometer equipped with  $\theta/\theta$  geometry, Cu radiation ( $\text{Cu } \lambda = 1.54056 \text{ \AA}$ ), a SSD136 PSD and an automated multi-position x-y sample stage. Samples were mounted on a 40-position sample plate supported on a polyamide (Kapton, 7.5  $\mu\text{m}$  thickness) film. Data was collected from each sample in the range of 4–35 °  $2\theta$  with a 0.017 °  $2\theta$  step size and 0.5 second per step count time. Samples oscillated in the x-y throughout data collection to maximise particle sampling and minimise preferred orientation effects. The obtained patterns of XRD were analysed using DIFFRAC.EVA software, Bruker.

## 2.9. Tensile strength

TA-XT2i texture analyser was used to measure the maximum stress that the prepared electrospun meshes can tolerate. The texture analyser was acquired with exponent stable micro systems software (Stable Micro Systems, UK) equipped with Exponent software. Small rectangular samples (2 $\times$ 5 cm) from empty and daunorubicin loaded meshes were cut and attached to the clamps of the texture analyser. A crosshead speed of 5 mm/min was used during the tensile strength measurement. The texture analysis tests were carried out until full breakage of the sample. The maximum stress value for each sample was expressed as the pressure which was resulted sample breaking. The measurements were done in triplicates the results were expressed as an average  $\pm$  SD.

## 2.10. Meshes water uptake

The water uptake ability of the prepared meshes with and without daunorubicin was measured by immersing the meshes in PBS and measure their weight at different time points and compared to their initial weight. A piece of 1  $\text{cm}^2$  from each mesh was cut and weighed then immersed in PBS at room temperature. At each time point (15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hrs, 24 hr), the meshes were taken out of the PBS and the excess PBS was removed by gentle wicking of the mesh with kimwipes and then weighed. The water uptake percentage was measured according to the following equation:

$$\text{Water uptake \%} = (\text{Wt}_s - \text{Wt}_0) / \text{Wt}_0 * 100\% \quad (1)$$

where  $\text{Wt}_0$  represent the weight of the mesh piece before immersion in PBS and  $\text{Wt}_s$  represent the mesh weight after immersion in PBS.

## 2.11. Drug release

The rate of daunorubicin released from the prepared meshes were evaluated by immersing each mesh in 3 ml of PBS in a 15 ml falcon tube and the tubes were placed in a rotator at 50 rpm at room temperature for 72 hrs. At specific time points (5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h), 0.5 ml of the release medium were taken for drug release evaluation and each sample was replaced with fresh PBS at the same volume to maintain constant release volume and sink conditions. The percentage of daunorubicin released at each time point of the experiment from each mesh was measured with high performance liquid chromatography (HPLC) using an Agilent Technologies 1260 Series system equipped with Clarity Chromatography software version 8.8.1.16. The measurements were done using C18 column (250  $\times$  3.0 mm) with the following chromatographic conditions: injection volume 40  $\mu\text{l}$ , flow rate 1 ml/min, mobile phase PBS: acetonitrile (70:30) (pH 1.9 adjusted with HCl). The measurements were taken at UV detection of 234 nm and the run time was 7 minutes. The concentration of the released daunorubicin from the electrospun meshes at each time point was calculated from a linear standard curve constructed using known daunorubicin concentrations.

## 2.12. Cytotoxicity

A2780 and A375 cells were seeded at  $1 \times 10^5$  in 24 well plates at 1 ml per well. 10 mg piece of each mesh was sterilised under UV for 12 hours then on the next day, media were replaced and meshes pieces were placed in each well for 24, 48, and 72 hours. Cytotoxicity was assessed using MTT assay. At 20, 44, and 68 hours after treatment, 100  $\mu\text{l}$  of 5 mg/ml MTT solution were added and the plates were incubated for further 4 hours. Media was then removed followed by the addition of 1 ml DMSO to dissolve the formed formazan crystals. The plates were then shaken for 10 minutes, and the absorbance measured at 570 nm using microplate reader (synergyMx BioTek, USA). The viability of the treated cells was expressed as a percentage by dividing the absorbance of the treated cells by the absorbance of the untreated cells.

## 2.13. Statistical analysis

Statistical analysis were done using Minitab software version 17 by calculating the One-way analysis of variance (ANOVA). In case of paired comparison, Tukey's multiple comparison test and *t*-test were performed. A *p* value < 0.05 was considered statistically significant.

## 3. Results and discussions

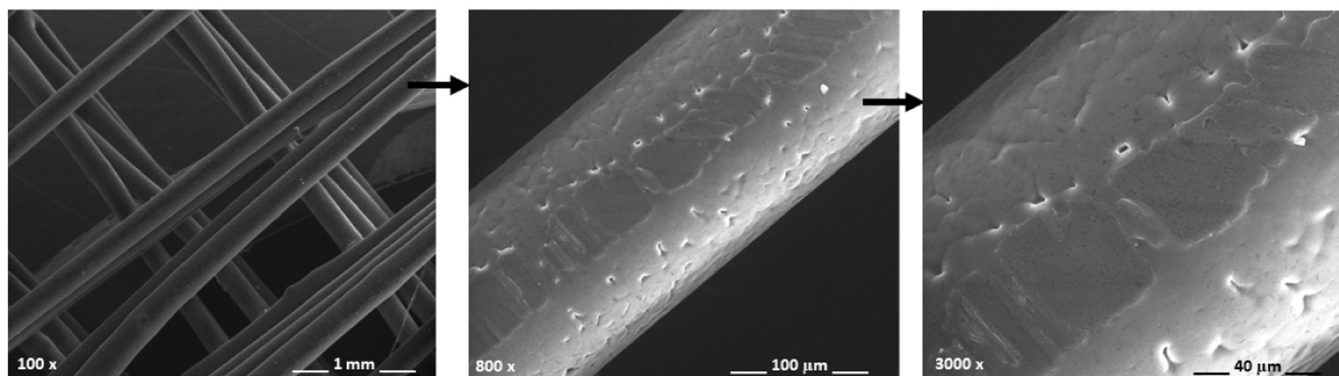
In this work, daunorubicin loaded polymeric meshes using PCL polymer were prepared using melt electrospinning method. This method is a solvent free method that is able to generate polymeric meshes with direct writing for various applications such as the preparation of polymeric meshes for controlled release of medications. Two different daunorubicin concentrations were used and the prepared meshes were characterised to evaluate their properties, drug release profile, and their cytotoxicity.

Melt electrospinning was able to incorporate daunorubicin into the PCL fibres which can be used to prepare meshes for controlled daunorubicin release for cancer treatment.

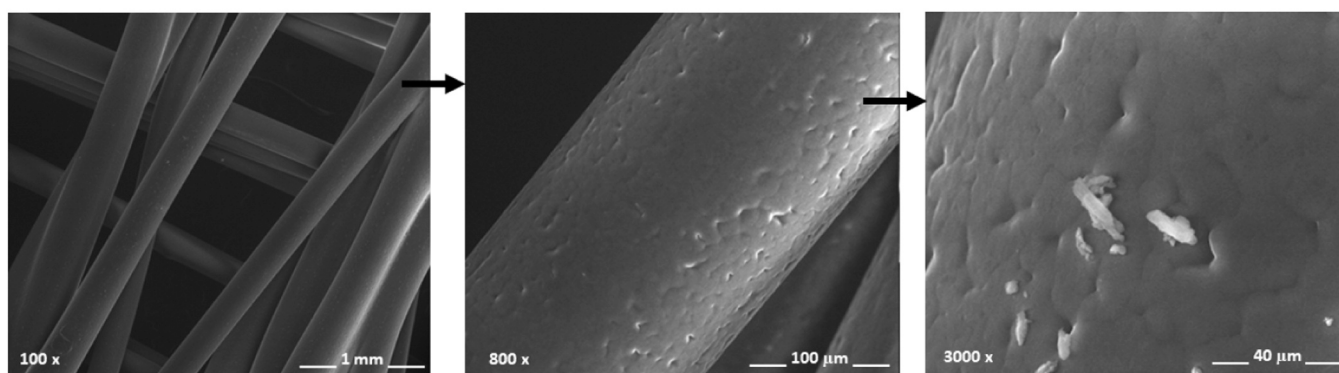
### 3.1. Meshes morphology

Morphologies of both empty and daunorubicin loaded meshes at different magnifications were evaluated using SEM and presented in Fig. 1. SEM images of the empty and daunorubicin loaded fibre meshes are smooth and uniform. The PCL fibre surfaces of D1 and D2 shows some crystals of daunorubicin which indicate that the drug was both mixed inside the polymer during the melt electrospinning and some of it

## Blank PCL mesh



## D1



## D2

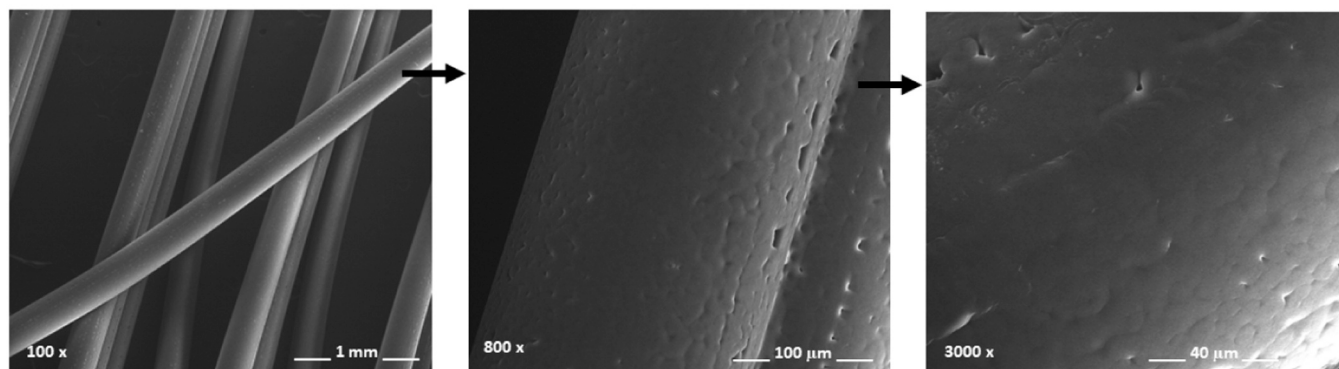


Fig. 1. SEM images of fibre meshes prepared by melt electrospinning using PCL with 0.05 % daunorubicin (D1) and 0.025 % daunorubicin (D2).

appears on the surface of the fibres.

During electrospinning, PCL was mixed with daunorubicin, and the mixture was heated to a temperature above the melting point of PCL which is still below the daunorubicin melting point. At this point, the solid daunorubicin will be mixed inside the melted PCL which can ensure the homogeneity of the fibres and the drug distribution throughout the fibre meshes. This is important to achieve predictable and controlled release profile of daunorubicin. This means that daunorubicin crystals on the surface of the fibre meshes will dissolve first followed by daunorubicin release from inside of the polymeric fibres by diffusion or erosion.

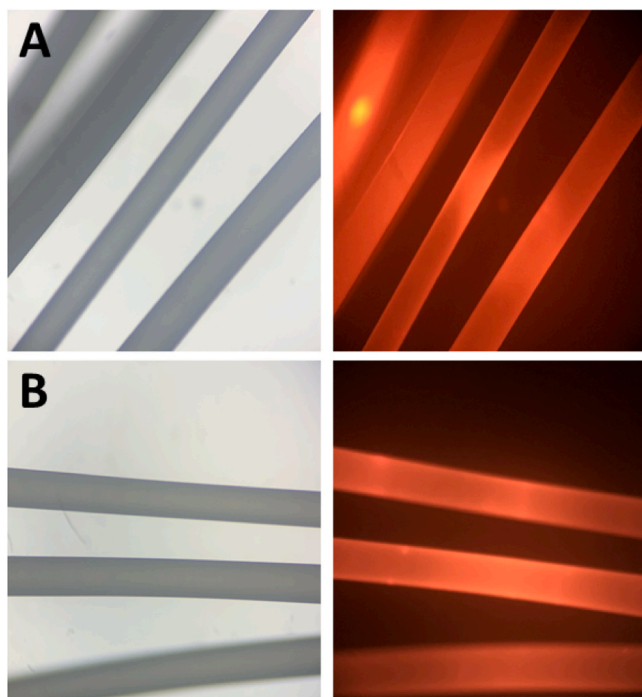
Previous work done in our lab demonstrated that antibacterial fibre meshes prepared for controlled release of ciprofloxacin had the same surface properties [20]. These properties were also reported in similar

reports in the literature [21].

In terms of the fibre diameter, the images indicates that all fibres looks similar in diameter which indicates that the daunorubicin inclusion into the PCL fibres does not significantly affect the fibre diameter compared to the empty meshes which means that the only factors that affect the fibre diameters are those related to the process of the electrospinning such as the nozzle size, the pressure, voltage, and the viscosity of the melted polymer.

### 3.2. Meshes fluorescence

Since daunorubicin is highly fluorescent, we can evaluate the dispersion of the drug throughout the polymeric meshes by measuring the fluorescence of the meshes. Fig. 2 represent D1 and D2 meshes in the



**Fig. 2.** Bright and fluorescence images taken by fluorescence microscopy for A: D1 meshes loaded with 0.05 % daurorubicin and, B: D2 meshes loaded with 0.025 % daurorubicin.

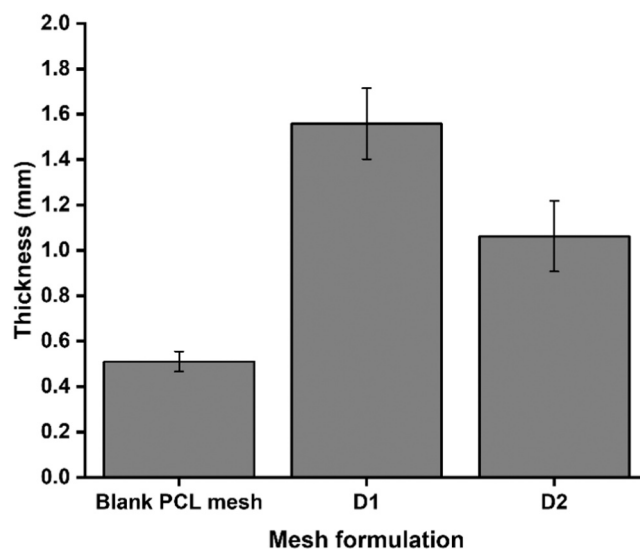
bright field and fluorescence field in the same position measured with fluorescence microscopy. The fluorescence of the polymeric fibres clearly show that the intensity of the fluorescence in Fig. 2A for D1 that contain 0.05 % daurorubicin is higher than the intensity of D2 in Fig. 2B that contain 0.025 % daurorubicin. This means that the fluorescence increased by increasing the daurorubicin concentration. He *et. al.* reported the same observations for increasing in the intensity by the increase of the drug concentration [22]. Moreover, the fluorescence in Fig. 2 indicate that the drug is distributed throughout the polymer fibres and not accumulated as bright spots which demonstrate the encapsulation of the drug inside the melt electrospun fibre with uniform dispersion.

### 3.3. Meshes thickness

Fig. 3 displays the thicknesses of empty and daurorubicin loaded PCL meshes. It is clearly presented that daurorubicin incorporation into the PCL fibres significantly increase the meshes thickness. The meshes thickness was also increased by the increase in the daurorubicin concentration were D1 that contain 0.05 % drug was significantly thicker compared to D2 which contains 0.025 % daurorubicin and both formulations were significantly thicker than the empty meshes. This can be explained by the possibility of daurorubicin to cause swelling in the polymeric matrix.

Another possible explanation for the increase in the meshes thickness by the addition of the drug is based on the fact that the addition of the drug to the melted polymer during the process of the melt electrospinning significantly increased the viscosity of the melted polymer. When this melted polymer pushed through the nozzle by the pressure, this higher viscosity of the polymer-drug mixture compared to the blank polymer will result in the formation of thicker fibres. The increase in the drug concentration will result in higher increase in the polymer viscosity and the formation of thicker meshes.

Previous reports indicate that drug loaded polymeric meshes can be prepared with melt electrospinning with various thicknesses depending on the polymer type and drug used [23]. The mechanical properties of



**Fig. 3.** Thicknesses of empty and daurorubicin loaded PCL fibre meshes prepared by melt electrospinning. Data presented the mean  $\pm$  SD (n=3).

the drug loaded meshes as well as the kinetics of the drug release can be affected by the fibre meshes thickness. Our results indicates that the increase in the drug content increases the meshes thickness which means that more drug can be loaded in thicker meshes. This means that gradual release kinetics of the loaded drug when these fibre meshes used for controlled drug delivery [22].

One more possible effect for the increase in the meshes thickness is the effect of this increase in the thickness on the fibre meshes pore size. The release of the drug from the fibre meshes can be increased by the increase in the pore size throughout the polymeric fibres that form the meshes as the thickness increase. Therefore, to achieve specific release profile, the meshes thickness should be properly optimized for specific release kinetics applications [24]. Daurorubicin loading into the PCL polymeric meshes during melt electrospinning could affect the melted polymer properties such as the viscosity which have significant effect on the thickness of the daurorubicin loaded meshes [25].

### 3.4. Differential scanning calorimetry (DSC)

Melt electrospinning involves heating to a temperature above the polymer melting point in order to be able to construct the meshes. Therefore, DSC was done to assess the thermal behaviour of the polymer and daurorubicin following the process of melt electrospinning.

Fig. 4 represents the DSC curves of free PCL, free daurorubicin, empty meshes and daurorubicin loaded meshes. DSC curves of PCL before and after electrospinning were the same and all expressed an endothermic peak around 64 °C. Free daurorubicin DSC curve showed a clear endothermic peak at 177.16 °C which represents the melting point of daurorubicin. For daurorubicin loaded meshes, there were clear endothermic peaks in D1 and D2 around 64 °C which represents the PCL. However, it can be noticed that the daurorubicin endothermic peak which represents its melting temperature at 177.16 °C disappeared in D1 and D2 following melt electrospinning. These results suggest that daurorubicin has successfully incorporated within the PCL chains.

Melt electrospinning involves melting the PCL polymer at 90 °C and then the daurorubicin was manually dispersed into the melted polymer to increase its distribution within the melted PCL as solid powder since it has much higher melting point than 90 °C. In this case, daurorubicin might be dissolved in the melted PCL. [26,27] This mixture of melted PCL and daurorubicin has a suitable viscosity to be formulated as polymeric meshes using electrospinning. This theory of daurorubicin dissolution into the melted polymer can explain the disappearance of the

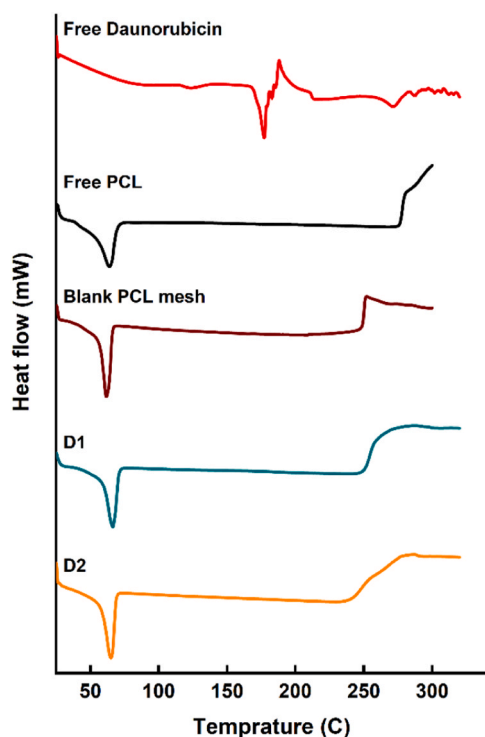


Fig. 4. DSC analysis of free PCL, free daunorubicin, and electrospun fiber meshes D1 and D2 loaded with varied percentages of daunorubicin.

endothermic peak on daunorubicin in both D1 and D2 meshes. One more explanation of the disappearance of daunorubicin endothermic peak in D1 and D2 the low concentration of daunorubicin in these formulations since D1 and D2 have 0.05 % and 0.025 % respectively which means that the drug concentrations in the daunorubicin loaded meshes were below the detection limits by the DSC analysis.

### 3.5. Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra of free PCL, free daunorubicin, empty, and daunorubicin loaded meshes prepared by melt electrospinning are shown in Fig. 5. Since melt electrospinning involve heating to polymer with the drug and casting the meshes using pressure and voltage, FT-IR analysis can be used to assess any interaction between the meshes components. Through the FT-IR spectra, characteristic peaks of each component can be determined before electrospinning and then the spectra for the prepared meshes can be evaluated after electrospinning to ensure the presence of each component in the meshes with no interactions. This can be detected by having similar spectra for each component before and after the melt electrospinning.

Analysis of the spectra revealed two characteristic peaks for free daunorubicin at  $1618\text{ cm}^{-1}$  and  $1576\text{ cm}^{-1}$  which are resulted from the stretching vibration of hydrogen-bonded quinone carbonyl groups and C=C respectively [28]. With regard to the PCL beads before electrospinning, characteristic peaks between  $2965$  and  $2991\text{ cm}^{-1}$  corresponding to the asymmetric  $\text{CH}_2$  stretching and symmetric  $\text{CH}_2$  stretching vibration respectively [29]. Another peak at  $1723\text{ cm}^{-1}$  for the vibration stretching of C=O in the ester bond while the stretching vibration of C-O-C resulted in distinct peaks for PCL at  $1160$  and  $1240\text{ cm}^{-1}$  [30]. These distinct peaks for free daunorubicin and PCL can be seen in the daunorubicin loaded PCL melt meshes. Moreover, the peaks in the daunorubicin loaded meshes were not observed to be shifted which indicates a lack of interaction between PCL and daunorubicin and no change can be seen in their chemical bonds following the process of melt electrospinning. This indicates that the different conditions that those components exposed to during the melt electrospinning

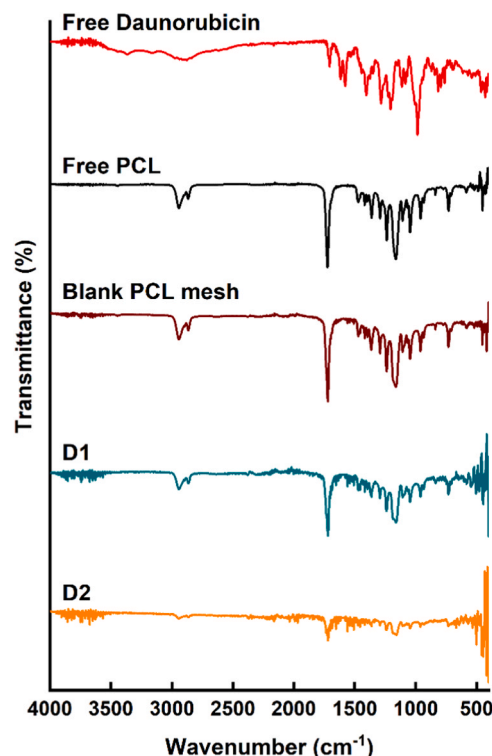


Fig. 5. FT-IR spectra of free daunorubicin hydrochloride, free PCL, empty meshes, and daunorubicin loaded meshes containing 0.05 % (D1) and 0.025 % (D2).

such as the high voltage and temperature did not initiate any chemical interactions among the meshes components and the possibility to prepare meshes for daunorubicin delivery without affecting its chemical structure.

### 3.6. X-ray diffraction (XRD)

PCL and daunorubicin were evaluated by XRD before the process of electrospinning and the results were compared to the XRD results of empty and daunorubicin loaded meshes and presented in Fig. 6. XRD spectra of free daunorubicin exhibited multiple peaks representing a typical crystalline nature of daunorubicin. PCL pellets exhibited sharp characteristic peaks at  $2\theta = 21.45^\circ$  and  $23.90^\circ$  which represents the PCL crystalline structure.

Blank PCL meshes has the same XRD spectra of the PCL polymer before the process of melt electrospinning with the same characteristic peaks indicating that the PCL crystalline properties didn't change by melt electrospinning. The same spectrum of the empty meshes can be seen for meshes loaded with 0.05 % and 0.025 % of daunorubicin (D1 and D2) with disappearance of the distinguished amorphous spectra of daunorubicin in these loaded meshes. This means that the conditions of the melt electrospinning process does not cause any change in the arrangement of PCL and daunorubicin which means the characteristics of these components were preserved followed the process of electrospinning.

### 3.7. Meshes tensile strength

In order to evaluate the suitability of the daunorubicin loaded meshes to be used for pharmaceutical application, the tensile strengths of the empty and daunorubicin loaded meshes (D1 and D2) was measured and expressed in Fig. 7 and ultimate strength ( $\text{kg/mm}$ ) vs the strain (%) when the meshes are stretched are presented in Fig. 8. According to our results, empty PCL meshes have a tensile strength of 6.42

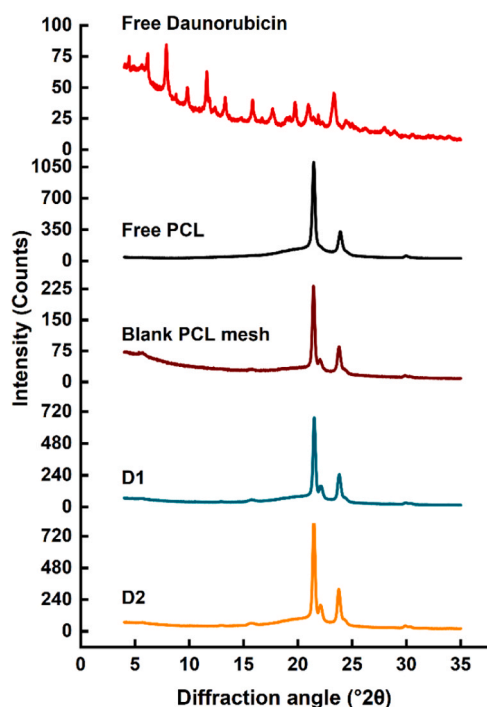


Fig. 6. XRD patterns of PCL pellets, free daurubicin, and electrospun fiber meshes as empty or loaded with various percentages of daurubicin.

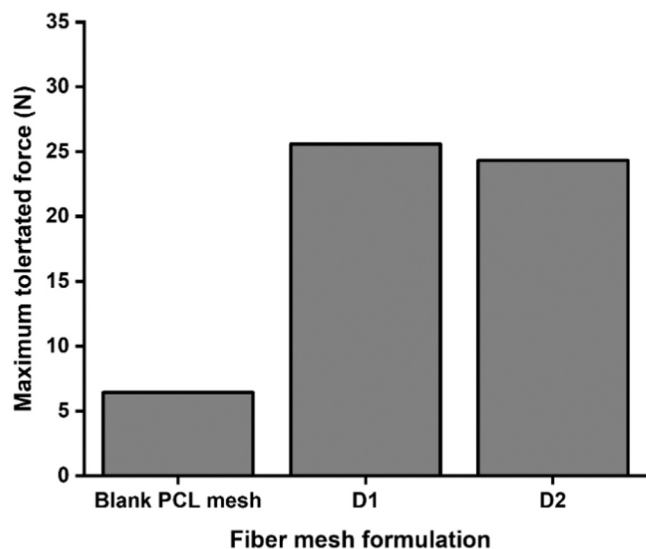


Fig. 7. The tensile strength of empty and drug loaded PCL meshes expressed as the maximum tolerated force ( $n=3$ ).

$\pm 0.06$  N. The incorporation of daurubicin in these meshes significantly ( $p < 0.05$ ) increase the tensile strength to  $25.60 \pm 0.01$  for D1 meshes that are loaded with 0.05 % daurubicin and to  $24.33 \pm 0.01$  for D2 meshes that are loaded with 0.025 % daurubicin. Moreover, D1 has non-significant ( $p > 0.05$ ) slightly higher tensile strength compared to D2 since it has higher drug concentration. These results could be explained by the fact that daurubicin was uniformly distributed throughout the melted polymer and when this mixture casted as meshes during the process of melt electrospinning, the resulted mesh will have higher tensile strength compared to the empty meshes because of the presence of the drug. Which means the incorporation of daurubicin within the polymer fibres increase the strength of the prepared meshes.

These results matches our previous results where the ciprofloxacin loading into the PCL meshes also significantly increases the tensile strength of the meshes [20]. This might be the case for any drug that is incorporated into these meshes. This means that the development of PCL based electrospun meshes for controlled drug release and delivery would have suitable strength to be used for pharmaceutical applications.

### 3.8. Meshes water uptake

The hydrophilicity of the prepared meshes was evaluated through the ability of the electrospun meshes to uptake water over specific period of time when soaked with PBS. Each mesh was immersed in PBS for 24 hours and the increase in the mesh weight was recorded at different time point. The water uptake percentage was calculated as a percentage of the weight of each mesh at each time point compared to the initial weight of the mesh before starting the experiment.

Water uptake by the polymeric meshes is required to initiate the process of drug release. Since the drug is incorporated inside the hydrophobic polymeric fibres of PCL and prepared as a polymeric monolithic system. Therefore, drug release rate mainly depends on the water diffusion rate and polymer swelling rather than polymer dissolution. This leads to drug dissolution first followed by the release of drug by diffusion [31]. The hydrophobic characteristics of PCL could retard the water diffusion into the polymer fibres and consequently the drug release from the matrix. This can be seen in Fig. 9 where the maximum increase in the meshes weight did not exceed 20 % for D2 after 24 h and 15 % for D1. The possible explanation for the observation where D2 absorbed more water compared to D1 could be attributed to the fact that the increase in the drug concentration will fill the spaces within the polymer chains and thus reduce the rate of water penetration.

### 3.9. Drug release

This work aims to prepare controlled release meshes of daurubicin. As these meshes are intended to be applied locally at the tumour site, maintaining relatively stable concentration of daurubicin at the tumour site will significantly improve the antitumour effect of the drug. Therefore, the release behaviours of the PCL electrospun meshes was evaluated by measuring the concentration of daurubicin following the immersion of the meshes in PBS for 72 h. Fig. 10 represents the release profile of PCL meshes D1 and D2. Daurubicin release from D2 meshes that are loaded with 0.025 % was gradual over time with the peak observed at 6 h followed by stable concentrations until 72 h. However, the increase in the drug release in D1 that contain 0.05 % drug resulted in gradual and higher release profile compared to D2 with peak release observed at 6 h followed by gradual decrease in the drug concentration. Returning to the SEM images of D1, drug crystals can be observed at the surface of the mesh fibres (Fig. 1 at 3000X) which can explain the higher profile of D1 or the burst release by the dissolution first of the drug localised at the fibre surface. However, most of the drug was imbedded inside the polymer fibres as demonstrated by the fluoresce images (Fig. 2) which can explain the subsequent gradual release in D1 and D2. After each sample, fresh solvent was added which explains the decrease in the concentration of the drug in D1 after 6 h where this dilution effect was higher than the rate of drug release. This was not the case in D2 since the release of the drug was gradual and consistent from the inside of the polymer as no drug crystals observed at the fibres surface.

In the process of melt electrospinning, polymeric fibres loaded with the drug are formed by cooling and casting as meshes and therefore, PCL crystals may form through the molecular chain rearrangement of PCL [32,33].

In comparison with the XRD results in Fig. 5, PCL was confirmed to have crystalline structure following melt electrospinning similar to that of the pure PCL pellets. Owing to the hydrophobic properties and the high degree of crystallisation of the melt electrospun PCL fibres, water penetration through the fibres was limited and thus the drug diffusion

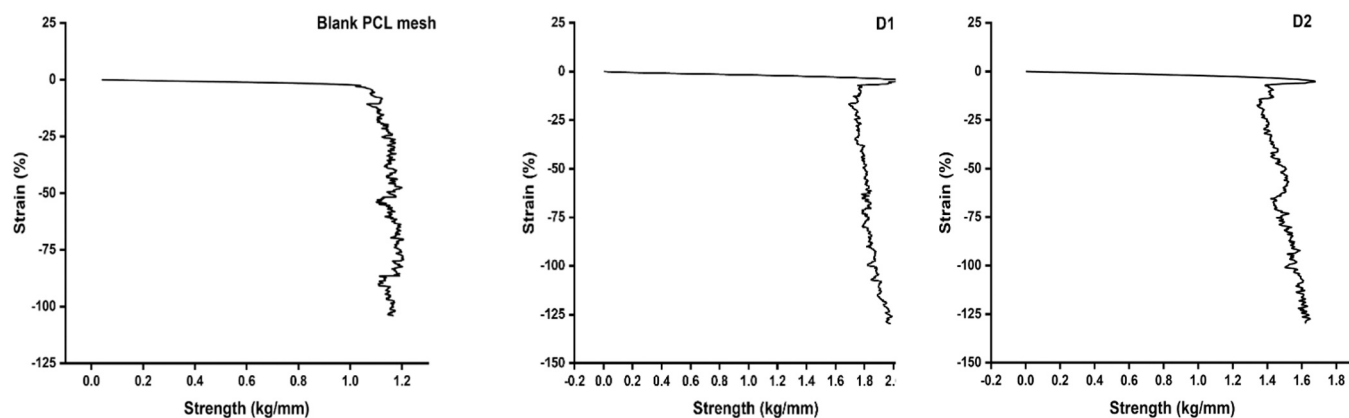


Fig. 8. The ultimate strength (kg/mm) vs the strain (%) when the meshes are stretched.

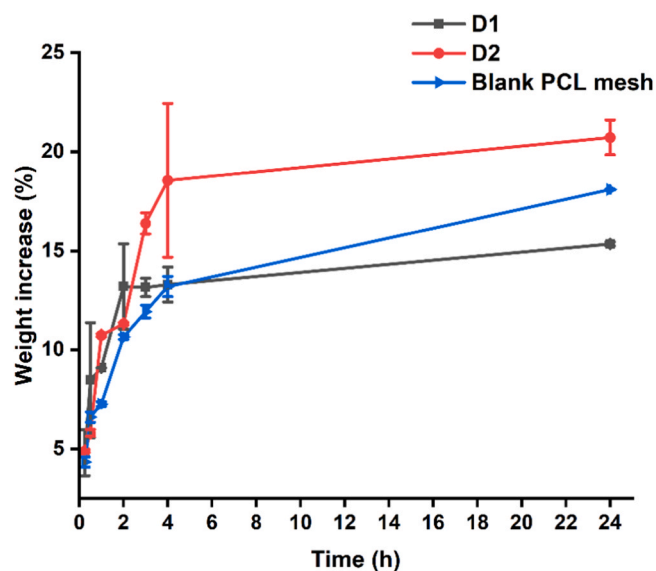


Fig. 9. Percentage of weight increase of D1, D2, and blank PCL meshes following immersion in PBS for 24 h (n=3).

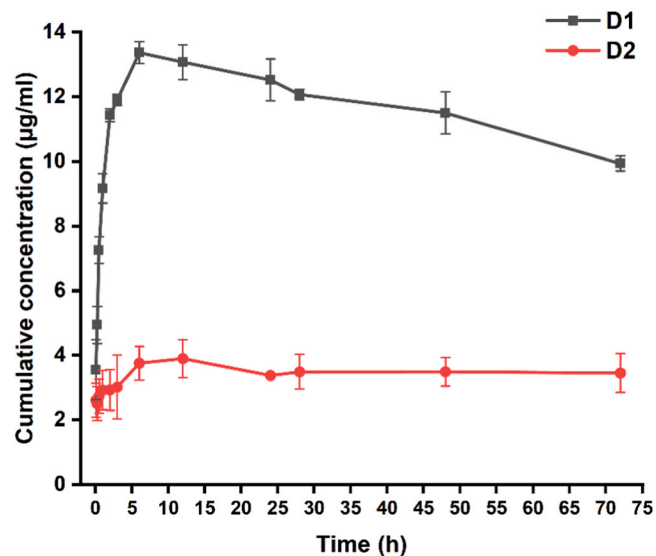


Fig. 10. Daunorubicin release behaviour from PCL melt electrospun meshes loaded with 0.05 % daunorubicin (D1) and 0.025 % (D2).

from the polymeric fibres which can explain the slow and gradual release profiles presented in Fig. 10 and with no burst release behaviour. After certain time, water can eventually diffuse through the fibres and increase the rate of drug release at controlled and slow rate [34].

### 3.10. Cytotoxicity

To evaluate the antitumour activity of the daunorubicin loaded meshes, two tumour cell lines (A375 and A2780) were cultured and incubated with empty PCL and with daunorubicin loaded meshes (D1 and D2) for 24, 48, and 72 h. MTT test was done after each incubation time to evaluate the cytotoxicity of the meshes.

As can be seen in Fig. 11, D1 and D2 meshes exhibited excellent antitumour activities with significant inhibition of the tumour cell growth on both cell lines with higher activity observed for D1 that is loaded with higher percentage of daunorubicin. In both cell lines, the increase in the incubation time resulted in more antitumour activity owing to the slow and gradual release of the drug from both types of meshes. Knowing that the empty PCL meshes were nontoxic at all incubation times in both cell line, it is clear that the observed cytotoxicity of D1 and D2 was due to the effect of daunorubicin release overtime. Again, since these meshes were intended to be applied locally to the tumour site, the cytotoxicity results can confirm the feasibility of these meshes to maintain constant and effective daunorubicin concentration at the tumour site following the gradual release which might be effective antitumour formulations. In this study, daunorubicin was selected to evaluate the possibility to prepare drug loaded meshes by melt electrospinning for controlled release of the drug at the disease site. However, several other antitumour drugs can also be loaded into the polymeric electrospun fibres or even a combination of drugs for better therapeutic outcomes.

## 4. Conclusions

In this work, PCL fibre meshes loaded with daunorubicin was successfully prepared by melt electrospinning which can be used for local applications of antitumour agents. The prepared meshes had smooth surface with uniform distribution of the drug throughout the polymer. Slow and controlled drug release was observed from the meshes owing to the hydrophobic and crystalline properties of the PCL fibres. Daunorubicin loaded meshes exhibited excellent cytotoxic effects on two tumour cell lines with antitumour activity increased by increasing the incubation time. Daunorubicin was selected to proof the concept of being able to prepare drug loaded meshes by melt electrospinning for controlled release of the drug while several other anticancer agents can also be used in this purpose. This can increase the therapeutic outcomes by facilitating the local application of the drug with slow and controlled release from the meshes. Therefore, the process of melt electrospinning



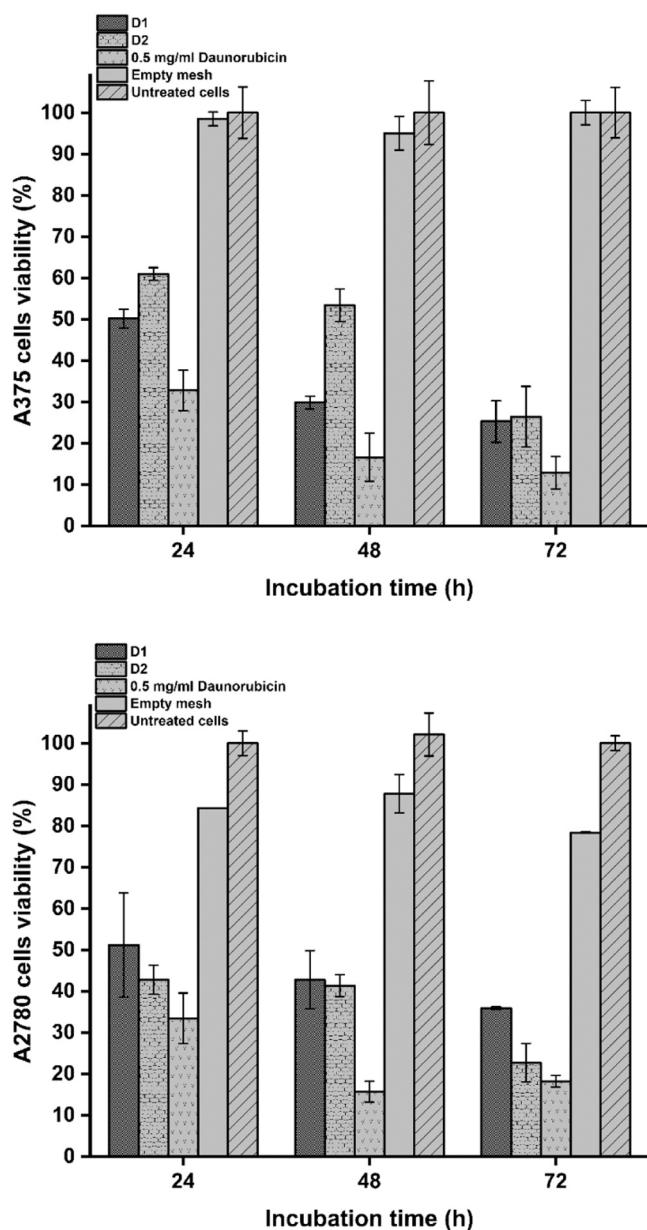


Fig. 11. MTT assay of A375 and A2780 cells following treatment with PCL melt electrospun meshes as empty or loaded with daunorubicin at 0.05 % (D1) or 0.025 % (D2).

presents a potential process to prepare slow and controlled release delivery systems for local administration of antitumour agents.

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#### CRediT authorship contribution statement

**Ibrahim Khadra:** Writing – review & editing, Supervision. **Yousef Abul-Haija:** Writing – review & editing, Validation. **Mohammad Obeid:** Writing – original draft, Methodology, Data curation, Conceptualization. **Lina Akil:** Visualization, Methodology, Conceptualization.

#### 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.

#### Data Availability

Data will be made available on request.

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