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Electrospun PCL/Fullerene Nanofibrous Scaffold for Sustained Paclitaxel Delivery and *In Vitro* Anticancer Evaluation in MCF-7 Breast Cancer Cells

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ABSTRACT

Purpose: To overcome the limitations of conventional chemotherapy, this study aimed to develop a novel electrospun nanofibrous drug delivery system by integrating poly(ϵ -caprolactone) (PCL) with fullerene (C60) as a platform for sustained paclitaxel (PTX) delivery and in vitro anticancer evaluation.

Methods: PCL C60 and PTX loaded PCL C60 (PCL C60 PTX) nanofibrous scaffolds were fabricated via electrospinning using a 13% (w/v) PCL solution containing C60 (8 μ g/mL). PTX (4 μ g/mL) was incorporated into the drug loaded formulation. Physicochemical characterization was performed using SEM, FTIR, XRD, contact angle measurements, swelling, and degradation studies. Drug release was quantified in PBS (pH 7.4). Hemocompatibility was assessed using a hemolysis assay. Anticancer efficacy against MCF 7 breast cancer cells was evaluated by MTT assay, flow cytometry (Annexin V/PI), and AO/PI staining.

Results: The scaffolds exhibited uniform nanofibrous morphology. PTX loading into PCL-C60 scaffolds reduced the mean fiber diameter from 312 ± 63 nm to 199 ± 98 nm and significantly decreased the water contact angle from $94.08 \pm 1.94^\circ$ to $87.47 \pm 0.43^\circ$ ($p = 0.023$). FT-IR and XRD analyses indicated preservation of the PCL crystalline structure after C60 and PTX incorporation. Swelling and degradation profiles showed no significant differences between PCL- C60 and PCL- C60-PTX scaffolds ($p > 0.05$). The PTX-loaded scaffold exhibited biphasic drug release ($18.55 \pm 0.63\%$ at 1 h and $74.36 \pm 0.50\%$ at 72 h), fitting the Korsmeyer-Peppas model ($R^2 = 0.987$, $n = 0.400$). Both scaffold groups exhibited hemolysis rates below 5%. In MCF-7 cells, the PCL-C60-PTX scaffold reduced viability to 56% at 72 h and induced apoptosis compared to untreated cells (control).

Conclusion: The developed PCL- C60-PTX nanofibrous scaffold exhibited sustained PTX release, acceptable hemocompatibility, and reduced viability in MCF-7 cells, supporting its potential as a localized drug-delivery platform for breast cancer studies. Further mechanistic and in vivo investigations are warranted to confirm its therapeutic applicability.

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Introduction

Cancer remains one of the leading causes of mortality worldwide, and conventional therapeutic strategies, particularly chemotherapy, are often limited by nonspecific toxicity, multidrug resistance, and adverse effects on healthy tissues.^{1,2} Nanotechnology-based drug-delivery systems have emerged as promising approaches for improving the therapeutic performance of anticancer agents by enhancing solubility, reducing systemic exposure, and enabling controlled drug release.³ Among these platforms, electrospun nanofibers have attracted considerable interest for localized chemotherapy owing to their high surface-area-to-volume ratio, tunable morphology, and capacity to provide sustained drug release.⁴

Among the polymers used in nanofiber-based drug-delivery systems, poly(ϵ -caprolactone) (PCL) has received considerable attention for sustained anticancer drug delivery because of its biocompatibility, biodegradability, and slow hydrolytic degradation.⁵ Its mechanical stability and affinity for hydrophobic drugs such as paclitaxel (PTX) make it an attractive matrix for the long-term controlled release of therapeutic agents from electrospun nanofibrous structures.⁶ Recent studies have demonstrated that PCL-based electrospun systems can effectively incorporate poorly water-soluble anticancer agents and modulate their release behavior through variations in fiber composition and architecture.⁷⁻⁹ Moreover, multifunctional PCL-based electrospun platforms incorporating functional nanomaterials have shown improved scaffold properties, preserved biocompatibility, and tunable drug-release performance, further supporting their potential for controlled and localized cancer therapy.^{10,11}

Beyond polymeric matrices, fullerene (C_{60}) has attracted considerable attention in drug-delivery research due to its unique carbon-cage structure, high surface area, and exceptional ability to interact with hydrophobic molecules.¹² Functionalized C_{60} derivatives have been extensively investigated for antioxidant, anti-inflammatory, and anticancer applications.^{13,14} More broadly, fullerene-based materials and fullerene/polymer hybrid systems have been explored as anticancer delivery platforms; however, their performance depends heavily on formulation design, surface chemistry, and the specific drug type.¹⁵ For instance, Raza et al. demonstrated that carboxylated C_{60} conjugated with docetaxel significantly enhanced drug solubility, cellular uptake, and cytotoxic efficacy.¹⁴ Within drug-delivery systems, C_{60} can serve as a multifunctional carrier for hydrophobic drugs through π - π and hydrophobic interactions.¹⁶ Earlier studies have also indicated the feasibility of fullerene-based carriers for PTX loading and hydrophobic drug delivery.^{17,18} These findings suggest that integrating C_{60} into polymeric nanofiber matrices may offer a promising strategy for formulating hydrophobic drugs within structurally versatile biodegradable systems.

PTX, a potent hydrophobic anticancer agent, remains challenging to deliver effectively as systemic administration is often associated with limited tumor-site accumulation and severe adverse effects. Consequently, electrospun fibrous scaffolds have emerged as promising platforms for localized drug delivery owing to their high surface-area-to-volume ratio, high porosity, and capacity to incorporate poorly water-soluble agents.¹⁹ Prior research has established that electrospun biodegradable scaffolds can serve as implantable platforms for localized PTX delivery, demonstrating robust in vivo antitumor activity. The release kinetics of such systems can be further tailored by modulating polymer composition, molar mass, hydrophilicity, and degradation behavior.^{20,21} Recent study has further shown that PCL-based electrospun PTX patches provide sustained release and favorable anticancer performance, underscoring their potential for localized breast cancer therapy.²² Collectively, these

findings highlight the versatility of electrospun polymeric scaffolds as tunable systems for the controlled delivery of hydrophobic anticancer agents.

To better position the present study within the existing literature, Table 1 summarizes recent PTX-loaded scaffold systems. Although many reported platforms show promise for localized drug delivery, achieving a balance among fabrication simplicity, controlled release, and acceptable biological performance remains challenging. In this context, the proposed electrospun PCL- C₆₀-PTX scaffold offers a simple fibrous platform with sustained PTX release, gradual degradation, and acceptable hemocompatibility under the tested conditions.

Table 1. Comparison of recent PTX-loaded scaffold systems with the present PCL- C₆₀-PTX nanofibrous scaffold.

Platform	Drug(s)	Design strategy	Main outcome	Reference
PGCL/PLGA scaffold	PTX	Scaffold-based local delivery combined with a brachytherapy-related approach	In vivo validation and high translational potential	²⁰
PDLGA nonwovens	PTX	Composition-tuned fibrous nonwoven system	Tunable release behavior with in vivo evaluation in a 4T1 model	²¹
PCL/PVA patch	PTX	QbD-optimized electrospun formulation	Systematic formulation optimization; in vitro evaluation using MDA-MB-231 cells	²²
PLA nanofiber-on-film composite	PTX	Electrospun PLA nanofibers deposited onto a spin-coated film	Improved structural integration and prolonged drug release; evaluation in HCT-116 cells	²³
Liposome-loaded chitosan/PCL core-shell nanofibers	PTX	Hybrid liposome-incorporated core-shell architecture	Sustained release for up to 30 days with in vitro and in vivo antitumor activity	²⁴
Tri-layer PCL/gelatin nanofibers	PTX + 5-FU	Multilayer dual-drug electrospun system	High drug-loading efficiency and controlled release over 72 h; tested on MCF-7 cells	²⁵
PCL/C60 electrospun scaffold	PTX	Fullerene-reinforced single-drug scaffold	Sustained PTX release and reduced MCF-7 cell viability	Present study

Electrospun PCL-based scaffolds have been widely investigated for localized drug delivery applications, and C₆₀-based nanocarriers have also attracted considerable interest for delivering anticancer agents such as PTX. However, the incorporation of C₆₀ into electrospun PCL nanofibrous scaffolds for PTX loading and sustained release has rarely been reported and remains insufficiently explored. In addition, the effects of integrating PCL, C₆₀, and PTX within a single electrospun platform on scaffold properties, degradation behavior, hemocompatibility, drug-release behavior, and anticancer activity have not been comprehensively evaluated. Therefore, the present study aimed to fabricate and characterize electrospun PCL- C₆₀ and PTX-loaded PCL- C₆₀ nanofibrous scaffolds and to systematically assess their physicochemical properties, degradation behavior, hemocompatibility, drug-release profile, and cytotoxic activity against MCF-7 breast cancer cells. The developed platform was designed as a localized drug-delivery system for sustained PTX and in vitro evaluation in breast cancer cells.

Materials and methods

Materials

Fullerene (C₆₀) and paclitaxel (PTX) were sourced from Sigma-Aldrich (USA). Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were provided by Gibco (USA). Poly(ϵ -caprolactone) (PCL) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (Germany). Chloroform, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were procured from Merck (Germany). The Acridine Orange/Propidium Iodide (AO/PI) assay kit and the flow cytometry kit were purchased from QMeDx.

Fabrication of electrospun PCL-C₆₀ and PCL- C₆₀-PTX nanofibrous scaffolds

Electrospun scaffolds were fabricated using a solvent system composed of chloroform and dimethylformamide (4:1 v/v ratio). To ensure reproducibility and define the formulation composition, C₆₀ nanoparticles were first dispersed in the solvent mixture at a fixed concentration of 8 μ g/mL under continuous magnetic stirring for 24 hours to obtain a homogeneous suspension. Subsequently, PCL was added to achieve a final polymer concentration of 13% (w/v) and allowed to dissolve for 3 hours. For the drug-loaded formulation (PCL-C₆₀-PTX), PTX was incorporated into the PCL-C₆₀ solution at an initial concentration of 4 μ g/mL and stirred for an additional 3 hours. The initial PTX concentration of 4 μ g/mL represents the feed concentration used during the electrospinning process. The amount of PTX incorporated into the scaffold was evaluated indirectly through the subsequent in vitro release study. The release profile was determined by quantifying PTX concentration in the release medium using a UV-Vis spectrophotometer based on a calibration curve. This approach allowed the assessment of PTX availability from the nanofibrous scaffold under physiological conditions.

The electrospinning process was conducted using a 22-gauge needle and a grounded aluminum foil-covered rotating drum collector. To maintain consistency, all scaffolds were fabricated under identical operational conditions: a tip-to-collector distance of 12 cm, a feed rate of 0.8 mL/h, an applied voltage of 18 kV, and a collector rotation speed of 300 rpm. The resulting nanofibrous mats were collected and stored in a desiccator until further physicochemical and biological analyses.

Characterization of electrospun PCL-C₆₀ and PCL-C₆₀-PTX scaffolds

The morphological and physicochemical properties of the electrospun PCL-C₆₀ and PCL-C₆₀-PTX scaffolds were comprehensively characterized. The surface morphology of the electrospun PCL-C₆₀ and PCL-C₆₀-PTX nanofibrous scaffolds was characterized by scanning electron microscopy (SEM; VEGA3, TESCAN, Czech Republic) following gold sputter coating. The obtained SEM images were analyzed to evaluate fiber morphology and structural uniformity. Fiber diameters were measured using ImageJ software based on approximately 75 randomly selected fibers from SEM micrographs of each sample. The mean fiber diameter and diameter distribution were then calculated and presented as histograms. The chemical composition and molecular interactions were investigated by Fourier-transform infrared spectroscopy (FTIR, Thermo Avatar) in the range of 600–4000 cm⁻¹, while the crystalline structure and phase composition were identified through X-ray diffraction (XRD, Philips PW1730) using Cu K α radiation ($\lambda = 0.1542$ nm) scanned over $2\theta = 4-99^\circ$. The wettability of the electrospun nanofibrous scaffolds was assessed by static water contact angle analysis. Briefly, scaffold specimens were cut into appropriate dimensions and mounted on glass slides. A 10 μ L droplet of distilled water was gently deposited onto the scaffold surface, and the droplet profile was immediately captured using a digital camera. The water contact angle was determined from the captured images using ImageJ software (version 1.52v). Measurements were performed in triplicate at different positions for each sample, and the results were expressed as mean $\pm$ standard deviation. The swelling behavior of the scaffolds was evaluated by incubating pre-weighed rectangular samples in PBS (pH 7.4) at 37°C for 6, 12, and 24 h. At each time point, the samples were removed and gently blotted with filter paper to remove excess surface moisture before weighing. The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio (\%)} = \frac{W_s - W_d}{W_d} \times 100$$

where W_d is the initial dry weight of the sample and W_s is the swollen weight at the specified time point. Data are presented as mean $\pm$ SD (n = 3).

The degradation behavior of PCL-C₆₀ and PCL-C₆₀-PTX scaffolds was evaluated in PBS (pH 7.4) at 37°C over 21 days. Pre-weighed samples were incubated and collected at predetermined time points (1, 2, 3, 5, 7, 14, and 21 days). At each time point, the samples were removed, gently rinsed, dried, and weighed. The percentage of degradation was calculated based on weight loss using the following equation:

$$\text{Weight loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the initial dry weight and W_t is the dry weight at each time point. Data are presented as mean $\pm$ SD (n = 3).

Evaluation of PTX release behavior

The in vitro PTX release profile from the PCL-C₆₀-PTX nanofibrous scaffold was investigated in PBS (pH 7.4) at 37 °C. Briefly, scaffold samples with a defined weight of 20 mg were immersed in 10 mL of release medium and incubated under gentle shaking at 37 °C. At predetermined time points (0, 1, 2, 6, 12, 24, 48, and 72 h), 1 mL aliquots of the release medium were withdrawn and analyzed using a double-beam UV–Vis spectrophotometer (JENWAY 6850, England) at 230 nm. An equal volume of fresh PBS was immediately added after each sampling to maintain a constant release volume. This sampling procedure was applied to minimize the accumulation of released PTX in the medium and reduce potential saturation effects during the release experiment. The selected release medium volume and continuous replacement of the withdrawn samples were considered appropriate to maintain suitable sink conditions throughout the study. PTX concentrations were quantified using a standard calibration curve, and the cumulative drug-release percentage was calculated accordingly. All experiments were performed in triplicate, and the results were reported as mean ± standard deviation (n = 3).

To evaluate the release kinetics, the experimental data were fitted to Zero-order, First-order, Higuchi, and Korsmeyer-Peppas models. Model fitting was performed using linear regression of the transformed equations, and the goodness of fit was evaluated based on the correlation coefficient (R²). For the Korsmeyer-Peppas model, only the initial stage of release corresponding to less than 60% cumulative release was considered for mechanistic interpretation.²⁶

Hemocompatibility assay

The hemocompatibility test was performed to evaluate the blood compatibility of the fabricated scaffolds, including PCL-C₆₀ and PCL-C₆₀-PTX. Healthy human blood anticoagulated with sodium citrate (3.8 wt.%) at a ratio of 9:1 (blood: anticoagulant) was diluted with PBS at a volume ratio of 4:5. Samples of each scaffold were placed separately in 15 mL centrifuge tubes containing 10 mL of PBS and pre-incubated at 37 °C for 30 min. Subsequently, 0.2 mL of the diluted blood was added to each tube, and the mixtures were incubated at 37 °C for 60 min. PBS and distilled water were used as the negative and positive controls, respectively. After incubation, all tubes were centrifuged at 3000 rpm for 5 min, and the supernatants were collected and transferred to 96-well plates for spectrophotometric measurement at 570 nm. All measurements were performed in triplicate for each group, and the mean hemolysis percentage was reported as the final result. Hemolysis percentage was calculated according to the equation:

$$HR(\%) = \frac{OD_s - OD_n}{OD_p - OD_n} \times 100$$

OD_s, OD_n, and OD_p represent the absorbance of the sample, negative control (PBS), and positive control (distilled water), respectively.

In vitro anticancer assessment

The anticancer potential of the electrospun PCL-C₆₀ and PCL-C₆₀-PTX scaffolds was assessed against MCF-7 human breast carcinoma cells cultured in DMEM supplemented with 10% FBS at 37 °C and 5% CO₂. Cells

(1×10^4 cells/well) were seeded in 96-well plates and allowed to attach for 24 h. The experimental groups consisted of: (1) Untreated cells cultured in complete medium as the control group, (2) cells exposed to PCL-C₆₀ scaffolds, and (3) cells exposed to PCL-C₆₀-PTX scaffolds. After incubation for 24, 48, and 72 h, cell viability was determined by MTT assay by adding 20 μ L of MTT solution (5 mg/mL) and incubating for 4 h, followed by dissolution of the formed formazan crystals in 100 μ L DMSO. Optical density was measured at 570 nm, and cell viability was expressed as a percentage relative to the untreated control group. Four replicate measurements were obtained for each group.

Flow-cytometric assessment of apoptosis

Flow-cytometric assessment of apoptosis was conducted using the Annexin V-FITC/PI assay. MCF-7 cells were divided into three groups: untreated control cells, cells treated with PCL-C₆₀ scaffolds, and cells treated with PCL-C₆₀-PTX scaffolds. After 24 h of incubation, cells from all groups were harvested, trypsinized, and stained with Annexin V-FITC and propidium iodide (PI) for 15 min in the dark. Samples were immediately analyzed by flow cytometry using FL1 for FITC and FL2 for PI detection to quantify viable, early apoptotic, late apoptotic, and necrotic cell populations. All flow cytometric analyses were performed in triplicate (n = 3) using three independent experiments.

Acridine orange/propidium iodide (AO/PI) staining

Acridine orange/propidium iodide (AO/PI) dual staining was performed to evaluate apoptosis and cell death in MCF-7 cells after exposure to the fabricated scaffolds. Three experimental groups were assessed, including untreated control cells, cells treated with PCL-C₆₀ scaffolds, and cells treated with PCL-C₆₀-PTX scaffolds. AO, a membrane-permeable fluorescent dye, stains viable cells green, whereas PI, which penetrates only cells with damaged membranes, stains late apoptotic or necrotic cells red. For staining, 10 μ L of the cell suspension was mixed with 2 μ L of the AO/PI working solution and incubated at 37 °C in the dark for 30 min. The stained cells were then observed under a fluorescence microscope to distinguish viable (green) cells from apoptotic or dead (red) cells. This assay was used as a qualitative method to compare scaffold-induced cytotoxicity among the experimental groups.

Statistical Analysis

All quantitative data are presented as mean $\pm$ standard deviation (SD). Comparisons among multiple groups were conducted using analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. A p value < 0.05 was considered statistically significant. Significance levels are indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001; ns denotes non-significant differences. Statistical analysis was performed using IBM SPSS Statistics version 29.0.

Results

Characterization of electrospun PCL-C₆₀ and PCL-C₆₀-PTX scaffolds

SEM micrographs revealed that both electrospun scaffolds exhibited continuous, randomly oriented, and bead-free fibrous structures. The drug-free PCL-C₆₀ scaffold (Figure 1A₁) showed relatively uniform fibers, whereas the PTX-loaded PCL-C₆₀-PTX scaffold (Figure 1A₂) exhibited thinner fibers with a broader diameter distribution and greater size variability. Quantitative analysis of SEM images showed that the average fiber diameter decreased from 312 ± 63 nm ($n = 74$) for the PCL-C₆₀ scaffold to 199 ± 98 nm ($n = 76$) for the PCL-C₆₀-PTX scaffold. These results suggest that PTX incorporation influenced jet stretching during electrospinning, resulting in reduced fiber diameter and lower diameter uniformity.

The FTIR spectra of PCL-C₆₀ (Figure 1B₁) and PCL-C₆₀-PTX (Figure 1B₂) scaffolds indicated the preservation of the characteristic chemical structure of PCL after incorporation of C₆₀ and PTX. Both samples showed the typical PCL bands, including the ester carbonyl stretching at ~ 1724 cm⁻¹, CH₂ stretching at 2953–2954 cm⁻¹, CH₂ bending at 1463 and 1400 cm⁻¹, and C–O/C–O–C stretching vibrations in the 1258–1066 cm⁻¹ region. Although no distinct new band corresponding to C₆₀ was clearly observed, this can be attributed to its low content, weak IR activity, and overlap with the dominant PCL peaks. However, slight changes in peak position and intensity suggest its successful incorporation into the polymer matrix. After PTX loading, additional changes appeared in the fingerprint region, particularly near 1599, 1531, and 1368 cm⁻¹, indicating weak intermolecular interactions between PTX and the PCL–C₆₀ scaffold.^{27,28} Overall, the FTIR results suggest that both C₆₀ and PTX were successfully incorporated, mainly through physical entrapment and non-covalent interactions.

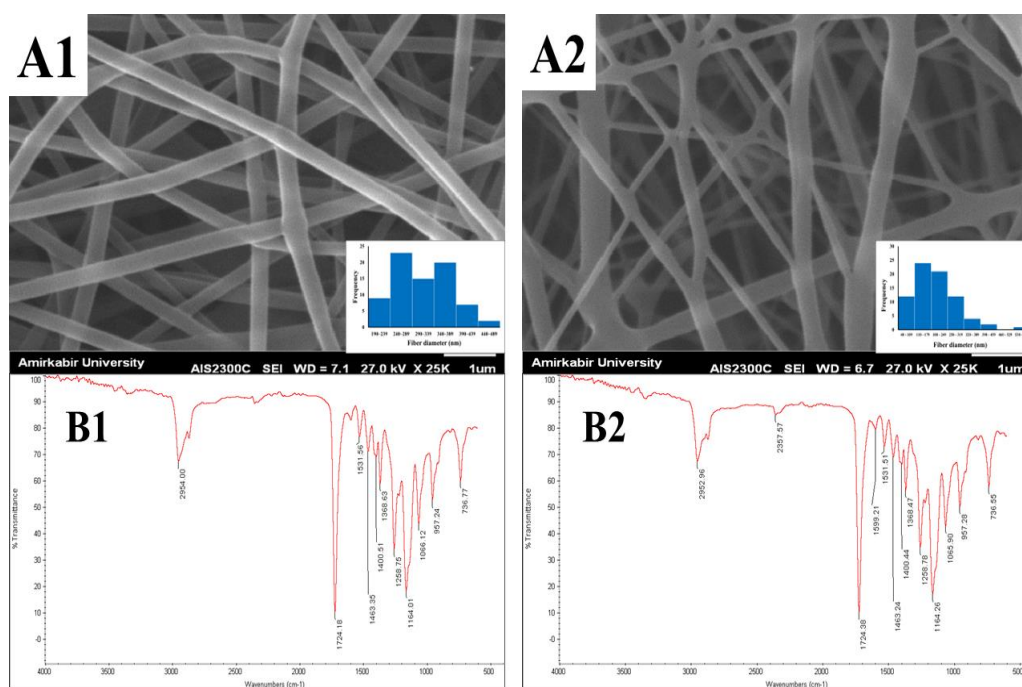


Figure 1. Morphological and chemical characterization of electrospun PCL–C₆₀ and PCL–C₆₀–PTX scaffolds. SEM micrographs of (A1) PCL–C₆₀ and (A2) PCL–C₆₀–PTX scaffolds showing continuous, randomly oriented, and bead-free nanofibrous structures; the insets represent the corresponding fiber diameter distributions. FTIR spectra of (B1) PCL–C₆₀ and (B2) PCL–C₆₀–PTX scaffolds indicating the preservation of characteristic PCL absorption bands and successful incorporation of C₆₀ and PTX mainly through physical entrapment and non-covalent interactions.

XRD patterns of PCL- C_{60} (Figure 2A₁) and PCL- C_{60} -PTX (Figure 2A₂) scaffolds showed the characteristic diffraction peaks of semicrystalline PCL at around 21.4° and 23.7° , corresponding to the (110) and (200) planes, respectively. These peaks indicated that the crystalline structure of PCL was preserved after incorporation of C_{60} and PTX. No distinct diffraction peaks related to C_{60} or PTX were observed, probably due to their low concentration, good dispersion, and overlap with the dominant PCL peaks. The sharper peak observed in the PCL- C_{60} -PTX scaffold may indicate slight changes in crystallinity or chain arrangement after PTX loading. Overall, XRD analysis indicated the semicrystalline nature of the scaffolds and suggested that PTX was mainly dispersed in an amorphous or non-crystalline state within the PCL- C_{60} matrix.²⁹⁻³¹

Water contact angle analysis showed that PTX incorporation reduced the contact angle from $94.08 \pm 1.94^\circ$ in the PCL- C_{60} scaffold (Figure 2B₁) to $87.47 \pm 0.43^\circ$ in the PCL- C_{60} -PTX scaffold (Figure 2B₂) ($p = 0.023$), suggesting improved surface wettability after drug loading. Although both scaffolds showed relatively hydrophobic behavior, the decrease in contact angle indicates a slight increase in the hydrophilic character of the PTX-loaded scaffold.

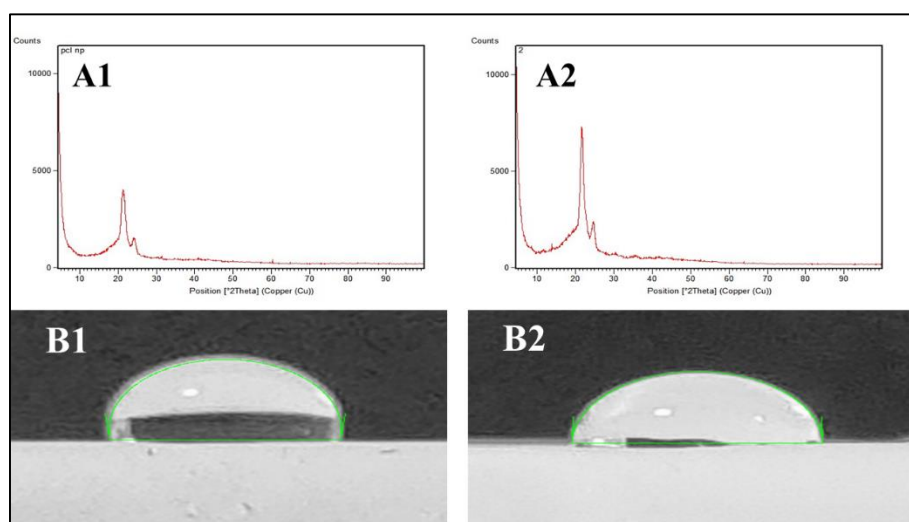


Figure 2. Structural and surface wettability characterization of electrospun PCL- C_{60} and PCL- C_{60} -PTX scaffolds. (A1) XRD pattern of PCL- C_{60} , (A2) XRD pattern of PCL- C_{60} -PTX, (B1) water contact angle image of PCL- C_{60} , and (B2) water contact angle image of PCL- C_{60} -PTX. The XRD results indicated the preservation of the semicrystalline PCL structure, while contact angle measurements showed improved wettability after PTX loading.

Swelling behavior analysis showed that both scaffold groups exhibited a time-dependent increase in swelling over 24 h. Although the PCL- C_{60} scaffold showed slightly higher swelling values than the PCL- C_{60} -PTX scaffold, the differences were not statistically significant ($p > 0.05$). Overall, both scaffold types demonstrated relatively low swelling under the tested conditions (Figure 3A).

The in vitro degradation profiles of PCL- C_{60} - and PCL- C_{60} -PTX scaffolds over 21 days are shown in Figure 3B. Both groups exhibited a time-dependent increase in weight loss, reflecting progressive degradation under the experimental conditions. A modest trend toward higher degradation was observed for the PCL- C_{60} -PTX scaffold at later time points; however, statistical analysis revealed no significant differences between the groups ($p > 0.05$). Data are expressed as mean $\pm$ SD ($n = 3$), indicating that PTX incorporation did not markedly alter the degradation behavior of the scaffold.

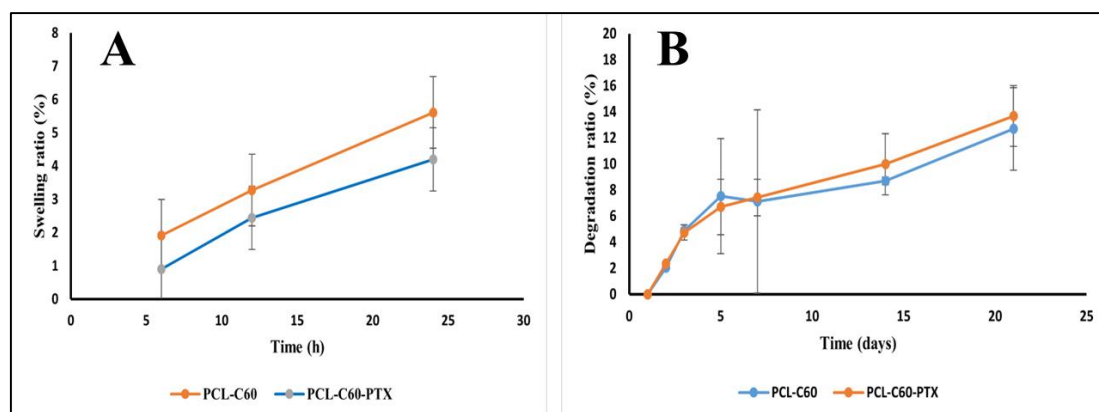


Figure 3. Swelling behavior and in vitro degradation profile of electrospun PCL-C₆₀ and PCL-C₆₀-PTX scaffolds. (A) Swelling ratio measured after 6, 12, and 24 h of incubation in aqueous medium, expressed as the percentage increase in scaffold weight relative to the initial dry weight. (B) In vitro degradation over 21 days, expressed as percentage weight loss. Data are shown as mean $\pm$ SD ($n = 3$). No statistically significant differences were observed between the two scaffold groups in either test ($p > 0.05$).

Evaluation of PTX release behavior

Although the initial PTX feed concentration was defined during scaffold fabrication, direct quantification of PTX loading efficiency in the final scaffold was not performed in the current study. The PTX release profile from the PCL-C₆₀-PTX scaffold (Figure 4) showed a characteristic biphasic pattern. Data are presented as mean $\pm$ SD ($n = 3$), and one-way ANOVA indicated a statistically significant difference in cumulative release across all investigated time points ($p < 0.0001$). An initial burst release was observed during the first hour, with $18.55 \pm 0.63\%$ of PTX released. This was followed by a more controlled, slower release phase. By 72 h, the cumulative release reached $74.36 \pm 0.50\%$, after which the release curve approached a plateau.

To characterize the release mechanism, the experimental data were fitted to various kinetic models. As summarized in Table S2, the Zero-order model provided the weakest fit ($R^2 = 0.650$), whereas the First-order and Higuchi models showed higher correlations ($R^2 = 0.925$ and $R^2 = 0.971$, respectively). Among all tested models, the Korsmeyer-Peppas model exhibited the best fit ($R^2 = 0.987$). The calculated release exponent ($n = 0.400$) suggests that the drug release mechanism is governed by Fickian diffusion from the nanofibrous matrix (Table 2).

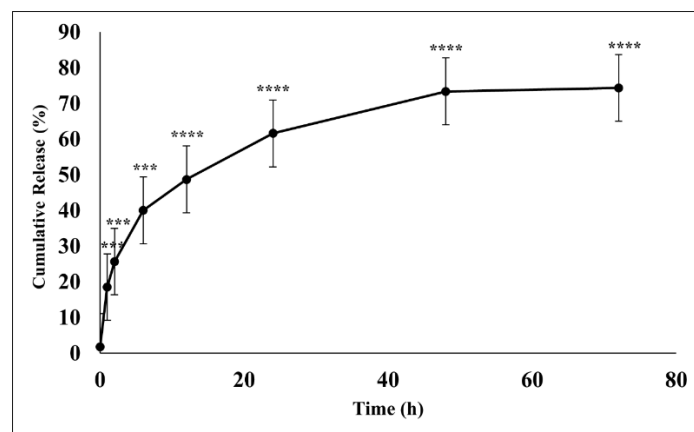


Figure 4. Cumulative in vitro release profile of PTX from the electrospun PCL-C₆₀-PTX scaffold over 72 h. The cumulative release percentage was measured at 0, 1, 2, 6, 12, 24, 48, and 72 h. Data are presented as mean $\pm$ SD ($n = 3$). Significance symbols indicate differences compared with 0 h: *** $p < 0.001$ (1, 2, and 6 h) and **** $p < 0.0001$ (12, 24, 48, and 72 h).

Table 2. Kinetic modeling parameters for PTX release from the PCL/C60/PTX electrospun nanofibrous scaffold.

Model	Linearized equation	R ²	Kinetic constant	Release exponent (n)
Zero-order	$Q_t = K_0t + b$	0.650	$K_0 = 0.7313$	—
First-order	$\ln(100 - Q_t) = -K_1t + b$	0.925	$K_1 = 0.0171$	—
Higuchi	$Q_t = K_H t^{0.5} + b$	0.971	$K_H = 8.1899$	—
Korsmeyer-Peppas*	$\log(M_t/M_\infty) = \log K + n \log t$	0.987	$K = 0.1880$	0.400

* Only the initial release data corresponding to less than 60% cumulative release were used for the Korsmeyer-Peppas fitting.

Hemocompatibility assay

The hemocompatibility of the electrospun scaffolds was evaluated using a hemolysis assay (Figure 5). The positive control produced marked hemolysis, reaching approximately 100%, and differed significantly from all other groups (****, $p < 0.0001$). In contrast, both PCL-C₆₀ and PCL-C₆₀-PTX scaffolds showed minimal hemolysis, with values below the 5% threshold defined by ASTM F756.³² No significant difference (ns, $p > 0.05$) was observed between the negative control and either scaffold group, or between the two scaffold groups.

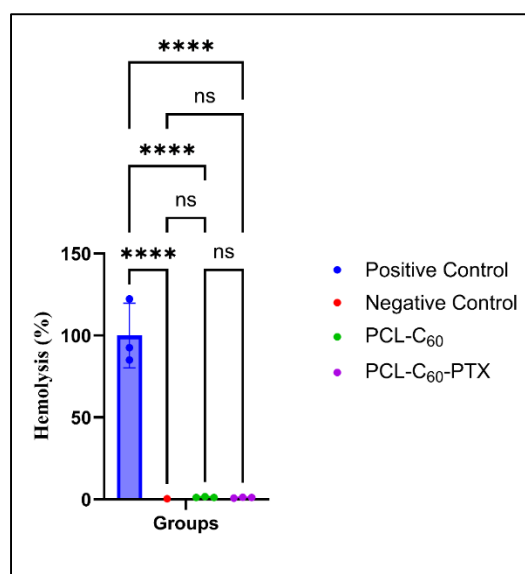


Figure 5. Hemolysis assay of the positive control, negative control, PCL-C₆₀, and PCL-C₆₀-PTX scaffolds. Data are presented as mean $\pm$ SD from three measurements. **** $p < 0.0001$; ns, not significant.

In vitro cytotoxicity assessment

The effects of the scaffolds on MCF-7 cell viability were assessed by MTT assay at 24, 48, and 72 h (Figure 6). A modest decrease in viability was observed in the PCL-C₆₀-PTX group at 24 h, whereas no significant difference was found between the two scaffold groups at 48 and 72 h. The PCL-C₆₀-PTX scaffold showed corresponding viabilities of 75.03%, 69.80%, and 56%. In both scaffold-treated groups, cell viability was lower than that of the

control group and decreased over time. The PCL-C₆₀-PTX group showed lower viability than the PCL-C₆₀ group at 24 h, whereas no significant difference was observed between the two scaffold groups at 48 and 72 h.

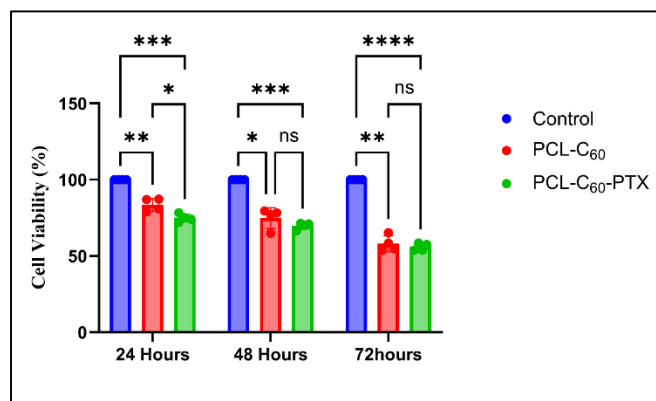


Figure 6. Cell viability of MCF-7 cells treated with PCL-C₆₀ and PCL-C₆₀-PTX scaffolds for 24, 48, and 72 h, as determined by MTT assay. The untreated control was normalized to 100% viability. Data are presented as mean $\pm$ SD from four measurements. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, not significant.

Flow-cytometric assessment of apoptosis

Flow cytometric analysis of apoptosis demonstrated significant differences among the experimental groups (Figure 7). Compared with the control group, both the PCL-C₆₀ and PCL-C₆₀-PTX groups exhibited a significant reduction in the percentage of viable cells ($P < 0.0001$). The percentage of early apoptotic cells was significantly increased in both scaffold-treated groups compared with the control ($P < 0.01$), while no significant difference was observed between the PCL-C₆₀ and PCL-C₆₀-PTX groups ($P > 0.05$). In addition, the proportion of late apoptotic cells was significantly elevated in both treatment groups relative to the control ($P < 0.0001$), with the PCL-C₆₀-PTX group showing a significantly higher percentage than the PCL-C₆₀ group ($P < 0.01$). Necrotic cells were also significantly increased following treatment compared with the control ($P < 0.01$), whereas the percentage of necrotic cells was significantly lower in the PCL-C₆₀-PTX group than in the PCL-C₆₀ group ($P < 0.001$). These findings indicate that incorporation of PTX into the PCL-C₆₀ scaffold was associated with increased apoptotic cell death, particularly late apoptosis, while reducing the proportion of necrotic cells.

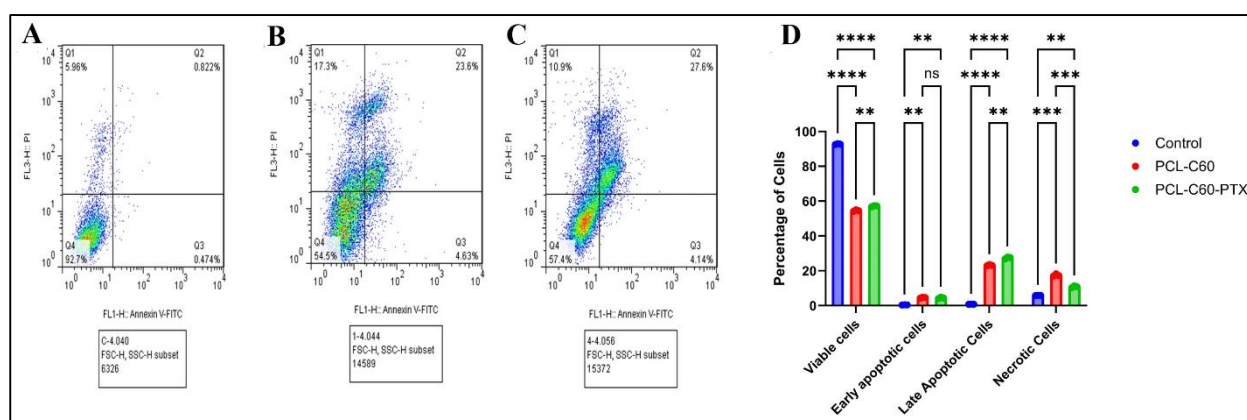


Figure 7. Flow cytometric analysis of apoptosis in MCF-7 cells. (A-C) Representative Annexin V-FITC/PI flow cytometry dot plots. (A) Control cells showing basal levels of apoptosis. (B) MCF-7 cells treated with PCL-C₆₀ scaffolds, and (C) MCF-7 cells treated with PCL-C₆₀-PTX scaffolds. Quadrants are defined as follows: Q4, viable cells (Annexin V⁻/PI⁻); Q3, early apoptotic cells (Annexin V⁺/PI⁻); Q2, late apoptotic cells (Annexin V⁺/PI⁺); and Q1, necrotic cells (Annexin V⁻/PI⁺). (D) Quantitative analysis of cell populations across the experimental groups. Data are presented as mean $\pm$ SD. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns, not significant ($p > 0.05$).

Acridine orange/propidium iodide (AO/PI) staining

Fluorescence imaging using AO/PI staining (Figure 8) showed that the control group predominantly contained green fluorescent cells, indicating a high proportion of viable cells, with only a few PI-positive nuclei. In the PCL- C_{60} group, an increased number of PI-positive cells together with reduced cell density was observed. A slightly higher number of red/orange fluorescent cells was observed in the PCL- C_{60} -PTX group. Overall, the AO/PI staining findings were consistent with the MTT and flow cytometry results, showing reduced cell density and increased apoptotic/PI-positive cell populations in the scaffold-treated groups relative to the control group, with a slight increase in PI-positive cells in the PCL- C_{60} -PTX group.

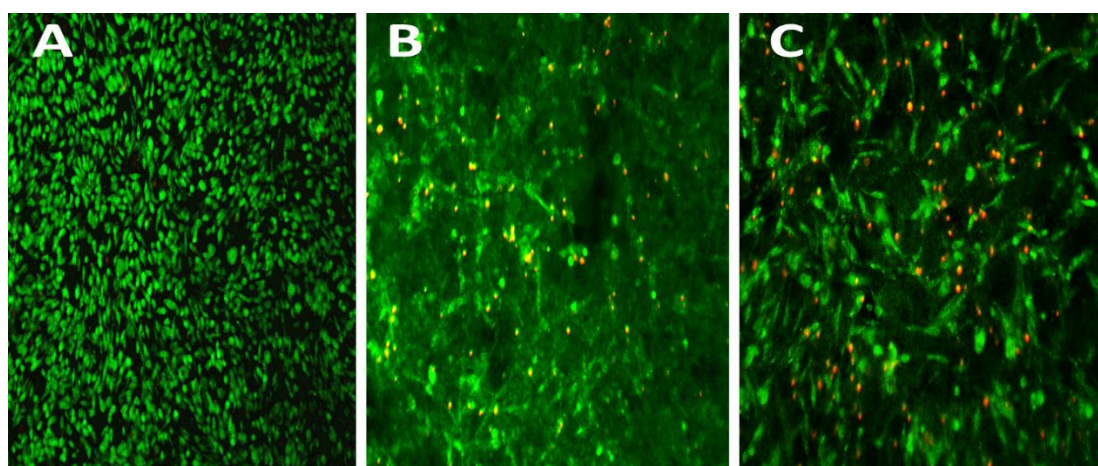


Figure 8. Fluorescence microscopy images of AO/PI-stained MCF-7 cells following different treatments. (A) Control cells, (B) Cells treated with PCL- C_{60} scaffolds, (C) Cells treated with PCL- C_{60} -PTX scaffolds. AO stains viable and early apoptotic cells green, whereas PI stains late apoptotic or necrotic cells red/orange.

Discussion

The rapid advancement of nanocomposite engineering has enabled the development of highly functional electrospun platforms for localized and sustained drug delivery, where carrier physicochemical properties may influence drug delivery behavior.^{1,33,34} PCL is widely used as a biocompatible, slowly degradable polymeric matrix, while C_{60} has been investigated in previous studies for properties such as antioxidant activity and drug-loading potential. However, their combined incorporation into a uniform nanofibrous system for the delivery of PTX has not been sufficiently explored.^{5,16,17,35} This gap is particularly important because incorporation of hydrophobic C_{60} nanoparticles into the PCL matrix may alter microstructural features, hydration behavior, and degradation kinetics, which in turn can influence the drug-release profile and subsequent biological responses in cancer cells. In this study, we designed and evaluated a PCL- C_{60} nanofibrous system aimed at providing controlled PTX release and evaluating its in vitro cytotoxicity against MCF-7 cells.

SEM imaging revealed noticeable morphological changes following PTX incorporation. The drug-free PCL- C_{60} scaffold exhibited relatively uniform, smooth, and bead-free fibers, whereas the PTX-loaded PCL- C_{60} -PTX scaffold showed thinner fibers with a broader diameter distribution and a rougher surface texture. Quantitative analysis indicated that the mean fiber diameter decreased from 312 ± 63 nm in the PCL- C_{60} scaffold to 199 ± 98 nm in the PCL- C_{60} -PTX scaffold. These changes may be attributed to PTX-induced alterations in the

physicochemical properties of the electrospinning solution, such as viscosity, conductivity, or polymer-drug interactions, which can affect jet stability and fiber formation. Consistent with the present findings, Chi et al. reported that PTX incorporation into a polylactic acid electrospinning solution resulted in finer fibers and altered surface roughness, which was associated with changes in solution viscosity.²³ This reduction in fiber diameter and increase in heterogeneity may have increased the effective surface area of the scaffold and influenced its interaction with the aqueous medium. As a result, these morphological changes may be associated with the observed swelling behavior, degradation profile, and the initial burst release of PTX.

The FTIR and XRD results together suggest that the incorporation of C₆₀ and PTX did not markedly alter the main chemical structure or semicrystalline characteristics of PCL. In the FTIR spectra, the characteristic absorption bands of PCL were preserved after loading, with no pronounced new absorption band or major peak shift. However, minor differences in band intensity and subtle changes in the fingerprint region were observed. Similarly, the XRD patterns retained the principal reflections of semicrystalline PCL, without distinct additional crystalline peaks attributable to PTX or C₆₀. These findings collectively indicate that both components were incorporated into the electrospun fibers without substantial disruption of the polymer matrix and are likely present in a physically dispersed state. These findings are consistent with those reported by Devi and Gayathri. Their comprehensive FTIR and FT-Raman study indicated that the major functional groups of PTX remained chemically intact in both solvent-based and polymeric nanoparticle formulations, with no clear spectroscopic evidence of interactions that substantially altered the drug structure.³⁶ Furthermore, Totiger and Hiremath reported findings consistent with PTX dispersion, as neither characteristic PTX absorption bands were detected in FTIR nor drug-related crystalline reflections were observed in XRD.³⁷ The FTIR spectra of cellulose-acetate and C₆₀ nanofibers showed preservation of the characteristic polymer functional groups, with no new absorption bands attributed to C₆₀, suggesting non-covalent incorporation within the electrospun matrix.¹³ Consistent with our observations, Liu et al. reported that C₆₀ can be incorporated into electrospun aliphatic polyester nanofibers without obvious evidence of new chemical bonds or crystalline phases, supporting the interpretation that they may remain physically dispersed within the polymer matrix.³⁸

The wettability and hydration behavior of the scaffolds clarify how PTX modulates surface interactions within the PCL-C₆₀ matrix. The higher contact angle of the PCL-C₆₀ scaffold (~94°) is consistent with the relatively hydrophobic character of fullerene-containing systems, which may contribute to reduced water spreading.²⁸ Since surface wettability is an important factor affecting protein adsorption and initial cell-surface interactions, the improved hydrophilicity of the drug-loaded scaffold may provide a more favorable interface for cellular attachment and biological response. Although both scaffolds remained within a moderately hydrophobic range, the decrease in contact angle after PTX loading indicates a meaningful modification of the scaffold surface properties. The reduction in water contact angle observed in PCL-C₆₀-PTX scaffold is consistent with the results reported by Chi et al., in which PTX incorporation similarly decreased the contact angle of polylactic acid (PLA) nanofibers. Despite differences in polymer chemistry (PLA vs. PCL), both studies suggest that PTX incorporation may affect surface wettability in electrospun matrices.²³ The lower early-stage swelling observed in PCL-C₆₀-PTX scaffolds may be associated with transient surface densification or partial pore obstruction around PTX-rich regions; however, these mechanisms were not directly examined in the present study. With prolonged immersion,

both scaffolds demonstrated progressive water uptake, suggesting that hydration increasingly involves diffusion through the polymer network over time.

The degradation results showed a gradual increase in mass loss in both scaffolds over the 21 days, with the PCL- C_{60} -PTX scaffold showing slightly higher values at several time points. The minimal mass change observed on the first day is in agreement with previous reports describing an initial phase of limited hydrolytic degradation in PCL-based materials.³⁹ From days 2 to 7, the PTX-loaded scaffold showed relatively higher mass loss, which may be related to differences in scaffold structure and water penetration within the nanofibrous matrix, as suggested in other modified PCL systems.⁴⁰ After day 14, both materials continued to exhibit a gradual degradation pattern consistent with the generally slow degradation behavior of PCL under physiological conditions.⁴¹ The slightly higher mass loss observed in the PTX-containing scaffold may also reflect the influence of drug incorporation on scaffold composition and fluid interaction, as reported for other polyester-based systems.⁴² Although C_{60} nanoparticles may affect surface-related properties and polymer-water interactions, the specific contribution of C_{60} to the degradation behavior in the present study cannot be determined from the current dataset.

The release profile exhibited an initial burst followed by a rapid release phase up to 12 h and a gradual plateau thereafter. The kinetic modeling results indicate that PTX release from the developed scaffold suggests that release was mainly governed by diffusion-related mechanisms. The strong correlation with the Higuchi model suggests that diffusion through the polymer matrix is a major factor controlling drug release. In addition, the best fit obtained with the Korsmeyer-Peppas model during the initial release stage, together with the release exponent value of $n = 0.400$, supports a Fickian diffusion mechanism. These findings suggest that the nanofibrous PCL-based matrix acts as the principal barrier regulating PTX transport, thereby contributing to the sustained-release profile of the scaffold.²⁶ These findings are consistent with the sustained-release behavior observed in the release profile and support diffusion-controlled drug transport through the nanofibrous matrix. Previous studies have suggested that C_{60} -containing systems may influence the behavior of hydrophobic drugs, potentially through changes in the local microenvironment. In the literature, covalently linked C_{60} -drug systems have generally been associated with slower release behavior because bond cleavage is required, whereas in physically loaded systems, release is more often interpreted in terms of diffusion-controlled processes.⁴³ Similarly, Zakharian et al. reported slower hydrolytic release in covalent systems, whereas non-covalent matrices were associated with more rapid diffusion-driven release behavior.¹⁷ In addition, Blazkova et al. described non-covalent interactions between oxidized C_{60} and DOX, suggesting that C_{60} -based materials may interact with hydrophobic drugs in a formulation-dependent manner.⁴⁴ Similar biphasic PTX release profiles have been reported in other PCL-based delivery systems, including those described by Chernonosova et al.⁴⁵ and Rezk et al., the latter of whom interpreted the sustained-release phase using a Fickian diffusion mode.⁴⁶ Furthermore, recent advances in biodegradable PCL-based delivery systems, such as composite PCL/chitosan/alginate implants, have also demonstrated similar biphasic profiles with sustained drug release over 15 days, further supporting the general suitability of PCL-containing matrices for prolonged localized chemotherapy.⁴⁷ However, the clinical relevance and therapeutic suitability of the observed release kinetics require further investigation through dose-response studies and in vivo evaluation.

The in vitro biological evaluations provided initial evidence of acceptable hemocompatibility under the tested conditions and demonstrated in vitro anticancer activity against MCF-7 cells. The observed hemolysis values

below 5% suggest favorable hemocompatibility of the scaffolds. These findings are consistent with previous reports describing the erythrocyte compatibility of pristine C₆₀,⁴⁸ the acceptable hemocompatibility of PTX-loaded PLGA nanoparticles,⁴⁹ and the blood compatibility of electrospun PCL-based vascular grafts.⁵⁰ Collectively, these observations suggest that incorporation of C₆₀ and PTX into the PCL matrix did not adversely affect hemocompatibility under the experimental conditions. Nevertheless, hemolysis assessment reflects only blood compatibility and does not by itself indicate overall biocompatibility, which also requires evaluation of inflammatory responses, compatibility with normal cells, and the potential effects of long-term degradation products. In contrast, the anticancer assays showed time-dependent in vitro cytotoxic effects against MCF-7 cells. The PCL-C₆₀ scaffold without PTX produced moderate inhibitory effects under the tested conditions. However, because a PCL-only scaffold was not included, the independent contribution of C₆₀ could not be determined, and the observed effect should therefore be interpreted with caution. PTX incorporation was associated with a greater reduction in cell viability at later time points, particularly at 72 h, which is consistent with sustained drug release from the scaffold. In addition, flow cytometry and AO/PI staining descriptively suggested increased apoptosis in the scaffold-treated groups, with a further apoptotic response observed in the PCL-C₆₀-PTX group. Overall, these findings support the in vitro anticancer activity of the PTX-loaded scaffold against MCF-7 cells under the present experimental conditions.

Recent studies on PTX-loaded PCL-based systems have reported controlled drug-release behavior together with in vitro anticancer effects in different experimental models. Hasanbegloo et al. developed core-shell chitosan/PCL nanofibers encapsulating liposomal PTX, which exhibited a biphasic release pattern and led to reduced MCF-7 cell viability and apoptotic features.²⁴ Similarly, a tri-layer PU-PTX-PCL membrane showed time-dependent cytotoxic effect against CT26 colon cancer cells, demonstrating effective PTX release from the composite scaffold over five days.⁴⁶ Likewise, tri-layered PCL/Gelatin nanofibers co-loaded with 5-fluorouracil and PTX were reported to decrease MCF-7 cell viability and increase the apoptotic cell population after prolonged incubation.²⁵ Additionally, a study on patch-based PCL nanofibers designed for localized breast cancer therapy demonstrated that incorporating PTX into the nanofibrous matrix supported sustained drug release and in vitro cytotoxicity against breast cancer cells.²² Additional PTX-loaded PCL-based delivery systems have also been summarized by Noori et al. in a broader review of nanostructured carriers.⁵¹ Collectively, these studies suggest that PCL-based formulations can support sustained PTX release and time-dependent in vitro anticancer effects, although outcomes depend on scaffold composition and experimental conditions.

The incorporation of C₆₀ into the electrospun PCL scaffold was motivated by its recognized potential as a nanocarrier for poorly water-soluble therapeutic agents. Recent studies have highlighted that carbon-based nanomaterials and other advanced nanocarriers can improve drug loading, enhance the delivery of hydrophobic compounds, and support controlled release behavior.^{14,52} Likewise, recent reviews have emphasized the continued interest in C₆₀-based systems as drug-delivery platforms owing to their unique nanostructure, large surface area, and versatility for cargo transport.³⁵ Consistent with these reports, the PCL/C₆₀ nanofibrous scaffold developed in the present study accommodated PTX and exhibited sustained drug release over the investigated period.

Conclusion

In the present study, electrospun PCL-C₆₀ and PCL-C₆₀-PTX nanofibrous scaffolds were successfully fabricated and characterized in terms of morphology, physicochemical properties, hemocompatibility, PTX release behavior, and in vitro anticancer activity against MCF-7 cells. The PCL-C₆₀ scaffold showed a relatively uniform fibrous structure, whereas PTX incorporation altered fiber morphology and resulted in a biphasic drug-release profile. Both scaffolds exhibited low hemolytic activity, indicating favorable hemocompatibility under the tested conditions. In biological assays, the PCL-C₆₀ scaffold was associated with a moderate reduction in MCF-7 cell viability under the present experimental conditions, whereas PTX-loaded scaffolds showed greater cytotoxic and apoptosis-associated effects. However, due to the absence of additional control groups, the individual contributions of fullerene and PTX cannot be conclusively distinguished. Overall, these findings suggest that the PCL-C₆₀-PTX scaffold could be considered a potential candidate for localized anticancer drug delivery. Further studies using pure PCL and free PTX controls are essential to clarify the respective roles of the scaffold components, investigate the underlying molecular mechanisms, and evaluate safety and therapeutic efficacy in more advanced in vitro and in vivo models.

Limitation

1- One limitation of the present study is the lack of additional control groups, including PCL-only, free paclitaxel (PTX), and vehicle controls. Although the current experimental design was focused on evaluating the overall antitumor performance of the developed PCL/PTX/fullerene-based system, the absence of these controls limits the ability to precisely determine the individual contribution of each component, including the scaffold, paclitaxel, and fullerene, to the observed biological effects. Future studies incorporating these control groups will be necessary to further clarify the underlying mechanisms and to better define the specific role of each component in the therapeutic response.

2- The exact PTX loading efficiency and the precise PTX concentration available to cells during biological assays were not directly quantified. Future studies should include quantitative drug loading analysis and release–response correlation to further clarify the relationship between scaffold composition and biological effects.

Authors Contributions

Asadollah Asadi, Arash Abdolmaleki, and Saber Zahri designed the study and supervised the data collection. Leila Taghizadeh and Arash Abdolmaleki analyzed and interpreted the data. Leila Taghizadeh conducted the investigation, collected the data, and prepared the original manuscript draft. Asadollah Asadi, Arash Abdolmaleki, and Saber Zahri reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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and compliance with academic standards. AI tools were not used for data generation, data analysis, study design, conceptual development, or the production of the scientific content of the manuscript. The authors take full responsibility for the originality, integrity, and accuracy of the final version of the submitted manuscript.

Ethical Approval

This study was approved by the Institutional Ethics Committee of the University of Mohaghegh Ardabili. Human blood samples were collected from a healthy, consenting volunteer and processed in accordance with institutional guidelines.

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Competing interests

The authors declare that they have no financial or non-financial competing interests related to this work.

Data availability

All data supporting the findings of this study are provided within the manuscript. Additional information may be obtained from the corresponding author upon reasonable request.

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