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Anti-cancer activity (A431 cancer cells) and cytotoxic efficiency (HaCaT skin cells) of Curcumin/Neem loaded polycaprolactone (PCL) nanofibres

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ABSTRACT

Presently, the anticancer activity with higher performance and lesser side effects remains a greater challenge in the scientific and medicinal research. We aim to fabricate natural ingredient or extract (curcumin/neem) loaded polycaprolactone (PCL) nanofibres. The fabricated nanofibre were characterised by Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscopy (SEM) and Static water contact angle measurement to investigate the chemical nature, surface morphology and hydrophilicity of the polymer surface. The PCL nanofibre diameter decreases considerably by the addition of neem extract than the addition of curcumin and curcumin/neem to PCL nanofibre, which is confirmed by SEM analysis. The fabricated nanofibres anti-cancer activity and cytotoxic efficiency were conducted on A431 cancer cells and HaCaT skin cells. PCL/Cur/Neem nanofibre showed higher anticancer activity and the PCL and PCL/Cur/Neem nanofibre showed lower cytotoxic activity against HaCaT skin cells.

Keywords: Anti-cancer activity, Curcumin, Nanofibre, Neem, Polycaprolactone.

INTRODUCTION

Synthetic biodegradable polyesters, such as, poly(lactic acid) (PLA), poly(glycolic acid), poly(ε-caprolactone) (PCL) and their copolymers, have been widely investigated for biomedical application because of their biodegradability, bioresorbability, and biocompatibility [1]. The ε-caprolactone and polycaprolactone are currently considered as harmless and tissue-compatible materials based on the large number of tests. Polycaprolactone high solubility, low melting point (59-64 °C), and exceptional ability to form blends has stimulated research on its application as a biomaterial. Polycaprolactone has a high penetrability to lots of drugs and degrades at a slower rate compared to PLA, hence prompting its use in drug delivery devices that stay active for over one year [2]. However, the PCL application for drug delivery has a disadvantage of slow degradation rate in vivo because of its high crystallinity and hydrophobicity [3].

It has been described that the PCL biodegradability can be improved by copolymerizing or blending with a variety of other polymers [4,5]. Polymer blending is an alternative way of tailoring hydrophilicity of the matrix without affecting its mechanical integrity significantly [6].Blending of two polymers is a method to develop novel biomaterials exhibiting combinations of properties that could not be attained by individual polymers [7]. Blending of PCL (synthetic polymer) with chitosan, a naturally derived polysaccharide can be investigated. The postulate is that blending PCL and chitosan will provide a superior biomaterial where the limitations of chitosan are complemented by PCL. Due to various advantages of chitosan, for instance, low cost, readily available, biocompatibility and antimicrobial activity has created huge attention [8].Therefore, this blend can be an enhanced biodegradable and biocompatible material can be utilized in control drug delivery systems.

The growth of new anti-cancer treatments with superior efficiency and lower side effects remains a greater challenge in the modern scientific and medical research. Over the last few decades there has been rapid development in the interest of consuming natural products for therapeutic applications. Curcumin, a natural polyphenol found in the dietary spice turmeric, has been showed to prevent existence and proliferation of cancer cell. Also, it induces apoptosis without promoting the development of side effects. Though, because of its sparing solubility and low bioavailability, until now curcumin has not been clinically used to treat cancer [9]. Furthermore, in several systems, curcumin has been chosen as a potent antioxidant and anti-inflammatory agent. Evidence has also been presented to propose that curcumin can suppress tumor initiation, promotion and metastasis. Pharmacologically, curcumin has been found to be harmless [10]. All of these reports propose that curcumin has vast potential in the prevention and therapy of cancer. Curcumin nanofibres were studied for their in vitro release profile [11] and biocompatibility using fibroblasts [12]. PCL nanofibres loaded with curcumin are studied for their anti-oxidant and anti-inflammatory properties [13], with an interest to apply as diabetic wound dressing material. Anti-tumor activity of curcumin-loaded nanofibres [14] was studied in vitro using rat 9L Glioma cells with an intention for the treatment of postoperative chemotherapy of brain cancers.

Azadirachtaindica (neem) extract has been studied for its anti-cancer activity [15,16] and anti-oxidant properties [17]. Nanofibres of neem extract [18] as a PCL combination were studied for their wound healing application and skin reconstitution. We intend to arrive at a natural extract recipe, having appropriate ingredients in combination with the synthetic biodegradable polymer PCL that can serve as a natural drug component eluting stent or implant for specific application to cancer. Hence in the present study, natural extract (curcumin, and neem) loaded PCL/chitosan blend nanofibres fabricated by the electrospinning method. The prepared samples were used as a biomaterial for treating anticancer activities.

MATERIALS AND METHODS

Natural extract-loaded Polycaprolactone (PCL) nano fibres were fabricated by the electrospinning method according to the literature [19].All chemicals were of analytical grade and used without further purifications.1g Polycaprolactone (PCL) was dissolved in 10% w/v (10 g) methanol: chloroform (3:7 ratio). 50 mg of Curcumin was separately loaded in PCL solution by dissolving its powder at the amount of 5 wt% with respect to PCL. To attain a homogenous solution, the mixture was stirred overnight at room temperature. Neem extracts were taken in the ratio of 1% in connection with PCL and dissolved to form the respective solutions.

Nanosized fibers were examined by using Fourier transform infrared (FTIR) Avatar 380 spectrometer, (Thermo Nicolet, Waltham, MA, USA) in the region of 4000–400 cm^{-1}.

The surface morphology and diameter of the electrospun fibers were observed and determined with the use of an optical microscope (Olympus BX51M, Japan) and a scanning electron microscope (FESEM) (Quanta 200F, FEI, Oregon, US.). Prior to imaging with the use of SEM, a small section of the fibers on the sample holder was sputter coated with gold (JEOL JFC-1200 fine coater, Japan). The SEM was then used to observe the samples at an accelerating voltage of 8 kV.

Nanofibres surface hydrophilicity and hydrophobicity were calculated by measuring static water contact angle measurement on the films (Rame hart Std 100, NJ, USA). For this, 2 µl drops of deionized water were placed on the films and contact angles were measured by using an attached CCD camera after reaching stable values.

The anticancer of samples on A431 cells was determined by the MTT assay. Fabricated nanofibers was cut into small pieces and placed it into the cell culture plate and kept it 2 hours under UV radiation for sterilization. After sterilization, A431 cells (5 × 103 cells per well) were seeded in 96 well plates of final volume of 100 µL and incubated for 24 h and 72 h. Later 20 µL of MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 3 h at 37 °C. The culture medium was removed and 50 µL of DMSO was added to each well to dissolve the purple-blue formazan crystals and optical density was measured at 570 nm using ELISA multi mode plate reader (SYNERGYMX, Bioteck).

Biocompatibility of nanofiber was assessed by MTT Assay. Briefly, fabricated nanofiber was cut into small pieces and placed it into the cell culture plate and kept it 2 hours under UV radiation for sterilization. After sterilization, HaCaT (immortalized human keratinocytes 20 K cells/well) cells were seeded onto cell culture plate and allowed it to grow for 24 hours and 72 hrs at 37^{o}C in a 5% CO_2 and 95% O_2 humidified incubator. After 24 hrs completed the spent medium was removed and cells were examined under phase contrast microscope and observed the cells morphology and photographed using Leica systems Germany. Subsequently, 0.5 mg/ml of MTT (Thiazolyl Blue

Tetrazolium Bromide salt) in 1X PBS (500µL/well) was added and kept it for 4 hours in a dark/ 37^{o}C. The MTT assay was carried out in Triplicates.

RESULTS AND DISCUSSION

PCL and natural extract loaded PCL nanofibres FT-IR spectrum were obtained by KBr method and the corresponding spectrum were shown in Figure 1. The IR vibration bands at 2944 cm^{-1} and 2866 cm^{-1} are corresponds to the CH_2 group asymmetric and symmetric stretching vibrations. The vibration band around at 1726 cm^{-1}due to the PCL nanofibers stretching vibrations of the ester carbonyl group C=O. The vibration band at 1290 $cm^{-1}$is assigned to the crystalline PCL C–C (backbone) and C–O stretching vibration. Hence, this band would be useful to investigate the degree of crystallinity of PCL [20]. The bands at 1238 cm^{-1} and 1180 cm^{-1} are corresponds to the C–O–C asymmetric and symmetric stretching vibrations [21]. The FTIR spectra of curcumin-incorporated PCL shows following characteristic vibrations at 1630 cm^{-1} and 1516 cm^{-1} which is a characteristic peak for C=O (enolic) and the presence of C–C group. The band at 3540 cm^{-1} shows the presence of OH group in the curcumin [22].Neem plant extract carbonyl group band combines with the PCL carbonyl and the presence of the strong peak alkyls of the extract can be observed from the CH stretching around 2925 cm^{-1}, 2860 cm^{-1} and 1470 cm^{-1}. The neem extract loaded nanofibres showed the small shifts in the curcumin bands corresponding to C=O and C-O-C around 1045 cm^{-1} [23].

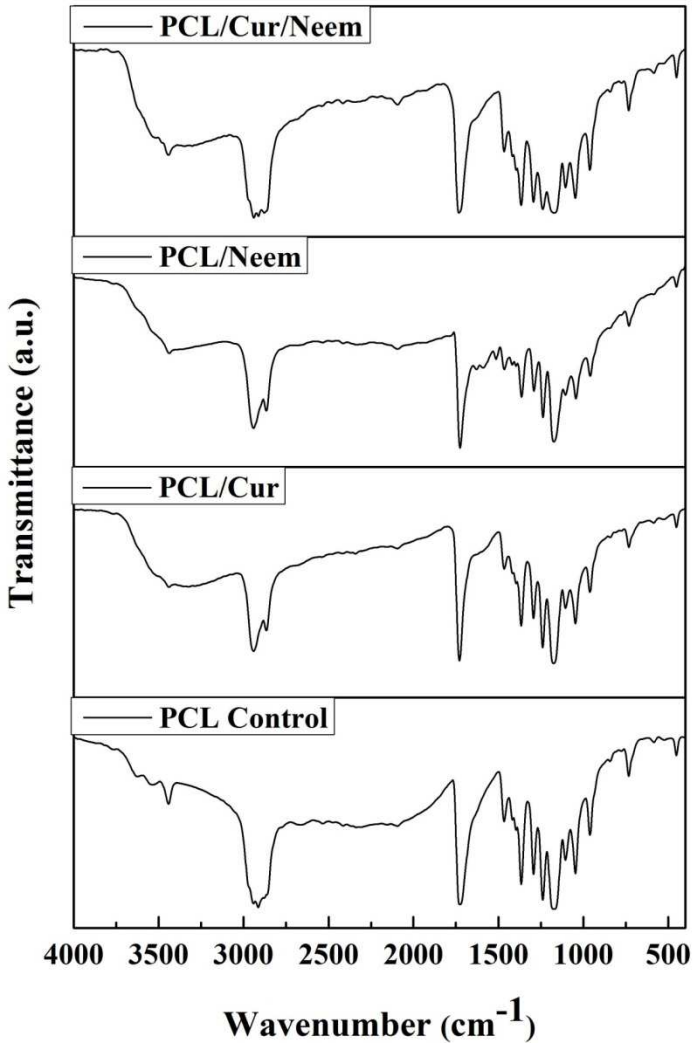

Figure 1: FT-IR spectrum of PCL and PCL/natural extract nanofibers

Scanning electron microscope (SEM) was used to investigate the surface morphology of the electrospun nanofibres. Figure 2a shows the SEM image of PCL nanofibre (control). Figure 2b-d were the SEM images of PCL/Cur, PCL/Neem and PCL/Cur/Neem nanofibres.Average fibre diameter was found to be 513 ± 173 nm for PCL nanofibre (control),though the addition of curcumin to PCL nanofibre increases the PCL fibre diameter to 624 ± 152 nm.

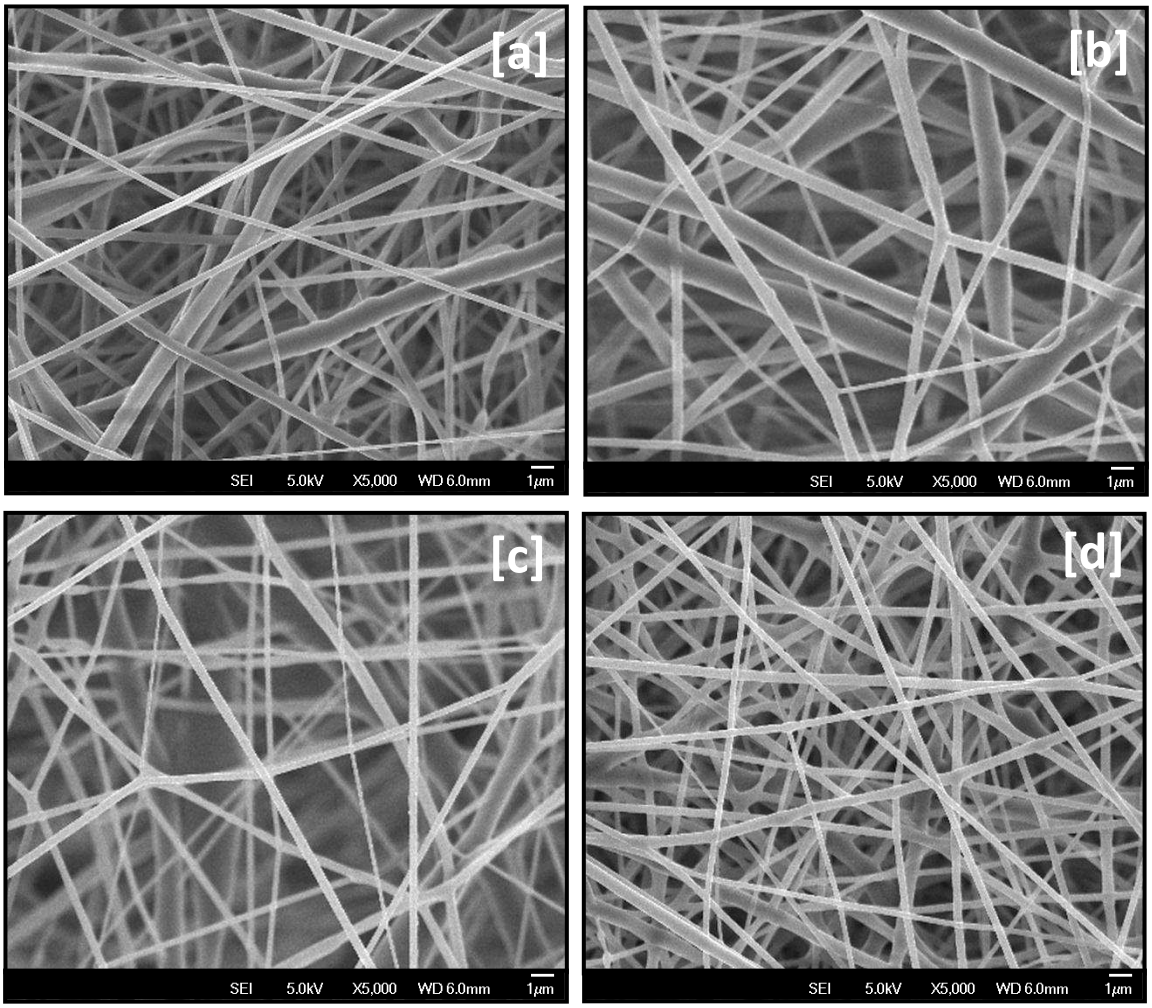

Figure 2: SEM images of (a) PCL (b) PCL/Cur (c) PCL//Neem and (d) PCL//Cur/Neem nanofibers

However, further addition of neem to PCL/Cur nanofibre decreases fibre diameter to 474 ± 93 nm. The PCL/Neem nanofibre average diameter was noted as 296 ± 45 nm, which have smallest fibre diameter compared to other samples. In conclusion, it was noted that the addition of curcumin, the PCL nanofibre diameter increases [13], while the fibre diameter decreases by the addition of neem. The PCL nanofibre diameter decreases drastically by the addition of neem extract compared to the addition of curcumin and curcumin/neem to PCL nanofibre. From the SEM results, we conclude that the addition of neem controls the size and the shape of the PCL nanofibres.

To evaluate the hydrophilicity of the PCL and natural extract loaded PCL nanofibers,the water contact angle measurements were carried out on the surface of the samples and the results were presented in Figure 3. Usually, if the water contact angle is larger than 90°, the sample is considered hydrophobic, and if the water contact angle is smaller than 90°, the sample is regarded as hydrophilic. In the initial PCL nanofibre showed hydrophobic nature with water contact angle of 93°. However, there were no considerable changes observed in the water contact angle even after 150 seconds (82°). Also, PCL/Cur/Neem sample exhibited hydrophobic nature in the initial with water contact angle of 90°. Though, hydrophilic property slightly improved after a while. The concluding contact angle for PCL/Cur/Neem sample was about 68°. PCL/Cur and PCL/Neem sample properties were slightly hydrophobic in the initial and turned hydrophilic within 60 min on contact with water. The final macroscopic contact angle for PCL/Cur and PCL/Neem samples was about 44° and 24°. Addition of neem to PCL nanofibre improves wetting and decreases the time needed for reaching the final apparent contact angle. Out of four samples neem-loaded PCL nanofibre observed a significant reduction in the water contact angle. This change in hydrophilicity in the PCL/Cur and PCL/Neem samples resulted in maximum release of the water soluble PCL nanofiber. The initial hydrophobic nature in the PCL/Cur and PCL/Neem samples were due to the PCL polymer which reduced gradually and became hydrophilic rapidly due to the surface treatment of PCL loaded natural plant extract. Those results have shown how efficient the neem treatment is in promoting the decrease in the interfacial tension between the liquid and the sample's surface.

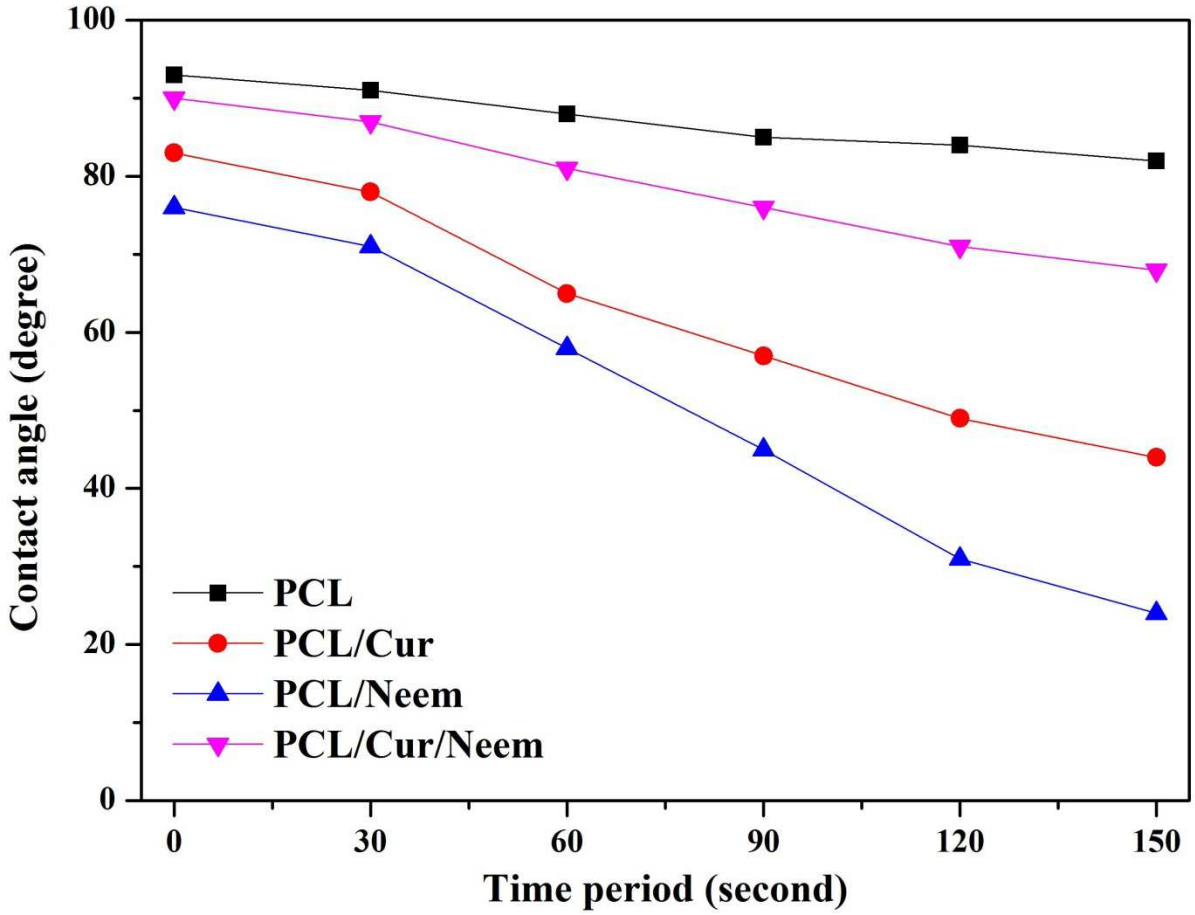

Figure 3: Water contact angle measurements of PCL and natural extract loaded PCL nanofibers

Some of the plant metabolites work as anti-oxidant and anti-cancer drugs because of their toxic effect toward cancer cells. Hence, anticancer activity of PCL and natural extract loaded PCL nanofibers were tested against A431 cancer cells and HaCaT skin cells for different times (24 h and 72 h). The A431 and HaCaT cells in vitro cancer cell viability results were compared along with the natural ingredient-incorporated PCL nanofibres and the results were shown in Figure 4 and Figure 5. Natural extract loaded PCL nanofibers showed significantly reduced cell proliferation than the PCL nanofibre due to enhanced cell death [24]. When the nanofibre treatment time increased to 72 h, the cell death was increased further. The PCL and natural extract loaded PCL nanofibers activity against A431 cell lines (72 h) morphological changes visualized under a microscope (Figure 4a-d). The morphological changes in the natural extract loaded PCL nanofibers by the number of cells reduced and gradually the cells became shrunken with the appearance of small bodies. The cancer cells morphological changes clearly defined which was due to the cell death [25]. The PCL/Cur/Neem nanofibre showed maximum anticancer activity against the A431 cancer cells and is shown in Figure 4e. This study gave the overall perception about importance of using PCL/Cur/Neem nanofibre as anticancer drug in medicine. The most important benefit of using PCL/Cur/Neem nanofibre against A431 cancer cells is it is a natural extract with no chemicals supplemented. Therefore, there might be no possibility of causing any side-effects to human body; another advantage was its inexpensive.

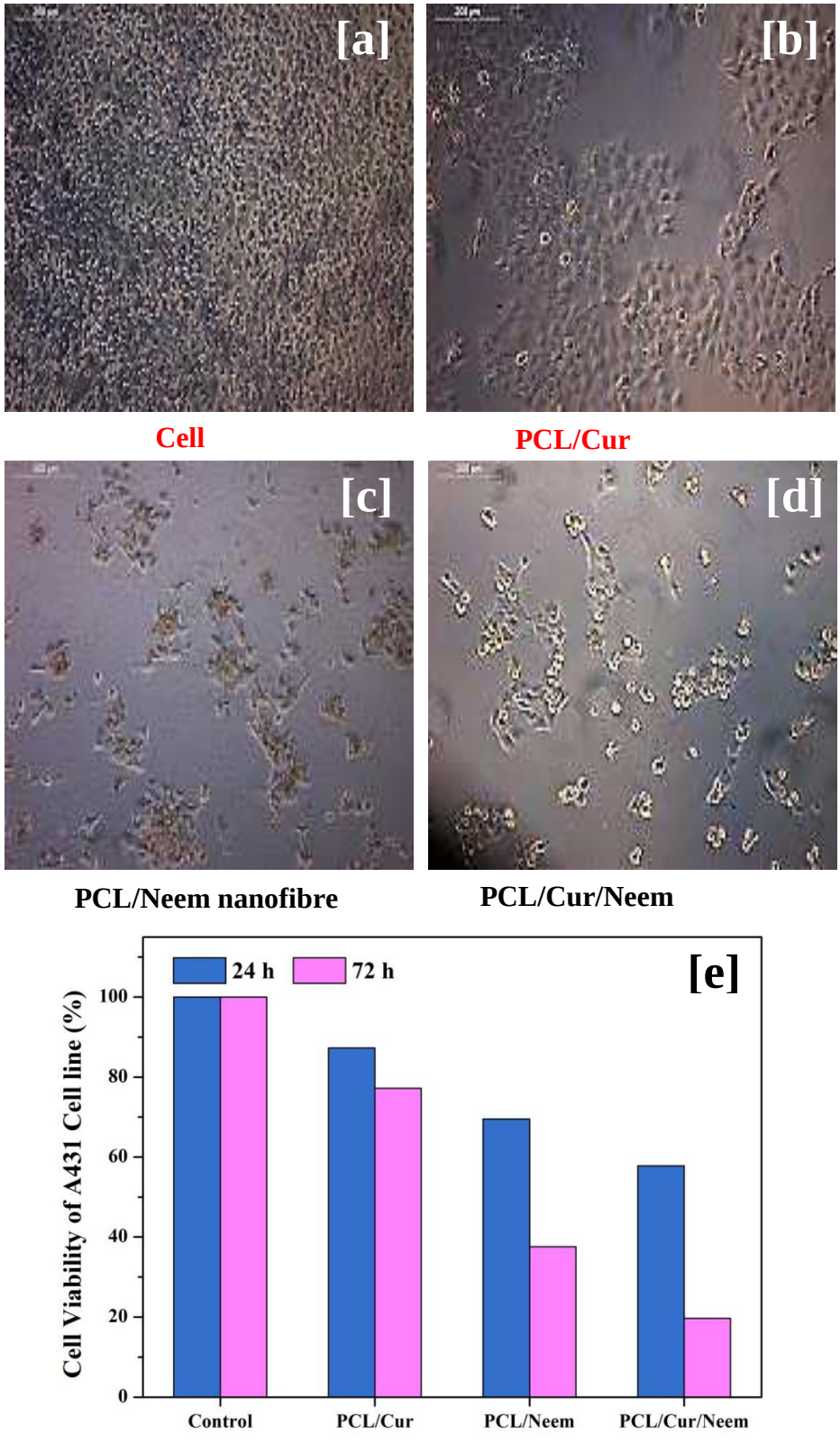

Figure 4:Comparison of in vitro anti-cancer activity of the PCL/natural extract nanofibers against A431 cancer cell lines

The cytotoxicity profiles of PCL and natural extract loaded PCL nanofibers against HaCaT cells were examined by MTT assay (Figure 5). There was no toxicity or lower toxicity observed in HaCaT cells after 24 h exposure to PCL and natural extract loaded PCL nanofibers. Compared to other samples, PCL/Cur sample showed higher toxicity against HaCaT skin cells. When HaCaT cells were exposed to PCL and natural extract loaded PCL nanofibers with the increasing time (72 h), the cell viability decreased for PCL/Cur and PCL/Neem samples. However, the HaCaT

cell viability not much affected for control and PCL/Cur/Neem samples which is shown in Figure 5e. The reduced cell viability was supported by images of cells morphology taken after exposure of 72 h. The PCL and natural extract loaded PCL nanofibers activity against HaCaT cell lines (72 h) morphological changes visualized under a microscope and is shown in Figure 5a-d. The result indicated that the PCL and PCL/Cur/Neem nanofibre did not affect the relative viability. In conclusion, the PCL/Cur/Neem nanofibre showed higher anticancer activity than other samples against A431 cancer cells and the PCL and PCL/Cur/Neem nanofibre showed lower cytotoxic activity against HaCaT skin cells. The new procedure requires, A431 cells were incubated with natural extract loaded PCL nanofibers for 72 h [26,27].

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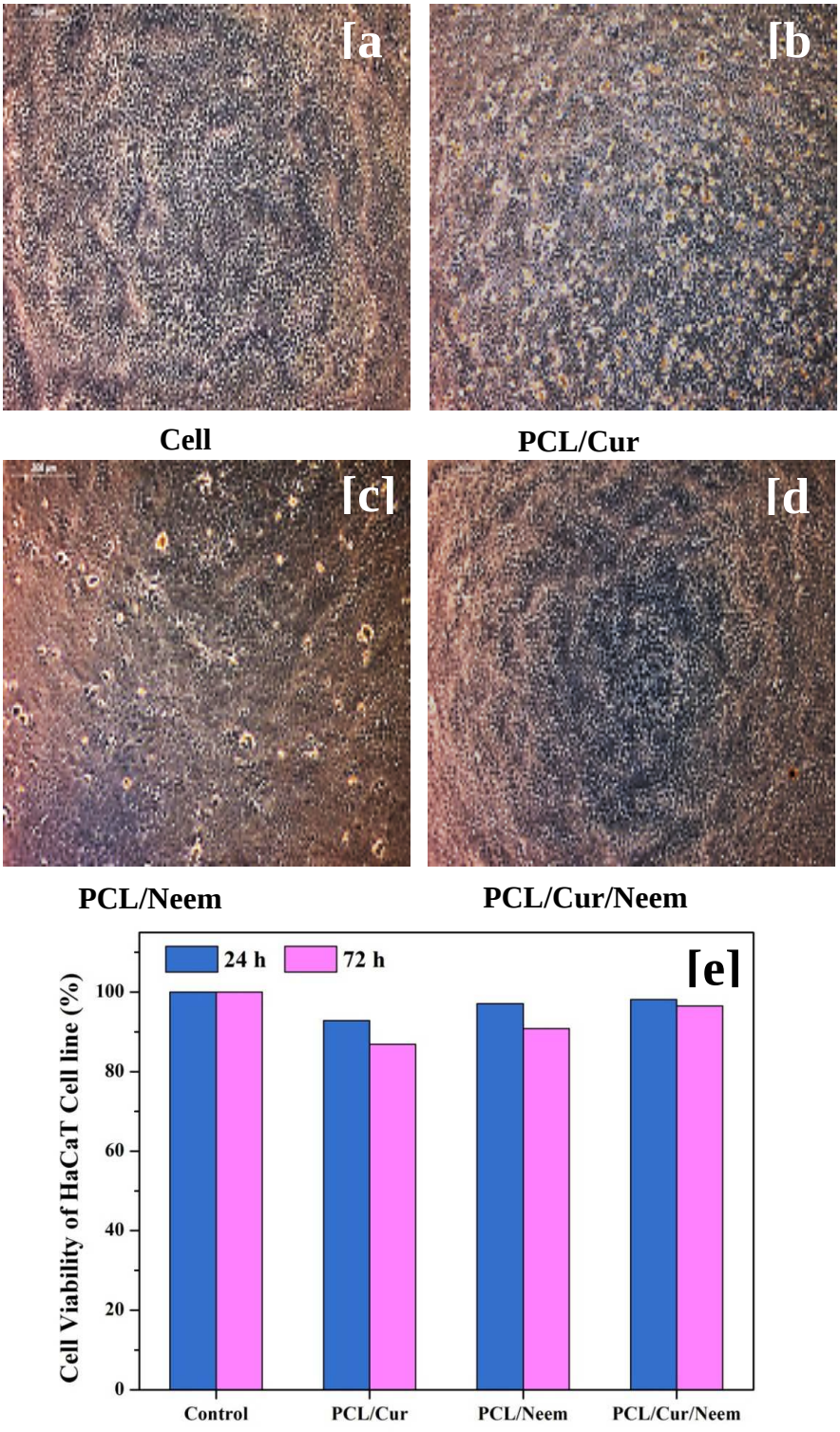

Figure 5:Comparison of cytotoxicity profiles of the PCL/natural extract nanofibers against HaCaT skin cell lines

CONCLUSION

Natural ingredient or extract (curcumin/neem) loaded polycaprolactone nanofibres were successfully fabricated by electrospinning method. Addition of neem controls the size and the shape of the PCL nanofibres, which is confirmed by scanning electron microscopy. Supplement of curcumin or neem to PCL increases hydrophilicity of nanofibre and decreases the time needed for reaching the final apparent contact angle. The in vitro anti-cancer activity of the natural ingredient or extract loaded PCL nanofibres showed effective inhibition against the A431 cancer cells. It was noted that the PCL/Cur/Neem nanofibres showed better inhibition against the A431 cancer cell lines compared to PCL, PCL/Cur and PCL/Neem nanofibres in 72 h. Also, the cytotoxic efficiency of nanofibers was conducted on HaCaT skin cells. No toxicity or lower toxicity observed in HaCaT cells after 24 h. However, the cell viability slightly decreased for PCL/Cur and PCL/Neem samples after 72 h. In conclusion, PCL/Neem nanofibres showed better anticancer and cytotoxic activities.

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