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Electrospun Poly(L-lactide) Nanofibers Loaded with Paclitaxel and Water-soluble Fullerenes for Drug Delivery and Bioimaging

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Abstract: Multifunctional electrospun composite nanofibrous scaffolds have attracted much interest as drug delivery vehicles and bioimaging applications for real-time tracing the whole process of postoperative therapy. Novel poly(L-lactide) (PLLA) composite nanofibers loaded with water-soluble fullerene C_{70} nanoparticles and paclitaxel were successfully fabricated. The nanofibers with the average diameters of fibers ranging from 350 to 750 nm were uniform and their surfaces were reasonably smooth. The nanofibers showed excellent hydrophilic surface and good mechanical properties. The in vitro release results demonstrated that the release rate of paclitaxel could be controlled by the content of C_{70} nanoparticles. With increase of C_{70} nanoparticles content, the drug release rate became faster with raised the total release amount. The composite nanofibers used as substrates for cytotoxicity and bioimaging in vitro were evaluated with human liver carcinoma HepG2 cells. Paclitaxel was released from the composite nanofibers without losing cytotoxicity. The drug-loaded composite nanofibers inhibited HepG2 cells proliferation effectively. Meanwhile, the fluorescent signal of C_{70} nanoparticles could be detected in HepG2 cells, which reflected the growth state of cells clearly. These results strongly suggested that these PLLA composite nanofibers could be used in the fields of tissue engineering, drug delivery and bioimaging.

Keywords: Electrospinning; Nanofibers; Fullerenes; Drug delivery; Bioimaging

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1 Introduction

Electrospinning has been considered as a facile and economic technique to produce nanofibers with high specific surface area, high porosity, 3D structures, flexible surface functionalities and superior mechanical durability.[1-3] Electrospun nanofibers has been widely used in biomedical field including, but not limited to, tissue engineering,[4-8] wound dressing[9-12] and drug delivery systems.[14-21] Particularly, electrospun nanofibers showed great potential in delivering anticancer drugs with effective drug loading capability, good stability and locally controlling drug release.[16-18] With the development of electrospinning techniques and the emergence of various biodegradable and biocompatible nanomaterials, construction of novel drug delivery system based on electrospun nanofibers and new nanomaterials remains a scientific challenge in the field of biomedical applications.[3-21]

In order to improve the fiber properties or introduce new functionalities to the fibers, small sized nanomaterials such as nanoparticles (NPs) or nanotubes (NTs) have been doped to form composite nanofibers with desired functionalities.[22-26] Fullerene fluorescent nanoparticles have offered a high potential for bioimaging application due to their unique properties such as non-blinking fluorescence emission, excellent water solubility, high cell permeability and good biocompatibility.[27,28] In our previous work, we have fabricated a fluorescent nanofibrous material consisting of water-soluble fullerene nanoparticles and poly(L-lactide) via a simple electrospinning method. The electrospun composite nanofibers used as substrates for bioimaging were evaluated with human liver carcinoma HepG-2 cells in vitro. The fullerene nanoparticles released from the fibers could penetrate into the HepG-2 cells for bioimaging, the fullerenes fluorescent signal displayed in every HepG-2 cell. This work had confirmed that photoluminescent fullerene nanoparticles/nanofibers had potential applications in bioimaging.

Inspired by the above results, we intend to construct novel multifunctional composite scaffolds for drug delivery and bioimaging application. It is anticipated to realize real-time tracing and monitoring the interaction between tumor cells and the drug-loaded nanofiber scaffolds, as well as the whole process of postoperative therapy. In this study, novel poly(L-lactide) composite nanofibers loaded with paclitaxel and water soluble fullerene C_{70} nanoparticles were successfully fabricated. The in vitro release results demonstrated that the release rate of paclitaxel could be controlled by the content of C_{70} nanoparticles. In vitro cytotoxicity and bioimaging of composite nanofibers was studied in detail, the drug-loaded composite nanofibers inhibited HepG-2 cells proliferation effectively, and the fluorescent signal of C_{70} nanoparticles in HepG-2 cells reflected the growth state of cells clearly. The results strongly suggested that this novel PLLA composite nanofibers loaded with paclitaxel and water-soluble fullerene C_{70} nanoparticles was able to provide a good alternative for cancer postoperative chemotherapy.

2 Materials and methods

2.1 Materials

C_{70} fullerene and paclitaxel (PTX) were purchased from Aldrich and used as received. Tetraethylene glycol (TEG), Lithium hydroxide and MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide) were purchased from Aladdin reagent and were used without further purification. Poly(L-lactide) (PLLA) (molecular weight 100K) was purchased from Shandong Jianbao biomaterials Ltd. (Jinan, China). Human liver carcinoma HepG-2 cell was purchased from Shanghai cell center (Chinese Academy of Sciences). RPMI 1640 and Newborn Calf serum were purchased from Shanghai Shichen Reagent Co. Ltd. Other reagents were commercially available and used as received.

2.2 Preparation of water-soluble fullerene C_{70} nanoparticles

Water-soluble fullerene nanoparticles C_{70}-TEGs was prepared according to the literatures.[27, 28] The fullerene nanoparticles C_{70}-TEGs was dialyzed against deionized water using dialysis tubing with a molecular weight cut-off of 3.5 kDa for 48 h, and then lyophilized to obtain water-soluble fullerene nanoparticles powders.

2.3 Preparation of PLLA composite nanofibers loaded with paclitaxel and water-soluble fullerene C_{70} nanoparticles

480 mg PLLA was dissolved in 3 mL chloroform by using bath sonicator (KQ-100, China) to prepare a 16 % (w/v) solution, respectively. 20 wt.% of C_{70}-TEGs with respect to the used polymer was dissolved with 1 mL DMF, and 5 wt.% of PTX with respect to the used polymer and C_{70}-TEGs was added to the solution. These two solutions were blended with continuous stirring to obtain homogeneous solutions. The mixture solution was then immediately electrospun. The nanofibers were collected on a target drum, which was placed at a distance of 14 cm from the syringe tip (inner diameter 22 μm). A voltage of 22-23 kV was applied to the syringe tip by a high

voltage power supply, and the flow rate of the solutions was 1.5 μL/min. All electrospinning experiments were carried out at about 25 °C in air. The nanofibers were dried in vacuum at 37 °C for 72 h to remove the residual solvent. The composite nanofibers obtained were abbreviated as PTX/PLLA@C_{70}-TEGs (0~20 wt.%) hereafter for simplicity.

2.4 Characterization of the fullerene C_{70} nanoparticles and electrospun composite nanofibers

The size distribution of C_{70}-TEGs was determined by DLS with laser fitted of 633 nm (Malvern, Nano ZS90, England). The scattering angle was fixed at 90°, and the measurement was carried out at a constant temperature of 25 °C. The photoluminescence (PL) spectrum of fullerene nanoparticles $_{70}$C-TEGs was determined by a fluorescence spectrophotometer (Hitachi F-7000). The surface morphologies and diameters of fullerene nanoparticles $_{70}$C-TEGs and electrospun PLLA composite nanofibers were observed by scanning electron microscope (SEM, FEI Quanta 200SEM) at an accelerating voltage of 20 kV. The distribution of fullerene nanoparticles $_{70}$C-TEGs in the electrospun nanofibers and interface nanostructure of composite nanofibers were analyzed using Laser scanning confocal microscopy (LSCM: ZEISS LSM 710, Germany) and transmission electron microscopy (TEM) (JEOL-2100F), respectively. Water contact angles of PLLA composite nanofiber mats were measured using contact angle instrument (JC2000A). Mechanical properties of different nanofiber mats were determined using SANS WDW universal test system with electronic data evaluation on specimens of 40 ×10 mm with a thickness in the range of 70 to 80 μm. Sample preparation and testing methods for the characterization of fullerene C_{70} nanoparticles and electrospun composite nanofibers were carried out according to our previous work.[28]

2.5 In vitro drug release

The composite nanofiber mats were cut into pieces of 50 mg mass, placed in a dialysis bag [cut-off, 3.5 kDa, Millipore Co., MA, USA] and suspended in 20 mL phosphate buffer solution (PBS, pH=7.4). Then, it was hermetically sealed and immersed into 80 mL PBS with magnetic stirring at 800 rpm at 37 °C. At predetermined time intervals, 1.0 mL released solution was withdrawn from the dissolution medium after incubation, while an equal amount of fresh PBS was added back to the incubation solution. The amount of PTX was detected by a PerkinElmer Lambda 750 UV-Vis spectrophotometer. A maximal absorption peak of 270 nm was observed for freshly prepared PTX in PBS and released within the designed period. For standard samples with a concentration from 0 to 50 μg/mL, a linear correlation (r^2 = 0.999) was determined between the absorption strength and PTX concentration[29]. Experiments were run in triplicate per sample. A profile showing the cumulative amount of drug release as a function of time was plotted.

2.6 Cell culture and MTT assay

HepG-2 cells were grown in RPMI 1640 medium containing 10% bovine calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. The cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2. Cells dissociated with 0.25% trypsin in PBS (pH=7.4) and centrifuged at 1000 rpm for 5 min at room temperature. The cells were collected and dispersed in 20 mL PBS. 200 μL of the dispersion was used for cell counting in a hemacytometer.

The cytotoxicities of composite nanofibers PTX/PLLA@C_{70}-TEGs against HepG-2 cells were evaluated by the MTT assay after treatment of cells with electrospun nanofibers, and the cytotoxicities of blank PLLA nanofibers and pristine PTX were also tested for comparison. Briefly, HepG-2 cells (8×10^3 cells/well) were seeded in 96-well plates and incubated overnight at 37 °C to allow cells to attach, then the medium was replaced with fresh medium and the medium containing 0.1 mg

composite nanofibers (PTX/PLLA@C_{70}-TEGs) at the total PTX concentration of 25 μg/mL. After incubation for another 24 h and 48 h, 20 μL of MTT solution (5 mg/mL) was added to each well and followed by incubation for another 4 h. After that, the solution in the wells was deserted completely and 200 μL DMSO was added to each well to dissolve the precipitate for 15 min. And subsequently the absorbance at 490 nm was detected using an ELISA microplate reader. The relative cell viability rate was calculated by dividing the optical density value of the test group by that of the control group.

Data were expressed as mean ± SD. Statistical significance was determined by the Students *t*-test. $P<0.05$ was considered statistically significant.

2.7 In vitro biological imaging and cell morphology observation

The potential use of the PLLA electrospun composite nanofibers loaded with water-soluble fullerene nanoparticles (C_{70}-TEGs) as substrates for bioimaging application was evaluated with human liver carcinoma HepG-2 cells in vitro. Briefly, HepG-2 cells (8×10^4 cells/well) were seeded in 6-well plates and incubated overnight at 37 °C to allow cells to attach; then the medium was replaced with fresh medium and the medium containing 1.0 mg composite nanofibers (PTX/PLLA@C_{70}-TEGs) at the total PTX concentration of 25 μg/mL. After incubation for another 24 h and 48 h, the cell morphology was observed using a laser scanning confocal microscopy (LSCM) (ZEISS LSM 710, Germany). The cell morphologies of blank PLLA nanofibers and PTX/PLLA nanofibers were also tested under bright filed for comparison.

3 Results and discussion

3.1 Characterization of the fullerene C_{70} nanoparticles

The maximum emission wavelength of fullerene nanoparticles C_{60}-TEGs and C_{70}-TEGs were 550 nm and 575 nm, respectively. According to the experiment results of in vitro biological imaging,[28] the photoluminescence intensity and fluorescence imaging effect of fullerene nanoparticles C_{70}-TEGs were superior to C_{60}-TEGs. Therefore, in this work, we selected the fullerene nanoparticles C_{70}-TEGs as bioimaging model. The highly water-soluble and photoluminescent fullerene nanoparticles C_{70}-TEGs was prepared by using TEG and C_{70} fullerene toluene solution at a concentration of 1 mg/mL with lithium hydroxide as a catalyst. The SEM micrograph of C_{70}-TEGs was shown in Fig. S1-A (in supported information). The image revealed that the nanoparticles showed uniformly spherical shape with the diameters ranging from 20 to 60 nm. To investigate the photophysical properties of fullerene nanoparticles C_{70}-TEGs, the photoluminescence spectrum of C_{70}-TEGs dissolved in water was shown in Fig. S1-B. The emission spectrum of the C_{70}-TEGs exhibited maximum emission wavelength at 657 nm (under 350 nm excitation). Fig. S1-C presented the size of the fullerene nanoparticles C_{70}-TEGs obtained by DLS. The mean diameter of C_{70}-TEGs was about 40 nm. It could be concluded that water-soluble fullerene nanoparticles C_{70}-TEGs was prepared successfully.[27, 28]

3.2 Characterization of the electrospun composite nanofibers

Multifunctional electrospun composite nanofibrous scaffolds have attracted much interest as drug delivery vehicles and bioimaging applications for real-time tracing the whole process of postoperative therapy.[21, 25, 26] In this study, novel poly(L-lactide) composite nanofibers loaded with paclitaxel and water-soluble fullerene C_{70} nanoparticles were successfully fabricated via a simple blend electrospinning method for drug delivery and bioimaging. The SEM micrographs of the electrospun

composite nanofibers were shown in Fig. 1. The nanofibers represented an identical morphology of PLLA fibers to those containing fullerene nanoparticles C_{70}-TEGs and PTX. The nanofibers were uniform and their surfaces were reasonably smooth, with the average diameters of fibers ranging from 350 to 750 nm. The average diameters of the nanofibers contained 0 wt.%, 5 wt.%, 10 wt.% and 20 wt.% C_{70}-TEGs were about 350, 520, 630 and 750 nm, respectively, increasing with the amount of C_{70}-TEGs. It might be that the addition of fullerene nanoparticles C_{70}-TEGs increased charge density of the jet during the electrospinning process, which resulted in decreasing of composite solution viscosity and the improvement of stretching force and the self repulsion.[30-32]

Fig. 1 SEM photographs of PLLA composite nanofibers: (A) PTX/PLLA, (B) PTX/PLLA@C_{70}-TEGs (5 wt.%), (C) PTX/PLLA@C_{70}-TEGs (10 wt.%) and (D) PTX/PLLA@C_{70}-TEGs (20 wt.%).

Fig. 2 showed LSCM images of the C_{70}-TEGs loaded by the electrospun composite nanofibers. The red and bright spots indicated C_{70}-TEGs or their aggregates distributed uniformly in the composite nanofibers. C_{70}-TEGs was linearly packed and aligned along the axis of the fibers. The fullerene nanoparticles C_{70}-TEGs were dispersed reasonably well in the resultant composite nanofibers owing to the homogeneity of the solution. With increasing the content of C_{70} nanoparticles, the red

spots were more and the average diameters of fibers were bigger, which was consistent with the SEM images.

Fig. 2 LSCM images of PLLA composite nanofibers: (A) PTX/PLLA@C_{70}-TEGs (10 wt.%) and (B) PTX/PLLA@C_{70}-TEGs (20 wt.%).

The internal structure of the PLLA composite nanofibers loaded with 10 wt.% C_{70}-TEGs and 20 wt.% C_{70}-TEGs were analyzed using TEM, which also confirmed the embedding of the C_{70}-TEGs into the composite nanofibers. As shown in Fig. 3, the dense fullerene nanoparticles C_{70}-TEGs in roughly spherical shape with the diameters ranging from 20 to 60 nm were uniformly dispersed in the PLLA nanofiber matrices. These results showed that the fullerene nanoparticles C_{70}-TEGs were successfully loaded into the PLLA composite nanofibers.

Fig. 3 TEM images of PLLA composite nanofibers: (A) PTX/PLLA@C_{70}-TEGs (10 wt.%) and (B) PTX/PLLA@C_{70}-TEGs (20 wt.%).

Surface wettability was important for optimal application of the electrospun fibers as drug carriers, tissue growth scaffolds, and wound dressing materials. Fig. 4 showed the optical observations of the water contact angles on the surface of electrospun composite nanofibers at about 1 s. The water contact angle of the composite nanofiber PTX/PLLA@C_{70}-TEGs (20 wt.%) immediately reached 0° (about 1 s). The water

contact angles of composite nanofibers PTX/PLLA@C_{70}-TEGs (5 wt.%) and PTX/PLLA@C_{70}-TEGs (10 wt.%) were between 74±0.5° and 30±0.5° at 30 s, while the nanofiber PTX/PLLA was higher than 120° and almost didn't change after 30 s. During the electrospinning process, fullerene nanoparticles C_{70}-TEGs and PLLA matrix happened micro-phase separation, some water-soluble fullerene nanoparticles C_{70}-TEGs transfered to the surface of PLLA matrix by forces of electric. The higher content of nanoparticles loaded in the nanofibers, the more nanoparticles came to the nanofibers surface. Consequently, it could improve the hydrophilicity properties of PLLA matrix because fullerene nanoparticles C_{70}-TEGs were much more hydrophilic than PLLA.

Fig. 4 Optical images of water contact angles on the surface of PLLA composite nanofibers: (A) PTX/PLLA, (B) PTX/PLLA@C_{70}-TEGs (5 wt.%), (C) PTX/PLLA@C_{70}-TEGs (10 wt.%) and (D) PTX/PLLA@C_{70}-TEGs (20 wt.%).

Mechanical strength should be considered in practical applications such as tissue engineering scaffold and implants. Mechanical properties of the composite nanofibers were determined and the mean value was summarized in Table 1. The modulus and the elongation at break of the composite nanofibers loaded with water-soluble fullerene nanoparticles C_{70}-TEGs were 128.0~142.4 MPa and 9.6~10.4%, respectively. Due to the poor phase compatibility between fullerene nanoparticles and PLLA, fullerene nanoparticles C_{70}-TEGs and PLLA matrix happened micro-phase

separation during the electrospinning process, the mechanical properties of composite nanofibers loaded with fullerene nanoparticles (C_{70}-TEGs) were poorer than that of blank PLLA nanofibers (the modulus, 148.6 MPa and the elongation, 114.3%). The mechanical properties of the composite nanofibers exhibited a similar tendency, the higher the fiber content in C_{70}-TEGs, the poorer mechanical properties of the nanofibers. However, the mechanical properties of composite nanofibers of PTX/PLLA@C_{70}-TEGs (20 wt.%) also reached the requirements of performance for tissue-engineered materials[33].

Table 1 Mechanical properties of PLLA composite nanofibers

PLLA nanofibers	Tensile Strength/ (MPa) ± SD	Elongation/ (%) ± SD	Modulus/ (MPa) ± SD
Blank PLLA	4.5±0.2	114.3±8.5	148.6±7.5
PTX/PLLA	4.7±0.3	118.5±7.0	154.1±7.2
PTX/PLLA @C_{70}-TEGs (5%)	4.2±0.3	104.4±6.5	142.4±8.6
PTX/PLLA @C_{70}-TEGs (10%)	3.8±0.2	101.9±6.8	135.6±8.5
PTX/PLLA @C_{70}-TEGs (20%)	3.5±0.3	99.6±6.2	128.0±5.4

3.3. In vitro drug release

The in vitro release profiles of PTX from the PTX loaded nanofibers were shown in Fig. 5. Obviously, in the whole drug release period, the release rate of PTX increased with increasing fullerene nanoparticles (C_{70}-TEGs) content. The release of 61% PTX from composite nanofibers of PTX/PLLA@C_{70}-TEGs (20 wt.%) was rapidly reached within 12 h, as compared with 48% from nanofibers of PTX/PLLA. Furthermore, the higher the C_{70}-TEGs content in the fiber, the higher the percentage of drug released from the PLLA nanofiber mats. Specifically, the sample with the highest C_{70}-TEGs content (sample D, 20 wt.%) released 83% of its total drug within 72 h, whereas the sample with the lowest C_{70}-TEGs content (sample A, 0 wt.%) released 72% of its entrapped drug. The release profile of PTX from the PLLA matrix was mainly controlled by diffusion of the drug through the matrix. PTX release obeyed a diffusion

mechanism at the early period.[16-18] This faster release behavior of PTX from the composite nanofibers loaded with fullerene nanoparticles C_{70}-TEGs could be attributed to more nanoparticles came out to the nanofiber surface as increasing the content of C_{70} nanoparticles. The nanofiber scaffolds PTX/PLLA@C_{70}-TEGs showed excellent hydrophilic surface, the fullerene nanoparticles-TEGs diffused from the PLLA matrix easily, forming a lot of diffusion passway, which resulted in accelerating the drug release rate. As increasing the content of nanoparticles C_{70}-TEGs loaded in the nanofibers, the hydrophilicity properties of PLLA matrix was improved greatly. Meanwhile, the network structure and mechanical properties of PLLA matrix were destroyed in a certain extent. As a result, the presence of fullerene nanoparticles C_{70}-TEGs could accelerate the degradation of PLLA matrix. Accordingly, the release rate and the total release amount of PTX would increase.

In conclusion, the in vitro release results demonstrated that the release rate of PTX could be controlled by the content of C_{70} nanoparticles. With increase of C_{70} nanoparticles content, the drug release rate became faster and the total release amount was more.

Fig. 5 Release profiles of PTX from PLLA composite nanofibers: (A) PTX/PLLA, (B) PTX/PLLA@C_{70}-TEGs (5 wt.%), (C) PTX/PLLA@C_{70}-TEGs (10 wt.%) and

(D) PTX/PLLA@C_{70}-TEGs (20 wt.%).

3.4 In vitro cytotoxicity against HepG-2 cells

To verify the pharmacological activity of the released drugs, the cytotoxicities of the composite nanofibers against the HepG-2 cells were evaluated by MTT assay after treated with different samples for 24 h and 48 h. The cytotoxicities of blank PLLA nanofibers and pristine PTX were also tested for comparisons. As shown in Fig. S2, the blank PLLA nanofibers did not display any cytotoxicity to HepG-2 cells. However, in the case of pristine PTX, it exhibited excellent cytotoxicity to HepG-2 cells, the cell growth inhibition rates were 65.3% at 24 h and 95.7% at 48 h.

The cytotoxicities of the composite nanofibers PTX/PLLA@C_{70}-TEGs were shown in Fig. 6. The cell growth inhibition rates of the composite nanofibers PTX/PLLA@C_{70}-TEGs contained 0 wt.%, 5 wt.%, 10 wt.% and 20 wt.% C_{70}-TEGs were about 37.5%, 43.4%, 46.8% and 48.2%, respectively, at 24 h. And these figures became 71.2%, 74.5%, 77.8% and 81.3%, respectively, at 48 h. These results suggested that PTX was released from the composite nanofibers PTX/PLLA@C_{70}-TEGs without losing cytotoxicity and had a relatively faster release in the drug delivery system with increasing the content of C_{70} nanoparticles, which was consistent with the in vitro drug release experimental results

Fig. 6 Cytotoxicities of the PLLA composite nanofibers to human liver carcinoma HepG-2 cells (PTX/PLLA@C_{70}-TEGs composite nanofibers were directly added to the tumor-cell-cultured well and incubated for 24 h and 48 h).

3.5 In vitro biological imaging and cell morphology

The composite nanofibers PTX/PLLA@C_{70}-TEGs loaded with PTX and fullerene nanoparticles C_{70}-TEGs used as substrates for cytotoxicity and bioimaging in vitro were evaluated with human liver carcinoma HepG-2 cells, the morphological changes of HepG-2 cells treated with different nanofibers for 24 h and 48 h were observed by CLSM. Blank PLLA nanofibers and PTX/PLLA nanofibers were also tested for comparison. Fig. S3 showed that HepG-2 cells adhered onto culture plate, and the cell morphology kept long spindle, nucleus integrity and cells plumping after treated with blank PLLA nanofibers, the cells grew very well, indicated good biocompatibility and low cytotoxicity of the blank PLLA nanofibers. However, the HepG-2 cells treated with PTX/PLLA nanofibers acquired a round shaped morphology and a sharply decreased cell number for 24 h. HepG-2 cells were in the state of differentiation and apoptosis and the sharp increase in the number of dead cells at 48 h. The results showed that PTX inhibited HepG-2 cells proliferation effectively after controlled release from the composite nanofibers.

Fig. 7 showed the fluorescence images of HepG-2 cells which co-cultured with PLLA composite nanofibers loaded with water-soluble fullerene nanoparticles C_{70}-TEGs at different time. After excitation at 405 nm and collection of 450~650 nm channel, the intense red fluorescence image of HepG-2 cells could be observed, implying a large number of fullerene nanoparticles C_{70}-TEGs were endocytosed by HepG-2 cells. Due to the excellent water-solubility of fullerene nanoparticles C_{70}-TEGs, the fullerene nanoparticles C_{70}-TEGs diffused from the PLLA matrix easily in a way that the fullerene nanoparticles C_{70}-TEGs behind always followed the pass-away of the front

one. The results suggested that C_{70}-TEGs released from the composite nanofibers and penetrating into HepG2 cells for bioimaging. When the composite nanofibers PTX/PLLA@C_{70}-TEGs (20 wt.%) incubated with HepG2 cells from 24 h to 48 h, the fullerene nanoparticles C_{70}-TEGs fluorescence signal enhanced greatly and almost displayed in every cell from the fluorescence images, which indicated that there were more fullerene nanoparticles C_{70}-TEGs released from the composite nanofibers and penetrating into HepG2 cells for bioimaging. Meanwhile, the merge images reflected the growth state of cells clearly, the HepG2 cells morphologies treated with composite nanofibers PTX/PLLA@C_{70}-TEGs (20 wt.%) were similar to that of nanofibers PTX/PLLA.

Fig. 7 LSCM images of HepG2 cells co-cultured with electrospun composite nanofibers PTX/PLLA@C_{70}-TEGs (20 wt.%) at different time.

4 Conclusions

In this work, novel poly(L-lactide) composite nanofibers loaded with paclitaxel and water-soluble fullerene C_{70} nanoparticles were successfully fabricated via a simple electrospinning method. The results showed that the dense fullerene nanoparticles in roughly spherical shape with the diameters ranging from 20 to 60 nm were uniformly dispersed in the fiber matrices. The in vitro release results demonstrated that the release rate of paclitaxel could be controlled by the content of water-soluble fullerene

C_{70} nanoparticles. In vitro cytotoxicity and bioimaging of composite nanofibers was studied in detail. While the drug paclitaxel inhibited HepG2 cells proliferation effectively after controlled release from the composite nanofibers, the fluorescent signal of C_{70} nanoparticles in HepG2 cells reflected the growth state of cells clearly. All the results strongly suggested that poly(L-lactide) composite nanofibers loaded with paclitaxel and water-soluble fullerene C_{70} nanoparticles could be used as scaffolds for tissue engineering, drug delivery and bioimaging application.

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