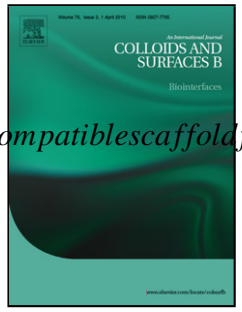


Title: Star poly(ϵ − *caprolactone*) − *basedelectrospunfibersasbiocompatiblescaffoldfordoxorubicinwithp*

Authors: Preethi Balakrishnan, Lorenza Gardella, Mahdi Forouharshad, Teresa Pellegrino, Orietta Monticelli

PII: S0927-7765(17)30750-6
DOI: https://doi.org/10.1016/j.colsurfb.2017.11.014
Reference: COLSUB 8965

To appear in: *Colloids and Surfaces B: Biointerfaces*

Received date: 5-6-2017
Revised date: 5-10-2017
Accepted date: 6-11-2017

Please cite this article as: Preethi Balakrishnan, Lorenza Gardella, Mahdi Forouharshad, Teresa Pellegrino, Orietta Monticelli, Star poly(ϵ-caprolactone)-based electrospun fibers as biocompatible scaffold for doxorubicin with prolonged drug release activity, Colloids and Surfaces B: Biointerfaces https://doi.org/10.1016/j.colsurfb.2017.11.014

Star poly(ε-caprolactone)-based electrospun fibers as biocompatible scaffold for doxorubicin with prolonged drug release activity

Preethi Balakrishnan,[a,b] *Lorenza Gardella,*[b] *Mahdi Forouharshad,*[b] *Teresa Pellegrino,*[a*] *Orietta Monticelli*[b*]

[a]Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genova, Italy.

[b]Dipartimento di Chimica e Chimica Industriale, Università di Genova, Via Dodecaneso, 31, 16146 Genova, Italy.

T. Pellegrino and O. Monticelli are both co-lasting and corresponding authors.

orietta.monticelli@unige.it

Teresa.Pellegrino@iit.it

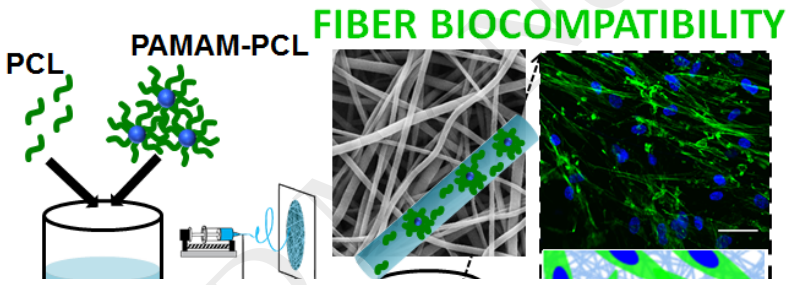
Number of tables: 0

Number of figures: 5

Number of words: 7204

Graphical abstract

Biocompatible star poly(ε-caprolactone)-based electrospun fibers as scaffolds with prolonged chemotherapeutic drug action.



Highlights

- A novel drug delivery system based on an hoc-synthesized star polymer is developed.
- The synthesized star polymer is made up of a PAMAM core and PCL branches.
- The synthesized star polymer is dispersible in PCL electrospun fibers.
- The star polymer allows the prolonged and controlled release of doxorubicin.

Abstract. In this work, a novel drug delivery system consisting of poly(ε-caprolactone) (PCL) electrospun fibers containing an ad-hoc-synthesized star polymer made up of a poly(amido-amine) (PAMAM) core and PCL branches (PAMAM-PCL) was developed. The latter system which was synthesized *via* the ring opening polymerization of ε-caprolactone, starting from a hydroxyl-terminated PAMAM dendrimer and characterized by means of ^{1}H-NMR, IR and DSC, was found to be compatible with both the polymer matrix and a hydrophilic chemotherapeutic drug, doxorubicin (DOXO), ~~used as drug model~~ the model drug used in this work. ~~Indeed, IR and ^{1}H-NMR measurements enabled assessing the successful preparation of the dendritic PCL star product, and the average length of the arms, which was found to be of about 2000 g/mol.~~ The preparation of the dendritic PCL star product with an average arm length of 2000g/mol was characterized using IR and ^{1}H-NMR measurements. ~~Moreover, t~~The prepared star polymer possessed a higher crystallinity and a lower melting temperature than that of the used linear PCL. Electrospun fibers were prepared~~,~~ starting from solutions containing the neat PCL as well as the ~~mixture~~ PCL/PAMAM-PCL mixture. ~~and~~ Electrospinning conditions were optimized in order to obtain defect free fibers, which was proven ~~as shown~~ by the structural FE-SEM study. PAMAM moieties enhanced the hydrophilicity of the fibers, ~~whose effect w~~as proved by comparing the water absorption for the PCL/PAMAM-PCL fibers ~~against~~ to that neat PCL fibers. The drug-loaded system PCL/PAMAM-PCL was prepared ~~built up~~ by directly introducing DOXO into the electrospinning solutions.~~,~~ The DOXO-loaded PCL/PAMAM-PCL showed a prolonged release of the drug with respect to the DOXO-loaded PCL fibers ~~only~~ and elicited ~~an~~ effective controlled toxicity over A431 epidermoid carcinoma, HeLa cervical cancer cells and drug resistant MCF-7 breast cancer cells. On the contrary, ~~killing action against cancer~~

~~cells. T~~the drug-free PCL/PAMAM-PCL scaffold ~~instead~~ demonstrated no toxic effects on ~~suitable biocompatibility when adult H~~human dermal fibroblasts, ~~were grown on the mats~~ suggesting the biocompatibility of the proposed system which can be used ~~use as~~ in cellular scaffold applications ~~material~~.

Keywords: PCL; electrospun fibers; star polymers; drug delivery; doxorubicin.

1. Introduction

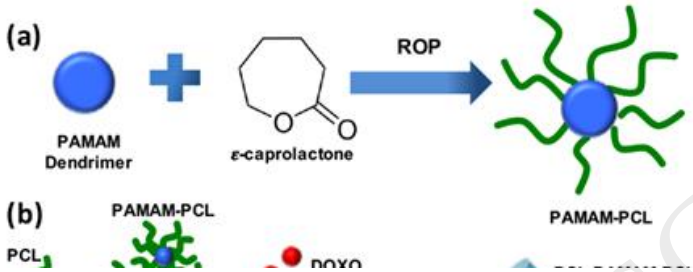
Among the methods used to limit the risk of tumor recurrence, the controlled/localized delivery of anticancer drugs has been proposed as a promising and ~~an~~ alternative approach to ~~the~~ classic~~al~~ applied procedures, such as radiation and chemotherapy. In general, ~~the~~ local drug delivery can be performed by applying various biodegradable polymer systems, including drug-eluting films [1], hydrogels [2,3], wafers [4], nanoparticles [5-6] and nanofibrous scaffolds [7-9]. In the latter case, the peculiar architecture of the support, which is characterized by a highest surface/contact area, ~~and its~~ that favors a robust drug loading capability ~~favors the~~ together with a prolonged drug release ~~with~~ that consequent ~~killing~~ kills of the tumor cells [10-12]. Moreover, the delivery of the drugs can be controlled by tuning the fiber morphology, porosity and composition. Electrospun polymer fibers have been widely applied as delivery devices of several anticancer drugs such as carmustine [~~10~~ 13], paclitaxel [7], cisplatin [8-9], polyphenol [~~11~~ 14], camptothecin [~~12~~ 15] and doxorubicin (DOXO) [~~13-17~~ 16-20] ~~(DOXO, that is the drug selected in the present work)~~. Despite the potentialities of these systems, the simple electrospinning of the drug and the polymer mixture leads to an undesirable initial burst release of the drug, which is

~~frequently~~ often inevitable [~~18~~ 21]. In order to overcome this limitation and attain a slow, sustainable release, several inorganic nanocarriers were inserted into the electrospun nanofibers. In particular, composite nanofibers were developed by combining the polymer with silica based nanoparticles [~~14-15~~ 17,18, ~~19-21~~ 22-24], ~~- in systems where the drug was either loaded into the pre-formed nanocarriers or introduced into the nanoparticles during their growth under controlled conditions -~~ and hydroxyapatite [~~13~~, ~~22~~ 16,25], as well as a combination of silica and hydroxyapatite [~~23~~ 26]. Even though such nanofillers can be considered ~~as~~ ideal drug carriers, mainly due to their high surface area and good biocompatibility, their application in designing composite nanofibers for ~~the~~ drug delivery ~~implies facing~~ has some drawbacks. Indeed, there are important issues to be taken into account: the ~~weak~~ interactions between the drug molecules and the inorganic nanoparticles are weak [~~13~~16] ~~as well as the not always easy~~ the dispersibility of the nanofillers into the polymer matrix is not always easy [~~24~~ 27] and their ~~slow~~ bio-absorption capacity is slow ~~are important issues to be taken into account~~. Clearly, the development of a nanofibrous scaffold ~~containing~~ that contains more specific drug carriers, as an alternative to classic~~al~~ inorganic nanofillers, that is characterized by a good affinity with both the drug molecules and the polymer matrix, represents an ideal target in the development of materials to be applied as a local drug delivery system. With this in mind, we have developed a novel drug delivery system, consisting of fibers, which is based on a biocompatible and re-absorbable polymer, ~~and~~ containing ~~as a drug carrier~~ an *ad-hoc*-synthesized star polymer as drug carrier, and was designed having taken~~ing~~ into account the specific characteristics of the polymer matrix, and the chosen drug~~,~~ that is DOXO. Star polymers are a class of regular branched polymers in which the branches radiate from a central core, having physio-chemical properties completely different from linear analogues [~~25~~ 28]. Thanks to their peculiar structure, ~~with~~ which has relatively short

chains but ~~still~~ also a high mass, a more compact shape and an increased concentration of functional end-groups with respect to linear polymer, star polymers are characterized by enhanced solubility, low viscosity, low crystallizability and thermal/hydrolytic stability [~~25~~ 28]. Star structures made up of a dendritic-type core and branches of biocompatible/bio-absorbable polymers, such as poly(lactide) [~~26-30~~ 29,30] and poly(ε-caprolactone) (PCL) [30-~~36~~ 33], have been addressed as potential systems for the encapsulation/controlled release of drug molecules, because of their amphiphilic core-shell structure. In particular, poly(amido-amine) (PAMAM) dendrimers, a family of dendrimers, built up ~~by~~ of polyamide branches with tertiary amines as focal point, have been used as multifunctional macro-initiators in the ring-opening polymerization of lactide [~~26-28,~~ 30] and ε-caprolactone [30, ~~34-36~~ 31-33], with the arm length being tuned through the monomer-to-initiator-molar ratio. As for the PCL/PAMAM system, which is the subject ~~object~~ of the present work, the dendrimer was mainly used to build up star block copolymers, whose arms consist of an inner block of PCL and an outer block of an hydrophilic polymer, such as poly(ethylene glycol) [30, ~~34-35~~ 31,32]. The above systems, used in micelle form, ~~was~~ were proven to solubilize hydrophobic molecules, such as pyrene, in an aqueous solution [30]. Besides PEG, poly(D-gluconamidoethyl methacrylate) (PGAMA) was also used to develop PAMAM/PCL/PGAMA star block copolymers [~~37~~ 34]. ~~Indeed, it~~ It is worth underling that ~~in all the above-mentioned works~~ PCL-based star polymers were used as micelles or particles in all the above mentioned works, as in their neat form they are totally unsuitable for exploitation as structural materials. Clearly, their formulation as self-standing materials, for example their insertion into an easily processable, biocompatible and bio-absorbable polymer support, represents an important advantage ~~for~~ regarding their applicability. In this work, the novel drug delivery system was developed by first synthesizing a star-like

polymer, made up of a PAMAM core and PCL arms (PAMAM-PCL), via ROP of ε-caprolactone starting from a hydroxyl-terminated PAMAM dendrimer (Figure 1). Then, ~~the preparation of~~ electrospun fibers ~~was set up~~ were prepared by mixing in solution the commercially available high molecular mass ~~commercially available~~ PCL with the here-synthesized star polymer. ~~Indeed, the~~ The chemical features and the peculiar architecture of the PAMAM-based macromolecule being characterized by arms whose chemical nature is identical to that of the polymer matrix may ~~favour~~ favor its dispersion. On the other hand, the hydrophilic PAMAM core may promote specific interactions with DOXO, which is a clinically used hydrophilic anticancer drug. Therefore, the insertion of the above molecule into PCL should allow us to combine the features of the star molecule with those of the processable polymer support. As shown in Figure 1, DOXO was directly introduced in the electrospinning solution and the obtained fibers were characterized in terms of morphology, thermal properties and water absorption. PCL alone ~~or~~ and in combination with other well know polymers such as PLA [~~37~~ 34], chitosan [~~38-39~~ 35,36], gelatin [~~40-43~~ 37-40], etc. ~~were~~ have been successfully used for the past few decades as three dimensional matrices for tissue engineering, especially in bone [~~37, 39, 44-46~~ 34,36] and nerve [~~38, 41~~ 35,38], regeneration and also in wound healing [~~42-43~~ 39,40] applications, used bared or loaded with stimulating drugs and growth factors. Only a few examples are available on their use as drug delivery agents in cancer therapeutics when made of complex responsive systems [~~17, 47-48~~ 20,41,42]. In this study, the biocompatibility of the here synthesized drug/free PCL/PAMAM-PCL scaffold was tested ~~when~~ ~~using~~ on adult human dermal fibroblasts (HDFa). On the DOXO-loaded scaffolds, cytotoxicity assays were conducted on three different human cancer cell lines and the PCL/PAMAM-PCL were found to exhibit a slower release profile than standard PCL fibers only. The biocompatibility of the material and

the drug release profile are features that are both interesting for the application of such drug deliver scaffolds in cancer therapy.



~~Figure 1. Scheme of the preparation of the PCL/PAMAM-PCL electrospun fibers containing doxorubicine drug (DOXO).~~

Scheme 1. Schematic representation of the preparation of PCL-PAMAM moiety (a) and DOXO-loaded PCL/PAMAM-PCL fibers prepared by electrospinning PCL, PAMAM-PCL and chemotherapeutic drug DOXO (b). PCL: Poly (ε-caprolactone), PAMAM: poly (amido amine); DOXO: doxorubicin; PAMAM-PCL: the star polymer synthesized containing PCL and PAMAM; ROP: ring opening polymerization.

2. Experimental

2.1. Materials

For the preparation of PAMAM-PCL: ε-caprolactone (97%), which was purchased from Sigma-Aldrich and ~~it was~~ distilled under reduced pressure; second generation PAMAM dendrimer containing 16 amidoethanol surface groups (structure reported in Figure 1S), commercialized from Sigma-Aldrich as a 20 wt.% solution in methanol, was evaporated under vacuum at 50 °C for 24 h prior to use, in order to completely remove the solvent. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) (95%; from Sigma-Aldrich) was used without further treatments. Anhydrous toluene (≥99.7%), chloroform and methanol were purchased from Sigma-Aldrich and used as received. For the preparation of the PCL (DOXO) and PCL/PAMAM-PCL (DOXO) nanofibers: PCL with an average molecular weight of 80.000, N,N-dimethylformamide (DMF), dichloromethane (DCM) and doxorubicin hydrochloride (DOXO) were purchased from Sigma-Aldrich.

2.2. Synthesis of PAMAM-PCL

Star-like PCL was synthesized by the ring-opening polymerization of ε-caprolactone, at 120 °C, using the hydroxyl-terminated PAMAM dendrimer as macroinitiator and $Sn(Oct)_2$ as a catalyst.

In detail, 1.14 ml of PAMAM solution (corresponding to 0.2 g of PAMAM) ~~were~~ was introduced into a 50-ml two-neck round-bottomed flask equipped with a magnetic stirrer and in-situ dried; then, 3 g of ε-caprolactone (which was previously distilled) ~~were~~ was charged into the flask under argon flow. The reaction vessel was immersed into a thermostatically controlled oil bath (initially set at 140 °C, in order to ~~favour~~ favor the solubilisation of the dendrimer in the monomer), under vigorous stirring. ~~as~~ As soon as the mixture was completely homogenized, about 0.3 ml of a freshly prepared solution of $Sn(Oct)_2$ in toluene ([ε-caprolactone]/[$Sn(Oct)_2$] = $5 \cdot 10^3$) was added through a micropipette. The reaction was then allowed to proceed for 24 hours at 120 °C, always ~~under~~ in an inert atmosphere. After this ~~time~~, the polymerization was stopped by cooling down the reaction mixture, which was subsequently dissolved in chloroform and poured into an excess of cold methanol. The as-purified polymer (PAMAM-PCL) was recovered as a fine white ~~fine~~ powder by filtration and dried in vacuum at 40 °C ~~till~~ until the powder reached constant weight.

2.3. Fiber preparation

Electrospinning solutions were prepared by dissolving 15 wt.-% of PCL or PCL containing 10 wt.-% of PAMAM-PCL (PCL/PAMAM-PCL fibers) in the DMF and DCM solvent mixture ~~DMF and DCM~~ with a 30:70 v/v ratio. ~~In the case of~~ Regarding the preparation of fibers containing the drug, the concentration of DOXO in the polymer solution was 0.015 mg/ml in order to have a final total amount of 0.5 μg in the electrospun mats, which were used ~~per~~ for each of the wells in the cytotoxicity tests~~, of 0.5 μg~~.

The fibers were electrospun using a conventional electrospinning system [~~49~~ 43]. The solutions were loaded in a syringe (model Z314544, diameter d=11.6 mm, Aldrich Fortuna Optima) and were placed in ~~the~~ a horizontal direction. A Gamma high-voltage research power supply (Model ES30P-5W) was used to charge the solution in the syringe with a positive DC voltage. The positive electrode was connected to the needle (diameter d=0.45mm) of the syringe and the negative electrode was attached to the grounded collector, an aluminium sheet wrapped on a glass cylinder (height 4 cm, diameter 14.5 cm). The distance between the tip and the collector was 20 cm. A syringe pump (Harvard Apparatus Model 44 Programmable Syringe Pump) was used to feed the needle. The needle tip and the ground electrode were contained in a hollow plastic cylinder (height 30.5 cm, inner diameter 24 cm, and thickness 3.5 mm), internally coated with a polytetrafluoroethylene sheet (thickness 1 mm). A glass Brooks rotameter was used to keep ~~constant~~ the air flow constant in the enclosed electrospinning space. The air flow was fed in the chamber at atmospheric pressure from an inlet placed behind the collector.

The established conditions were: voltage tension = 15 kV, tip-collector distance = 20 cm, air flow = 2 l/min and temperature =21 °C.

2.4. Measurements

FTIR spectra were recorded on a Bruker IFS66 spectrometer in the spectral range 400-4000 cm^{-1}. ^{1}H NMR spectra were collected with a Varian NMR Mercury Plus instrument, at a frequency of 300 MHz, in $CDCl_3$ solutions containing tetramethylsilane as internal standard.

Differential scanning calorimetry (DSC) measurements were performed with a Mettler-Toledo TC10A calorimeter calibrated with high purity indium and operating under a flow of nitrogen.

The sample weight was about 5 mg and a scanning rate of 10 °C/min was employed in all the runs. The samples were heated from 25 °C to 100 °C, ~~at which temperature~~ then the melt was allowed to relax for 1 minute at this temperature~~, then~~ before being cooled down to -100 °C, and finally heated up again to 100 °C (second heating scan). The degree of crystallinity (Xc) was calculated by using an enthalpy of fusion for a 100% crystalline PCL of 136 J/g [~~50~~ 44].

The water absorption was measured by immersing electrospun mat specimens having area of 1.5 × 1.5 cm^2 in distilled water at room temperature for 48 h, and expressed as increase in weight percent according to the formula [~~51~~ 45]:

$$\text{water adsorption } (\%) = [(W_{wet} - W_{dry_0})/W_{dry_0}] \times 100 \quad (1)$$

~~where~~ in which W_{wet} is the wet weight measured (immediately – to avoid evaporative losses) after withdrawing the films from water and gently wiping off the surface water with a tissue, and W_{dry0} is the initial weight of the specimens, measured after vacuum-drying the films for 24 h at 40 °C.

~~To study~~ With regards to the sample surface morphology, a Zeiss Supra 40 VP field emission scanning electron microscope (FE-SEM) was used to examine the fiber morphologies. All samples were thinly sputter-coated with carbon using a Polaron E5100 sputter coater. The fiber diameters and their distribution were measured using an image analyzer, with ImageJ 1.41 software.

Confocal analysis was performed to check the presence of DOXO in the PCL and PCL/PAMAM-PCL mats. The DOXO-loaded-PCL and DOXO-loaded-PCL/PAMAM-PCL mats were cut into 1 x 1 cm^2 pieces. Each piece was then mounted on a flat sample holder and viewed under a confocal ~~microscope~~ Nikon Eclipse Ti microscope equipped with an argon laser source

with an excitation and emission wavelength of 488 and 530 nm, respectively, to view the doxorubicin loading in the electrospun mats.

2.5. In-vitro viability assay for testing of toxicity caused by pristine and DOXO-loaded PCL or PCL/PCL-PAMAM electrospun mats

Three different cancer cell lines were used: - A431 epidermoid carcinoma cells (ATCC® CRL-1555™), HeLa-WT cervical cancer cells (ATCC® CCL-2™) and MCF-7 breast cancer cells (ATCC® HTB-22™) ~~were used~~. All these cell lines were cultured using DMEM media supplemented with 10% Fetal Bovine Serum, 2% Penicillin-streptavidin and 1% L-Glutamine (Gibco™) under 37ºC, 5% CO_2 and 95% relative humidity. These cells were then harvested and plated in 24 multi-well-plate at a cell number of 100,000 cells in 1 ml cell media per well and allowed to adhere overnight. Given the interference of phenol-red in the optical read out of the cytotoxicity assay~~,~~ prior to performing the viability assays applied in this work, the media in the well plates was replaced with 1 ml of freshly prepared Phenol-Red free DMEM media supplemented with 10% Fetal Bovine Serum, 2% Penicillin-streptavidin and 1% L-Glutamine. Meanwhile, the electrospun mats, pristine PCL, pristine PCL/PAMAM-PCL, DOXO-loaded-PCL and DOXO-loaded-PCL/PAMAM-PCL were cut into 1x1 cm^2 pieces (each single piece of scaffold was manipulated to contain 0.5 µg DOXO during the electrospinning process). These 1x1 cm^2 scaffold pieces were peeled off from their aluminum substrate and dropped on top of the media in the 24 multi-well-plate that contained~~ing~~ the cells and incubated for 1, 3, 5, 7 and 9 days. After the respective incubation periods, PrestoBlue® and AlamarBlue® Cell Viability

Reagents (Molecular Probes™) were used to access the viability of the cancer cells. For each condition, the assays were performed three times ~~in triplicate~~.

2.6. PrestoBlue® Viability Assay

The 24 multi-well-plates containing the cells incubated with the scaffolds floating in their media, ~~after their incubation periods~~ were washed after their incubation period with Phosphate Buffer Saline (PBS) and incubated with fresh Phenol-Red free media containing 10% PrestoBlue® reagent for 30-40 minutes at 37 °C. Then 100 µl of supernatants from each well were transferred in a new 96 multi-well-plate and absorbance was read using a Multiskan™ GO Microplate Spectrophotometer (ThermoFisher Scientific™) at 570 and 600nm. The viability of each experimental condition was normalized to the viability of the cells cultured under the same conditions and never exposed to the materials which were considered ~~control untreated cells~~ to be 100% viable. The experiment was done four times and the mean value with its deviation (SD) was plotted using GraphPad.

2.7. AlamarBlue® Viability Assay

After the respective incubation periods previously ~~as~~ mentioned ~~earlier~~, 100µl of AlamarBlue reagent (10% AlamarBlue reagent in media) was added to each of the 24 multi well-plate containing the cells incubated with the floating scaffolds. The well plates were then incubated at 37ºC for 4h. Next, 100 µl of supernatants from each well were collected in a new 96 multi well-plate and absorbance was read using a Multiskan™ GO Microplate Spectrophotometer

(ThermoFisher Scientific™) at 570 and 600nm (Ab_{570}-Ab_{600}). The viability of each experimental condition was normalized to the viability of the control ~~untreated~~ cells ~~considering~~ which were considered ~~them~~ to be 100% viable. The experiment was done four times and the mean value with its deviation (SD) was plotted using GraphPad.

2.8. Confocal Microscopy characterization to study the attachment and proliferation of adult human dermal fibroblasts (HDFa) on PCL/PAMAM-PCL electrospun scaffolds

The HDFa Adult human dermal fibroblasts (ThermoFisher Scientific™ C0135C) were grown in special fibroblast media, Medium 106 (M106500) supplemented with 10 mL low serum growth supplement (LSGS_S00310). The cells were maintained in culture at 37ºC in a sterile incubator ~~and~~ with 5% CO_2 and 95% relative humidity. These cells were then harvested using 0.1% trypsin-EDTA, counted and plated in the 24 MW-plate containing the PCL/PAMAM-PCL scaffolds at density of 10,000 cells/well in 1mL complete media to check for the ability of this normal and healthy cell line to attach and proliferate on the drug free-PCL/PAMAM-PCL scaffolds. In order for the scaffolds to remain at the bottom of the well, a soft hand-made PDMS ring ~~prepared by us~~ was placed over the scaffold. The scaffolds used were neither physically (heat, plasma treatment, etc.) nor chemically (using proteins or adhesion factors) pre-treated. These cells ~~after 24h~~, were washed carefully with PBS after 24 h and fixed using 4% sterile formaldehyde (Sigma) for 10min, then washed thoroughly and permeated with 0.1% Triton X-100 for 2-3min. ~~Followed by~~ Following fixation and permeation, the scaffolds containing the cells were washed 3~~X~~ times with PBS. They were the incubated with 1% bovine serum albumin (BSA) for 30-40min~~;~~, which ~~this step~~ provides a blocking step for the upcoming F-actin staining.

~~Then~~ Next, they were stained using Alexa Fluor® 488 phalloidin (Invitrogen™ A12379) for 30min, washed with PBS 3~~X~~ times and stained using NucBlu® Fixed Cell ReadyProbes® Reagent (Invitrogen™ R37606) for 15 min. Then the scaffolds containing the cells were washed and carefully inverted in the same 24 MW-plate and filled with 1mL fresh PBS for the purpose of imaging using a Confocal Microscope (Nikon) at an excitation and emission wavelength of 495/518nm for F-actin phalloidin and 360/460nm for nuclei DAPI. The same was repeated after 48 and 72h. The analysis was done three times ~~in triplicate~~.

3. Results and discussion

3.1. Synthesis and characterization of dendritic star-like PCL (PAMAM-PCL)

In this work, at first, the star-like polymer, made up of a PAMAM core and PCL arms, was synthesized via ROP of ε-caprolactone using ~~as macro-initiator~~ the generation 2 PAMAM (fitted with 16 hydroxyl end groups) as macro-initiator (Figure 1S).

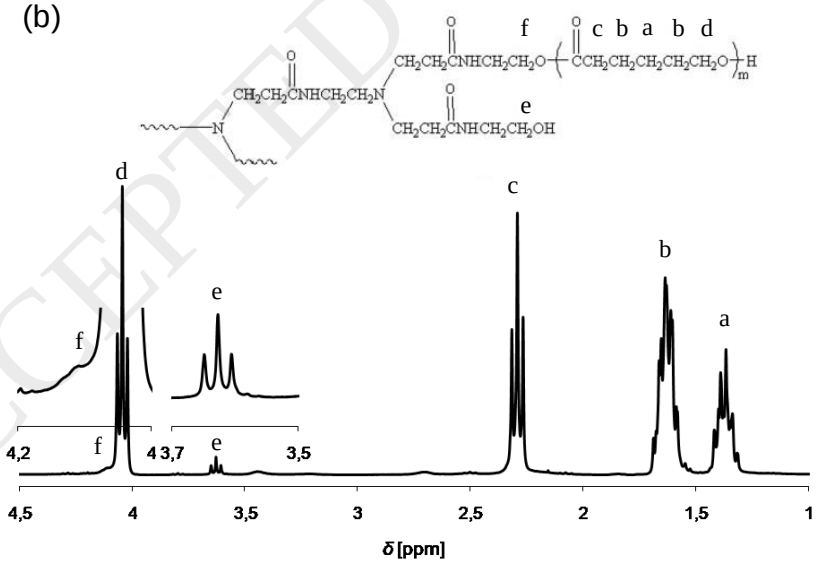
The dendrimer-graft architecture of the obtained product was established through spectroscopic measurements. Figure ~~2~~1a shows the FTIR spectrum of PAMAM-PCL: together with the intense absorption band for the stretching of the ester carbonyl at 1720 cm^{-1}, which is characteristic of PCL, two new peaks with a ~~of~~ lower intensity can be observed at 1650 and 1545 cm^{-1} (corresponding to amide carbonyl stretching and amide N-H bending, respectively) [~~52~~ 46], which is ~~to be~~ related to the poly-(amidoamine) structure of the PAMAM molecule. Since the sample was purified by precipitation in methanol, which is a good solvent for PAMAM, the

presence of these peaks is indicative of the successful incorporation of PAMAM as the core onto which the PCL arms were grown.

This was further confirmed by means of ^{1}H NMR spectroscopy (Figure ~~2~~1b), ~~where~~ in which the characteristic peaks of PCL (at δ 1.40, 1.65, 2.31, 3.64 and 4.06 ppm – assigned to CH_2-CH_2-C**H**$_2$-CH_2-CH_2, CH_2-C**H**$_2$-CH_2-CH_2-CH_2, CO-C**H**$_2$, terminal C**H**$_2$-OH, and C**H**$_2$-O-CO protons, respectively) are accompanied by several signals in the region ~~at~~ of δ 2.60-3.60 ppm, which is ascribable to the presence of the PAMAM units [~~52~~ 46]. Furthermore, the triplet at δ 3.60 ppm for the terminal methylene protons of unbound PAMAM becomes hardly discernible, while a signal appears at δ ~4.12 ppm (see the insets of Figure ~~2~~1b), which is consistent with the resonance shift upon the formation of ester linkages. This confirms that the formation of the desired star-like PCL with the ROP started from the hydroxyl end groups of PAMAM, and also indicates that all of these groups were active initiators, thus giving rise to a dendritic star-like PCL having on average 16 polyester arms. Finally, ^{1}H NMR spectroscopy was exploited to calculate the mean degree of polymerization of the PCL arms~~,~~ by comparing the peak integral of the methylene protons in the PCL chain with those at the chain end (at δ 2.31 and δ 3.64 ppm, respectively), ascertaining it is about 18. Thus, the spectroscopic measurements enabled us to assess~~ing~~ the successful preparation of a dendritic PCL star product, ~~having~~ with 16 arms, each ~~one with~~ of which has an average length of about 2000 g/mol, and thence an overall number average molecular weight of about 35000 g/mol – corresponding to a PAMAM content of about 9 wt.%.

(a)

(b)



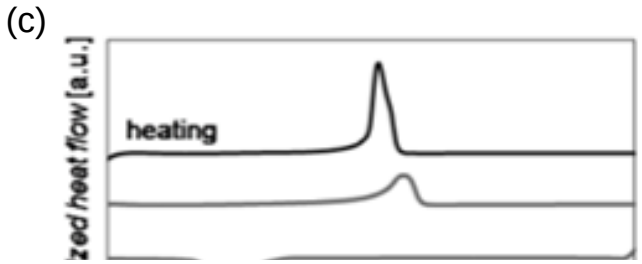


Figure ~~2~~ 1. (a) FTIR spectra of the sample PAMAM-PCL in the spectral region 1900-900 cm^{-1}, (b) ^{1}H NMR spectrum of PAMAM-PCL (region at δ 4.5-1.0 ppm) with enlargement insets (at δ 3.7-3.5 and 4.2-4.0 ppm) and (c) DSC thermograms of PAMAM-PCL (black lines) and PCL (grey lines) on cooling and subsequent heating at 10 °C/min after one minute relaxation in the melt at 100 °C (the heat flow is normalized to the PCL content).

Figure ~~2~~1c presents the DSC cooling and (second) heating curves of PAMAM-PCL, together with those of the linear high-molecular-weight PCL employed for electrospinning. In spite of the branched nature of PAMAM-PCL, which would be expected to disturb ordering, its crystallizability is enhanced with respect to PCL: the melt-crystallization is anticipated, as well as the final crystallinity is found higher (Xc ~55% against ~37%) than that of the linear polymer. As reported in the literature [47], ~~T~~this suggests that the improved chain mobility is promoted in the short-armed star system, ~~as~~ compared to high-molecular-weight PCL and prevails over the hindering effect caused by branching. Contrariwise, the temperature of melting is lower for the PAMAM-PCL than for the linear PCL (Tm ~51 °C against ~56 °C), ~~—that~~ which is a general

observation for star polymers, and it would be imputed to the short arm length and branched architecture of PAMAM-PCL, both of which are factors that negatively affect crystal thickness and perfection.

3.2. Preparation and characterization of PCL, PCL/PAMAM-PCL, PCL/PAMAM-PCL/DOXO and PCL/PAMAM-PCL/DOXO fibers

As shown in Figure ~~1~~ 2, DOXO was directly introduced ~~in~~ to the electrospinning solution and the obtained fibers were characterized in terms of morphology using SEM imaging technique and confocal microscopy.

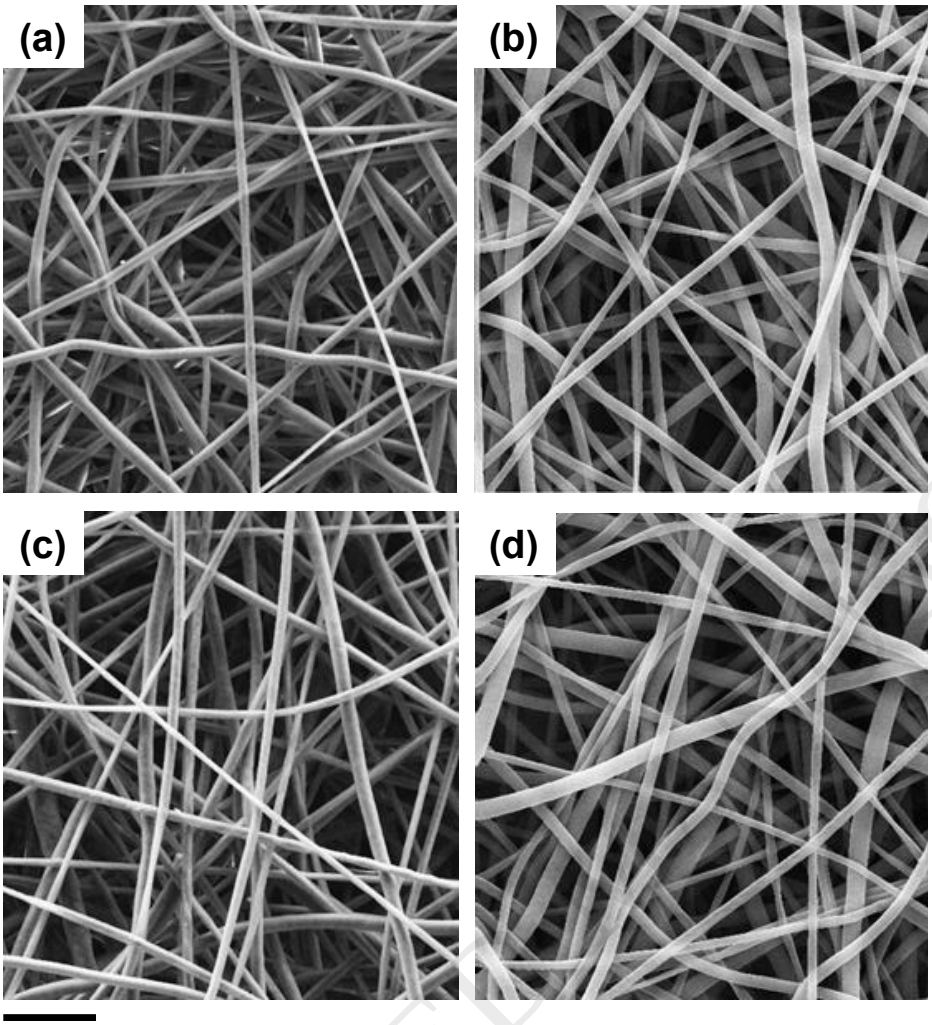
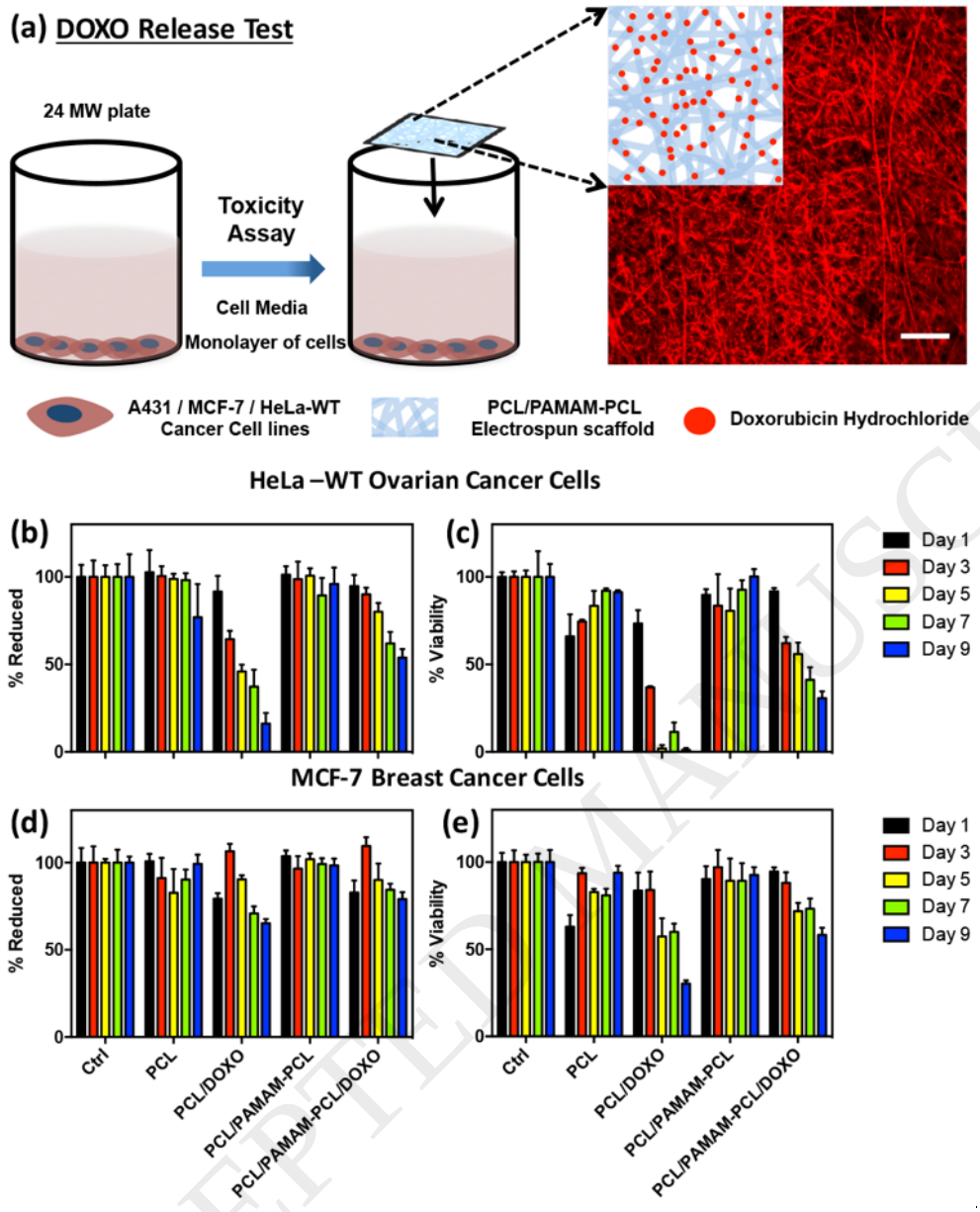


Figure ~~3~~ 2. FE-SEM micrographs of: (a) PCL, (b) PCL/PAMAM-PCL, (c) PCL/DOXO and (d) PCL/PAMAM-PCL/DOXO fibers. Scale bar represents 5 μm.

The electrospinning conditions were tailored in order to obtain defect-free and homogeneous fibers. Indeed, in Figure 3a, a typical SEM micrograph of a PCL mat ~~having~~ with an average diameter of 1.5 μm, prepared by applied the optimized electrospinning conditions, is shown. Also, the addition of PAMAM-PCL (10 wt.-%) turned out not to modify the fibers morphology, as by applying the same conditions used in the preparation of the neat PCL fibers ~~having~~ with an average diameter similar to that of the PCL fibers were obtained (Figure 3b). The addition of

DOXO within both the PCL and PCL/PAMAM-PCL mats did not change the fiber morphology, as shown in Figures 3c and 3d. The incorporation of the hydrophilic PAMAM moieties into the hydrophobic PCL fibers is expected to enhance the hydrophilicity of the resulting materials, which should be reflected in their capability to absorb water and, at the same time, interact with hydrophilic drug molecules like DOXO. The percentage of water absorbed by the mats containing PAMAM-PCL resulted ~~to be~~ in being 30 %, while the neat PCL fibers do not show any tendency to uptake water. This finding ~~points out~~ demonstrates the capacity of the star PAMAM-PCL polymer to modify the performances of the fibers, increasing their affinity for hydrophylic molecules, which is a feature ~~turns out to~~ that happens be relevant for the formulation of the novel drug delivery system based on the hydrophylic DOXO.

The DOXO loading in the PCL/PAMAM-PCL (Figure 4a) and PCL (Figure 2S a and c) fibers ~~were~~ was analyzed using confocal microscopy at an emission wavelength of 620 nm. Considering the confocal microscopy images, the DOXO-loaded fibers result ~~to be~~ in being characterized by a uniform red fluorescence, thus indicating a fine dispersion of the drug into the mats.



Replaced with

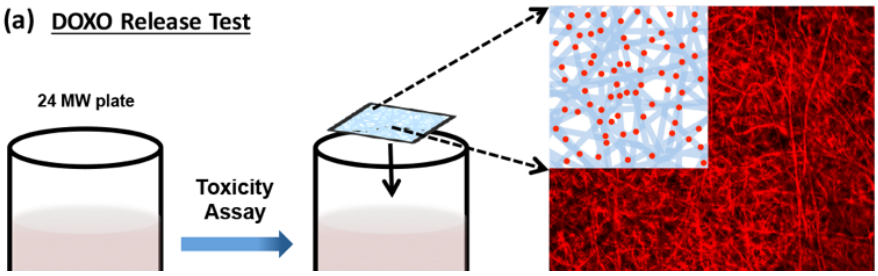


Figure 4 3. Scheme representing the drug release assay when using the mats as drug scaffolds and typical confocal imaging of a red fluorescent DOXO loaded PCL/PAMAM-PCL fibers ((a) - scale bar corresponds to 50µm). AlamarBlue® Viability assay on HeLa-WT (b) and MCF-7 (d) tumor cells treated with pristine and DOXO-loaded PCL or PCL/PCL-PAMAM mats. PrestoBlue® Viability assay on HeLa-WT (c) and MCF-7 (e) tumor cells treated with pristine and DOXO-

loaded PCL or PCL/PCL-PAMAM mats. (Error bar represents Sd and n=4 for all groups studied).

To test the drug release from the various mats, an indirect cell viability study was performed on different cancer cell lines (A431 adenocarcinoma, HeLa-WT cervical cancer and MCF-7 breast cancer cells). The cytotoxicity effects of the DOXO ~~was~~ were tested by using two sensitive resazurin based-viability assay kits (PrestoBlue® and AlamarBlue®), that assess the mitochondrial activity of cells exposed to the scaffolds at different time points post-incubation (1, 3, 5, 7 and 9 days). To demonstrate that the toxicity was caused by the release of DOXO and not by the materials constituting the electrospun mat itself, the pristine PCL and PCL/PAMAM-PCL fibers were tested in parallel with the corresponding DOXO-loaded fibers (PCL/DOXO and PCL/PCL-PAMAM/DOXO). ~~From~~ As can be seen in Figure ~~4~~ 3, PCL and PCL/PAMAM-PCL showed good biocompatibility with all the cancer cells lines studied, for up to 9 days of incubation. The HeLa-WT cells showed an abrupt reduction in viability from day 1 to 9 when treated with PCL/DOXO fibers whereas the same cell-line treated with PCL/PAMAM-PCL/DOXO showed a subtler profile in the reduction of viability over a few days (Figure ~~4b~~ 3b and ~~4c~~ 3c). Controls were untreated cells grown in cell media and kept under the same culture conditions. In case of A431 cancer cells, also a similar trend was also observed (Figure 3S). The prolonged and slow release of the new fibers could be due to the fact that the amphiphilic nature of the PAMAM-PCL~~,~~ contained in the mats~~,~~ increases the interactions and good mixing of the hydrophilic drug that in turn, was released in a more controlled manner over a few days, with respect to the most hydrophobic PCL mats. Indeed, the latter released DOXO at a much higher rate causing such sudden decrease in viability. We also tested the scaffolds on a DOXO resistant MCF-7 cell line, which ~~and when compared to the other two cell lines studied, the MCF-7 cells~~

showed much more resistance to toxicity caused by the release of DOXO in comparison to the other two cell lines studied, but a gradual reduction in viability was also observed which followed ~~following~~ the same comparative trends as for the other two cell lines (Figure ~~4d~~ 3d and ~~4e~~ 3e). Overall the cytotoxicity study suggests that the PCL/PCL-PAMAM scaffolds enable a slower release of the drug with respect to the PCL mats thus allowing a prolonged drug treatment. When considering the drug release profile with other hydrid-based fibers prepared by electro-spun techniques, we could assess that in our study we ~~had a look at~~ looked at longer periods of drug release (up 9 days) than ~~the case of~~ studies on polyethylene oxide/chitosan/graphene oxide fibers (up to 48 hours) [~~47~~ 41] and to the polymeric-based fibers (72 h) [~~48~~ 42]. The drug release profile of our hybrid system was ~~more~~ closely in line with that of multiwall carbon nanotubes/poly(lactic-co-glycolic acid) polymer fibers in which ~~several days of~~ release over several days has~~ve~~ been also achieved ~~considered~~ (up to 42 days) [~~53~~ 48] This prolonged release feature makes the nano-based systems appealing as an implantable post-surgical drug delivery scaffold.

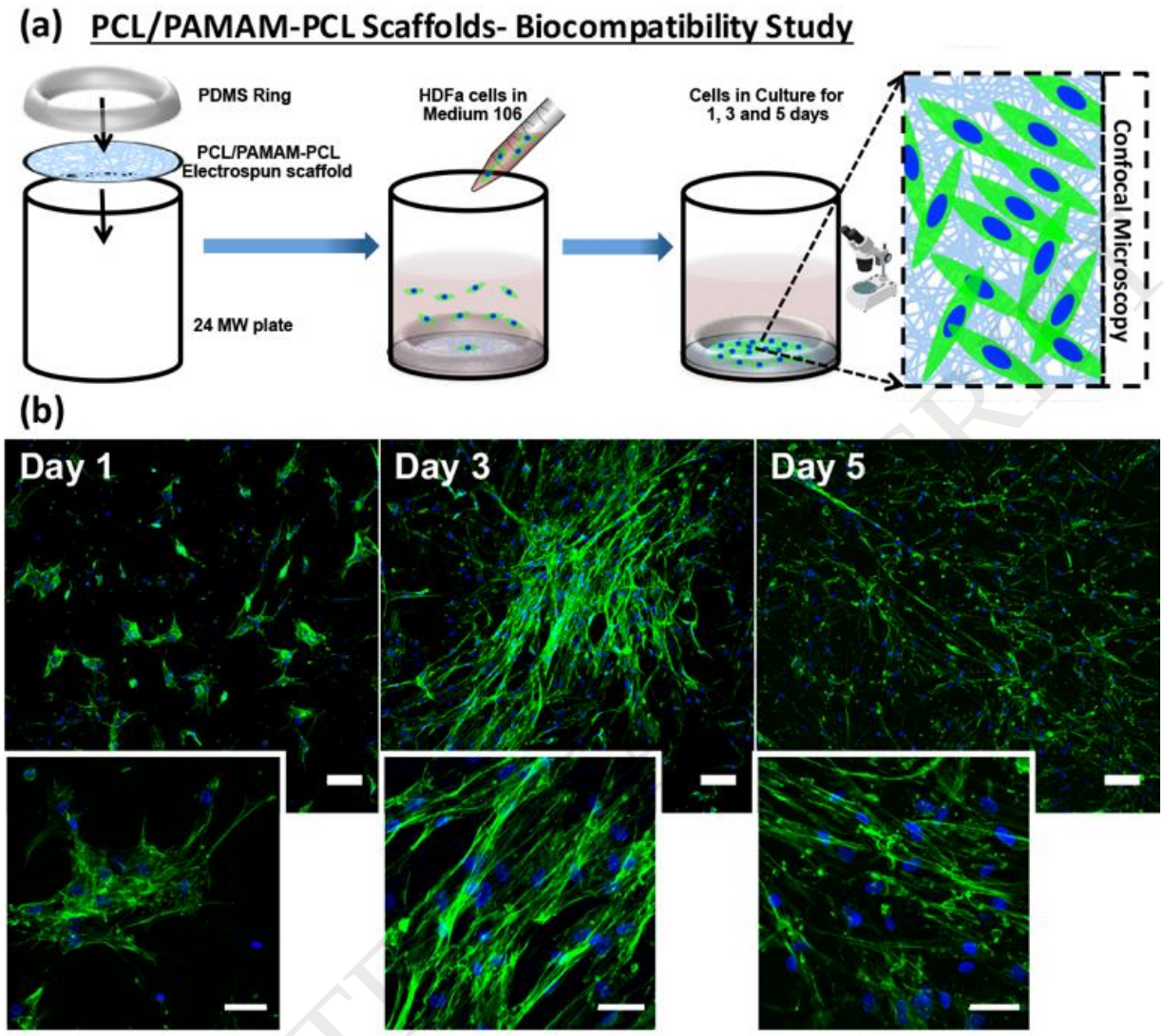


Figure ~~5~~ 4. Scheme representing the culturing of healthy dermal fibroblast (HDFa) cells on PCL/PAMAM-PCL scaffold (a). Confocal images showing the attachment and growth of HDFa cells on a PCL/PAMAM-PCL scaffold between 1 and 5 days (b). Scale bar represents 100µm (50µm in the inserts). F-Actin stained using Alexa Fluor® 488 Phalloidin (green) and nuclei stained with DAPI (blue).

The drug-free PCL/PCL-PAMAM scaffolds did not show any toxicity towards ~~all~~ any of the tumor cell lines. This prompted us to investigate the biocompatibility of the mats with healthy cells ~~in the view to apply~~ with the idea of applying such mats as tissue scaffolds. To this aim adult human dermal fibroblasts (HDFa) were grown directly on PCL/PAMAM-PCL scaffolds. Confocal imaging characterization was done to study the attachment and growth of these cells (Figure ~~5~~ 4 and Figure 4S). It was evident that HDFa cells attached firmly already after day 1 of incubation, ~~being~~ which were characterized by their multiple finger-like projection (F-actin stained in green by Alexa Fluor® 488 phalloidin, Figure ~~5b~~ 4b). By day 3 these cells were proliferating rapidly and by day 5, they spread well and formed their typical bipolar shape. An interesting note is that~~,~~ these cells were able to penetrate deep into the PCL/PAMAM/PCL scaffold, as shown in the zeta-stack analysis of the fibers (Figure 5S), even without any physical or chemical pre-treatment of the scaffolds, which might be contributed to the hydrophilic PAMAM moiety in the scaffold.

4. Conclusions

In this work, a composite polymer material designed to be applied as a localized/sustainable delivery system of anticancer drugs was developed. The system consists of poly(ε-caprolactone) (PCL) electrospun fibers, a material which combines the suitable features of the polymer for its implantation in the body, such as the bioabsorbibility and biocompatibility, with those of the fibrous architecture, ~~that are~~ for example the high surface area and the easy application at the targeted area. In order to enhance the affinity of the fibers for the drug, doxorubicin, and tune its

release, an *ad-hoc*-synthesized star polymer, made up of a poly(amido-amine) (PAMAM) core and PCL branches (PAMAM-PCL), was added into the electrospun fibers. ~~Conversely~~ In contrast to the inorganic nanofillers, which are commonly applied as drug carriers in the polymer fibers, our star system ~~is characterized by~~ has a good affinity with PCL, since the PAMAM-branched PCL has ~~being characterized by~~ arms whose chemical nature is identical to that of the polymer matrix. Moreover, the PAMAM core of the star polymer, which renders the fibers more hydrophilic, by interacting with the chosen hydrophilic drug (DOXO), ~~turns out to ameliorate~~ improves its dispersion in the fibers and allow its prolonged and controlled release (we have here extended the study to 9 days). These fibers~~, as implantable patches~~ can be used for delivery systems as implantable patches with a prolonged release period, to avoid the development of drug resistance in cancer therapy due to an unnecessary burst and uncontrolled release of the anti-cancer drug used in conventional chemotherapies. The described PCL/PAMAM-PCL system could be used in various applications just by replacing the therapeutic agents incorporated within the fibers. Both hydrophilic as well as hydrophobic drugs can be incorporated into this system due to ~~its~~ their amphiphilic nature, which makes them more versatile than most available systems. Remarkable~~y~~, the biocompatibility study showing the attachment and growth of adult Human Dermal Fibroblast (HDFa) studied on PCL/PMAM-PCL scaffolds~~,~~ suggests their use~~d~~ as transdermal drug delivery patches for wound healing and faster regeneration of the damaged tissue (stem cell delivery with appropriate signaling molecules) [~~54-56~~ 49-51], as biodegradable patches to be used post-surgery to avoid infections when loaded with antibiotics (Biteral®, gentamicin sulphate, etc.) [~~57-58~~ 52,53] or eventually ~~when~~ as bandages when loaded with immuno-suppressants ~~to be used as bandages~~ to prevent organ rejection in in post organ transplantation.

Acknowledgment

This work was funded by the Italian AIRC (Contract No. 14527), the Cariplo foundation (Contract No. 2013 0865) and the European Research Council (ERC) (starting grant ICARO project, Contract No. 678109) funds.

References

1 Liu, R.; Wolinsky, J. B.; Catalano, P. J.; Chirieac, L. R.; Wagner, A. J.; Grinstaff, M. W.; Colson, Y. L.; Raut, C. P. Paclitaxel-Eluting Polymer Film Reduces Locoregional Recurrence and Improves Survival in a Recurrent Sarcoma Model: A Novel Investigational Therapy. *Ann. Surg. Oncol.* **2012,** *19* (1), 199-206.

2. Vashist, A.; Vashist, A.; Gupta, Y. K.; Ahmad, S. Recent advances in hydrogel based drug delivery systems for the human body. *J. Mater. Chem. B* **2014,** *2* (2), 147-166.

3. Lei, N.; Gong, C. Y.; Qian, Z. Y.; Luo, F.; Wang, C.; Wang, H. L.; Wei, Y. Q. Therapeutic application of injectable thermosensitive hydrogel in preventing local breast cancer recurrence and improving incision wound healing in a mouse model. *Nanoscale* **2012,** *4* (18), 5686-5693.

4. Wolinsky, J. B.; Colson, Y. L.; Grinstaff, M. W. Local drug delivery strategies for cancer treatment: Gels, nanoparticles, polymeric films, rods, and wafers. *J. Controlled Release* **2012,** *159* (1), 14-26.

5. Karimi, M.; Ghasemi, A.; Zangabad, P. S.; Rahighi, R.; Basri, S. M. M.; Mirshekari, H.; Amiri, M.; Pishabad, Z. S.; Aslani, A.; Bozorgomid, M.; Ghosh, D.; Beyzavi, A.; Vaseghi, A.; Aref, A. R.; Haghani, L.; Bahrami, S.; Hamblin, M. R. Smart micro/nanoparticles in stimulus-responsive drug/gene delivery systems. *Chem. Soc. Rev.* **2016,** *45* (5), 1457-1501.

6. Ang, C. Y.; Tan, S. Y.; Zhao, Y. L. Recent advances in biocompatible nanocarriers for delivery of chemotherapeutic cargoes towards cancer therapy. *Org. Biomol. Chem.* **2014,** *12* (27), 4776-4806.

7. Ma, G. P.; Liu, Y.; Peng, C.; Fang, D. W.; He, B. J.; Nie, J. Paclitaxel loaded electrospun porous nanofibers as mat potential application for chemotherapy against prostate cancer. *Carbohydr. Polym.* **2011,** *86* (2), 505-512.

8. Zong, S.; Wang, X.; Yang, Y. P.; Wu, W. B.; Li, H. J.; Ma, Y.; Lin, W. H.; Sun, T. T.; Huang, Y. B.; Xie, Z. G.; Yue, Y.; Liu, S.; Jing, X. B. The use of cisplatin-loaded mucoadhesive nanofibers for local chemotherapy of cervical cancers in mice. *Eur. J. Pharm. Biopharm.* **2015,** *93*, 127-135.

9. Xie, J. W.; Tan, R. S.; Wang, C. H. Biodegradable microparticles and fiber fabrics for sustained delivery of cisplatin to treat C6 glioma in vitro. *J. Biomed. Mater. Res. Part A* **2008,** *85A* (4), 897-908.

10. Qiu, K.; He, C.; Feng, W.; Wang, W.; Yin, X. Z. Z.; Chen, L.; Wang H.; Mo. X. Doxorubicin-loaded electrospun poly(L-lactic acid)/mesoporous silica nanoparticles composite nanofibers for potential postsurgical cancer treatment. *J. Mater. Chem.* B **2013**, *1*, 4601-4611.

11. Chen, C.; Lv, G.; Pan, C.; Song, M.; Wu, C.; Guo, D.; Wang, X.; Chen B.; Gu. Z. Poly(lactic acid) (PLA) based nanocomposites—a novel way of drug-releasing. *Biomed. Mater*. **2007**, *2*, L1-L4.

12. Irani, M.; Sadeghi, G.M.M.; Haririan I.. Electrospun biocompatible poly(ε-caprolactonediol) based polyurethane core/shell nanofibrous scaffold for controlled release of temozolomide. *Int J Polym Mater.* **2017**, in press.

13. Xu, X. L.; Chen, X. S.; Xu, X. Y.; Lu, T. C.; Wang, X.; Yang, L. X.; Jing, X. B. BCNU-loaded PEG-PLLA ultrafine fibers and their in vitro antitumor activity against glioma C6 cells. *J. Controlled Release* **2006,** *114* (3), 307-316.

14. Shao, S. J.; Li, L.; Yang, G.; Li, J. R.; Luo, C.; Gong, T.; Zhou, S. B. Controlled green tea polyphenols release from electrospun PCL/MWCNTs composite nanofibers. *Int. J. Pharm.* **2011,** *421* (2), 310-320.

15. Yohe, S. T.; Herrera, V. L. M.; Colson, Y. L.; Grinstaff, M. W. 3D superhydrophobic electrospun meshes as reinforcement materials for sustained local drug delivery against colorectal cancer cells. *J. Controlled Release* **2012,** *162* (1), 92-101.

16. Zheng, F. Y.; Wang, S. G.; Shen, M. W.; Zhu, M. F.; Shi, X. Y. Antitumor efficacy of doxorubicin-loaded electrospun nano-hydroxyapatite-poly(lactic-co-glycolic acid) composite nanofibers. *Polym. Chem.* **2013,** *4* (4), 933-941.

17. Zhou, X. J.; Chen, L.; Wang, W. Z.; Jia, Y. T.; Chang, A. N.; Mo, X. M.; Wang, H. S.; He, C. L. Electrospun nanofibers incorporating self-decomposable silica nanoparticles as carriers for controlled delivery of anticancer drug. *Rsc Adv* **2015,** *5* (81), 65897-65904.

18. Qiu, K. X.; He, C. L.; Feng, W.; Wang, W. Z.; Zhou, X. J.; Yin, Z. Q.; Chen, L.; Wang, H. S.; Mo, X. M. Doxorubicin-loaded electrospun poly(L-lactic acid)/mesoporous silica nanoparticles composite nanofibers for potential postsurgical cancer treatment. *J. Mater. Chem. B* **2013,** *1* (36), 4601-4611.

19. Ignatova, M. G.; Manolova, N. E.; Toshkova, R. A.; Rashkov, I. B.; Gardeva, E. G.; Yossifova, L. S.; Alexandrov, M. T. Electrospun Nanofibrous Mats Containing Quaternized Chitosan and Polylactide with In Vitro Antitumor Activity against HeLa Cells. *Biomacromolecules* **2010,** *11* (6), 1633-1645.

20. Xu, X. L.; Chen, X. S.; Ma, P. A.; Wang, X. R.; Jing, X. B. The release behavior of doxorubicin hydrochloride from medicated fibers prepared by emulsion-electrospinning. *Eur. J. Pharm. Biopharm.* **2008,** *70* (1), 165-170.

21. He, C. L.; Huang, Z. M.; Han, X. J. Fabrication of drug-loaded electrospun aligned fibrous threads for suture applications. *J. Biomed. Mater. Res. Part A* **2009,** *89A* (1), 80-95.

22. Zhang, S. L.; Chu, Z. Q.; Yin, C.; Zhang, C. Y.; Lin, G.; Li, Q. Controllable Drug Release and Simultaneously Carrier Decomposition of SiO2-Drug Composite Nanoparticles. *J. Am. Chem. Soc.* **2013,** *135* (15), 5709-5716.

23. Song, B. T.; Wu, C. T.; Chang, J. Dual drug release from electrospun poly(lactic-co-glycolic acid)/mesoporous silica nanoparticles composite mats with distinct release profiles. *Acta Biomater.* **2012,** *8* (5), 1901-1907.

24. Song, B. T.; Wu, C. T.; Chang, J. Controllable delivery of hydrophilic and hydrophobic drugs from electrospun poly(lactic-co-glycolic acid)/mesoporous silica nanoparticles composite mats. *J. Biomed. Mater. Res., Part B* **2012,** *100B* (8), 2178-2186.

25. Zheng, F. Y.; Wang, S. G.; Wen, S. H.; Shen, M. W.; Zhu, M. F.; Shi, X. Y. Characterization and antibacterial activity of amoxicillin-loaded electrospun nano-hydroxyapatite/poly(lactic-co-glycolic acid) composite nanofibers. *Biomaterials* **2013,** *34* (4), 1402-1412.

26. Chen, M. X.; Feng, W.; Lin, S.; He, C. L.; Gao, Y.; Wang, H. S. Antitumor efficacy of a PLGA composite nanofiber embedded with doxorubicin@MSNs and hydroxycamptothecin@HANPs. *Rsc Adv* **2014,** *4* (95), 53344-53351.

27. Monticelli, O.; Putti, M.; Gardella, L.; Cavallo, D.; Basso, A.; Prato, M.; Nitti, S. New Stereocomplex PLA-Based Fibers: Effect of POSS on Polymer Functionalization and Properties. *Macromolecules* **2014,** *47* (14), 4718-4727.

28. Inoue, K. Functional dendrimers, hyperbranched and star polymers. *Prog. Polym. Sci.* **2000,** *25* (4), 453-571.

26. Zhao, Y. L.; Qing, C.; Jing, J.; Shuai, X. T.; Bei, J. Z.; Chen, C. F.; Fu, X. Synthesis and thermal properties of novel star-shaped poly(L-lactide)s with starburst PAMAM-OH dendrimer macroinitiator. *Polymer* **2002,** *43* (22), 5819-5825.

27. Zhao, Y. L.; Shuai, X. T.; Chen, C. F.; Xi, F. Synthesis and characterization of star-shaped poly (L-lactide)s initiated with hydroxyl-terminated poly(amidoamine) (PAMAM-OH) dendrimers. *Chem. Mater.* **2003,** *15* (14), 2836-2843.

28. Cai, Q.; Zhao, Y. L.; Bei, J. Z.; Xi, F.; Wang, S. G. Synthesis and properties of star-shaped polylactide attached to poly(amidoamine) dendrimer. *Biomacromolecules* **2003,** *4* (3), 828-834.

29. Zhang, C. X.; Wang, B.; Chen, Y.; Cheng, F.; Jiang, S. C. Amphiphilic multiarm star polylactide with hyperbranched polyethylenimine as core: A systematic reinvestigation. *Polymer* **2012,** *53* (18), 3900-3909.

30. Wang, F.; Bronich, T. K.; Kabanov, A. V.; Rauh, R. D.; Roovers, J. Synthesis and characterization of star poly(epsilon-caprolactone)-b-poly(ethylene glycol) and poly(L-lactide)-b-poly(ethylene glycol) copolymers: Evaluation as drug delivery carriers. *Bioconjugate Chem.* **2008,** *19* (7), 1423-1429.

31. Cordova, A.; Hult, A.; Hult, K.; Ihre, H.; Iversen, T.; Malmstrom, E. Synthesis of a poly(epsilon-caprolactone) monosubstituted first generation dendrimer by lipase catalysis. *J. Am. Chem. Soc.* **1998,** *120* (51), 13521-13522.

32. Liu, Y.; Samsonova, O.; Sproat, B.; Merkel, O.; Kissel, T. Biophysical characterization of hyper-branched polyethylenimine-graft-polycaprolactone-block-mono-methoxyl-poly(ethylene glycol) copolymers (hy-PEI-PCL-mPEG) for siRNA delivery. *J. Controlled Release* **2011,** *153* (3), 262-268.

~~33. Li, Z. B.; Li, J. Control of Hyperbranched Structure of Polycaprolactone/Poly(ethylene glycol) Polyurethane Block Copolymers by Glycerol and Their Hydrogels for Potential Cell Delivery. *J. Phys. Chem. B* **2013,** *117* (47), 14763-14774.~~

31. Wang, F.; Bronich, T. K.; Kabanov, A. V.; Rauh, R. D.; Roovers, J. Synthesis and evaluation of a star amphiphilic block copolymer from poly(epsilon-caprolactone) and poly(ethylene glycol) as a potential drug delivery carrier. *Bioconjugate Chem.* **2005,** *16* (2), 397-405.

32. He, H.; Wang, Y.; Wen, H.; Jia, X. R. Dendrimer-based multilayer nanocarrier for potential synergistic paclitaxel-doxorubicin combination drug delivery. *Rsc Adv* **2014,** *4* (7), 3643-3652.

33. Dai, X. H.; Zhang, H. D.; Dong, C. M. Fabrication, biomolecular binding, in vitro drug release behavior of sugar-installed nanoparticles from star poly(epsilon-caprolactone)/glycopolymer biohybrid with a dendrimer core. *Polymer* **2009,** *50* (19), 4626-4634.

34. Yao, Q. Q.; Cosme, J. G. L.; Xu, T.; Miszuk, J. M.; Picciani, P. H. S.; Fong, H.; Sun, H. L. Three dimensional electrospun PCL/PLA blend nanofibrous scaffolds with significantly improved stem cells osteogenic differentiation and cranial bone formation. *Biomaterials* **2017,** *115*, 115-127.

35. Cooper, A.; Bhattarai, N.; Zhang, M. Q. Fabrication and cellular compatibility of aligned chitosan-PCL fibers for nerve tissue regeneration. *Carbohydr. Polym.* **2011,** *85* (1), 149-156.

36. Neves, S. C.; Teixeira, L. S. M.; Moroni, L.; Reis, R. L.; Van Blitterswijk, C. A.; Alves, N. M.; Karperien, M.; Mano, J. F. Chitosan/Poly(epsilon-caprolactone) blend scaffolds for cartilage repair. *Biomaterials* **2011,** *32* (4), 1068-1079.

37. Ghasemi-Mobarakeh, L.; Prabhakaran, M. P.; Morshed, M.; Nasr-Esfahani, M. H.; Ramakrishna, S. Electrospun poly(epsilon-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials* **2008,** *29* (34), 4532-4539.

38. Alvarez-Perez, M. A.; Guarino, V.; Cirillo, V.; Ambrosio, L. Influence of Gelatin Cues in PCL Electrospun Membranes on Nerve Outgrowth. *Biomacromolecules* **2010,** *11* (9), 2238-2246.

39. Chong, E. J.; Phan, T. T.; Lim, I. J.; Zhang, Y. Z.; Bay, B. H.; Ramakrishna, S.; Lim, C. T. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomater.* **2007,** *3* (3), 321-330.

40. Zhang, Y. Z.; Ouyang, H. W.; Lim, C. T.; Ramakrishna, S.; Huang, Z. M. Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *J. Biomed. Mater. Res., Part B* **2005,** *72B* (1), 156-165.

~~44. Chakrapani, V. Y.; Kumar, T. S. S.; Raj, D. K.; Kumary, T. V. Electrospun Cytocompatible Polycaprolactone Blend Composite with Enhanced Wettability for Bone Tissue Engineering. *J. Nanosci. Nanotechnol.* **2017,** *17* (4), 2320-2328.~~

~~45. Yoshimoto, H.; Shin, Y. M.; Terai, H.; Vacanti, J. P. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. *Biomaterials* **2003,** *24* (12), 2077-2082.~~

~~46. Sill, T. J.; von Recum, H. A. Electro spinning: Applications in drug delivery and tissue engineering. *Biomaterials* **2008,** *29* (13), 1989-2006.~~

41. Ardeshirzadeh, B.; Anaraki, N. A.; Irani, M.; Rad, L. R.; Shamshiri, S. Controlled release of doxorubicin from electrospun PEO/chitosan/graphene oxide nanocomposite nanofibrous scaffolds. *Mater. Sci. Eng., C* **2015,** *48*, 384-390.

42. Salehi, R.; Irani, M.; Rashidi, M. R.; Aroujalian, A.; Raisi, A.; Eskandani, M.; Haririan, I.; Davaran, S. Stimuli-responsive nanofibers prepared from poly(N-isopropylacrylamide-acrylamide-vinylpyrrolidone) by electrospinning as an anticancer drug delivery. *Des. Monomers Polym.* **2013,** *16* (6), 515-527.

43. Cozza, E. S.; Monticelli, O.; Marsano, E. Electrospinning: A Novel Method to Incorporate POSS Into a Polymer Matrix. *Macromol. Mater. Eng.* **2010,** *295* (9), 791-795.

44. Forouharshad, M.; Gardella, L.; Furfaro, D.; Galimberti, M.; Monticelli, O. A low-environmental-impact approach for novel biocomposites based on PLLA/PCL blends and high surface area graphite. *Eur. Polym. J.* **2015,** *70*, 28-36.

45. D570-98(2010)e1, A. Standard Test Method for Water Absorption of Plastics. *ASTM International, West Conshohocken, PA, 2010, www.astm.org* **2010**.

46. Gardella, L.; Basso, A.; Prato, M.; Monticelli, O. On stereocomplexed polylactide materials as support for PAMAM dendrimers: synthesis and properties. *Rsc Adv* **2015,** *5* (58), 46774-46784.

47. Choi, J.; Kwak, S.-Y. Architectural effects of poly(ε-caprolactone)s on the crystallization kinetics. *Macromolecules* **2004**, *37*, 3745–3754.

48. Qi, R. L.; Tian, X. J.; Guo, R.; Luo, Y.; Shen, M. W.; Yu, J. Y.; Shi, X. Y. Controlled release of doxorubicin from electrospun MWCNTs/PLGA hybrid nanofibers. *Chin. J. Polym. Sci.* **2016,** *34* (9), 1047-1059.

49. Lim, S. H.; Mao, H. Q. Electrospun scaffolds for stem cell engineering. *Adv. Drug Delivery Rev.* **2009,** *61* (12), 1084-1096.

50. Discher, D. E.; Mooney, D. J.; Zandstra, P. W. Growth Factors, Matrices, and Forces Combine and Control Stem Cells. *Science* **2009,** *324* (5935), 1673-1677.

51. Kataria, K.; Gupta, A.; Rath, G.; Mathur, R. B.; Dhakate, S. R. In vivo wound healing performance of drug loaded electrospun composite nanofibers transdermal patch. *Int. J. Pharm.* **2014,** *469* (1), 102-110.

52. Bolgen, N.; Vargel, I.; Korkusuz, P.; Menceloglu, Y. Z.; Piskin, E. In vivo performance of antibiotic embedded electrospun PCL membranes for prevention of abdominal adhesions. *J. Biomed. Mater. Res., Part B* **2007,** *81B* (2), 530-543.

53. Teo, E. Y.; Ong, S. Y.; Chong, M. S. K.; Zhang, Z. Y.; Lu, J.; Moochhala, S.; Ho, B.; Teoh, S. H. Polycaprolactone-based fused deposition modeled mesh for delivery of antibacterial agents to infected wounds. *Biomaterials* **2011,** *32* (1), 279-287.