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STUDY OF THE PROPERTIES AND CELLS GROWTH ON ANTIBACTERIAL ELECTROSPUN POLYCAPROLACTONE/CEFUROXIME SCAFFOLDS

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Abstract:

Electrospun materials are good candidates for the design of tissue regeneration scaffolds as they can simulate the natural surroundings of tissue cells. The study proposes electrospun polycaprolactone (PCL)/cefuroxime (CFU) scaffolds for human cell culture and investigates the influence of the antibiotic content on scaffold morphology, thermal and mechanical properties. The increase in the CFU concentration resulted in the reduction of fiber diameter and number of deformations. It also influenced the reduction of scaffold thermal enthalpies and improved scaffold break strength. With regard to cell growth, the scaffolds showed precedence in greater colonization of the HeLa cells. Finally, these scaffolds showed compatibility with standard human cell lines, and thus they can be used for the repair of damaged tissues.

Keywords:

Electrospun, scaffolds, PCL, Cefuroxime, SEM, DSC, tensile behavior, human cells

1. Introduction

Electrospinning is a simple and promising technique for the design and production of biomedical products, among which are tissue engineering scaffolds. Due to the severe lack of organ donors and possible tissue inflammation after transplantation, electrospun scaffolds are promising alternative substitutes that can provide tissue repair and potential organ in vitro growth [1, 2] (Figure 1). In electrospinning, a polymer solution is stretched under the influence of an electric field and further bended to finally form ultrafine filaments collected on an opposite electrode in the shape of a nonwoven material [1, 3].

The advantage of an electrospun scaffold comes from the nanofibrous structure with large surface area for proteins adsorption, thus providing more binding sites for tissue cell receptors [5]. The porous structure also provides release and exchange of biomolecules and nutrients [6]. In wound healing, it can also prevent wound desiccation by liquid exudation, controlled evaporation, excellent oxygen permeability and promoted fluid drainage capacity, as well as microorganism invasion inhibition [7]. Apart from the good physical structure of the electrospun scaffolds, the same can be further functionalized for additional cell support. One of the functionalizations includes incorporation of a medicine for controlled therapies. The investigations reported both: drug effect and cultured cells, adhesion, growth and proliferation. When polycaprolactone (PCL)/poly(trimethylene carbonate) (PTMC) was electrospun with shikonin, the drug experienced fast release initial stage, followed by a gradual release that sustained for over 48 h. The shikonin release rates also depended on the PTMC and its own concentration [8]. Another study reported on the encapsulation of the drug paclitaxel in electrospun poly-(D,L-lactide-co-glycolide) (PLGA) for the treatment of malignant glioma. The electrospun PLGA/paclitaxel showed sustained release of the drug for over 80 days with small initial burst effect. The release effect was faster in the case of submicrofibers compared with the microfiber materials. In vivo studies of mice model with glioma showed inhibition of the tumor cells as well [9]. Similarly, electrospun polylactide (PLA)/pearl powder was loaded with the antitumor drug doxorubicin hydrochloride (DOX). It was reported that the presence of the pearl powder due to higher hydrophilicity resulted in more rapid release of the drug. Moreover, at certain pearl concentration (compared with the neat PLA), an improved antitumor efficacy was reported when the materials were seeded with HeLa tumor cells [10]. DOX was also loaded in poly(L-lactide-co-D,L-lactide) (coPLA), and a synergistic action was reported when it was used in combination with quaternized chitosan (QCh) for the inhibition of human cervical tumor cell line HeLa. DOX easily penetrated the cells membranes due to the opposite charges of the tertiary amino groups of QCh and the areas of the tumor

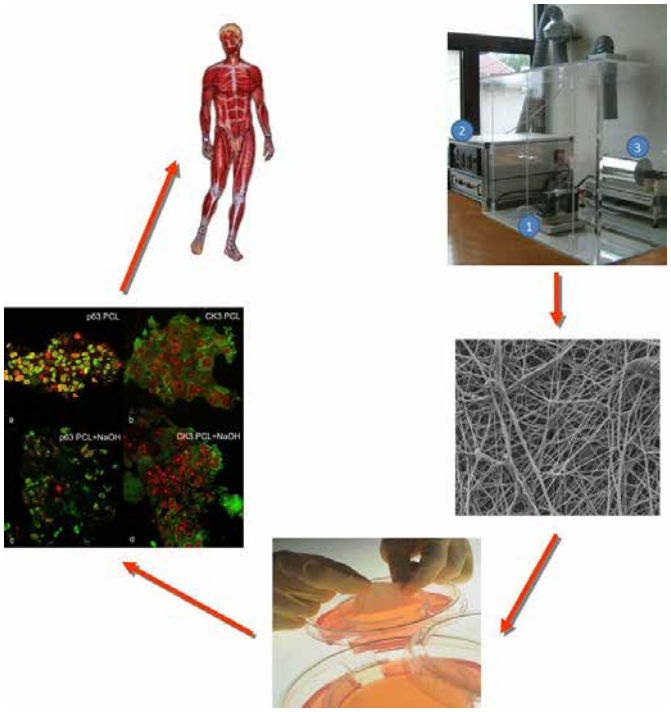

Figure 1. Stages in electrospun scaffold preparation and cell culture for tissue repair [4].

cells membranes that lead to their destruction [11]. Emulsion electrospun hydroxycamptothecin (HPCT)/poly(dl-lactic acid)-poly(ethylene glycol) (PELA) fibers were used for the inhibition of the activity of cancer cell line HepG2. It was reported that during 72-h incubation, the incorporated drug showed 20 times higher inhibition against HepG2 cells than the free HCPT [12]. For skin wound healing, electrospun PCL/collagen scaffolds were loaded with androgen receptor (AR) inhibitor called ASC-J9. The study showed that the loaded active molecules can enhance skin cell growth, migration, differentiation and wound closure. Thus, the sustained release of the biofactor over 28 days improved fibroblasts adhesion and ingrowth, and it also accelerated wound closure by improved keratinocytes migration [13].

In this work, electrospun PCL scaffolds were loaded with the antibiotic cefuroxime (CFU) to be used in the therapeutic repair of eye tissues, especially after eye surgery. The scaffolds morphology, as well as their thermal and mechanical properties were evaluated depending on the loaded drug concentration. To verify the biocompatibility of the electrospun scaffolds, growth and viability of two different human cell lines were tested (HeLa and Hep G2). For that purpose, a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was applied after cell seeding and incubation on PCL scaffold samples. The assay is based on the conversion of soluble yellow tetrazolium salt into purple insoluble formazan crystals in the presence of metabolically active cells. In this relatively easy and fast way, variously produced scaffolds can be tested for their potential biomedical application. Similar approach of PCL-based scaffold testing has been carried out recently by some other researchers [14, 15]. Apart from medicine support function of the developed electrospun scaffold, this work contributes concerning comparison of the metabolic activity of the two forth-mentioned human cell lines.

2. Experimental

2.1. Materials and methodology

Materials used in this work were PCL with Mn = 80,000 (Lach:ner), antibiotic CFU (Astro Pharm) and solvents: glacial acetic acid and acetone (Ru-Ve). 10% PCL polymer solution was prepared by dissolving the polymer in glacial acetic acid and acetone with the volume ratio of 8:2 with a constant stirring for at least 24 h. The antibiotic CFU was added with the concentrations of 0.5%, 1%, 1.5% and 2% in 100 ml of the polymer solution with further stirring and heating at 50°C. As prepared, blend polymer/antibiotic solutions were electrospun on the electrospinning device NT-ESS-300, NTSEE Co. Ltd., South Korea. The processing conditions were as follows: electrical voltage of 15–16 kV, needle tip to collector distance of 15 cm, volume flow rate of 1 ml/h and electrospinning time of 4 h. The polymer was contained in a BD plastic syringe, with 21-G blunt needle.

2.2. Characterization techniques

2.2.1. Electrospun surface structure observation

Material surface structure was observed under scanning electron microscopy (SEM) FEG QUANTA 250, FEI. The samples were coated with a thin layer of gold/palladium to provide electrical conductivity before imaging. Fiber diameters and area of the pores were measured using ImageJ by randomly selecting fibers and pores on the surface of the SEM-imaged samples.

2.2.2. Thermal property analysis

The thermal properties of the electrospun materials were evaluated using differential scanning calorimetry (DSC, Mettler Toledo DSC 822e). The samples (about 9–10 mg) were first heated from room temperature to 100°C (first run) under nitrogen stream, at a standard heating/cooling rate of 10°C min^{-1}, annealed at 100°C for 5 min to erase thermal history. After heating, cooling was done using liquid nitrogen from100°C to −100°C at 10°C min^{-1} and then second heating from −100°C to 100°C. Measurement was conducted in a nitrogen stream at a flow rate of 40 ml/min. The DSC curves were used to determine characteristic transitional temperatures and heat enthalpies.

2.2.3. Tensile testing

Materials tensile tests were conducted on dynamometer (Statimat M, Textechno). The test conditions were a maximal force of 100 N, a grip distance of 75 mm and a test speed of 25 mm/min. The samples were cut in 10 mm x 100 mm and tested in triplicates.

2.2.4. Cell seeding

Two standardly used human cell lines were applied in this work for PCL scaffold biocompatibility testing: HeLa and Hep G2. Both cell lines are of epithelial type and have originated from human cancerous tissue, cervix and liver, respectively. The cell inoculum for scaffold seeding was produced in Petri dish with DMEM culture medium and 10% fetal bovine serum, incubated at 37°C and humidified atmosphere composed of 95% air and 5% CO_2. Before cell seeding, the produced scaffold sheet was cut into circular-shaped discs that were embedded in wells of 24-well plate. The scaffold sheets were cleaned there and sterilized by soaking in 70% ethanol and exposed to UV light under laminar-flow hood for 60 min. The ethanol was removed from the plate, and discs were washed twice in culture medium with serum. The cell inoculum was harvested form Petri dish using 0.25% trypsin (Sigma) and set to concentration of 100,000 cells/ml. Into each well, 0.5 ml of cell inoculum was added. The plate was left static inside the incubator for cells to attach. After 6 h, the culture medium with unattached cells was replaced with the fresh one. The plate was incubated inside the incubator for the next 72 h. After 24- and 72- h postseeding, three discs of both types (PCL and PCL+CFU) were taken out and underwent standard MTT assay [16]. The discs were subsequently visually inspected for the development of purple color. The control discs were not seeded with cells but were incubated in culture media and MTT assay treated.

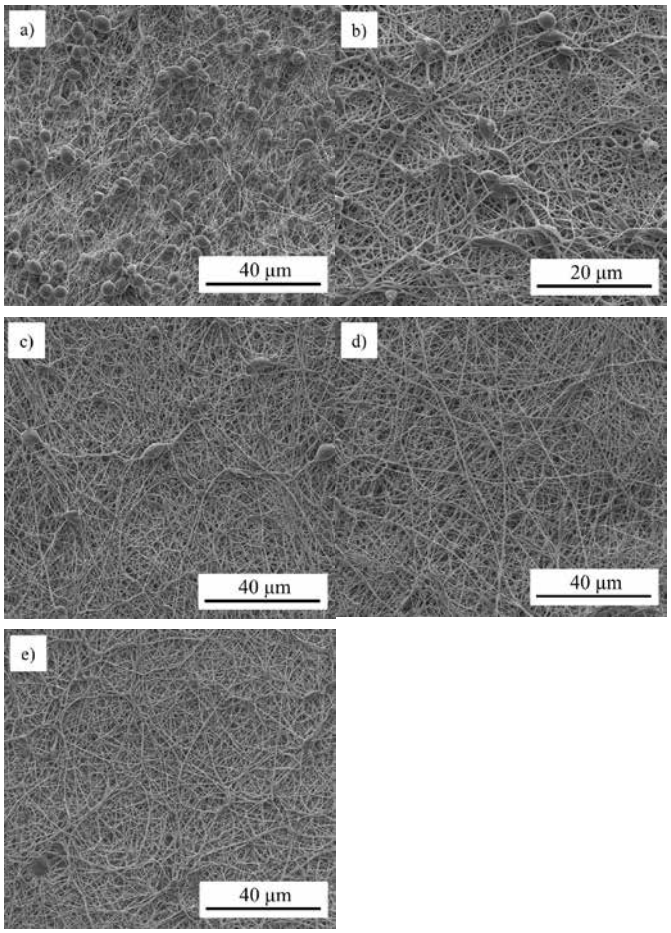

Figure 2. SEM images of the electrospun scaffolds: (a) neat PCL, (b) PCL/0.5% CFU, (c) PCL/1% CFU, (d) PCL/1.5% CFU and (e) PCL/2% CFU.

3. Results and discussion

3.1. Scaffold morphology depending on the added antibiotic

In scaffold design, one of the first factors affecting cell adhesion is their surface and thus fiber morphology and pore sizes. These two parameters need to be in compliance with seeded cell sizes to enable their adhesion and further migration inside the scaffolds. Figure 2 shows the SEM images of the electrospun neat PCL and blended PCL/CFU scaffolds.

The scaffolds were successfully electrospun at all antibiotic concentrations, and thus fibers were collected for the neat PCL as well as PCL with 0.5%–2% added CFU. Generally, the observed SEM images showed cylindrical fibers with smooth surfaces. Some fibers also showed deformations or beads along their lengths with spherical shapes and stretched or elliptical shapes. The number of random spherical beads was the highest for the neat PCL (Figure 2a). The increase in the antibiotic concentration reduced the number of beads and resulted in bead elongation or even disappearance in the case of the PCL/2% CFU scaffolds (Figure 2e).

Figure 3a and b shows the distribution of the fiber diameter and pore area. Generally, most of the fiber diameters were distributed between 100 nm and 700 nm with few fibers having diameters up to 1.6 μm. The increase in the CFU concentration reduced the fibers distribution, and thus the mean fiber diameter reduced gradually with the increase in the antibiotic concentration, i.e., from 750 nm (neat PCL) to 300 nm (PCL/2% CFU). A study reported on the electrospun poly(L-lactic acid) (PLLA)/riluzole fibers showed that there was a decrease in fiber diameter, which is due to the fact that the diameter was dependent on the drug small molecules prior to the solution concentration. Thus, electrospinning is affected by both drug chemical properties and drug size (hydrodynamic volume) [17]. In contrast, the distributions of the area of the pores mostly overlapped for all electrospun PCL and PCL/CFU scaffolds, and generally, the area of the pores was in the range between 1 μm^2 and 7 μm^2. Some pores were measured to have areas above 8 μm^2 to almost 14 μm^2. Finally, it was concluded that the addition of the CFU did not affect the changes in the area of the pores, which means that the area of the pores measured was all between 3 μm^2 and 4 μm^2.

3.2. Scaffold thermal behavior depending on the added antibiotic

The DSC curves during heating and cooling of the electrospun neat PCL and PCL/CFU scaffolds are shown in Figure 4a and b, respectively. Table 1 shows the characteristic temperatures (melting temperature [T_m] and crystallization temperature [T_c]) as well as enthalpy of melting (ΔH_m) and enthalpy of crystallization (ΔH_c).

PCL as a semicrystalline polymer contains amorphous and crystalline phases, and depending on the parameters in the electrospinning process, the level of these phases will change with the electrostatic forces during electrospinning that affect

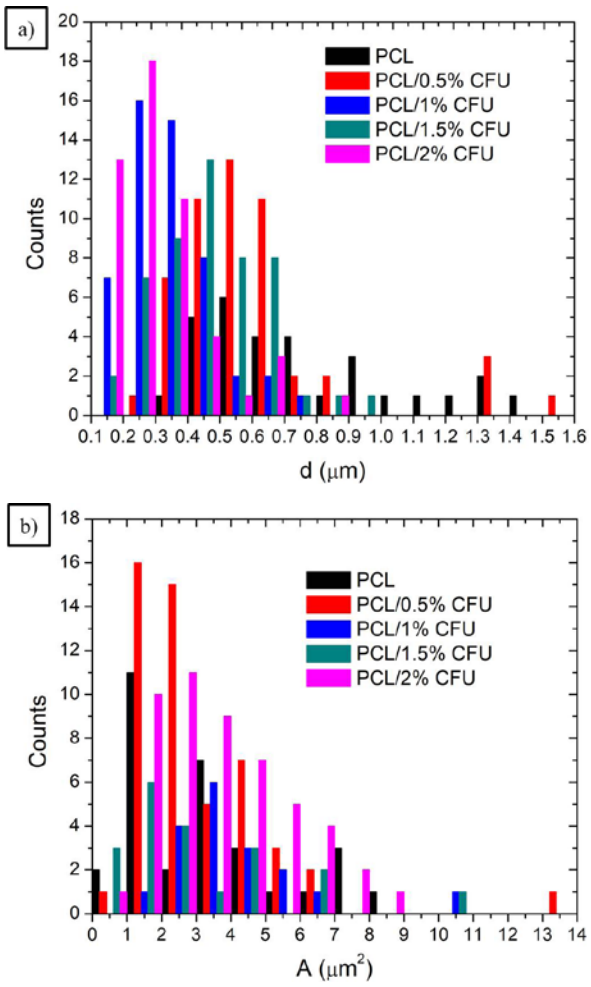

Figure 3. (a) Fiber diameter and (b) area of the pores distribution of the electrospun neat PCL and PCL/CFU scaffolds.

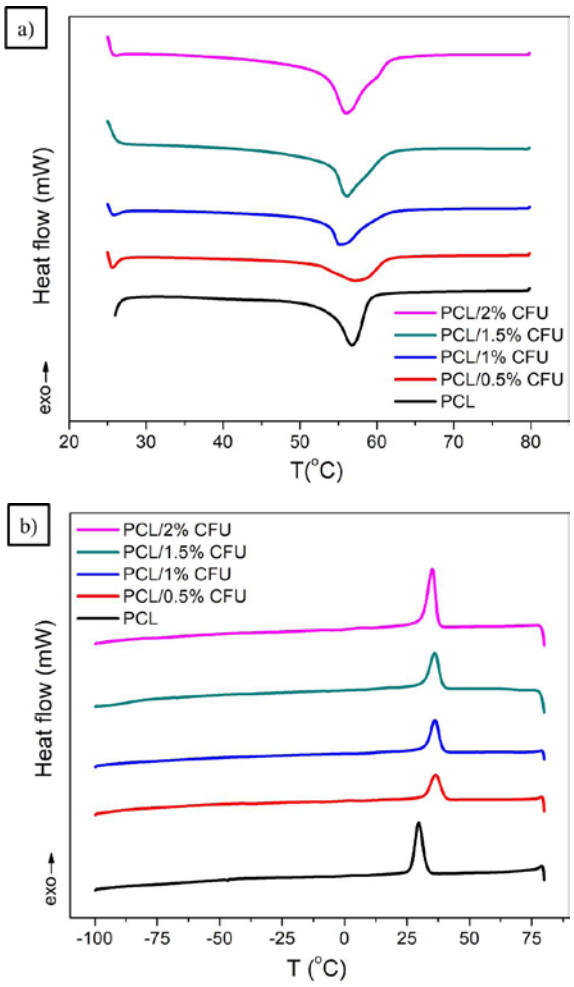

Figure 4. (a) DSC curves of the electrospun PCL and PCL/CFU scaffolds during melting and (b) crystallization.

the degree of stretching of the PCL fibers. Similarly, a study reported on the effect of the electrical voltage change at different nozzle tip to collector distances on the degree of crystallinity of the electrospun PLLA fibers [18]. The addition of the antibiotic CFU in the electrospun PCL generally resulted in very small (<1°C) change in the melting temperature (T_m) of the PCL crystalline phase, with an exception for the PCL/1% CFU where the decrease in T_m was from 56.78°C to 53.00°C. Similarly, no changes in the T_m were reported when dexamethasone was encapsulated in PCL electrospun fibers [19].

This suggests that the highest decrease in the order of the PCL crystalline structure was achieved at 1% of the added antibiotic. In contrast, the higher shift (increase) in the crystallization temperature (T_c) was observed for all antibiotic concentrations with no significant changes when the antibiotic concentration increased. This increase in T_c suggested on the faster crystallization with the addition of antibiotic CFU.

Figure 5 gives the heating DSC curve of the antibiotic CFU where the T_m of the antibiotic was observed at 174.66°C. In the electrospun PCL/CFU scaffolds, no peak appeared around the T_m of the antibiotic, and thus the heating was conducted up to 80°C. This suggests that the antibiotic is fully miscible with the polymer as confirmed by the single endothermic peak observed.

The addition of the antibiotic has significantly affected the scaffold enthalpies; although there was no regular trend with regard to the increased CFU content, the presence of the antibiotic has decreased the melting ΔH_m and crystallization ΔH_c enthalpies of the scaffolds. The highest decrease in the

Table 1. Temperatures and enthalpies of melting and crystallization

Electrospun scaffolds	T_m (°C)	T_c (°C)	ΔH_m (J/g)	ΔH_c (J/g)
PCL	56.78	29.72	84.29	72.36
PCL/0.5% CFU	57.30	36.37	47.84	40.33
PCL/1% CFU	53.00	36.21	63.57	50.84
PCL/1.5% CFU	56.10	36.22	73.84	46.46
PCL/2% CFU	56.09	35.07	58.26	42.94

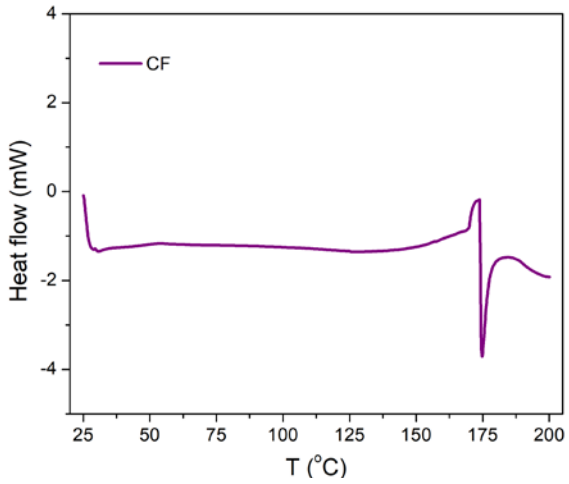

Figure 5. DSC curve of the cefuroxime during melting.

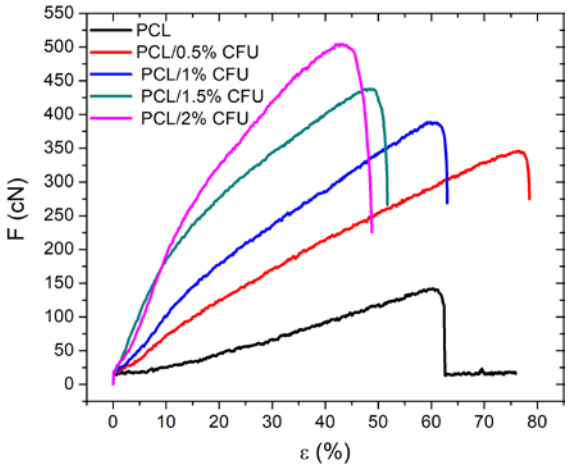

Figure 6. Tensile test behavior of the electrospun PCL/CFU scaffolds.

enthalpies was observed for the lowest CFU content, i.e., from 84.29 Jg^{-1} to 47.84 Jg^{-1} and from 72.36 Jg^{-1} to 40.33 Jg^{-1} for melting and crystallization, respectively. The lowest ΔH_m measured indicates highest reduction in the crystalline phase of the PCL. As the content of the CFU did not show a trend in the enthalpy change, this suggests that the distribution of the antibiotic in the polymer fibers differs with regard to homogeneity.

3.2.1. Scaffold tensile behavior depending on the added antibiotic

Figure 6 shows the tensile test behavior of the electrospun PCL and PCL/CFU scaffolds depending on the added antibiotic. Table 2 lists the break force, break strain and break stress of the electrospun PCL/CFU scaffolds. The force–strain curves of the electrospun scaffolds showed small breaks before materials final collapse due to the fiber slippage during each test. The mechanical integrity of the electrospun PCL/CFU scaffolds is very important for the scaffolds to withstand acting forces during cell growth or tissue formation. The results showed that the increase in the antibiotic concentration required higher force for the final collapse of materials. Highest break force was measured for the electrospun PCL/2% CFU scaffolds of 505 cN, while the neat electrospun PCL scaffolds collapsed at 117 cN. The addition of the antibiotic also increased the break strength, with the highest of 6.824 MPa for the PCL/1% CFU scaffolds or eight times higher than the break strength of the neat PCL.

The strain change showed an opposite trend, and thus the increase in the antibiotic after the concentration of 0.5% CFU has reduced the scaffold strain, down to 42.72%. The irregular trend of change in the break strain and break strength suggests that the encapsulation of the antibiotic inside the fibers may vary with regard to homogeneous CFU distribution along the fibrous structure. In a study of electrospun PCL/PLGA (20/80), fibers loaded with a small molecule hydrophilic drug, tenofovir, the modulus and the tensile strength were not affected by the drug content up to 40 wt% of the drug loaded [20].

Table 2. Break force, break strain and break stress of the electrospun PCL/CFU scaffolds

Electrospun scaffolds	F (cN)	ε(%)	σ(MPa)
PCL	117	64.80	0.842
PCL/0.5% CFU	346	76.27	3.932
PCL/1% CFU	389	59.31	6.824
PCL/1.5% CFU	438	48.43	5.763
PCL/2% CFU	505	42.72	3.389

3.2.2. Comparison of the growth of two human cell lines

Figure 7 shows PCL scaffolds seeded with two cell lines, HeLa (a) and Hep G2 (b) and a control disc without cells (c). All scaffolds are treated with MTT assay, and the resulting purple color indicates cell density and cell viability. Evidently both cell lines were successfully attached to the scaffold surface. However, HeLa cells showed somewhat better metabolic activity and faster growth rate and hence the development of more intense coloration. The seeding cell density was enough to cover most of the disc surface within 72 h.

Therefore, the cells did not have much of leftover surface available to spread, and thus the increase of viable cell quantity over 72 h was not significant (i.e., not visually detectable). The presence of antibiotic CFU in scaffold composition expectedly did not affect cell attachment and survival. All these facts show that PCL scaffolds produced in aforementioned manner are compatible for growth of human cells and can be easily considered for further tests to improve their applicability in tissue repairment.

4. Conclusion

In this work, the preparation of electrospun PCL scaffolds incorporating antibiotic CFU to be used for human cell growth was investigated. The effect of the CFU content on the scaffolds properties was evaluated through scaffold morphology and thermal and mechanical properties. It was noted that the increase in the CFU content resulted in fiber

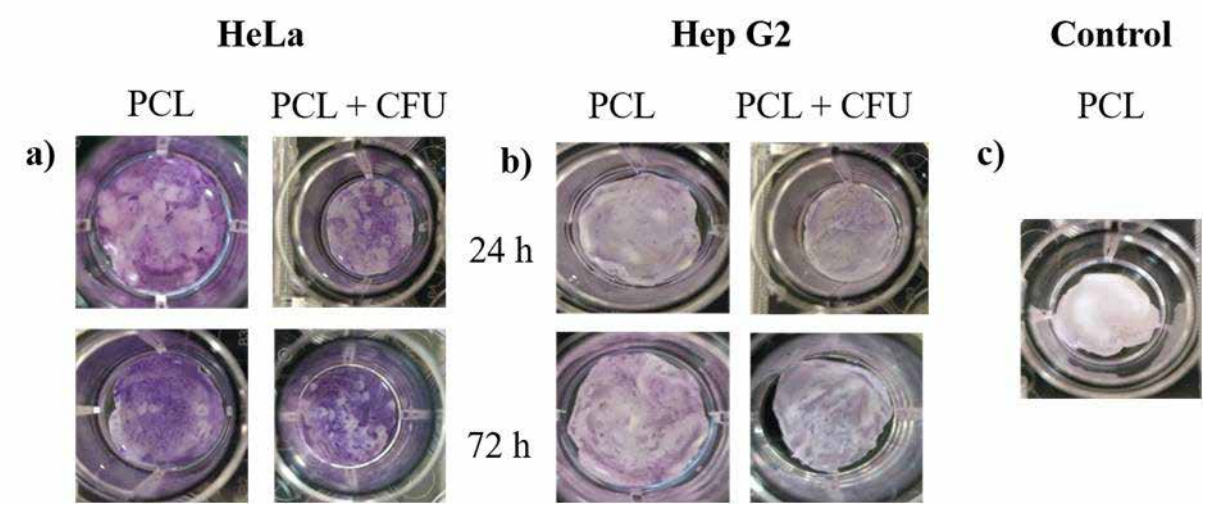

Figure 7. Testing PCL scaffolds with and without CFU for human cell growth. Two human cell lines were seeded on scaffold surface: (a) HeLa and (b) Hep G2. A scaffold without cells was used as (c) a control specimen. Standard MTT assay was applied for visual detection of cell viability and density 24 and 72 h after the seeding.

beads disappearance, fiber diameter distribution reduction, and thus fiber mean diameter decreases from ~750 nm (neat PCL) to ~300 nm (PCL/2% CFU), while in the case of area of the pores, no influence on the same was noted (area of the pores remained between 3 μm^2 and 4 μm^2). With regard to scaffold thermal properties, the addition of the CFU confirmed miscibility of the antibiotic and the polymer, and the reduction of the enthalpy of melting to almost half of the initial value suggested reduction of the fiber crystalline phase. The addition of the CFU improved scaffold break strength, with the highest measured of 6.824 MPa for the electrospun PCL/1% CFU scaffolds. This suggests that the mechanical integrity of the scaffolds is not compromised by the addition of the antibiotic, thus will withstand the forces acting on it during tissue growth. The model cells used, HeLa and Hep G2, were both supported with regard to cell adhesion and spreading, and thus the PCL/CFU scaffolds provided compatibility for human cells growth, with precedence in greater colonization of the HeLa cells. This model will be further used for the design of scaffolds for tissue repair.

Acknowledgements

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