Original Article

Fabrication and *In Vitro* Evaluation of A Chondroitin Sulphate-Polycaprolactone Composite Nanofibrous Scaffold for Potential Use in Dermal Tissue Engineering

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Abstract

Objective: Poly(ε-caprolactone) (PCL) has considerable mechanical and biological properties that make it a good candidate for tissue engineering applications. PCL alongside proteins and polysaccharides, like gelatin (GEL) and chondroitin sulphate (CS), can be used to fabricate composite scaffolds that provide mechanical and biological requirements for skin tissue engineering scaffolds. The aim of this study was fabricating novel composite nanofibrous scaffold containing various ratios of GEL/CS and PCL using co-electrospinning process.

Materials and Methods: In this experimental study, PCL mixed with a GEL/CS blend has limitations in miscibility and the lack of a common solvent. Here, we electrospun PCL and GEL/CS coincide separately on the same drum by using different nozzles to create composite nanofibrous scaffolds with different ratios (2:1, 1:1 and 1:2) of GEL to CS-PCL, and we mixed them at the micro/nanoscale. Morphology, porosity, phosphate-buffered saline (PBS) absorption, chemical structure, mechanical property and *in vitro* bioactivity of the prepared composite scaffolds were analysed.

Results: Scanning electron microscopy (SEM) images showed beadless nanofibres at all ratios of GEL to CS-PCL. The composite scaffolds (2:1, 1:1 and 1:2) had increased porosity compared to the PCL nanofibrous scaffolds, in addition to a significant increase in PBS absorption. The mechanical properties of the composite scaffolds were investigated under different conditions. The results demonstrated that all of the composite specimens had better strength when compared with the GEL/CS nanofibres. The increase in PCL ratio led to an increase in tensile strength of the nanofibres. Human dermal fibroblasts (HDF) were cultured on the fabricated composite scaffolds and evaluated by 3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) analysis and SEM. The results showed the bioactivity of these nanofibres and the potential for these scaffolds to be used for skin tissue engineering applications.

Conclusion: The fabricated co-electrospun composite scaffolds had higher porosity and PBS absorption in comparison with the PCL nanofibrous scaffolds, in addition to significant improvements in mechanical properties under wet and dry conditions compared to the GEL/CS scaffold.

Keywords: Chondroitin Sulphate, Co-electrospinning, Nanofibres, Polycaprolactone, Tissue Engineering

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Introduction

Polymeric nanofibrous scaffolds simulate the native extracellular matrix (ECM) in skin tissue and offer compelling advantages (1, 2). Selection of scaffold architecture is important because the dimensions of the ECM protein fibre (collagen) are smaller than the cell dimensions, and they can easily contact the cells directly in three dimensions. The micro-environmental features of a tissue-engineered scaffold should be considered in order to restore tissue function and exchange proper signals between cells and other cells or environments (3). Therefore, preparation of nanoscale fibres that resemble the environmental conditions of a tissue could be of benefit when developing tissue-engineered scaffolds that have nanoscale structures (4-6).

Electrospinning is a flexible, reliable method to produce nanofibres that have various applications for tissue engineering (7). Both natural and synthetic polymers are good candidates for production of electrospun nanofibres. Natural polymers have good biocompatibility and biological properties because their structure mimics native cellular environments; however, they often have low mechanical properties when compared to synthetic polymers (8, 9). Therefore, the combination of natural and synthetic polymers is considered a promising option when preparing nanofibrous scaffolds for tissue engineering applications. Access to a range of polymers could be beneficial for improving special properties such as mechanical or biological features; however, there are limitations. For example, natural polymers mostly have

polar structures and are easily soluble in polar solvents like water, whereas synthetic hydrophobic polymers are soluble in nonpolar organic solvents. Finding a common solvent is difficult; otherwise, researchers must use toxic or aggressive solvents (10). Poly(ε-caprolactone) (PCL)/chitosan blends, for instance, are dissolved in trifluoroethanol (TFE), hexafluoro-2-propanol and trifluoroacetic acid (11-13). In order to overcome these solvent problems, natural and synthetic polymers can be fabricated separately on the same collector by a co-electrospinning process.

Widespread use of GAGs and gelatin (GEL) for preparation of scaffolds is an effective approach used to replace natural ECM components (14, 15). GEL is derived from collagen and can improve the wound healing process by inducing cell migration, adhesion, growth and organization (16, 17). Chondroitin sulphate (CS) plays an important role in tissue regeneration and wound healing, and is one of the main components of the ECM. CS supports cell proliferation, attachment and migration. Therefore, GEL/CS is a suitable biomaterial for tissue engineering and wound healing applications, and its nanofibrous structure has tremendous potential to mimic the ECM in tissue engineering (18, 19). The results of different studies show that both mechanical properties and cellular activities of proteins, like collagen and GEL, can be improved by incorporation of polysaccharides such as CS or chitosan (15, 20). Furthermore, PCL is a biodegradable and a biocompatible material that has noticeable mechanical properties and spinnability. PCL has been used to fabricate nanofibrous scaffolds by electrospinning (21, 22). There are many reports about the use of natural polymeric nanofibres for tissue engineering applications; however, low mechanical performance, particularly under wet conditions, has limited their clinical applications. Skin is usually subjected to tensile stresses. Moreover, the scaffolds used as substitutes for skin tissue regeneration have high area/thickness ratios; therefore, the tensile mechanical performances of scaffolds are important factors that can influence both the clinical operations and wound healing processes (8, 9, 16). The co-electrospining process can be a promising technique for fabrication of nanoscale/microscale composite scaffolds that have enhanced physical properties. In this study, we intend to fabricated a new composite nanofibrous scaffold that contains various ratios of GEL/CS and PCL by co-electrospinning. The fabricated composite nanofibrous scaffolds were crosslinked using N-(3-dimethylaminopropyl)-N'-ethyl carbodiimide hydrochloride (EDC) as a zero-length crosslinker. The effect of PCL ratio on physical, chemical and biological properties of these composite scaffolds was investigated. To the best of our knowledge, there is no published study about composite scaffolds that contain CS and PCL, and crosslinked using EDC for skin tissue engineering applications.

Materials and Methods

Fabrication of electrospun nanofibres

In this experimental study, we prepared a PCL solution (MW: 80 000, Sigma Aldrich, USA) in a co-solvent system of formic/acetic acid (9:1 ratio, Merck, Germany) with a concentration of 12.5% (w/v) for fabrication of the PCL. The GEL solution that contained CS was prepared as previously reported (20, 23). Briefly, GEL type B (Sigma Aldrich, USA) and CS type A from bovine trachea (Sigma Aldrich, USA) blend solutions were prepared in a solvent system of TFE: water (equal volume ratio, Merck, Germany) and mixed overnight at room temperature in order to have a homogeneous blend solution with a GEL/CS ratio of 85:15. We prepared five samples that consisted of neat PCL, GEL/CS and three different composite nanofibres (GEL/CS-PCL). The composite scaffolds consisted of the following ratios: one syringe of GEL/CS to one syringe of PCL (1:1); two syringes of GEL/CS to one syringe of PCL (2:1); and one syringe of GEL/CS to two syringes of PCL (1:2). The prepared solutions were subjected to electrospinning using a 5 ml syringe and a cylindrical collector covered with aluminum foil in a horizontal system (Co881007 NYI, ANSTCO, Iran). The electrospinning process was performed at a process condition of 0.6 ml/h flow rate and 19 kV applied voltage for the GEL/CS samples. PCL was electrospun at a 0.9 ml/h flow rate and 25 kV applied voltage. The collector was placed 100 mm of the needle tips. Table 1 summaries the compositions of the electrospun scaffolds.

Table 1: Different combinations of GEL/CS- PCL composite electrospun scaffolds

Scaffold	Number of nozzles and feeding rate (ml/h) for GEL-CS	Number of nozzles and feeding rate (ml/h) for PCL
GEL/CS	1 (0.6 ml/h)	0 (0.9 ml/h)
GEL/CS-PCL (2:1)	2 (0.6 ml/h)	1 (0.9 ml/h)
GEL/CS-PCL (1:1)	1 (0.6 ml/h)	1 (0.9 ml/h)
GEL/CS-/PCL (1:2)	1 (0.6 ml/h)	2 (0.9 ml/h)
PCL	0 (0.6 ml/h)	1 (0.9 ml/h)

PCL; Poly(ε-caprolactone), GEL/CS; Gelatin/chondroitin sulphate, and h; Hour.

Crosslinking and sterilization

The chemical crosslinking agent to the nanofibrous scaffolds consisted of 0.02 g EDC (Merck, Germany) in which 10 ml ethanol (Merck, Germany) was added for 24 hours, followed by sterilization in 70% ethanol for 4 hours. The scaffolds were washed several times in phosphate-buffered saline (PBS) to remove any residual ethanol.

Morphology of the fibres

A scanning electron microscope (VEGA, TESCAN, Czech), at an operating voltage of 15 kV, was used to investigate the morphology of the GEL/CS-PCL electrospun nanofibres. The samples were mounted on aluminum stubs and coated with a thin layer of gold.

Measurement of porosity and phosphate-buffered saline absorption

Dry scaffolds (W_d) were plunged in ethanol for two hours and the weights of the samples in ethanol were noted as W_l. The ethanol was removed from the surface of scaffolds by a filter paper and the wet scaffold weights were measured as W_w. The porosity of the electrospun nanofibres was calculated by following formula (8, 24):

$$\text{Porosity } (\%)=(W_w-W_d)/(W_w-W_l)\times 100$$

The PBS solution absorbed of prepared composite nanofibres was calculated by placing the crosslinked electrospun composite scaffolds in PBS (pH=7.4) at 37˚C for 24 hours as reported previously (24, 25):

$$\text{PBS absorption } (\%)=(w_1- w_0)/w_0\times 100$$

Where: w_0 and w_1 are the weights of the scaffolds before and after soaking in PBS, respectively. The values are shown as the mean ± standard error (n=3).

Mechanical properties

Mechanical performance of the prepared composite nanofibres was analysed as previously reported using a mechanical tester STM-20 (SANTAM, Iran) and rectangular shape (3×1 cm^2) with an approximate thickness of 50 µm under a 10 mm/min deformation rate at room temperature (8, 26, 27). The tensile strength and elongation at the break of the composite scaffolds were expressed as the mean ± standard error (n=3). The tensile strength of samples was investigated under three conditions: non-crosslinked (as spun) dry state, crosslinked dry state and wet state. For the wet conditions, the nanofibres were immersed in 50 ml of PBS (pH=7.4) for two hours at room temperature.

Fourier transform infrared analysis

The GEL/CS-PCL nanofibrous scaffolds were analysed by infrared spectroscopy to identify their chemical structures (EQUINOX 55, Bruker, Germany). Fourier transform infrared (FTIR) spectra were scanned in the spectral range of 400-4000 cm^{-1}.

Cell culture study

Human dermal fibroblasts (HDF) were purchased from Royan Cell Bank Services (code no: RSCB0179, Ethics Code: IR.IUMS.REC.1396.32153). The medium consisted of DMEM/F12 (Gibco, Canada) with 1% L-glutamine (Gibco, Canada), 10% foetal bovine serum (FBS, Gibco, Canada) and 1% penicillin/streptomycin (Gibco, Canada). A flask tissue culture (T-75) was used to culture the HDF. In all of the experiments, we used cells that were between four and six passages. The cells were maintained at 37˚C, 5% CO_2 and 95% humidity. The culture medium was refreshed every two days. Prior to cell seeding, 0.05% trypsin/EDTA (Gibco, Canada) was used to dissociate the cells, which were subsequently centrifuged and resuspended in medium.

Analysis of cell proliferation and morphology on the composite scaffolds

The scaffolds were sterilized and the samples were incubated overnight in culture medium. The HDF were resuspended in culture medium (1×10^4 cells/cm^2) with 10% FBS which loaded on the scaffolds in 24-well culture plates. The medium was changed every two days. All experiments were performed in triplicate. 4′-6-diamidino-2-phenylindole (DAPI, Sigma Aldrich, USA) staining, 3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and scanning electron microscopy (SEM) were used to analyse the samples. The MTS assay is a standard method to evaluate cell proliferation and metabolic activity on scaffolds. The MTS (Promega, G5421) assay was performed according to the manufacturer's instructions. Briefly, HDF cells were seeded on the scaffolds in medium, at a density of 1×10^4 cells/cm^2 (n=3). The medium was replaced every two days. At 1, 3, 7 and 14 days after seeding, we added the MTS solution to each well. Then, the plates were incubated in the dark at 37°C for three hours. Absorption of the solution was measured at 490 nm.

On days 1 and 7, the samples were fixed with 10% formaldehyde for two hours, stained with DAPI and washed with PBS in order to assess the amount of cells that adhered to the nanofibres and to visualise their nuclei. The images were taken using a fluorescence microscope (Olympus, IX71, Japan) to determine the location and distribution of the cell nuclei.

SEM was used to study the morphological characteristics of the cells cultured on the nanofibres. The samples were cultured for one and seven days, and then the scaffolds were harvested. The samples were washed with PBS and fixed overnight with 2.5% glutaraldehyde at 4˚C to remove any non-adherent cells. These samples were then dehydrated by a graded series of alcohol (30, 50, 70, 80, 90, 96 and 100%) and subsequently vacuumed-dried overnight. The scaffolds were coated with gold and observed by SEM at 15 kV.

Statistical analysis

All of the experimental data are presented as mean ± standard error. Statistical analysis for elucidation of differences in the measured properties between the groups was accomplished using one-way analysis of variance (ANOVA) in SPSS 16.0 software (America, IBM) followed by Tukey's HSD post hoc test.

Results

Morphology of the nanofibres

Figure 1 shows the SEM micrographs of the electrospun GEL/CS, PCL and GEL/CS-PCL composite scaffolds with different ratios of PCL. Nanofibrous structures were attained in all of the scaffolds and no beads were observed.

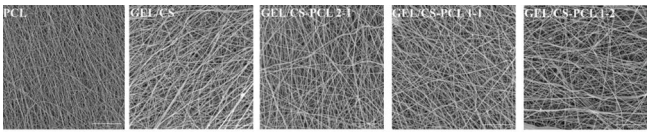

Fig.1: SEM micrographs of the PCL, GEL/CS and composite scaffolds with different rations of PCL (GEL/CS-PCL 2:1, GEL/CS-PCL 1:1 and GEL/CS-PCL 1:2). SEM; Scanning electron microscopy, PCL; Poly(ε-caprolactone), and GEL/CS; Gelatin/chondroitin sulphate (scale bar: 10 µm).

Porosity and phosphate-buffered saline absorption

Figure 2A shows the physical and chemical properties of the PCL, GEL/CS and GEL/CS-PCL hybrid nanofibrous scaffolds. The GEL/CS nanofibrous scaffolds had the highest value (96 ± 0.5%), whereas the PCL scaffold was 82 ± 2%. Co-electrospining with different ratios of PCL and GEL/CS (2:1, 1:1 and 1:2) led to an increase in porosity compared to pure PCL.

PBS absorption of all specimens was performed to evaluate the exudate drainage ability of the nanofibrous scaffolds (Fig.2B). It is important that wound exudate can be steadily captured by the scaffold during the dermis regeneration process. Therefore, one of the effective dermal scaffold properties should be the ability to have high water absorption, which helps with better cell activity.

Hydrophilicity of the scaffold's components (e.g., GEL) can help to absorb the wound exudates (28). As shown in Figure 2, the GEL/CS scaffold had the highest absorption (around 900 ± 100%) compared to the composite scaffolds. By taking into consideration the higher feeding rate of PCL, in comparison with GEL/CS, the composite GEL/CS-PCL 1:2 scaffold displayed the lowest PBS absorption among the co-electrospun samples because of the high mass ratio of PCL and hydrophobicity of the final composite.

Mechanical properties

Figure 2C, D show tensile strength and elongation at the break of the composite scaffolds. The GEL/CS based hybrid scaffolds with different concentrations of PCL were fabricated in three ratios (2:1, 1:1, 1:2). Mechanical performance of the prepared composite scaffolds was analysed under three different conditions: spun dry state, crosslinked dry state, and crosslinked wet state. Figure 2C shows that pure PCL has significant mechanical properties compared to pure GEL/CS; therefore, by increasing the proportion of PCL in the composite, the amount of stress needed to break the sample increased for the specimens under all conditions. The highest level was observed in the GEL/CS-PCL 1:2 in the crosslinked dry state. Furthermore, we observed that the composite scaffolds containing PCL fibres, had higher elongation at break and more capablity to resist rupturing (Fig.2D).

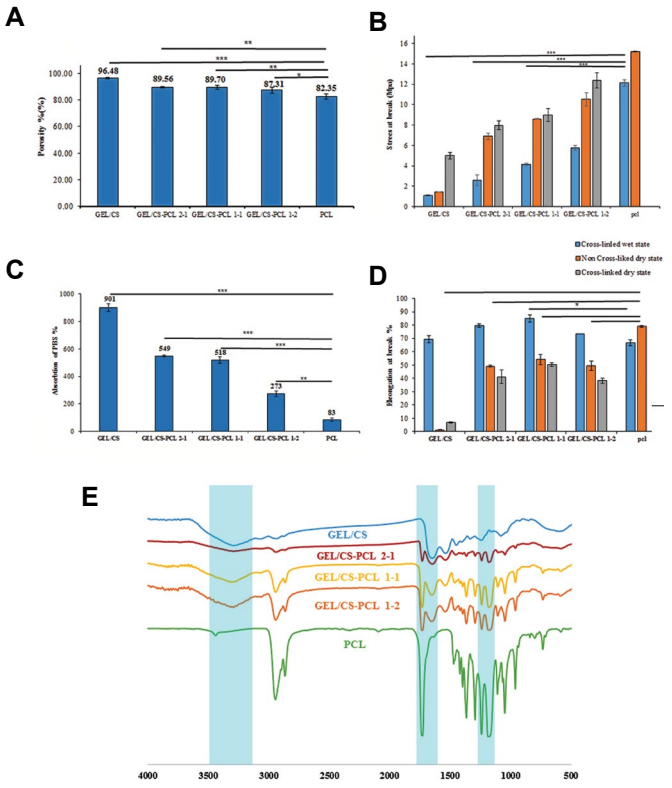

Fig.2: Physical and chemical properties of electrospun PCL, GEL/CS and composite scaffolds with different ratios of PCL (GEL/CS-PCL 2:1, GEL/CS-PCL 1:1 and GEL/CS-PCL 1:2). The values are presented ase mean ± standard error (n=3). **A.** Porosity analysis, **B.** Swelling ratio, **C, D.** Mechanical analysis and **E.** FTIR spectra (the X-axis of the spectrum is the wavenumber [cm^{-1}]). *; P<0.05, **; P<0.01, ***; P<0.001, ****; P<0.0001 indicate statistical significance, PCL; Poly(ε-caprolactone), GEL/CS; Gelatin/chondroitin sulphate, and FTIR; Fourier transform infrared spectra.

Fourier transform infrared analysis

Chemical structure analysis of the specimens was carried out by FTIR at 400-4000 cm^{-1} (Fig.2E). Carbonyl stretching of C=O is the dominant peak of PCL (1727 cm^{-1}). Moreover, peaks at 2942 and 2865 cm^{-1} are ascribed to C-H stretching vibration. The C=O and C-C had stretching peaks at 1293 cm^{-1} and 1240 cm^{-1}, respectively, while symmetric C-O-C stretching peaked at 1170 cm^{-1} (29). Because GEL/CS has alcoholic groups in its structure, 3460 cm^{-1} was the stretching vibration of OH. The C-O-C stretching vibration at 1070 cm^{-1} was related to the saccharide structure of GEL/CS. The peaks at 1430, 1400 and 1427 cm^{-1} resulted from coupling C-O stretching and O-H variable angle and the S=O stretching vibration (SO4-groups of GEL/CS), respectively. Other peaks at 750 cm^{-1}, 860 cm^{-1} and 940 cm^{-1} were related to C-O-S vibration (30-32).

In vitro cell adhesion and proliferation

In order to assess the biocompatibility of these GEL/CS-PCL scaffolds, we investigated proliferation, distribution

and adhesion of the HDF onto the composite nanofibres. Figure 3 shows the DAPI staining results. The cell nuclei remained intact during seven days of cell culture.

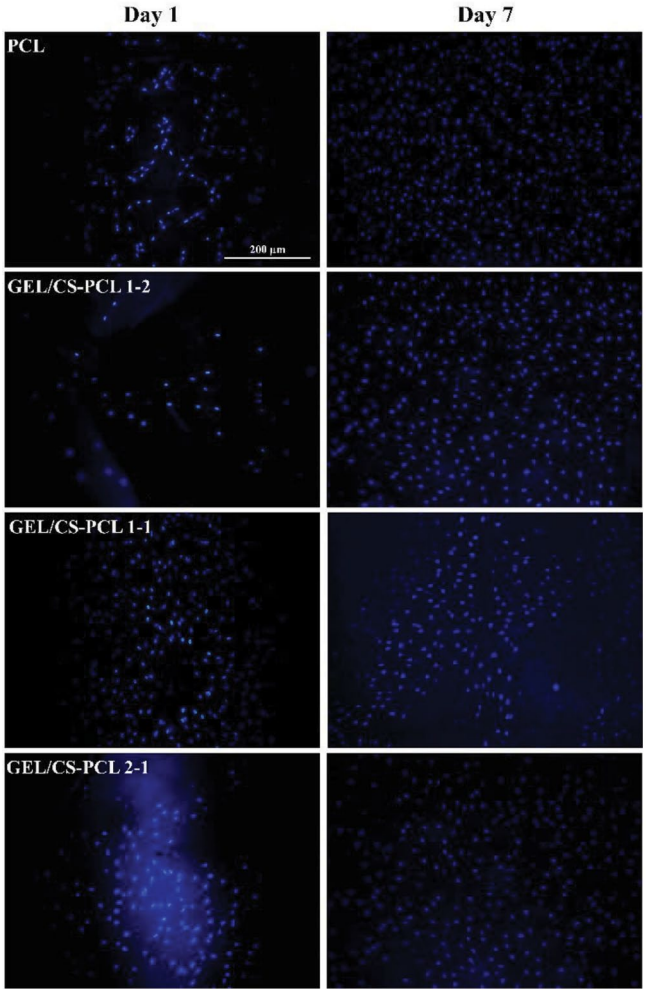

Fig.3: DAPI staining of HDF cells on electrospun PCL and composite scaffolds (GEL/CS-PCL 2:1, GEL/CS-PCL 1:1 and GEL/CS-PCL 1:2) after one and seven days of cell culture (scale bar: 200 µm for all DAPI images). DAPI; 4'-6-diamidino-2-phenylindole, PCL; Poly(ε-caprolactone), GEL/CS; Gelatin/chondroitin sulphate, and HDF; Human dermal fibroblasts.

Suitable cell attachment and proliferative behaviour were seen for all composite scaffolds. SEM analysis was performed on days 1 and 7 to obtain adequate precision in assessing cell adhesion and distribution, and their interactions with the composite scaffolds. Figure 4 depicts the different magnifications of the SEM results for the HDF cells cultured on prepared composite scaffolds that contained different ratios of PCL. We observed spreading and attachment of the cells on all of the composite scaffolds. DAPI staining images, by comparing the population of cells at days 1 and 7, indicated that the cells had high proliferation and proper distribution on the nanofibrous substrates. This finding confirmed the SEM results.

The results of the MTS assay for HDF cell viability for 14 days in cells cultured on scaffolds is shown in Figure 5. The absorbances of the composite GEL/CS-PCL scaffolds compared to the PCL and GEL/CS nanofibres showed that co-electrospinning these polymers improved cell proliferation during 14 days. According to the MTS assay, the best proliferation for HDF cells was noted in the GEL/CS-PCL 1:1 and 2:1 composite nanofibrous scaffolds (Fig.5). Fibroblast cells in scaffolds that contained high ratios of GEL/CS in their composition (GEL/CS-PCL 1:1 and 2:1) had accelerated adhesion and proliferation.

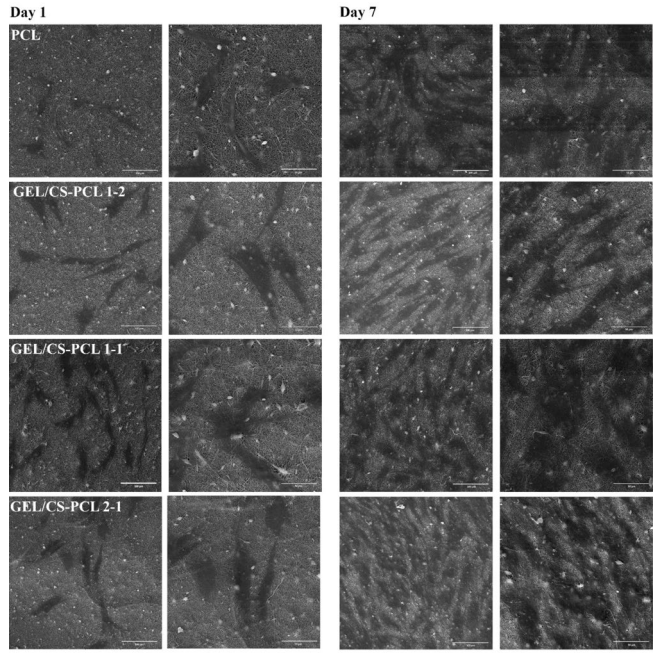

Fig.4: SEM micrographs of HDF cells on electrospun PCL and composite scaffolds (GEL/CS-PCL 2:1, GEL/CS-PCL 1:1 and GEL/CS-PCL 1:2) after one and seven days of culture visualised at diffrent magnifications [scale bar:100 µm (left) and 50 µm (right)]. SEM; Scanning electron microscopy, PCL; Poly(ε-caprolactone), GEL/CS; Gelatin/chondroitin sulphate, and HDF; Human dermal fibroblasts.

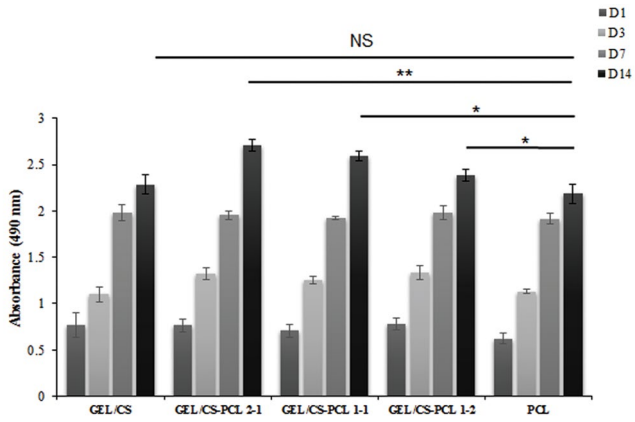

Fig.5: 3-(4,5-dimethylthiazol-2-yl)-5-(3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay after 1, 3, 7 and 14 days for PCL, GEL/CS and composite scaffolds (GEL/CS-PCL 2:1, GEL/CS-PCL 1:1 and GEL/CS-PCL 1:2). Tthe values are presented as the mean ± standard error (n=3). *; P<0.05, **; P<0.01 indicate statistical significance, NS; Not significant, PCL; Poly(ε-caprolactone), and GEL/CS; Gelatin/chondroitin sulphate.

Discussion

Co-electrospinning is a fascinating technique used to fabricate nanofibrous structures from completely different polymers and solvent systems. In this study, we took into consideration the bioactivity of a GEL/CS blend and the remarkable mechanical properties of PCL to prepare nanofibrous composite scaffolds that contained different ratios of PCL and GEL/CS by co-electrospinning. Two different solvent systems were used for the electrospinning process because PCL is a hydrophobic polymer and the GEL/CS blend are hydrophilic polymers.

In order to investigate the effect of PCL in the scaffold in terms of mechanical, chemical and biological properties, we assessed various ratios of PCL simultaneously combined with GEL/CS on one collector. In order to control the deposition of PCL and GEL/CS on the surface of the collector, three nozzles were used simultaneously. For example, in the GEL/CS-PCL 2:1 scaffold, two nozzles injected the GEL/CS solution and the third nozzle injected the PCL solution toward the collector.

SEM micrographs revealed that all of the prepared composite nanofibrous scaffolds were bead-free, and had uniform and highly porous morphologies. Scaffold porosity plays a key role in the penetration of nutrients and oxygen, waste removal, and drainage of wound exudate during dermis regeneration. Our results revealed that all of the GEL/CS- PCL composite electrospun scaffolds were similar in terms of porosity. Changing the PCL ratio, at the range used in this study, had no significant effect on composite scaffold porosity. Different ranges of porosity (between 60% and 90%) are often used for tissue engineering applications (9, 33, 34). In this study, prepared GEL/CS-PCL composite scaffolds had more than 80% porosity, which was comparable with different scaffolding methods like salt leaching, phase separation, fibre bonding, and three-dimensional printing (33, 35). Electrospun scaffolds have both high porosity and interconnected pore morphology; therefore, the efficient mass transfer of oxygen and nutrients would enable better migration and ECM formation of cells (34, 36, 37).

PBS absorption by the scaffolds was evaluated as an indication for exudate drainage ability of the composite scaffolds. Hydrophilicity of scaffold components like GEL and CS can help to absorb wound exudates (24, 28). We observed that all composite specimens were approximately equal in terms of porosity; therefore, the effect of using a hydrophilic material in the final electrospun scaffold is significant. Increasing the proportion of GEL/CS in the composite nanofibres (e.g., comparing GEL/CS-PCL 2:1 and 1:2) resulted in enhanced PBS absorption, whereas PCL, because of its hydrophobic structure, had the least PBS absorption. Co-electrospinning of GEL/CS with PCL at an equal number of nozzles (1:1) could improve PBS absorption to 524%.

Skin is usually exposed to tensile stresses; therefore, significant factors such as elongation at break and tensile strength of scaffolds for dermal regeneration applications with high area and thickness ratios can impact only wound healing and the clinical operation. PCL is a common material with high tensile strength and elongation at break, and it is used to fabricate different kinds of scaffolds. We previously demonstrated (23, 24) that GEL and CS blend nanofibres have good potential for tissue engineering applications. We added PCL and used the co-electrospinning technique to enhance the mechanical properties of our GEL/CS nanofibres. The mechanical properties of these composite scaffolds were analysed under three different conditions: spun dry state, crosslinked dry state, and crosslinked wet state. The crosslinked wet state showed the effect of hydrated conditions on the mechanical property of the scaffold and simulated physiological conditions of the body. A comparison of GEL/CS and GEL/CS-PCL 1:1 in the crosslinked wet state showed that co-electrospinning of PCL with GEL/CS at equal nozzles (1:1) improved the tensile strength to 283% and elongation at break to 23%.

Crosslinking, as well as the addition of PCL to specimens, can improve the mechanical properties of scaffolds. In the absence of PCL fibres, crosslinking plays an important role in enhancing mechanical properties. Stress at break increased in the pure GEL/CS from 1.44 ± 0.02 MPa to 4.98 ± 0.31 MPa (245%) in the non-crosslinked dry state and crosslinked dry state, respectively. This boosting effect in the hybrid composition was less compared to pure GEL/CS. Under wet situations, although PBS can act as a plasticizer and lead to a longer elongation at break and lower tensile strength, compared to the dry states. Composite scaffolds that have a higher ratio of GEL/CS show less stress at break, which could be due to increased absorption of PBS. The results in this study demonstrated a significant effect of PCL in improving the GEL/CS mechanical properties under all conditions.

The biocompatibility of the composite nanofibres were evaluated using the MTS assay, SEM and DAPI staining. SEM micrographs of growing HDF cells demonstrated a good interaction that surrounded the fibres and attachment to the surface by filopodia. Cells stretched completely on the nanofibres by a spindle-like shape. The cells had a good adhesion and a characteristic spindle shape, as HDF cells, in which the scaffolds maintained the phenotype of the fibroblast cells. The SEM images also showed interconnection of neighbouring cells, which secreted filopodia. Different cellular activities were expected with the electrospun GEL/CS- PCL scaffolds that had different ratios of PCL because of the differences in chemical compositions in terms of hydrophobicity and functional groups of the natural and synthetic polymers. Absorbances of the composite GEL/CS- PCL scaffolds with PCL and GEL/CS nanofibres according to the MTS assay revealed that co-electrospinning of these polymers improved both the mechanical properties and cell proliferation during 14 days. Although HDF cell adhesion and proliferation were considerable in all specimens, both the GEL/CS-PCL 1:1 and GEL/CS-PCL 2:1 scaffolds had the best performance

for culturing HDF. Fibroblast cells in scaffolds that contain high ratios of GEL/CS in their composition have accelerated adhesion and proliferation.

Conclusion

The high mechanical performance of PCL was used to significantly improve the mechanical properties of GEL/CS nanofibrous scaffolds, as a bioactive structure for tissue engineering applications, by co-electrospinning. SEM images showed beadless nanofibrous structures for all PCL ratios. The fabricated co-electrospun composite scaffolds had better porosity and PBS absorption (524%) in comparison with the PCL nanofibrous scaffolds. Mechanical properties of the composite nanofibrous GEL/CS-PCL scaffolds were also investigated under wet and dry conditions. The results demonstrated that all composite specimens had better strength and elongation at break in comparison with GEL/CS nanofibres; increasing the PCL ratios led to increased tensile strength of the nanofibres. We compared the mechanical properties of the scaffolds in the crosslinked wet state and noted that co-electrospinning of PCL with GEL/CS at equal nozzles (1:1) improved both tensile strength and elongation at break in the GEL/CS to 283% and 23%, respectively. HDF cells cultured on fabricated composite scaffolds, along with MTS analyses and SEM micrographs, showed bioactivity of all the composite scaffolds; however, the GEL/CS-PCL 1:1 scaffold appeared to have the most potential for skin tissue engineering applications.

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Authors' Contributions

M.P.-M.; Supervision, conceptualization, and writing the original draft. M.A., D.E., A.S.; Methodology, investigation, validation, and writing the original draft. M.Z.; Design, methodology, and validation. T.G.; Methodology and validation. S.R.; Supervision, methodology, and statistical analysis. All authors performed editing and approved the final version of this manuscript for submission.

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