Journal of Fiber Bioengineering & Informatics 5:1 (2012) 33–40
http://www.jfbi.org | doi:10.3993/jfbi03201202

Study on Cell Adhesion Detection onto Biodegradable Electrospun PCL Scaffolds

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Abstract

The polycaprolactone (PCL) known for its slow biodegradability shows potential applications in the field of skin scaffold production. In the current study PCL mats with micro scaled structure were produced via electrospinning and treated with NaOH solution for enhanced cell adhesion. The scaffolds were evaluated as an efficient stem cell growing material by human skin keratinocytes and fibroblasts culture. The cell viability assay (CellTiter-Blue) of the as spun scaffolds was compared with previously prepared PU electrospun scaffolds, fibrin scaffolds and amniotic membrane scaffold. Significant difference in cell adhesion was evident between the natural and synthetic scaffolds. As far as the electrospun scaffolds were concerned the significance between, as well as for the NaOH treated, samples was less evident. The repetition of different donor cells fluorescence within the same scaffold material was also present.

Keywords: PCL; NaOH Treatment; Keratinocytes; Fibroblasts; Cell Culture

1 Introduction

Concerning tissue regeneration (e.g. human skin, or any other vital organs failure), medicine faces huge problems due to shortage of adequate transplant donors, possible recipient's immunological system rejection, with potential risk of infection, tumor development or any other unwanted side effects. From this point of view tissue engineering, as biological substrates developer that can restore and maintain the tissue function, has received impressive attention in the medical science

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and wider [1]. Synthetic biodegradable biomaterials designed as scaffolds for cell culture are already known for successful functionality and special attention in the past decades has been paid to electrospun scaffolds. The advantage of these types of scaffolds is in their architecture influencing cell attachment due to the larger surface area of the nano-scaled structure to absorb proteins that will promote more binding sites [2].

Human embryonic stem cells (hESCs) cultivated on polyurethane electrospun scaffolds showed successful attachment and neuronal differentiations. The authors reported on a significant increase of cell growth after day 5 until day 18 where the cell number stabilized [3].

In a study of electrospun PCL and PCL/PEO scaffolds, seeded human dermal fibroblast showed dependence upon the scaffold architecture. Significant influence on cell proliferation was reported by the pore size, as bigger pore sizes, obtained by PEO removal due to its solubility in water, provided cells bridging instead of aligning upon single fibers [4].

By modulating the in-plane elasticity of an electrospun scaffold by means of Cu electrodes, orienting the electrospun fibers into orthogonal directions, adult stem cells adhesion and proliferation were obtained for cardiac tissue engineering, confirming material cytocompatibility. These types of fiber oriented scaffolds are particularly promising in the engineering of skeletal muscle tissues, ligaments, blood vessels and articular cartilages, where one prefers improved tissue organization and function [5, 6]. Studies reported on the successful implementation of electrospun (via nanospider technology) copolymer PA 6/12 scaffolds for limbal (LSC) and Mesenchymal Stem Cells (MSC) growth for treatment of ocular surface injuries. The cell morphology, proliferation and metabolic activity were reported as comparable with cells grown on plastic surfaces [7].

PCL scaffolds revealed to be suitable for trilaminar, cell growth for composite tissue engineering. The study discussed simultaneous culture of fibroblasts, keratinocytes and periosteal cells for bone, tissue and periosteum reconstruct. A drawback reported of the coculture was inability to provide temporary barrier between the cell layers until separate tissue type organization [8].

In this study PCL scaffolds were developed on the bases of studies reported previously [9], with the main focus on cell growth detection.

2 Materials and Methods

Polycaprolactone (PCL) was purchased from *Aldrich Chemistry*, with molecular weight Mn = 70 000-90 000. The polycaprolactone solution was prepared by 2 g of polymer dissolved in a solvent mixture of N, N dimethyl formamide and tetrahydrofuran with a volume ratio of 1:1. The PCL fibrous mats were produced by electrospinning on a high voltage electrospinning system NT-ESS-300, following the processing conditions of 1 ml/h, 15 cm and 12 kV, of volume flow rate, needle tip to collector distance and electrical voltage respectively. As spun PCL fibrous mats were dried in vacuum desiccators 48 h for residual solvent removal. The PCL morphology was delivered using a scanning electron microscope type: SEM-FE MIRA II LMU with Pd/Au sample coating preparation, 3 series for 180 sec. *Image J (NIH)* software was used for the fiber diameters and pores area measurement. The SEM photo micrographs of the cell cultured PCL scaffolds were obtained on a scanning electron microscope type ESEM Philips XL30 with no sample coating preparation.

2.1 Cell Culture

The as spun PCL webs were decontaminated under UV light for 30 min on both sides and hydrated in a series of 70%, 50% and 25% of ethanol, sterile distilled water and Hanks' Balanced Salt Solution (HBOS). Half of the samples were treated with 1N NaOH solution for 1 hour and washed in a Dulbeccov Phosphate Buffered saline (DFPO) before cell culture.

Two types of cell culture were carried on the as prepared PCL scaffolds. Keratinocytes isolated from human skin epidermis and fibroblasts isolated from human skin dermis were seeded on the electrospun PCL and electrospun PCL treated with NaOH in the second passage. The second passage cells were cultured 10 days onto nutritious death mice fibroblast layer (3T3 cell lines). Cell attachment evaluation was performed by cell viability assay (CellTiter-Blue), where cell metabolic capacity was indicated by fluorescent signal generation due to resuzurin reduction into resorufin. The fluorescence measurement was delivered for the PCL scaffolds and compared to fibrin, amniotic membrane and electrospun polyurethane (PU) seeded scaffolds prepared at the Tissue Bank, Clinic of Traumatology. The number of seeded cells on each scaffold was 15 000 in 96-well plates, as for the control wells no scaffold was present but the same number of cells.

3 Results and Discussion

The morphological characteristics of the electrospun PCL scaffolds were evaluated on the bases of representative SEM photo micrographs. Fig. 1 shows SEM image of a sample before cell culture. The uniformness of the fibers in the scaffolds was very low, as was evident from the variation in the fiber diameter in Fig. 2. During scaffold fabrication it was noted that the polymer solution flow was multiple, to insure jet stability the electrical voltage was increased above 12 kV, but no fiber formation was noticed at higher values, except for a single fluid flow.

Thus, the value of 12 kV was taken as optimal. The structure obtained gave multivariate fiber diameters and surface deformations which are reported as an improvement in cell surface attachment. The deformations obtained were recorded as diverged fibers on the main axis, grooved

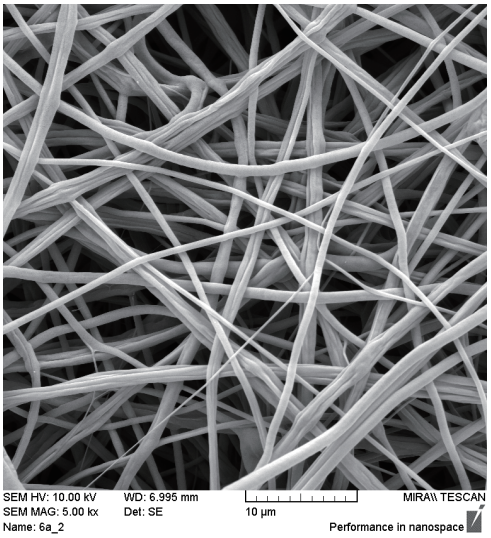

Fig. 1: SEM photo micrographs of the electrospun PCL scaffolds before cell culture.

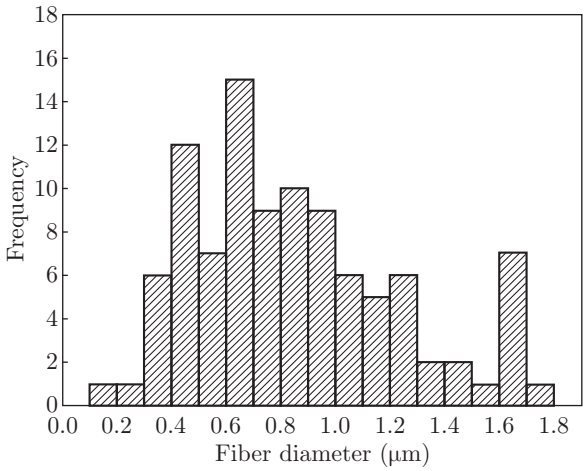

Fig. 2: PCL fiber diameter distribution

fiber surfaces and fiber merges. Fig. 2 presents the diameter fiber distribution of the electrospun PCL scaffolds. The range of the fiber diameter present was dually scaled, between 200 nm and almost 2 microns.

The pore area values in the PCL scaffold, given in Fig. 3, showed skewed distribution, with a wide range of pore sizes present, from 2 to almost 30 μm^2.

The micro scale diameters of the fibers present in the scaffolds are preferred for cell culture, since human cell sizes are in the range of 10 μm in width. Thus, thicker fibers will result in wider pores inter formations, which will facilitate the cell attachment and further penetration in the inner structure.

Figs. 4-7 show the SEM photo micrographs of the cultured human skin keratinocytes and fibroblasts on the electrospun PCL scaffolds without and with the NaOH treatment, respectively.

As previously reported in literature the NaOH treatment of as spun scaffolds not only ensures scaffold cell adhesive properties enhancement but also the hydrolyzed PCL surface provides 3D scaffold structure environment favorable for cell proliferation and interactions [10]. In all figures the cell culture was recorded after 9 days of cell seeding. As evident in Figs. 5 and 7, the

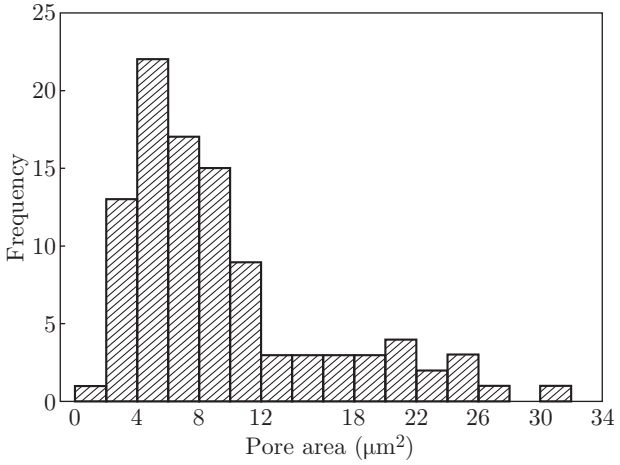

Fig. 3: PCL pore area distribution

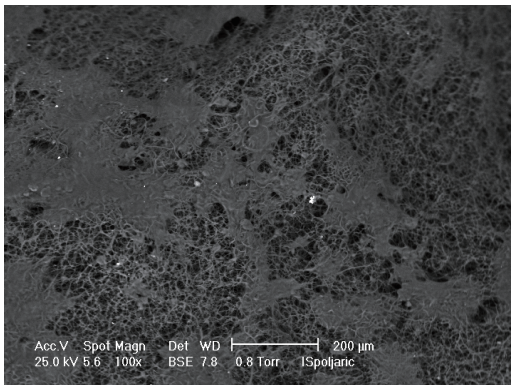

Fig. 4: Keratinocytes cultured on PCL electrospun scaffolds

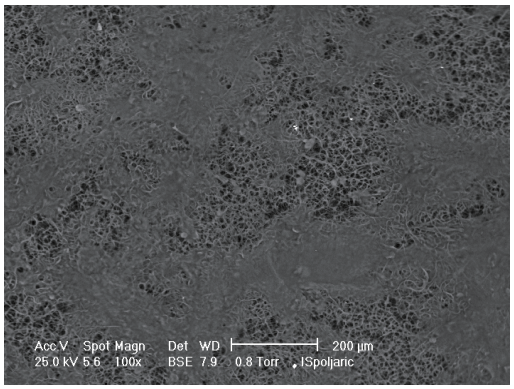

Fig. 5: Keratinocytes cultured on NaOH treated electrospun PCL scaffolds

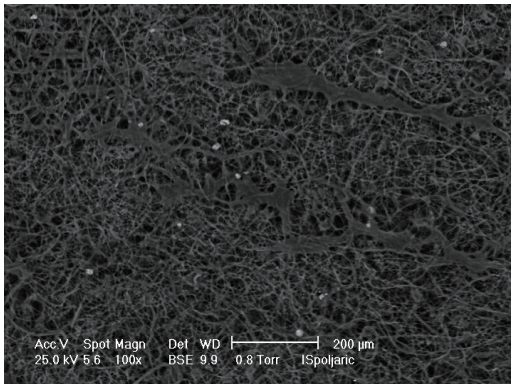

Fig. 6: Fibroblast cultured on PCL electrospun scaffolds

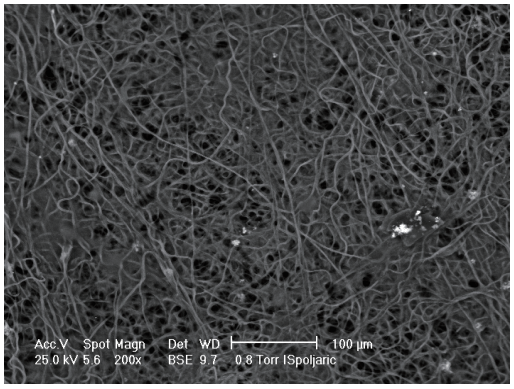

Fig. 7: Fibroblast cultured on NaOH treated electrospun PCL scaffolds

hydrolyzed PCL scaffold showed keratinocytes and fibroblasts wider area covered unlike the non treated scaffold for the same period of time. The area covered in the case of the keratinocytes is evidently bigger, since these types of cells are well known for their high level of fast development and colonies formation.

For the cell adhesion evaluation the CellTiter-Blue assay was undertaken at day 3 and day 10 for the fibroblasts and keratinocytes cells, respectively.

Both types of cells were cultured without any scaffold material for the control as indicated in Figs. 8 and 9. The fluorescence results given were carried out for the PCL scaffolds as well as the materials prepared previously, electrospun PU scaffolds, fibrin and amniotic membrane scaffolds. All of the measurements were conducted for each material by 3 donors of both cell types. The results are shown as averaging the whole population of the individual scaffolds materials.

Statistical analysis was carried out by Anova following Post-hoc (Bonferroni) tests for significant means difference evaluation. For both cell types the significant difference ($p<0,05$) was evident for the synthetic electrospun materials compared to the control (no scaffolds) and the natural fibrin material. In the case of the keratinocytes cells this difference was present for the amniotic membrane as well.

As for the electrospun PCL and PU scaffolds without and with NaOH treatment no significant

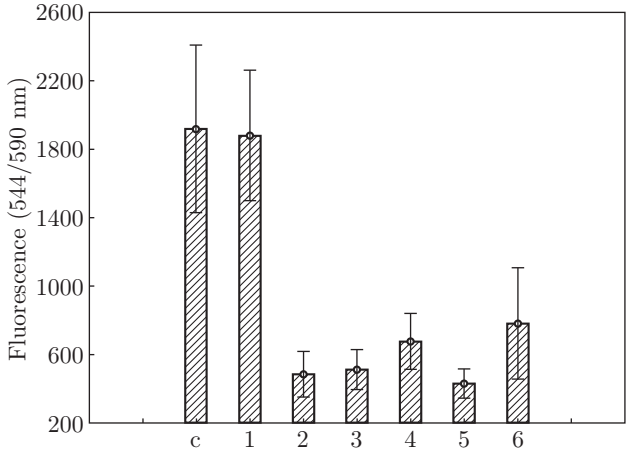

Fig. 8: Fibroblast viability assay on the electrospun scaffolds after 3 days of cell seeding: C-cells only, 1-fibrin, 2-amniotic membrane, 3-PU, 4-PCL, 5-PU+NaOH, 6-PCL+NaOH (Error bars: ± standard deviation)

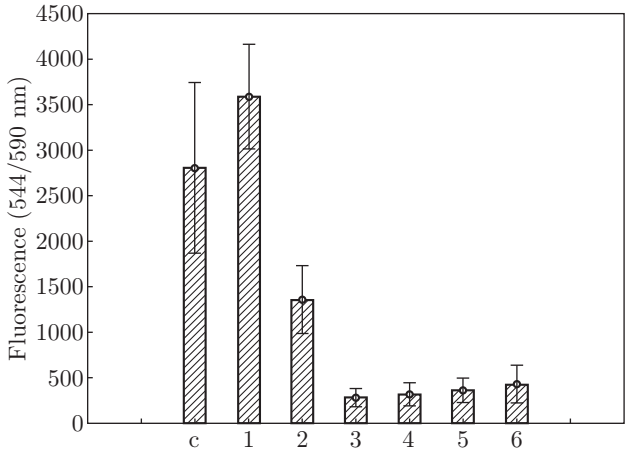

Fig. 9: Keratinocytes viability assay on the electrospun scaffolds after 3 days of cell seeding: C-cells only, 1-fibrin, 2-amniotic membrane, 3-PU, 4-PCL, 5-PU+NaOH, 6-PCL+NaOH (Error bars: ± standard deviation)

difference was noticed, even though a slight change or increase in the fluorescence was present for the NaOH treated scaffolds.

The results shown in this study were quite expected as it is well known that the natural materials scaffolds are better substrates for cell attachment and improved metabolic activity..

As for the separate cell donor comparisons, Fig. 10, the repetitiveness of the cell adherence on the scaffold binding cites for the same material was noticed. A slight difference in the cells fluorescence within the same scaffold material is due to individual characteristics of the donors.

4 Conclusion

As described in previous chapters the study has been focused on the cell adhesion detection onto electrospun biodegradable PCL scaffolds. The as prepared scaffolds were evaluated for

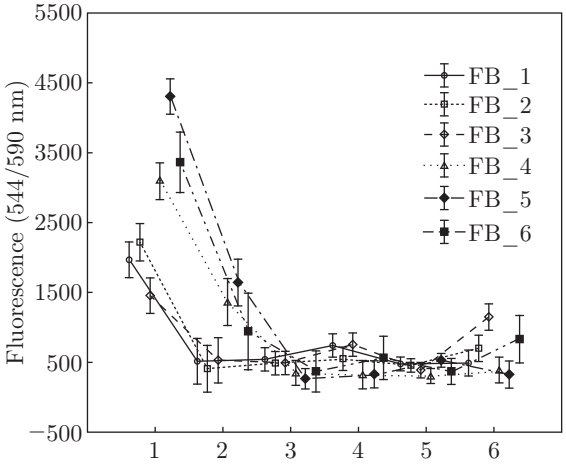

Fig. 10: Fluorescence comparison of the seeded cells, for both FB_1-3 and KC_4-5 donors separately

keratinocytes (isolated from human skin epidermis) and fibroblasts (isolated from human skin dermis) adhesion for 3 different donors each. The viability assay (CellTiter-Blue) was carried out for the PCL scaffolds and compared to previously prepared electrospun PU scaffolds, fibrin and amniotic membrane scaffolds all seeded with same number of FB and KC cells.

As expected the metabolic activity indicated by the highest fluorescence measurement was evident for the natural fibrin scaffolds, as it is quite known for cell adhesion and proliferation. The second one was the amniotic membrane, both showing significant difference in fluorescence for the KC cell type. As for the FB cells, the difference between the amniotic membrane and the synthetic electrospun scaffolds was less evident.

Comparing the cells attachment on the biodegradable PCL and PU scaffolds, no significant difference, but only a slight change in the cell adhesion for the NaOH treated samples was noticed. As for the different type of donors, cells adhesion was confirmed with repetitiveness for the same material for both FB and KC cells.

Future work will be directed on the development of an optimal scaffold by a combination of natural and synthetic biodegradable polymers as to ensure performance benefit from the two materials simultaneously.

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