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Peculiarities of Cell Seeding on Electroformed Polycaprolactone Scaffolds Modified with Surface-Active Agents Triton X-100 and Polyvinylpyrrolidone

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We compared the capability of human fibroblasts to populate porous polycaprolactone (PCL) scaffolds modified during fabrication with surface-active agents Triton X-100 (type 1 scaffold) and polyvinylpyrrolidone (type 2 scaffold). The mean fiber diameter in both scaffolds was almost the same: 3.90±2.19 and 2.46±2.15 μ, respectively. Type 1 scaffold had higher surface density and hydrophilicity, when type 2 scaffold was 1.6 times thicker. The cells were seeded on the scaffolds by the dynamic seeding technique and then cultured in Petri dishes with nutrient medium in a humid atmosphere. During 3-day culturing, no cell release from the matrix was noted. DAPI staining proved the presence of cells in both scaffolds. However, in type 1 scaffold the cells populated the whole thickness, while in type 2 scaffold, the cells were present only in the superficial layer. These findings suggest that PCL scaffolds modified with Triton X-100 or polyvinylpyrrolidone are not cytotoxic, but the structure of the scaffold treated with Triton X-100 is more favorable for population with cells.

Key Words: *tissue engineering; synthetic scaffold; Triton X-100; polyvinylpyrrolidone; cell culture*

The progress in regenerative medicine and in fabrication of artificial tissues and organs for substitutive surgery depends, among other things, on the development of available 3D structures for cell seeding [4,6]. Fabrication of scaffolds similar to biological tissues by their 3D structure is the most promising approach [11]. The problem of fabrication of these scaffolds is largely solved due to the development of electrospinning technology [1,5]. This method allows producing fibrous scaffolds from various synthetic materials with predetermined structural characteristics and strength properties close to biological tissues [5]. The need in scaffolds promoting more efficient population with cells and preserving their proliferative activity and the capacity to direct differentiation steadily increases [8]. A promising approach to improving scaffold characteristics is addition of bioactive or surface-active agents [5] to the basic substance used for scaffold fabrication; these additives can essentially modify the properties of scaffold fibers and, eventually, its whole structure. Previously, scaffolds for implants for reconstructive and substitutive surgery were fabricated from polycaprolactone (PCL) by using electrospinning technology [2]. It was noted that surface properties of the PCL fibers could be improved by using of PCL composite with surface-active agents [9].

The aim of the study is to compare hydrophilicity of PCL scaffolds electroformed in the presence of Triton X-100 or polyvinylpyrrolidone (PVP) and their suitability for population with cells.

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MATERIALS AND METHODS

Two types of scaffolds fabricated from PCL (Sigma-Aldrich; Mn=80,000) were studied. The scaffolds were manufactured by using electroforming process on an experimental single-capillary apparatus engineered at the National Research Center "Kurchatov Institute". We used a rotating metal electrode (diameter 63.6, mm; rotation speed 22 rpm). Forming solution was supplied through a metal capillary with a resistance of 30 mm H_2O using a DSH-08 syringe pump (Wisma-Planar). High voltage generated by a Spellman SL130PN30 source was applied to the capillary. The forming solution was supplied at a rate of 90 ml/h (type 1 scaffold) or 18 ml/h (type 2 scaffold). The interelectrode distance and voltage in both cases were 30 cm and 15.0-18.8 kV.

Forming solution with following content was used: 9.5% PCL, 81.5% chloroform, and 9% ethanol. When forming type 1 scaffold, Triton X-100 (Sigma-Aldrich) was added at a rate of 0.32 g per 100 g of solution. When forming type 2 scaffold, PVP (Sigma-Aldrich, M_w=40,000) was added to ethanol at a rate of 15% solution weight. The solutions of Triton X-100 and PVP in ethanol were prepared separately, and then added dropwise to the PCL solutions. All solutions were prepared in sealed vessels while stirring on a magnetic mixer at room temperature. All solvents were at least of chemically pure grade.

After synthesis, the scaffolds are washed in 96% ethanol for 24 h and then in running distilled water and dried at room temperature. This procedure allows reducing the presence of Triton X-100 and PVP in the scaffold to trace amounts.

The structure of the scaffolds was analyzed under a Versa 3D DualBeam scanning electron microscope in high vacuum mode. The images were obtained using a secondary electron detector at a low accelerating voltage (1 kV), which made it possible to enhance the topographic contrast, to avoid accumulation of electric charge on the samples, and to increase spatial resolution [12]. The diameter of the fibers in the scaffold was assessed using Fiji and Origin software [14]. The mean diameter of fibers was calculated from the results of measurements of 70-100 fibers.

The porosity of samples was determined by the difference in the specific weight of the fibrous sample and the initial polymer. For measurements, a Rycobel thickness gauge and Sartorius AG CPA24S scales were used [9]. Contact wetting angle was determined with Drop Shape Analyzer KRUSS DSA30E (liquid phase — water, drop volume — 5 μl).

Suspension of human skin fibroblasts was used as the cell material. The fibroblasts were derived by migration from the skin fragments obtained during cosmetic plastic surgery. Prior to cell seeding, the scaffolds were soaked with deionized water 3 times for 30 min. A suspension of passage 4-6 cells (3×10^4 cells/ml) was used. The cells were seeded on the scaffolds by the dynamic seeding method [7]. Previous studies have demonstrated that this method ensures effective population of electrospun scaffolds with cells [2,3]. To this end, a closed silicone tube loop including a special chamber with rigidly fixed scaffold was used. Prior to cell seeding, the chamber and tubes were sterilized by filling with 70% ethanol for 15 min. After that, the loop was washed with phosphate buffer twice to remove ethanol and filled with cell suspension. The cell suspension was pumped through the loop and through the scaffold with a peristaltic pump at a speed of 50 ml/min over 20 min. Preliminary studies have shown that this circulation mode did not cause mechanical cell damage and did not affect their proliferative activity. After cell seeding, the scaffold was transferred to a Petri dish with DMEM supplemented with 10% fetal serum and incubated for 3 days in a humid atmosphere with 5% CO_2 at 37°C [10]. All manipulations with scaffolds and cell material were carried out aseptically in a laminar flow cabinet.

The efficiency of cell seeding was evaluated by assessing cell release from the scaffolds on the bottom of the Petri dishes during incubation [7] under an inverted microscope.

After 3-day incubation, the scaffolds were fixed in 10% buffered formalin. The presence of the cells in the scaffolds was evaluated with fluorescent microscopy. To this end, the scaffolds were paced in DAPI solution for 45 min for visualization of cell nuclei, washed with PBS, transferred on glass, and examined under an AxioImager M2 microscope (Carl Zeiss) at λ_{ex}=353 nm, λ_{em}=465 nm, ×400. At the 2D mode a cell number was calculated on the area of 348.79×263.03 μ. The cells were counted in 2D mode in 10 randomly selected fields of view (348.79×263.03 μ).

The results were processed using the Student's *t* test Statistica 10.0 (StatSoft, Inc.). The differences were significant at $p<0.05$.

RESULTS

Visually, type 2 scaffold looked denser. However, instrumental measurements showed that the mean porosity of type 1 and 2 scaffolds differed insignificantly: 83.04±5.20 and 90.60±5.05%, respectively. Moreover, the fibers of type 2 scaffold have a more pronounced surface relief.

The ranges of fiber diameters in type 1 and type 2 scaffolds were different (0.48-12.3 and 0.34-5.8 μ, respectively; Table 1), while the mean diameter of fibers was similar (3.90±2.19 and 2.46±2.15 μ, respec-

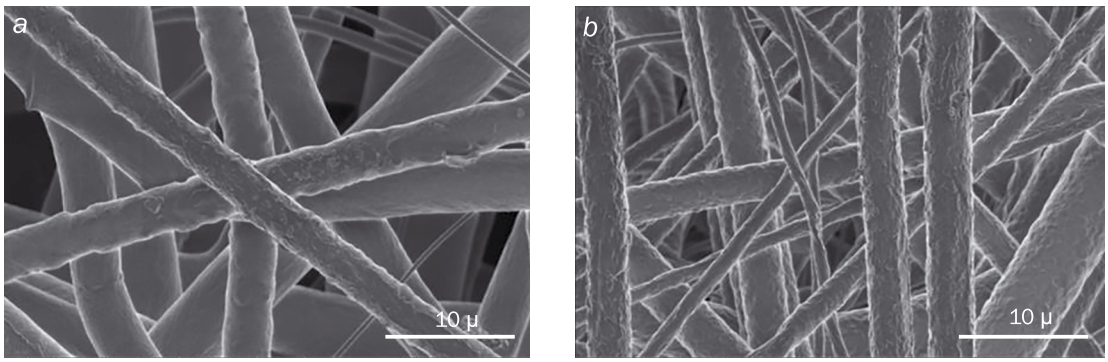

Fig. 1. Surface morphology of PCL fibers synthesized in the presence of Triton X-100 (*a*) and PVP (*b*), ×5000.

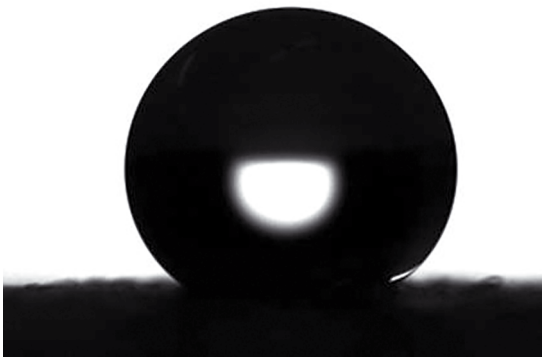

Fig. 2. A water drop applied on type 2 scaffold surface.

tively). Type 2 scaffold was thicker by 1.6 times, but had lower surface density. Analysis of surface properties of the obtained samples showed that type 1 scaffold absorbed the applied water drops almost instantly, whereas water drop applied on the surface of type 2 scaffold retained its round shape (Fig. 2). The mean contact angle was 137.0±1.5°. Hence, introduction of Triton X-100 into the scaffold structure ensured its hydrophilicity, while the scaffold containing PVP exhibited properties close to superhydrophobic. Thus, the use of Triton X-100 or PVP as additives at the stage of fabrication allows obtaining scaffolds with different surface properties. This difference could affect susceptibility of scaffolds to colonization with cell material.

To test this hypothesis, the scaffolds were dynamically seeded with cells. Subsequent culturing of the scaffolds revealed no cells on surface of the Petri dishes close to the sample (Fig. 3). The cells in the scaffolds were detected using fluorescent microscopy. The cells were detected in both types of scaffolds, but their distribution considerably differed. In type 1 scaffold, the cells populate the whole thickness of the scaffold, but were more numerous in the surface layer of the upper side of the scaffold (Fig. 4). In type 2 scaffold, the cells were seen only in the surface layer of the upper side of the sample (Fig. 5). Quantitatively, the mean number of cells in 2D images of type 1 scaffold was 81.4±6.0, while for type 2 scaffold, this parameter was lower by 4.2 times and did not exceed 19.3±9.2 ($p<0.05$). Even more pronounced differences were revealed when studying 3D images of the scaffolds: the mean number of cells in type 1 and type 2 scaffolds differed by more than 9 times: 215±32 and 23±8, respectively ($p<0.05$).

Both Triton X-100 and PVP were used to modify the structure of the fibers synthesized from PCL to obtain scaffolds with good wettability. This parameter determined the uniform population of the deep layers of the scaffold with cells [13]. However, satisfactory wettability was observed only for the scaffold modified with Triton X-100. It can be hypothesized that poor wettability of PCL scaffold modified with PVP determines less intensive population of this scaffold with fibroblasts.

Thus, we can conclude that electrospun scaffolds fabricated from PCL modified with surface-active agents Triton X-100 and PVP are not cytotoxic and can be seeded with human fibroblasts. However, the PCL scaffold modified with Triton X-100 is more preferable for cell seeding. The revealed peculiarity

TABLE 1. Characteristics of the Used Scaffolds

Parameter	Scaffold	
	type 1 (Triton X-100)	type 2 (PVP)
Porosity, %	83.04±5.20	90.60±5.05
Mean fiber diameter, μ	3.90±2.90	2.46±2.15
Range of fiber diameters, μ	0.48-12.30	0.34-5.78
Thickness of the scaffold, mm	0.48	0.78
Surface density, g/m^2	92	81
Contact angle, degrees	Instant absorption of water drops	137.0±1.5

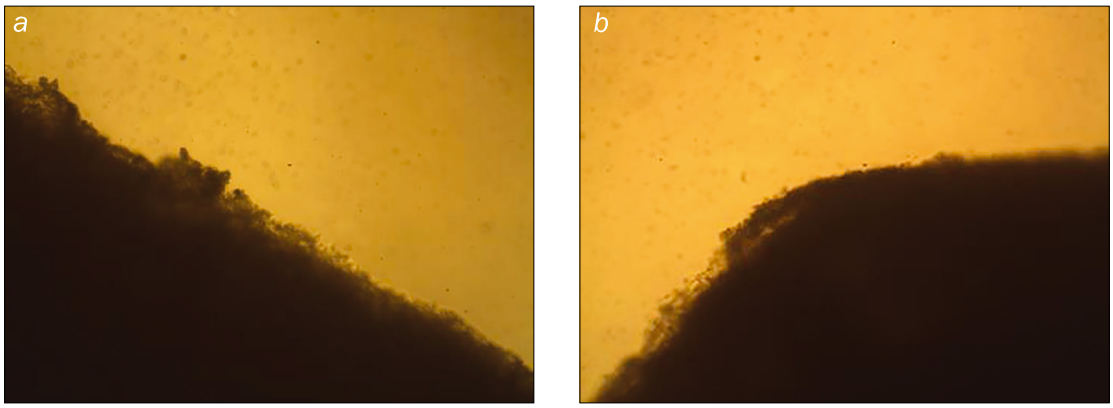

Fig. 3. Surface of a Petri dish with PCL scaffold populated by human skin fibroblasts on day 1 (*a*) and day 3 (*b*) of culturing, ×10.

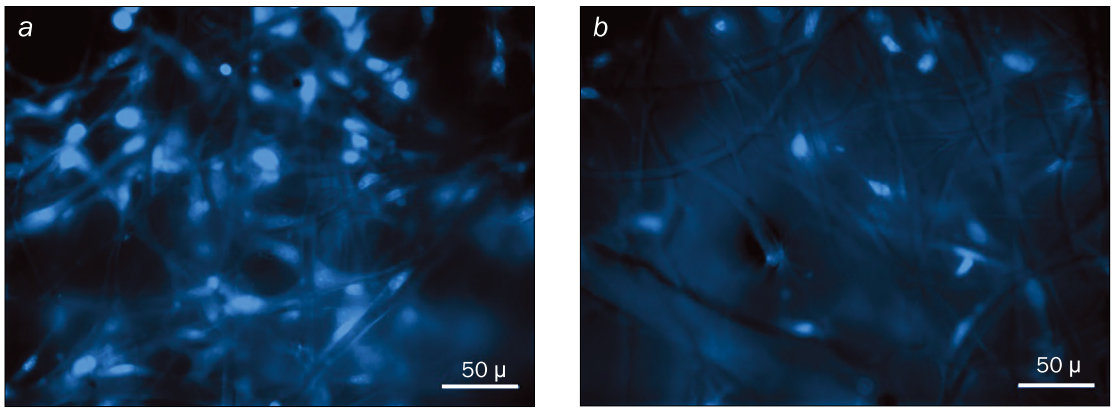

Fig. 4. Typical view of type 1 scaffold populated with cells after staining with nuclear dye DAPI. *a*) Surface layer of the upper side of the scaffold; *b*) surface layer of the lower side of the scaffold.

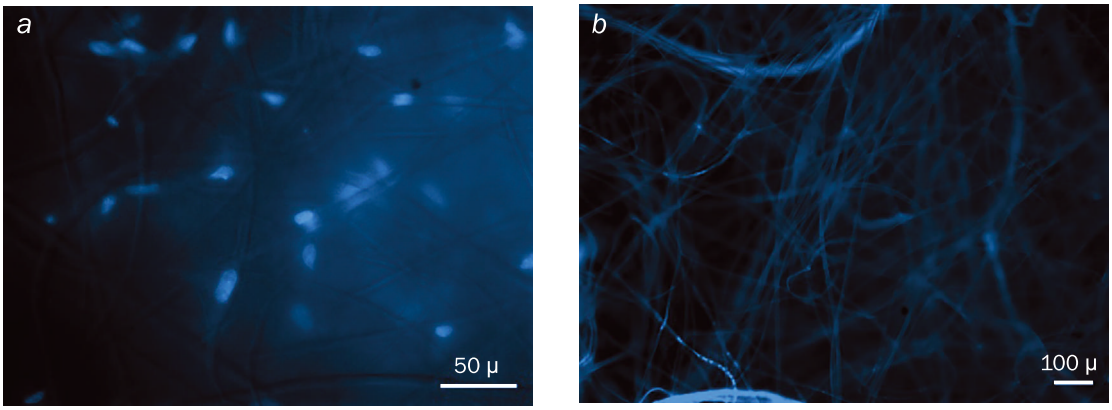

Fig. 5. Typical view of type 1 scaffold populated with cells after staining with nuclear dye DAPI. *a*) Surface layer of the upper side of the scaffold; *b*) surface layer of the lower side of the scaffold.

of cell population in the studied scaffolds should be considered when modeling artificial multilayer tissue-engineering constructs.

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