Piezoelectric Polyvinylidene Fluoride Nanofibers as a Scaffold used for Bone Tissue Engineering

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Abstract—The field of bone tissue engineering deals with the issue of developing compatible and non-toxic scaffolds, which provide the growing bone cells with the necessary mechanical support and suitable stimulation for proper growth and proliferation. Polyvinylidene fluoride (PVDF) nanofibers are considered to be a suitable scaffold due to their biocompatibility, fibrous structure, and characteristic piezoelectric properties, which have proven to be an important factor in cell regeneration. For this work, flexible PVDF nanofibers were successfully spun, from which the scaffolding needed for the subsequent settling of cultivated osteoblasts was created. Thereafter the structure and compatibility of this nanomaterial were assessed in relation to the study of cell adhesion to individual nanofibers.

Keywords—bone tissue engineering; polyvinylidene fluoride; piezoelectric properties; scaffolds; osteoblasts.

1. INTRODUCTION
At a time of ever-increasing incidence of musculoskeletal disorders due to obesity, sedentary work, and unhealthy or poor physical activity we are looking for the most suitable ways to replace damaged bone tissue and support its regeneration and healing. The cells needed for this therapy are grown on special scaffolds that simulate the natural extracellular matrix that cells make for their support. This scaffold also works as a network for signalization and interaction between each cell or whole tissue.

Each scaffold must meet certain requirements such as good biocompatibility and flexibility and should have hydrophilic, fibrous, porous, and piezoelectric properties. It was found that piezoelectric materials can generate and deliver an electric stimulus as a result of the application of mechanical stress without any external power source. Human bone tissue produces electric charges due to the deformation of extracellular fibrous protein - collagen, which is crucial for the right function of cellular membrane channels, therefore cell communication and growth [1]. To mimic this behavior, it was discovered that a fabrication technique called electrospinning is the most effective and low-cost method for achieving both fibrous structure and piezoelectricity in PVDF scaffolds [2].

The cells chosen for this work are osteoblasts that make up the bone tissue. Osteoblasts specialize in the synthesis of bone matrix and secretion of collagen which is needed for the bone to adjust to current load and mechanical tension. Osteoblasts are mostly found in a layer on the surface of bone structures. When new cells completely bury mature osteoblasts, they transform into osteocytes which are chiefly involved in metabolism [3].

2. MATERIALS AND METHODS

2.1 PVDF FABRICATION
The method chosen for fabricating a flexible fibrous PVDF scaffold for this experiment is called electrospinning. The basic principle of electrospinning is that an electrical potential is applied between an emitter - the spinneret and an electrically conductive collector for the produced nanofibers. The newly formed electric field stretches the electrically conductive solution which is forced to take a highly specific shape called the Taylor cone. When the electrical voltage is increased and reaches the critical value when the surface tension is no longer enough to maintain the Taylor cone, the polymer is drawn.

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to the surface of the spinning collector and begins to form a thin layer [4].

The electrospinning device used for this experiment was a 4SPIN (Contipro, Dolní Dobrouč, Czech Republic) with a cylindrical collector wrapped with aluminum for better separation of the formed nanomaterial from the cylinder at the end of the making process. The emitter was in a form of one needle, which the solution was passed through at a flow rate of 30 μl per minute. The voltage between the emitter and the collector was 50 kV. The used solution was a 20% solution of PVDF with a molecular weight of 275,000 g/mol in a solvent of dimethyl sulfoxide/acetone in a ratio of 7:3, which was heated for 24 hours on a stirrer at a temperature of 80 °C and a speed of 200 rpm.

2.2 CELL SAMPLE PREPARATION

The preparation of cell samples is a standardized procedure. Modifications were chosen according to existing experiments that used cultured osteoblasts [5]. Human osteosarcoma Saos-2 cells (ATCC® HTB-85™) were maintained in complete Dulbecco’s Modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum and 5% penicillin/streptomycin (50 UI · mL⁻¹ and 50 μg · mL⁻¹) at 37 °C in a humidified 5% CO₂ incubator. Cells were harvested by trypsinization with 0.25% trypsin-EDTA solution. Saos-2 cells were seeded at the density of 1 × 10⁴ · mL⁻¹ onto the sterile PVDF samples (1 × 1 cm) placed in a polystyrene microplate.

The interaction of cells with PVDF fibers was visualized by immunocytochemistry in 48 hours. Paraformaldehyde - fixed Saos-2 cells were permeabilized with a solution of 0.5 % Triton-X 100 supplemented with 2 % bovine serum albumin for 2 hours and thoroughly washed in deionized water. The actin fibers were stained with ActinGreen™ 488 (Invitrogen™) for 30 min at RT. The stained cells were imaged using a confocal microscope (CLSM) Zeiss LSM 880 with a 488 nm laser. All chemicals were purchased from Sigma Aldrich.

To observe the sample on a Lyra3 (Tescan, Brno, Czech Republic) scanning electron microscope (SEM), it was necessary to metalize the surface of the preparate. The metal coating does not only dissipate the charge, which then does not accumulate on the surface but also fixes the fibers that would be otherwise affected by the electron beam and began to twist. Thanks to this, they do not move, and it is possible to take a good picture. Gold was used to metalize the samples, which is also good for energy-dispersive X-ray spectroscopy (EDS) because gold does not disrupt the peaks on the spectrum. The EDS detector used was X-Max 50 (Oxford Instruments, Oxford, United Kingdom). An instrument called coater EM ACE600 (Leica, Wetzlar, Germany) was used for metal coating and the gold layer was 7 nm thick.

3. RESULTS

3.1 STUDYING QUALITY OF PVDF SCAFFOLD

For making a good scaffold for cells to grow on is also important the overall layout of nanofibers. It is known that for some types of cells the alignment of nanofibers is essential. The right layout can be achieved during the process of electrospinning. The polymer jet has a chaotic trajectory therefore the collected fibers exhibit various orientations. The fibers can be directed during spinning by controlling the rotation speed of the collector or adjusting the electric field between electrodes [1].

Two nanofiber materials with different fiber orientations were created by this method, which were then cut down into small samples. The nanofibers were successfully spun with no greater imperfections. The occurrence of beads on the fibers was not common thanks to the right ratio of dimethyl sulfoxide and acetone. For this observation were used two types of samples. The first sample had nanofibers that were spun at a speed of 300 rpm, and the second sample had nanofibers spun at a speed of 2000 rpm. Both samples were after proper preparation and gold coating imaged by SEM under a voltage of 5 kV.

The biggest difference in samples is the very arrangement of individual fibers, which are in a non-directed sample in Figure 1a very chaotic and variously twisted and bent. On the contrary in Figure 1b can be seen fibers highly aligned and stretched in the same direction. The next important difference is the thickness of fibers which varies dramatically in the non-directed sample. The thickness difference between the widest and thinnest nanofibers in Figure 1a is 1872,79 nm. Meanwhile, Figure 1b shows directed nanofibers that were spun at a much higher speed, where the thickness of individual fibers is smaller and more consistent. The biggest thickness difference there is only 603,87 nm.
3.2 OBSERVATION OF CELL ADHESION

To evaluate the degree of adhesion of individual osteoblasts to the surrounding nanofibers, a confocal microscope was chosen, in which, thanks to the green staining, it was possible to distinguish the cells from the fibers very well and observe their various attachments. The cells adhered to the fibers very nicely and could be seen using the fibers as mechanical support.

An interesting difference in cell attachment can be seen through SEM in samples with different fiber orientations. A better result is shown on the directed nanofibers in Figure 3b, where the osteoblasts are oriented mostly parallel to the fibers, the cells are elongated, and they are in larger equally aligned groups. While on undirected nanofibers in Figure 3a the cells oriented randomly, took on more various shapes, and didn’t form a greater pattern due to their random location and orientation.

Figure 1: Examples of a) undirected PVDF nanofibers and b) directed PVDF nanofibers. Imaged by SEM.

Figure 2: Stained osteoblasts on directed PVDF nanofibers imaged by CLSM.

Figure 3: Examples of a) osteoblast seated on undirected PVDF nanofibers and b) osteoblast seated on directed PVDF nanofibers. Imaged by SEM.
3.3 EDS MAP ANALYSIS

The gold-coated sample was subjected to EDS, which confirmed the elemental representation in the nanofibers and the cell itself. The main elements forming the whole sample are carbon, oxygen, and fluorine. In Figure 4b can be seen an independent representation of oxygen which is the second most represented component in the sample right after carbon and is part of both osteoblasts and PVDF nanofibers. On the other hand, in Figure 4c, it is shown that fluorine is only a part of nanofibers and does not influence or pollute the cells.

![Image](image1.jpg)

**Figure 4:** Results from EDS: a) example of colored sample according to the representation of carbon (purple), oxygen (green), and fluorine (red) in the spectrum, b) highlighted oxygen, and c) fluorine.

4. CONCLUSION

In this work a PVDF nanomaterial was created through the process of electrospinning. This material proved to be suitable for making a biocompatible scaffold for settlement of cultivated osteoblasts. Through examining the samples with SEM and CLSM, it was proven that increasing rotation speed over 2000 rpm during the process of electrospinning reduces the fiber diameter and porosity of the scaffolds. It directs the fibers to the same course, which enhances the piezoelectric properties of the material which makes the material more preferable for the right attachments of cells. The osteoblasts attached well and overall seem to exploit the potential of nanofiber support without any difficulties. The EDS map confirmed the expected elemental composition of the sample. It is a fact that PVDF is a hydrophobic material, which could be counterproductive in means of proper cell adhesion. If I were to continue this line of work, I would deal with the combination of PVDF with other materials such as polyamide 6, which is considered highly hydrophilic. I would examine the differences in cell adhesion also in combination with different cell types.

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REFERENCES


