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Book of Abstracts

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Influence of process-material conditions on the phase composition, architecture and biological properties of electrospun polyvinylidene fluoride fibers

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The discovery of electric fields in biological tissues has led to efforts in developing technologies utilizing electrical stimulation for therapeutic applications. Native tissues, such as cartilage and bone, containing collagens and glycosaminoglycans (GAGs) exhibit piezoelectric behavior, with electrical activity generated due to mechanical deformation through physiological movement. However, the use of piezoelectric materials in tissue engineering has largely been unexplored.

Piezoelectric properties of polyvinylidene fluoride (PVDF) are highly dependent on supermolecular structure, which in turn is governed by conditions of material formation [1,2]. The relations between the conditions of formation, supermolecular structure and piezoelectricity of PVDF will be discussed. The nanofibrous structure of PVDF scaffolds is expected to mimic part of extracellular matrix (ECM). Finally, our results of cellular in vitro investigations, using PVDF nanofibrous scaffolds under ultrasound stimulation together with recent achievements reported in the literature will be shown.

The electrospinning equipment for nanofibers formation, consisting syringe pump, collector and high voltage generator, connected with positive terminal to a stainless steel needle and grounded to the rotating drum collector (diameter 40 mm) was operated in horizontal mode. PVDF (SigmaAldrich) with Mw = 400 000 g/mol was used. Mixture of dimethylformamide (DMF) with acetone (4:1 weight ratio) were used as a solvent at polymer concentration 15%. Distance between needle and collector was 180 mm, flow rate of solution was 0,2 mL/h, and needle with 3 mm inner diameter was used. Our results indicate that the minimum collector rotational speed needed to induce preferred fiber arrangement is 500 rpm which corresponds to linear velocity 10 m/s. Comparison of SEM images of fibers obtained at 300 and 500 rpm is shown in Fig. 1.

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PVDF nanofibers formed by electrospinning were subjected to *in vitro* cellular studies. In the stimulation experiments, fibroblasts L929 cells cultured on the piezoelectric PVDF scaffolds collected at various rotational speeds of the collector, were exposed to ultrasounds for 30 minutes, one time per day, for 7 days. Ultrasound stimulus of power 20 and 80 mW at frequency 1.7 MHz was applied. In order to confirm the piezoelectric effect of the PVDF scaffolds on fibroblasts activity, piezoelectric PVDF scaffolds without ultrasonic stimulation were used for reference.

Activity of cells have been analyzed using MTT assay. The preliminary data indicate positive effect of piezoelectricity on the cells activity under mechanical stimulation. This effect was more pronounced for samples formed at higher rotational speed of the collector, as fiber mats had more preferred orientation (Fig. 2A). The observations using SEM imaging verified the cell morphology and proliferation to the scaffolds. On day 1 the cells had more rounded morphology, while on day 7 their morphology was more elongated and spread-out (Fig. 2B).

![Fig. 1. SEM images of PVDF nanofibers formed at different rotational collector speed, 300 rpm (A) and 500 rpm (B, C)](image1)

![Fig. 2. Viability of fibroblasts L929 using ultrasound stimulation with different power(A); SEM images of PVDF nanofibers with fibroblasts L929 cell culture on day 7 (rotational speed of collector: 300, frequency: 1,7 MHz) (B)](image2)
In summary, the preliminary results show that using piezoelectric PVDF stimulated by ultrasounds increase the cells activity, what is very promising from the perspective of tissue engineering applications.

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References