NEW ALIGNED MICROFIBERS FOR TISSUE ENGINEERING

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Abstract

The present work deals with preparation and subsequent modification of aligned polycaprolactone microfibers made by new semiautomatic drawing technique. This advanced scaffold for tissue engineering combines 3D architecture, large surface area-to-volume ratio, alterable conductivity which closely imitates native extracellular matrix and actively modulating cell functions. New synthetic approach has been chosen for derivatization of aligned polymer fibers, which lack any surface functional groups for immobilization of biomolecules, grow factors or specific amino acid sequences. To overcome this drawback the thin conductive polypyrrole layer was applied on the polycaprolactone fibers by chemical oxidative polymerization from the solution. Thus, in the one simple synthetic step we can modify original fibers with various functional groups presented in the β-position of the pyrrole. Our future goal is to functionalize microfibrous surface with cyclodextrin units using highly efficient copper free click reaction. Finally we gain an aligned microfibrous scaffold covered by immobilized cyclodextrin macrocycles that allow inclusion of proteins during cell cultivation. These nonbonding interactions are relatively weak but the cumulative effect makes them efficient in the complexation of various neutral or ionic molecules. Moreover better environment for cell adhesion and proliferation is being provided.

Keywords: Polycaprolactone, polypyrrole, tissue engineering, drawing, cyclodextrin

1. INTRODUCTION

Nanotechnology is a rapidly emerging cross-discipline program that manipulates assorted synthetic and naturally occurring materials in nanoscale dimensions. Large surface area-to-volume ratio of nanomaterials enables revolutionary advances in almost every field of research [1]. There is currently a great variety of nanomaterials available used in many applications. Among them, nanofibers found their use in drug delivery, biomedical and biotechnological applications, sensors, batteries and tissue engineering.

Nanofibrous scaffolds mimic the nanodimensional features of the native extracellular matrix (ECM), which in turn directs numerous aspects of cellular organization and survival. ECM has two main components: polysaccharides and fibrous proteins. As such, nanofibrous constructs have been used extensively as potential tissue engineering platforms. It is generally hypothesized that a close imitation of the ECM by nanofibers will provide environmental or physical cues to cells due to features including high surface area-to-volume ratio, alterable porosity, and three-dimensional architecture.

For specific tissue engineering applications, such as controlling the guidance and alignment of nerve cell growth, the most common approach is to use aligned electrospun fibers rather than randomly orientated fibers [2]. Many studies have proved that neurons cultured on aligned electrospun fibers have longer neurite lengths than those on random fibers [3]. These results are intuitive because topographical cues come from the scaffold features rather than material chemical properties. Also, enhanced neurite outgrowth has been observed on aligned conducting nanofibers using external electrical stimulation.

2. METHODOLOGICAL BASES

2.1. Fibers preparation

There are several methods available for the fabrication of aligned nanofibers: electrospinning [4], rotary jet spinning [5], self-assembly [6], sol-gel methods [7], supersonic drawing [8], melt-drawn protocols [9] and fiber drawing [10]. The latter process is a technology of mechanical pulling of individual fibers proceeded without the need of applying an electrical field [11]. Though, the fiber drawing method is capable of making more precise scaffolds with defined order of fibers. For this purpose the unique “Micromanipulator” device was recently developed by engineers from our university. This device pulls a single fiber (with a diameter in μm scale) out of the polymer solution droplet and is able to make individual aligned microfibers (**Fig. 1**).

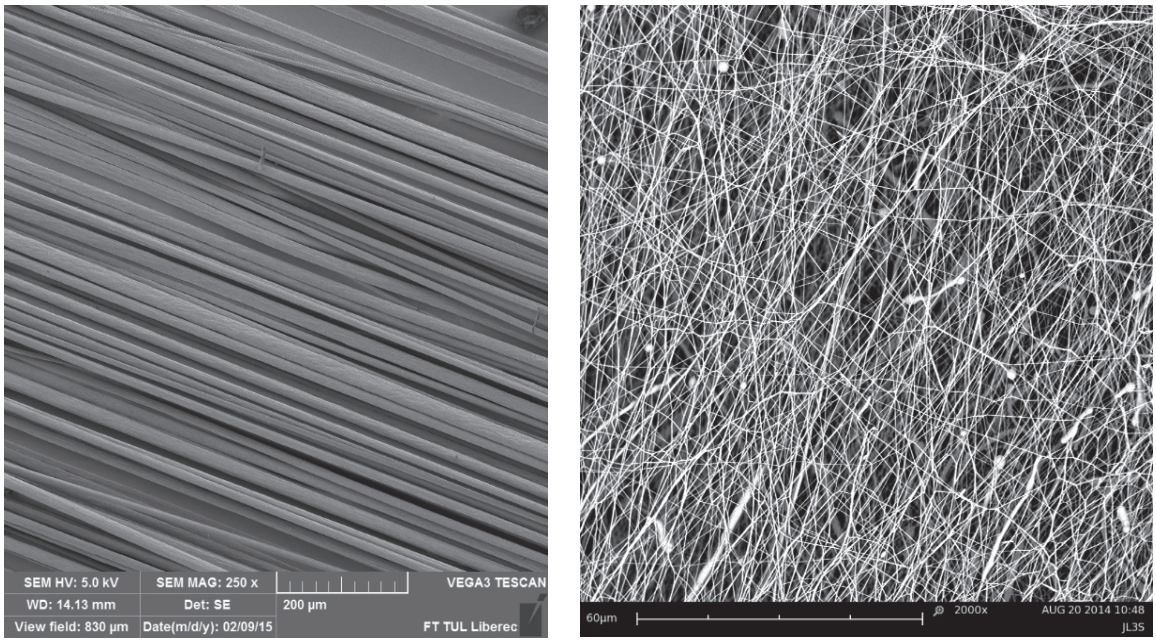

Fig. 1 SEM images of polycaprolactone fibers made by drawing *(left)* and electrospinning technique *(right)*

2.2. Polypyrrole synthesis and derivatization

Classically, electrically conducting materials and nanofibrous scaffolds have been studied separately. It was only recently that electrically conducting nanofibers drew attention as tissue engineering scaffolds [12]. They contain both nanofibrous features and electrical activity and they are valuable in actively modulating cell functions. Among the conductive polymers for biological applications, polypyrrole (PPy) is the most thoroughly investigated one because of its high electrical conductivity, flexible method of preparation, ease of surface modification, excellent environmental stability, ion exchange capacity, and its ability to support cell adhesion and growth of a number of different cell types [13].

PPy is generally synthesized by chemical [14] or electrochemical [15] technique. Chemical pathway uses polymerization from the solution, where monomer, oxidant (e.g. $FeCl_3$, $(NH_4)_2S_2O_8$) and dopant (e.g. *p*-toluenesulfonic acid (PTSA) or hyaluronic acid) react together in the appropriate molar ratio. The resulting doped PPy is almost insoluble blue-black powder or thin layer [16].

In order to extend possibilities of tissue engineering on conductive nanofibrous surfaces it is necessary to modify them with suitable derivatives. In the case of PPy, it is possible to synthesize a pyrrole derivative and subsequently cover ready-made nanofibers by polymerization of such pyrrole. Substituted PPys are usually obtained by polymerization of modified monomer unit. Pyrrole needs to be substituted at β-position to preserve α-position for subsequent polymerization. However, pyrrole is attacked by electrophilic reagents in the most cases at α-position. This can be avoided by substitution of nitrogen atom with bulky functional group like

triisopropylsilyl (TIPS) or tosyl (Ts), which is sterically/electronically blocking α-carbons [17-20]. One versatile option, how to prepare the functionalized PPy, is to synthesize cyclodextrin (CD) modified PPy derivatives.

3. EXPERIMENTAL PART

Aligned polymer fibers clamped on the PMMA circlet made by drawing technique were coated by conductive PPy layer of various thicknesses. Reaction conditions (temperature, type of solvent, concentration of reagents, reaction time) have been optimized to obtain the best modified scaffold for initial cell culturing study. The key aspect of this functionalization was the appropriate monomer concentration as well as the slow polymerization rate tuned by methanol / water content, otherwise the clusters have been arisen instead of smooth PPy layer formation (**Fig. 2**). Mild oxidizing agents $FeCl_3$ in combination with PTSA as a doping molecule proved to be an ideal combination. Reaction mixture was stirred at room temperature for 12, 24 and 36 hours (various PPy thickness), then washing steps using water and methanol took place. Thoroughly PCL / PPy microfibers were put into vacuum desiccator for several days.

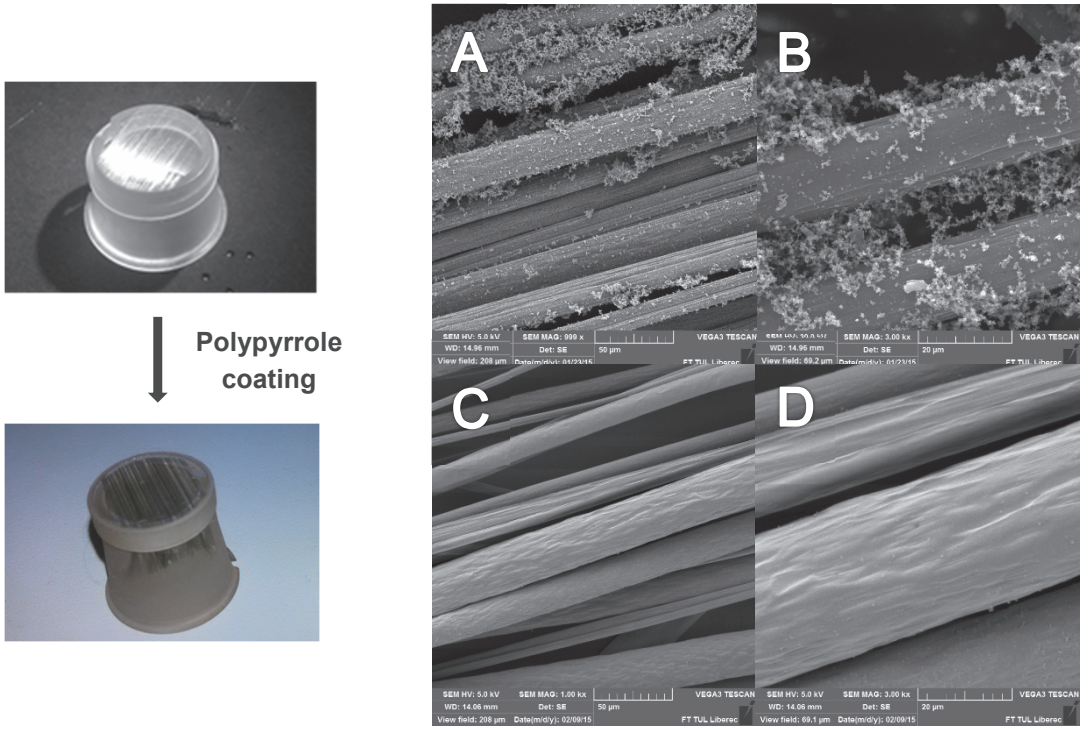

Fig. 2. (*left*) PCL fibers made by drawing technique tailored on the PMMA circlet, (*right*) SEM images of PCL fibers coated by PPy layer *(a, b)* clusters formation *(c, d)* optimized reaction conditions

Several β-substituted pyrroles have been prepared according published literature [20]. The first step was protection of aromatic nitrogen using bulky TIPS group, which sterically hinder α-position and directs electrophilic substitution into the β -position. The reaction of pyrrole (**1**) was carried out at 0 °C in dry DMF, sodium hydride was used for deprotonation and then TIPS-Cl was added dropwise. The reaction mixture was separated between DEE and water, organic phase was washed several times with water, dried over sodium sulphate and then solvent was evaporated. The final vacuum distillation of residue gave and oily product (**2**) with yield over 90 %. Second step was the iodination of N-blocked pyrrole (**2**) using N-iodosuccinimide as halogenation agent. The reaction was carried out at -78 °C for 6 h, workup and vacuum distillation yielded product (**3**) 73 %. This iodo-pyrrole (**3**) was undergoing Sonogashira cross-coupling reaction using 5 mol % of palladium catalyst. Great advantage of this synthetic approach is possibility of selectively removing silyl functional group at the end of the procedure using fluoride anion as mild deprotecting agent (**Fig. 3**). Compound (**3**) is general the platform for click type reaction which will be discussed in the next section.

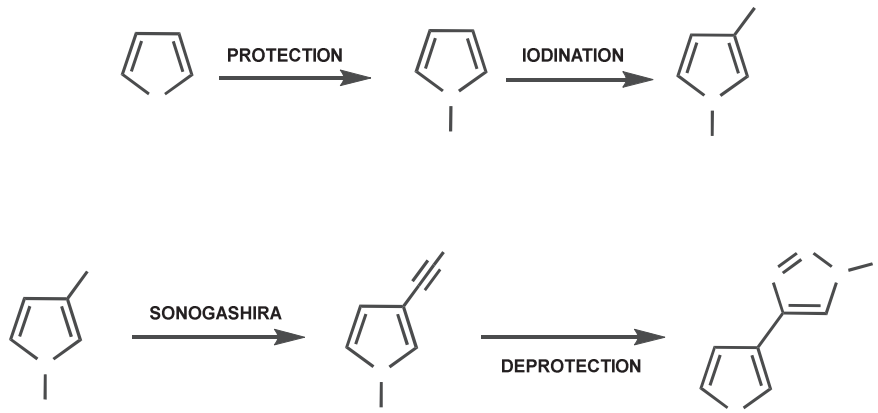

Fig. 3 Synthesis of alkynyl substituted pyrrole for click reaction

4. RESULTS AND DISCUSSION

The CD-PPy covered nanofibers would open the way for versatile conductive material which can bind on its surface biomolecules enhancing cell adhesion, proliferation, activity or possessing other desired features. These nanofibers could be modified by including lipophilic motives of helpful biomolecules into cyclodextrin cavities without the need of covalent bond attachment (**Fig. 4**). Such an approach has many advantages, including better attachment of biomolecules (in comparison with adsorption to plain PPy), easy process of modifying the surface, and stability of included biomolecules. Prepared CD-PPy coated fibers will be loaded with various biomolecules occurring in the culture cell medium (DMEM) enriched by fetal bovine serum (FBS) and antibiotics (penicillin, streptomycin, and amphotericin B). Cyclodextrins immobilized on the PPy surface will include proteins side chains from the culture medium (e.g. alkyl groups, branched alkyl groups or benzene rings) into the hydrophobic cavity and provide highly ECM mimicking environment for cell adhesion. The amount of absorbed biomolecules will be studied by spectroscopic methods and the dependence on cyclodextrin type as well as type of attachment between CD and pyrrole will be studied. These preliminary results will serve as a guideline for CD-PPy scaffold design. Such versatile scaffolds will be thus desired by tissue engineers for above mentioned advantages.

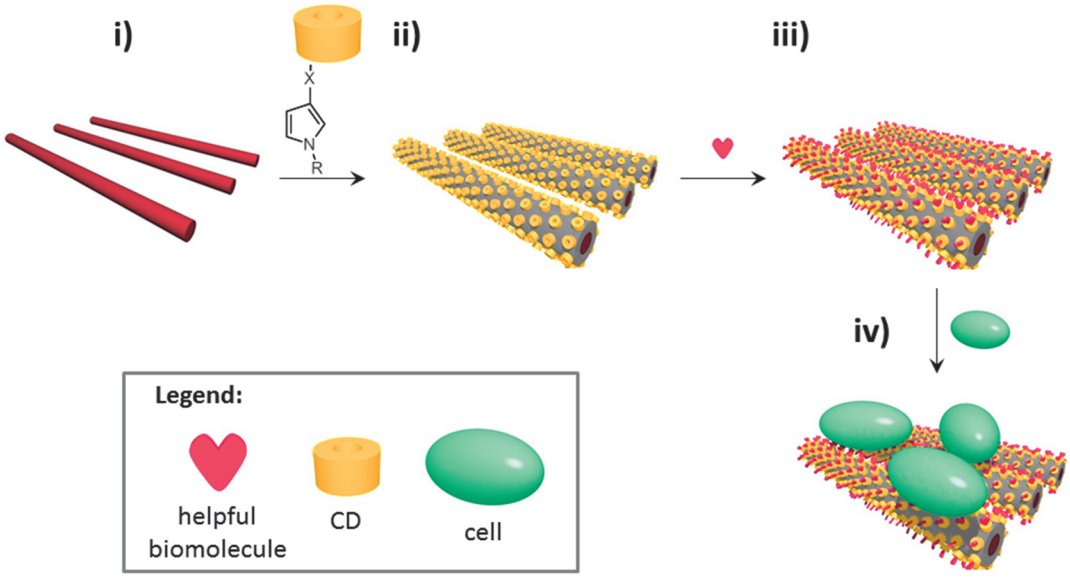

Fig. 4 (*i*) PCL drawing (*ii*) polymerization of Py-CD (*iii*) inclusion of molecules into CD cavity, (*iv*) cell growing

5. CONCLUSION

The fundamental aspect of this research is to create a new and highly sophisticated molecular scaffold for tissue engineering. To fulfil this scientific intention, four main follow-up procedures will be applied i) nanofiber drawing optimization; ii) synthesis of functionalized Py and PPy; iii) modifying PPy derivatives covered nanofibers by inclusion complexes with biomolecules enhancing cell adhesion, proliferation or activity; iv) testing of cell adhesion and viability on prepared scaffolds. During our ongoing research we have successfully completed first two steps and we are ready for immobilization CD-units on the pyrrol using Huisgen [3+2] dipolar cycloaddition reaction. Our research team developed a new method for preparation of aligned PCL micro/nano fibers, using unique semiautomatic „Micromanipulator“ device. We optimized polymerization conditions to produce smooth conductive PPy layer on the PCL fibers, moreover biocompatibility, cell adhesion, proliferation of 3T3 mouse fibroblasts on this combined polymer surface were also evaluated.

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