Biofunctionalised nanofibers based on resorbable poly(ethyleneglycol)-*b*-polyesters for tissue engineering

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Statement of Purpose: Surface immobilized nano-structures with bioactive molecules have attracted great interest for the next generation of biomedical devices. In particularly electrospun cell-nanofibrous structures are promising scaffolds for tissue engineering and regenerative medicine. To further control the interface between the nanofiber and cells, we plan to decorate the fiber surfaces with biological active peptide sequences while minimizing non-specific interactions. A functionalised diblock copolymer was synthesised in a three step synthesis based on biodegradable polyester and poly(ethylene oxide). To promote cell attachment, we covalently coupled the GRGDS peptide sequence to the hydrophilic poly(ethylenoxide) part of the block copolymer. Electrospinning of these copolymers results in nanofibers which suppress unspecific protein adsorption, and enhance cell adhesion. In the present we demonstrate novel electrospun nanofibers based on bioactive block copolymers, which can specifically interact with cells through functional surfaces.

Methods: 3,3-Diethoxipropanol, ε-caprolacone, ethylene-oxide, toluene, methanol, chloroform and diethylether were purchased from Aldrich (Milwaukee, WI, USA). GRGDS was supplied by Bachem (Germany). The biologically functionalized block copolymeres have been synthesized in a three step synthesis (1). (resulting polymer see figure 1).

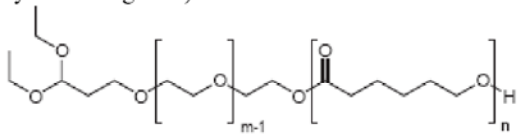

Figure 1: Structure of the protected block copolymer

The polymers were deprotected at acidic conditions (pH 2 for 3 hours) and GRGDS coupled. All polymers were characterised by ^{1}H-, ^{13}C- NMR and GPC. The polymers were dissolved in a mixture of chloroform and methanol (75/25 v/v) to make 2 or 10% solutions for electrospinning. The polymer solution was pumped to the 18-gauge, flat-tipped, stainless steel spinneret at a rate of 0.15 mL/h. The fibers were collected on an aluminium cylinder (diameter 80 mm, length 25 mm), which rotated at 200 rpm, in a 200 mm distance from the tip of the spinneret. A voltage of 25 kV was applied to the spinneret with a Series 205B high voltage power supply (Bertan, NY, USA). The samples were imaged with a Cambridge SEM (S360, Leica) with an accelerating voltage of 15 kV. Rat fibroblasts were seeded onto the electrospun fiber substrate for 24 hours and later stained with mayers haemalaun. Microscope images were taken with a Zeiss Stemi 2000-C.

Results/Discussion: Block copolymers with low polydispersities and molecular weights from 10 to 30 kDa were synthesised (see table 1). The conversion of the monomers into polymer and the complete removal of the acetal group was confirmed with ^{1}H-NMR spectroscopy.

Polymer	*M^{*}_{n} (PEG) /KDa*	*M^{*}_{n} (block copolymer) /KDa*	*M^{+}_{n} (block copolymer) /KDa*	*PDI*	*Yield /%*
1	10	13	10.4	1.37	88
2	10	26	19.8	1.66	86
3	13	22	13.3	1.31	89
4	13	24	15.9	1.32	88
5	13	27.5	24.5	1.72	91

Table 1: Overview of block copolymers, composition and yield (molecular weight M^{*}_{n} determined by NMR, and M^{+}_{n} by GPC with a PMMA standard)

For electrospinning chain entanglement, both the molecular weight and concentration play key roles in producing nano fibers. We investigated different molecular weights copolymers, compositions (hydrophobic and hydrophilic block length) and concentrations for electrospinning these polymers. Solutions with 10% of the block copolymers with molecular weights higher than 26 kDa resulted in homogeneous fibers (figure 2 A). Contact angle measurements suggest that the hydrophilic block is present at the fiber surface. While no cells adhered to the pure copolymer mats (figure 2 B), the bioactive functionalised non-woven showed cell attachment (figure 2 C). We propose that the cell-adhesion promoting peptide is therefore located on the fiber surface of the electrospun fibres.

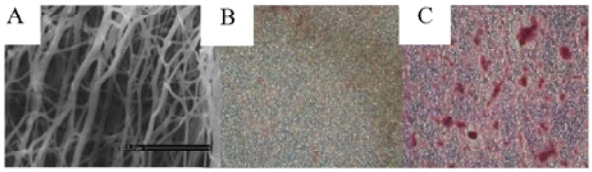

Figure 2. A: SEM-image of block copolymer fibers B, C: microscope images of non-wovens in cell culture (B: without GRGDS, C : with GRGDS)

Conclusions: We produced bio-functionalised electrospun fibers with specific peptide sequences that are suitable for artificial tissue engineering scaffolds.

References: Otsuka H.: Biomacromelecules, 2000;1:39-48.

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