# Biofunctional Star PEG Coatings on 3 Dimensional Polyvinylidene fluoride Scaffolds for Specific Cell Adhesion

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**Statement of Purpose:** One of the main problems of textile scaffolds for tissue engineering or textile implants are unspecific interactions of proteins and cells at the biomaterial surface. Concerning the controlled interaction of polymeric scaffolds with cells and living tissue, a rational design of biomimetic surfaces will provide boil-ogical signals to control the adhesion and proliferation of cells and simultaneously prevents unspecific protein adsorption. Hydrogel coatings are widely used to suppress the unspecific interaction of proteins and cells. Otherwise the design of biofunctional surfaces with incorporated RGD-peptide sequences is often described to achieve better cell attachment to biomaterial surfaces. In the presented work unmodified and GRGDS modified six arm PEG star based hydrogels (star PEG) have been applied as a multi-functional easy to handle coating system for textile Polyvinylidene fluoride (PVDF) scaffolds, which either prevents unspecific protein and cell adsorption or controls specific cell adhesion.

**Methods:** PVDF (Solvay) is used as 3D textile mesh or as PVDF spin coated 2D glass substrate to develop the coating system. First microwave induced ammonia plasma treatment was performed to introduce amine groups onto the PVDF surface. For immobilisation of the hydrogel onto the amino functionalised PVDF solutions of water/THF (9ml/1ml) containing 100 mg six arm star shaped prepolymers consisting of statistically polymerised ethylene oxide and propylene oxide in the ratio 4 to 1 and functional isocyanate endgroups ($M_n$ = 12 kDa) were prepared and reacted with the PVDF surface [1]. Spin coating was used for 2D amino functionalised substrates, dip coating for 3D meshes. GRGDS modifcation of the star PEG layer was performed by treatment with a water solution of GRGDS (50 mg/ml) directly after star PEG coating. GRGDS is immobilised to the star PEG layer by its residual isocyanate endgroups. All modification steps were verified by means of X-ray photoelectron spectroscopy (XPS). GRGDS modified surfaces were characterised by TOF-SIMS. Protein adsorption studies were done by surface-MALDI-TOF-mass spectroscopy with insulin, lysozyme and albumin and by fluorescence microscopy with fluorescent labeled proteins (Avidin Texas Red®, Albumin Rhodamine B). The influence on cell adhesion and proliferation was investigated by means of *in vitro* studies of human dermal fibroblasts on 2D- and 3D PVDF substrates for 1, 4 and 9 days. Cell attachment was evaluated by light and fluorescence microscopy after cells were stained by life/dead and/or haematoxylin staining.

**Results/Discussion:** The star PEG coating has been successfully applied to ammonia plasma treated PVDF surfaces. The water coating process results in a covalent binding to surface amino groups and a crosslinking of the PEG chains via urea groups. In comparison to amino-functionalised PVDF surfaces star PEG grafted surfaces show an increase in nitrogen and a decrease in fluorine by XPS. After immobilisation of GRGDS onto the star PEG layer positive and negative fragments of the immobilised RGD-sequence are detected by TOF-SIMS. Surface-MALDI-TOF-mass spectra show no adsorption of insulin, lysozyme and albumin onto pure star PEG and GRGDS functionalized star PEG coated 2D-PVDF substrates. Additionally GRGDS functionalised star PEG coated 2D- and 3D-PVDF-substrates show severely decreased fluorescence intensity after treatment with Avidin Texas Red® and Albumin Rhodamine B. Long term cell culture experiments with human dermal fibroblasts on GRGDS star PEG modified 2D- and 3D-substrates show advanced cell adhesion and proliferation while on pure star PEG coated surfaces no cell adhesion and on untreated PVDF surfaces slow cell adhesion was observed (see Figure 1)

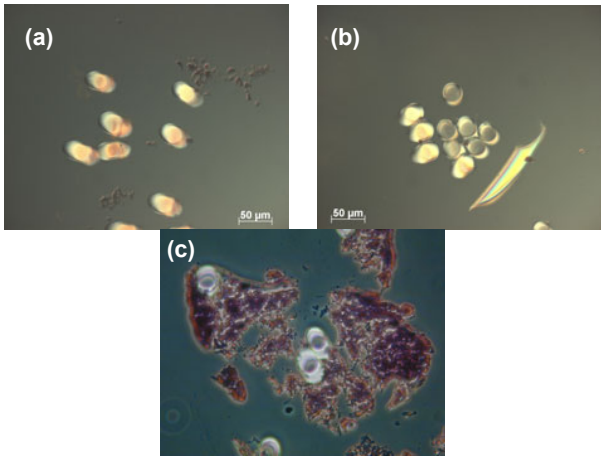


Figure 1. Micrographs of cross sections of PVDF mesh filaments after 9 days cell seeding of human fibroblasts: (a) untreated PVDF, (b) $NH_3$ plasma and star PEG coated PVDF, (c) $NH_3$ plasma and GRGDS modified star PEG coated PVDF

**Conclusions:** The easy application of the star PEG coatings as well as the combination of protein and cell repellent properties and their specific biofunctionality provides large potential for further applications in surface modification of 3D-scaffolds for implantation.

**References:** [1] Groll J., J. Biomed. Mat. Res. Part A. 2005; 75A, 4: 607-617.

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