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P123 (EI0199)

ANALYSIS OF CERAMIC AND POLYMERIC MEMBRANES USING FLUORESCENT-LABELED ENDOTOXIN

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Objectives: Endotoxins are components of the bacterial cell wall and are found in almost all fluids even in those which are poor of nutrients. This is a huge problem for medical and pharmaceutical applications, because already small amounts of endotoxin cause strong immune reactions. Endotoxin removal is possible by e.g. filtration. Therefore normally polymer membranes are used. The disadvantage of these membranes is their short life time. An alternative are ceramic membranes because they are inert and long-lasting. This work investigates polymeric and ceramic membranes concerning their ability of endotoxin removal from aqueous solutions e.g. dialysis water. Beside the analysis of endotoxin removal with conventional LAL-test fluorescence-labeled endotoxins and microscopic analysis of the membranes have been done.

Methods: Several polymeric adsorber membranes and ceramic membranes were investigated concerning their endotoxin removal ability. Therefore membranes were loaded with aqueous endotoxin solutions (0-1000 EU/mL) and tested in cross-flow and dead-end modus. The permeat samples were analyzed by the Limulus Amoebocyte lysate (LAL) test. The endotoxin removal of a membrane was classified as sufficient at permeat endotoxin levels under 0.25 EU/mL. For microscopic analysis the membranes were loaded with fluorescence-labeled endotoxin, embedded and analyzed via fluorescence microscopy.

Results: Although adsorber membranes showed good endotoxin binding capabilities the endotoxin removal was insufficient. Ceramic membranes showed significant better endotoxin separation. Microscopic analysis showed that in polymeric membranes endotoxin could penetrate until a depth of 25µm, whereas in ceramic membranes the penetration depth was only 4µm.

Conclusions: Microscopic analysis of endotoxin filtration gives a deeper understanding in the separation behavior of the investigated membranes. Further fouling and capacity of the membranes can be observed directly. In the future a quantitative correlation of the endotoxin amount on the membranes should be established by measuring the fluorescence intensity of the labeled endotoxin.

P124 (EI0197)

ELASTOMERIC PHOTOPOLYMERS BY THIOL-ENE POLYMERIZATION FOR VASCULAR TISSUE ENGINEERING

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Objectives: Diseases of the cardiovascular system account for a significant number of morbidities and mortalities in developed counties. Hence, the need of suitable materials for artificial replacements for damaged blood vessels arose over the last decades, especially in the field of narrow blood vessel substitutes. This research focuses on the fabrication of blood vessel substitutes based on elastomeric photopolymers.

Methods: Additive manufacturing technologies (AMTs) such as digital light processing are very capable techniques for the fabrication of constructs with complex geometries and high resolution mimicking the cellular structures of biological materials. For the application as blood vessel substitutes, polymers possessing urethane groups are interesting candidates since they exhibit elastic properties and also show good biocompatibility. Various urethane oligomer acrylates were tested in combination with different monofunctional acrylates as reactive diluents. In order to match the material properties of native blood vessels, a combination of low crosslink density and high urethane group concentration was desirable. To accomplish this, dithiols were added to formulations allowing the resulting thiol-ene reaction to compete with acrylate homopolymerization and thus lower crosslink density.

Results: The high content of urethane groups caused a high density of reversible crosslinks due to H-bonds. With this polymer architecture the material had elastomeric properties comparable to native vascular tissue and exhibited similar tensile strength and suture tear resistance tests. Due to the hydrolytically cleavable ester bonds along the back bone and the branches, these polymers possess an inherent degradability similar to that of poly(lactic acid). The polymers also exhibited good endothelial cell attachment, which is crucial for the long-term performance of the vascular grafts.

Conclusions: Structuring photopolymers by means of AMT enables the fabrication of artificial grafts with complex geometries. Implementation of the thiol-ene concept leads to materials with both highly elastomeric mechanical behavior and good biocompatibility.

P125 (EI0368)

2D BI-LAYER SCAFFOLDS OF POLYCAPROLACTONE AND CHITOSAN β-GLYCEROL-BASED FILM FOR BLOOD VESSEL CONSTRUCTS

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Objectives: The objective of this study was to develop a scaffold model aiming at fabricating small diameter blood vessel grafts with distinct surface properties. This study was designed to evaluate the influence of the scaffold properties on endothelial and smooth muscle cells.

Methods: The scaffolds consisted of either a polycaprolactone (PCL) nanofibre mesh (NF) layer fabricated by means of electrospinning or a PCL membrane fabricated by solvent casting (SC); and a second layer prepared from a mixture of β-glycerol phosphate salt (GP) and chitosan (Ch). Scaffold characterization was performed in terms of surface topography (SEM) and mechanical properties (tensile, Young's tensile and yield stress; and strain at break). For the biological evaluation endothelial and smooth muscle cells isolated from the vein of human umbilical cord (HUVECs and HUVSMCs) were used. Single cell cultures were established for both cell types and both scaffolds up to 7 days. Cell behavior was evaluated after DNA quantification, alkaline phosphatase activity, methylene blue staining and SEM.

Results: The tensile strength values for both SC PCL and NF PCL scaffolds exceeded the one of natural artery (15MPa vs. 3MPa vs. 1MPa). As expected no alkaline phosphatase activity was detected in the cultures. Moreover, HUVECs attachment and proliferation rate was significantly higher on the SC PCL layer while for HUVSMCs the opposite was observed and the NF PCL layer was the preferable substrate for adherence and growth.

Conclusions: Scaffolds with mechanical properties capable of withstand the physiological vascular conditions were obtained. The GP layer did not cause any sign of calcification, which constitutes a good indicator for its incorporation within the blood vessel scaffold. The selective response of each cell type to a specific surface topography allows the definition of the design of a blood vessel graft combining HUVECs and HUVSMCs in the opposite layers.

P126 (EI0270)

ORIENTATION OF ELECTROSPUN FIBERS BY MINIMIZING JET INSTABILITIES

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Objectives: Basic electrospinning setups suffer from random fiber deposition conditions thus limiting their applications. The aim of this work was to minimize the bending instability of the jet in order to produce scaffolds with predesigned geometries.

Methods: Polyurethane was electrospun on a horizontal oscillating and rotating conductive aluminum mandrel. The required electrostatic field essential to overcome the surface tension of the polymer solution was complemented by an auxiliary gradient field. This was generated by two additional electrodes that were symmetrically positioned around the spinning nozzle and operated with adjustable high voltage. The effect of the auxiliary electric field was characterized by comparing fiber deposition at different surface velocities, spinning times, with and without auxiliary electrodes.

Results: Without the auxiliary electrodes only poor fiber alignment was possible. By introducing the auxiliary gradient field it was possible to minimize jet instabilities and improve fiber alignment. Fiber deposition took place in a focusable plane between the auxiliary electrodes. After 5 minutes' spinning on a rotating mandrel the fiber deposition could be focused to a 3mm width area. Oriented fiber deposition was achieved with the auxiliary electrodes at target velocities starting from 1m/s for the first deposition layer. For longer lasting spinning durations surface velocities up to 6m/s were necessary to achieve aligned and straight fibers.