DEVELOPMENT OF POLYESTER COATINGS ON POLY(ETHYLENE TEREPHTALATE) PROSTHESES FOR VASCULAR TISSUE ENGINEERING

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Introduction
Cardiovascular diseases are a serious problem nowadays, causing the highest percentage of deaths each year in the world. Despite successful application of commercially available vascular prostheses made of expanded polytetrafluoroethylene (ePTFE) or poly(ethylene terephthalate) (PET), there is a need of their surface modification, to improve cell adhesion and neointima creation [1]. Currently, innovative polyesters of citric acid and diols (polyalkylene citrates, PAC) are intensively investigated due to their biocompatibility with human tissues, controllable mechanical properties and degradation kinetics. The use of such polyesters could significantly improve functionality of vascular prostheses [2]. The aim of this study was to develop an optimal modification of vascular prostheses made of PET with poly(octamethylene citrate) (cPOC) in order to improve their biological properties.

Materials and Methods
Poly(octamethylene citrate) (POC) was synthesized in polycondensation reaction of citric acid and 1,8-octanediol in a molar ratio of 2:3, at 140°C for 40 min, and later it was purified and freeze-dried, to obtain POC prepolymer. Samples of PET prostheses (1 cm x 1 cm) were covered with an undiluted prepolymer (100%) as well as 30%, 15%, 7.5% and 2.5% ethanolic prepolymer solutions using dip-coating method and cross-linked at 80°C, 200 mbar for 10 days, resulting in crosslinked POC (cPOC) coatings on prostheses. The samples were weighed before and after the prepolymer application, as well as after cross-linking process to determine the final ratio of the cPOC coating. The effectiveness of polymeric coverage was tested using SEM and FTIR-ATR methods. For further studies involving L929 cells, the sample covered with 2.5% POC solution was chosen. Cells at a density $15x10^3$ were seeded on the samples unmodified and modified with cPOC, and also on the control sample (TCPS), and incubated at 37°C, 5% CO_2. After 1, 3 and 7 days the live-dead staining was performed and the cells were observed using fluorescence microscopy (Axiovert Zeiss 40 CFL). Metabolic activity test (Alamar Blue) was performed, and data were statistically analysed with t-test.

Results and Discussion
The weight of the cPOC layer on samples after crosslinking decreased significantly, what was caused by evaporation of the solvent and by elimination of water molecules during polycondensation of the prepolymer. Coating with more concentrated POC solution resulted in formation of thick layers comprising up to 40% of the total sample weight, what negatively affected the flexibility and shape of the prosthesis. 2.5% POC was found to be the optimal solution concentration for prosthesis coverage, resulting in a layer comprising of 10% of the total sample weight. FTIR-ATR studies confirmed the presence of cPOC on PET prosthesis. SEM pictures revealed that the coatings were inhomogeneous in some places. Biological studies with L929 cells showed no cytotoxic effect of the samples coated with cPOC polymer. The pH of the culture medium during incubation of the samples remained constant within the correct physiological range (~7). It suggests that potential release of POC unreacted monomers or degradation products did not cause acidification of the surrounding environment. Live-dead staining revealed a huge amount of living cells on the surface of the modified and unmodified protheses. The results of Alamar Blue test showed that cells grown on a modified sample exhibited slightly higher metabolic activity than those on the unmodified one. In addition, cells found on the bottom of the well were developing correctly, indicating that the presence of the samples did not have a negative and cytotoxic effect on cell proliferation.

Conclusions
In this work, an attempt to develop a stable cPOC coating on PET vascular prosthesis using dip-coating method was made. The best concentration of POC in ethanol to prepare coating was chosen and then used to produce the samples for biological studies with L929 cells. The polymer synthesis and the process of prosthesis modification resulted to be simple to carry out and the necessary reagents were cheap and easily available. SEM and FTIR-ATR studies confirmed presence of the coating on the PET prosthesis samples. Experiments with L929 cells showed no cytotoxic character of examined cPOC layer, however, there is a need to improve the process of seeding the cells on the material due to its hydrophobic character. Future research will be focused on cPOC modification aimed on the introduction of anti-oxidant properties.

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