NOVEL APPROACH FOR UTILIZATION OF POLY(α-ESTERS) AND MESENCHYMAL STEM CELLS IN VASCULAR TISSUE ENGINEERING

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Introduction
Mesenchymal stem cells (MSCs) are one of the promising stem cells type that may be used in biomedical applications. MSCs are characterized by e.g. huge proliferative and multi-lineage differentiation capacity. Moreover, their low immunogenicity potential makes them suitable for auto- and allo-transplantation with low risk of rejection. Recently, innovative approaches which improve regeneration process, are a wide of interest. They involve utilization of scaffolds composed with MSCs and biomaterials. Thus, the aim of the study was to evaluate the potential of FDA approved poly(α-esters) such as polylactide (PLA) and polycaprolactone (PCL) as a biocompatible and nontoxic substrate for MSCs in *in vitro* culture.

Materials and Methods
In the study we investigated the influence of bioresorbable PLA and PCL polymer-based substrate on morphology, biology and functions of MSCs derived from human umbilical cord Wharton's jelly (hUC-MSCs).
5% polymer solutions were prepared by dissolution of PCL and PLA in glacial acetic acid and dioxane, respectively. Polymers were characterized with scanning calorimetry, X-ray diffractometry and atomic force microscopy techniques. Thin layers of PLA and PCL solutions were poured into culture plate and left for 96h to evaporate the solvents. Prior to cell culture, the PCL and PLA films were rinsed with cell culture medium supplemented with antibiotics.
hUC-MSCs were cultured in DMEM/F12 medium supplemented with 10% FBS, at 37°C with 5% CO_2. The morphology of cells cultured on PLA and PCL surfaces, was examined by several microscopy techniques: light microscopy, fluorescence microscopy and scanning electron microscopy. Cell motility was quantitatively examined via on-live movie recording during cell culture. Time-lapse images were acquired every 10 min up to 30 hrs. Proliferation rate was assessed for every 24 hrs, up to 72 hrs. Cells viability and apoptosis were analyzed by flow cytometry analysis. Furthermore, the influence of PLA and PCL on hUC-MSCs angiogenic differentiation was evaluated through gene expression level by real-time PCR technique.

Results and Discussion
The results demonstrated that both analyzed polymers (PCL and PLA) constitute non-toxic substrate for hUC-MSC growth. Microscopic analysis of hUC-MSCs morphology indicated that presence of PLA and PCL slightly induced stress fibres formation in hUC-MSCs. Data revealed that proliferation rate was partially reduced but analyzed polymers do not affect cell viability. It may be directly associated with properties of culture surfaces e.g. surface topography, crystallinity, wettability and mechanical properties. Moreover, analysis of cells trajectories revealed, that PCL stimulate hUC-MSCs motility by increasing cell speed and total length of cell distance.
Interestingly, the results strongly indicated that the physicochemical properties of culture surfaces play crucial role in hUC-MSCs differentiation process. Our observations suggested that PLA and PCL polymers may preferentially promote spontaneous differentiation of hUC-MSCs towards angiogenic cells. It was confirmed by gene expression analysis of angiogenic cell markers.

Conclusions
In this study we revealed that PLA and PCL constitute suitable substrates for hUC-MSCs culture. Furthermore, we indicated that analyzed polymers may induce spontaneously differentiation of hUC-MSCs into angiogenic cells. We propose novel approach of utilization of scaffolds combined with PLA and PCL polymers and hUC-MSCs in vascular tissue reconstruction.

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