DIFFERENT BEHAVIOUR OF ADIPOSE TISSUE-DERIVED STEM CELLS AND VASCULAR SMOOTH MUSCLE CELLS ON MODIFIED POLY(L-LACTIC ACID) FOILS

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Introduction

Nowadays, damaged blood vessels can be replaced by biological grafts or synthetic prostheses. Due to the limited availability of biological grafts, current vascular tissue engineering focuses on various modified polymers with mechanical and biological properties similar to blood vessels. Poly(L-lactic acid) (PLLA) is a biocompatible and slowly degradable polyester; however its relatively high hydrophobicity can impair cell-material interactions as well as later positive interactions with surrounding tissues in human body [1].

Vascular smooth muscle cells (VSMCs) are a major component of the *tunica media* (i.e. a middle layer of blood vessels). Adipose tissue-derived stem cells (ADSCs) are mesenchymal stem cells which can be harvested in large quantities and can be later differentiated towards VSMCs by medium composition, biomaterial properties and/or dynamic culture conditions [2].

The aim of our work was to study the growth, behaviour and differentiation of ADSCs and VSMCs on variably modified PLLA foils which can be later used for vascular tissue engineering purposes.

Materials and Methods

Biopolymer PLLA (crystallinity of 60-70%, density of 1.25 g.cm^{-3}, the thickness of 50 µm (± 20 %), purchased from Goodfellow, UK) was used. The PLLA were variably modified and the studied samples were as follows: pure PLLA, plasma-treated PLLA (PLLA240), plasma-treated PLLA grafted with polyethylene glycol (PEG), plasma-treated PLLA grafted with dextran (Dex), and control tissue culture polystyrene (PS).

The samples were sterilized in 70% ethanol for 1 hour, washed with PBS, and then the ADSCs or VSMCs were seeded in a growth medium, i.e. DMEM + 10% foetal bovine serum (FBS). The growth medium was partly changed with differentiation medium on day 4 (for ADSCs) or on day 7 (for VSMCs). The differentiation medium contained DMEM + 2% FBS, TGF-ß1, and BMP4, and was changed twice a week with an overall culture time of 3 weeks.

The metabolic activity of the cells was measured by resazurin conversion assay. The immunofluorescence staining was used to visualise initial cell morphology and to detect the early, mid-term and late markers of differentiation towards VSMCs, i.e. α-actin, calponin and SM-myosin heavy chain (SM-MHC). The differentiation was quantified by RT-qPCR.

Results and Discussion

The initial metabolic activity of ADSCs was similar on all samples. Moreover, this tendency was the same in later time intervals when the cells were cultured in the growth medium. However, when the differentiation medium was added to ADSCs, the metabolic activity was higher on PLLA240 and control PS than on pure PLLA, PEG and Dex samples. The initial metabolic activity of VSMCs was the lowest on pure PLLA. The PLLA240 and control PS samples showed higher metabolic activity of VSMCs than the other samples. The addition of differentiation medium to VMSCs decreased the metabolic activity; however the trend of metabolic activity values remained the same. On day 1, the cell morphology revealed almost the same morphology characteristics of ADSCs on all samples (FIG. 1). In contrast, the VSMCs were visibly round and showed higher circularity on pure PLLA and Dex samples than on PLLA240, PEG, and control PS (FIG. 1).

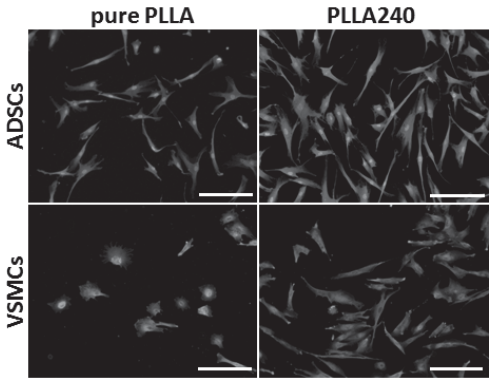

FIG. 1. The morphology of ADSCs and VSMCs on pure PLLA and plasma treated PLLA (PLLA240) on day 1 after seeding, scale bar 100 µm.

The addition of differentiation medium to ADSCs supported the differentiation towards VSMCs on all samples which was proved by the positive immunofluorescence staining of α-actin and calponin, and sporadically also by positive staining of SM-MHC. These results were quantified by increased gene expression of α-actin, calponin and smoothelin.

Conclusions

In our study, we proved the biocompatibility of pure and modified PLLA in three-week cell culture. The modification of pure PLLA improved the adhesion, metabolic activity, and growth of the cells. With appropriate medium composition, the differentiation of ADSCs towards VSMCs was successful on all samples. We observed different behaviour of ADSCs than of VSMCs on samples and also different behaviour within one cell type depending on the medium composition.

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