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

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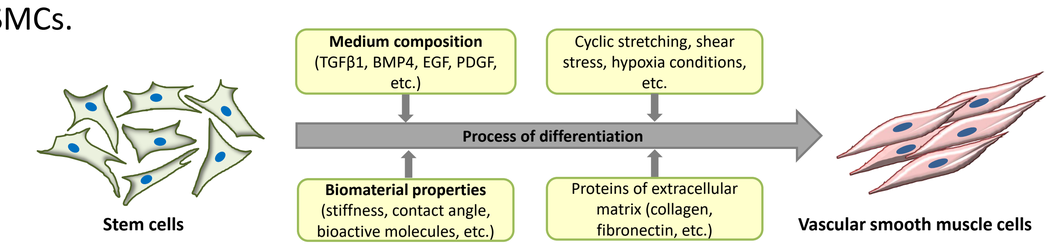
INTRODUCTION

Poly (L-lactic acid) (PLLA) is a biocompatible and slowly degradable polyester. **Plasma treatment** can increase the cell attachment and cell growth by lowering the contact angle of materials.

Polyethylene glycol (PEG) and **dextran (Dex)** have several positive properties (i.e. non-thrombogenicity, non-immunogenicity, low-toxicity, hydrophilicity, suitable mechanical properties).

Vascular smooth muscle cells (VSMCs) are a major component of the *tunica media* (i.e. the middle layer of blood vessels).

Adipose tissue-derived stem cells (ADSCs) are a promising cell source for vascular tissue engineering, because they can be relatively easily differentiated towards VSMCs.



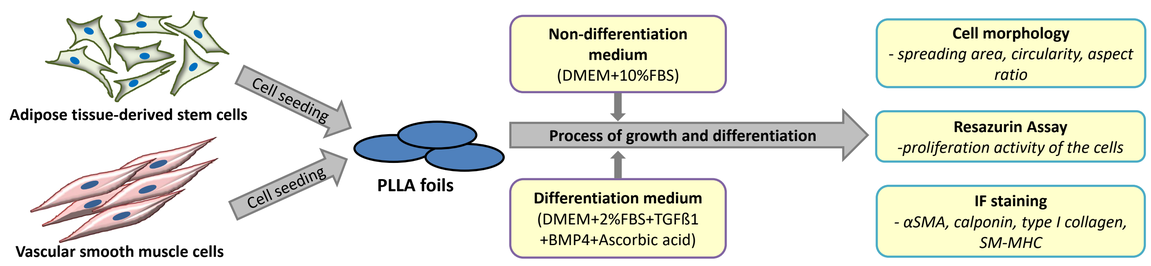
Scheme 1. Culture conditions influencing the differentiation of ADSCs towards VSMCs

AIM OF THE STUDY

In the present study, we focused on ADSCs and VSMCs behavior on modified PLLA foils in combination with appropriate differentiation medium. These findings can be later used in vascular tissue engineering.

MATERIALS AND METHODS

- The tested samples were as follows:
  - Pure PLLA (**PLLA**)
  - Plasma-treated PLLA (**PLLA240**)
  - Plasma-treated PLLA grafted with PEG (**PEG**)
  - Plasma-treated PLLA grafted with Dextran (**Dex**)
  - Tissue culture polystyrene (**PS**)
- The ADSCs were seeded in DMEM medium with 10% of FBS and 10 ng/mL of FGF2. The VSMCs were seeded in DMEM with 10% FBS.



- From day 4 (ADSCs) or day 7 (VSMCs), the medium was changed either for non-differentiation (_nd) or differentiation (_d) one. The differentiation experiment was adjusted for 21 days (ADSCs) and for 24 days (VSMCs), and the medium was being replaced twice a week.
- The **cell morphology** (spreading area, circularity) was measured by Image J software according to microphotographs taken on day 1.
- The **metabolic activity** was evaluated by resazurin assay. The fluorescence of a resazurin conversion to resorufin was measured.
- To visualize the **protein expression** of markers of VSMCs differentiation, the cells were immunostained for **αSMA** and **calponin,** the early and mid-term markers of differentiation. The cell nuclei were counterstained with Hoechst 33342.

RESULTS

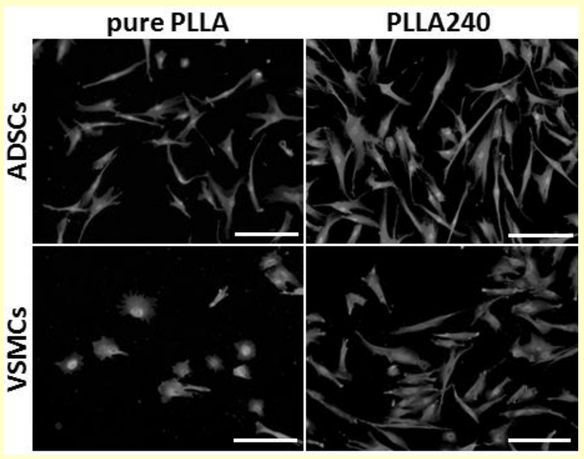


Fig. 1: Cell morphology on day 1, representative images of pure PLLA and plasma-treated PLLA (PLLA240). The ADSCs and VSMCs were stained with Texas Red. Scale bar 200 µm.

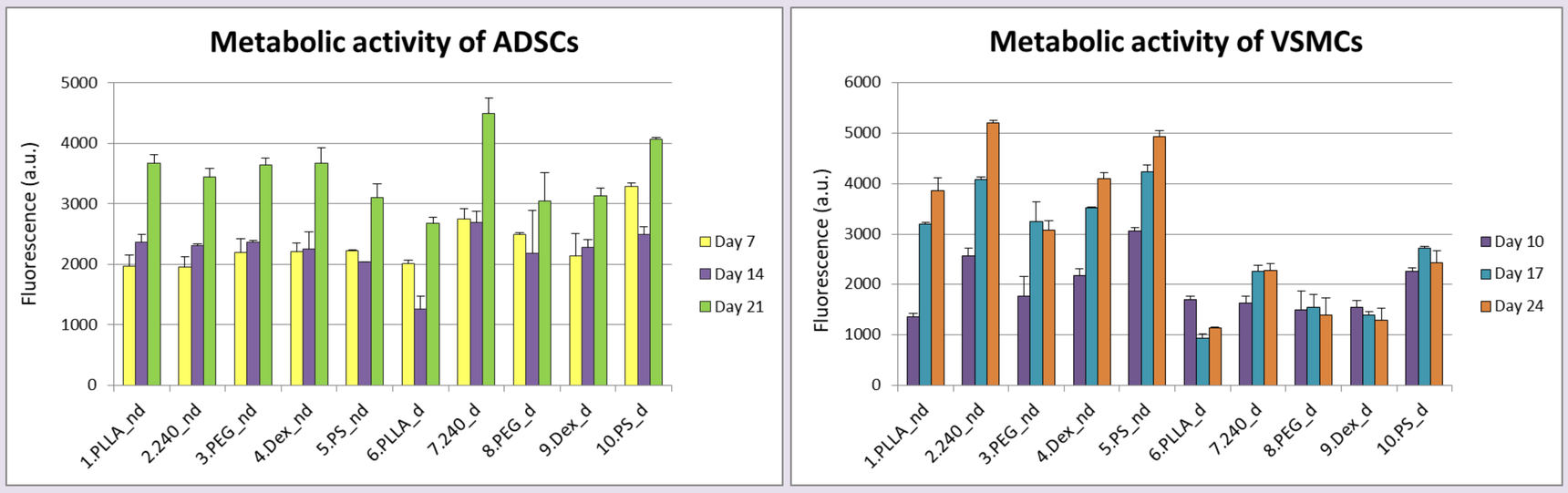


Fig. 2: The **metabolic activity** of the cells estimated by resazurin conversion on days 7, 14 and 21 (for ADSCs) and on days 10, 17 and 24 (for VSMCs). The cells were cultured on the tested materials either in the non-differentiation medium (_nd) or in the differentiation medium (_d). Mean+SD.

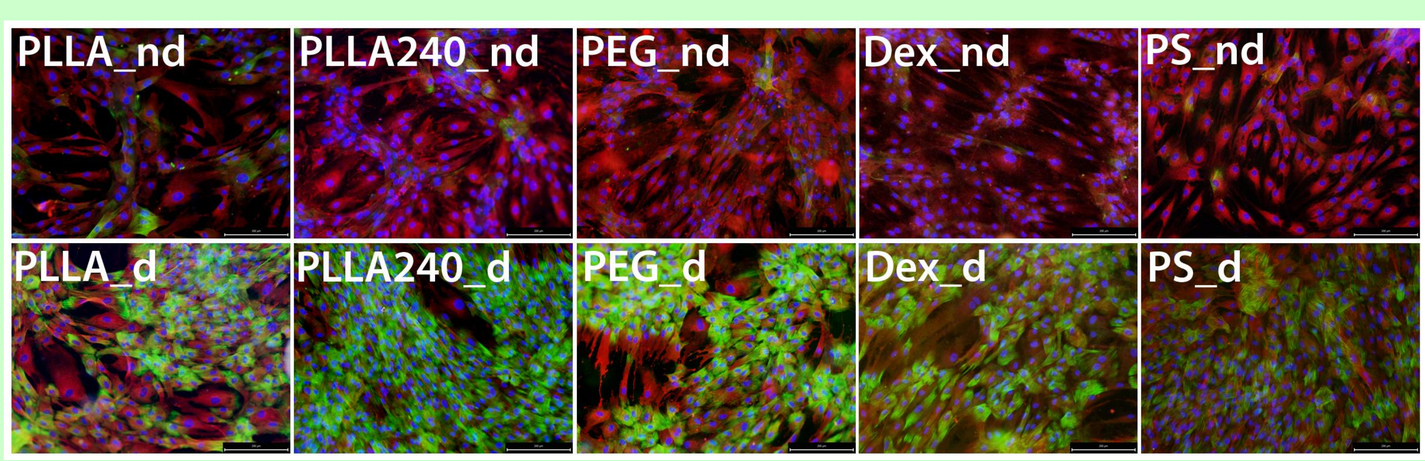


Fig. 3: The **immunofluorescence staining** of **αSMA** (red), **calponin** (green), and cell nuclei (blue) in ADSCs on day 7 of the culture (i.e. 3 days of differentiation). Pristine PLLA (PLLA), plasma-treated PLLA (PLLA240), plasma-treated PLLA grafted with PEG (PEG), plasma-treated PLLA grafted with Dextran (Dex), and control tissue culture polystyrene (PS). The cells were cultured either in the non-differentiation medium (_nd) or in the differentiation medium (_d). Olympus IX 71, objective ×10, scale bar 200 µm.

CONCLUSION

- **The morphology of ADSCs and VSMCs was almost the same on all tested samples except for pristine PLLA.**
- **The metabolic activity of ADSCs was the same on all samples when cultured in the non-differentiation medium. However, it was higher on PLLA240 and PS than on pristine PLLA, PEG and Dex when cultured in the differentiation medium.**
- **The metabolic activity of VSMCs was the lowest on pristine PLLA in both culture media.**
- **The differentiation medium quickly supported the expression of αSMA and calponin at the protein level in ADSCs, which was stable till the end of the cell culture (i.e. day 21) on all the tested samples.**

All tested modifications seem to be promising for vascular tissue engineering.

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