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Qualification of labeled Endothelial Progenitor cells for tracking in the context of tissue engineering

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Abstract

In order to track location and distribution of endothelial cells (ECs) within scaffolds, we chose lentiPGK-TdTomato transduction of human Endothelial Progenitor Cells (EPCs). Because transduction could have a functional impact on cell behaviour, we checked different parameters for qualification of labeled- EPCs. After isolation and expansion, EPCs were transduced with the lentiviral vector containing the TdTomato protein gene. Conventional karyotyping, differentiation capacity, viability, proliferation assays and functional assays were performed with labeled and unlabeled EPCs. Results show that cell labeling did not affect cell adhesion nor induce cell death. Cell labeling did not induce more chromosomal aberrations. Phenotypical characterization was not affected. In the context of tissue engineering applications, labeled EPCs maintained their ability to line scaffolds, withstand physiological arterial shear stress and form tubular networks in co-cultures with human osteoblast progenitor cells. So it is possible to label human EPCs with TdTomato without affecting their behaviour by the transduction procedure. This creates an important tool for vascular and bone tissue engineering.

key-words: Biomaterials, Molecular Imaging, Optical Imaging.

II. MATERIALS AND METHODS

II.1 Isolation and expansion of human cells derived from mononuclear cell (MNC) cultures

EPCs were isolated from cord blood and cultured as previously described by [3].

II.2 EPCs lentiviral transduction (EPCs labeling)

The lentiviral vector contained the TdTomato protein gene under the control of the Phosphoglycerate kinase (PGK) promoter. For viral transduction, $2 \times 10^5$ freshly trypsinized EPCs in suspension were mixed with $6 \times 10^6$ viral particles (MOI: 30). Expression of TdTomato was observed under fluorescent microscope (excitation 554 nm and emission maxima 581 nm).

II.3 Cytogenetic analysis

labeled and unlabeled EPCs were cultured to evaluate chromosomal abnormalities at early and late passages using conventional karyotyping.

II.4 Assessment of endothelial phenotype in EPCs

The following criteria were used for characterization and to control the stability of the endothelial phenotype during the expansion of labeled or not EPCs: i) cellular uptake of UEA-1 lectin, ii) immunofluorescent stainings and flow cytometric analysis for CD31 and vWF according to [2]. For analysis of capillary tube formation, labeled or not EPCs were seeded onto Matrigel\textsuperscript{\textregistered} and observed after 48 h in culture.

II.5 Viability assay

At days 1, 2, 3, 6 and 9 neutral red and MTT assays were performed with cells from 3 different donors at the same passages labeled or not.
II.6 Applications of labeled EPCs for tissue engineering
Several features were checked relating potential applications of EPCs in tissue engineering. To determine whether labeled EPCs maintained their ability to line prosthesis, we used the synthetic vascular graft INTERGARD™ or biological Chitosan-based hydrogels [4]. These prostheses were filled with the labeled or not EPCs. Prosthesis samples endothelialized with unlabeled EPCs were observed in epifluorescence (Live/Dead). To determine the effects of laminar shear stress (1.2 Pa) on labeled EPCs, we used a parallel plate culture flow chamber seeded with the labeled or not EPCs. The angle deviation of cells was quantified. Then, cells were co-cultured with Human Osteoprogenitors (HOPs). Intracellular alkaline phosphatase (ALP) and immunofluorescent stainings for vWF were checked respectively in HOPs and EPCs.

II.7 Statistical analysis
Comparisons between unlabeled and labeled cells were performed by GraphPad Prism® 5: two way ANOVA and Bonferroni post test for proliferation assay and angle deviation, Wilcoxon test for cytometry analysis and Mann-Whitney test for cytogentic analysis. P values less than 0.05 were considered significant.

III. RESULTS

III.1 Assessment of endothelial phenotype in labeled or not EPCs
According to immunofluorescent stainings and cytometry, there was no difference between labeled and unlabeled EPCs (UEA-1+, vWF+, CD31+). Labeled and control cells formed tube-like structures on Matrigel®.

III.2 Cytogenetic analysis
Chromosomal abnormalities were noticed for TdTomato cells but labeling did not induce more chromosomal aberrations.

III.3 Viability assay
Proliferative capacity is not significantly affected by TdTomato labeling.

III.4 Applications of labeled EPCs for tissue engineering
Ability to line prosthesis
Labeling did not alter the propensity of TdTomato EPCs to cover the luminal side of Chitosan-based hydrogels and INTERGARD™ prosthesis (Figure 1).

Figure 1: Confocal microscopy of vascular prosthesis INTERGARD™ seeded with labeled EPCs and unlabeled cells revealed by Live/Dead test.

Effects of shear stress
After 24 hours under shear stress conditions, cells elongated and became orientated compared with static conditions(a), confirmed with angle deviation(b).

Figure 2: microscopic appearance (A to H) of the TdTomato-labeled EPCs (A, C, E, G) and unlabeled EPCs (B, D, F, H) under static (A, B, E, F) or shear stress conditions (C, D, G, H) after 6 hours (A, B, C, D) or 24 hours (E, F, G, H). Angle deviation analysis in each condition (I). **: *p<0.01.

Functionality
In direct co-culture conditions between labeled or unlabeled EPCs and HOPs, a tubular-like network occurred.

IV. DISCUSSION – CONCLUSION
From the present study, it is possible to label human EPCs with TdTomato without affecting the viability and capacities of these cells, suggesting no gross modification of the cell-specific properties by the transduction procedure. This creates an important tool for numerous applications. Our results provide a qualification of labeled EPCs for vascular and bone tissue engineering.

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