SHORT COMMUNICATION

O-13. CHARACTERIZATION OF LABELED PROGENITOR DERIVED ENDOTHELIAL CELLS FOR TISSUE ENGINEERING APPLICATIONS

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Tissue Engineering, Endothelial progenitor cells, labeled cells.

Introduction
Bone Tissue engineering aims to develop a tissue substitute combining osteoprogenitor cells within a three-dimensional matrix (3D) able to promote bone reconstruction. Because vascularization is a crucial process during the growth and development of bone [1], the prevascularization of biomaterial by an association with endothelial cells could enhance angiogenesis and bone regeneration. The inclusion of PDECs (Progenitor Derived Endothelial Cells) to tissue-engineering constructs has become a point of focus not only in vascular tissue engineering [2] [3] but also in bone tissue engineering [4], [5]. However, the delivery and traceability of the cells in 3D matrix represent a problem. Fluorescent labeling by lentiviral infection could be a method to better track their migration and distribution within the matrix. However, infection could influence the viability, proliferation and differentiation capacity of PDECs, which would limit their use. Therefore, the aim of this study was to evaluate the influence of lentiPGK-TdTomato infection on the viability and differentiation capacity of human PDECs for their use in tissue engineering.

Materials and methods
Isolation and expansion of human cells derived from mononuclear cell (MNC) cultures
PDECs were isolated and cultured as previously described by Thébaud et al. 2010 [3] with some modifications.

PDECs lentiviral transduction (PDECs labeling)
The lentiviral vector contained the tdTomato protein gene under the control of the Phosphoglycerate kinase (PGK) promoter. For viral transduction, PDECs (between subculturing 1 and 3) were mixed with 6.106 viral particles (MOI: 30) and cultured with standard procedures. Expression of tdTomato was observed under fluorescent microscope (Zeiss Axiovert 25 CFL microscope, excitation and emission maxima equal 554 nm and 581 nm, respectively).
Characterization of PDECs labeled versus non-labeled
For the different experiments, cells were used until subculturing 3 and 7. The following criteria were used for characterization and to control the stability of the endothelial phenotype during the expansion of these labeled or not PDECs: (1) cellular uptake of UEA-1 lectin, (2)immunofluorescent stainings for CD31, VE-cadherin and von Willebrand factor (vWF) according to [6], (3) Flow cytometric analysis for CD31, CD45 and vWF according to Thébaud et al. [3].
Proliferative assay
Cells from 3 different donors labeled or not were seeded in quadruplicate into tissue culture treated polystyrene 48-well microplates coated with type I collagen at seeding density 10,000 cells/cm^2 at 37 °C. At days 1, 2, 3, 6 and 9 neutral red and MTT assay were performed according to [2] and [3].
Functional characterization
To observe functionality of PDECs labeled or not and to evaluate the capacity of the labeled cells to form cord-like structures as unlabe-

led cells are able to do, cells were cocultured with Human Osteoprogenitors (HOPs). HOPs were obtained according to [7].

Results

The microscopic appearance of the tdTomato-labelled EPCs (Fig 1A) did not differ from that of the unlabelled EPCs (Fig 1B) when cultured, even after subculturing.

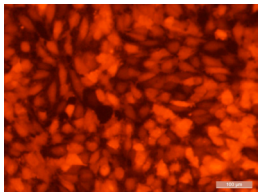

Figure 1A

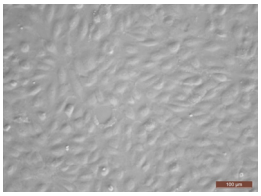

Figure 1B

Characterization of Labeled PDECs

For each PDECs obtained from 3 different donors, the percentage of labeling cells is between 93 and 98% (Flow cytometry analysis). Fluorescence microscopy observations shown there is no difference between PDECs labeled or not for cellular uptake of UEA-1 lectin and immunofluorescent stainings for CD31, VE-cadherin and von Willebrand factor. Flow cytometry analysis confirmed that the tdTomato labeling did not affect the baseline characteristics of EPCs. The surface marker CD45 was found at less than 0.5% of both labeled and unlabeled cells in culture, indicating that the cells were non-haematopoietic. CD31 was found at more than 85% of cells (means 92% and 85% for unlabeled or labeled cells respectively) and vWF was found at more than 90% of cells (means 91 and 94% for unlabeled or labeled cells respectively).

Proliferative assay

Results obtained with MTT or neutral red essay shown that proliferation is not affected by tdTomato labelling.

Functional characterization

Co-culture between HOPs and labeled PDECs promote cord-like structures like it is observed with un-labeled cells.

Discussion

PDECs tdTomato labeling do not alter the expression of specific endothelial markers, proliferation and capacity to form cord-like structures in co-cultures with HOPs.

Conclusions

It is possible to label human PDECs from cord blood with tdTomato protein and to use them for tissue engineering in vitro experiments. Our results provide an improved qualification of PDECs for vascular and bone tissue engineering.

References

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