

Labeling and tracking goat adipose stem cells (g-ASC) with QDOT® Nanocrystals

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Abstract

The use of cell therapy is an alternative to the treatment of various chronic and degenerative diseases unresponsive to conventional treatments. The aim of this study was to evaluate the labeling of subcutaneous goat adipose stem cell (g-ASC) and tracking these cells after infusion in the mammary glands of goats affected by chronic mastitis. The subcutaneous fat was collected on chest of discarded animals. The g-ASC was isolated by mechanic and enzymatic dissociation with collagenase type I (1 mg/mL). The medium used in culture was DMEM-F12 (Gibco®). The g-ASC differentiation in osteogenic, adipogenic and chondrogenic was performed using standardized medium of differentiation (StemPro®) according to the instructions of the manufacturer. Flow cytometry was performed using CD 90, CD 45 and CD 105. For labeling cells was used Qdot Fluorescence Nanocrystals (Qtracker® 655) according to the protocols of the manufacturer. After intracytoplasmic Qdot® inclusion in g-ASC, these cells were prepared for infusion at a concentration of 4×10^6 g-ASC with nanocrystals (g-ASC-Nac) per microliter direct in left mammary gland. A control was performed by injecting phosphate buffered saline (PBS) in the right mammary gland. Biopsies were performed 30 days after infusion. Primary cultures showed high cellularity after 72 hours in culture and 95% of variability. The fibroblastoid morphology was present in 20 days of culture. The study of plasticity showed positive results in all cell types studied. Osteogenic assay demonstrated calcified matrix labeled by alizarin red. Adipogenic study showed lipid granules labeled by Oil Red. The chondrogenic assay was performed in monolayer and showed cell aggregate nodule-like, although Alcian Blue did not stain it. Flow cytometry assay demonstrated only 30% positive for CD45 and CD 90, and negative for CD105. These inconclusive results need to be repeated. The g-ASC-Nac was applied in goat mammary gland at 4×10^6 /mL. Histological biopsies were prepared in paraffin blocks. The processing in paraffin did not prevent the tracking of cells applied in the mammary gland. The g-ASC-Nac injected were tracked by fluorescence microscopy. The process of Qtracker® cell inclusion in the g-ASC is easy to perform, not requiring adjustments in the protocol provided by the manufacturer. The tracing of g-ASC-Nac injected in mammary gland was possible even after 30 days of application, demonstrating a long-term labeling. It is not necessary to perform frozen cuts for tracking cells containing intracytoplasmic Qdots®.

Ethics Committee: UFPI/ CEAC nº 041/11; SISBIO nº 33058/ CEEA-UFPI nº 037/2012.

