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Olfactory mucosa stem cells differentiate into neuron-like cells

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The human body has over two hundred different cell types that are organized into tissues and organs and derived from a single totipotent cell, the zygote, which provides all the functions required for its viability and reproduction. Stem cells are a distinct class of cells, since they preserve to varying extents, their potential for multi-lineage differentiation, depending on the type (embryonic or somatic stem cells). These cells are characterized by their self-renewal and differentiation ability, since they are able to unlimitedly generate daughter cells, similar to themselves but with specific properties. In adults, there are several pools of somatic stem cells, in which the olfactory mucosa derived stem cells are included. In the olfactory mucosa, we find two populations of stem cells, one localized in the epithelium and the other one in the lamina propria.

In the present work we isolated olfactory mucosa stem cells from both rat epithelium and lamina propria as previously described by Girard et al. [1] and Carvalho [2]. Briefly, after isolation of both tissues, they were cultured in adequate culture medium and neurosphere formation was induced by culturing the cells in DMEM/F12 culture medium supplemented with 1% ITS-X, 50ng/ml EGF, 50ng/ml FGF2 and 1% Pen/Strep (Figure 1). These neurospheres are multipotent cells that can have different fates depending on the growing factors present in culture medium and the surrounding cellular microenvironment. Here we differentiated neurospheres using neurobasal medium supplemented with 50ng/ml NGF, 1x B27, 2mM glutamine, 0,025 mM glutamate and 1% Pen/Strep, for 37 days. During this period, several microphotographs were taken at each day of differentiation (Figure 2A) and a morphometric analysis of these cells was achieved by measuring the number and the length of the neuritis (Figure 2B). The total number of the cells in each preparation was also determined. These differentiated cells were fixed and immunolabelled with β-tubulin III and DAPI, and examined as presented in Figure 3.

Our results indicate that we efficiently isolated olfactory stem cells from both epithelium and lamina propria. The stemness properties of the isolated stem cells were confirmed by their ability to generate neurospheres when cultured in an adequate culture medium. Our detailed morphometric characterization of stem cells, cultured in neurobasal medium, indicates that we efficiently differentiated neuropheres into neuron-like cells, since the length of neuritis significantly increased up to the 6^{th} day in culture. The confirmation of the neuronal phenotype induction was achieved by immunostaining of these neuron-like cells with β-tubulin III. These results are of paramount importance since the stem cells represent a very attractive model system for the study of several neuropathologies such as Alzheimer's disease.

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[1] Girard, S.D. *et al.*, *Journal of Visualized Experiments*, **54**, 1-5, 2011.
[2] Carvalho, S.D.B.O., Master Thesis, University of Aveiro, 2012.

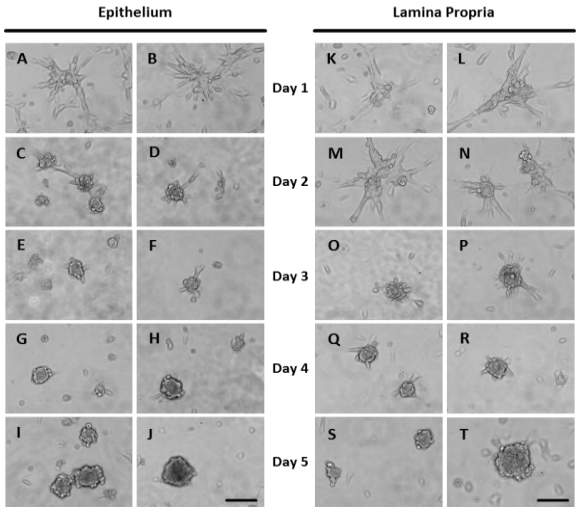

Figure 1 - Induction of olfactory neurospheres formation from epithelium and lamina propria primary stem cultures. Phase-contrast photomicrographs of epithelium and lamina propria derived neurospheres were taken at day 1 (A, B, K and L), day 2 (C, D, M and N), day 3 (E, F, O and P), day 4 (G, H, Q and R) and day 5 (I, J, S and T). Scale bar = 100 µm

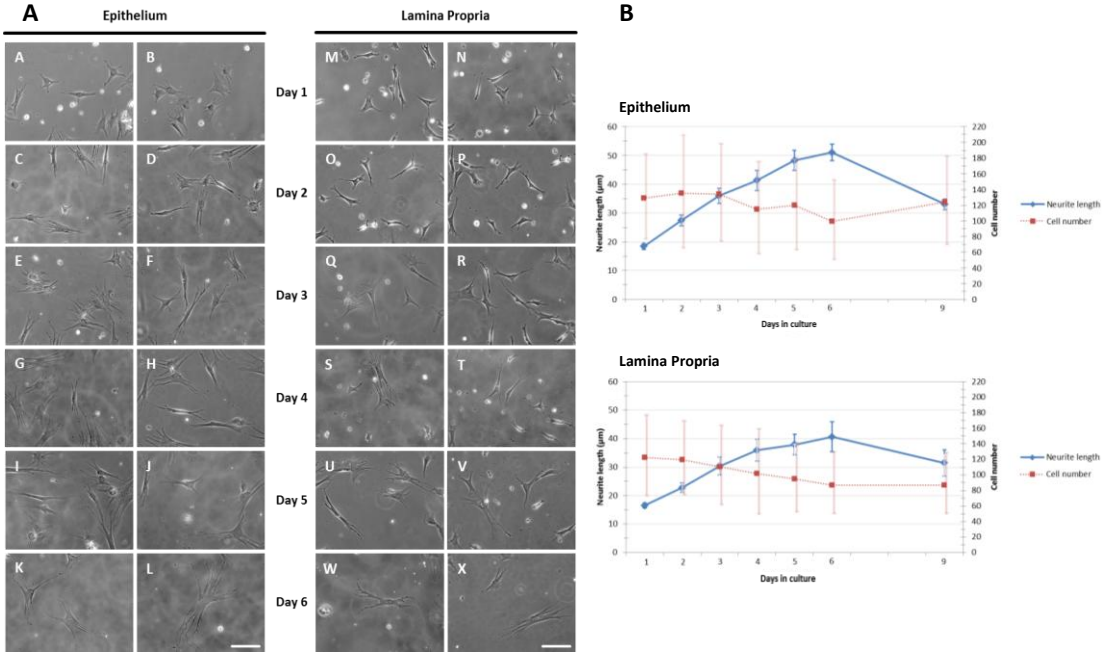

Figure 2 - Neurospheres differentiation into neuron-like cells. A - Trypsin-disaggregated neurospheres were plated with neurobasal medium on 35 mm culture dishes and phase-contrast photomicrographs of EPI and LP derived neuron-like cells were taken at day 1 (A, B, M and N), day 2 (C, D, O and P), day 3 (E, F, Q, R), day 4 (G, H, S and T), day 5(I, J, U and V) and day 6 (K, L, W and X). Scale bar = 100 µm. **B -** Morphometric analysis of neuron-like cells derived from epithelium (A) and lamina propria(B). Images from 10 independent experiments from each cell type were analyzed in terms of neurite length and cell number. Error bars represent the quotient of standard deviation and square rout of the number of samples (in neurite length series) and average deviation (in cell number series).

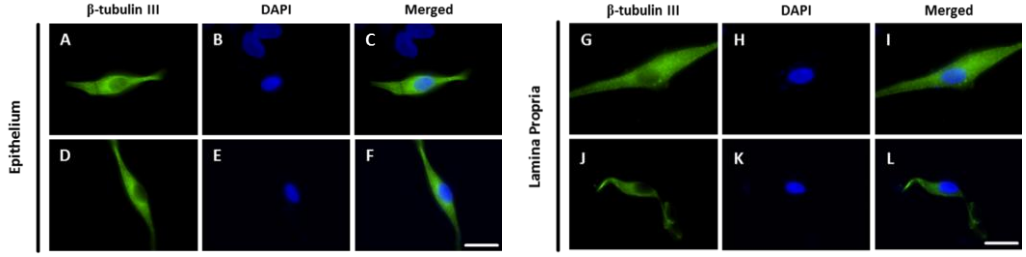

Figure 3 - β-tubulin III immunolocalization in neuron-like cells derived from epithelium (A and D) and lamina propria (G and J) neurospheres. Cell nuclei were simultaneously stained with DAPI (B and E; H and K). The merged images are also presented (C and F; I and L). Scale bar = 20 µm

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