Cultured cells from olfactory epithelium of dogs as a source to regeneration of nervous system

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Adult tissue-specific stem cells have the capacity to self-renew and functional rehabilitation of lost cells through the life. Actually, numerous diseases such as Parkinson's and Alzheimer's disease have been treated using cell therapy and satisfactory results have been obtained. Olfactory epithelium was obtained from cribriform ethmoidal lamina at necropsy of 10 dogs around 1 to 2 hours postmortem. Olfactory epithelium was washed six times with sterile saline solution 0.9%, and transferred (with minimal dissection) into 35 mm Petri dishes (Corning, New York, N.Y., USA) with 100 $U.mL^{-1}$ penicillin, 100 $g.mL^{-1}$ streptomycin (Invitrogen, Cat. # 15140-122). Sections were cut into pieces of about 1 mm^3. Prior to enzymatic treatment, the tissue was passed five times through a pipette (10 mL) and centrifuged (1,000 rpm, 4 °C, 5 minutes). Incubation with trypsin (0.25%; Invitrogen, Cat. #25200-114) in DMEM/F-12 medium (Invitrogen, Cat. #10565-018) was performed for 30 min at 37 °C. The cells were seeded on culture flasks of 25 cm^2 (TPP, Switzerland). Cultures were maintained under standard conditions (5% CO^2, >37 °C) in DMEM Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 (1:1, Invitrogen, Carlsbad, Calif., USA) supplemented with 10% fetal bovine serum (FBS, HyClone, Logan, Utah, USA), supplemented with fetal calf serum (FCS, PAA) and penicillin/streptomycin (1%; PAA). After 7 days theses cells were evaluated to verify the adherence to the bottom of the culture flasks. Cell culture based on isolated cells from the epithelium showed rapid growth when successively cultured. Approximately 24 hours after plating the cells of olfactory biopsies, phase-bright spheres cells were found floating from fragments attached on culture flasks. Within the first week the cell culture was submitted to exchange of the culture medium. Most of the viable cells attached to the surface and assumed a division process that reveals a large potential of growing (image). After 10 day of culture, these cells were collected from the monolayer, washed twice in PBS, dissociated in a 0.25% trypsin solution, and seeded at 104 cells per 25 cm 2 flask. Both proliferation and expansion were noted during the subsequent confluent culture following trypsinization with olfactory cells assuming the tendency to form isolated colonies (image). A large heterogeneous population not be showed, whichever the homogeneity of the colonies composed by cells ellipsoids cells although that showed different sizes, the same morphology. Unlike, Bipolar, fusiform, stellate or spherical shapes. Olfactory stem cell showed to be a potential source to progenitor cells that generate daughter cells with morphological characteristics seen into the Nervous System.