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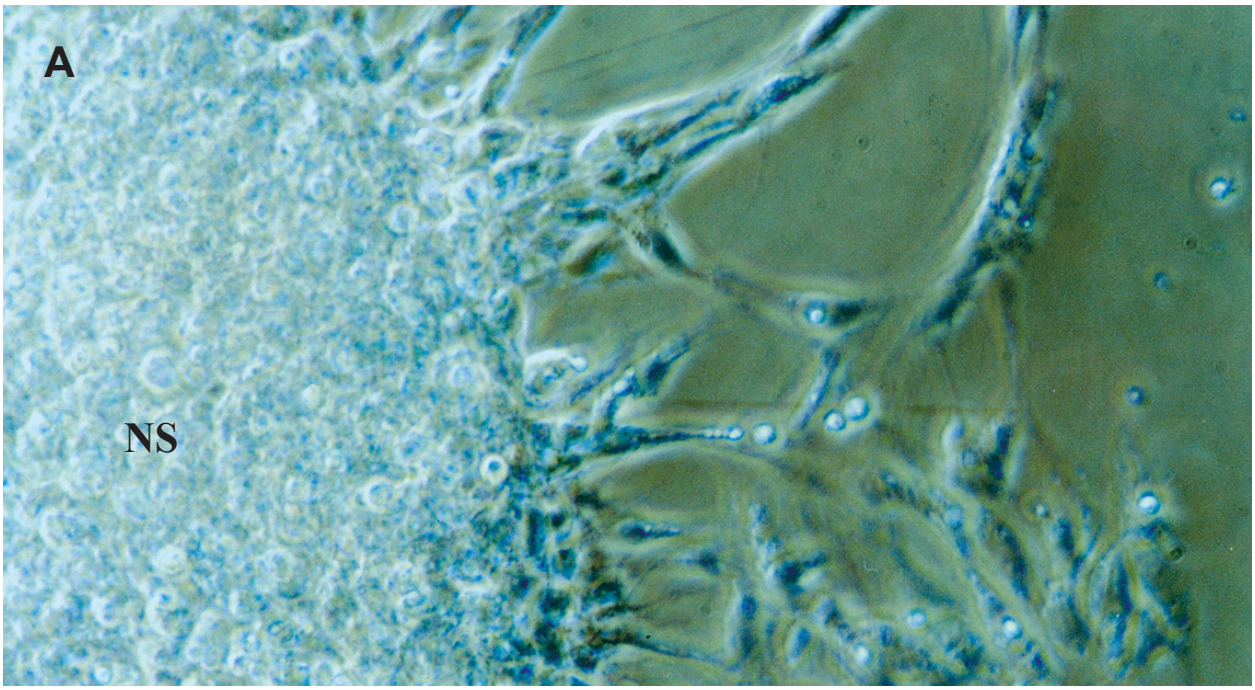
In vitro differentiation of human embryonic neural stem cells

Human neural stem cells not only provide an important source of cells for *in vitro* studies, but they are also the hopeful candidates for cell replacement therapy. Isolation and long term culturing of neural stem/progenitor cells have been advanced by the findings that mitogenic growth factors, epidermal growth factor (EGF) and basic fibroblast growth factor (FGF2), have proliferative effects on these cells.

Neural stem/progenitor cells can proliferate as a monolayer population or non-adherent neurospheres and can produce both neurons and supporting glial cells when *in vitro* plated on coated surfaces [1, 2].



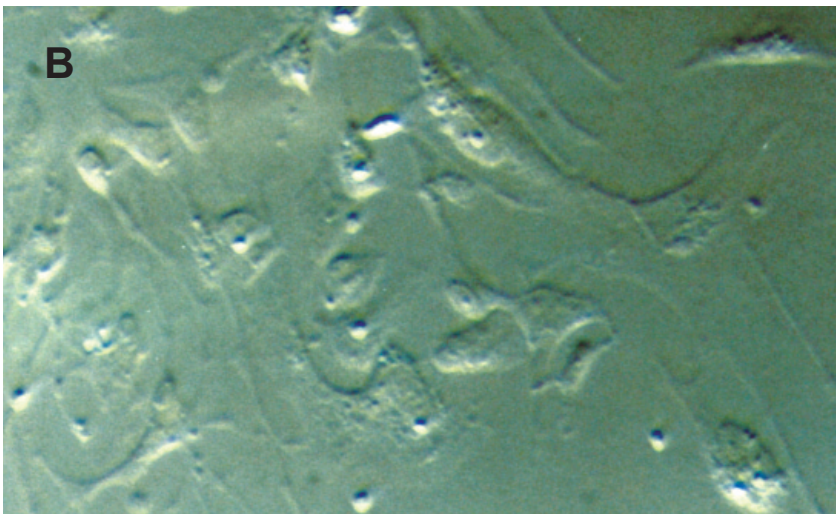


Fig. 1 Phase contrast microscopy. Neural stem/ progenitor cells from developing brain of human embryo (8 weeks, ectopic pregnancy), proliferated in culture (DMEM, FGF-2 (20ng/ ml), EGF (20ng/ml), 10%FCS, L-glutamine, PEST) as non-adherent **neurosphere (NS)** (A) or **monolayer population** (B). After 7 days, cells were induced to differentiate into neurons and/or astrocytes by plating on poly-L-lysine coated plates (DMEM, 10%FCS, L-glutamine, PEST) in the absence of growth factors. Ob. 40x.

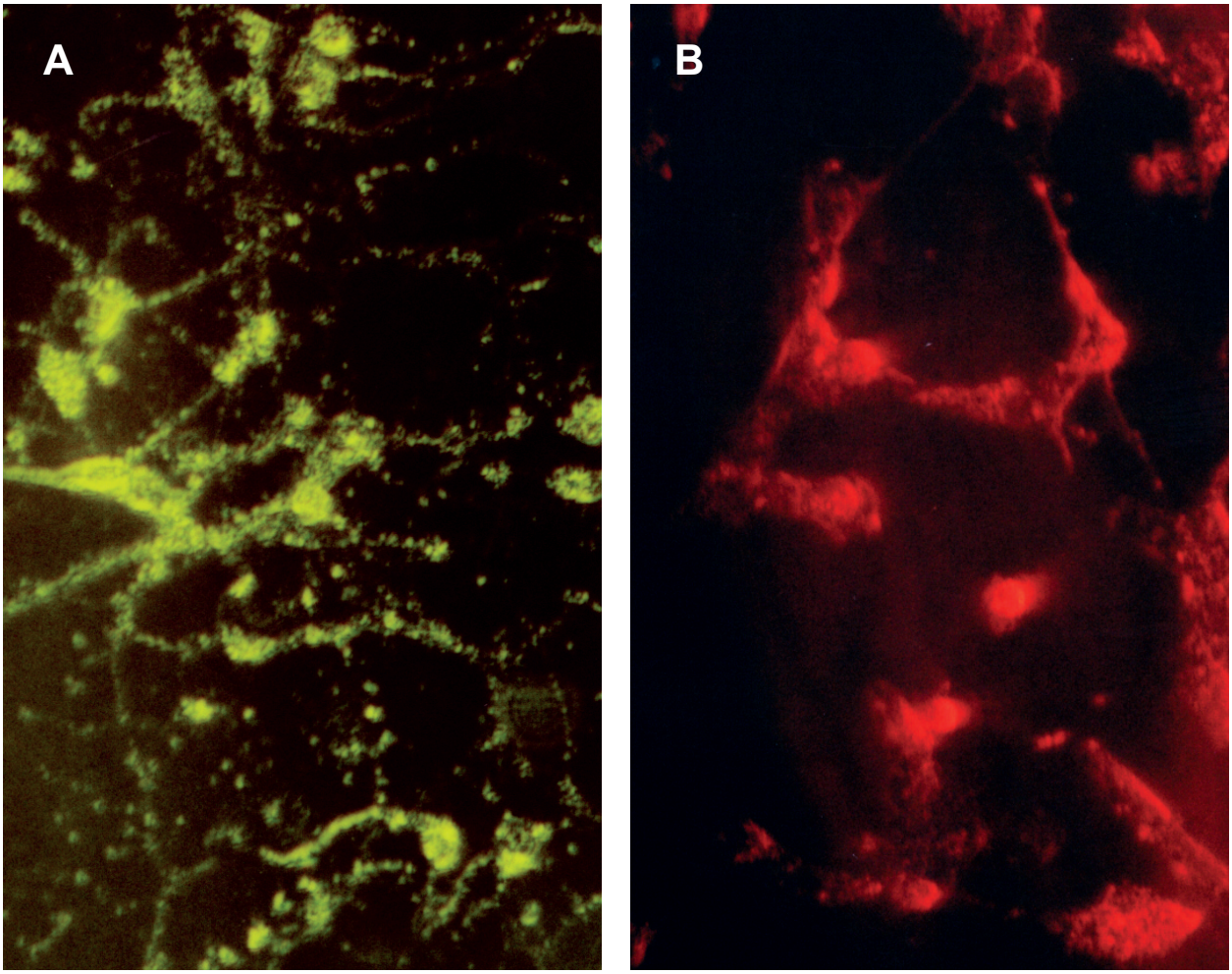


Fig. 2 Fluorescence microscopy. Neural stem/ progenitor cells from developing brain of human embryo (8 weeks, ectopic pregnancy) were induced to differentiate into neurons and/or astrocytes by plating on poly-L-lysine coated plates, in the absence of growth factors, and stained in culture, after 4 days, for **βIII tubulin (green)** (A) **for marking neurons** and **glial fibrillary acidic protein (GFAP)** (red) (B) **for marking astrocytes**. Ob. 40x.

Keywords: neural stem cells - progenitor cells - human embryo - neurospheres - neurons - astrocytes

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