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Effect of growth matrices and growth factors on proliferation and viability of a neural stem cell line derived from the brain of an adult rat

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ABSTRACT

Neural stem cells (NSCs) are self-renewing, multi-potent cells that generate neurons, astrocytes, and oligo-dendrocytes in the nervous system. NSCs have been isolated from both the developing fetal nervous system (CNS) and the post-natal brain. Neural stem cells and progenitors may be cultured *in vitro* as so called neurospheres. Moreover, adult neural stem cells can be differentiated *in vitro* into neuronal and glial cells, such as astrocytes and oligodendrocytes. Expansion of NSCs in *in vitro* cultures is carried out using serum-free media containing a serum-supplement and the growth factors EGF and bFGF. *In vivo* neural stem cell proliferation and differentiation is regulated by a complex interaction of cues including (i) soluble factors (growth factors and cytokines), (ii) insoluble factors (extracellular matrix) and (iii) cell-cell contacts in a specialized microenvironment known as the stem cell niche. Hence, the question must be posed as to whether culture conditions would influence growth and differentiation behavior of NSCs *in vitro*. In addition to the requirement of growth factors the presence of matrices might be important. In this regard one has to keep in mind that the extracellular matrix (ECM) is constituting a highly dynamic and complex environment *in vivo*, which is characterized by biophysical, mechanical and biochemical properties specific for each tissue and able to regulate cell behavior.

The focus of my master thesis was to investigate the effects of medium composition and growth matrices on *in vitro* growth, viability and stemness of adNSCs. For this purpose, I used the neural stem cell line rNSC-1, which was isolated from the brain of an adult rat. I investigated the requirements of the growth factors EGF and bFGF in the presence of components of the extracellular matrix and biopolymers. To accomplish this objective I plated rNSC1 cells on different matrices and analyzed proliferation (BrdU ELISA) and vitality (MMT assays) in the presence of distinct media. Moreover, rNSC1 grown on distinct substrates and in the presence of distinct media were stained for the markers Nestin, GFAP and MAP2. The matrices tested were fibronectin (FN), fibronectin/poly-ornithine (FN/PO), fibronectin/matrigel (FN/Mt), fibronectin/laminin (FN/Lm), fibronectin/gelatin (FN/Ge), fibronectin/poly-lysine (FM/PL). The changes in medium composition were (i) depletion of EGF or bFGF, or both and (ii) addition of increasing concentrations of fetal calf serum.

It was observed that either EGF or bFGF or both were required to permit efficient growth of rNSC1 on different matrices. Whereas full proliferative activity on fibronectin required both EGF and bFGF, bFGF alone was sufficient to permit growth on the mixed matrices containing matrigel (FN/Mt), gelatin (FN/Ge) and laminin (FN/Lm). On the contrary, bFGF alone could not induce efficient growth on fibronectin/poly-lysine (FN/PL) or fibronectin/poly-ornithine (FN/PO). Rather EGF alone mediated a higher growth rate than EGF plus bFGF on the FN/PO substrate. The results were less clear for poly-lysine (FN/PL), as improved growth with EGF alone was only observed on day d6 but not on day d4. Glial differentiation was induced with fetal calf serum (FCS) on all matrices tested and was most efficient on fibronectin/poly-lysine (FN/PL). Also growth factor depletion was inducing glial differentiation, though in a matrix-dependent manner. For example, depletion of EGF induced glial differentiation on matrigel (FN/Mt) and laminin (FN/Lm). In addition, depletion of bFGF or both EGF and bFGF induced glial differentiation on laminin (FN/Lm). In conclusion, growth factor requirement and glial differentiation was affected by the matrices the rat NSC-1 line was grown on.