

**The Effect of Hemodynamically-Stimulated Endothelial Cell-Produced
Extracellular Matrix on Adult Neural Stem Cells**

by

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ABSTRACT

Every year in the United States, over 80,000 cases of stroke, 10,000 cases of spinal cord injury, and 200,000 cases of traumatic brain injuries will occur (Madri et al 2009). These events impact not only the patients, but also their families and our healthcare infrastructure as a whole. In 1995, the economic impact of spinal cord injury was estimated to amount to \$7.7 billion (DeVivo et al 1997). The economic impact of neurodegenerative diseases is enormous, with over \$100 billion spent per year on research and treatment of Alzheimer's disease alone (Meek et al 1998). It is important to consider that a large portion of these costs stem from complications related to their injuries or disease, which can amount to up to an estimated \$1,000,000 per patient throughout their lifetime (DeVivo et al 1997).

Neural stem cells are a multi-potent cell that could be used as an autologous cell-source for cell-mediated therapy that could both address traumatic injuries or neurodegenerative diseases by replacing the injured or degenerated cells and restore a patient's long-term quality of life (Baizabal et al 2003, Madri 2009). In vivo, neural stem cells generally reside and proliferate in close proximity to blood vessels, suggesting that endothelial-produced factors may influence NSC fate to some degree (Shen et al 2008). In vitro, soluble factors released by endothelial cells (EC) were shown to stimulate self-renewal and inhibit differentiation of embryonic NSC (Shen et al 2004). However, EC products (soluble and insoluble) by the local hemodynamic environment have not been studied.

In this thesis, we developed an in vitro model to investigate the role of EC-produced ECM (insoluble) on NSC. A cell-mediated lysis technique was developed to isolate ECM substrates from murine endothelial cells (mbEnd.3; chapter 2), the ECM substrates produced by both static and hemodynamically stimulated (10 dyne/cm²) EC were qualitatively characterized (chapter 3), and used as a substrate to examine NSC studies (chapter 4).

Differences were observed between ECM obtained from statically or dynamically cultured endothelial monolayers. Both fibronectin and laminin were observed to increase following hemodynamic stimulation in contrast to paired static controls and type IV collagen increased to a lesser extent following exposure to dynamic culture. The EC-

produced ECM was collected and applied onto glass at various concentrations for subsequent NSC studies. Adult primary murine NSC were isolated from the sub-ventricular zone, cultured as neurospheres, were dissociated and seeded onto ECM substrates. In the dynamically produced ECM, the NSCs were observed to attach, spread, and infiltrate into the matrix, while the statically produced ECM instead generated only small neurospheres at the ECM surface in the presence of growth-promoting medium. This observation suggests that differences in EC-ECM produced under hemodynamic flow significantly alter NSC response relative to statically-produced ECM.

In future work, the extent of the differentiation in response to these ECM substrates will be quantified via immunofluorescent staining in both growth-promoting and differentiation medium. In addition to insoluble factors, the EC-produced soluble factors are produced to varying degrees and their release will be characterized under static and hemodynamic conditions. These EC cues can be examined both alone and in conjunction with one another to gain a better understanding of the neural stem cell niche. Ultimately, the *in vitro* elucidation of the role of soluble and insoluble EC-produced factors such as the ECM in the determination of NSC fate is critical (Baizabal et al 2003, Tavazoie et al 2008). Fully understanding environmental cues *in vivo* will eventually allow for regulation of NSC proliferation and fate for the development of future *in vivo* regenerative cell-based therapies, and prevent adverse outcomes of improper stem cell signaling during therapy such as the generation of uncontrollable tumor formation (Baizabal et al 2003, Eriksson et al 1998, Goh et al 2003).