Biologic Scaffolds Derived from Mammalian CNS for Regenerative Medicine

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Statement of Purpose: The adult human central nervous system (CNS) has limited regenerative capacity despite the presence of neural stem cells in brain and spinal cord tissues (Dromard; Nunes). Decellularized xenogeneic tissues have been used both clinically and preclinically as biologic scaffolds that facilitate constructive remodeling rather than scar tissue formation in a variety of tissues (Badylak). Tissue-specific biologic scaffolds can facilitate constructive remodeling for some tissues (Zhang); it is possible that neural tissue-derived biologic scaffolds may be the most appropriate for CNS repair. The present study reports the preparation and characterization of biologic scaffolds from porcine CNS tissues and their *in vitro* effects on the viability, proliferation, and migration of cells that may be critical to constructive CNS remodeling, including human neural stem cells from cortical neuroepithelium and human perivascular stem cells.

Methods: Porcine brain and spinal cord were decellularized by agitating in a series of enzyme, detergent, and acid baths, with intermediate and terminal water and saline rinses. Growth factors were extracted and quantified by ELISA (R&D Systems, Minneapolis, MN). ECM prepared from whole brain (WBM), spinal cord (SCM), and urinary bladder (UBM) were compared. ECMs were solubilized using pepsin for stem cell culture and migration assays. Viability was tested using Live/Dead Assay (Invitrogen Corp., Carlsbad, CA) to assess cytotoxicity of ECMs. Mitogenesis was evaluated using a colorimetric BrdU assay (Roche Diagnostics Corp., Indianapolis, IN) to indicate whether ECMs might increase endogenous stem cell proliferation *in vivo*. Chemotaxis was assayed using micro chambers and polycarbonate PFB filters with 8.0 µm pore size (Neuro Probe Inc., Gaithersburg, MD) to determine whether ECMs could recruit endogenous stem cells *in vivo*.

Results: WBM and SCM contained <1.5% of the DNA of their native tissue counterparts. WBM and SCM contained 16% and 44% of the bFGF of their respective native tissues. SCM contained 84% of the VEGF of spinal cord tissue. Similar to UBM, SCM formed a gel at 37°C. Human stem cell viability was unaltered by any of the ECMs (data not shown). WBM, SCM, and UBM elicited positive mitogenic and chemotactic responses from human cortical stem cells (Fig. 1) and from human perivascular stem cells (Fig. 2).

Conclusions: Biologic scaffolds can be derived from porcine CNS tissues. The scaffolds contain <1.5% of DNA compared to native tissues while retaining growth factors. Human neural and perivascular stem cells showed positive mitogenic and chemotactic responses to WBM, SCM, and UBM *in vitro*, suggesting that these biologic scaffolds are likely to cause proliferation and migration of endogenous stem cells *in vivo*. Preclinical studies are required to assess the potential of these materials for improving post-injury CNS remodeling and function.

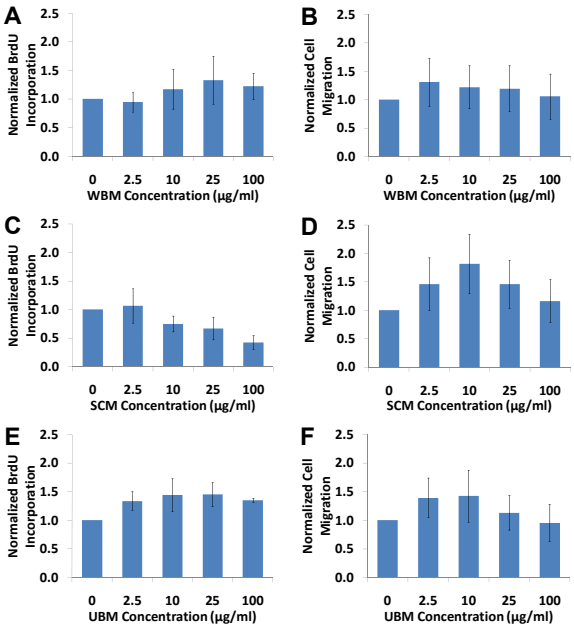

Figure 1. WBM (A-B), SCM (C-D), and UBM (E-F) induced positive mitogenic (left) and chemotactic (right) responses in human cortical stem cells with the exception of mitogenesis when cultured with SCM (C).

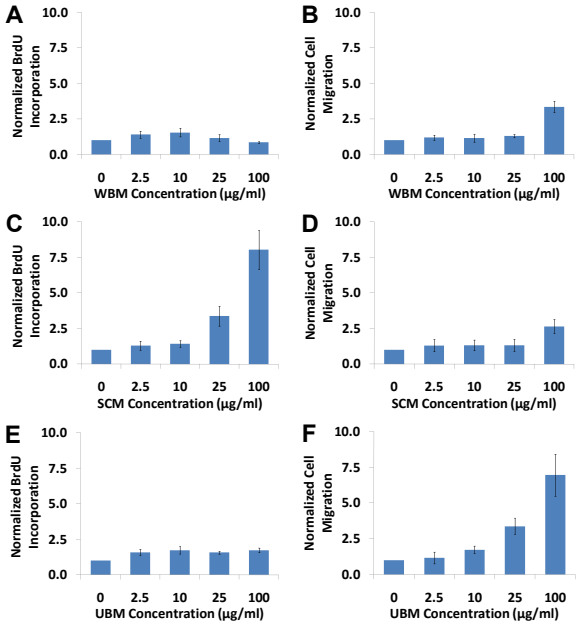

Figure 2. WBM (A-B), SCM (C-D), and UBM (E-F) induced positive mitogenic (left) and chemotactic (right) responses in human perivascular stem cells.

References:

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