Decellularized Porcine Brain Matrix as a Cell Culture Platform and Tissue Engineering Scaffold

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Statement of Purpose: The extracellular matrix (ECM) plays an important role in many cell behaviors, but in conventional cell culture and tissue engineering strategies, single proteins are frequently used, which does not mimic the complex extracellular environment seen *in vivo*. In the case of brain tissue, the ECM contains fibrous proteins such as collagen and laminin, and is highly rich in glycosaminoglycans. Decellularization techniques have been used to extract the extracellular matrix from the native tissue, but have not been performed on the brain. With this study, we report for the first time, a method to decellularize brain tissue using detergents. This decellularized matrix is rich in glycosaminoglycans and contains extracellular matrix proteins such as a variety of collagens as well as laminin, and can be further processed into a liquid form to be used as a cell culture coating or as an injectable tissue engineering scaffold.

Methods: Porcine brains from Yorkshire pigs (~30-45 kg) were decellularized using 0.1% wt/vol of sodium dodecyl sulfate (SDS) in phosphate buffered saline with 1% penicillin/streptomycin. The supernatant containing the cellular remnants were decanted and refilled to the start volume daily until the tissue was decellularized, and then rinsed with deionized water through centrifugation to remove residual SDS. DNA removal was assessed using a DNeasy assay (Qiagen, Valencia, CA), and through hematoxylin and eosin (H&E) staining of sections frozen in Tissue Tek O.C.T. Brain matrix was solubilized through enzymatic digestion using pepsin (1 mg/ml in 0.1M hydrochloric acid) for ~48 hours under agitation. Glycosaminoglycan (GAG) content was measured with a colorimetric Blyscan assay (Biocolor, United Kingdom) with rat tail collagen as a control. Immunohistochemistry of decellularized brain sections was used to determine ECM protein retention using antibodies against collagen I, collagen III, collagen IV and laminin.
To test the material as a cell culture coating, 96 well imaging plates were coated with 20 µg/ml Polyornithine (PO) at 37°C overnight. Next day, the wells were rinsed twice with sterile DI water to remove unattached PO. Plates were coated with the liquid brain matrix diluted to 1 mg/ml using 0.1 M acetic acid for 1 hour at 37°C and finally rinsed twice with sterile PBS prior to use. Matrigel was diluted to 0.25 mg/ml in DMEM/F12 and incubated for the same period of time. Neurons derived from induced pluripotent stem cells (iPSC) from adult skin biopsies were plated at 0.625 million cells/cm^2 in neuron differentiation media. Media was changed every 3 days, and cells were fixed and stained at 7 or 14 days for GABA, bIII-tubulin and synapsin.
Brain matrix was brought to a physiological pH through the addition of sodium hydroxide (NaOH) and PBS, and kept on ice prior to use. Female C57 mice were anesthetized under isoflurane whereupon 100 µl of brain ECM was injected through a 27 gauge needle subcutaneously into the dorsal region. Twenty minutes post injection, the site of injection was excised, and gels were fresh frozen in Tissue Tek O.C.T. for histological analysis or prepared for scanning electron microscope (SEM) analysis.

Results: The brain matrix was decellularized using SDS detergent buffered in PBS with antibiotics. H&E staining demonstrated the absence of intact nuclei, and DNeasy quantified DNA removal with a clearance ratio of 95.7% as there was 0.13±0.04 ug DNA content/mg lyophilized native brain tissue and 0.0069±0.002 µg DNA content/mg in the post-processed brain matrix. Immunohistochemistry demonstrated the retention of collagens as well as laminin post-processing. Additionally, retained GAG content was measured to be 34.7 ± 0.32 µg sulfated GAG/mg dry weight brain matrix, with none found in rat tail collagen. Neurons derived from iPSC were FACS sorted, and cultured on Matrigel or decellularized brain matrix coatings. Both materials were able to support mature neurons, as the iPSC-neurons were able to attach, extend dendritic processes, and were positively stained for the neuronal marker βIII-tubulin. In culture, some neurons cultured on the brain matrix exhibited a distinct morphology with complex dendritic processes (**Figure 1 a,b**). These highly arborized dendrites were only identified on the pig brain matrix coated wells, and not found on the Matrigel substrates. Additionally, synapsin expression, a protein found in mature synapses increased from one week to two weeks, indicating maturation of the neurons over time in culture. The brain was also able to form a gel *in vivo* upon injection, and reassembled into a fibrous network as seen through SEM.

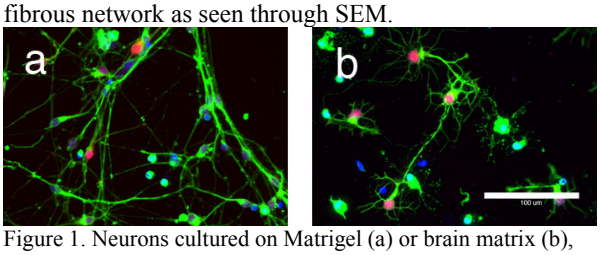

Figure 1. Neurons cultured on Matrigel (a) or brain matrix (b), stained for bIII-tubulin (green) as well as GABA (red), scale bar 100 µm.

Conclusions: Brain tissue is able to be decellularized and still retains extracellular matrix protein components and glycosaminoglycans, which may be used as a cell culture platform as induced pluripotent stem cell-derived neurons are able to grow and mature on the brain matrix. The brain matrix is also able to self-assemble and gel *in vivo* forming a nanofibrous scaffold demonstrating feasibility as an injectable, brain-specific tissue engineering scaffold.