Fabrication and Characterization of Neurospheres with Novel Method for Suppression of Immune Response in Vivo

Xiaowei Li [1], Xiaoyan Liu [1], Laila C. Roudsari [1], Jeffrey Morgan [2], Ning Zhang [1,3], Xuejun Wen [1,3]
[1]Clemson - MUSC Bioengineering Program, Department of Bioengineering, Clemson University, Clemson, SC, 29634
[2]Department of Medical Science and Engineering, Brown University, Providence, RI 02912
[3]Department of Orthopaedic Surgery, Cell Biology & Anatomy, Medical University of South Carolina, Charleston, SC, 29425

Statement of Purpose: Consequences of CNS physical injury and neurodegenerative disease are severe because the CNS has limited capacity to replace neuronal loss after injury or disease. Neural stem cells (NSCs) are the most versatile and promising cell source for the regeneration of injured and diseased CNS. However, cell therapy faces many difficulties related to cell survival, control of cell fate and proper cell engraftment after transplantation[1]. Cell survival is one of the most challenging technical issues as only a small percentage of implanted cells can survive after transplantation. As survival is poor, most probably due to an ongoing immune response that may not have been fully suppressed by the immunosuppressant, improved immuno-suppressive strategies or generation of syngeneic and thus immunologically tolerated NSCs must be found. Mesenchymal stem cells (MSCs) have been demonstrated to possess immunosuppressive properties[2,3]. We hypothesized that the survival of transplanted NSCs may be improved in cell aggregate format such as neurospheres, the size of neurospheres may affect the viability after transplantation and coating of MSCs on the surface of neurospheres may further improve the viability by suppressing the host immune response at the transplantation site.

Methods: Two types of NSCs were used in this study: human NSCs and GFP labeled mouse NSCs. Agarose-based microwells (35 and 96 wells) were applied to fabricate neurospheres with controlled size. Human MSCs coating on neurospheres were conducted through microwells and co-culture shaking flask. Neurospheres were transplanted into the striatum of rat brain to investigate the effects of size and MSCs-coating on neurosphere viability and differentiation in vivo.

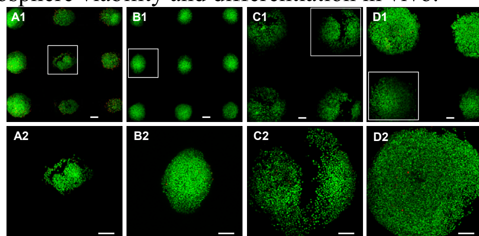

Fig. 1: LIVE/DEAD staining (green for LIVE and red for DEAD) of hNeurospheres in agarose microwells (A, B: 96 wells; C, D: 35 wells). A: 100µm; B: 200µm; C: 300µm; D: 400µm. Scale bar: 100µm.

Results: Neurospheres with different sizes were fabricated through adjusting cell seeding concentrations in agarose microwells as shown in Fig. 1. NSCs with enough quantity aggregated and formed 3D spheroids (Fig. 1 B and D). When co-culturing with neurospheres in shaking flask, hMSCs invaded into neurospheres easily (Fig. 2). The reason of invasion lies in the fact that NSCs come from the ectoderm and MSCs come from the mesoderm. Coating neurospheres with a barrier layer of hydrogel such as gelatin before co-cultured with hMSCs can prevent MSCs invasion. We optimized hMSCs concentration to achieve hMSCs-coated neurospheres with shell-core structure (Fig. 3). Neurospheres were transplanted into striatum of rat brain. Neurospheres survived in the injection sites. Most NSCs have differentiated into neurons rather than astrocytes (Fig. 4). The in vivo study is still ongoing. Uniformly sized neurospheres can be a good model to study neurite outgrowth in different biomaterial structures. Figure 5 shows the high viability and extensive neurite outgrowth within hydrogels of 25Pa. When hydrogels are too stiff or too soft, neurite outgrowth is very limited or does not even occur as in 370Pa hydrogels (Fig. 5A). The reason of the difference may be due to these hydrogels with different permissiveness or pore size.

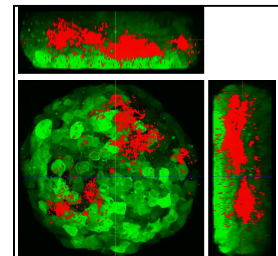

Fig. 2: Immunocytochemisty of hMSCs invided mNeurospheres. Green for GFP labeled mNeurospheres and red for hMSCs stained with human mitochondria antibody.

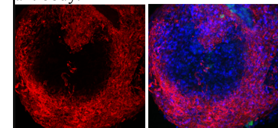

Fig. 3: Immunocytochemisty of hMSCs coated on the surface of mNeurospheres. Red for human hMSCs stained with human mitochondria antibody.

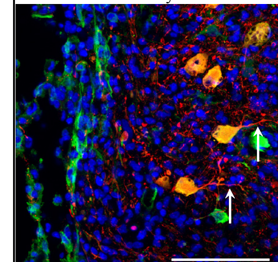

Fig. 4: Immunohistochemistry of mNeurospheres transplanted into striatum of rat brain. Green for GFP labeled Neurospheres. Red for beta 3 tubulin positive neurons. Scale bar: 100µm.

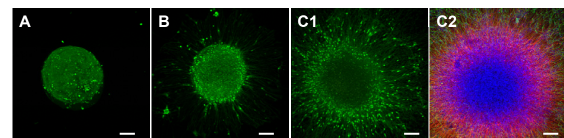

Fig. 5: LIVE/DEAD staining (A, B, C1) and immunostaining (C2, red for βIII tubulin positive neurons, green for GFAP positive astrocytes and blue for nuclei stained with DAPI) of hNeurospheres encapsulated in hydrogels of different stiffness for 21 days. A: 370Pa; B: 75Pa; C: 25Pa. Scale bar: 100µm.

Conclusions: Neurite outgrowth is larger on less stiff hydrogels because these hydrogels undergo hydrolysis more quickly. Co-culture hMSCs with gelatin-coated neurospheres results in hMSCs-coated neurospheres with shell-core structure because gelatin prevents hMSCs invading. Neurospheres transplanted into striatum of rat brain can survive and differentiate into neurons.

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References: 1. Nature 441:1094 (2006), 2. TRENDS in Neurosciences 25:131-134 (2002), 3. Blood 110:3499-3506 (2007)

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