Immune Modulatory Effects of Fetal Surgical Repair with Placenta-Derived Mesenchymal Stromal Cell in an Ovine Model of Myelomeningocele.

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Introduction

Myelomeningocele (MMC), or spina bifida, results from incomplete closure of the spinal cord and spinal canal during gestation, leaving the spinal cord unprotected. Intrathecal chemical and mechanical trauma to the exposed spinal cord results in lifelong lower limb paralysis, bowel and bladder dysfunction, musculoskeletal deformities, and severe cognitive disabilities due to hindbrain herniation in afflicted children. It is the most common congenital cause of lifelong paralysis in the United States, and approximately four children a day are born with this devastating congenital defect.

Previously, we lab discovered that treatment with early gestation placenta-derived mesenchymal stem cells (PMSCs) seeded on a clinical grade extracellular matrix (ECM) during in utero repair of MMC functionality cures paralysis in a rigorous ovine model, resulting in 75% improvement over standard in utero repair.

Methods

 Immunofluorescence staining was performed 20 µm thick transverse sections of spinal cord at the epicenter of insult (most compressed). To block nonspecific binding, the slides were incubated with PBS containing 5% goat serum and 0.2% Tween 20 for 1 hr at room. The slide was washed with PBS and then incubated with primary antibody overnight at 4°C. The primary antibodies were used anti-GFAP (1:1000, Sigma), anti-vimentin (1:1000, Sigma). Immunofluorescence detection was performed with Alexa Fluor 488 goat anti-mouse or Alexa Fluor 555 goat anti-rabbit (1:10000, Invitrogen, Carlsbad, CA) and DAPI (ThermoFisher Scientific, Carlsbad, CA) for the nuclear staining. Adjacent tissue sections where the primary antibody was omitted served as negative controls.

Microscope images were acquired with an Axio Observer 200M inverted microscope and a monochromatic camera and arc lamp illumination using selective filters for fluorescence. Images were analyzed using Image-J software. Fluorescence was quantified as the integrated fluorescence intensity per linear µm from 10 randomly acquired microscopy (200X) software. Fluorescence was quantified as the integrated fluorescence intensity of sections stained with primary antibodies to the negative controls were used for statistical analysis.

Results

![Image](https://via.placeholder.com/150)

Figure 1. PMSCs seeded at different densities on ECM improved motor function at birth in the fetal ovine model of MMC

The neurorestorative effects of mesenchymal stem cells are well documented and include promoting neurite outgrowth, sparing apoptotic and neuropeptide factors, and regulating inflammation [6, 7]. In an in vitro neuronal protection assay, we found that PMSCs can rescue apoptotic neurons with their robust paracrine secretions [1]. Previously, we had conducted neurotrophic assays, and in vitro, we found that PMSCs can rescue apoptotic neurons with their robust paracrine secretions. Previously, we had conducted in vitro neuronal protection assay, we found that PMSCs can rescue apoptotic neurons with their robust paracrine secretions.

Results Continued

![Image](https://via.placeholder.com/150)

Figure 4. Representative GFAP Staining which highlights distinct GFAP expression around the periphery of a blood vessel (A). No distinct pattern was discernable in the negative control (B).

![Image](https://via.placeholder.com/150)

Figure 5. Representative Vimentin Staining which produced a similar pattern to the GFAP stain (A). No distinct pattern was discernable in the negative control (B).

Conclusions

Our study indicate insignificant changes in inflammatory marker expression in spinal cord tissue treated with PMSCs. Immunofluorescence patterns suggest no difference in inflammatory response by glial cells, however, notable changes did exist:

- Fluorescence staining is susceptible to artifacts of variable fixation and tissue section thickness, as well as procedural details including consistent washing and sample handling.
- RBCs and clots contribute significant autofluorescence that can create false positive fluorescence and should be avoided during imaging.
- euthanization at 45 days post surgery may be optimal to observe inflammation in these studies. Timing of the inflammatory response needs to be further investigated.
- Antibodies for sheep tissue are not well characterized and are very difficult to titer.
- Other pathways of immune modulation may exist that are not encompassed by this study

In conclusion, methodological approaches need to be further optimized, however preliminary data suggests no difference in the inflammatory response in spinal cord tissue treated with PMSCs in an ovine model of MMC.

References


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