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Fetal surgical repair with placenta-derived mesenchymal stromal cell engineered patch in a rodent model of myelomeningocele

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ABSTRACT

Purpose: The purpose of this study is to determine the feasibility of fetal surgical repair of myelomeningocele (MMC) in a rodent model using human placental mesenchymal stromal cells (PMSCs) seeded onto extracellular matrix (ECM) and to characterize the resulting changes in spinal cord tissue.
Methods: Fetal rodents with retinoic acid (RA) induced MMC underwent surgical repair of the MMC defect using an ECM patch on embryonic age (EA) 19 and were collected via caesarean section on EA 21. Various seeding densities of PMSC-ECM and ECM only controls were evaluated. Cross-sectional compression (width/height) and apoptotic cell density of the lumbosacral spinal cord were analyzed.
Results: 67 dams treated with 40 mg/kg of RA resulted in 352 pups with MMC defects. 121 pups underwent MMC repair, and 105 (86.8%) survived to term. Unrepaired MMC pups had significantly greater cord compression and apoptotic cell density compared to normal non-MMC pups. Pups treated with PMSC-ECM had significantly less cord compression and demonstrated a trend towards decreased apoptotic cell density compared to pups treated with ECM only.
Conclusion: Surgical repair of MMC with a PMSC-seeded ECM disc is feasible with a postoperative survival rate of 86.8%. Fetal rodents repaired with PMSC-ECM have significantly less cord deformity and decreased histological evidence of apoptosis compared to ECM only controls.

Myelomeningocele (MMC) is the most common neural tube defect, and is characterized by exposure of the meninges and neural elements through a defect in the spinal column and soft tissue of the back [1]. Despite the promising results of fetal intervention in patients with MMC to decrease the risk of hindbrain herniation and need for cerebrospinal fluid shunting, improvements in motor function are still limited [2]. Fetal intervention on MMC provides tissue coverage for the fetal spinal cord to prevent further intrauterine damage; however, it does not appear to repair injury that has already occurred throughout early gestation. There, lies the potential for cell-based regenerative therapies to augment fetal repair of MMC.

Mesenchymal stromal cells (MSCs) can be isolated from a variety of tissues and may have significant therapeutic potential derived from their ability to secrete paracrine factors to improve endogenous healing [3]. Previous work in our laboratory [4] has demonstrated that placenta-derived mesenchymal stromal cells (PMSCs) have distinct neuroprotective properties and can mediate significant distal motor function rescue in an ovine MMC model. PMSCs can also be banked in large quantities in order to generate cell banks that are free from contamination facilitating their translation into large scale clinical use [5]. Comparative studies have also suggested that MSCs derived from the placenta may have a superior neuroprotective secretory profile compared to other sources of MSCs [6–8]. We hypothesize that human PMSCs seeded onto porcine small intestine submucosa derived extracellular matrix (SIS-ECM) are an ideal product for use in fetal MMC repair. SIS-ECM is an optimal delivery vehicle since it is already approved by the Food and Drug Administration (FDA) for clinical use as a dural replacement device thus expediting translation into human use. Notably it has also been shown to support MSC growth and delivery [9,10]. Thus, the purpose of this study is to assess the feasibility of performing fetal surgical repair of MMC in a rodent model using a bioengineered scaffold of PMSCs seeded onto SIS-ECM in order to establish a cost effective, high throughput model for cell dosing studies.

1. Methods

1.1. Ethics statement

This study was approved by the University of California (UCD) Institutional Animal Care and Use Committee (IACUC) and performed

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according to the criteria provided by the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals. Deidentified human tissue was collected from discarded placenta. Protected health information was neither stored nor disclosed and thus this study was exempt by the Institutional Review Board.

1.2. Isolation and culture of PMSCs and preparation of PMSC-ECM

A sample of tissue from a 15-week gestational age human placenta was collected. PMSCs were isolated using an explant culture method [4,11,12] and cryopreserved in liquid nitrogen. The cell line used was manufactured and fully characterized as MSCs as previously reported [13]. PMSCs were transduced with green fluorescent protein-containing lentiviral vector (UCD Institute of Regenerative Cures Vector Core Facility, Sacramento, CA) and seeded onto 6 mm diameter circular discs of SIS-ECM (Cook Biotech Inc., West Lafayette, IN) at the following densities (cells/cm^2); 5×10^4, 1×10^5, 2×10^5, and 3×10^5. Cell seeding was confirmed using a Carl Zeiss AxioObserver Z1 Microscope (Fig. 1F). All ECMs (PMSC seeded and non cell seeded ECM only controls) were exposed to the same culture conditions and timeline.

1.3. Animal experiments

Time mated Sprague–Dawley rats (Envigo, Livermore, CA) were dosed with 40 mg/kg of all-trans retinoic acid (RA) (Sigma-Aldrich Chemical, St Louis, MO) on embryonic age (EA) 10 days between 15:39 and 18:08 PCT via oral gavage. Survival surgery was performed on EA19 using an operating microscope at 0.8× magnification. Anesthesia was induced with inhalational 5% Isoflurane and maintained with 1%–3%. Dams were placed supine on a temperature regulated warming pad set to 38 °C measured by rectal probe. After sterile preparation, a midline laparotomy was performed. The uterus was fully exteriorized and the total number of live pups as well as the presence of previous intrauterine fetal demise was noted. Each pup was examined for presence of MMC and/or anencephaly. Selection for operative intervention was determined by the presence of MMC, absence of anencephaly and/or other obvious congenital defects, and optimal positioning of the MMC defect within the uterine sac. Pups were repaired one at a time while the remaining uterine sacs were replaced intraabdominally. Treatment groups included PMSC-ECMs seeded at the following densities (cells/cm^2); 5×10^4, 1×10^5, 2×10^5, 3×10^5, and ECM only controls.

The selected pup was manipulated and gently restrained within the sac to allow optimal visualization of the MMC defect through the uterine wall. A 6-0 polypropylene suture (PDS-II, Ethicon, Somerville, NJ) was used to make a purse-string through the uterine wall over the lumbosacral area of the underlying pup. A hysterotomy was made exposing the MMC defect. The amniotic sac was opened with a 30 Gauge needle to expose the pup and MMC defect. Two 8-0 nylon sutures (AROSurgical, Newport Beach, CA) were used to secure the lateral sides of the 6 mm ECM disc to the fetus (Fig. 1A). After confirming that the ECM provided adequate coverage of the MMC defect, lost amniotic fluid was replaced with warm sterile saline. The hysterotomy was closed with the previously placed purse-string suture (Fig. 1B) and the uterine sac was returned to the abdomen. The procedure was repeated for the remaining pups selected for repair. Upon completion of all repairs, intraperitoneal warm saline was administered and the abdomen was closed in layers. All postoperative dams were treated once daily via oral gavage with 40 mg/kg cyclosporine for immunosuppression for the remainder of gestation. Pain medication (Buprenorphine, 0.05 mg/kg) was administered twice a day for 48 h after surgery.

Pups were collected via terminal cesarean section on EA21 (Fig. 1C). All pups in the litter were examined prior to termination to confirm accurate identification of MMC defect, anencephaly and other congenital defects. Normal and unrepaired MMC pups from the same litter were collected as controls. Collected pups underwent intracardiac perfusion with phosphate buffered saline and were fixed in 10% formalin. A selection of pups (n = 3) had ECM discs removed from the MMC defect post collection. These discs were imaged separately to assess PMSC retention.

1.4. Histological analysis

Whole pup specimens were dehydrated in 30% sucrose for seven days prior to being frozen in Optimal Cutting Temperature compound (Fisher Healthcare Tissue-Plus, Waltham, MA). 9 μm serial cross sections were taken through the lumbosacral portion of the pup starting from the approximate midportion of the MMC defect.

Cresyl Violet staining was performed on the serial cross sections of the lumbosacral portion for each collected pup. Each slide was analyzed for the presence of spinal cord tissue to use for analysis. Samples without adequate spinal cord tissue were excluded from further analysis. Three consecutive cross sections were analyzed per animal. The cross-

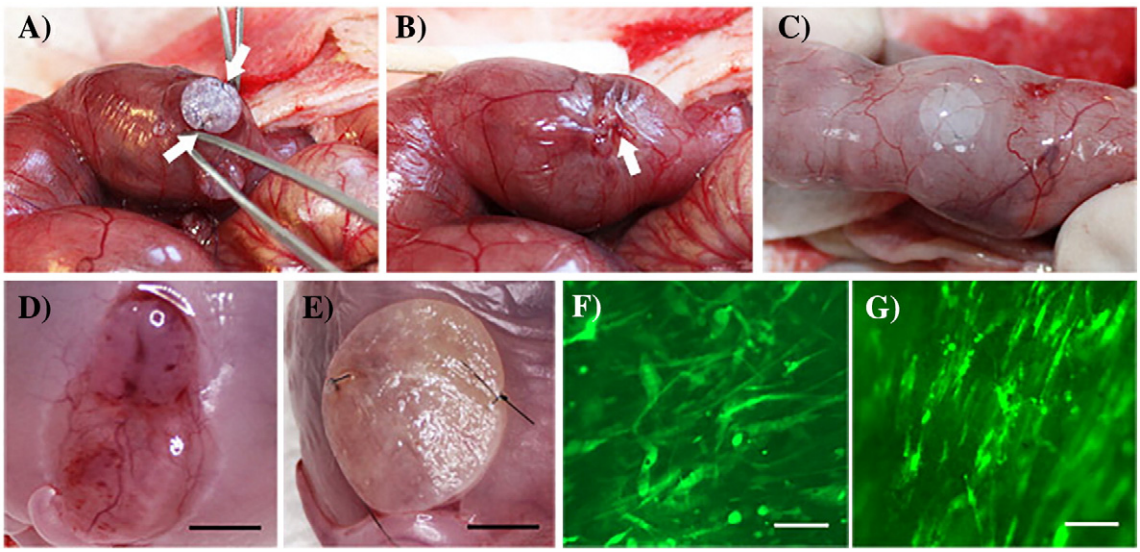

Fig. 1. Operative images of the 6 mm extracellular matrix patch sutured over the myelomeningocele defect on the rodent fetus; arrows correspond to suture sites (A). The patch remains in place after closure of the hysterotomy site (arrow) during the initial surgery (B) and at the terminal laparotomy on embryonic age (EA) 21 (C). Representative images of the myelomeningocele defect under the operating microscope (D, E). GFP-tagged PMSC seeding onto the extracellular matrix is confirmed prior to use (F). PMSCs can be visualized on the matrix on EA21 (G) indicating their ability to survive on the matrix for the remaining duration of gestation Scale = 2 mm for (D) and (E), Scale = 100 μm for (F) and (G).

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