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Isolation of myogenic progenitor cell population from human placenta: A pilot study

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ABSTRACT

Purpose: The purpose of this study was to demonstrate a method of isolating myogenic progenitor cells from human placenta chorionic villi and to confirm the myogenic characteristics of the isolated cells.
Methods: Cells were isolated from chorionic villi of a second trimester male placenta *via* a combined enzymatic digestion and explant culture. A morphologically distinct subpopulation of elongated and multinucleated cells was identified. This subpopulation was manually passaged from the explant culture, expanded, and analyzed by fluorescence *in situ* hybridization (FISH) assay, immunocytochemistry, and flow cytometry. Myogenic characteristics including alignment and fusion were tested by growing these cells on aligned polylactic acid microfibrous scaffold in a fusion media composed of 2% horse serum in Dulbecco's modified Eagle medium/high glucose.
Results: The expanded subpopulation was uniformly positive for integrin α-7. Presence of Y-chromosome by FISH analysis confirmed chorionic villus origin rather than maternal cell contamination. Isolated cells grew, aligned, and fused on the microfibrous scaffold, and they expressed myogenin, desmin, and MHC confirming their myogenic identity.
Conclusion: Myogenic progenitor cells can be isolated from human chorionic villi. This opens the possibility for translational and clinical applications using autologous myogenic cells for possible engraftment in treatment of chest and abdominal wall defects.

Congenital diaphragmatic hernia (CDH) requires diaphragmatic replacement with a synthetic or biologic patch in up to 51% of the cases [1–3]. Morbidities associated with the use of a patch for CDH repair are not insignificant and can include recurrence [4,5], small bowel obstruction [6], and chest wall deformities [7]. Large CDH defects are typically repaired with nonabsorbable synthetic patches [3]. Biologic patches and autologous muscle flaps have been used as alternatives in an effort to decrease morbidity, however, no differences have been identified in complication rates between the various types of patches currently available [5,8,9].

Tissue engineering is an innovative alternative currently under investigation [10–12]. Cells from various sources have been induced to differentiate into myogenic cells. Sources of myogenic cells include skeletal muscles [13–15], embryonic stem cells [16], blood vessels [17], amniotic fluid [18], adipose tissue [19], and bone marrow [20]. Studies investigating the placenta as a potential source of myogenic progenitor cells are limited [21,22]. In those studies, placenta-derived cells were induced to differentiate into myogenic progenitors by treatment with 5-azacytidine, an inhibitor of DNA methylation, and growth in differentiation medium. Our laboratory has identified a population of myogenic progenitor cells growing spontaneously from human chorionic villi explant culture. The potential benefit of utilizing myogenic progenitor cells isolated from the placenta is creating the possibility of a tissue engineered patch using autologous cells [23]. Our lab has employed electrospinning technology to generate microfibrous scaffolds using biodegradable poly (ε-caprolactone) (PCL) and poly (L-lactic acid) (PLLA) polymer blend, as previously described [24–28], for tissue engineering of a muscle patch.

The purpose of this study is to report a method of isolation and characterization of a population of myogenic progenitor cells derived from human chorionic villi.

1. Materials and methods

1.1. Dissection of chorionic villi tissue

Chorionic villi (CV) were dissected from a discarded, de-identified, second trimester male placenta. 7.5 g of CV tissue was washed with a

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solution containing 1× phosphate buffered saline (PBS) and 100 U/mL penicillin–100 μg/mL streptomycin (Penicillin/Streptomycin, Thermo Fisher Scientific). The weighed CV tissue was minced and suspended in 30 mL of enzymatic dissociation solution: 1× Hank's Balanced Salt Solution (Thermo Fisher Scientific) containing 1 mg/mL of collagenase type I (Thermo Fisher Scientific), 0.2 mg/mL of DNase I (Roche Diagnostics GmbH), 0.1% trypsin (Thermo Fisher Scientific), and 8 mM magnesium sulfate. The tube containing minced CV tissue and enzyme solution was incubated at 37 °C, 5% CO2 for 15 min with manual mixing every 5 min.

The CV tissue was allowed to settle for 5 min, the supernatant was decanted, and the remaining CV tissue was washed once with complete culture medium: Dulbecco's modified Eagle medium/high glucose (DMEM/HG) containing 5% fetal bovine serum (Hyclone, Thermo Fisher Scientific), Penicillin/Streptomycin, 20 ng/mL recombinant human basic fibroblast growth factor (R&D Systems), and 20 ng/mL recombinant human epidermal growth factor (R&D Systems).

1.2. Isolation and expansion of myogenic progenitor cells

The CV tissue was resuspended in 7 mL of complete culture medium and transferred to a T150 flask for explant culture. The culture medium was exchanged every 3–4 days. A subpopulation of elongated and multinucleated cells was identified under phase contrast microscopy. The location of this subpopulation was demarcated for further isolation and separation from surrounding placental mesenchymal stromal cells (PMSCs). Cells in the area of interest were scraped gently with a cell scraper and transferred to a 35 mm tissue culture-treated dish precoated with 10 μg/mL laminin (Roche). Selected cells were cultured and expanded in the culture medium described above.

1.3. Fluorescence in situ hybridization (FISH)

Cells were plated in 8-well glass slide (ibidi) at a density of 20,000 cells/cm^2. FISH analysis was performed using Biotin Detection/Paint kit with a Y Chromosome Probe labeled with Texas Red (Cambio) according to the manufacturer's instructions. Cells were imaged using Carl Zeiss Axio Observer D1 inverted microscope.

1.4. Immunocytochemistry (ICC) for myogenic progenitor markers

Cells at passage 4 were seeded at a density of 10,000 cells/cm^2 in tissue culture-treated 24-well plates in fusion media composed of 2% horse serum in DMEM/HG. After 5 days, the cells were fixed with 10% formalin for 15 min, washed twice with 1× PBS, permeabilized with 0.5% Triton X-100 for 5 min, blocked with 0.2% gelatin for 1 h and incubated overnight at 4 °C with primary antibodies: myogenin 2 μg/mL (Santa Cruz), desmin 2 μg/mL (Abcam), and myosin heavy chain (pan-MHC) 2.3 μg/mL (MF-20, DSHB). Secondary antibodies Alexa Fluor 488 and Alexa Fluor 546 (Thermo Fisher Scientific) were used at a dilution of 1:500. Nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI). Cells were imaged using Carl Zeiss Axio Observer D1 inverted microscope.

1.5. Flow cytometry

The expression of integrin α-7 (ITGA-7), a laminin-1 receptor highly expressed in skeletal and cardiac muscles, was analyzed by flow cytometry. Cells at passage 4 were detached using Accutase (Thermo Fisher Scientific) and viable cell count was obtained using Trypan Blue exclusion. Analyzed samples were first stained with Near infrared LIVE/DEAD™ Fixable Dead Cell Stain (Thermo Fisher Scientific). Cells were separated into vials containing 1 × 10^6 cells each and stained either with FITC ITGA-7 antibody (Novus Biologicals) or isotype control (BD Biosciences). All samples were fixed in 10% formalin for 30 min prior to analysis. Samples were analyzed using a BD Fortessa LSR Cell Analyzer. Further analysis of the data was completed using FlowJo software (FlowJo LLC).

1.6. Tissue engineering of biodegradable patch

An electrospun aligned microfibrous scaffold was prepared as previously described by Zhu et al. [25]. In summary, PLLA (MW 67,400, Sigma Aldrich) and PCL (MW 2000, Polysciences) polymer blends were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Aladdin) and electrospun at 800 rpm to generate 200 μm-thick microfibrous scaffolds. A 1 cm × 1.5 cm piece of the scaffold was placed in a 35-mm suspension culture dish and secured to the dish on two ends with double sided tape. The scaffold was soaked in 70% ethanol for 1 h and exposed to ultraviolet light overnight. The scaffold was then coated in a solution of 1 μg/mL collagen I (B.D. Biosciences) and 10 μg/mL laminin for 1 h at 37 °C and washed once with 1× PBS prior to cell seeding. Cells at passage 4 were seeded on the scaffold at a density of 300,000 cells/cm^2 in 15 μL cell suspension and placed in the incubator for 1 h to allow adhesion of cells to the scaffold. After 1 h, 2.5 mL of pre-warmed complete culture media was added and the dish returned to the incubator. The following day, media was changed to DMEM/HG with 2% horse serum, in which the cells were cultured for 5 days on the scaffold prior to fixation. Cells were stained with desmin, MHC, and DAPI as described above and imaged using Carl Zeiss Axio Observer D1 inverted microscope.

2. Results

In this modified tissue explant culture, we observed a morphologically distinct subpopulation of elongated and multinucleated cells suggestive of myogenic progenitor cells (Fig. 1A). This subpopulation was manually passaged and expanded and continued to display its distinct morphology (Fig. 1B). Cells from this distinct population were positive for Y-chromosome by FISH assay (Fig. 1C) confirming their origin from chorionic villi rather than the result of maternal contamination.

These isolated cells were positive for myogenic markers including myogenin, desmin, and MHC on immunocytochemistry (Fig. 2). They were uniformly positive (>99%) for integrin α-7 (Fig. 3).

Cells were successfully seeded onto a biodegradable poly (ε-caprolactone) and poly (L-lactic acid) microfibrous scaffold and demonstrated alignment and fusion after culture for 5 days. Expression of myogenic markers desmin and MHC by these cells on the scaffold was confirmed using ICC (Fig. 4).

3. Discussion

We have shown that a population of myogenic progenitor cells can be isolated and expanded from human chorionic villi. These cells were confirmed to originate from the chorionic villi rather than from maternal cell contamination by identifying Y-chromosome through FISH analysis. This population of cells was confirmed to have myogenic characteristics as the cells demonstrated uniform expression of ITGA7 by flow cytometry and commonly used myogenic markers (myogenin, desmin, and MHC) by ICC. These cells behaved similarly to myogenic cells; they aligned and fused when seeded onto a tissue engineered biodegradable microfibrous scaffold and expressed the myogenic markers desmin and MHC.

Our lab has extensive experience isolating placenta-derived mesenchymal stromal cells by explant culture of human chorionic villus tissue [23,29,30]. In this modified tissue explant culture, we observed a distinct and spontaneously growing population of elongated and multinucleated cells that appeared to be myogenic. The myogenic properties of these cells were confirmed by both ICC and flow cytometry. Myogenin is a well-known transcription regulator involved in myogenesis [31,32]. Desmin, a myocyte-specific intermediate filament protein, is a known myogenic marker in both cardiac and skeletal muscles [33–35]. Myosin heavy chains are motor protein components of thick filaments in