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Novel Methods for Delivery of Cell-Based Therapies

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Background. **Pulmonary hypoplasia (PH) is found in 15% to 20% of all neonatal autopsies, accounting for 2850 deaths yearly. Development of engineered tissue substitutes that could functionally restore damaged tissue remains a unique opportunity for biotechnology. Recently, we isolated and characterized murine fetal pulmonary cells (FPC) and engineered 3-D pulmonary tissue constructs *in vitro*. Our goal is to devise a reliable and reproducible method for delivering FPC into a live animal model of PH.**

Materials and methods. **Three methods of delivery were explored: intraoral, intratracheal, and intrapulmonary injection. Adult Swiss Webster mice were anesthetized and fluorescent labeled microspheres (20 μm diameter) were delivered by intraoral and intratracheal injection. Subsequently, labeled FPC (Cell Tracker, CMTPX; Molecular Probes, Eugene, OR) were delivered by the same methods. In addition, direct transpleural intrapulmonary injection of FPC was performed. Outcome analysis included survival, reproducibility, diffuse *versus* confined location of the injected substance, and adequacy of delivery. Routine histological examination, fluorescent microscopy, and immunostaining were performed.**

Results. **Microspheres: We demonstrated reproducible, diffuse instillation via tracheotomy into the distal alveoli. Intraoral delivery appeared less reliable compared to direct intratracheal injection. FPC: Intratracheal injection was a reliable method of delivery. Labeled FPC showed transepithelial migration after 7 d of *in vivo* culture. Intrapulmonary injection led to local accumulation of cells in sites of injection.**

Conclusions. **We demonstrate that delivery of FPC is feasible with intratracheal injection giving the most reliable, diffuse delivery throughout the lung. This represents the first step toward translational research with site-specific delivery for a cell-based therapeutic approach toward PH and similar pulmonary diseases.**

Key Words: **cell-based therapy; pulmonary hypoplasia; murine fetal pulmonary cells; intratracheal injection; intraoral injection; intrapulmonary injection; transepithelial migration.**

INTRODUCTION

Preterm delivery with resultant developmental lung abnormalities (i.e., pulmonary hypoplasia [PH]) is a major problem in neonatology and accounts for more than 70% of perinatal mortality [1]. The pathology of PH includes reduced lung mass, insufficient surfactant production, poorly differentiated alveolar epithelium, and a reduction of alveolar gas exchange [2].

The loss or failure of lung tissue is one of the most frequent, devastating, and costly problems in health care. The most frequently used and successful method of therapy is transplantation. However, the severe scarcity of donor organs, especially in the pediatric population, is a major limitation and has thus stimulated investigation into selective cell transplantation and other molecular-based therapies [3–8].

Because of advances in tissue engineering, novel cell-based therapies are emerging as available treatment modalities for damaged tissues [7, 8]. Delivery of engineered cells into hypoplastic lung tissue has the potential to improve underdeveloped lung tissue and aid in restoring the process of natural tissue development. Drug delivery alone does not have the ability to achieve both of these effects.

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We have recently been able to demonstrate histiotypic tissue construct formation from a mixed population of fetal pulmonary cell culture *in vitro* [9, 10]. After characterization of the cells, we further examined methods of introduction into the lung parenchyma. We began by investigating three different delivery methods for the *in vivo* administration of fetal pulmonary cells (FPC) into the pulmonary system.

MATERIALS AND METHODS

The Institutional Animal Care and Usage Committee (IACUC no.16150) at Drexel University approved all animal protocols in compliance with the Guide for the Care and Use of Laboratory Animals. Adult Swiss Webster female mice (Charles River Laboratories, Wilmington, VA) were injected with different substrates between March 2006 and December 2006.

Methods of Injection (Fig. 1)

Intraoral Injection

Adult Swiss Webster mice were anesthetized with isofluorane. Direct laryngoscopy with a small spatula was performed and a 24 gauge angiocathether was inserted into the oropharynx to deliver either microspheres (100 μL suspension, microsphere solution diluted 1:1 in 10X phosphate-buffered saline [PBS]) or fluorescently labeled fetal pulmonary cells (10 million CMTPX CellTracker labeled FPC suspended in 100 μL of medium as described below).

Intratracheal Injection

Adult Swiss Webster mice were anesthetized with isofluorane. A small incision was made over the anterior neck in a transverse fashion. Blunt dissection was used to identify the trachea and a 27 gauge needle was inserted between the tracheal rings. Microspheres or labeled FPC (10 million labeled FPC suspended in 100 μL of medium) were delivered as described below. Adequate injection was evidenced by visualization of the suspension through the tracheal tissue with minimal reflux of suspension back through the nose. Pain relief was obtained with buprenorphine (0.2 mL Buprenex diluted 1:100 in 10X PBS) injected subcutaneously before termination of the procedure. The incision site was closed with 4-0 silk sutures.

Intrapulmonary Injection

Adult Swiss Webster mice were anesthetized with isofluorane and a skin incision was made over the right chest. The muscle layers were dissected sharply until the lung was visualized through the intercostal spaces. With a 27 gauge needle 10 million labeled FPC in 100 μL of 1 mg/mL collagen Type 1 solution (BD Biosciences, San Jose, CA) were injected through the intercostal space directly into the lung parenchyma. The collagen solution was used as a delivery vehicle to localize the distribution of the engrafted cells, as the collagen solution gels rapidly at 37°C. The skin was closed in an interrupted fashion with 4-0 silk sutures. Buprenorphine was administered subcutaneously for pain relief as described above.

Spheres and Labeled FPC (Fig. 1)

Sphere Delivery

One hundred μL of a solution containing fluorescent microspheres (microspheres diluted 1:1 in 10X PBS, yellow; 20 μm diameter, Polysciences, Warrington, PA) were administered with a 24 gauge angiocatheter intraorally or with a 27 gauge needle intratracheally. After the procedure, the animals were housed overnight. Lungs were harvested on the next day, washed in 10X PBS and fixed in paraformaldehyde overnight. Tissue was embedded in OCT compound (Triangle Biomedical Sciences, Durham, NC), snap-frozen at −80°C and stored at −80°C. Thirty micron sections were prepared with a cryostat. Slides were mounted with Vectashield mounting medium containing DAPI (Vector Laboratories, Burlington, CA) for nuclear counterstaining.

Cellular Delivery

Labeled FPC were administered intraorally, intratracheally or intrapulmonary, as described above.

FPC Isolation

Murine FPC were obtained from the lungs of timed pregnant Swiss Webster mouse fetuses at gestational day 18 (Charles River Laboratories) as previously described [9–11]. Briefly, fetal lungs were surgically removed, rinsed in Hanks balanced salt solution (HBSS; Cellgro, Herndon, VA) and minced. Following mincing, the tissue was first triturated in and then digested with 0.5% trypsin in PBS for 5 and 20 min, respectively. Following quenching of the trypsin with Dulbecco's modified Eagle medium containing FBS (Cambrex, East Rutherford, NJ) and filtration through a 70 μ filter (BD Falcon, San Jose, CA), the cell suspension was pelleted for 5 min at 800 rpm. The pellet was resuspended for 30 s in 900 μL distilled water to remove the RBCs by hypotonic lysis, followed by the addition of 100 μL of 10 × PBS (Cellgro). The cells were washed once more in 1 × Ca^{2+}/Mg^{2+} containing PBS, and resuspended in complete medium (DMEM +10% fetal bovine serum + antibiotics). A suspension of 10 million CMTPX CellTracker labeled FPC in 100 μL of medium or 100 μL of 1 mg/mL collagen Type 1 solution was injected.

Labeling

To identify the injected FPC *in vivo*, the cells were labeled *in vitro* with Cell Tracker (CMTPX; Molecular Probes, Eugene, OR), a fluo-

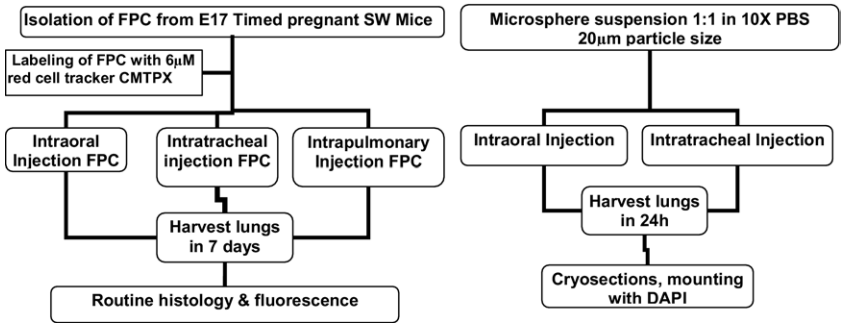

FIG. 1. Schematic depicting the delivery of fluorescent microspheres and labeled FPC, harvesting and sample processing times.