

# Stem Cell Transplantation Effectively Occludes Bronchopleural Fistula in an Animal Model

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**Background.** Bronchopleural fistula after lung resection still represents a challenging life-threatening complication for thoracic surgeons. Considering its extremely high mortality rate, an effective treatment is urgently required. Our project investigated the hypothesis of experimental bronchopleural fistula closure by bronchoscopic injection of autologous bone marrow-derived mesenchymal stem cells into the cavity of the fistula, evaluating its feasibility and safety in a large animal model.

**Methods.** An experimental bronchopleural fistula was created in 9 goats after right upper tracheal lobectomy. The animals were randomly assigned to two groups: one received autologous bone marrow-derived mesenchymal stem cell bronchoscopic transplantation; the other received standard bronchoscopic fibrin glue injection.

**Results.** All animals receiving bronchoscopic stem cell transplantation presented fistula closure by extraluminal fibroblast proliferation and collagenous matrix development;

none (0%) died during the study period. All animals receiving standard treatment still presented bronchopleural fistula; 2 of them (40%) died. Findings were confirmed by pathology examination, computed tomography, and magnetic resonance imaging.

**Conclusions.** Bronchoscopic transplantation of bone marrow-derived mesenchymal stem cells effectively closes experimental bronchopleural fistula by extraluminal fibroblast proliferation and collagenous matrix development. Stem cells may play a crucial role in the treatment of postresectional bronchopleural fistula after standard lung resection. Although these results provide a basis for the development of clinical therapeutic strategies, the exact mechanism by which they are obtained is not yet completely clear; further studies are required to understand exactly how stem cells work in this field.

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Lung cancer is the leading cause of cancer death worldwide [1]. For patients with limited disease, surgical resection is the most effective method of controlling the primary tumor and provides the best opportunity for cure. Therefore every patient with non-small cell lung cancer is approached as a surgical candidate [2].

Postresectional bronchopleural fistula (BPF) is a pathological connection between the airway (bronchus) and the pleural space that may develop after lung resection. In lung cancer operations, the incidence of BPF ranges from 1% to 4%, but its mortality ranges from 12.5% to 71.2% [3]. Bronchopleural fistula may be caused by incomplete bronchial closure, impediment of bronchial stump wound healing, or stump destruction by residual neoplastic tissue [3].

The clinical effect of failure of the bronchial stump to heal after anatomic lung resection may culminate in a

life-threatening septic and ventilatory catastrophe [4]. For many patients with empyema, the presence or absence of a fistula makes the difference among recovery, chronicity, or death [2].

We investigated experimental BPF closure by bronchoscopic injection of autologous bone marrow-derived mesenchymal stem cells (MSC) into the cavity of the fistula, evaluating its feasibility and safety in a large animal model.

## Material and Methods

The choice of a goat model was based on the anatomical similarities of the pleural cavity and mediastinum with those of human beings to reproduce all the aspects of clinical BPF as closely as possible: infection, suture technique, vascular ligation, bronchial dissection, and bronchial dehiscence.

## Technology

Animals were treated according the requirements of the European Union Directive 86/609 regarding the protection of animals used for experimental or other scientific

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purpose and the Council of Europe Convention for the protection of vertebrate animals used for experimental and other scientific purpose (ETS 123).

The present study was performed in nine 22- to 26-month-old female goats with a mean initial weight of 35 kg ( $\pm 2.5$  kg). Four animals entered the study group and 5 the control group. Each study group animal received a bone marrow biopsy of the iliac crest by a Snarecoil bone marrow biopsy needle (Ranfac Corp; Avon, MA). Autologous bone marrow–derived MSC were then isolated and expanded according to previous reports [5]. In detail, 10 mL bone marrow was aseptically collected in sterile heparinized tubes. The whole marrow washouts were layered onto Ficoll-Paque and centrifuged at 400g for 30 min at room temperature. A mononucleate cell ring was then removed and centrifuged then supplemented with ammonium chloride solution to eliminate red blood cells. Cells were cultured in Dulbecco's modified Eagle Medium with 20% fetal bovine serum with the addition of HEPES.

As MSC profiles vary among animals, the “stemness” of isolated cells was assessed by inducing osteogenic, chondrogenic, and adipogenic differentiation as described by Pittenger et al. [5], rather than immunogenic detection of antigens, differing from one animal to another. The same procedure was performed in the control group with medium and fibrin sealant delivery without MSC.

Cell growth in a fibrin substrate (Evicel; Ethicon; Somerville, NJ) was evaluated using MSC infected with a green fluorescent protein (GFP)-expressing lentiviral vector (Fig 1).

### Technique

Standard right upper tracheal lobectomy was performed on a goat model under general anesthesia without the need for single lung ventilation.

Right lateral muscle-sparing thoracotomy was performed; mediastinal vessels for the upper lobe were isolated and then transected after double manual ligation. The right upper bronchus was then exposed, transected, and clamped close to the tracheal takeoff to avoid gas and oxygen leakage during intraoperative ventilation. Lobectomy was completed by fissure manual division similarly to human right upper fissureless lobectomy.

Bronchial stump closure was performed by single interrupted 3/0 polypropylene stitches (Prolene nonabsorbable monofilament; Ethicon; Somerville, NJ); the medial edge of the stump was left open and the caliber of the fistula homogeneously created by a standard 4-mm caliber probe left inside the bronchial lumen at the time of last stitch application. Water submersion test under standard airway pressure of 15 cm H<sub>2</sub>O was then performed to confirm the bronchial fistula and exclude other sites of air leakage.

Mean whole bronchial stump caliber was  $10.1 \pm 1$  mm in the transplanted group and  $9.9 \pm 1$  mm in the control group without any significant discrepancy among animals ( $p > 0.05$ ), the created fistula thus accounting for almost one-third of the entire stump caliber. Then

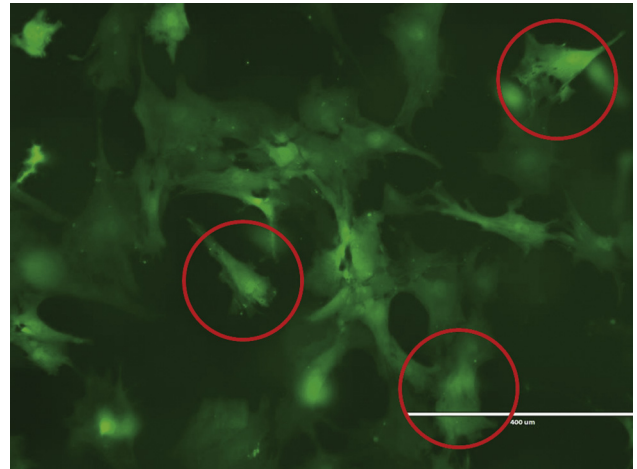


Fig 1. Mesenchymal stem cells infected with a green fluorescent protein (GFP)-expressing lentiviral vector (encircled) growing in a modified fibrin sealant substrate.

24 Fr/Ch (8.0 mm) silicone tubular drainage (Kendall Argyle-Tyco Healthcare; Tullamore, Ireland) was placed and connected to a unidirectional Heimlich valve (Laboratoires Pharmaceutiques VYGON; Ecouen, France) before chest closure. The chest tube was routinely removed on postoperative day 1 because of the high risk of incidental removal during stable housing of the animals and consequent external infection. A “purse string” suture on the chest drain insertion orifice was then tied to work as a Heimlich valve in case of major air leaks to avoid tension pneumothorax.

On postoperative day 7 cervical tracheostomy was performed under general anesthesia; a fiberoptic bronchoscope was inserted, the bronchial stump was inspected, and the bronchial fistula identified. A transbronchial aspiration needle (Olympus SmoothShot 19G  $\times$  13 mm; Olympus; Zoeterwoude, The Netherlands) was inserted through the bronchoscope operative channel; 5 mL medium with modified fibrin glue (Evicel; Ethicon; Somerville, NJ) containing  $2 \times 10^6$ /mL MSC were injected into the fistula and in the submucosal aspect of the bronchial stump. The same procedure was performed in the control group with medium and fibrin glue delivery without MSC.

On postoperative day 28, all animals were euthanized by intravenous injection of pentobarbital sodium. Re-do ipsilateral thoracotomy was performed and the right tracheobronchial system was harvested and then frozen. Autoptic evaluation and pathology examination together with computed tomography (CT) and magnetic resonance imaging (MRI) were also performed on the specimens.

## Results

### Clinical Experience

All animals in both groups still presented BPF on postoperative day 7, at the time of operative bronchoscopy and injection of MSC or fibrin sealant alone, proven by

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