



## Closure of a Recurrent Bronchopleural Fistula Using a Matrix Seeded With Patient-Derived Mesenchymal Stem Cells

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### ABSTRACT

Management of recurrent bronchopleural fistula (BPF) after pneumonectomy remains a challenge. Although a variety of devices and techniques have been described, definitive management usually involves closure of the fistula tract through surgical intervention. Standard surgical approaches for BPF incur significant morbidity and mortality and are not reliably or uniformly successful. We describe the first-in-human application of an autologous mesenchymal stem cell (MSC)-seeded matrix graft to repair a multiply recurrent postpneumonectomy BPF. Adipose-derived MSCs were isolated from patient abdominal adipose tissue, expanded, and seeded onto bio-absorbable mesh, which was surgically implanted at the site of BPF. Clinical follow-up and postprocedural radiological and bronchoscopic imaging were performed to ensure BPF closure, and *in vitro* stemness characterization of patient-specific MSCs was performed. The patient remained clinically asymptomatic without evidence of recurrence on bronchoscopy at 3 months, computed tomographic imaging at 16 months, and clinical follow-up of 1.5 years. There is no evidence of malignant degeneration of MSC populations *in situ*, and the patient-derived MSCs were capable of differentiating into adipocytes, chondrocytes, and osteocytes using established protocols. Isolation and expansion of autologous MSCs derived from patients in a malnourished, deconditioned state is possible. Successful closure and safety data for this approach suggest the potential for an expanded study of the role of autologous MSCs in regenerative surgical applications for BPF. *STEM CELLS TRANSLATIONAL MEDICINE* 2016;5:1375–1379

### SIGNIFICANCE

Bronchopleural fistula is a severe complication of pulmonary resection. Current management is not reliably successful. This work describes the first-in-human application of an autologous mesenchymal stem cell (MSC)-seeded matrix graft to the repair of a large, multiply recurrent postpneumonectomy BPF. Clinical follow-up of 1.5 years without recurrence suggests initial safety and feasibility of this approach. Further assessment of MSC grafts in these difficult clinical scenarios requires expanded study.

### INTRODUCTION

Postoperative bronchopleural fistula (BPF), particularly following pneumonectomy, remains a feared complication. Although surgical management has improved over time, incidence of postpneumonectomy BPF in large series remains approximately 2%–15% [1–3]. Timing of onset and recognition of BPF are highly variable, but median time of onset is typically on postoperative day 20 [1, 2]. A variety of techniques and devices have been used to treat BPF; standard surgical management typically requires pleural

drainage, management of sepsis, and closure of the open airway, frequently with vascularized tissue coverage. Current surgical approaches for BPF closure, although extremely heterogeneous, are not reliably or uniformly successful (10%–78% recurrence and 3%–71% perioperative mortality) [4–11]. Reports describe the use of autologous mesenchymal stem cells (MSCs) for treatment delivered bronchoscopically in animal models and recently in clinical cases [12–14]. Fistulas in these experimental models were lobar [15]; in the patients bronchoscopically injected, the fistulas were either small or

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tracheomediastinal fistulas associated with malignancy [12, 13]. We report closure of a large right mainstem postpneumonectomy BPF using a matrix graft seeded with autologous mesenchymal stem cells after multiple failed attempts at repair.

## METHODS

A 63-year-old woman was referred for treatment of a large right mainstem BPF (approximately 1.5 cm) temporized with an Eloesser window and wound packing. She had previously undergone a right pneumonectomy and chest wall resection for a T3N0M0 squamous cell carcinoma and developed a BPF approximately 30 days after the initial operation. This was temporized with an Eloesser flap, further chest wall resection, and dressing changes for control of sepsis. Despite best management, her BPF subsequently recurred. Combined, approximately 30 bronchoscopic and surgical attempts at either closure or management of complications of the resulting BPF were unsuccessful, including primary repair, omental flap coverage, tracheal stent placement, and bronchoscopic approaches. After exhausting therapeutic options, and considering the patient's declining health, we moved toward using an autologous MSC-seeded matrix graft in combination with a surgical procedure to repair the defect. The protocol and approach were based on an ongoing trial investigating this method to treat anal fistulas in Crohn's disease. Mayo Clinic Institutional Review Board (#14-004663) and Food and Drug Administration (FDA) Investigational New Drug classification (#16018) compassionate use exemption were granted.

### Adipose-Derived Mesenchymal Stem Cells

The patient underwent an abdominal wall adipose biopsy during bronchoscopic evaluation of the fistula. A small incision was made on the right side of the patient's abdominal wall, and under sharp dissection, 0.6 g (approximately 0.67 ml) of aseptically obtained adipose tissue was transferred sterilely to a container. Immediate isolation of MSCs was performed on the aseptically biopsied tissue. Briefly, tissue was washed in Dulbecco's phosphate-buffered saline (D-PBS), centrifuged, minced, and incubated in a 0.075% collagenase in D-PBS solution for 60 minutes. The solution was neutralized with MSC medium (PL5% medium), comprising advanced minimal essential medium (Thermo Fisher Scientific Life Sciences, Waltham, MA, <http://www.thermofisher.com>), GlutaMAX (Thermo Fisher Scientific Life Sciences), 5% platelet lysate (PLTMax; Mill Creek Life Sciences, Rochester, MN, <http://www.millcreeks.com>), and 2 IU/ml heparin.

The expanded MSCs were tested for cell-surface markers via Gallios flow cytometry (Beckman-Coulter, Danvers, MA, <https://www.beckmancoulter.com>) and found to be positive for CD90, CD73, CD105, CD44, and human leukocyte antigen (HLA)-ABC and negative for CD14, CD45, and HLA-DR [16]. Release testing included karyotype analysis and phenotype, mycoplasma, endotoxin, and sterility testing. The MSCs were found to be negative for all release criteria and were not differentiated before administration.

### Matrix and Cellular Seeding

Approximately 5 days before surgery, a matrix of synthetic bioabsorbable poly(glycolide:trimethylene carbonate) copolymer (BIO-A Tissue Reinforcement; Gore Medical, Newark, DE, <https://www.goremedical.com>) was seeded with  $2.5 \times 10^7$  autologous MSCs at passage 3 and placed in a bioreactor with fresh medium replaced daily (Fig. 1). The matrix was washed in lactated Ringer's solution and transferred to the operating room on the day of the procedure. Total time in bioreactor and cell seeding was 3 days and 22.5 hours (Fig. 2A, 2B). All procedures were performed under Good Manufacturing Practices consistent with FDA guidance.

### Surgical Approach for Fistula Closure

A redo right thoracotomy was performed, and the right mainstem bronchus was identified and dissected free to expose and resected flush with the carina (Fig. 2B). The airway was closed using interrupted 3-0 Vicryl sutures (Fig. 2C). Before placement, the MSC-seeded matrix was trimmed for optimal fit into the affected area. The matrix was anchored in position over the closure site with 3-0 Vicryl sutures (Fig. 2D). An abdominal free flap was harvested and used to obliterate the pleural space. Repeat bronchoscopy was performed, confirming integrity of the closure and airway patency.

### In Vitro Lineage Differentiation of MSCs

Patient MSCs underwent directed differentiation to characterize the differentiation capacity. MSCs were maintained in PL5% medium before adipocyte, chondrocyte (Human MSC Functional Identification Kit; R&D Systems, Minneapolis, MN, <http://www.rndsystems.com>), or osteogenic (Lifeline Cell Technology, Frederick, MD, <http://www.lifelinecelltech.com>) differentiation, using manufacturer-supplied protocols.

## RESULTS

### MSC Differentiation

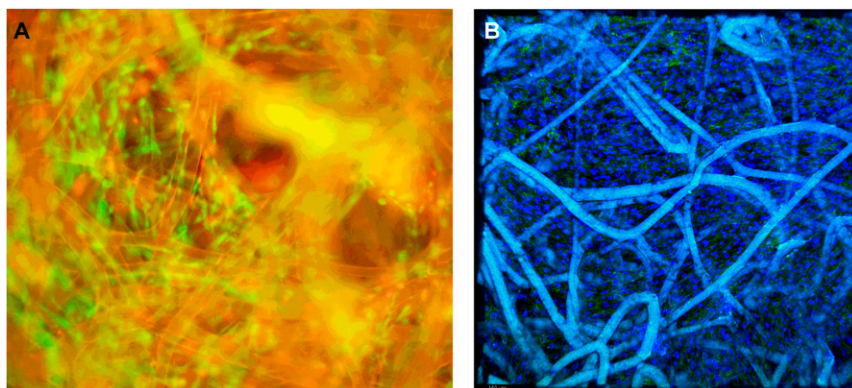
Patient-specific MSCs were capable of differentiating into adipocytes, chondrocytes, and osteocytes using established protocols consistent with International Society for Cellular Therapy criteria [16].

### Clinical Follow-Up

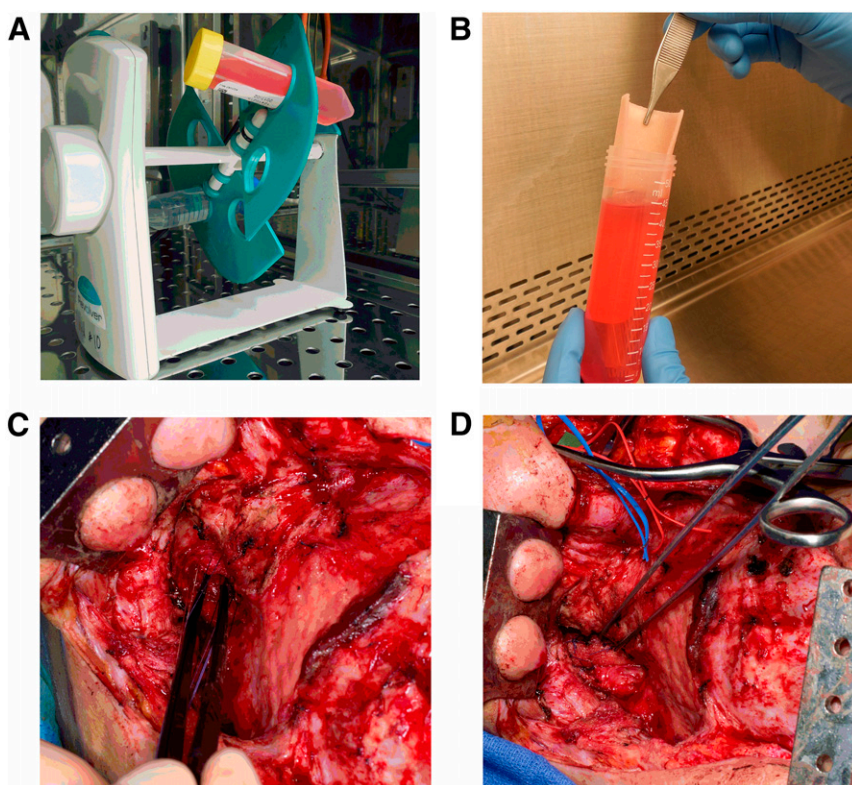
After surgical closure, the patient underwent redo tracheostomy to facilitate postoperative ventilation and secretion management. She was decannulated on postoperative day 17 and discharged home in good condition on postoperative day 25. Surveillance bronchoscopy (3 months) (Fig. 3), computed tomography scan of the chest (16 months), and clinical follow-up at 18 months have demonstrated the fistula to be well healed. The patient is doing clinically well, uses positive pressure ventilation at night, and has resumed her activities of daily living.

## DISCUSSION

To our knowledge, this case represents the first in-human report of a surgically placed MSC graft for repair of a large,



**Figure 1.** Microscopic assessment of matrix cell seeding. **(A):** Ethidium bromide (red) and Syto-13 (green) costain demonstrating live and dead cells on mesenchymal stem cell seeding on matrix. **(B):** Confocal microscopy with CD90 (Thy-1) fluorescein isothiocyanate (green) and Hoechst 33342 (trihydrochloride trihydrate) (blue) fluorescent nuclear staining. These images were captured using a  $\times 20$  objective and a  $\times 10$  eyepiece, for a combined magnification of  $\times 200$ . Scale bar =  $150 \mu\text{m}$ .

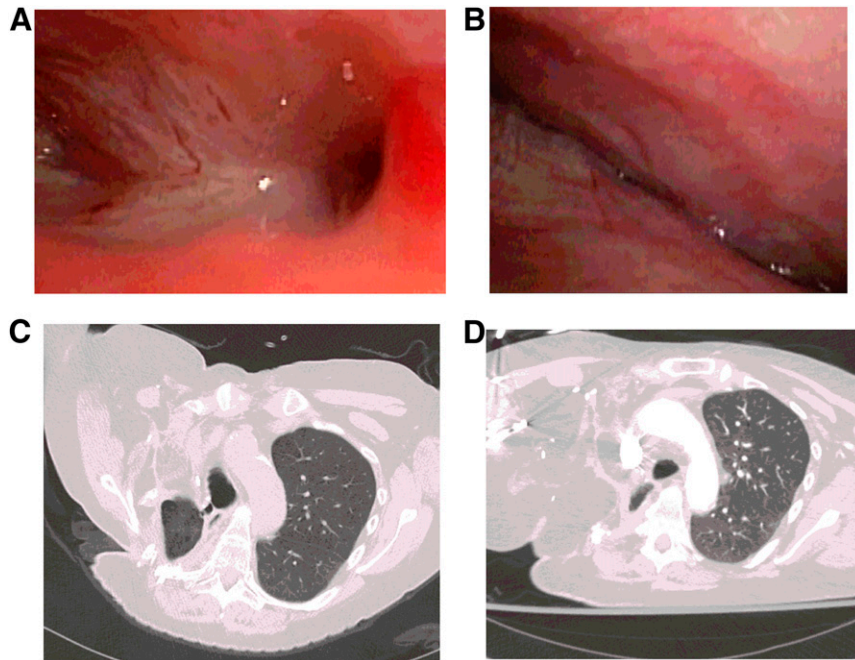


**Figure 2.** Manufacturing the mesenchymal stem cell matrix under good-manufacturing-practices conditions and operative implantation. **(A):** Before surgery, synthetic bio-absorbable matrix was MSC-seeded and placed in the bioreactor, and medium was changed daily. **(B):** On the day of surgical implantation, the matrix was rinsed and transported sterilely to the operating room. **(C):** Redo thoracotomy exposure and resection of the bronchial stump was performed (forceps in bronchial defect). **(D):** The bioengineered MSC-seeded matrix was implanted over the closed fistula site (forceps indicate mesh location).

multiply recurrent BPF. Although the results demonstrate the safety and feasibility of using MSC-seeded grafts in this difficult scenario without adverse events, it is unclear to what degree the graft itself contributed to successful healing of the fistula. Although surgical closure and tissue flap coverage are standard approaches to postpneumonectomy BPF, the failure of previous surgical interventions, the extreme deconditioning of the patient, and the resultant definitive closure suggest

the possibility that the intervention played some role in recovery.

Our results also demonstrate that patient-derived MSCs can be successfully harvested from patients with compromised functional and nutritional status and may be proliferated, expanded, and seeded on an MSC-loaded graft for surgical reconstruction. We have further shown that these patient-derived MSC populations have potential to be differentiated in vitro



**Figure 3.** Preoperative imaging showing size and location of fistula, and postoperative imaging demonstrating disease resolution. **(A):** Preoperative bronchoscopy demonstrating large bronchopleural fistula (BPF) cavity and lateral extension of fistula tracts. **(B):** Postoperative bronchoscopy (3 months) demonstrating progressive healing of BPF site. **(C):** Preoperative computed tomography scan demonstrating large BPF with connection to atmosphere (additional axial slices inferiorly). **(D):** Postoperative computed tomography scan (16 months) demonstrating resolution of BPF.

into a number of nonepithelial lineages potentially required for airway regeneration consistent with MSC definitions [16]. Prior large animal studies by Petrella et al. [15] proposed that the regenerative potential of MSCs in BPF may be through fibroblast proliferation and deposition of collagen matrix materials. It is unclear whether the MSCs themselves serve this role and provide the matrix deposition and differentiation, or whether the mechanism of airway regeneration instead is signaled through MSC-secreted molecules in vivo [17, 18]. Future directions aimed at determining the mechanistic steps of BPF resolution using animal models are warranted, as is further patient experience studying safety and efficacy.

#### CONCLUSION

We have demonstrated that repair of a large, recurrent BPF in a functionally compromised patient was accomplished through use of autologous MSCs and cellularization of synthetic materials with surgical implantation. The approach was well tolerated, suggesting the potential for expanded use of autologous MSCs in regenerative surgical applications.

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#### AUTHOR CONTRIBUTIONS

J.M.A.: literature search, collection of data, data analysis and interpretation, figure creation, manuscript writing, final approval of manuscript; A.B.D., W.A.F., and D.A.W.: study design, data analysis and interpretation, manuscript writing, critical revision and final approval of manuscript; D.J.R.: data analysis and interpretation, manuscript writing, final approval of manuscript; G.W.B.: study design, data analysis and interpretation, final approval of manuscript; M.T.: data analysis, figure creation, manuscript writing, critical revision and final approval of manuscript; T.J.N.: data analysis and interpretation, critical revision and final approval of manuscript; B.T.C. and S.D.C.: study design, collection of data, data analysis and interpretation, final approval of manuscript; Z.T.R.: collection of data, data analysis and interpretation, figure creation, manuscript writing, critical revision and final approval of manuscript.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

A.B.D. has compensated intellectual property rights, is an uncompensated consultant for, and has uncompensated ownership interest in Mill Creek Life Sciences. The other authors indicated no potential conflicts of interest.

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