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

JOHNATHON M. AHO,1,2,3 ALLAN B. DIETZ,5,6 DARCE J. RADEL,1 GREG W. BUTLER,5 MATHEW THOMAS,4 TIMOTHY J. NELSON,6 BRIAN T. CARLSEN,1 STEPHEN D. CASSIVI,2 ZACHARY T. RESCH,6 WILLIAM A. FAUBION,4 DENNIS A. WIGLE3

Key Words. Bronchopleural fistula • Mesenchymal stem cells • Cell transplantation • Cellular therapy • Clinical translation • Stem cell transplantation

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

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 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 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.

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 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.
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 steriley 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 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.

Matrix and Cellular Seeding

Approximately 5 days before surgery, a matrix of synthetic bio-absorbable poly(glycolide:trimethylene carbonate) copolymer (BIO-A Tissue Reinforcement; Gore Medical, Newark, DE, https://www.goremedical.com) was seeded with 2.5 × 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.rndsystem.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, postpneumonectomy BPF using a matrix graft seeded with autologous mesenchymal stem cells after multiple failed attempts at repair.

©AlphaMed Press 2016
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.
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.

ACKNOWLEDGMENTS

J.M.A. is supported by a grant from the National Heart, Lung, and Blood Institute (T32 HL105355). These funders had no role in the design or conduct of the study, collection, management, analysis, or interpretation of the data, or preparation, review, or approval of the article.

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; 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.

REFERENCES

1. Deschamps C, Bernard A, Nichols FC 3rd et al. Empyema and bronchopleural fistula after pneumonectomy: Factors affecting incidence. Ann Thorac Surg 2001;72:243–247; discussion 248.
2. Panagopoulos ND, Apostolakis E, Koletis E et al. Low incidence of bronchopleural fistula after pneumonectomy for lung cancer. Interact Cardiovasc Thorac Surg 2009;9:571–575.
3. Sonobe M, Nakagawa M, Ichinose M et al. Analysis of risk factors in bronchopleural

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.
fistula after pulmonary resection for primary lung cancer. Eur J Cardiothorac Surg 2000;18:519–523.

4 Cardillo G, Carbone L, Carleo F et al. The rationale for treatment of postresectional bronchopleural fistula: Analysis of 52 patients. Ann Thorac Surg 2015;100:251–257.

5 Hollaus PH, Lax F, Janakiev D et al. Endoscopic treatment of postoperative bronchopleural fistula: Experience with 45 cases. Ann Thorac Surg 1998;66:923–927.

6 Varoli F, Roviaro G, Grignani F et al. Endoscopic treatment of bronchopleural fistulas. Ann Thorac Surg 1998;65:807–809.

7 Scappaticci E, Ardisone F, Ruffini E et al. Postoperative bronchopleural fistula: Endoscopic closure in 12 patients. Ann Thorac Surg 1994;57:119–122.

8 Fruchter O, Kramer MR, Dagan T et al. Endobronchial closure of bronchopleural fistulae using amplatzer devices: Our experience and literature review. Chest 2011;139:682–687.

9 Boudaya MS, Smadhi H, Zribi H et al. Conservative management of postoperative bronchopleural fistulas. J Thorac Cardiovasc Surg 2013;146:575–579.

10 Asamura H, Naruke T, Tsuchiya R et al. Bronchopleural fistulas associated with lung cancer operations. Univariate and multivariate analysis of risk factors, management, and outcome. J Thorac Cardiovasc Surg 1992;104:1456–1464.

11 Bazzocchi R, Bini A, Grazia M et al. Bronchopleural fistula prevention after major pulmonary resection for primary lung cancer. Eur J Cardiothorac Surg 2002;22:160.

12 Petrella F, Spaggiari L, Acocella F et al. Airways fistula closure after stem-cell infusion. N Engl J Med 2015;372:96–97.

13 Alvarez PD-A, Garcia-Arranz M, Georgiev-Hristov T et al. A new bronchoscopic treatment of tracheomediastinal fistula using autologous adipose-derived stem cells. Thorax 2008;63:374–376.

14 Diaz-Agero Álvarez PJ, Bellido-Reyes YA, Sánchez-Girón JG et al. Novel bronchoscopic treatment for bronchopleural fistula using adipose-derived stromal cells. Cytotherapy 2016;18:36–40.

15 Petrella F, Toffalorio F, Brizzola S et al. Stem cell transplantation effectively occludes bronchopleural fistula in an animal model. Ann Thorac Surg 2014;97:480–483.

16 Dominici M, Le Blanc K, Mueller I et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315–317.

17 Weiss DJ. Concise review: Current status of stem cells and regenerative medicine in lung biology and diseases. STEM CELLS 2014;32:16–25.

18 Tropea KA, Leder E, Aslam M et al. Bronchoalveolar stem cells increase after mesenchymal stromal cell treatment in a mouse model of bronchopulmonary dysplasia. Am J Physiol Lung Cell Mol Physiol 2012;302:L829–L837.