Influence of Mesenchymal Stem Cells on Cryopreserved Tracheal Allografts in Rabbits

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Background: Ischemic injury and the rejection process are the main reasons for graft failure in tracheal transplantation models. To enhance the acceptance, we investigated the influence of mesenchymal stem cells (MSCs) on tracheal allografts. **Methods:** Extracted tracheal grafts from New Zealand white rabbits were cryopreserved for 4 weeks and orthotopically transplanted (control group A, n=8). In group B (n=8), cyclosporin A (CsA, 10 mg/kg) was injected daily into the peritoneal cavity. In group C (n=8), MSCs (1.0×10^7 cells/kg) from the same donor of the tracheal allograft, which had been pre-cultured for 4 weeks, were infused intravenously after transplantation. In group D (n=8), MSCs were infused and CsA was injected daily. Four weeks after transplantation, gross and histomorphological assessments were conducted for graft necrosis, measuring the cross-sectional area of the allograft, determining the degree of epithelization, lymphocytic infiltration, and vascular regeneration. **Results:** The morphologic integrity of the trachea was retained completely in all cases. The cross-sectional areas were decreased significantly in group A (p=0.018) and B (p=0.045). The degree of epithelization was enhanced (p=0.012) and the lymphocytic infiltration was decreased (p=0.048) significantly in group D compared to group A. The degree of vascular regeneration did not differ significantly in any of the groups. There were no significant correlations among epithelization, lymphocytic infiltration, and vascular regeneration. **Conclusion:** The administration of MSCs with concurrent injections of CsA enhanced and promoted epithelization and prevented lymphocytic infiltration in tracheal allografts, allowing for better acceptance of the allograft.

Key words: 1. Mesenchymal stem cells
              2. Trachea
              3. Cryopreservation
              4. Transplantation

INTRODUCTION

Treatments for long segmental and circumferential tracheal defects remain a challenge. Although synthetic and bio-synthetic flaps [1] and reconstitution of the trachea through tissue engineering [2,3] have been investigated, their clinical applicability is questionable because of functionality, biomechanics, and viability. In tracheal transplantation models, the restoration of epithelization and vascularization, as well as immunosuppression, are necessary to enhance the acceptance of an allograft.

Tracheal revascularization and epithelial regeneration are concomitant processes and an adequate blood supply to the tracheal allograft plays a great role in preventing mucosal

disintegration [4]. The tracheal blood supply has been restored by revascularization from tracheal vessels [5] and a pedicled flap of omentum or muscles [6,7]. Since the airway complications after lung transplantation are not affected by the presence or absence of a flap wrapped around the bronchial anastomosis [8,9], and the immunologic process is not related to the degree of revascularization, the histological alterations seen in tracheal allograft are therefore produced by activation of the immune response rather than transient ischemia by itself [10].

The trachea is subject to the same immunological principles of transplantation as all other transplanted tissues [11,12]. The human tracheal mucosa expresses HLA class II molecules, and this may be the major antigenic structure that is responsible for the immunologic response against allogenic tracheal transplants [13,14]. To prevent the mucosal rejection mediated by the mononuclear cells [4], continuous immunosuppression with cyclosporin A (CsA) is necessary to maintain an optimal condition for tracheal allograft survival [15]. However, CsA does not alter the injuries sustained by the allograft through the phases of early ischemia [16], and the resulting immunosuppression may increase the risk of infection or aggravate a malignant process; therefore, attenuation of tracheal allograft antigenicity is required, which can be greatly reduced by removing the tracheal epithelium and by cryopreservation [17]. However, if the inner surface of the trachea is not remodeled by the recipient's epithelium, fibro-proliferative tissue can enter the tracheal lumen and will lead to tracheal obstruction [18], emphasizing the role of the epithelium in controlling airway obliteration [19] and affecting the graft's antigenicity [20].

Another method to reduce the antigenicity is the induction of chimerism by injection of mesenchymal stem cells (MSCs). Mixed allogenic chimerism induces donor-specific tolerance and prevents the development of fibro-proliferative obstruction [21] of the tracheal allograft by improving epithelial regeneration [22] and neovascularization [23], as well as modulating the recipient's immune response [24].

To develop a transplantation method to enhance the acceptance of an allograft, we conducted a study to determine the influence of MSCs on the survival of cryopreserved tracheal allograft in rabbits.

METHODS

1) Animal

Male New Zealand White rabbits weighing 3.0 to 3.5 kg were purchased from Samtacho Inc. (Osan, Korea) and cared for in accordance with the international guideline for laboratory animal care.

2) Harvesting and culture of mesenchymal stem cells of donor rabbits

The donor rabbits were sedated with Zoletil 50 (Tilentamin ＋Zolazepam; Virbac Laboratories, Carros, France) and then sacrificed with an intravenous injection of KCl. Under sterile conditions, both femurs were dissected and disarticulated. Both ends of each femur were excised and the marrows were flushed out with 5 mL of low-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco/BRL, Grand Island, NY, USA) and 1 mL (5,000 IU) heparin. The heparinized bone marrow in the DMEM medium was transferred into a 50 mL Falcon tube and centrifuged at 600 g for 10 minutes, and the supernatant was discarded. The resulting cell pellet was re-suspended with 7 mL low-glucose DMEM medium and 4% acetic acid. The nucleated cells were adjusted to a density of 1×10^5 cell/mL and were cultured in low-glucose DMEM medium with 10% fetal bovine serum (FBS; Gibco/BRL) and 1% antibiotic solution (penicillin-streptomycin; Gibco/BRL) at $37^{\circ}C$ in a humidified atmosphere containing 5% CO_2. When the cultures were near confluence, the cells were detached by a treatment with 0.05% trypsin EDTA (trypsin ethylenediaminetetraacetic acid; Gibco/BRL) and replated. After 4 weeks of two to three passages (Fig. 1), MSCs were adjusted to 1.0×10^7 cells/mL and resuspended with HBSS solution (Hank's Balanced Salt Solution; Sigma, St. Louis, MO, USA).

3) Harvesting and cryopreservation of trachea allograft

After harvesting the bone marrow, the whole trachea was harvested through a median cervical incision. The harvested trachea was then trimmed off to a 2 to 3 cm segment. The diameter of the graft was measured and then stored in a cryogenic vial filled with the freezing solution containing low-glucose DMEM, 10% dimethyl sulfoxide (DMSO; Sigma), 40% FBS, and 1% antibiotic solution. The tracheal graft was
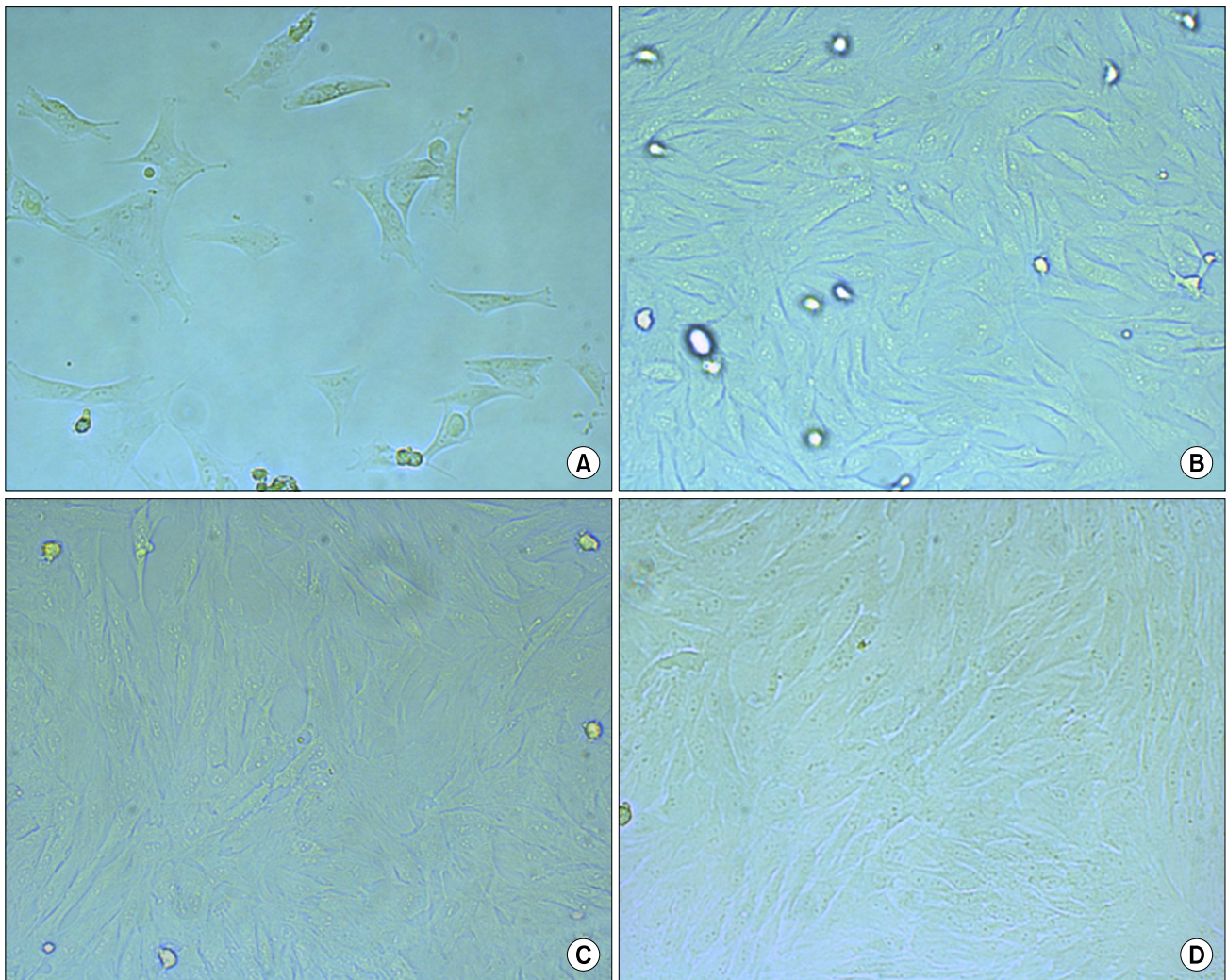
Fig. 1. Mesenchymal stem cell culture and proliferation. (A) Day 6 with passage 0; (B) day 12, passage 1; (C) day 16, passage 2; (D) day 21, passage 2. On day 21 with passage 2, the other marrow components had disappeared and the mesenchymal stem cells proliferated predominantly (Phase contrast, ×100 for all figures).

frozen in a programmed freezer (Cryo Med 1010; Forma Scientific, Marietta, OH, USA) which cooled at a rate of 1°C/min until -90°C was reached and was then kept in liquid nitrogen (-196°C) for 4 weeks. The cryopreserved allograft was thawed in normal saline at 37°C for 15 minutes. The freezing solution was washed out using DMEM with 5% DMSO for 5 minutes, and then with DMEM only for an additional 5 minutes.

4) Tracheal transplantation

A weight-matched male New Zealand White rabbit was in-

jected intramuscularly with 0.2 mg/kg atropine sulfate (Dai Han Pharm Co., Ansan, Korea), 50 mg/kg Flumarin Inj (flomoxef sodium; Ildong Pharm Co., Seoul, Korea), and 10 mg/kg Zoletil 50. The deeply sedated but spontaneously breathing recipient rabbit was placed in a supine position. A midline cervical incision was made and the whole cervical trachea was exposed. A 1-cm segment of the cervical trachea was excised. The diameter of the recipient's native trachea was measured and compared to that of the allograft and any difference was noted. Orthotopic transplantation was performed in an end-to-end continuous fashion using absorbable

monofilament suture (5-0 Mexon, Syneture; Tyco, Norwalk, CT, USA) and the allograft was wrapped with adjacent muscle and lymphoid tissues. The wound was closed with 3-0 Ethilon (monofilament non-absorbable suture; Johnson & Johnson Co., New Brunswick, NJ, USA).

5) Experimental design

The cryopreserved tracheal grafts were transplanted orthotopically, and the transplant recipient rabbits were divided into four groups. In group A (n=8), no additional interventions were performed. In group B (n=8), 10 mg/kg Sandimmun (cyclosporin A [CsA]; Novartis Korea, Seoul, Korea) was injected daily into the peritoneal cavity starting from day 0. In group C (n=8), MSCs (1.0×10^7 cells/kg) from the same donor of the tracheal allograft, and which had been cultured for 4 weeks, were infused intravenously to the corresponding recipient following completion of the transplantation procedure on day 0. In group D (n=8), MSCs were infused and 10 mg/kg CsA was injected daily.

6) Assessment of the tracheal allograft

Four weeks after transplantation, the recipients were sacrificed with intravenous injection of KCl, and the tracheal allografts were extracted en bloc including the native trachea. Macroscopic assessments were conducted by checking for graft necrosis and measuring the cross-sectional area of the allograft. The cross-sectional area (mm^2) of the allograft was estimated by using the formula ($\pi \times$long diameter [mm]/2$\times$ short diameter [mm]/2). The specimens were then fixed in buffered formalin (4%) and stained with hematoxylin-eosin for histomorphological assessments for evaluating the degree of graft epithelization, lymphocytic infiltration, and vascular regeneration.

The pathologic changes were assessed using the following scales. The degree of epithelization (Fig. 2) was estimated by the layers of epithelium (grade 0=no epithelization; grade 1=less than 20% epithelization with a single layer of epithelium; grade 2=20% to 40% epithelization with several layers; grade 3=40% to 60% epithelization with columnar epithelium; grade 4=60% to 80% epithelization with full layers of columnar epithelium; grade 5=more than 80% epithelization with complete columnar epithelium). The degree of lym-

phocytic infiltration (Fig. 3) was estimated by counting the lymphocytes in the submucosa (grade 0=infiltration absent; grade 1=limited [less than 30%] infiltration; grade 2=local [30% to 70%] infiltration; grade 3=diffuse [more than 70%] infiltration). The degree of vascular regeneration (Fig. 4) was estimated by the number of capillaries in the submucosa filled with erythrocytes (grade 0=no vascular regeneration, grade 1=vascular regeneration less than that of the native trachea; grade 2=vascular regeneration similar to that of the native trachea; grade 3=vascularization more than that of the native trachea).

7) Statistical analysis

SPSS ver. 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The continuous variables, that is, the cross-sectional areas of the allograft and native trachea, were expressed as mean±standard deviation and compared using the Wilcoxon rank sum test. The degree of epithelization, lymphocytic infiltration, and vascular regeneration were expressed as mean±standard deviation and estimated by comparison of the Fisher's exact t-test and correlation analysis of the Pearson coefficient. A p-value of less than 0.05 was considered statistically significant.

RESULTS

1) Macroscopic assessments of tracheal allograft

Four weeks after transplantation, the morphologic integrity of the extracted tracheal allograft had been completely retained in all cases without dehiscence or necrosis. The vessels from adjacent wrapped tissues had regenerated well in all of the cases. The morphologic figures of the tracheas had been preserved well in groups C and D, but were shrunken and had become O-shaped in groups A and B.

The sizes of the tracheal allografts estimated before transplantation were about 5×7 mm in rabbits weighing 3.0 to 3.5 kg and there were no differences between the proximal and distal ends, or between each of the groups. The cross-sectional areas tended to have decreased in all of the groups after transplantation, but they had decreased significantly in groups A (p=0.018) and B (p=0.045) (Table 1).
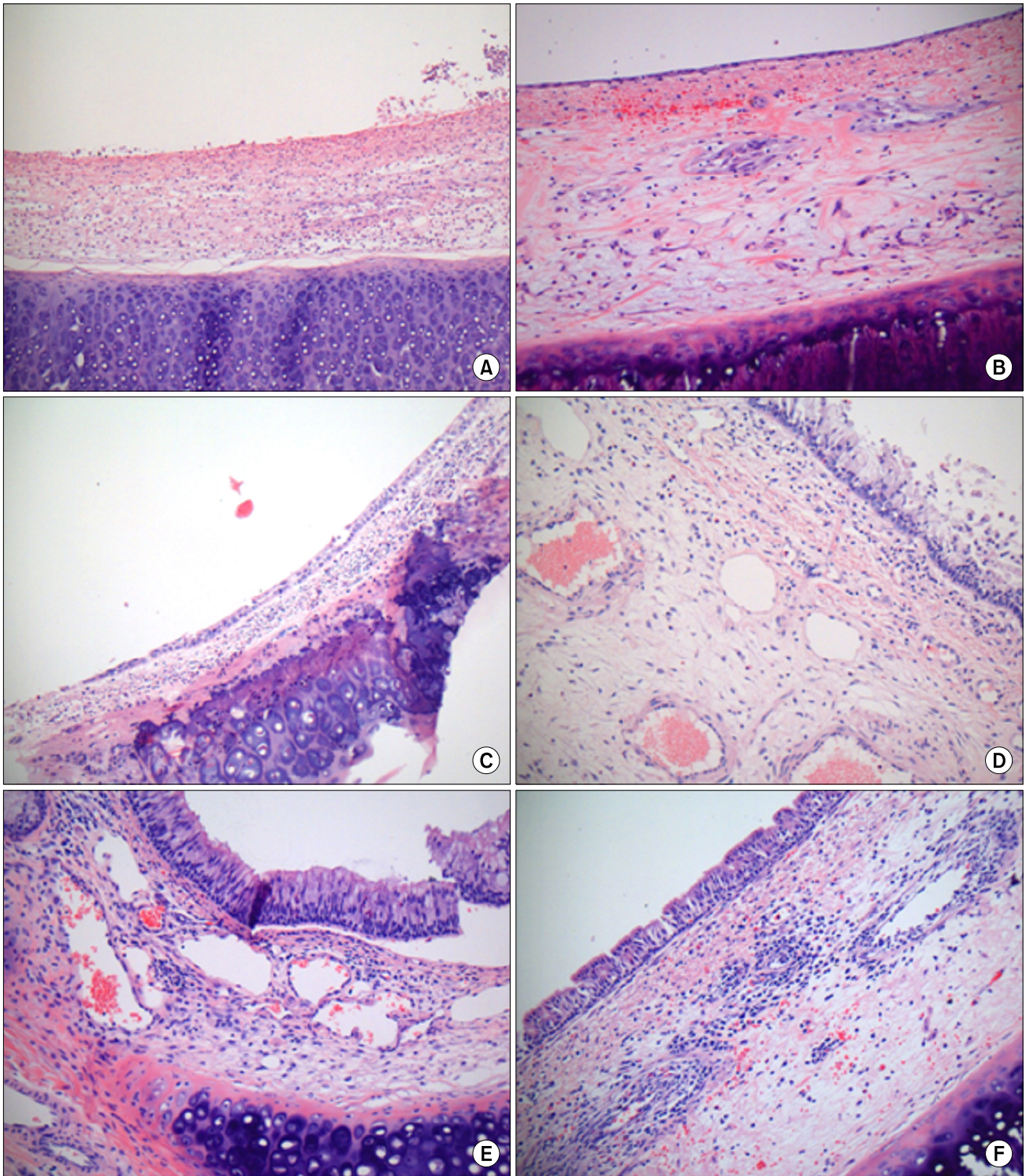
Fig. 2. Microscopic grading of the degree of epithelial regeneration. (A) Grade 0, the epithelium was not regenerated. (B) Grade 1, epithelization was less than 20% with a single layer of epithelium. (C) Grade 2, epithelization was between 20% and 40% with several layers of epithelium. (D) Grade 3, epithelization was between 40% and 60% with columnar epithelium. (E) Grade 4, epithelization was between 60% and 80% with full layers of columnar epithelium. (F) Grade 5, epithelization was more than 80% with complete columnar epithelium (H&E stain, ×100 for all figures).
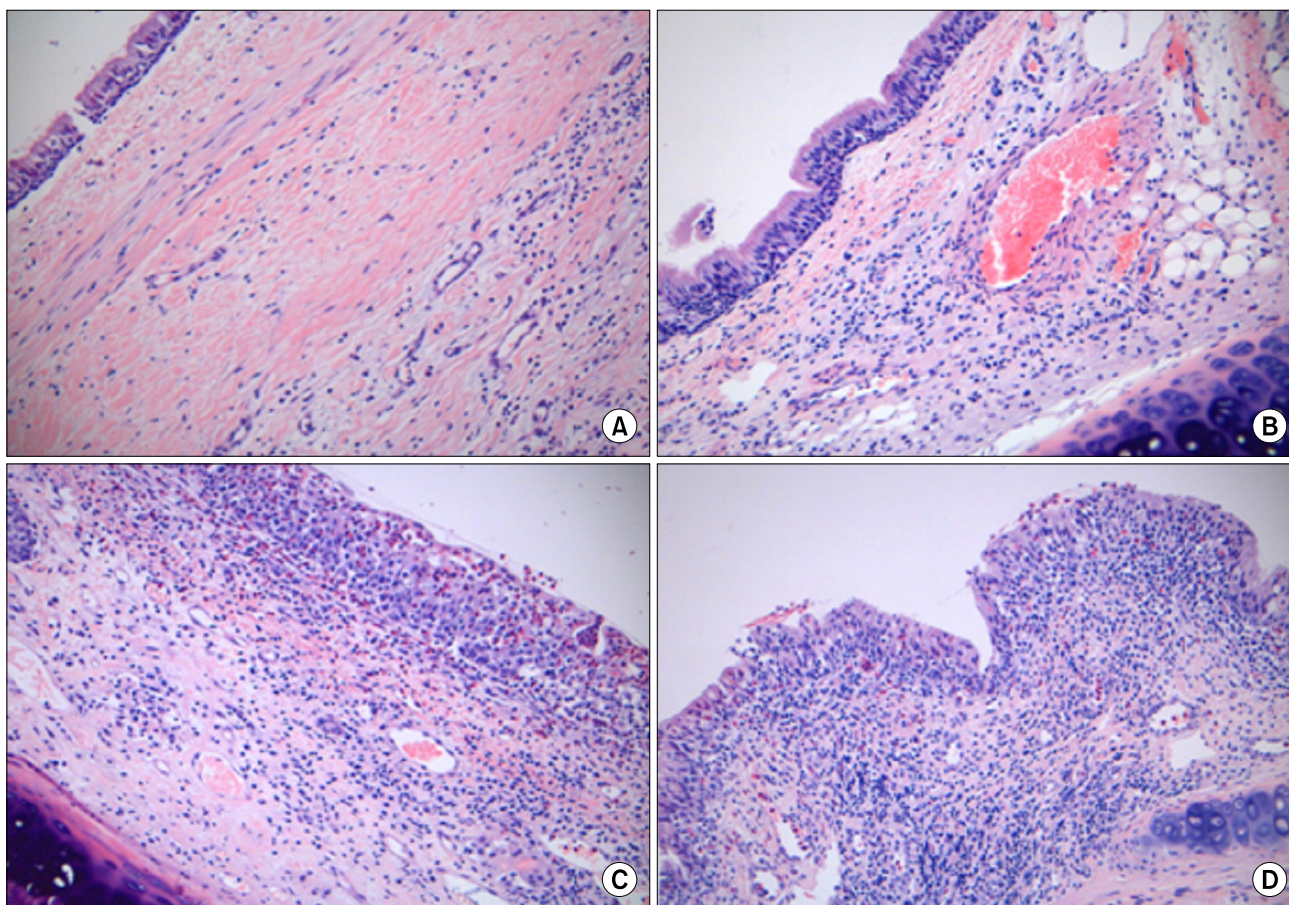
Fig. 3. Microscopic grading of the degree of lymphocytic infiltration. (A) Grade 0, lymphocytic infiltration was not detected. (B) Grade 1, lymphocytic infiltration was limited in the field to less than 30%. (C) Grade 2, lymphocytes had infiltrated locally between 30% and 70%. (D) Grade 3, lymphocytes had infiltrated diffusely by more than 70% (H&E stain, ×100 for all figures).

2) Microscopic assessments of tracheal allografts

Four weeks of cryopreservartion caused the epithelium of the allograft to disappear or the epithelial cells to change from columnar to cuboidal in appearance. The regenerated epithelium had the appearance of columnar epithelium with cilia. The degree of epithelial regeneration was 1.25 ± 1.04 in group A, 2.13 ± 1.36 in B, 3.38 ± 1.41 in C, and 3.88 ± 1.36 in D. In group A, columnar epithelium was detected in only 1 case, but a full layer or complete ciliated columnar epithelium was not detected. Although the full layers of columnar epithelium were found in 1 case from group B, complete ciliated columnar epithelium was not detected. Complete ciliated columnar epithelia were detected in 2 and 3 cases from groups C and D, respectively. The degree of epithelization in group D was significantly enhanced ($p=0.012$) only when compared to group A (Fig. 5). The degree of lymphocytic infiltration was 2.38 ± 0.74 in group A, 1.38 ± 0.92 in B, 1.25 ± 1.04 in C, and 0.88 ± 0.83 in D. Diffuse or local lymphocyte infiltrates were detected in 7 of 8 cases in group A, 3 cases each in groups B and C, and 2 cases in group D. The degree of lymphocytic infiltration in group D was significantly less than in group A ($p=0.048$) (Fig. 5). Even though the vessels regenerated to a level similar to or more than that of the native trachea in groups C and D, the degree of the vascular regeneration did not differ significantly in any of the groups (Fig. 5). The degree of vascular regeneration was 0.88 ± 0.64 in group A, 1.25 ± 0.71 in B, 1.63 ± 1.19 in C, and 1.75 ± 0.71 in D. There was no statistical correlation between epithelization, lymphocytic infiltration, and vascular regeneration.
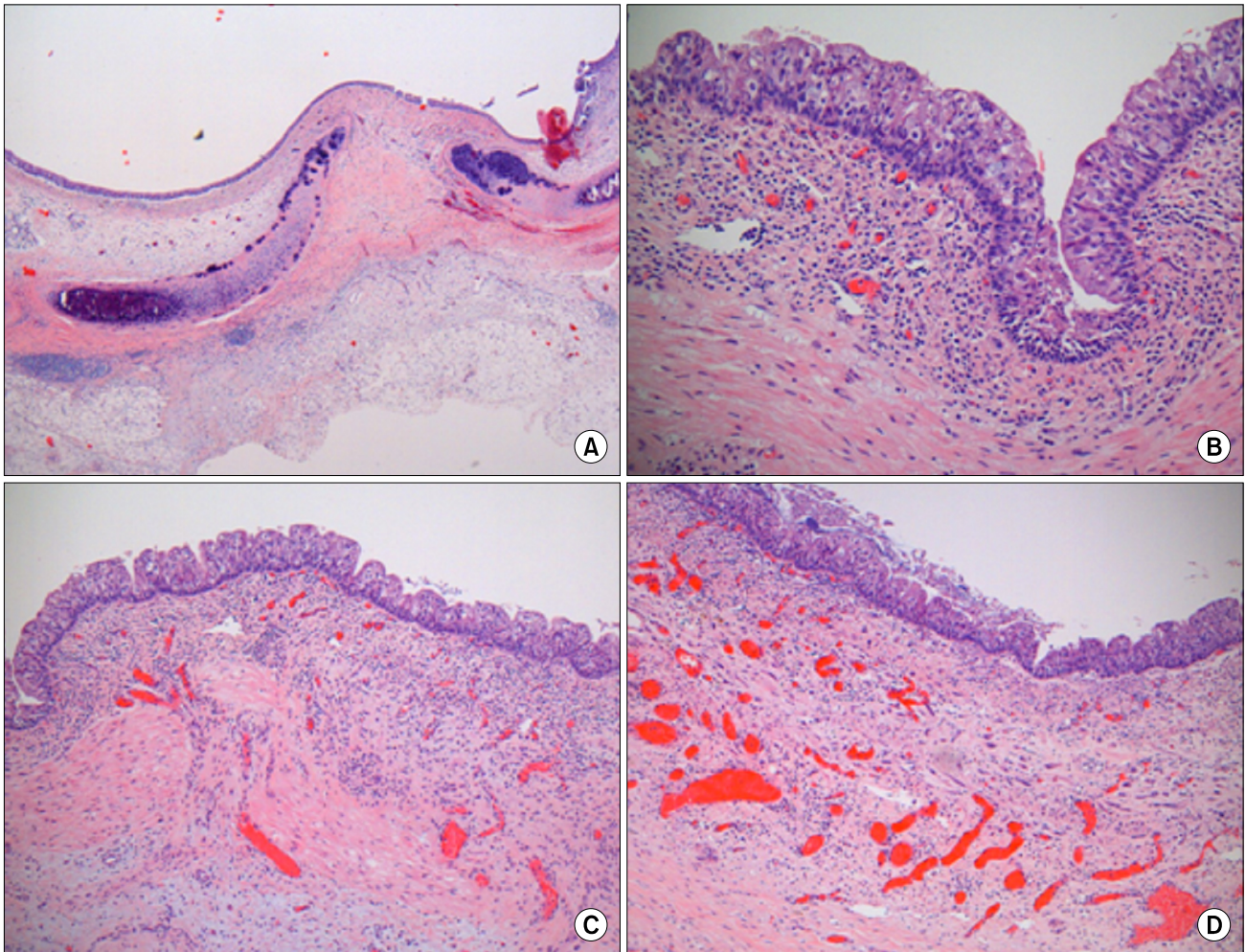
Fig. 4. Microscopic grading of the degree of vascular regeneration. (A) Grade 0, vascular regeneration was not detected. (B) Grade 1, vascular regeneration was detected to be less than 30%. (C) Grade 2, vascular regeneration was detected between 30% and 70%. (D) Grade 3, vascular regeneration was detected to be more than 70% (H&E stain, ×100 for all figures).

The rabbits (3 cases in the control group, 2 in B, 2 in C, and 1 in D) died of severe tracheal stenosis on the 7th to 9th day after transplantation. The biopsy revealed that the epithelial regeneration was poor, squamous metaplasia had developed, dense fibrous tissues grew into the lumen, and some portion of the tracheal wall was infarcted or had disappeared. The degree of epithelial regeneration was lower (p＜0.001) and lymphocytic infiltration was higher (p＜0.001), but the degree of vascular regeneration did not differ significantly (p=0.059) compared to the rabbits that survived. Other causes of death were intratracheal bleeding in one animal of group C and respiratory failure without tracheal stenosis in one of group D. None of the animals that died before 4 weeks were included in this study.

DISCUSSION

Although various approaches have been proposed to reconstructing tracheal defects affecting more than half of the trachea's total length, optimal methods have not been established because the substitute for bridging the defect must be airtight, rigid, have longitudinal flexibility, have an epithelial lining, and should be vascularized [1]. Tracheal allotransplantation is one of the better methods of repairing long segmental tracheal injury, but major obstacles to successful transplantation remain the restoration of blood supply, epithe-

Table 1. Cross-sectional areas of tracheal allografts before and after TPX

	Cross-sectional area of tracheal allograft (mm^2)		p-value
	Before TPX	4 weeks after TPX	
Group A	26.5±1.82	18.3±5.40	0.018
Group B	25.5±2.10	21.6±5.14	0.045
Group C	26.5±1.82	23.9±3.43	NS
Group D	26.0±2.03	24.1±2.70	NS

In group A and B, the lumina of the tracheal allografts were significantly decreased in size and their walls were shrunken, but in group C and D, the lumina and the walls were preserved well. There were no significant differences in the cross-sectional area among group C and D before and after TPX.

NS, not significant.

lization of the tracheal allograft, and immunosuppression of the recipient.

In order to improve the restoration of blood supply to the tracheal allograft, we wrapped the allograft just with the adjacent muscles and lymphatic tissues. Four weeks after transplantation, neovascularization from the adjacent wrapped tissues was well established and no dehiscence or necrosis of the allograft was evident. This means that our wrapping method was sufficient to restore the blood supply for preventing transient ischemia of the allograft until optimal revascularization was established. Microscopically, the degree of revascularization did not correlate with the degree of epithelization or lymphocytic infiltration and did not differ significantly in the eight cases that died of tracheal stenosis compared to the others. This suggests that transient ischemia was not the single factor responsible for the histologic alterations seen in tracheal allografts [10], and additional procedures, such as
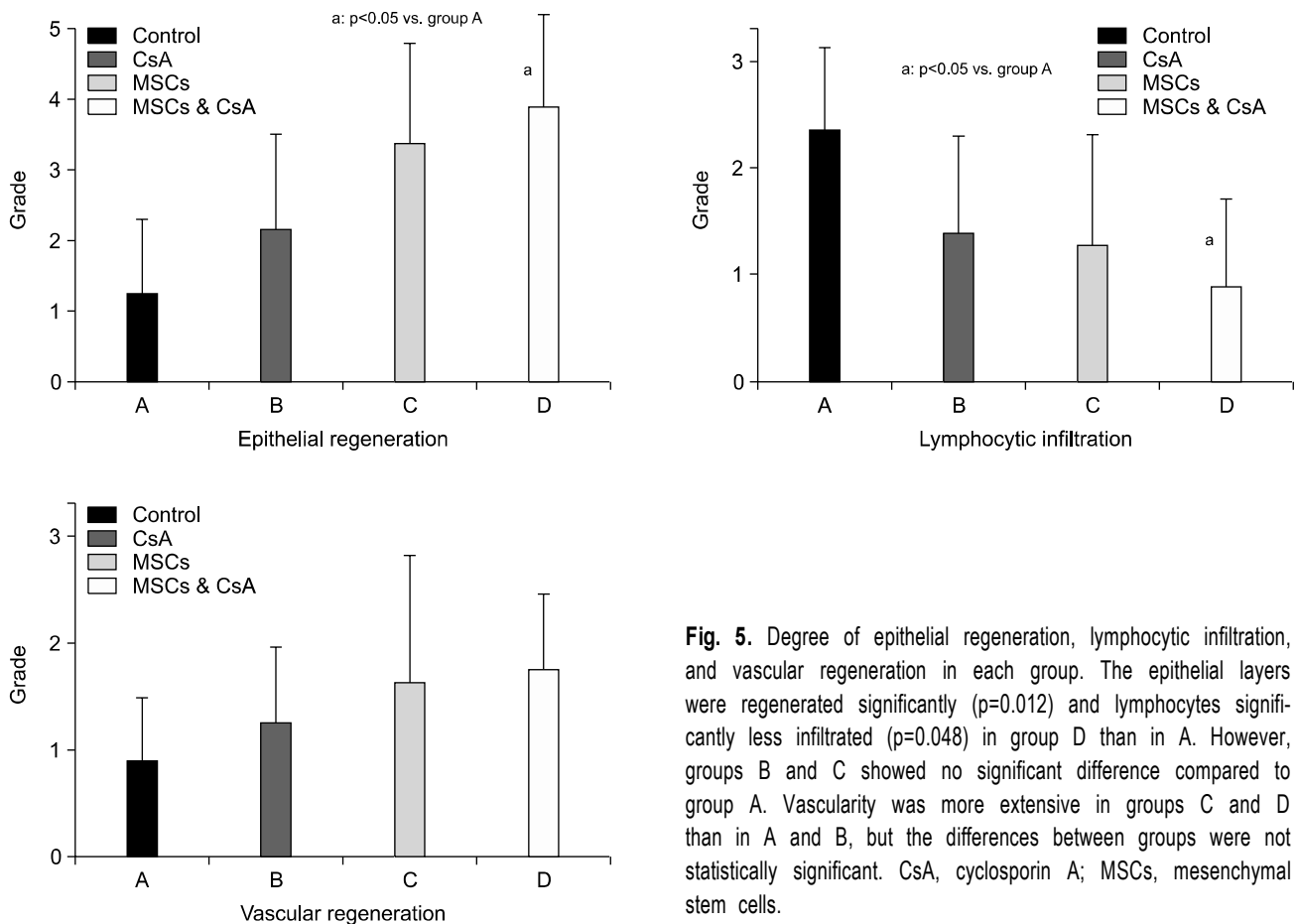
Fig. 5. Degree of epithelial regeneration, lymphocytic infiltration, and vascular regeneration in each group. The epithelial layers were regenerated significantly (p=0.012) and lymphocytes significantly less infiltrated (p=0.048) in group D than in A. However, groups B and C showed no significant difference compared to group A. Vascularity was more extensive in groups C and D than in A and B, but the differences between groups were not statistically significant. CsA, cyclosporin A; MSCs, mesenchymal stem cells.

direct or indirect revascularization, are no longer essential for improving the acceptance of tracheal allografts in orthotopic transplantations. Moreover, mononuclear cells provided by the revascularized submucosal vessels can attack the lamina propria and destroy the vessel wall, resulting in thrombosis and blockage of microcirculation and subsequent mucosal degeneration [4]. To prevent this subsequent reaction in the vascularized allograft and to improve the acceptance of the tracheal allograft, suppression of lymphocytic infiltration and enhancement of epithelization are necessary.

The trachea was long believed to have weak antigenicity, but it is subject to the same immunological laws of transplantation as all other transplanted tissues [11,12]. Human tracheal mucosa, which expresses HLA class II molecules, may be the major antigenic structure responsible for triggering immunologic action in allogenic tracheal transplantation [13,14]. This major histocompatibility complex (MHC)-oriented alloreactivity plays an important role in acute rejection. Epithelial cells can function as antigen-presenting cells when they express MHC class I and II antigen on their surface [25]. Lymphocytic infiltration is the precursor of fibrous airway obliteration, which is aggravated by the initial ischemic phase, and followed by complete epithelial loss and fibrous obliteration [26]. Therefore, immunosuppression is necessary to preserve the optimal morphologic and functional conditions in order to, in turn, improve the viability of tracheal allograft epithelium [27].

CsA, a widely accepted immunosuppressant, inhibits activation and proliferation of T-lymphocytes. It can preserve the viability of a transplanted trachea [4,10,28], markedly reduces the development of fibroproliferation in the allograft [29], and inhibits the infiltration of monocytes/macrophages in the acute phase [30]. An adequate dose of CsA for intravenous injection in rabbits is 10 mg/kg [4,15], and continuous immunosuppression is necessary to maintain optimal conditions for tracheal allograft survival [15]. On the other hand, King et al. [29] reported that CsA does not reduce inflammation or airway epithelial cell injury, and Neuringer et al. [16] reported that although CsA can delay airway fibrosis, it does not alter the progression of the allograft through the phases of early ischemic injury. In this study, we found that the tracheal lumen shrunk significantly in the CsA group.

Epithelization, lymphocytic infiltration, and vascular regeneration did not improve compared to the control group. We suggest that CsA may or may not reduce the severity of acute rejection, but it does not prevent the fibrous proliferation and luminal obliteration that can be protected by epithelial regeneration. If epithelization is interrupted by other causes, such as ischemia or infection, fibrous proliferation will eventually develop. Moreover, CsA may increase the risk of infection, aggravate the malignant process, or cause renal toxicity. Therefore, attenuation of tracheal allograft antigenicity is required in addition to immunosuppression.

The antigenicity of a tracheal allograft can be greatly reduced by removing the epithelium [17], and various methods has been investigated, such as cryopreservation [31,32], irradiation [33], photodynamic therapy [34], and detergent treatment [35]. Cryopreservation has been the most favorable technique to maintain long-term tissue viability and structural integrity. It can also reduce the immune response and the acute rejection process by depletion of the antigenic donor epithelia [17,20,32,36-39]. It has been postulated that the freezing and thawing processes involved in cryopreservation [36,40] as well as long-term cryopreservation of more than one month [40] results in the loss of class II antigen expression. In this study, we found the cryopreservation of tracheal grafts for 4 weeks led to the reduction of the epithelium from a ciliated multilayer to a monolayer type or transformation of the epithelial cells from a columnar to cuboidal shape. These changes regenerated back to a ciliated columnar appearance after transplantation. This de-epithelization effect of cryopreservation may have concealed additional effects of CsA or MSCs, because the control, CsA, and MSC groups had no statistical differences in the degree of lymphocytic infiltration.

Even though acute rejection was reduced by cryopreservation, mild but chronic rejection responses continued, resulting in occlusion of the transverse intercartilaginous arteries and consequent progressive atrophy [38]. To reduce allograft antigenicity further, the inner surface of the trachea should be remodeled and replaced by the recipient's epithelium [20]. This can prevent chronic rejection even after withdrawal of immunosuppression [41]. If the denuded lining of the tracheal allograft is not promptly covered by the recipient's epithelium,

fibroproliferative tissues will enter the tracheal lumen via the membranous part [18] and cause airway obliteration and also cause the rejection process to continue unchecked. These processes are more pronounced in a heterotopic transplant model than in an orthotopic model because the growth of the recipient's epithelium over the denuded donor trachea does not occur [18,19]. Epithelial re-growth from the host limits the progression of obliterative airway disease [19], which can be explained by an experiment showing that co-culturing with epithelial cells leads to deregulation of contact inhibition of the normal fibroblasts [2].

Another method for increasing the acceptance of an allograft is the formation of chimerism that can lead to an acquired donor-specific tolerance [42]. Mixed allogenic chimerism through infusion of MSCs induces donor-specific tolerance and prevents development of the fibroproliferative obstruction of a tracheal allograft in a heterotopic transplant model [21]. Simultaneous administration of donor MSCs following reduced intensity conditioning prevented tracheal graft rejection by preserving the lining epithelial cells and reducing lymphocytic infiltration. It was found that allograft acceptance is dependent on the co-administration of donor MSCs rather than the preconditioning of bone marrow [22,24]. MSCs can also increase the neovascular response in a limb ischemia model [23] and myocardial infarction model [43]. Therefore, we hypothesized that co-administration of MSCs may further enhance the acceptance of a tracheal allograft by increasing epithelial and vascular regeneration in addition to inhibiting lymphocytic infiltrations. However, we found the MSCs themselves had no statistical superiority over cryopreservation or CsA, although the MSCs could preserve the luminal areas of the trachea well. The MSCs in conjunction with CsA enhanced epithelial regeneration and inhibited lymphocytic infiltration significantly. We also found that well differentiated regenerated epithelium was detected on day 5 in the MSC and CsA group, which suggests that the MSCs with CsA were able to shorten the period of epithelization.

This study has brought several issues to light concerning the methods associated with using MSCs to enhance allograft survival. Further research with a larger sample size and longer follow-up will clarify these issues. First, it is important to understand whether intravenous administration of MSCs is su-

perior to local implantation. Although intravenously administered MSCs are capable of homing into the sites of tissue damage or inflammation [43,44], further study will be needed to compare this capability in a tracheal transplantation model with that in other models.

Second, the effective dose of MSCs has to be determined. Le Blanc et al. [45] reported that a high concentration of MSCs induced the inhibition of lymphocyte proliferation, whereas less suppression or a marked lymphocyte proliferation occurred with a low concentration of MSCs. In contrast, Bartholomew et al. [46] reported that the administration of an increased number of MSCs did not extend skin graft survival. In our study, two rabbits that received less than 0.5×10^7 MSCs/kg died of tracheal stenosis that showed severe lymphocytic infiltration. These two rabbits were not included in this study because of the low MSC density. However, in the 16 rabbits that received the dose of 1.0×10^7 MSCs/kg, only two rabbits died of tracheal stenosis (p=0.039). Therefore, the infusion of 1.0×10^7 MSCs/kg or more may be needed to enhance the acceptance of tracheal allografts in rabbits in conjunction with CsA. MSC infusion is, however, not sufficient to enhance graft acceptance without immunosuppressing agents.

Third, whether multiple infusions of MSCs have any advantages in enhancing the acceptance of tracheal allograft over a single high dose should be determined. A single infusion of MSCs may be adequate to subdue the lymphocytic response but may not be sufficient to affect the recruitment of additional inflammatory cells towards the graft [46]. Pham et al. [24] reported that lung transplantation patients receiving multiple infusions of MSCs had significantly longer graft survival than those receiving a single infusion. Our study showed that a single dose of MSCs was no better than CsA or cryopreservation. MSCs can suppress the initial lymphocytic proliferation, but the effect may be transient or incomplete in enhancing graft acceptance.

The results suggest that early tracheal graft failure was due to acute rejection characterized by lymphocytic infiltration and epithelial degeneration rather than ischemic injury. The administration of MSCs with concurrent injections of CsA enhanced and promoted epithelization and prevented the lymphocytic infiltration of tracheal allografts. The route of injection, dosage,

and frequency of administration should be investigated further to enhance the acceptance of tracheal allografts without immunosuppressive agents.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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