



Does Conditioning the Airway Epithelium Improve Stem Cell Transplantation?

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Introduction:

Airway disease is a primary cause of morbidity and early mortality for patients with cystic fibrosis. Cell transplantation therapy has proven successful for treating some immune disorders and this approach has potential to correct the airway disease phenotype associated with cystic fibrosis. This project assessed whether conditioning the mouse nasal airway with the agent povidone-iodine (PDOC) enhanced the level of cultured human airway basal cell (HABC) transplantation.

Methods:

To be able to track delivered cells, normal HBEC (LONZA, USA: CC-2540S) were seeded onto collagen-coated flasks, expanded to 75% confluency, and exposed to a lentiviral vector containing the luciferase (Luc) transgene at a MOI of 10. To prepare the nasal airways for cell transplantation the airway epithelium was first exposed to 2% PDOC, which disrupts most of the epithelial cell layer. Normal female C57Bl/6 mice received 4 μ l of either PBS (control (n=5-10)) or PDOC (n=5-10) into the right nostril. Two, or twenty four hours later three 10 μ l aliquots of gene-transduced cells (HABC-Luc) were delivered to the same nostril of those mice. Bioluminescence imaging (BLI; Xenogen, IVIS) was performed at 1, 3, 5 and 8 weeks to assess HABC engraftment via luminescence of Luc-expressing cells.

Results:

Not all mice that received HABC-Luc cell transplantation 2 hours after PDOC airway epithelial disruption survived the procedure. Three of the seven remaining mice showed significant luminescence for up to 3 weeks (p<0.01, RM ANOVA vs PBS control) as detected by BLI (Figure 1).

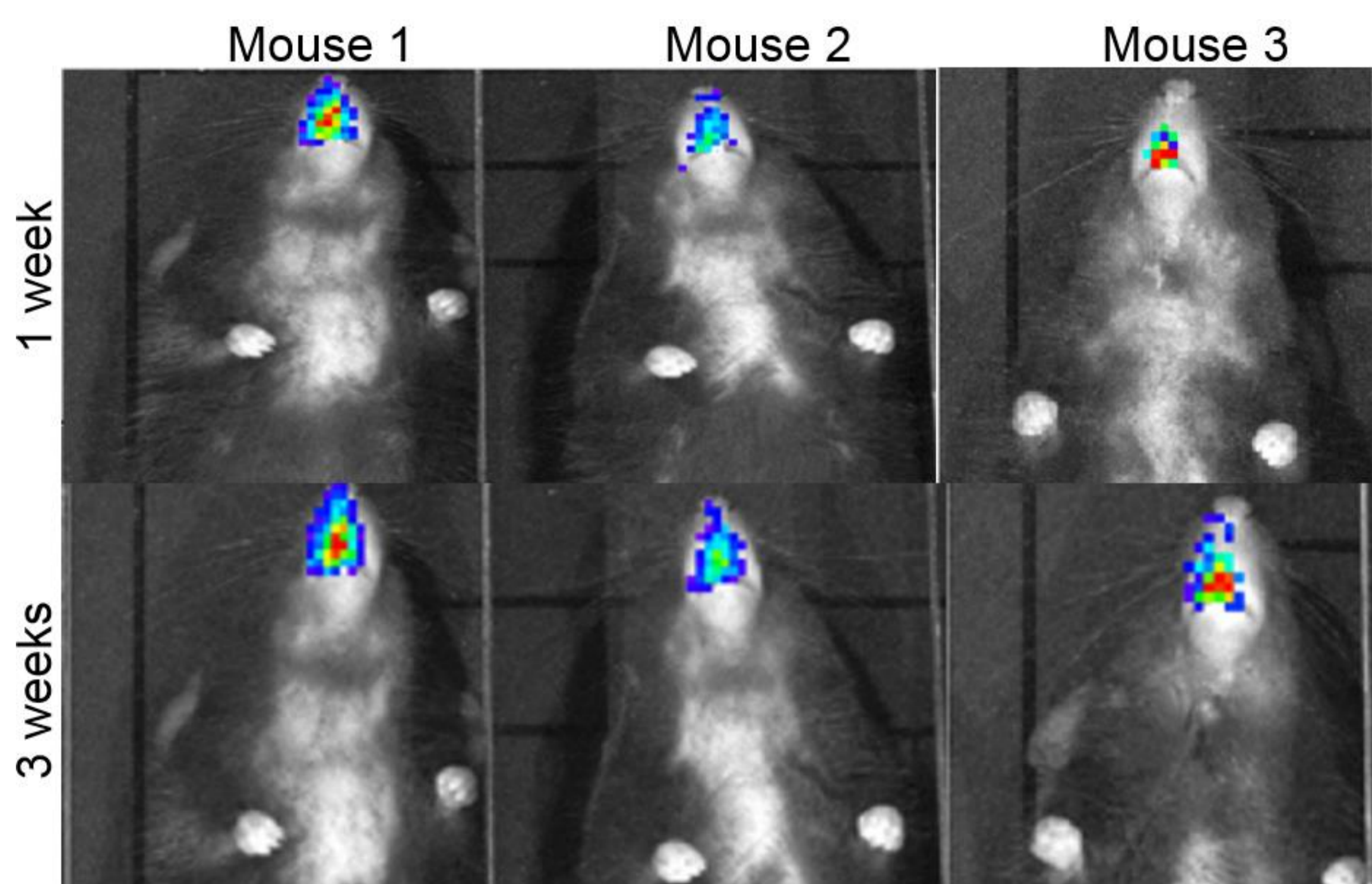


Figure 1: Bioluminescent *in vivo* imaging of 2 hour PDOC/HABC-Luc delivery revealed luciferase reporter gene expression in the nasal airways of three mice at 1 week and 3 weeks following transplantation.

Luciferase gene expression declined by the 5 week time point, and was below detectable levels by 8 weeks. No luciferase expression was detected by BLI at any time point in the PBS conditioned (control) animals (Figure 2).

2 Hour PDOC Conditioning: HABC-Luc

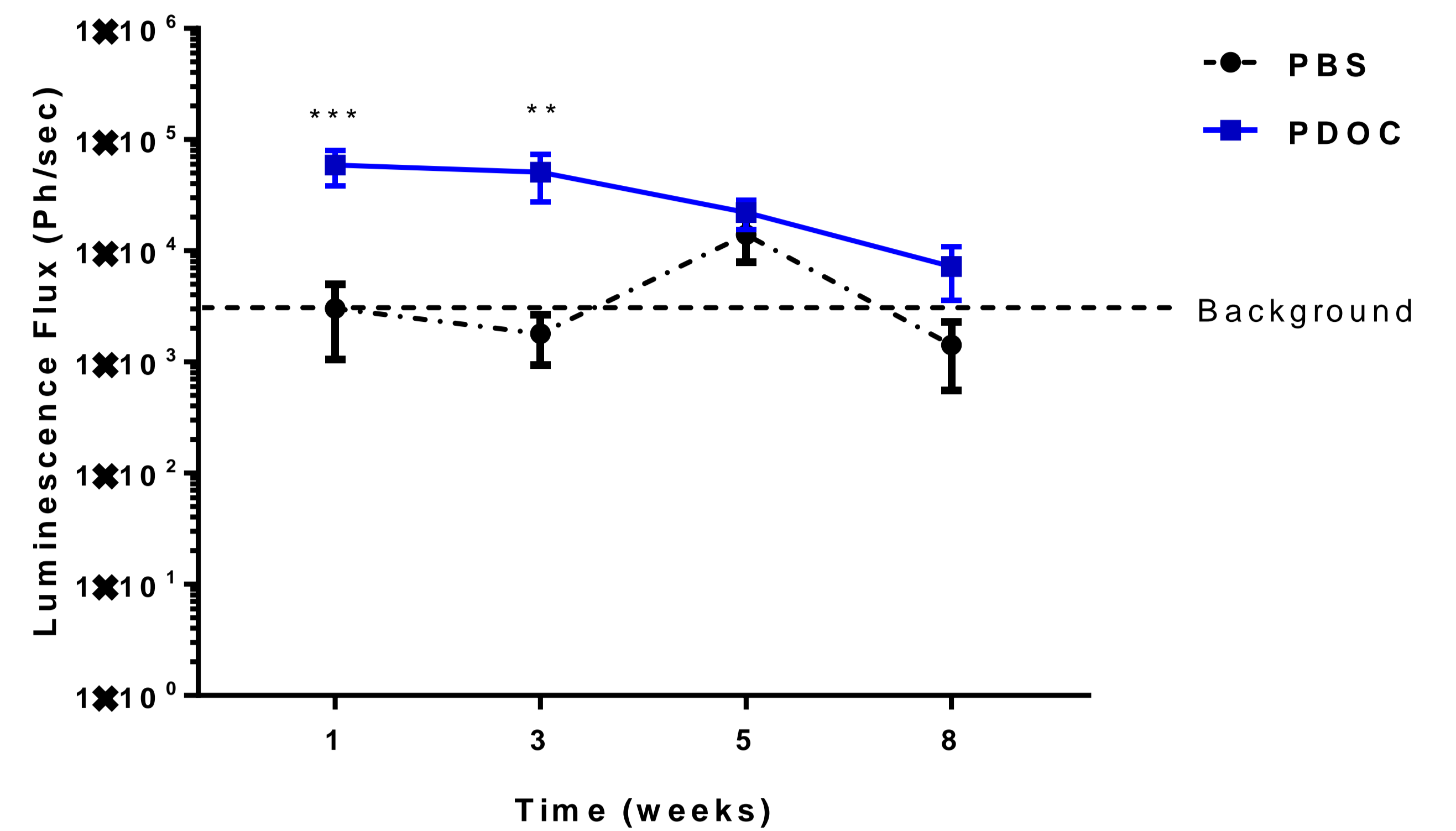


Figure 2: PDOC treatment enabled luciferase expression in 3 out of 7 mice for at least 21 days compared to control animals (**p<0.01, ***p <0.001 2-way-ANOVA, Sidak's multiple comparison, n=7-10/group) at 1 week and 3 weeks).

In contrast, mice that received HABC-Luc cell delivered 24 hours after PDOC airway conditioning showed no luminescence, indicating a failure for HABC-Luc to transplant (Figure 3).

24 Hour PDOC Conditioning: HABC-Luc

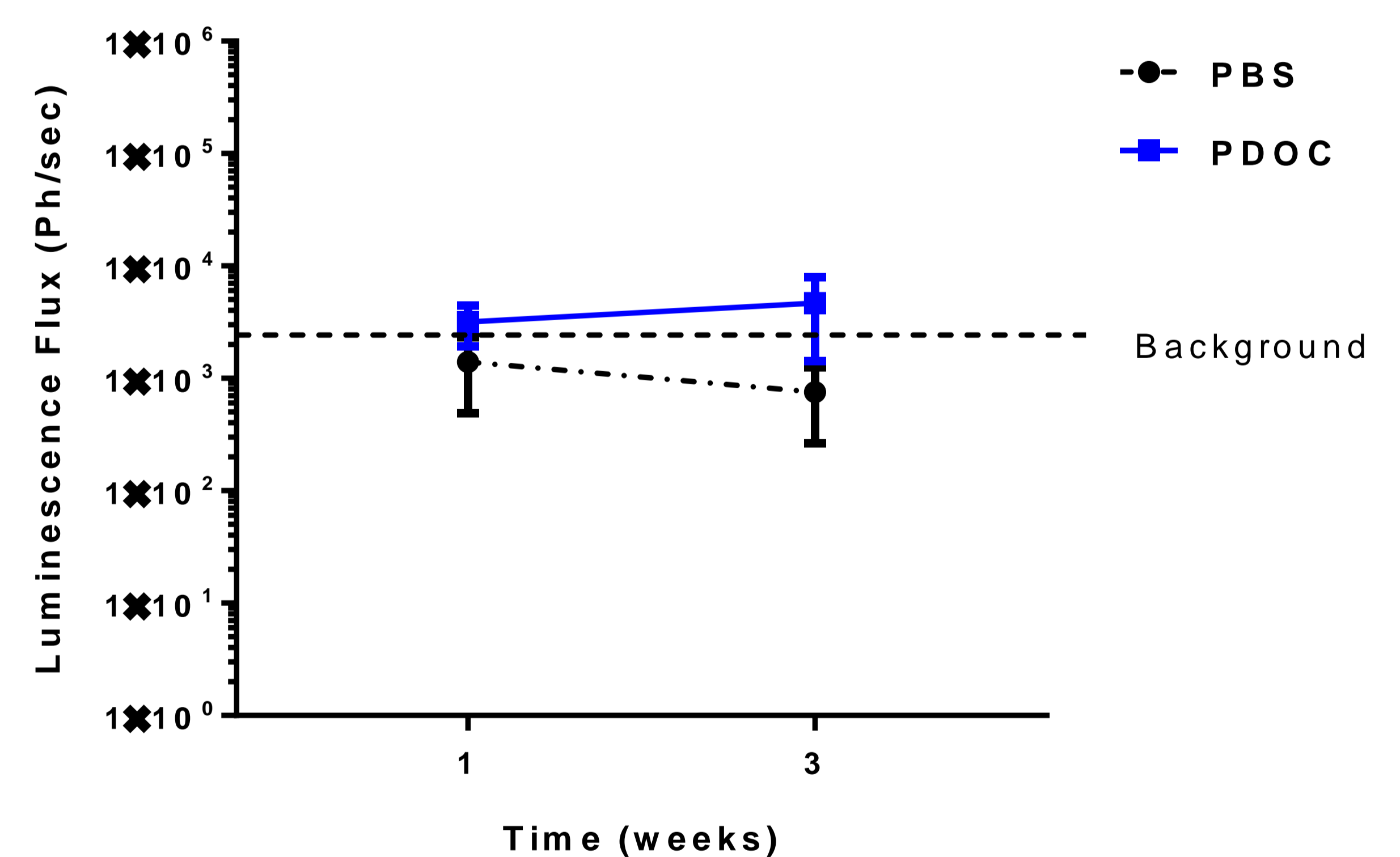


Figure 3: There was no significant difference in luciferase reporter gene expression in the nasal airways following 24 hr PDOC disruption after either a) hAEC-Luc or b) HABC-Luc delivery compared to PBS at both 1 and 3 weeks following cell therapies. (n.s. 2-way-ANOVA, Sidak's multiple comparison, n=5/group)

Conclusion:

These encouraging findings show that the PDOC airway conditioning procedure can produce initial and persisting Luc-expressing cell transplantation in the nasal airways. Future studies will need to assess the potential for the protocols presented here in both autologous and allogeneic cell transplantation into CF disease animal models with functional assays to determine the therapeutic value of the procedure.

Acknowledgments

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