Bioartificial pleura using allogenic cell sheet for closing of lung air leakage

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CENTRAL MESSAGE

Because the ACSs used to close PALs survived on the lung surface for at least 7 days, transplanted ACSs can prevent persistence of PALs.

VIDEO 1. Cell sheet engineering. Under normal culture conditions at 37°C, various types of cells can attach, spread, and proliferate on these surfaces, similar to cells on ordinary tissue culture dishes. However, by simply reducing the culture temperature to 20°C, the cultured cells with their deposited extracellular matrix (ECM) can be noninvasively harvested as intact sheets without the need for proteolytic enzymes. Video available at: https://www.jtcvs.org/article/S2666-2507(20)30544-7/fulltext.

Transplanted allogenic cell sheets can prevent persistence pulmonary air leaks.
every 2 days. At 7 days after being treated with 0.05% trypsin-ethylenediaminetetraacetic acid for 5 minutes at 37°C, LDCs were harvested, subsequently dissociated with 0.05% trypsin-ethylenediaminetetraacetic acid, seeded on a 24-mm square-patterned temperature-responsive dish at a density of 2 \times 10^5 cells/cm^2, and cultured for additional 7 days. After being incubated at 20°C, LDCs were harvested as a CS (Figure 1, A), which shrank and had 2 to 4 cell layers with slight extracellular matrix (ECM) (Figure 1, B and C).

For transplantation, F-344 athymic (n = 4) and Brown Norway (BN) rats (n = 4) were anaesthetized with 2% inhaled isoflurane under mechanical ventilation. Rats were positioned in the right lateral decubitus position, and a left-lateral thoracotomy was performed. Based on a rat air leak model reported previously, a 5 \times 2-mm incision was created by surgical scissors for making pleural injury (Figure 1, D and E), which was confirmed by observing air bubbles from the lung. Pleural injury was successfully closed with the transplantation of CS (Figure 1, F, and Video 2) in all animals, and due to difficulty in managing chest tube drainage in rats, no chest tubes were placed. After transplantation, no immunosuppressants were used in both groups. To investigate the cell viabilities of luciferase expressing CSs, CS transplanted rats were anaesthetized in the same fashion at postoperative days (PODs) 7 and 14,
and 150 mg/kg D-luciferin firefly potassium was injected into the dorsalis penis vein 15 minutes before imaging analysis with an in vivo bioluminescence imaging system. Bioluminescence images showed dynamic changes in the intensity for both rats at POD 7 and POD 14 (Figure 2, A). Compared with the athymic rats, BN rats seemed to

**FIGURE 2.** Bioluminescence images of transplanted allogenic cell sheet (ACSs) in rats. A, Typical images show luminescence signals emitted from luciferase-expressing cell sheets transplanted in F-344 athymic rat at 7 postoperative days (POD) and 14 POD, and Brown Norway (BN) rat at 7 POD and 14 POD. Compared with the athymic rats, BN rats showed a lower intensity at 14 POD. The viability of cell sheet in the athymic rats was higher than that of BN rats at 14 POD. The upper and lower columns of images represent F-344 athymic rat and BN rat, respectively. B, The graph shows changes in photon flux emitted from ACSs in the thoracic cavity, and the flux indicates the luminescence signal intensity. A dynamic change in the intensities of both rats is clearly shown by the numerical data. At 7 POD and 14 POD, mean ± standard deviation photon flux values were 4.17 ± 1.88 to 4.53 ± 2.03 × 10^6 photon/sec in athymic rats and 2.79 ± 1.43 to 0.53 ± 0.49 × 10^6 photon/sec in BN rats, respectively. Although there were no significant differences in intensities between both groups, the differences between athymic rats and BN rats increased from 7 POD to 14 POD. BN rats had a larger decrease in the photon flux value than the athymic rats at 14 POD.
show a lower intensity at POD 14. A dynamic change in the intensities was clearly shown by the numerical data where the mean $/C_6$ standard deviation intensities of the athymic rats at POD 7 and POD 14 were 4.17 $/C_6$ 1.88 and 4.53 $/C_6$ 2.03 $10^6$ pho- tons/second, respectively (Figure 2, B). The differences between athymic and BN rats increased from POD 7 to POD 14, and the athymic rats showed a higher cell viability than BN rats at POD 14. All animals survived for the follow-up period. The thoracic cavity was reopened at POD 28, and the transplanted CS was observed to attach to the lung surface (Figure 1, G). After the left lung was resected, the transplanted CS adhered tightly to the lung, and ECM increased inside CS (Figure 1, H and I).

Although most PALs disappear within 2 to 3 PODs, approximately 5% to 10% of patients continue to have PALs after POD 5. For performing intraoperative PAL closure, various biological sealants are used, resulting in marginal success due to their poor adhesive stability, biocompatibility, and inflexibility. Although autologous CS is used as a durable long-term PAL sealant, a long culture period for creating CS is a problem. After being closed with short-life adhesives or materials, postoperative PALs disappear within several days, requiring no long-life materials, and this study focused on allogeneic CSs. Although transplanted allogenic CSs could be rejected by immune response, the transplanted CS survived on the lung surface at least 7 days without immunosuppressants with rich ECM. Additionally, transplanted allogeneic CSs closed PALs in all the animals. Allogeneic CSs may be an alternative to autologous CSs for closing PALs.

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APPENDIX 1: CELL SHEET ENGINEERING

Cell sheet engineering uses temperature-responsive culture dishes. By covalently grafting a temperature-responsive polymer, poly(N-isopropylacrylamide), the surfaces of conventional culture dishes, various types of cells can be cultured on the dishes and harvested as cell monolayers by simply reducing the incubation temperature instead of typical proteolytic harvest methods.

Under normal culture conditions at 37°C, various types of cells can attach, spread, and proliferate on these surfaces, similar to cells on ordinary tissue culture dishes. However, by simply reducing the culture temperature to 20°C, the cultured cells with their deposited extracellular matrix can be noninvasively harvested as intact sheets without the need for proteolytic enzymes (Video 1). During these spontaneous cell detachment processes, cell sheets slightly shrink due to cytoskeletal reorganization but can re-expand by external force while maintaining their functions and intact structures. Because extracellular matrix is maintained on the basal surfaces of the cell sheets, they can stably attach to other surfaces.

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