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Functional evaluation of the tissue engineered diaphragm after orthotopic transplantation in rats

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Objective

To evaluate the function of decellularized diaphragm scaffolds reseeded with mesenchymal stem cells in rats after orthotopic transplantation.

Material and methods

15 adult male Lewis rats, weighing 250±20 g were used in this study after ethical requirement. 80% of the left hemi-diaphragm was replaced with decellularized and reseeded diaphragmatic scaffolds. Three groups of animals were analyzed: untreated; sham (resection 80% of the left hemi-diaphragm and autologous re-implantation of the resected, native diaphragm), and treated (resection 80% of the left hemi-diaphragm and replacement with tissue engineered diaphragm). All functional outcomes were evaluated before and 21 days after the transplantation. Electromyography (EMG) was performed on each group to detect and compare the electrical muscular activity of the bilateral hemi-diaphragms by NeuroBioLab (NeuroBioLab LTD, Russia). Chest X-ray and cone-beam computed tomography (CT) were performed using Rayscan Symphony V (Samsung Electronics, South Korea) and X-ray apparatus (Axion Icon R 200, Germany). Respiratory function was evaluated using Spirometer Power Lab 8/35 (ADInstruments, Australia). Blood gas samples were analyzed using a Radiometer ABL800 Flex (Radiometer Medical ApS, Denmark) to evaluate oxygenation and ventilation.

Results

Spirometry revealed flow-volume loops with similar patterns for all groups with no significant differences in respiratory rate and tidal volume between groups. Blood gas analyses revealed no statistically significant difference in PvCO₂, PvO₂, pH, haemoglobin (Hb) and saturated oxygen (sO₂) levels between native and transplanted rats (p<0.05). Significant differences between native and sham as well as between sham and transplanted were noted in PvO₂, sO₂ and Hb level were lower in the sham surgery group compared with native and transplanted rats (p<0.05, p<0.001). EMG was performed to assess the neurological conduction and muscle responses of the neo-diaphragm. Analyses of the EMG data were used to quantify the difference between the signals from the two hemi-diaphragms, including spectral content, temporal delay, signal range and signal root-mean-square amplitude. The analyses revealed marked differences among the groups in terms of the mean values of the comparison measures. The analysis of the full EMG sequence revealed that electrical activity on both hemi-diaphragms of the transplanted and native rats started simultaneously, however in the sham group the operated hemi-diaphragm showed a time lag compared to the intact one. CT and X-ray evaluations were used to assess the anatomic integrity of the tissue engineered constructs. On CT scan, there were no signs of abdominal organ herniation into the left pleural cavity, residual pneumothorax or pneumoperitoneum, free abdominal fluid, or diaphragmatic-related lung dysfunction (atelectasis) were observed. The position of the heart remained normal and there was no bronchial or tracheal deviation or other organ displacement. Both sides of the diaphragm in the transplanted group had equal diaphragmatic excursion on X-ray.

Conclusion

Three weeks after tissue engineered rat diaphragms were implanted into an orthotopic position, the animals exhibited functional evaluations similar to native rats. In light of these promising results, the future focus will be on replicating this study in a large animal model before attempting this in a clinical setting.