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Thoracic | Abstract | Airway

Scaffold-free trachea regeneration by tissue engineering with bio-three-dimensional printing

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There are general limits for safe tracheal resection, i.e. half of the tracheal length in adults and one-third in small children. Thus, safe and dependable techniques for tracheal replacement are being developed. There are many approaches for reconstructing the trachea, including regeneration with tissue engineering; however, no standard procedures for tracheal transplantation/regeneration, particularly circumferential replacement, have been developed. In the current situation, most artificial airway organs still require scaffolds to maintain the strength and stiffness of the airways. However, scaffolds for artificial organs have some issues, such as risk of infection, irritation, lower biocompatibility, and time-dependent degradation. Here, we aimed to assess circumferential tracheal replacement strategies using scaffold-free trachea-like grafts made by bio-three-dimensional (bio-3D) printing technology with the isolated cells in an inbred animal model.

Chondrocytes and mesenchymal stem cells were isolated from three-week-old F344 male rats. Rat lung microvessel endothelial cells (RLMVECs) were purchased and used as a cell source. After the preparation of multicellular spheroids, trachea-like tube structures were prepared by bio-3D-printing. The structure was matured in a bioreactor and transplanted into eight-week-old F344 male rat as tracheal grafts under general anaesthesia.

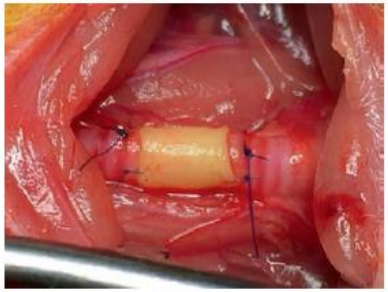

Figure 1. Photograph of the surgical field after transplantation of the scaffold-free graft made by bio-3D-printing technology.

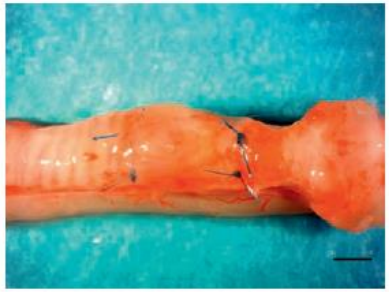

Figure 2. Day 7 post operation. Some amount of connective tissue with microvessels surrounding the tracheal graft was observed. Scale bar = 2 mm.

Trachea transplantation was performed using the silicone stent and followed up for 11 postoperative days (POD). The generated scaffold-free trachea-like structures showed around two-thirds the tensile strength of native adult trachea. The bio-3D printed structures were easy to handle with surgical forceps and had sufficient strength to transplant into tracheas using silicon stents (Figure 1). After sacrifice and resection of the transplanted trachea, all tracheal grafts maintained shape and stiffness (Figure 2). Some connective tissue with microvessels surrounding the tracheal grafts was observed. Histologically, glycosaminoglycan (GAG) production was assessed by Alcian blue staining, and GAG deposits were found in the bio-3D-printed structures after the maturation period; GAGs persisted until 11 POD. Immunohistochemistry showed that collagen II was observed in structures after maturation and maintained after tracheal transplantation. Our findings showed that cartilaginous tissue was formed during the maturation period after the bio-3D printing and maintained after transplantation. Some small capillary-like tube formations consisting of CD31-positive cells were observed in the structures, and the number of these structures increased over time. These results showed that appropriate vasculogenesis could be obtained in scaffold-free trachea transplantation with our bio-3D printing technique.

This work demonstrated our initial experience of tracheal tissue engineering with bio-3D printing technology using a scaffold-free approach. The artificial trachea fitted and matured in situ after transplantation. The structures produced by the bio-3D printer with isolated rat cells could be transplanted via allogenic trachea transplantation in an inbred animal model. This technology could give the opportunities for the patients with tracheal tumour, tracheomalacia or tracheal stenosis to have another option for better quality of life.