Fabrication of Bi-layered PU Scaffold with PEG-grafted Surface for Reconstruction of Tracheal Defects

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INTRODUCTION: The purpose of this study was to design and create a matrix scaffold which is suitable for clinical applications in reconstructive therapies for tracheal defects. A bi-layered Polyurethane (PU) matrix was devised to closely mimic the microenvironment and to overcome existing limitations, such as the complexity of surgical procedures involved in autologous transplantation. To one side of porous PU matrix, non-porous dense PU film is integrated to form outer layer of the tracheal duct. This is to provide sufficient compliance to the matrix to withstand any forces that result from air flow. Polyethylene glycol (PEG) is grafted onto the other side of matrix, which will form the inner layer of the tracheal duct. These grafted PEG molecules are intended to form a hydrophilic layer, thereby inhibiting the adhesion of adjacent cells and granulation formation.

MATERIALS AND METHODS:

FABRICATION OF SCAFFOLD:

1 .Preparation of Porous matrix: PU was prepared as described previously. (Cha, JM., et al., Artif. Organs 2006;30(4):250–258)

2. Surface Ozonation and PEG grafting: Ozone was generated by passing dry gas oxygen through an ozone generator. The matrix was ozonated for an hour in an ozone chamber. PEG (mPEG-AM 2000, SunBio) was dissolved in distilled water to form 10% solution(w/w), and the ozonated scaffold was submerged in the PEG solution and was left for 24 hours at 50 °C.

3. Addition of dense film membrane to the porous matrix: PU was dissolved in chloroform to form 12% solution on which the porous matrix was floated. The solution was allowed to dry for 24 hours at 25 °C. The prepared scaffold was sterilized in a UV chamber for 4 hours.

SCAFFOLD CHARACTERIZATION: Morphology of the complete scaffold was observed by scanning electron microscopy (SEM). In order to confirm whether the PEG was grafted onto PU matrix surface, the chemical composition and C_{1s} spectra of the matrix surface were assayed with X-ray photoelectron spectroscopy (XPS).

SCAFFOLD IMPLANTATION: All the experimental procedures and animal management were executed in compliance with the animal laboratory regulations of Ehwa Womans University College of Medicine. 3 male beagles weighing around 10kg were operated for implantation after being anesthetized with 100 mg of ketamine chloride and 24 mg of xylazine chloride. Enflurane, which is an inhalant anesthetic, and NO and O_2 gases were used to maintain anesthesia during the implantation. A 2cm by 2 cm sized scaffold was implanted into anterior tracheal ring after resection of tracheal cartilages. Vicryl was used for suture and fibrin glue was spread over the site of implant.

IN VIVO MORPHOLOGICAL AND HISTOLOGICAL ASSAYS: Segmental gross specimens of the scaffold implantation were retrieved along with adjacent tissues and cartilages 4, 8, and 12 weeks after the scaffold implantations. The morphological changes and the formation of mucosa were examined with SEM, and the histology of the specimens was assayed via hematoxylin-eosin (H-E) staining.

RESULTS AND DISCUSSION: SEM observation of the scaffold revealed that thickness of the porous matrix was about 3.2 mm with pore size of around 200μm, which is a suitable size for adjacent cells to infiltrate. The dense film was about 260μm thick. Firm attachment between the two different layers was also confirmed. Data from XPS measurements showed a 6% increase in oxygen composition on the PU surface after ozonation, and this confirmed ozone oxidation. After grafting PEG onto the surface, carbon composition of the surface was increased by 0.5% and a subsequent decrease in oxygen composition was shown. C_{1s} spectra analysis also revealed that a peak corresponding to -O-C=O (Bond energy: 289.0 ± 0.2 eV) group appeared, indicating the formation of these bonds after PEG was grafted.

In vivo histological analysis showed total biological integration of the implanted scaffold with the tracheal environment. The fibrous connective tissue was observed at the previous site of normal tracheal hyaline cartilage after 8 weeks. After 12 weeks, infiltration of fibrous tissue into the porous matrix was observed. On the PEG- grafted layer of implanted scaffold, which forms the inner layer of tracheal duct, respiratory mucosa was sufficiently recovered and fibrous tissue was formed at the previous site of normal trachea. The hydrophilic ends of PEG grafts may be responsible for preventing growth of granulations and cell adhesions, ensuring enough time for mucosa to form around the implanted scaffold. Through SEM observation, the formation of respiratory mucosa and fibrous tissues was re-confirmed, and a plenty of cilium was also noted, indicating sufficient reconstruction.

CONCLUSIONS: *In vitro* characterization of the bi-layered PU matrix presented the evidence that the bi-layered PU matrix with PEG grafted surface was successfully fabricated. *In vivo* study demonstrated that the implanted scaffold was totally integrated with the trachea, assuring its biocompatibility and performance. Further research to investigate chronic effects of implantation may be necessary before full clinical application is possible.

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