# Material Composition Gradients and Protein-Loaded Electrospun Scaffolds: An Animal Model Study for Repair of Tracheal Defects

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**Introduction:** Infants and toddlers can be born with or develop a narrowed airway (larynx and/or trachea), resulting in labored breathing. The preferred surgical 'Standard of Care' is augmentation of the airway with autologous rib cartilage. We invented a polymeric, multi-layered scaffold that has the following properties: air-tight, resorbable, suturable, eliminates morbidity associated with a second surgical site, and is mechanically reinforced to prevent restenosis. Two pilot studies (8 rabbits) were initially performed, to determine *in vivo* functionality and effect size so that a properly powered study could be rationally designed, with much success. Thus a large scale *in vivo* study was undertaken to determine statistical significance. Using 5 as the effect size, a power of 0.80 and α of 0.05, the present study was designed.

**Materials and Methods:** Scaffolds composed of radial gradients of polycaprolactone (PCL) and poly(lactic-co-glycolic acid) (PLGA) were fabricated using an electrospinning technique. Scaffold treatments included growth factor (TGF-β3) encapsulation in the PLGA and seeding with rabbit bone marrow mesenchymal stromal cells. Elliptical-shaped scaffolds were implanted into induced elliptical defects in New Zealand White rabbits (30 rabbits, n=5) for six and twelve weeks. *In vivo* constructs were analyzed by bronchoscopy, histological and IHC staining, and microCT imaging methods.

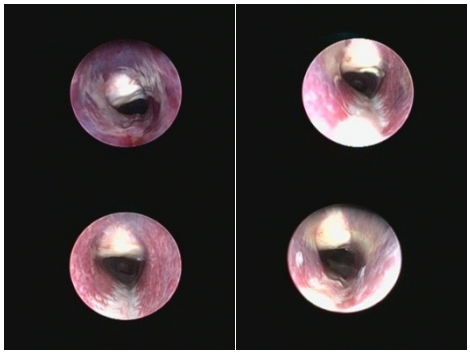

**Figure 1.** Bronchoscopy images from one subject (control group, ID #3961) at 3, 6, 9, and 12 weeks post-surgery (top left to bottom right).

**Results:** The studies demonstrated that our scaffolds maintained a robust, airtight trachea allowing a majority of the animals to be free of apparent breathing distress. At the conclusion of the study, there was a 77% survival rate with adverse events occurring in all groups and caused by complications pertaining to tracheal stenosis. Periodic bronchoscopies (3, 6, 9, and 12 weeks) revealed epithelial tissue and blood vessels migrating over the scaffold (Figure 1). The microCT data was reconstructed and the internal volume of the trachea was quantified (Figure 2). This analysis was performed to quantify the level of stenosis through the scaffolded region. Preliminary statistical analysis of this data revealed that the tracheal internal volume was slightly decreased as compared to a normal tracheal internal volume. The control scaffold (no cell and no growth factors) had a larger volume than the scaffolds with cells (Figure 3).

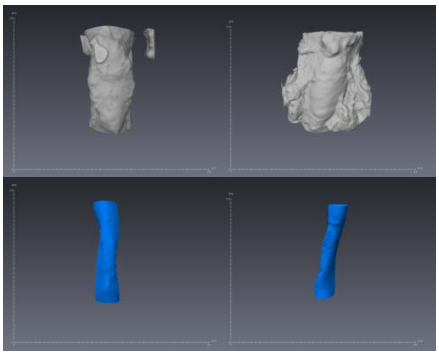

**Figure 2.** microCT reconstruction of trachea (top row) and lumen void (bottom row) (control group, ID #3983 and cell group, ID#3977, left to right).

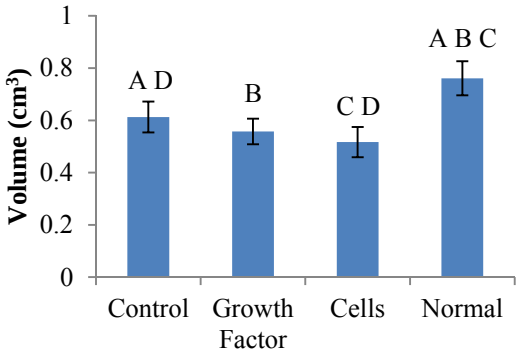


**Figure 3.** Tracheal lumen volume quantification. Common letters indicate a statistically significant difference ($p<0.05$) (i.e. [A] denotes significance between Control and Normal groups).

**Conclusions:** These studies successfully demonstrated the feasibility of applying electrospun scaffold technology to tracheal repair. Furthermore, we established that our scaffolds could be a possible alternative to the current autograft scaffolds prepared from rib (costal) cartilage tissue.

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**Disclosures:** The authors have nothing to disclose.