# Spectroscopic Analysis of Decellularized Human Tracheal Scaffolds

Derrick Tint, MD[1]; Collin Stabler, PhD[2]; Arash Hanifi, PhD[2]; Ahmed Soliman, MD[1]; Nancy Pleshko, PhD[2]

[1] Head and Neck Institute, Lewis Katz School of Medicine at Temple University, Philadelphia, PA

[2]Department of Bioengineering, Temple University, Philadelphia, PA

## ABSTRACT

Objectives:

Implement mid-infrared (mid-IR) spectrometry during the process of creating a decellularized human tracheal scaffold.

Analyze the changes spectroscopic signatures to monitor scaffold degredation and decellularization of tracheal lumen.

Validate spectroscopic data by comparing to various histologic stainings.

Methods

A decellularized tracheal scaffold was created cadaveric human trachea using a detergent-enzymatic method (DEM). Slides were stained with hemotoxylin and eosin (H&E) and DAPI stain for DNA to monitor for successful decellularization. Mid-IR spectra were collected from the lumen of the scaffold after 0, 1, 10 and 25 DEM cycles. A multivariate assessment of second-derivative spectra was performed using principle component analysis (PCA).

Results

H&E and DAPI staining of tracheal scaffold after 1 DEM cycle showed complete loss of nuclear material from respiratory epithelium and submucosa. There was incomplete removal of nuclear material from within lacunae of the hyaline cartilage after 25 cycles. PCA of second derivative spectra show significant separation of normal trachea and decellularized trachea along PC-2.

Conclusion

Mid-IR spectroscopic imaging is a novel method of monitoring the process of creating a tracheal scaffold. The emergence of collagen and connective tissue spectral peaks can be serially monitored non-destructively using this method.

## INTRODUCTION

Advanced disorders of the trachea, including tracheal stenosis and tracheal tumors, require segmental resection of diseased tracheal tissue with primary anastomosis. However, the amount of trachea that can be safely resected is limited to approximately 6 centimeters, even with mobilization and releasing procedures. The optimal treatment for longer segments of disease requires some method of reconstruction. Advances in tissue engineering have provided various methods to create tracheal scaffolds that can be used to reconstruct the airway.

One method to engineer a tracheal scaffold is to decellularize cadaveric trachea and repopulate it with recipient cells. This method is theoretically advantageous because it retains the cartilaginous framework of human trachea, while at the same time preventing transplant antigenicity by removing all donor cells. However, the current method of monitoring the decellularization process requires sacrificing viable tissue and examining it under the microscope. [1]

Fourier Transform Infrared (FTIR) Spectroscopy is a method of characterizing tissue composition by measuring the absorbance of light of specific molecules in the infrared spectral region. We hypothesize that this method can be applied in a non-destructive matter using a fiber optic probe and can characterize progression of the decellularization process without sacrificing sections of the scaffold for histology. [3]

## METHODS

**Engineered Tracheal Scaffold**

6 decellularzed tracheal scaffolds were created by decellularizing human tracheal tissue using the detergent-enzymatic method (DEM). [1] Each scaffold was:

1.Rinsed in Milli-Q water + antibiotic and antimycotic (ABAM) for 72 hours.
2.Incubated in 4% sodium deoxycholate solution in Milli-Q water + ABAM for 4 hours at room temerature
3. Incubated in 2000 KU (Kunitz Units) Dnase in 1 M NaCl, 2mM $CaCl_2$, 1.3 m M $MgSO_4$ + ABAM
4.Stored in PBS 1x + ABAM at 4oC
5.Steps 2-4 repeated for 25 cycles.

**Histology**

Tissue samples were collected and fixed in formalin after Step 1 (Day 0), 1 DEM Cycle (Day 1), and 25 Cycles (Day 25). These samples were stained with:

1.Hemotoxylin, Eosin and Alcian Blue
2.4', 6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI)

**Mid-IR (MIR) Fiber Optic Probe Data Acquisition**

Infrared spectral data were collected from the luminal surface of the tracheal scaffold. Three spectra were collected at Day 0, 1, 10 and 25 for each scaffold in the MIR region of 1500-900 $cm^{-1}$ at 4$cm^{-1}$ spectral resolution with 32 co-added scans using a Thermo Fisher infrared spectrometer (Waltham, MA) equipped with a silver halide attenuated total reflectance (ATR) loop probe.

**MIR Data Processing**

MIR spectra were processed using Unscrambler 10.1 (CAMO, Woodbridge, NJ, USA). First, the spectra were visually compared to see if peak differences were visible in the raw spectrum. Second derivative spectra were then analyzed (Savitzky Golay, 3 point smoothing) to improve peak resolution of the 1336 peak. In addition, a principal component analysis (PCA) was performed for a multivariate assessment of second derivative spectra.

## RESULTS

**Histology**

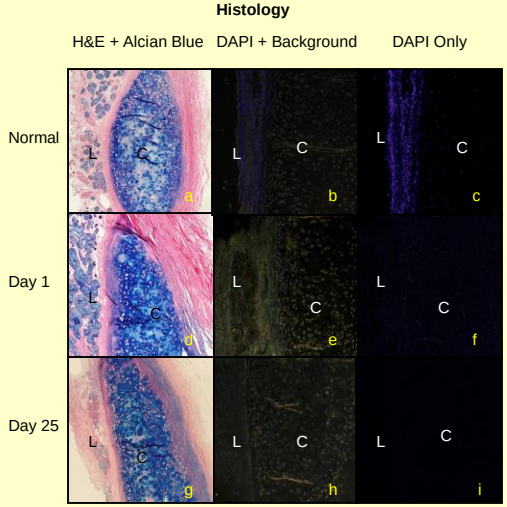


**Fig 1.** Characterization of tracheal scaffold. (a-c) Normal tracheal tissue. (d-f) Tracheal scaffold after 1 DEM cycle. (g-i) Tracheal scaffold after 25 DEM cycles. H+E + Alcian blue stain (a,d,g) H&E and Alcian Blue staining shows that the cartilaginous structure (C) and extracellular matrix of the tracheal scaffold is essentially unchanged after 25 DEM cycles. (b,c,e,f,h,i) nuclear staining with DAPI shows loss of cellular elements on the luminal (L) side of trachea after 1 DEM cycle. Tracheal cartilage (C) is almost completely decellularized after 25 DEM cycles.

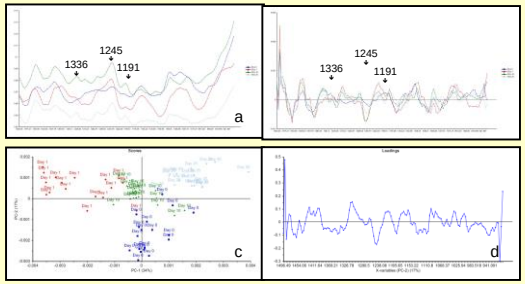


**Fig 2.** (a) MIR spectra in the region of 1500-900 $cm^{-1}$ of luminal surface of trachea at Day 0, 1, 10 and 25. (b) Second derivative of MIR spectra from (a). (c) PCA of second derivative spectra showing separation of Day 0 along (d) principle component 2 (PC-2)

## DISCUSSION

Decellularization of trachea allows for the creation of a scaffold that retains the original framework of normal trachea and removing its antigenicity. A method to confirm of the decellularization process is imperative. If unsuccessful, the tracheal scaffold would retain its antigenic potential could lead to rejection and require lifelong immunosuppression medication.

The most superficial layer of tracheal lumen is composed of pseudostratified respiratory epithelium. The height of a single pseudostratified epithelial cell is 20-50μM. The MIR fiberoptic probe measures the absorbance of tissue surface with a depth of penetration of 2-10μM, less than a single cell layer. [2]

The lumen of the tracheal scaffold is completely decellularized after 1 DEM cycle as demonstrated by the loss of DAPI staining in Fig. 1 (c,f). Therefore the MIR probe is measuring absorbance of surface molecules of respiratory epithelium in normal trachea, and surface molecules of extracellular matrix once the scaffold is decellularized.

PCA analysis of second derivative spectra shows separation of normal trachea from decellularized trachea (Day 1, Day 10, and Day 25) along PC-2. 1336 $cm^{-1}$ , 1245 $cm^{-1}$ , 1191 $cm^{-1}$ represent significant peak values that separate these spectra. The 1336 peak contribution is from collagen and its lack of presence in Day 0 and emergence in Day 1, 10 and 25 reflects the successful removal of respiratory epithelium, because the probe is now measuring the absorbance of collagen in the underlying submucosal connective tissue.

## CONCLUSION

MIR fiberoptic probe may represent a viable method that can be used to monitor the decellularization of tracheal lumen during the creation of a tracheal scaffold.

## REFERENCES

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Head & Neck Institute
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