Advancing Localized Therapy and Disease Modeling: Trachea-On-A-Chip Model for Targeted Treatment of Idiopathic Subglottic Stenosis (iSGS)

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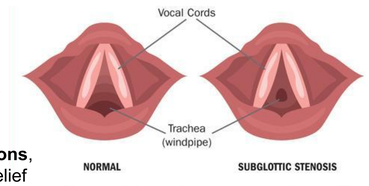
We strive to create innovative, patient-centered solutions through rigorous, detail-oriented research to advance regenerative medicine.

Introduction

Project goal: Develop an OoC model of iSGS to facilitate research into more **effective and less invasive therapies**.
Idiopathic subglottic stenosis (iSGS) is a rare and progressive narrowing of the airway below the vocal cords due to excessive scar tissue formation.

This narrowing causes **partial airway obstruction**, leading to symptoms such as:

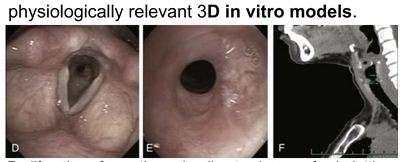
- Dyspnea (shortness of breath)
- Stridor (high-pitched wheezing)
- Voice changes
- In severe cases, **life-threatening airway restriction**



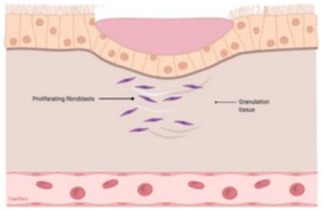
Closing of the trachea due to scar tissue. (Flynn 2025)

Current treatments (e.g., **endoscopic dilations**, **surgical resections**) offer only temporary relief and do not resolve the underlying **fibrosis**, leading to frequent recurrences.

Organ-on-a-chip (OoC) platforms enable researchers to study diseases in more physiologically relevant 3**D in vitro models**.

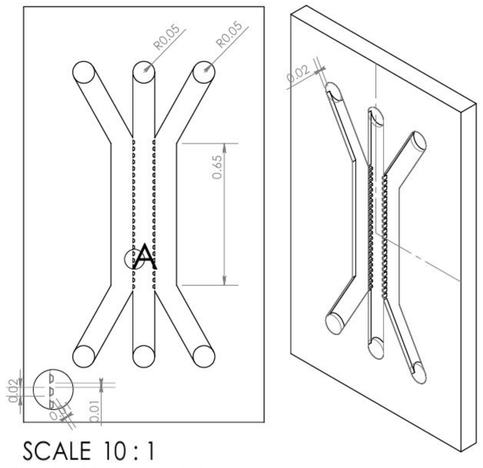


Proliferation of scar tissue leading to closure of subglottic tracheal opening. ("Idiopathic Subglottic Stenosis", 2015)



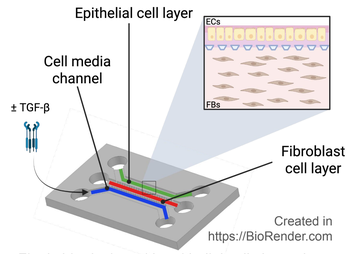
Depiction of the nature of scarring in the dermal layers. (Carpenter et. al. 2022)

Concept and Design



Blueprint of final product design.

Designed a microfluidic organ-on-a-chip (OoC) platform to **model the subglottic environment** of the trachea. Integrated key structural and cellular features to better **replicate in vivo conditions** compared to traditional culture methods.



Final chip design with epithelial cell channel (green), fibroblast channel (blue), and cell media channel (red).

Developed a three-channel layout:

- **Central channel** representing the fibrotic region, containing fibroblasts.
- **One flanking channel** representing the local tissue, containing epithelial cells.
- **One flanking media channels** to support nutrient diffusion and biomolecule exchange.

Incorporated trapezoidal micro-posts to:

- Provide structural stability.
- Enable controlled hydrogel retention in the central cell channel

Experiment & Analysis

Study Design:

Cell Type(s)	Condition	Number of Chips	
		1 mg/ml	4 mg/ml
Epithelial + Fibroblast Donor 1	Collagen	4	4
	Collagen + TGF-β	4	4

Summary of experimental conditions.

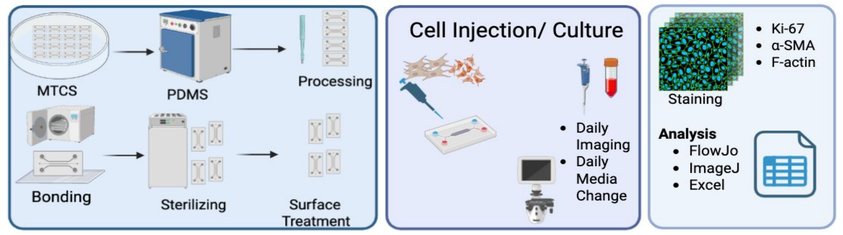
Purpose:
Conducted experiment to **optimize the chip platform** for modeling fibrosis.
- Aimed to observe how **collagen stiffness (1 mg/mL vs. 4 mg/mL)** affects cell behavior and matrix remodeling.

Cell Types Used:
- **Epithelial cells**: immortalized cell line.
- **Fibroblasts**: primary cells isolated from a **patient donor**.

Conditions Tested:
- **Collagen only**
- **Collagen + TGF-β** to induce fibrotic signaling

Experimental Setup:
Two collagen concentrations: **1 mg/mL** and **4 mg/mL**
- **4 chips per condition and concentration combination**
- **Total:16 chips used**

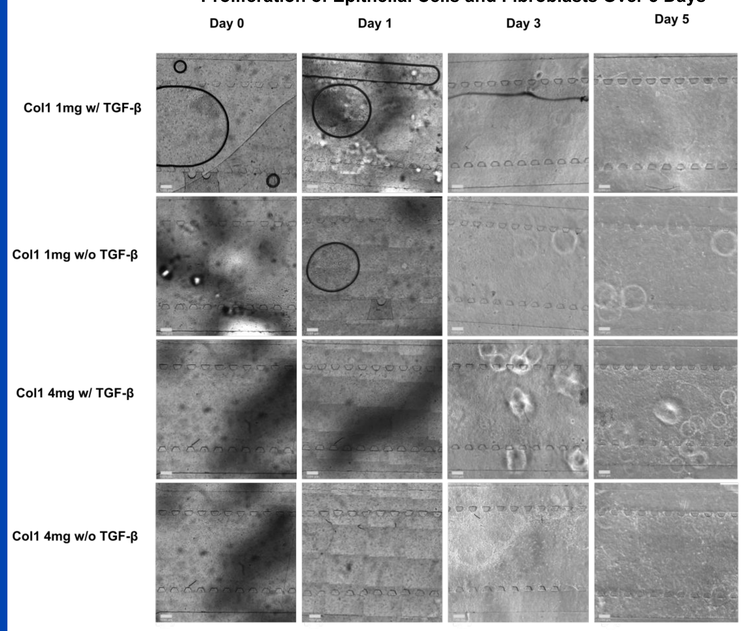
Experimentation:



Overview of experimental timeline. Created in https://BioRender.com

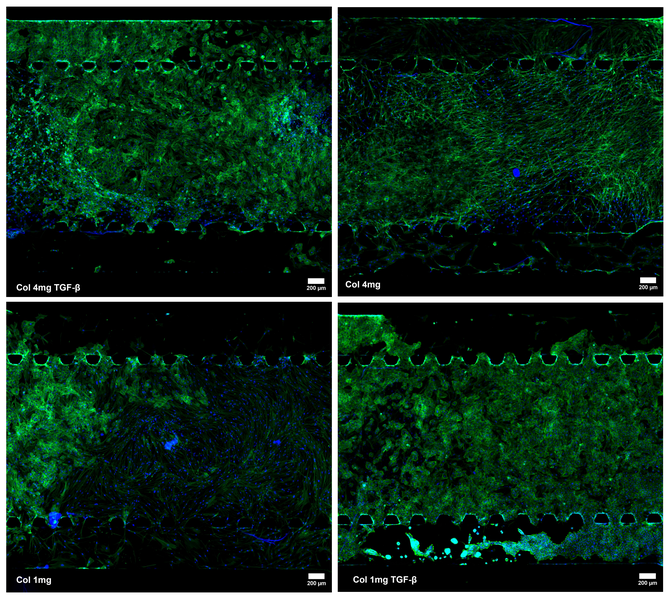
Verification and Validation

Proliferation of Epithelial Cells and Fibroblasts Over 5 Days



Light microscopy (10X magnification) images of the chip at each day of seeding ECs and FBs.

Ki67, α-SMA, and F-actin staining: 4mg/mL Collagen Provides Successful Outcome



Staining characterizes cell proliferation over time in each condition.

- Fibroblasts **proliferate** through the channel **with TGF-beta**.
- **1 mg/ml collagen** is **not a strong enough concentration** to hold fibroblasts within channel.
- **4 mg/ml collagen** provides **structural support** while **maintaining** a higher level of **differentiation**.
- **4 mg/ml collagen** allows fibroblasts to proliferate **without seeping** into surrounding channels.

Future Work

Cell Type(s)	Condition	Number of Chips	
		1 mg/ml	4 mg/ml
Epithelial + Fibroblast Donor 1	Collagen	2	2
	Collagen + TGF-β	2	2
Epithelial + Fibroblast Donor 2	Collagen	2	2
	Collagen + TGF-β	2	2
Epithelial + Fibroblast Donor 3	Collagen	2	2
	Collagen + TGF-β	2	2

Summary of future experimental conditions.

Expand **donor variability** by incorporating fibroblasts from three patient donors to improve model relevance and **capture biological heterogeneity.**

Focus on 4 mg/mL collagen concentration, based on initial optimization results.

Introduce Matrigel into the hydrogel matrix to better mimic native extracellular matrix (ECM) composition.

Explore crosslinking strategies:
- Vary crosslinking duration to systematically increase hydrogel stiffness.
- Assess impact on cellular behavior and matrix remodeling.

Begin alignment with **FDA regulatory pathways**:
- Explore qualification through the **ISTAND** (Innovative Science and Technology Approaches for New Drugs) Pilot Program.
- Position the chip as a potential drug development tool for fibrotic airway diseases by aligning with regulatory expectations.

Acknowledgements

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