Decellularized Meniscus Scaffolds for Laryngotracheal Reconstruction in a Porcine Model

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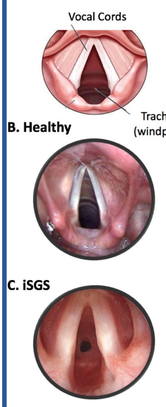
MOTIVATION



Subglottic stenosis (SGS) is the narrowing of the airway caused by scar tissue build-up leading to difficulty breathing

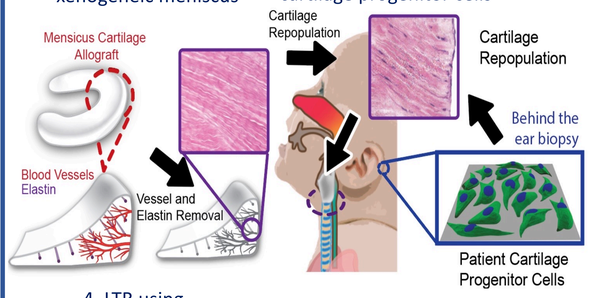
In **pediatric patients** each day of intubation increases the risk of SGS by 50%. Particularly prevalent issue in the NICU.

Standard of care for severe SGS is laryngotracheal reconstruction (LTR) using autologous cartilage. Drawbacks of this approach include donor site morbidity and limited size of available cartilage.

These shortcomings can be averted by the creation of a **decellularized meniscus scaffold (MEND)** for LTR

APROACH

1. Decellularization of xenogeneic meniscus
2. Reinvasion with cartilage progenitor cells
3. MEND differentiation in chondrogenic media
4. LTR using differentiated scaffolds

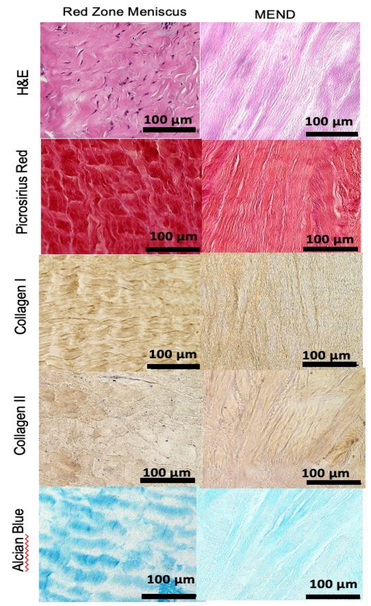


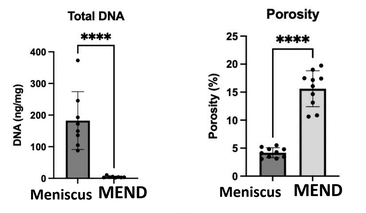
EXPERIMENTAL PLAN

1. Decellularization of xenogeneic meniscus
2. Characterization of acellular MEND
3. Optimization of cell population for airway cartilage engineering
4. MEND Invasion and Differentiation
3 weeks
4. LTR in 4 -month-old Yukatan Miniature pigs using MEND
12 weeks
5. Airway excision for analysis

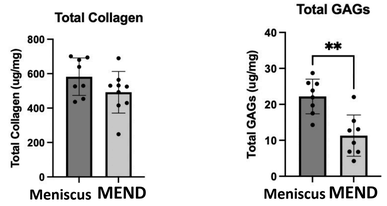
MEND LTR in a juvenile porcine model

MENISCUS DECELLULARIZATION



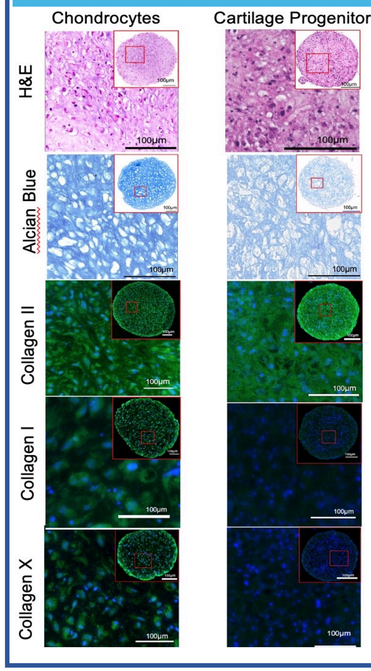


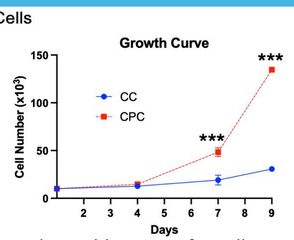
Decellularization **removed** porcine **genetic material** and allowed for **channel creation**



DMMB and hydroxyproline assays showed **no change in collagen** content and **reduction in glycosaminoglycans** after decellularization

OPTIMIZING CELL POPULATION FOR CARTILAGE ENGINEERING



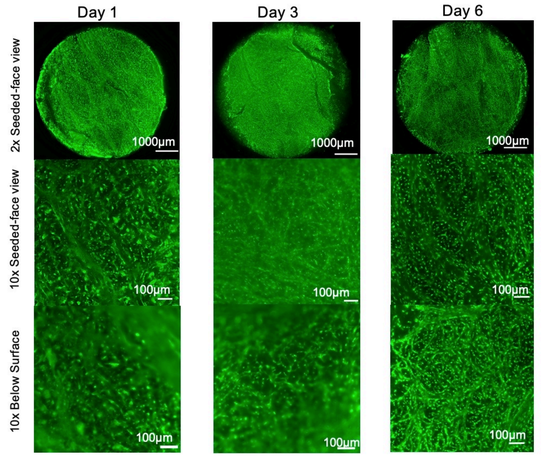


Alamar blue assay for cell metabolism used to quantify proliferation

Cartilage marker collagen II was highly visible in pig **cartilage progenitor cell (CPC)** pellets after immunofluorescent imaging

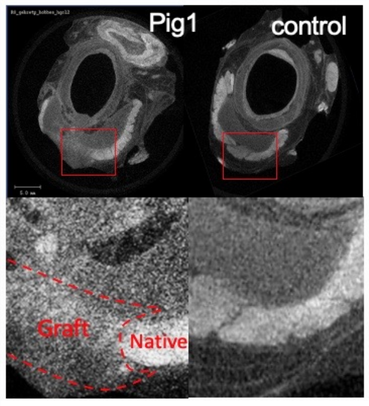
Cartilage progenitor cells had **higher proliferation and less hypertrophy** than chondrocytes (CCs) and are thus **ideal for airway cartilage engineering**

IN VITRO MEND CHARACTERIZATION

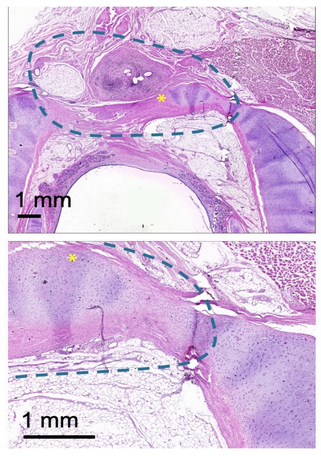


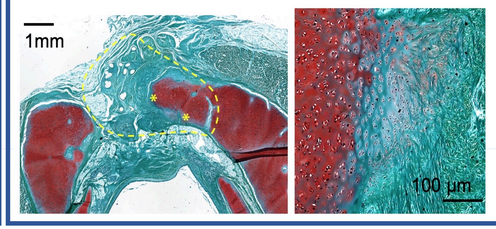
Calcein staining demonstrated **cell invasion below the surface** of MEND scaffolds **after 6 days**. This was then followed by 3 weeks of differentiation in chondrogenic media

MEND LTR



uCT showed **integration of MEND graft with native cricoid cartilage**





Safranin-O and H&E confirmed **new cartilage formation** after MEND LTR

CONCLUSIONS

Meniscus decellularization increased porosity and successfully removed all genetic material whilst mostly maintaining meniscal biochemical properties. Due to their lack of hypertrophic markers and rapid growth CPCs are an optimal cell type for cartilage engineering. MEND is fully penetrated by CPCs 6 days after invasion. MEND used in pig LTR demonstrated integration with native cricoid as well as neocartilage formation.

NEXT STEPS

Remaining work:
-PCR validation of CPC and CC pellet findings
-Histological analyses of native airway
-Mechanical testing of MEND after *in vivo* study
-**Future studies:**
-Understanding which cells in native airway integrate into MEND *(in vivo)*
-Investigation of immune response following MEND implantation

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REFERENCES

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