Channel-Laden Decellularized Meniscus Scaffold for Meniscus Tissue Engineering

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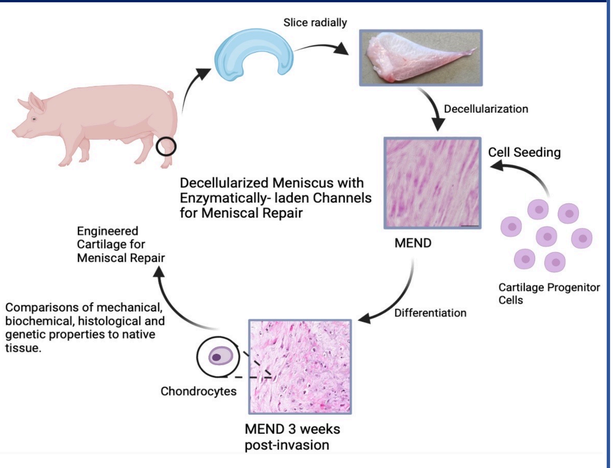
MOTIVATION

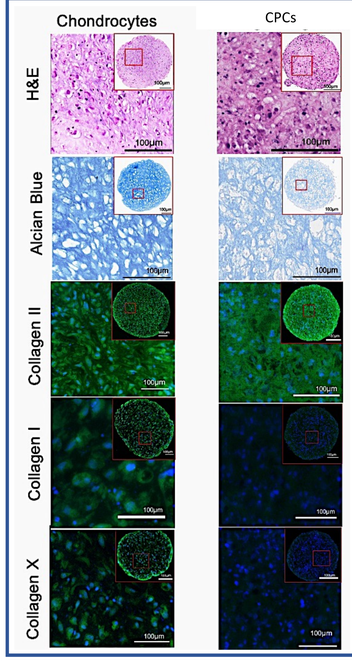
The meniscus protects articular cartilage from load-induced injury in the knee. Damage to the meniscus causes erosion of articular cartilage leading to pain, disability, and possible osteoarthritis. Traditionally, a meniscectomy is performed to remove the damaged portion of the meniscus; however, this procedure leads to increased contact forces between the tibia, femur, and subsequent cartilage. Cadaveric allografts have shown promise but are limited by donor availability, imperfect mechanical matching, and limited repopulation with autologous cells causing a nearly 30% revision rate. To circumvent these shortcomings, we employed an *innovative tissue engineering approach* to use decellularized allogeneic meniscus in which we have created microchannels repopulated with cartilage progenitor cells (CPCs) obtained from a minimally invasive biopsy from ear cartilage. Cartilage progenitor cells were selected for their high proliferation rate and formation of superior cartilage for meniscus engineering. Our approach leverages the native meniscal blood vessels and elastin fibers to overcome the limitations of previous cartilage decellularization techniques. By selectively removing the elastin fibers and blood vessels uniquely present in the fibro-elastic cartilage of allogeneic meniscus we were able to form microchannels that support effective recellularization and subsequent differentiation. We hypothesize that combining our meniscal decellularized scaffold (MEND) and cartilage progenitor cells will create cartilage with biochemical, mechanical, and phenotypic properties that will integrate into an *ex vivo* meniscal tear model.

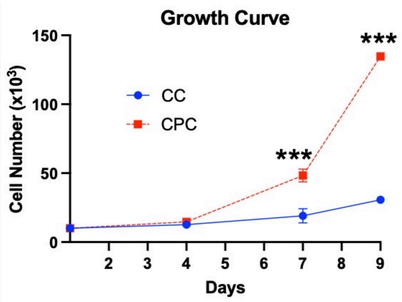
APPROACH



Porcine menisci were sliced radially into triangular sections before freeze thaw cycles. Scaffolds were then washed with pepsin and elastase for decellularization as well as creation of channels which aid alignment of cells following invasion. Decellularized scaffolds were invaded with cartilage progenitor cells and expanded for six days. Each day scaffolds were stained with Live staining to examine cell penetration. This followed a pellet study conducted to determine optimal cell type for cartilage engineering. Differentiation occurred for a six-week period during which scaffolds were cultured In chondrogenic media. Thereafter engineered cartilage was compared mechanically, histologically, biochemically and genetically to native tissue. Given prior difficulties in repair of white zone meniscus, MEND will be critically compared to both regions of native meniscus tissue

CPCs ARE AN IDEAL CELL TYPE FOR CARTILAGE ENGINEERING

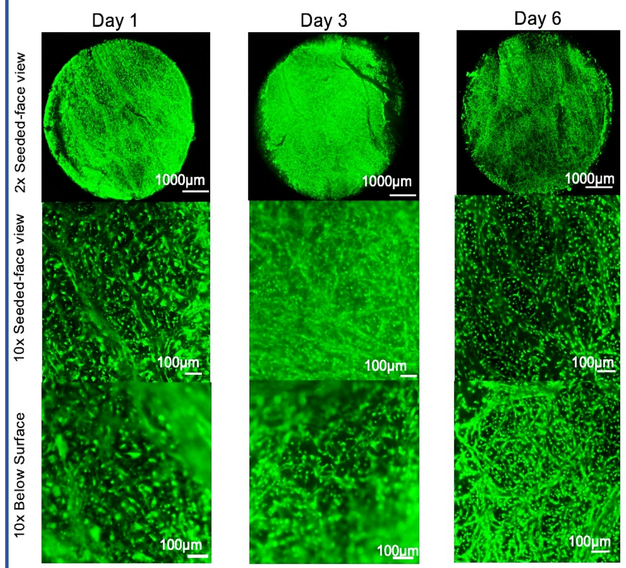




Data represented as n=X+- standard deviation, *p<0.1 **p<0.01***,p<0.001 ,****p<0.0001

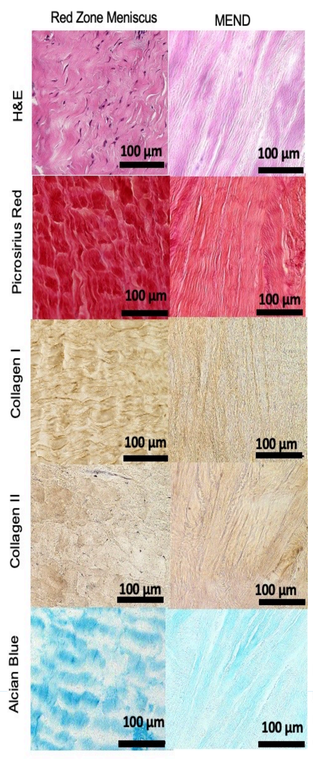
CPCs proliferated faster than chondrocytes (above). Despite lowered glycosaminoglycans, **CPCs had more collagen II deposition** than chondrocytes (left). Immunofluorescence of Collagen I and X on cell pellets indicated **more hypertrophy in chondrocytes** than in CPCs (left) . Given proliferation and ECM markers, **cartilage progenitor cells** provided an **ideal cell type for cartilage tissue engineering**.

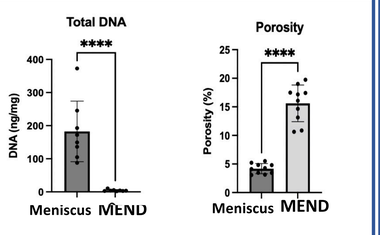
MEND INVASION



CPCs were invaded for 6 days using a serum gradient and stained with calcein (green) at day 1,3 & 6 to visualize post-seeding invasion. Scaffold was imaged at seeded surface as well as below the surface to demonstrate **even cell penetration** through the scaffold.

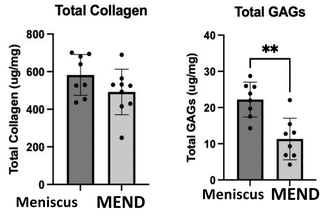
MENISCUS DECELLULARIZATION





Data represented as n=X+- standard deviation, *p<0.1, **p<0.01***,p<0.001 ****p<0.0001

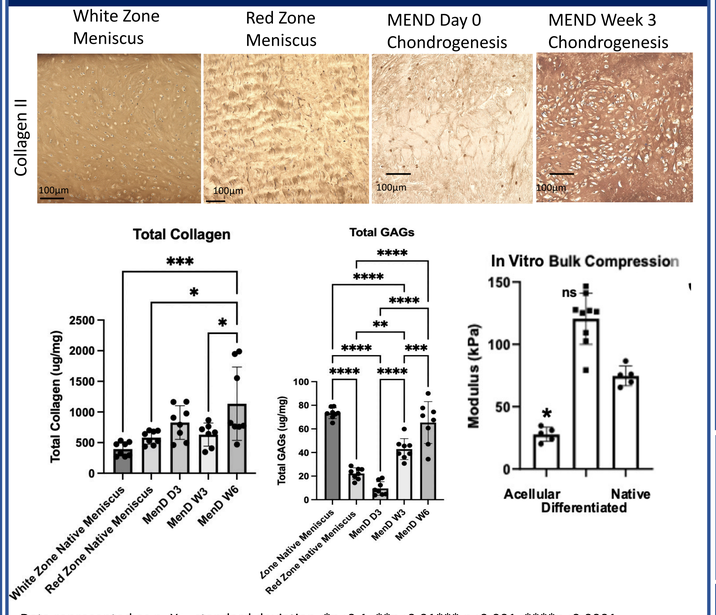
Decellularization **removed** porcine **cells below acceptable threshold of 50ng/mg dry tissue** and allowed for **channel creation** (above and left)



Data represented as n=X+- standard deviation, *p<0.1,**p<0.01, ***p<0.001 ,****p<0.0001

MEND showed similar collagen, but **reduced glycosaminoglycans** content in comparison to native meniscus following decellularization (above and left)

MEND REMODELING DURING CPC DIFFERENTIATION



Data represented as n=X+- standard deviation, *p<0.1, **p<0.01***,p<0.001 ,****p<0.0001

DMMB and Hydroxyproline assays revealed **collagen and glycosaminoglycan** content that **increased with chondrogenic differentiation** to levels **higher than both white zone and red zone meniscus** (above left). Collagen II immunohistochemistry further validated biochemistry findings (above). Compression testing showed that MEND had **bulk modulus similar to native tissue** (above right).

CONCLUSIONS

Cartilage Progenitor Cells are a superior cell type for meniscal engineering.

-Meniscus decellularization successfully removed genetic material whilst maintaining meniscus collagen content.

-Following 6 days of expansion CPCs had fully invaded decellularized scaffolds.

-Chondrogenic differentiation of MEND scaffolds produced cartilage biochemically and mechanically similar to native meniscus.

NEXT STEPS

Integration Testing:
Culturing MEND between sections of native meniscus to observe scaffold integration

Immune Response:
Co-culture of immune cells and MEND for quantification of inflammatory cytokine release

In Vivo Studies:
Surgical implantation of MEND into meniscus tissue following meniscal injury

Drug Delivery:
Investigating MEND release profiles for anti-inflammatories to reduce immune response at implantation site

Rate of Chondrogenesis:
To further optimize cell population for cartilage engineering, conducting ELISAs on pellets throughout differentiation can allow for quantification of rate of differentiation

ACKNOWLEDGEMENTS

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REFERENCES

[1] Logerstedt *et al.*, *J Orthop Sports Phys Ther*. 2010, [2] Buma *et al.*, *Biomaterials*. 2004, [3] Vaquero *et al.*, *Musc Lig Tend J*. 2016, Figures Created using BioRender Software