Rapid Hyaline-like Cartilage Formation via Scaffold-free Layering of Juvenile Chondrocyte Sheets

Nicolás F. Metzler[1,2], Adam Ford[1], Makoto Kondo[2], David W. Grainger[1,2], Teruo Okano[1,3]

[1]Cell Sheet Tissue Engineering Center (CSTEC), Department of Pharmaceutics and Pharmaceutical Chemistry; [2]Department of Biomedical Engineering University of Utah, Salt Lake City, UT; [3]Institute for Advanced Biomedical Engineering and Science, Tokyo Women's Medical University (TWIns), Tokyo, JAPAN.

Background

Poor native articular cartilage repair capacity requires development of regenerative medicine approaches for treating articular cartilage injury.

Requirements for cartilage defect filling and restoration of joint mechanics commonly uses biomaterials to produce 3-dimensional tissue-engineered constructs. However, biomaterials may complicate healing by introducing biocompatibility challenges and disrupting the dense cell-cell network present in native chondrogenic development[1]. In contrast, human juvenile cartilage-derived chondrocyte (JCC) cell sheets are scaffold-free, cell-dense constructs prepared using thermo-responsive cultureware with promising chondrogenic potential[2].

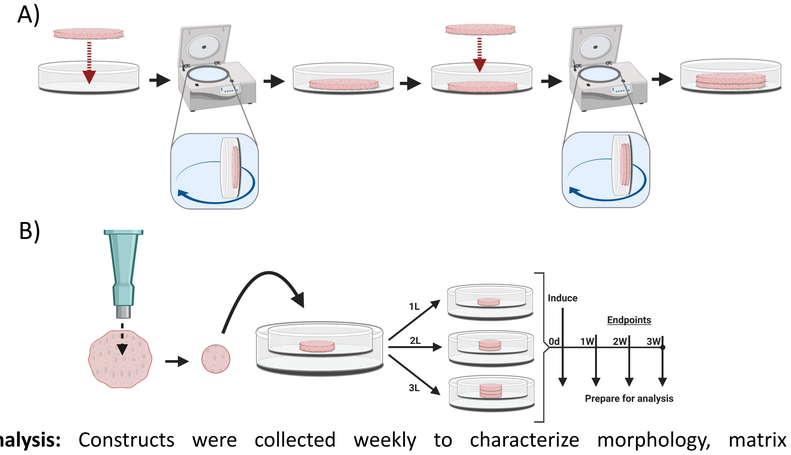
Layering of cell sheets, enabled by retained native extracellular matrix and cell surface proteins, forms thick, tissue-like constructs, increasing cell-cell interactions that support chondrogenesis[3] and reducing needs for exogeneous stimuli, such as expensive recombinant cytokines, thus further increasing potency of the overall construct.

As such, the ***aims of this study focused on human chondrocyte cell sheet layering to improve in vitro differentiation capacity of JCC sheets with the ultimate goal of developing a focal chondral defect treatment.***

Methods

Cell Sheet Construct Fabrication: Human JCC cell sheets were prepared via culture on UpCell thermo-responsive culture dishes (35 mm) for 2 weeks. Resulting cell sheets were detached from culture dishes after 20 minutes at room temperature and layered up to three layers (1L, 2L, 3L) via centrifugation on insert well culture membranes (Fig. 1A) and returned to growth media culture.
Differentiation: Three days after layering, cell sheet constructs were cut using an 8mm biopsy punch and cultured in hypoxia (5% O_2) in chondrogenic media for up to 3 weeks (Fig. 1B). Chondrogenic media was supplemented with 10 ng/mL TGFβ-3 (T+ condition) and with or without additional 200 ng/mL BMP6 (B+ condition), i.e., T+/B+ or T+/B-.

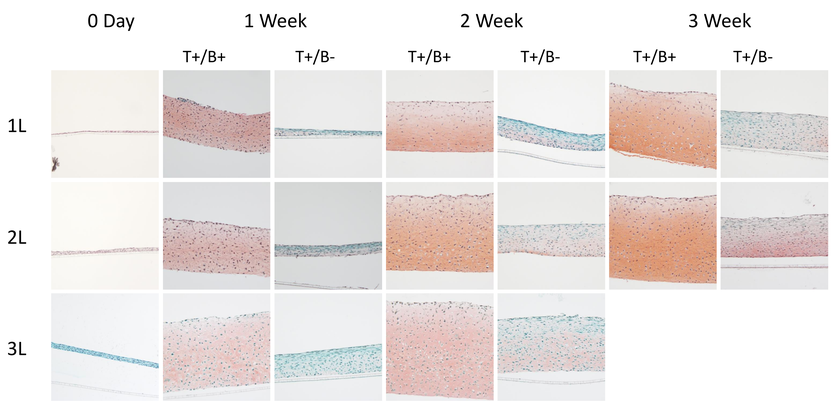
FIGURE 1. Schematic of JCC construct fabrication sequence:



Analysis: Constructs were collected weekly to characterize morphology, matrix composition and chondrogenic gene expression. Sulfated proteoglycans, collagen II (COL2) and collagen I (COL1) were stained via safranin O and immunohistochemistry using horseradish peroxidase respectively. Construct thickness was measured histological slides using imageJ. Gene expression of COL2A1 and COL1A1 was determined using qRT-PCR. Fold change expression and COL2/COL1 ratio calculated using the $\Delta\Delta C_T$ method.

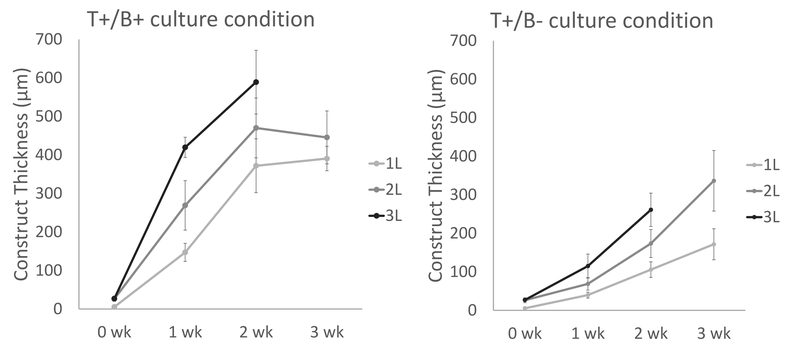
Results

FIGURE 2. Safranin-O stained cell sheet layered constructs throughout differentiation



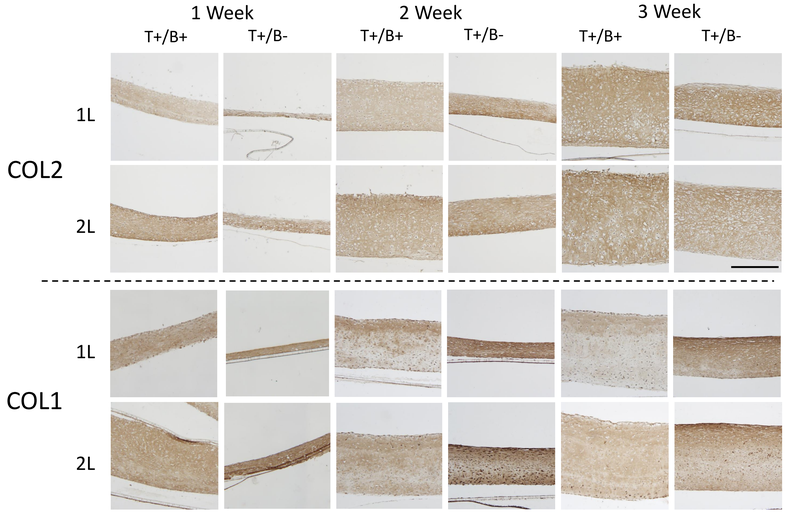
JCC cell sheet constructs rapidly accumulate proteoglycan (red) by one week and develop hyaline-like morphology (lacuna, low cell density) when cultured with TGFβ-3+/BMP6+ media. In TGFβ-3+/BMP6- media, proteoglycan accumulation is greatly diminished; however, safranin O staining increased with increasing construct layers.

FIGURE 3. JCC sheet construct layered thickness throughout differentiation



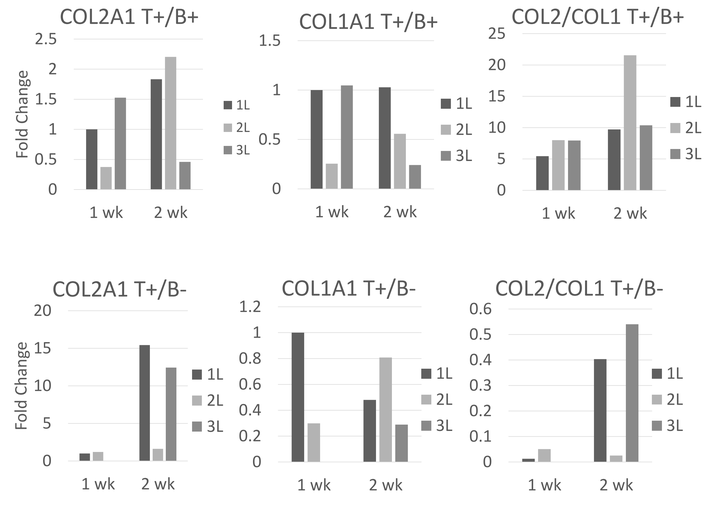
T+/B+ cultured 1L and 2L constructs reach equilibrium thickness of ~400µm by 2 weeks; however, P3L sheets reach and exceed that by 1 week. T+/B- cultured constructs maintain the trend of additional layers increasing construct thickness at all timepoints.

FIGURE 4. Cell sheet IHC for collagen II and collagen I in layered constructs



COL2 stains homogenously throughout all cell sheet constructs at all time points suggesting that TGFβ-3 alone is sufficient to drive deposition. Collagen I COL1 is present at all timepoints, but becomes less homogenously distributed, skewing towards the apical sheet surface during continued culture

FIGURE 5. Relative gene expression of COL2 and COL1



Increasing COL2/COL1 ratios from 1 to 2 weeks indicate increasing differentiation, with substantially higher magnitude in T+/B+ culture reflecting more hyaline-like phenotype.

Discussion

- JCC cell sheet constructs demonstrate robust and rapid differentiation into hyaline-like cartilage when cultured in TGFβ-3 and BMP6 containing (T+/B+) media.
- T+/B- cultured constructs do not reach ~400µm by 3 weeks, suggesting longer culture is needed for differentiation.
- Spatial reorganization of COL1 may be in part due to substrate stiffness or the dissolved oxygen concentration gradient inducing construct polarity
- Cell sheet layering may improve cell sheet therapies for in vivo hyaline cartilage regeneration.

Acknowledgements

- CSTEC group members
- UTAG (State of Utah), CSTEC Center support through HSC, SOM and CoP
- Schematic produced in BioRender

References

[1] DuRaine G.D., *Ann. Biomed. Eng.*, **43** (2015) 543**;**
[2] Kondo M., *npj Regen. Med.*, **6** (2021) 65**;**
[3] Thorp H., *Regen. Ther.*, **18** (2021) 487.