

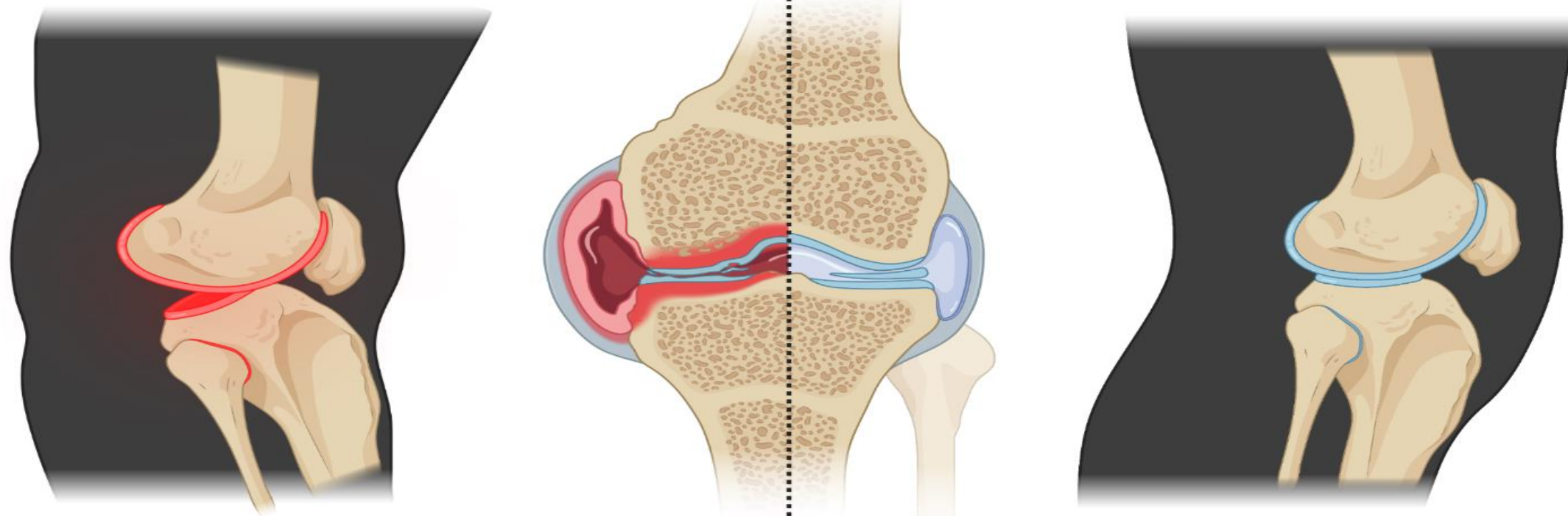
# Evaluation of cultureware effects on human-derived chondrocyte sheets

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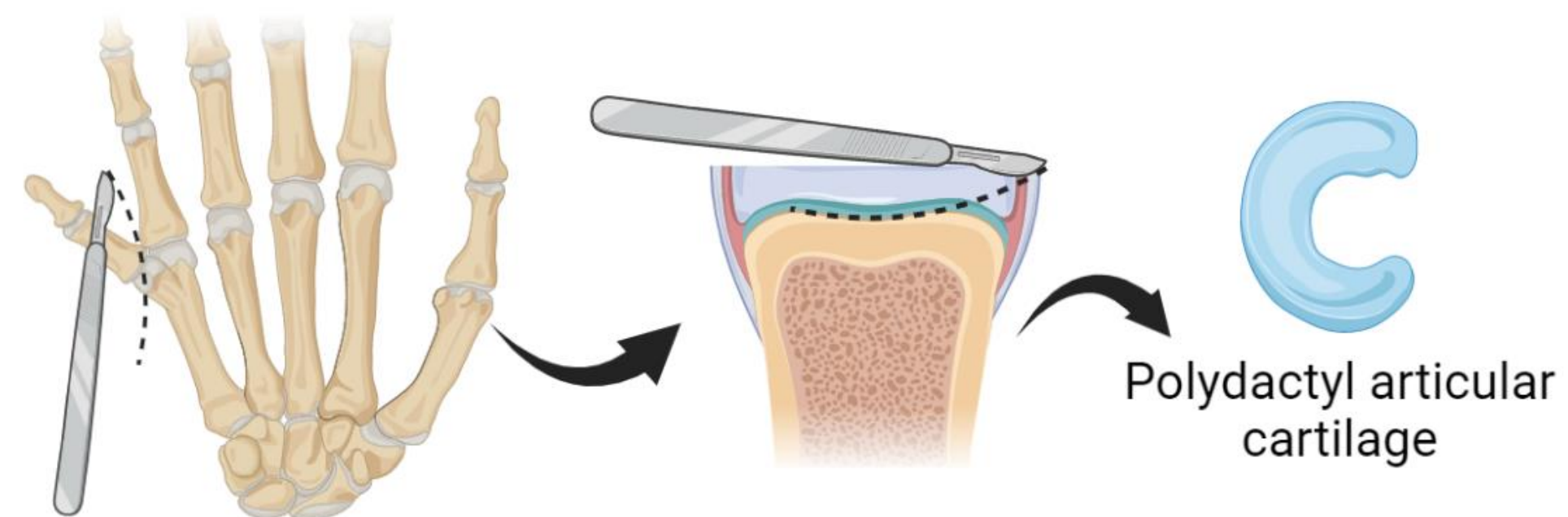
## Motivation



### Human chondrocyte-derived cell sheets to treat osteoarthritis

32.5 million American adults suffer from osteoarthritis (OA). Articular cartilage does not regenerate naturally<sup>[2]</sup>; joint replacement is risky and expensive<sup>[2]</sup>. Allogeneic, shelf stable, readily available human chondrocyte cell sheets attempt to solve current OA treatment deficiencies.

## Background



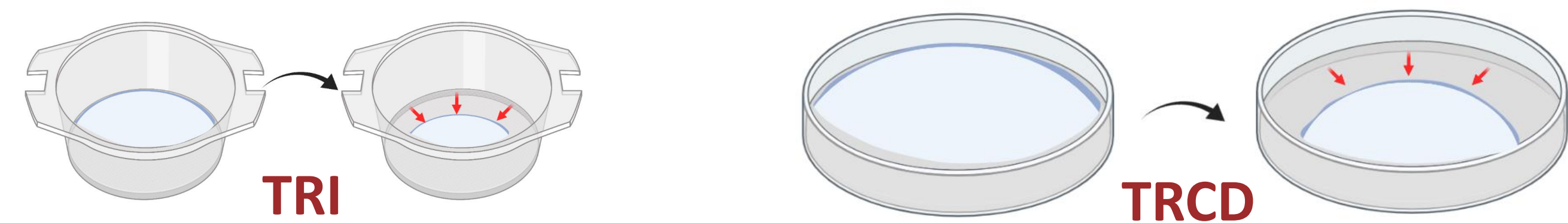
Polydactyl articular cartilage

### Utilization of surgical discards as potent chondrocyte source

Polydactyl surgical discards provide human-derived chondrocytes (PDCs). These cells show excellent proliferative and differentiation characteristics<sup>[2]</sup>. A single donor bank can produce cell sheets to treat thousands of patients at high passages without severe immune response. Cell characteristics may suffer at high passages.

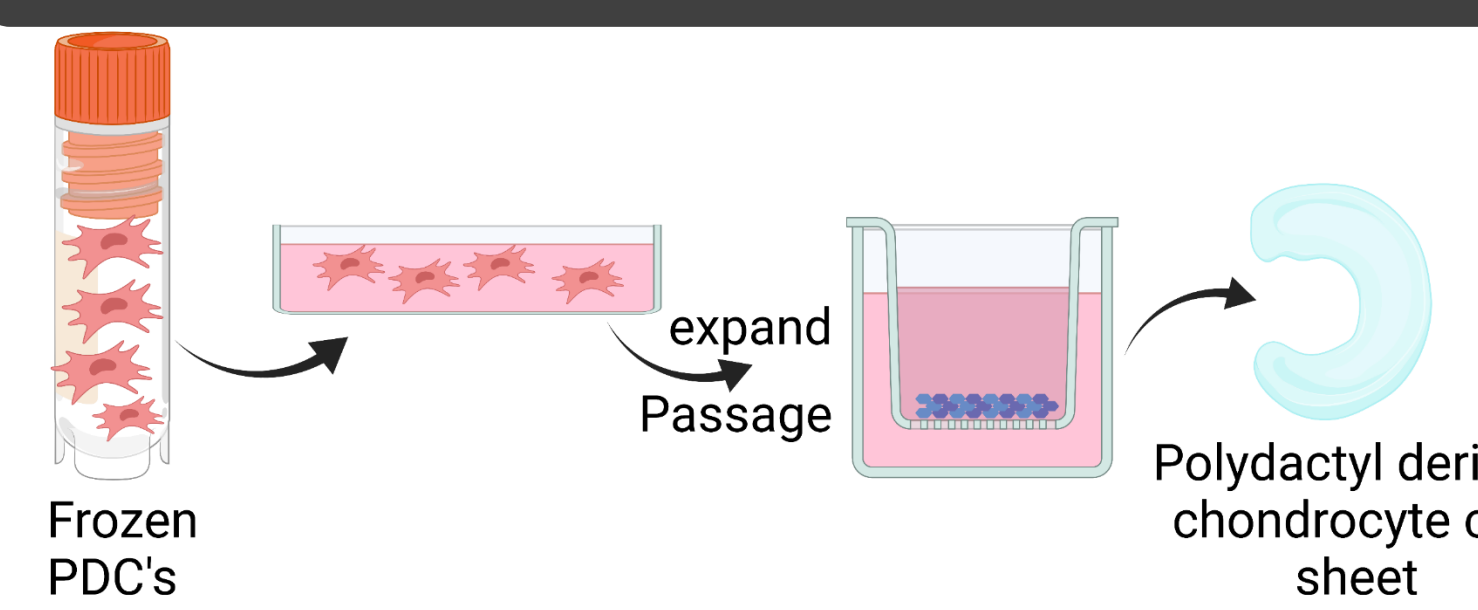
### Characterization of cell sheet cultureware is needed

Cell sheet contraction after detachment from culture surfaces has been reported as a predictor of chondrogenic potential<sup>[1]</sup>. The effect of cell sheet culture surface on sheet contraction and gene expression as a function of passage is unexamined. Newer thermally responsive culture dishes offer substantial advantages over thermally responsive inserts.



**Aim: distinguish TRI and TRCD cultureware effects on human polydactyl derived chondrocyte cell sheets vs. passage number**

## Materials & Methods



### A. Culture method

PDCs seeded onto thermally responsive culture dish (TRCD) and insert (TRI). Cell sheet detached from surface at room temperature after culture.

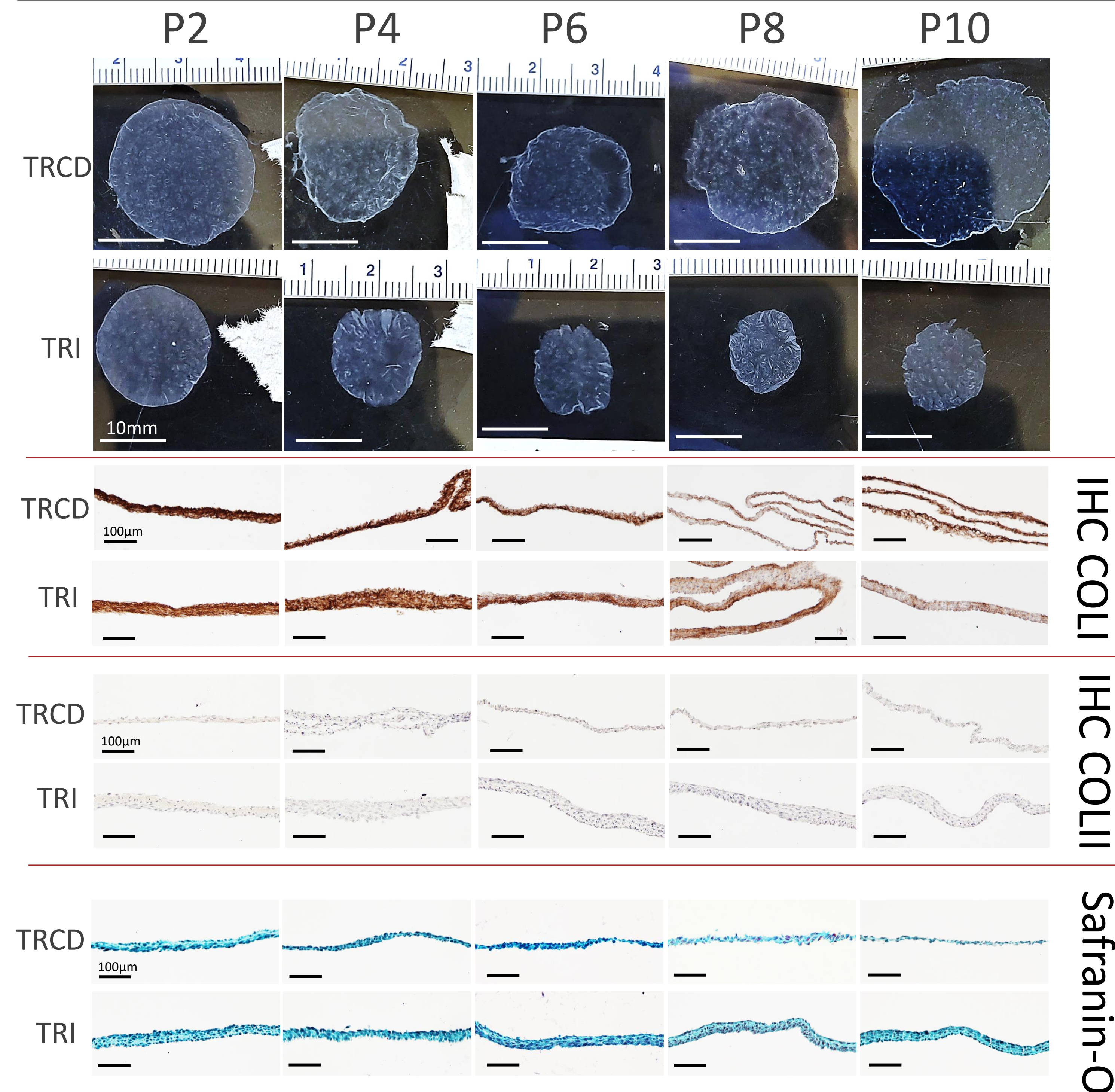
### B. Cell sheet characterization

Half of cell sheet used for IHC staining of collagen types 1 and 2, Safranin-O stain for nuclei and proteoglycans. Other half used for PCR for SOX9 expression

### C. PDC culture media

- DMEM/F12
- 20% Fetal Bovine Serum
- 1% Anti-Anti
- Ascorbic acid to conc. 100µg/mL

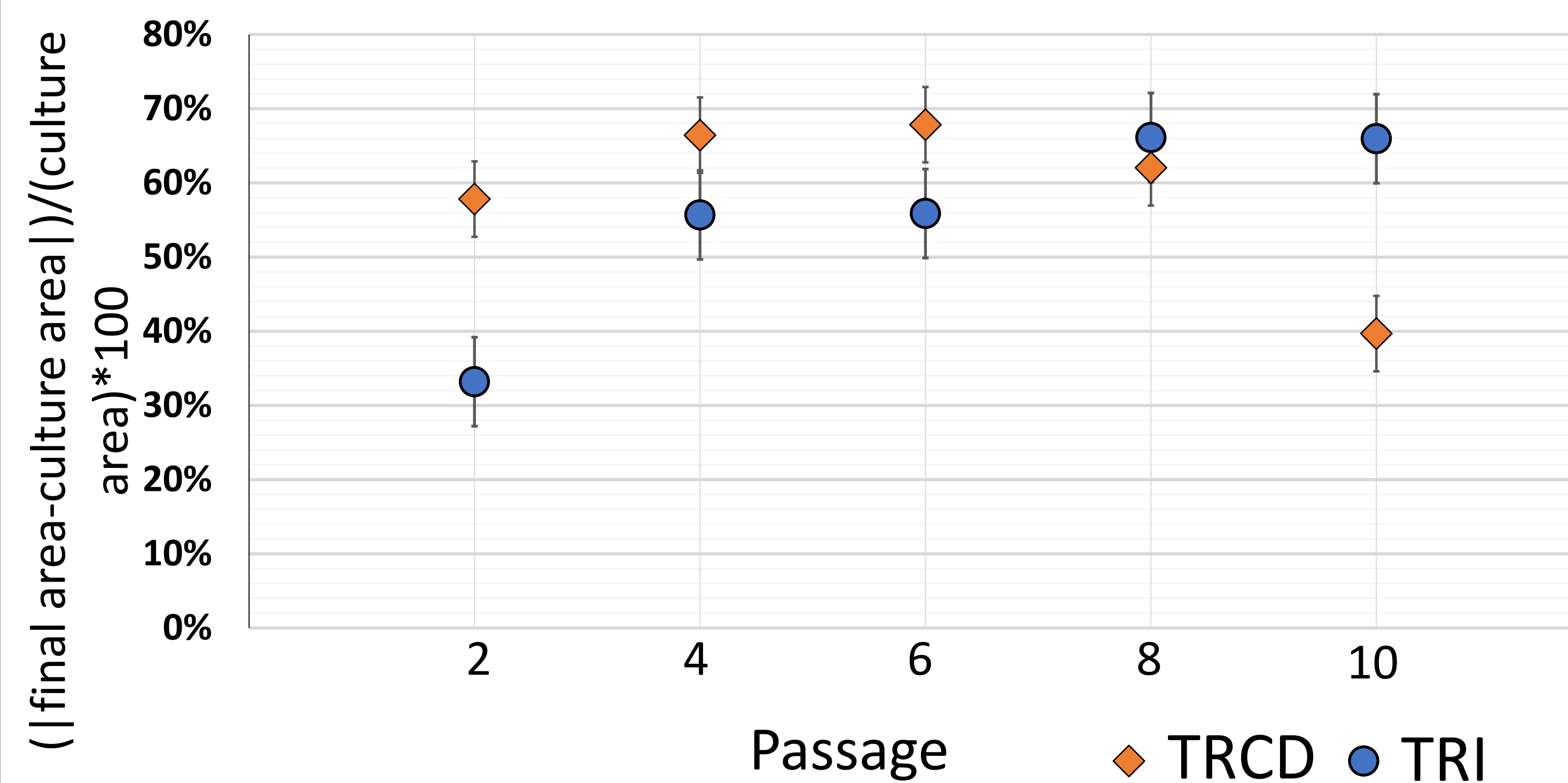
## Results



### Figure 1: Chondrocyte cell sheet histology data vs passage

Macro images show greater consistency in TRI sheets. TRCD sheets change gradually. Cell sheets are COL1 positive, COL2 negative, Saf-O negative. TRI sheets are thicker at all passages

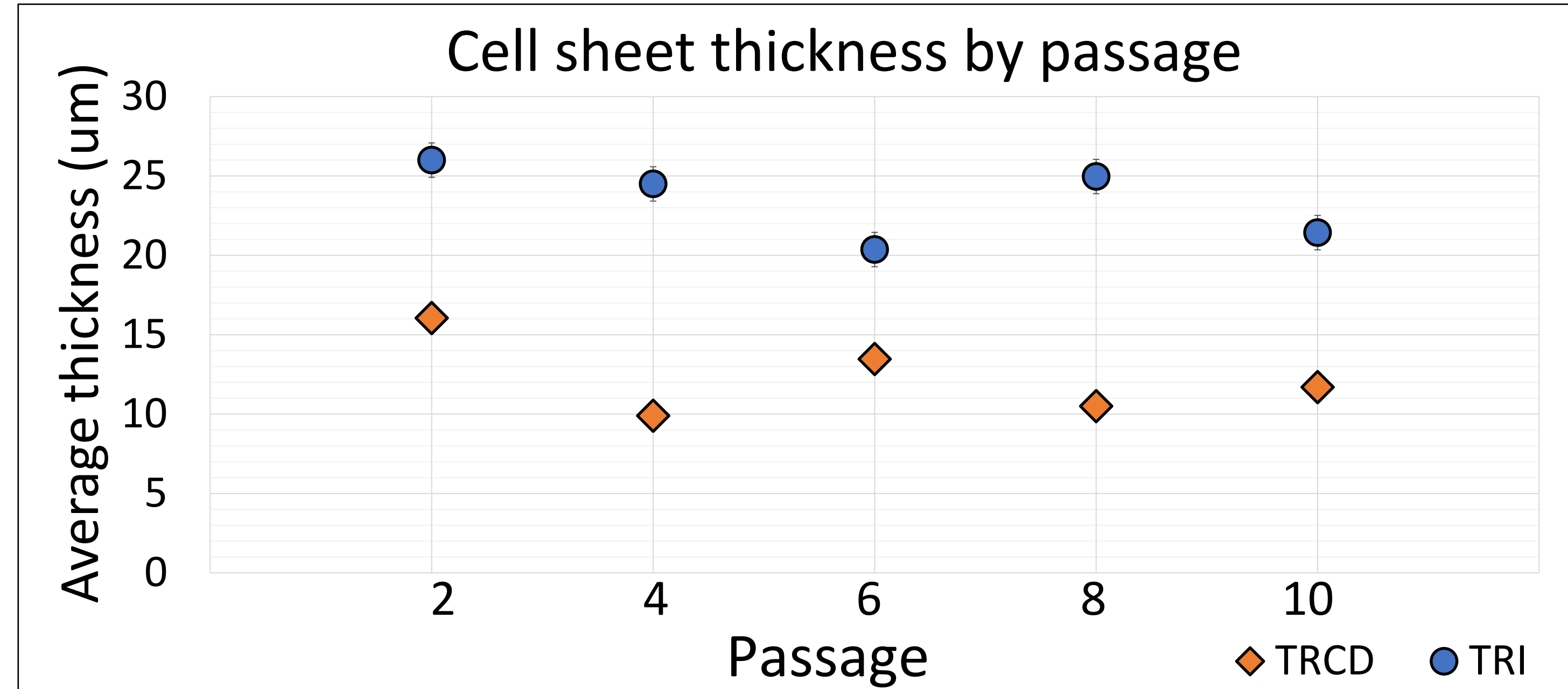
## Cell sheet contraction after harvest



### Figure 2: Cell sheet contraction data vs passage

TRCD and TRI sheets show substantial contraction differences. Peak contraction occurs at P6 for TRCD, and P8 for TRI sheets. TRCD sheets show greater contraction until P8

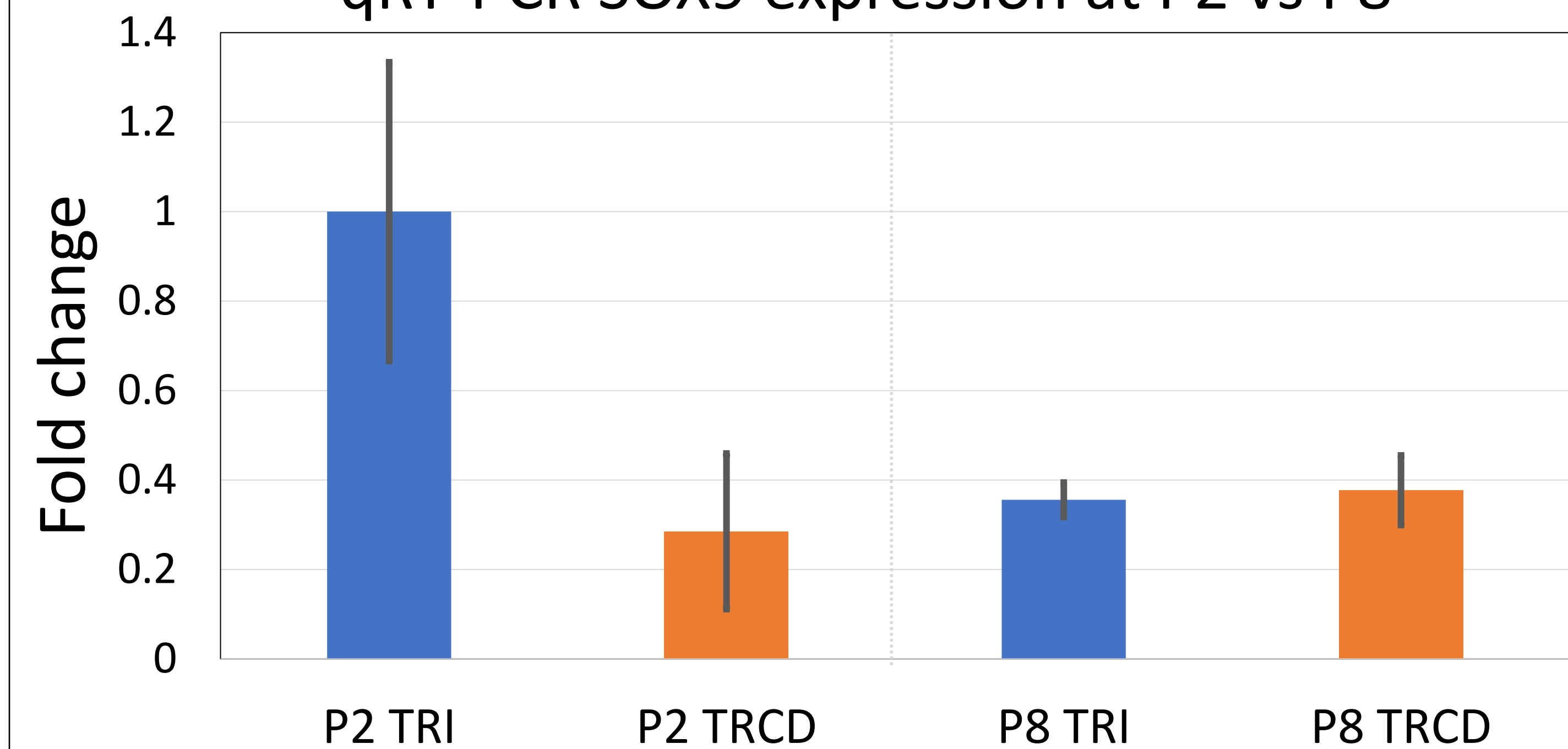
## Results



### Figure 3: Cell sheet thickness vs. passage

TRI sheets are consistently thicker vs. TRCD sheets at all passages.

## qRT-PCR SOX9 expression at P2 vs P8



### Figure 4: Sox9 gene expression at P2 and P8

P2 TRI PDC sheets exhibit the highest gene expression of chondrogenic transcription factor SOX9. However, at passage 8, SOX9 gene expression in the TRI group is similar to the TRCD group. SOX9 expression remains low across passage numbers in the TRCD group.

## Conclusions

TRI versus TRCD cultureware:

- Influences sheet thickness; TRCD thinner on average
- Influences contraction; TRI sheet contraction unaffected by passage
- Does not influence COL1, COL2, Saf-O staining
- Influences sheet thickness; TRI thicker in every passage
- Influences SOX9 expression, P2 TRI sheets highest expression

## Acknowledgements & References

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- Contact (AF): [u1038910@utah.edu](mailto:u1038910@utah.edu)
- Graphics composed using Biorender

[1] Thorp H et al. (2020) *Sci Rep.* 10.1038/s41598-020-77842-0

[2] Kondo M et al. (2021) *npj Regen Med.* 10.1038/s41536-021-00173-9

