Type V Collagen Regulates Nascent Matrix Templating and Cell-Mediated Reorganization in Embryonic Meniscus

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INTRODUCTION: The meniscus is essential for providing knee joint stabilization, load redistribution and energy dissipation functions, which are endowed by its specialized fibrocartilaginous extracellular matrix (ECM) [1]. Currently, there is a paucity of knowledge on the molecular and cellular mechanisms that drive the formation and maturation of the meniscus ECM. Our studies indicate that inner-to-outer regional specification and rapid matrix templating take place during early embryonic development prior to the onset of weight bearing [2, 3]. Furthermore, we show that collagen V regulates meniscal cell mechanosensing *in vivo* by modulating the integrity of meniscus pericellular matrix (PCM) [4], which is beyond the canonical function of collagen V in regulating collagen I fibrillogenesis [5]. This study aims to elucidate the roles of collagen V in mediating the meniscus matrix templating and reciprocal cell-matrix interactions using embryonic porcine meniscal cells, which will enable the translational applications of collagen V activities in meniscus regeneration.

METHODS: Meniscal cells were harvested from the knee joints of Yorkshire pigs at embryonic day E84. Knockdown of *Col5a1* was induced via 48-hour transfection of custom designed *Col5a1*-siRNA primers (Horizon Discovery), and assessed by qPCR and immunofluorescence (IF) staining at days 3 and 7. Additional IF was performed to assess focal adhesion formation (*Paxillin* [6]) and cell proliferation (*Ki-67* [7]) at timepoints. Cell-derived matrices (CDMs) were generated by culturing meniscal cells for 7 and 10 days following the knockdown. The resultant matrix architecture was assessed by NHS-ester IF staining for primary amines, followed by quantification of fibril alignment via CT-Fire. AFM-nanoindentation was applied to quantify the micromodulus of CDMs, following established protocols ($R \approx 5$ μm, $k \approx 0.03$ N/m, ≥ 15 positions from $N = 3$ replicates) [8]. Next, cell-mediated matrix contraction was assessed by measuring the contraction of meniscal cell-embedded collagen I gels at 2×10^5 cells/gel during an 8-day culture after knockdown. Finally, cells were embedded at a concentration of 5×10^4 cells/gel, and fibril reorganization at the cell-matrix interface was evaluated at day 7 via second harmonic generation (SHG). Two sample student's *t*-test was applied to detect differences in gene expression, fibril alignment, fibril density, and modulus at $\alpha = 0.05$.

RESULTS: Following transfection of *Col5a1*-siRNA, the expression of collagen V was significantly reduced at both transcriptional and protein levels at day 3, but recovered to the baseline level by day 7 (Fig. 1a,b). Despite this reduction, we did not notice appreciable changes in paxillin (Fig. 1c) or Ki-67 staining (data not shown) at either day 3 or 7, suggesting the temporal knockdown of collagen V did not have a pronounced effect on meniscal cell-substrate focal adhesion or cell proliferation. In contrast, the temporal collagen V knockdown showed substantial impacts on the formation of nascent matrices. At day 7, despite the recovery of collagen V expression and content, CDMs exhibited reduced volume fraction, degree of alignment and had a 28.6 ± 3.5% lower micromodulus (mean ± 95% CI) compared to the control (Fig. 2). At day 10, although CDMs for both groups showed similar volume fraction, likely due to the abundance of deposited ECM (Fig. 2), the CDM formed following *Col5a1*-siRNA transfection still showed 36.00 ± 2.9% modulus reduction, illustrating impaired ECM formation. In alignment with the impaired matrix formation, *Col5a1*-siRNA transfection also resulted in reduced contraction of ring-shaped collagen I gels over an 8-day culture (Fig. 3a,b), evidencing disrupted cell-mediated matrix force transmission. Meanwhile, *Col5a1*-siRNA transfected cells showed reduced intensity and alignment of collagen fibers at the cell-matrix interface, as assessed by SHG (Fig. 3c). This suggested that temporal loss of collagen V impaired cell-mediated fibril reorganization in the pericellular domain, corroborating the localization of collagen V in the pericellular domain of meniscal cells at the early stage of matrix formation (Fig. 1).

DISCUSSION: This study highlights that collagen V is essential for the nascent matrix templating and cell-mediated matrix reorganization. Given that siRNA transfection only leads to temporary collagen V knockdown for the initial 3-5 days (Fig. 1a,b), the pronounced impairment in CDM modulus (Fig. 2c), cell-mediated contraction (Fig. 3a,b) and pericellular matrix fibril reorganization (Fig. 3c) at longer durations underscores the necessity of collagen V in the proper initial matrix templating. Loss of collagen V leads to disrupted assembly of the collagen I fibril template, which in turn, perturbs the cell-fibril interactions at the cell-matrix interface and thus reduces the meniscal cell capacity to reorganize the nascent matrix structure. Therefore, our observation that the later recovery of collagen V content does compensate for its initial reduction suggests that the initial templating is a crucial, rapid phase of matrix establishment mediated by collagen V. Furthermore, contrasting the impaired matrix reorganization versus lack of changes in paxillin staining suggests that collagen V is more likely to directly regulate the initial matrix fibril architecture, rather than cell-matrix adhesion. These results corroborate our earlier findings showing that collagen V is highly expressed during embryonic development, and joint-specific *Col5a1* deletion results in impaired meniscus development and premature OA in mice [9]. Building on these findings, our ongoing studies aim to uncover how collagen V regulates the nanostructure of initial matrix templates and integrin profile [10] in embryonic cells, and delineate its impact on embryonic versus adult meniscal cells and inner versus outer zone cells. We expect these findings to enable us to improve meniscus repair by modulating initial collagen V-mediated matrix assembly.

SIGNIFICANCE/CLINICAL RELEVANCE: This study highlights the crucial role of collagen V in the initial matrix templating and cell-mediated reorganization at cell-matrix interface, supporting collagen V as a promising therapeutic target for improving meniscal regeneration.

REFERENCES: [1] Makris+ 2011. [2] Kwok+ 2023. [3] Tsinman+ 2021. [4] Wang+ 2025. [5] Wenstrup+ 2004 [6] López-Colomé+ 2017 [7] Gerdes+ 1983. [8] Li+ 2017 [9] Kwok+ 2025. [10] Zoppi+ 2004.

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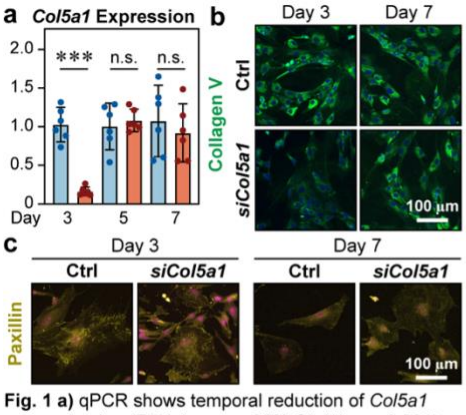

Fig. 1 a) qPCR shows temporal reduction of *Col5a1* expression by siRNA (mean ± 95% CI, ***: $p < 0.001$). **b,c)** IF of collagen V (green, blue: DAPI) and c) Paxillin (yellow, magenta: DAPI) at days 3 and 7.

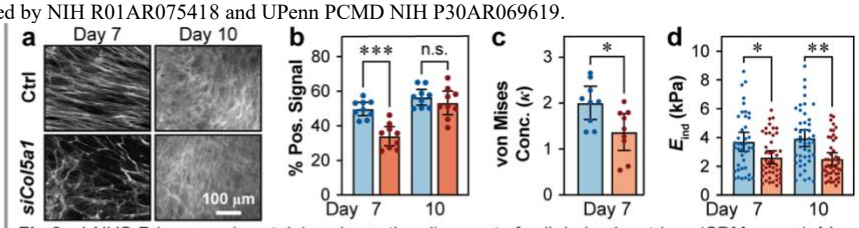

Fig 2. a) NHS-Primary amine staining shows the alignment of cell derived matrices (CDMs, gray). **b)** Matrix density represented by signal fraction (mean ± 95% CI, ***: $p < 0.001$). **c)** CDM fibril alignment, as measured by von Mises concentration, κ, at day 7 (mean ± 95% CI, *: $p < 0.05$). **d)** Indentation Modulus, E_{ind} of CDMs at days 7 and 10 (mean ± 95% CI, *: $p < 0.05$, **: $p < 0.01$).

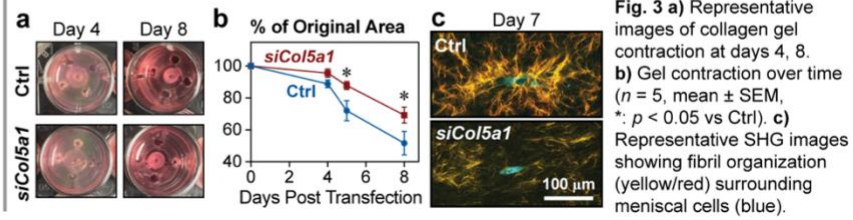

Fig. 3 a) Representative images of collagen gel contraction at days 4, 8. **b)** Gel contraction over time ($n = 5$, mean ± SEM, *: $p < 0.05$ vs Ctrl). **c)** Representative SHG images showing fibril organization (yellow/red) surrounding meniscal cells (blue).