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ESI - Establishment of a human three-dimensional chip-based chondro-synovial co-culture joint model for reciprocal cross talk studies in arthritis research

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Content: ESI Table 1-3, ESI Fig. 1-7

Tables

ESI Table 1: Overview over fluorescent staining panels for flow cytometry analysis.

Staining panel 1	***Staining panel 2***
CD90-PerCP-Cy5.5	CD45-PECy7
CD34-FITC	CD3-APC-Cy7
Podoplanin-PE	CD4-FITC
CD45-PECy7	CD8-PE
	CD14-PerCP-Cy5.5
	CD19-APC

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ESI Table 2: Primer pair sequences for qPCR.

Gene	*Forward (5´- 3´)*	*Reverse (5´- 3´)*
Col1a1	CACTGGTGATGCTGGTCCTG	CGAGGTCACGGTCACGAAC
Col2a1	AGCTGGCAACCTCAAGAAGG	CGATAACAGTCTTGCCCCACTT
ACAN	GGCGAGCACTGTAACATAGACAT	GCATGTGAAAGAGTCGATGGC

ESI Table 3: Staining and imaging data for different cell tracker dyes from Thermo Fisher.

Cell Tracker™	*Volume (µl/ml)*	*incubation time I (min)*	*incubation time II (min)*	*Ex. (nm)*	*Em. (nm)*
Green CMFDA	2.5	30	30	492	517
Orange CMRA/CMTPX	2.5	30	30	548	576
Deep Red	2	15	-	630	660

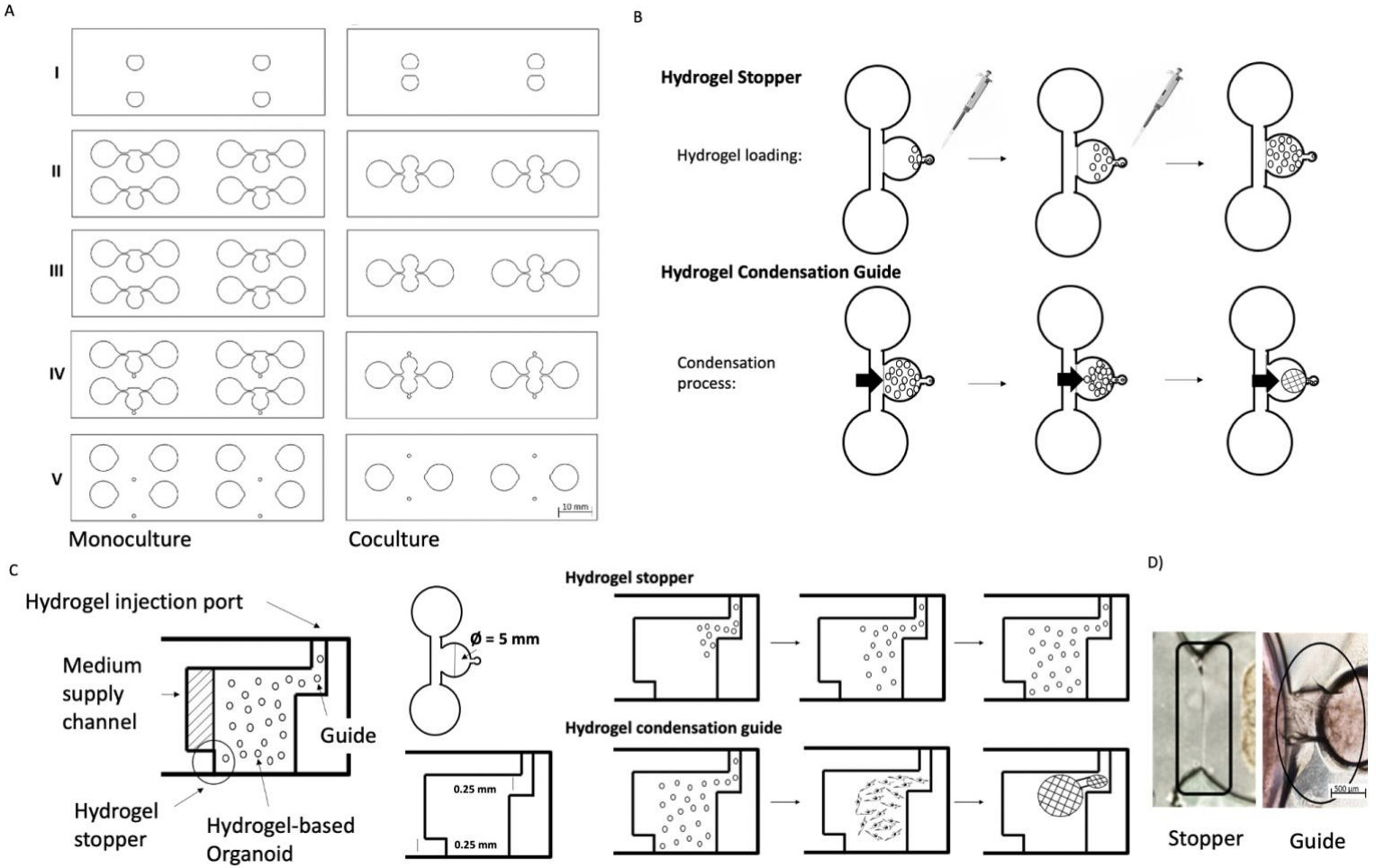
ESI Table 4: Positioning precision analysis of the organoid biochip system.

	Distance to anchor in mm mean ± SD (% RSD)	*Distance from center in µm mean ± SD*
Overall d1 (n = 32)	2223±147 (6.6)	276±147
Overall d7 (n=32)	2148±257 (11.9)	351±242
Overall d21 (n=32)	2002±648 (32)	497±399

ESI Table 5: Morphometric parameters of synovial organoids triplicates produced from three RA patient tissue sample.

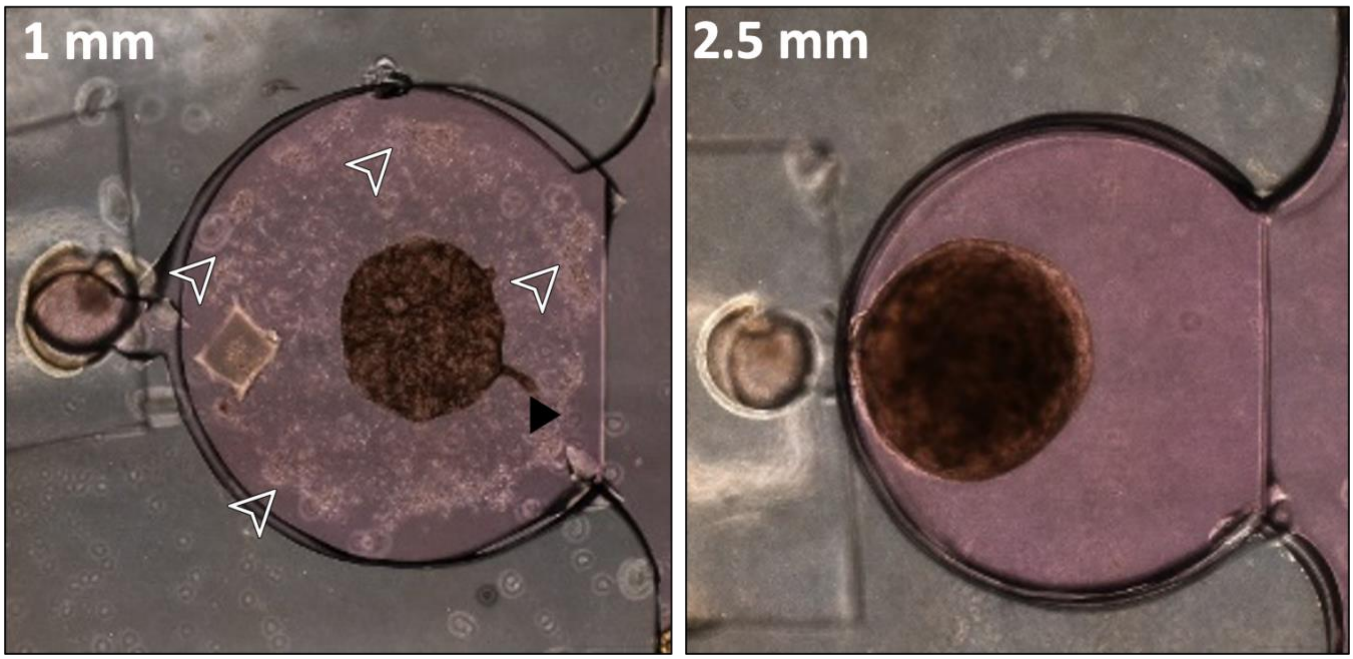
Tissue origin	*Solidity (±SD)*	*Perimeter (in mm ±SD)*	*Roundness (±SD)*
RA (n=3)	**0.97±0.03**	**8.93±0.89**	**0.85±0.05**
Best theoretical values	1	N/A	1

Figures

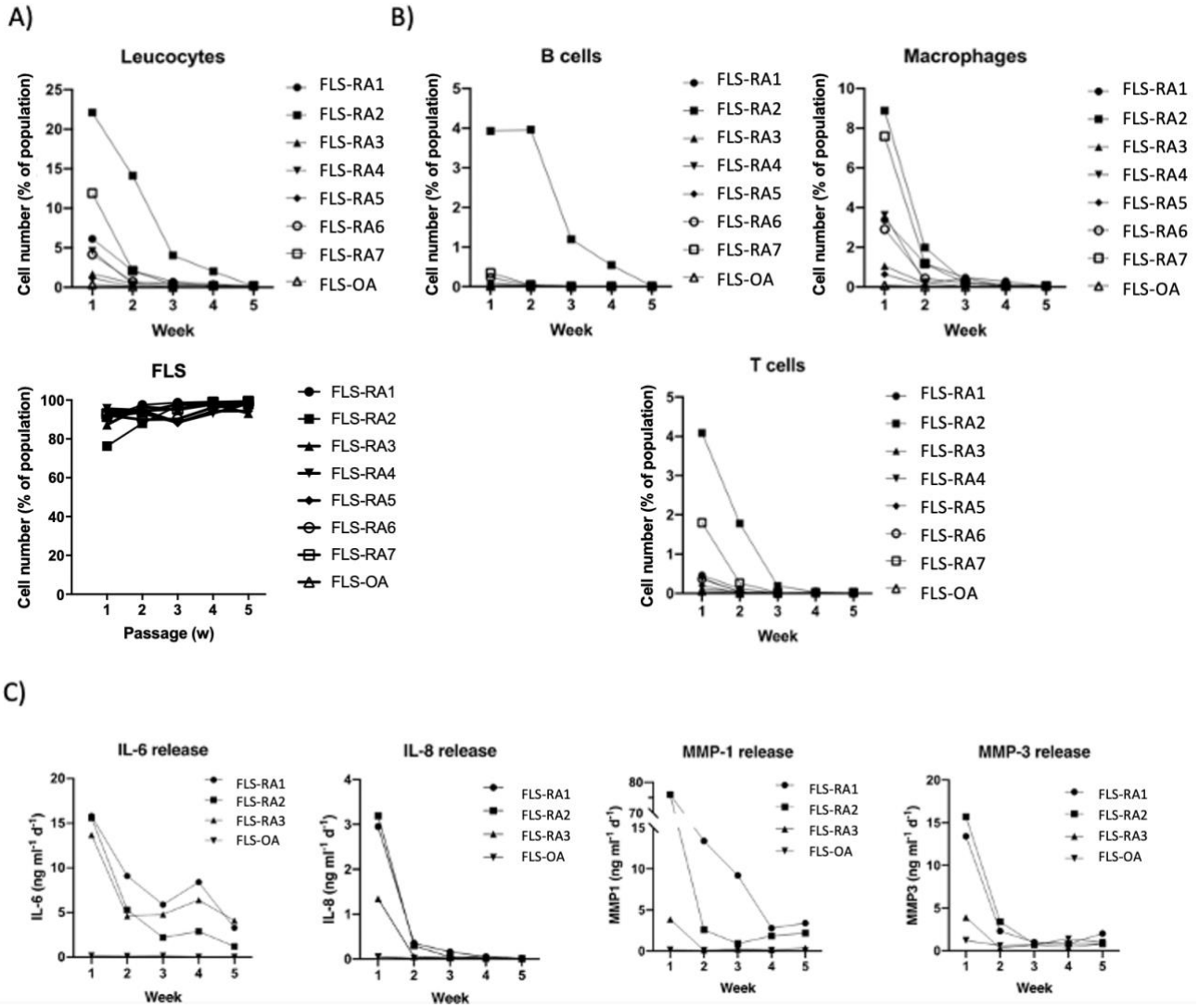


ESI Fig. 1. A) CAD design of the individual structured chip layers from bottom (layer I) to top (layer V) for organoid monocultures (left panel) or cocultures (right panel). B,C) Top and side-view of the hydrogel stopper and condensation structures within an individual organoid mono-culture unit during seeding (top row) and organoid formation (bottom row) with D) representative microscopic images.

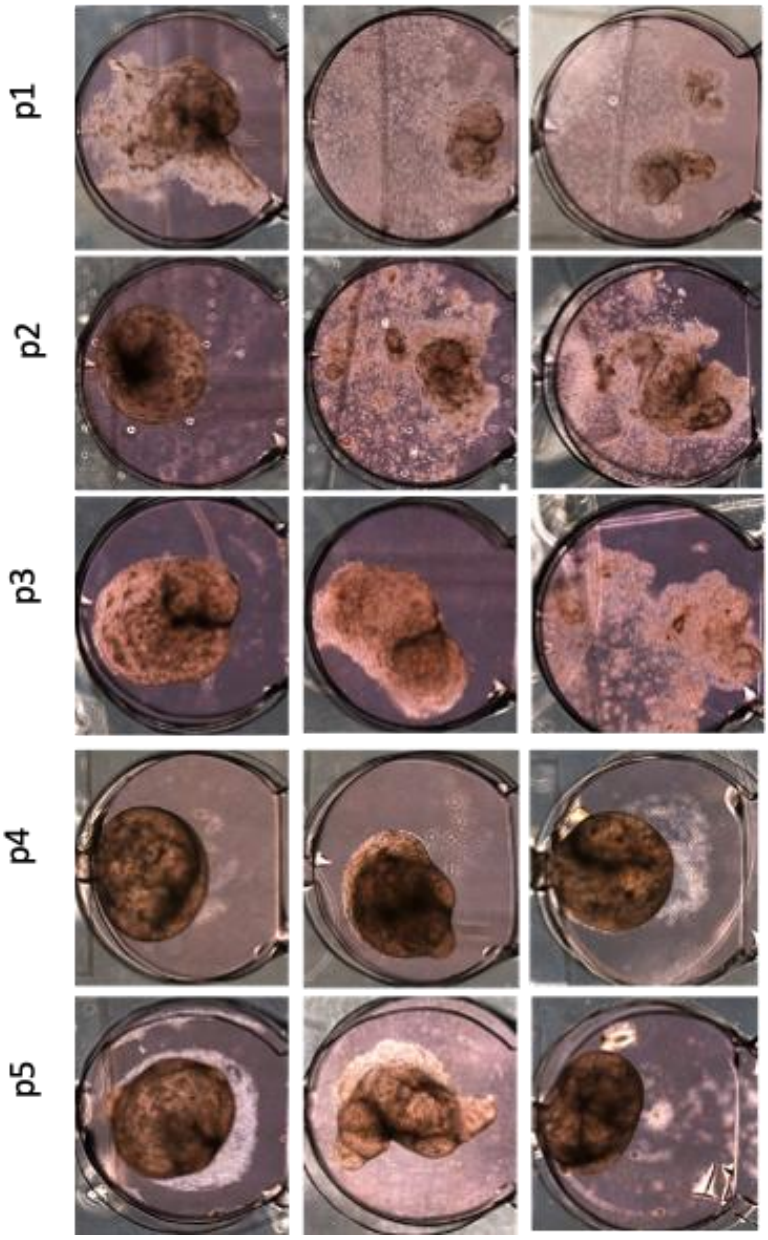
ARTICLE



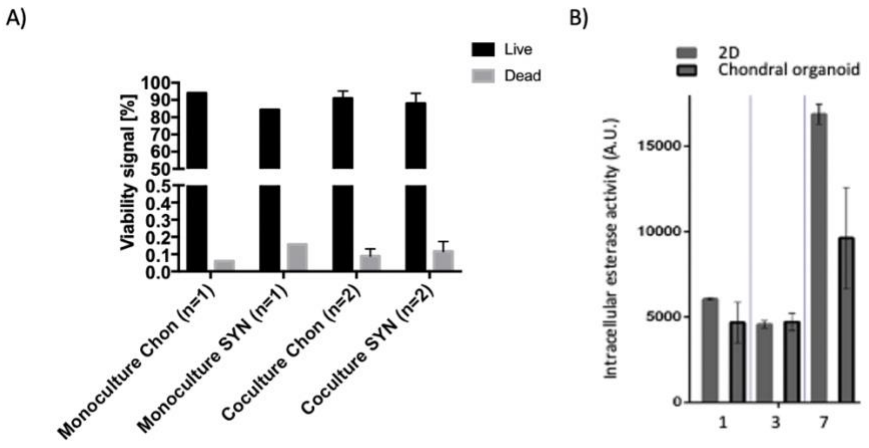
ESI Fig. 2 Fragmentation (black arrowhead) and cell dispersion (white arrowheads) artifacts during FLS organoid maturation in hydrogel chambers of 1mm (left) compared to 2.5 mm (right) height after 28 days post-seeding.



ESI Fig. 3 Characterization of patient-derived cell populations using flow cytometric and cytokine analysis. A,B) Cell distribution for patient-derived fibroblast-like synoviocytes throughout five passages of subcultivation. (RA; Rheumatoid arthritis. OA; osteoarthritis specimen as control. Data is expressed as % of total gated cell population for n=8 individual patient-derived samples. See ESI Table 1 for information of staining panels.) C) Secretion of proinflammatory cytokines and degradative enzymes throughout five subcultivation passages in 2D culture using Luminex multiplex technology. (RA; Rheumatoid arthritis. OA; osteoarthritis. n=4 individual patient-derived synovial samples)



ESI Fig. 4 Representative microscopic images of cultured synovial organoids of different FLS passages up to 5 weeks at day 28 post-seeding.



ESI Fig. 5 A) Calcein-AM/Ethidium bromide cell exclusion assay quantification of chondral and synovial mono- in comparsion to cocultures. B) Esterase activity analysis of human articular chondrocytes in 2D and chip-based organoid culture over a week of culture using Presto Blue resazurin to resarrufin conversion capacity. (Data is expressed as mean ± sdev for n=3 organoids of n=1 donors)

Author contributions

AF, EIR, IOC, and SSch: experiments and data analysis; editing and reviewing – original draft. FS, JH and RW,: surgical preparation of primary patient tissue; reviewing – original draft.. SS, BB, WH and HR: assistance with fibrin-based organoids, editing and reviewing – original draft. HPK: reviewing and editing – original draft. RAB: experiments; data analysis; reviewing and editing – original draft. MR: supervision; experiments; data analysis; methodology; supervision, writing – original draft, review and editing. ST: writing – original draft, review and editing. PE: supervision; writing, review and editing – original draft; funding acquisition.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

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