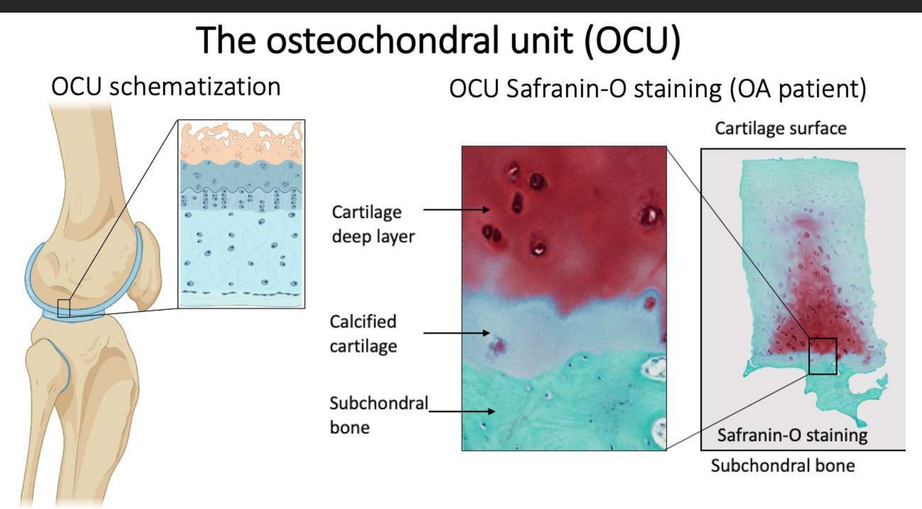
Modelling the Joint-on-a-chip: a mechanically active microfluidic system to engineer 3D multi-layer osteochondral tissues and investigate osteoarthritis processes to a single cell level

Andrea Mainardi[1,2], Paola Occhetta, [2] Martin Ehrbar[3], Ivan Martin[1], Marco Rasponi[2], Andrea Barbero[1]

[1]Department of Biomedicine, University Hospital Basel, Basel, Switzerland-Department of Electronics, Informatics and Bioengineering, Politecnico di Milano, Milano, Italy-Department of Biomedical Engineering, University of Basel, Allschwil, Switzerland [2]Department of Biomedicine, University Hospital Basel, Basel, Switzerland-Department of Biomedical Engineering, University of Basel, Allschwil, Switzerland [3]Department of Electronics, Informatics and Bioengineering, Politecnico di Milano, Milano, Italy [4]Department of Obstetrics, University Hospital Zurich, Zurich, Switzerland [5]Medical Additive Manufacturing Research Group (Swiss MAM), Department of Biomedical Engineering, University of Basel, Basel, Switzerland

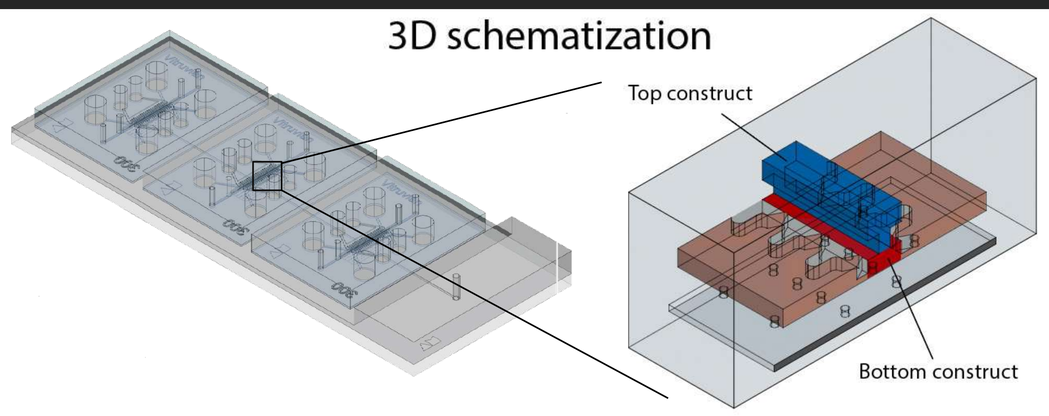
Background

Osteoarthritis (OA), the most prevalent musculoskeletal disease, is a degenerative disorder affecting mainly load bearing joints. Correlated with mechanical dysregulation, OA leads to cartilage degeneration, to its vascular invasion, and to subchondral bone alterations[1]. No disease modifying OA treatment is presently available. This is partly because of the absence of representative *in vitro* models that incorporate the subchondral layers permitting to dissect the pathological triggers. In this work we propose a new Organ-on-Chip (OoC) concept that allows for the easy generation of stacked, directly interfaced, multi-tissue, 3D microconstructs. Leveraging on this technology we pioneer a new mechanically active Joint-on-a-chip comprehensive of both a cartilage and a subchondral compartment.



Methods

Building upon our Cartilage-on-chip model[2] we used multi-layer photolithography to engineer a mechanically active device with a valve system that pins a suspended fluid so that a multi-layer construct can be realized with two subsequent injections. A co-culture joint model was realized with chondrocytes (hACs) for the cartilage layer and mesenchymal stromal cells (MSCs) for the mineralized subchondral compartment. GFP expressing hACs were used to evaluate the effect of the compression in the different layers through immunofluorescence and RT-qPCR. Cells were embedded in an enzymatically formed, metalloproteinase (MMP) sensitive PEG hydrogel[3].



Results

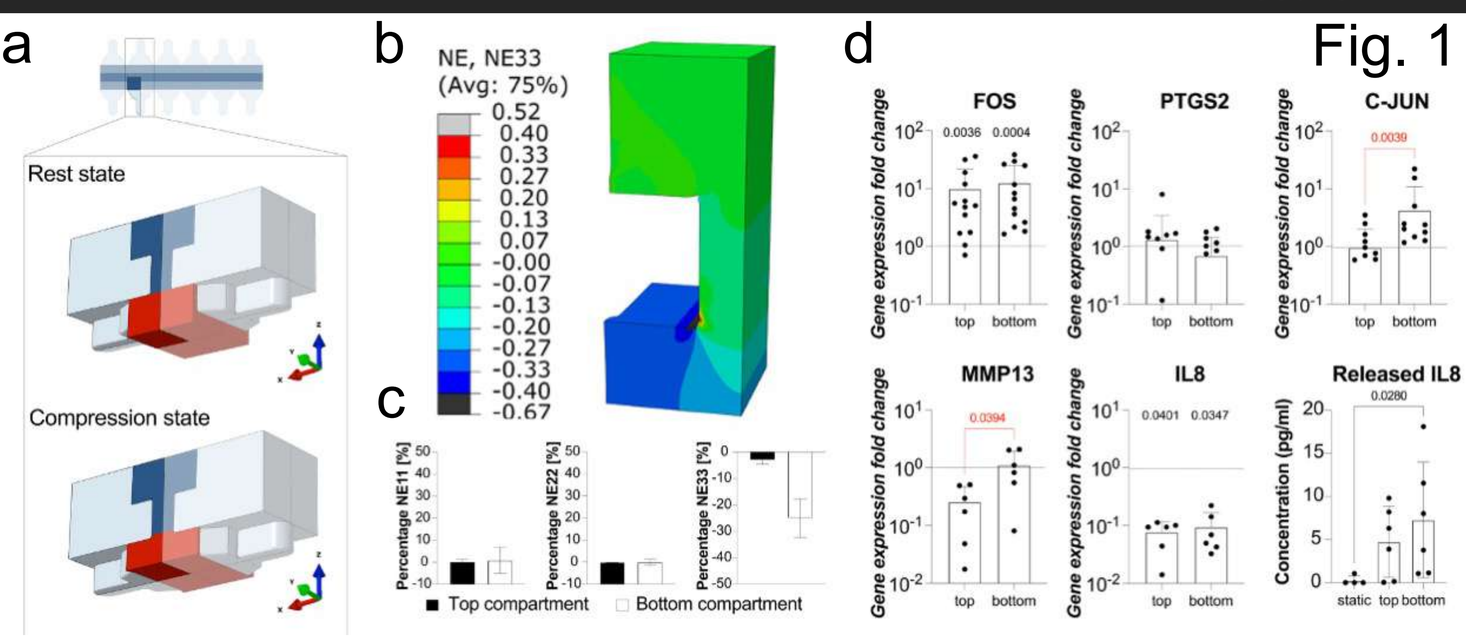


Fig. 1

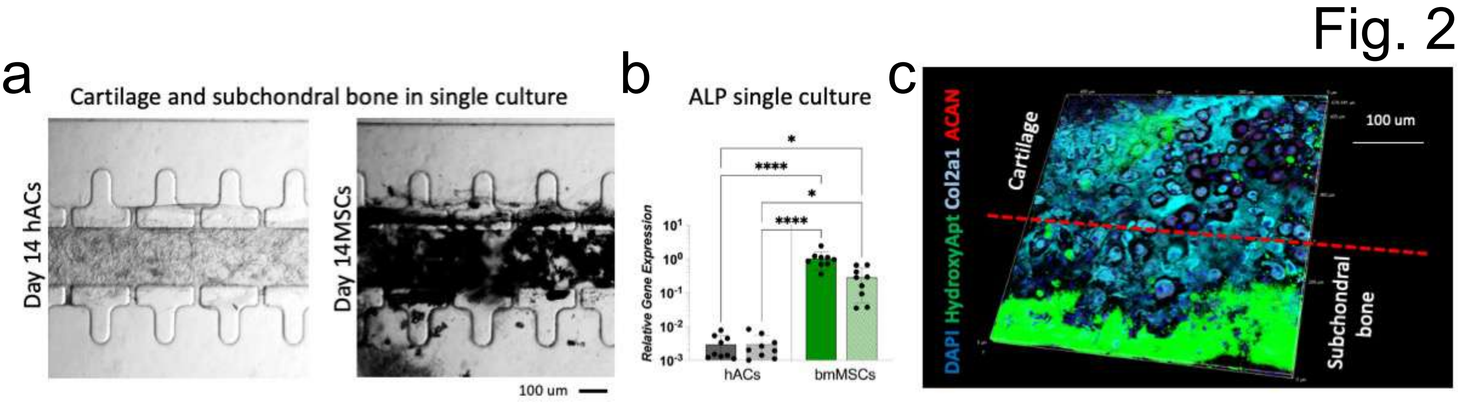


Fig. 2

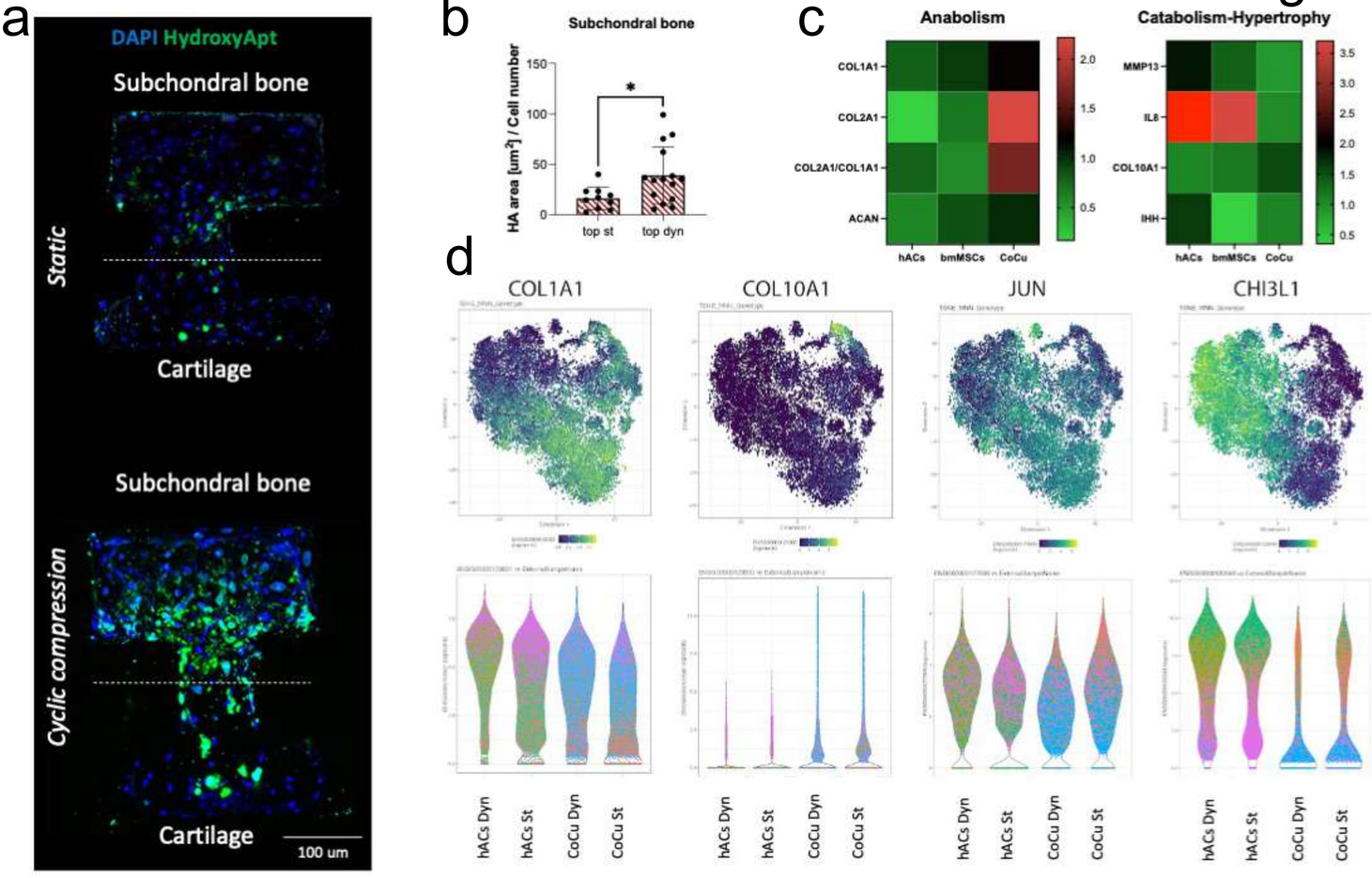


Fig. 3

The device was validated demonstrating its production feasibility and the functioning of the vertical valve concept. Computational simulations adopted to estimate the complex strain field revealed a strain gradient ranging from 30% to 0.3% reminiscent of that of *in vivo* OA joints (Fig. 1a-c).
Furthermore, it was demonstrated that the microconstructs hosted in the two device compartments are truly exposed to distinct compression levels as indicated by the differences of expression of the mechanotransduction related genes FOS, C-JUN, and MMP13, and by the release of the OA-related inflammatory cytokine IL8 (Fig. 1d).
Addition of beta-glycerophosphate to the chondrogenic medium does not affect hACs behavior that differentiate in cartilage-like constructs positive for ACAN and COL2A1, while it drives bmMSCs to form hydroxyapatite (HA) rich constructs highly expressing bone markers genes such as ALP (Fig. 2a, b). Notably, in co-culture, these tissues form a direct interface with osteochondral-like features (Fig. 2c).
Hyperphysiological compression (HPC) on the osteochondral tissues leads to an increase in HA deposition (Fig. 3a, b); moreover, osteochondral constructs respond differently from single cultures to mechanical compression in terms of various genes involved in OA pathogenesis (e.g. COL2A1, IHH, DKK1) demonstrating a cross talk between cartilaginous and subchondral compartments (Fig. 3c).
We further demonstrated at a single cell level that the osteochondral model is effective in maintaining various hACs subpopulations and possesses an early-OA-like genetic signature (Fig. 3d).

Discussions and conclusions

The introduction of this new vertical, fluid pinning OoC concept paves the way for more representative Joint models where phenomena such as cartilage degradation and subchondral bone sclerosis can be dissected *in vitro* and innovative OA drug targets and regenerative therapies tested in a human relevant context .

Additional Information

References

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Contacts: andrea.mainardi@unibas.ch