Efficacy assessment of the novel combined anti-OA treatment SYN321 in an advanced mechanically active osteoarthritis-on-chip model

Palma C.[1], Piazza S.[2], Visone R.[2], Bermejo Gómez A.[3], Rasponi M.[1], Occhetta P.[1,2]

[1]Department of Electronic, Information and Bioengineering, Politecnico di Milano, Piazza Leonardo Da Vinci 32, 20133, Milano, Italy; [2]BiomimX S.r.l., Viale Decumano 41, MIND - Milano Innovation District, 20157 Milano, Italy; [3]Synartro AB, Vasagatan 28, 111 20, Stockholm, Sweden

Intra-articular injection of nonsteroidal anti-inflammatory drugs (NSAIDs) to relieve osteoarthritis (OA) pain and inflammation is challenging due to rapid clearance[1].

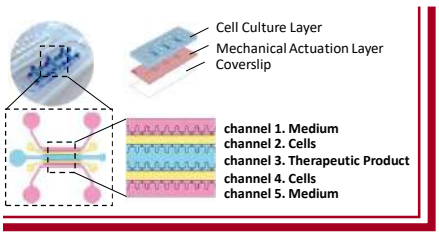
Thus, focus is on developing **polymer-drug conjugates** able to guarantee **NSAID sustained release[2].**

In this scenario, **organs-on-chip** provide an innovative solution to unravel mechanisms of such combined therapeutic products in physiologically relevant in vitro models.

AIM OF THE STUDY

Here, we developed a **novel microfluidic platform aimed at recapitulating OA-like 3D cartilage microtissues (namely uKnee model**), that offers the possibility to **test injectable therapeutic formulations**. The platform was qualified with **SYN321, a novel intra-articular drug candidate based on diclofenac linked to a modified sodium hyaluronate (NaHa) backbone.**

Design of the uBeat® platform

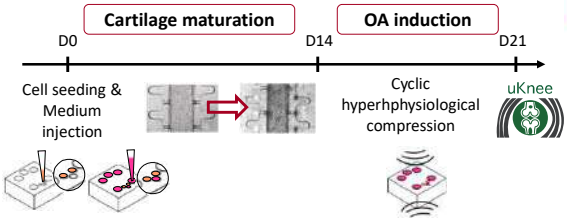


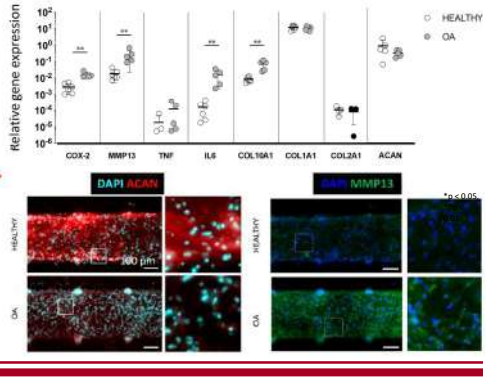
The microfluidic platform comprises three cell culture chambers, each composed by **5 channels**, i.e. two channels delimited by rows of T-shaped hanging posts conceived to host **3D cartilage micro-constructs**, a central channel for **therapeutic product injection**, and two outermost channels for medium supply.

The presence of an actuation layer allows to apply a hyperphysiological compression (HPC)[3] to the constructs by exploiting the **uBeat® technology**.

Generation of the uKnee model

Human articular chondrocytes (hACs) embedded in fibrin gel were cultured in the device for two weeks in static conditions in **chondrogenic medium**. A **one-week cyclic HPC** was then applied to the cartilage micro-constructs to to induce a shift in cartilage homeostasis towards catabolism and inflammation, generating the uKnee model.



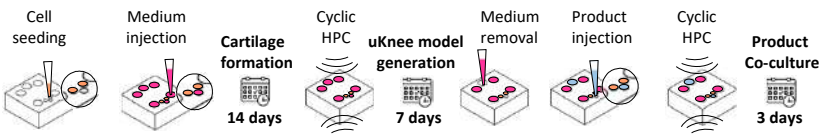


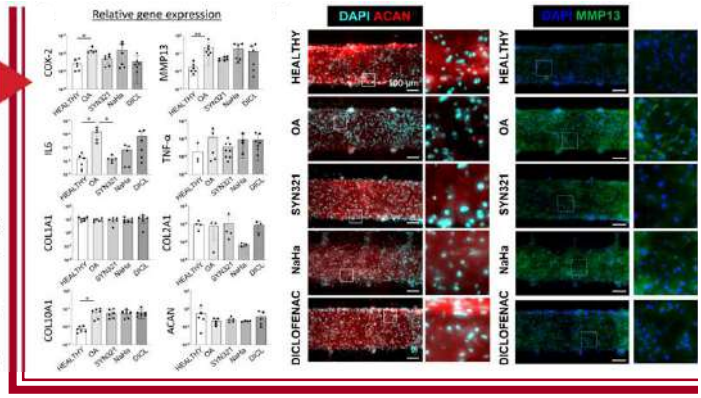
- A shift towards an OA phenotype was triggered due to HPC, as demonstrated by a **significant increase in the expression of *MMP13* and pro-inflammatory genes** (i.e. *COX-2* and *IL6*), as compared to static healthy controls.
- **Up-regulation of *COL10A1***, which is correlated to the onset of a hypertrophic cartilage phenotype, was also detected.
- Immunofluorescence staining showed a **reduced aggrecan expression** in the ECM of OA samples to healthy controls, whereas MMP13 was highly expressed.

Qualification of the uBeat® platform with SYN321

SYN321[4] is a **novel therapeutic drug candidate** for intraarticular injections, consisting of **diclofenac bounded to a NaHA backbone as the active ingredient**. These two moieties are conjugated through a **spacer containing ester functionalities**: the *in vivo* hydrolysis of the ester bonds of the molecule in the synovial fluid is expected to guarantee a slow release of diclofenac.

uKnee model was exploited to investigate SYN321 efficacy and unravel SYN321 effect at a cellular level. In detail, SYN321 was injected in the central channel of the device after uKnee model generation and its effect on the constructs was assessed after three days of treatment. SYN321 effects on the uKnee model were compared to the administration of NaHA and diclofenac only.





- SYN321 treatment exhibited an **anti-inflammatory effect in the OA cartilage-on-chip model,** decreasing the expression of *TNF-α, COX-2* and *IL-6*, as compared to OA controls
- The **expression of *IL6* in SYN321-treated samples was significantly reduced** and it was comparable with healthy condition expression level. The downregulation of these pro-inflammatory genes was less marked in the positive controls (i.e., NaHA and diclofenac).
- SYN321 played a role in **reducing matrix degradation** both at gene and protein level, **reducing *MMP13* expression** as compared to the OA control.
- **Aggrecan matrix deposition**, a prominent component of the extracellular matrix in growth plate cartilage was higher as compared to OA control.

CONCLUSIONS

The here presented microfluidic platform enables for the first time to **test the effect of injectable therapeutic products on an OA cartilage model (namely uKnee), generated upon hyperphysiological mechanical stimulation**. In particular, the therapeutic formulation can be injected in the platform, cultured in direct contact with 3D OA cartilage microtissues and mechanically stimulated together with them, **resembling the *in vivo* environment**.

The platform was successfully qualified with **SYN321**, a novel drug candidate based on modified NaHA bounded to a diclofenac derivative, that was demonstrated to have a beneficial effect in reducing OA traits in vitro.

REFERENCES

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cecilia.palma@polimi.it
paola.occhetta@biomimx.com
antonio.bermejo@synartro.com