PROJECT DELIVERABLE REPORT

Project Title:

Advanced personalised, multi-scale computer models preventing osteoarthritis
SC1-PM-17-2017 - Personalised computer models and in-silico systems for well-being

Deliverable number	**D8.2**
Deliverable title	OA in vitro models
Submission month of deliverable	M16
Issuing partner	RIMED
Contributing partners	CERTH, LEITAT
Dissemination Level (PU/PP/RE/CO):	PU
Project coordinator	University of Nicosia (UNIC)
Tel:	**+357 22 841 528**
Fax:	**+357 22 357481**
Email:	**felekkis.k@unic.ac.cy& giannaki.c@unic.ac.cy**
Project web site address	www.oactive.eu

Revision History

Version	Date	Responsible	Description/Remarks/Reason for changes
1.0	30/04/2019	RIMED	First draft
1.1	02/05/2019	RIMED	Revision first draft
1.2	12/05/2019	RIMED	Final version

Table of Contents

1 Summary

Ri.MED's osteochondral bioreactor is positioned between currently employed *in vitro* single tissue models and *in vivo* animal models. Our device can exploit a tissue engineering approach using cells from humans, either differentiated cells (i.e., chondrocytes and osteoblasts) or adult stem/progenitor cells (e.g., mesenchymal stem cells, MSCs). The tissue engineering approach allows for a higher throughput than possible with native human tissues, thus allowing for a screening approach that could be relevant for therapy development and human health. Overall, the osteochondral bioreactor allows to separately control media input and analysis of media output for the chondral and osseous compartment to develop engineered OC constructs and study their response to stress or therapies. Moreover, this approach will allow the study of processes of OA development, homeostasis, and pathogenesis in response to environmental stresses, toxins or treatments, using either native osteochondral plugs or engineered osteochondral constructs. The osteochondral bioreactor has already been validated as an effective tool to generate engineered osteochondral constructs from a common adult mesenchymal stem cell (MSC) source, as well as to assess the effect of inflammation on engineered bone and cartilage and identify communication between the two compartments.

2 Introduction

A first osteoarthritis *in vitro* model was developed based on native porcine osteochondral tissues. The inflammatory environment of osteoarthritis was simulated using chemokine inducers. Specifically, we tested the response to two pro-inflammatory cytokine such as IL-1β and TNF-α. The results for the induced OA model will be compared to those obtained in Task 8.1 for native OA tissues to assess which OA induction method better reproduces *in vitro* the *in vivo* disease phenotype. Furthermore, we realized two *in vitro* osteochondral model based on human MSCs engineered constructs, to be employed as models for native osteochondral units to study the cellular response to OA inducing stimuli. Using an engineered construct approach, it will be possible to obtain a higher number of identical human cells constructs allowing for higher throughput and to build a library of cellular response profiles.

3 OA in vitro models

3.1 OA native model

Our experimental design was based on the realization of a native OA model, which is then highly biomimetic. In fact, native tissue shows stronger cell-matrix and cell-cell interactions, possess more realistic molecular transport dynamics and is characterized by a more natural gene expression. We realized our OA model based on native tissue using healthy porcine osteochondral plugs due to the limited availability of human donor tissue, especially not derived from OA joints. More in detail, we harvested osteochondral plugs using the techniques developed within the experimental activities included in deliverable 8.1. We collected tissue from knee joints of juvenile pigs and cultured within bioreactors using osteo- and chondro-specific media. The inflammatory response was induced by supplementing pro-inflammatory cytokines such as TNF-α or IL-1β at 1.0 and 10.0 ng/ml. The negative control was cultured with only osteo- and chondro-specific media. Following treatment, we measured the relative changes in gene expression for three anabolic chondrogenic genes, aggrecan (ACAN), SOX-9 (SOX9), and collagen II (COL2A1), using quantitative real-time polymerase chain reaction (qRT-PCR), and fold changes of mRNA expression were analyzed relative to day 0. We noticed a more relevant inflammatory response in the osteochondral plugs cultured with IL-1β supplement. Furthermore, the stimulation of tissue with IL-1β tends to downregulate the expression of anabolic chondrogenic genes. At 10.0 ng/mL, ACAN is significantly downregulated compared to the negative control ($p<0.05$), and COL2A1 is significantly downregulated compared to the negative control

($p<0.01$) and compared to 1.0 ng/mL ($p<0.05$) (Figure 1).

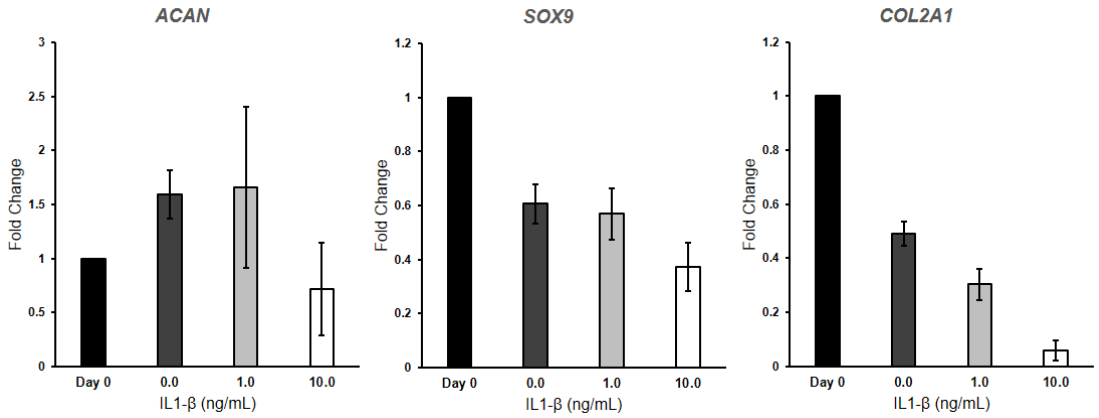

Figure 1: Expression of ACAN, SOX9, and COL2A1 mRNA following stimulation of osteochondral plugs with IL-1β

We also measured the relative changes in gene expression for four anabolic osteogenic genes: osteocalcin (OC), osteopontin (OPN), collagen I (COL1), and bone sialoprotein (BSP), however we did not found results statistically significant. TNF-α was ineffective in inducing a catabolic gene expression profile at the concentrations tested.

3.2 RECAPP scaffold

To reproduce the bone structure, we tested two different highly porous materials. The first one was made of an inorganic proprietary mixture of calcium and phosphate (ReCaPP), main components of the osseous inorganic extracellular matrix *in vivo*. More in detail, the ReCaPP powder is composed of α-$Ca_3(PO_4)_2$ (80% w/w), $CaSO_4$ (10% w/w), and $CaCO_3$ (10% w/w), meanwhile a nano-CaP solution containing 0.2 M $CaCl_2$ was used as a mixing liquid. The ReCaPP is formulated as a cement, and this feature allowed us to produce compact and resistant plugs suitable to support cell

adhesion and proliferation in a bone-biomimetic environment. The microporous scaffold was realized by compression of the powder mixed with a porogen of paraffin microspheres with controlled diameter (150 – 200 nm), and was shaped in a stiff cylinder that fit well within the inner insert of the bioreactors. We optimized the operative conditions adjusting systematically the ratio of microsphere added to the ReCaPP powder to realize a scaffold with an adequate porosity, maintaining at the same time the mechanical resistance of the construct (Figure 2).

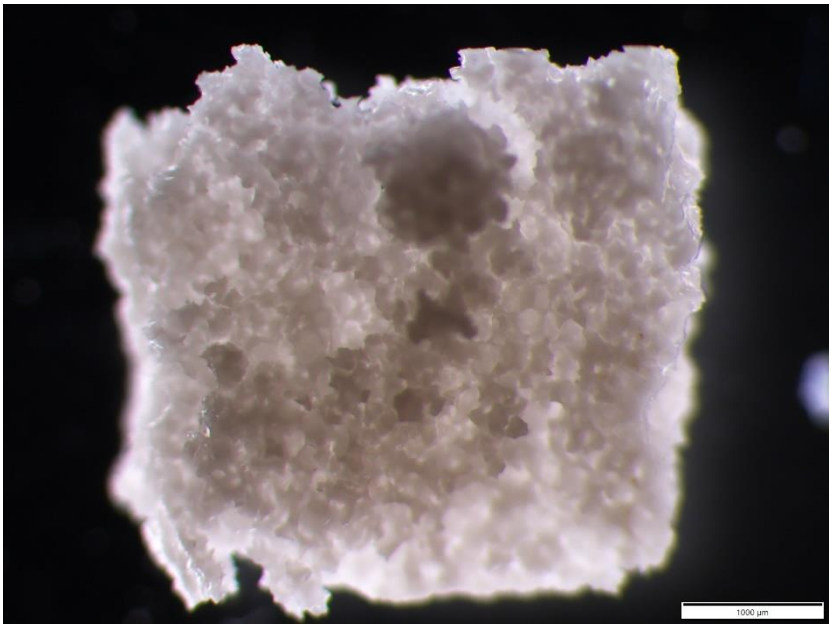

Figure 2: Transversal section of a ReCaPP/Paraffin microporous scaffold

The cellularization of the scaffold was achieved by seeding MSCs before insertion in the bioreactors with a specific seeding procedure tailored on the scaffold structure. We performed different seeding tests by dispensing a cellular suspension from the top, the bottom, the side, or from all sides. Furthermore, we noticed an irregular cell distribution, with a higher concentration in the outer portion of the scaffold and a low distribution in the inner portion (Figure 3).

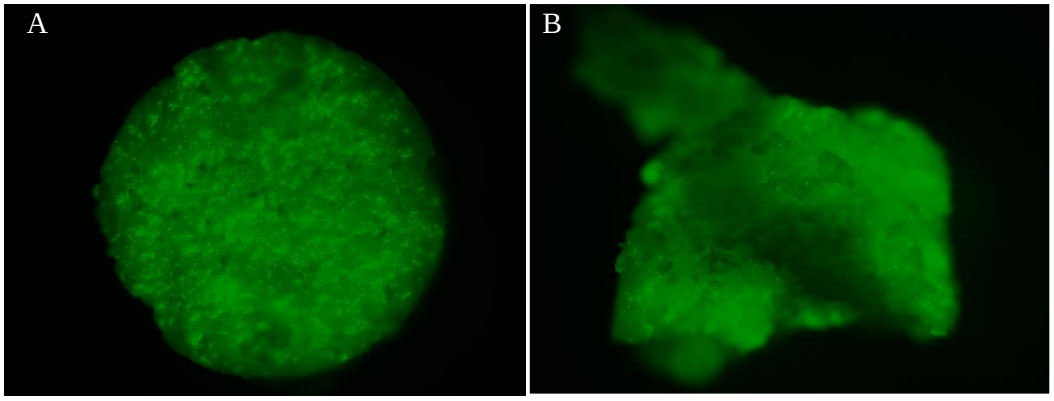

Figure 3: Top view (a) and section (b) of a ReCaPP/Paraffin scaffold after cells seeding. Calcein M staining

To overcome this problem, we attempted to modify the seeding technique, and the scaffold was soaked in a cellular suspension for 2 hours under slow shaking. The cellular distribution appeared more regular, and we observed more cells in the core of the construct (Figure 4).

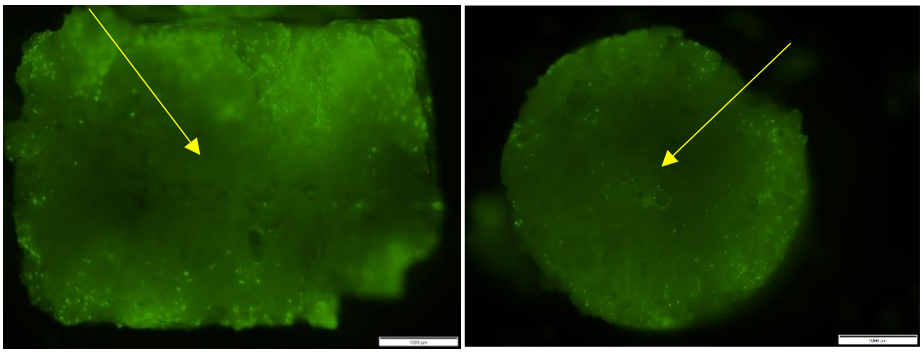

Figure 4: Transversal (a) and longitudinal (b) section of a ReCaPP scaffold seeded with “soaking” technique. The yellow arrows highlight the cells in the core of the scaffold

3.3 Gelatin/GelMA-HAMA scaffold

The second engineered construct, is based on a biocompatible organic matrix composed of gelatin crosslinked with genipin and realized by 3D-printing, and this approach provided the best cell distribution within the scaffold matrix. The gelatin-genipin scaffold tended to swell slightly during seeding, which then resulted in scraping of the outer part of the construct on the inner wall of the bioreactor during insertion. Therefore, we opted for post-insertion seeding to avoid loss of cellular population during assembly of the constructs within the bioreactors. The cell distribution was notable, with a regular distribution also in the inner area of the construct (Figure 5)

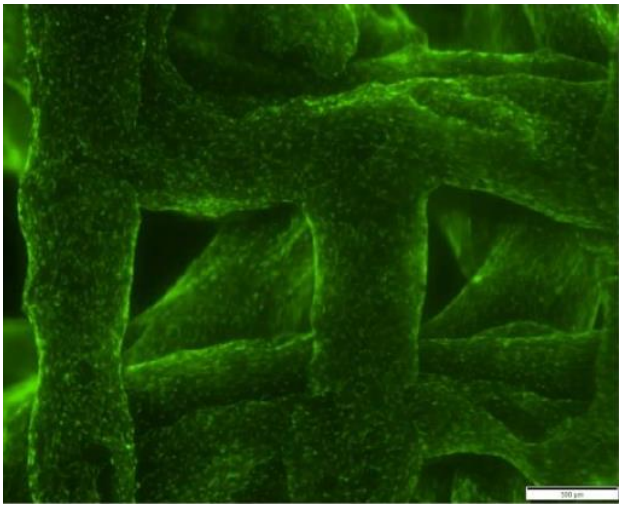

Figure 5: Cell distribution in the Gelatine/Genepin scaffold

Osseous scaffolds were completed by adding a synthetic construct mimicking the overall properties of the native cartilaginous tissue. For this, we employed a composite hydrogel made of gelatin methacrylate (gelMA) and hyaluronic acid methacrylate (HAMA), highly biocompatible polymers that confer good elastic properties to the constructs and support chondrogenesis. Furthermore, the methacrylic moieties allow a UV-mediated crosslinking by near UV light source that does not affect cells viability.

Hence, it was possible to embed cells directly in the hydrogel matrix prior to photopolymerization, obtaining a high cells density, close to that of native cartilage (figure 6).

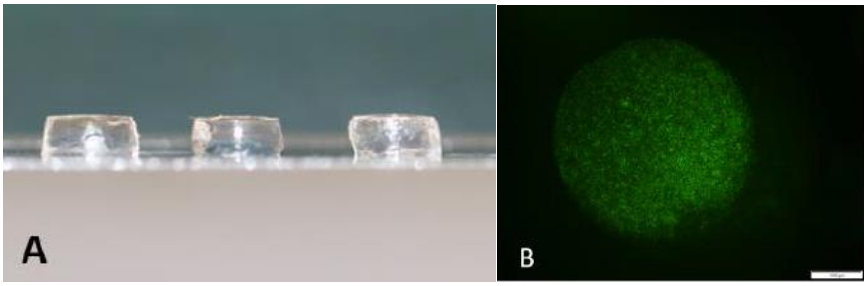

Figure 6: GelMA/HAMA scaffold immediately after fabrication (a) and after cell seeding (b)

We cultured the scaffolds within our bioreactor with osteo- and chondro-specific media and analyzed by qRT-PCR anabolic osseous gene expression in the bone component as Alkaline phosphatase (ALP), collagen type I (COL 1), bone sialoprotein II (hBSPII), osteocalcin (OCN), runt-related transcription factor 2 (RUNX2) (figure 7). The results was encouraging, and showed an overall increase in fold change of all genes analyzed.

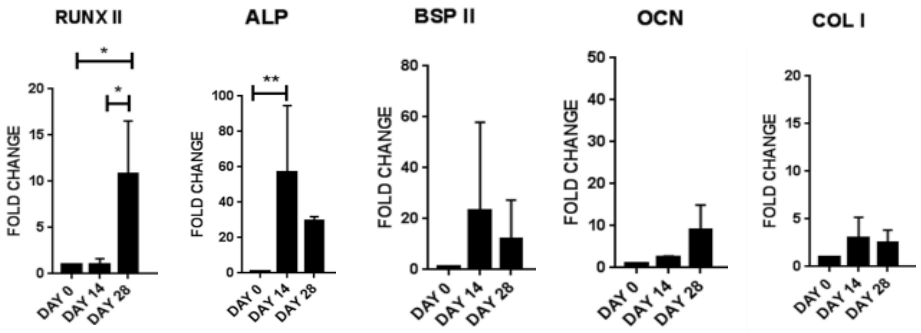

Figure 7: qRT-PCR analyses of anabolic gene of osseous tissue

4 Conclusions

We successfully realized an OA native model, and our data suggest that IL-1β decreases anabolic activity in chondrocytes and potentially induces cartilage catabolism, a prominent feature of OA. Thus, IL-1β may be leveraged to induce inflammatory features of the disease in our engineered tissue cultured inside our bioreactor, which is designed to target cytokines specifically to either bone or cartilage, or to both tissues simultaneously. We realized two engineered tissue cellularized with MSCs. The Gelatin/GelMa-HAMA construct was successfully cultured within our bioreactor and MSCs were able to differentiate in cartilage and osseous tissue, as suggested by results obtained from qRT-PCR analyses.

The realization of the *in vitro* model is still on going, and will be compared to the native OA model.