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	**D8.3**
Deliverable title	Effects of hormones during OA
Submission month of deliverable	M11 - M18
Issuing partner	RIMED
Contributing partners	LJMU
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	13/05/2019	RIMED	Final version

Table of Contents

1 Summary 4

2 Introduction 5

3 Effects of hormones during OA 6

4 Conclusion 9

1 Summary

OA is a leading cause of disability in the US, afflicting nearly 27 million and over 33% of individuals over 65. The OA rates generally increase continuously with age however, women after menopause face higher risks than age-matched men, with a prevalence that can be as much as 50% higher. In support of this clinical finding, ovariectomy (OVX) animal models that simulate the effects of menopause consistently exhibit increased incidence and severity of OA. Thus is likely to hypothesize a protective role of sex hormones, and in particular for estrogens, on the health of cartilage. Several studies support this hypothesis, and show as in *in vitro* cartilage explants and chondrocytes cultures, estrogen treatment has been shown to increase anabolic response (by promoting proteoglycan synthesis) and to decrease catabolic response (increasing MMP production). Furthermore, estrogen treatment has also been shown to have protective effects against reactive oxygen species and against mechanical injury-related cell death. The effect of progesterone was less investigated, and available literature is still opposing. If on the one hand some studies suggest that progesterone may not be directly chondroprotective *in vitro*, on the other hand *in vivo* models would instead suggest a protective role of progesterone.

Today, the results of the Women's Health Initiative of the NIH in the USA indicate that hormone replacement therapy (HRT, generally consisting of the continuous administration of estrogen or estrogen and progestin), rescues the risk of osteoporosis but not that of OA.

2 Introduction

Our activities was addressed to assess how the administration of a hormonal sequence simulating that of the menstrual cycle could be protective from OA and, conversely, whether interruption of the menstrual cycle could constitute a risk factor. With this scope, native OA osteochondral tissues derived from tissues from surgical wastes of knee replacements in post-menopausal women not subject to hormone replacement therapy, were harvested employing the techniques developed in the context of the activities described in Task 8.1. Furthermore, we realized an engineered construct to assess the response both to direct and indirect supplementing of sex hormones. We cultured both native and engineered osteochondral plugs within our bioreactor, with or without the supplementation of cycling hormonal concentrations.

Our bioreactor was essential to realize the experimental design, allowing the supplementing of sex hormones in the upper/lower chamber separately. Outcomes were analyzed to evaluate differential cellular response profiles in the two osteochondral plugs to highlight the different metabolic response ascribable to hormone cycling.

3 Effect of hormones during OA

The experimental activities in deliverable 8.3 was aimed to study how the administration of a hormonal sequence simulating that of the menstrual cycle could be protective against OA and, conversely, whether interruption of the menstrual cycle could constitute a risk factor, and with this aim we tested two tissue models. The first one was based on osteochondral plug from human donor and was employed as native tissue model. The osteochondral plugs were harvested from surgical waste obtained from postmenopausal female patients undergoing total joint replacement, and we exploited the technique developed during the activities included in the deliverable 8.1. The second model was realized by cellularization of a substrate composed of a photopolymerizable gelatin gel. Moreover, we used primary chondrocytes and primary osteoblasts obtained from post-menopausal women in 10% photopolymerizable gelatin with the addition of 1% hyaluronic acid (cartilaginous component) and 0.5% hydroxyapatite (osseous component). Both osteochondral models were cultured within our bioreactor in which either the chondral or the bone components were exposed to hormonal stimulation, and the full osteochondral plug model was then examined to assess bone-cartilage interaction, using histology, microCT, and qRT-PCR. More in detail, the menstrual cycle was simulated by supplementing the media with a sequence of four concentrations of estradiol (E2) and progesterone (P4) mimicking the physiological hematic level of the sex hormones, each lasting 1 week (w1=0.1 nM E2; w2=1 nM E2; w3=1 nM E2 + 10 nM P4; w4= 0.1 nM E2 + 50 nM P4) (figure 1).

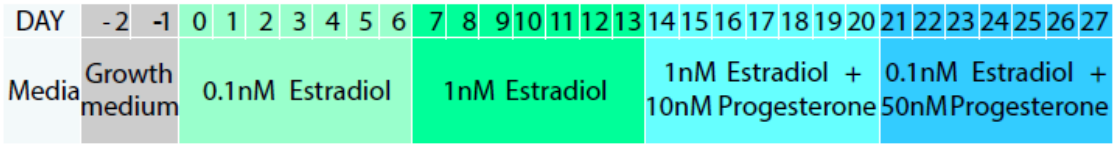

Figure 1: Hormonal sequence to mimic the restoration of the menstrual cycle

We evaluated the difference between hormone treated and not treated native osteochondral plugs in term of loss of bone mass. This approach was possible because our bioreactors material is X-ray translucent, thus we were able to perform microcomputed tomography (µCT) analysis of the samples without remove them from the bioreactors' slots. Representative µCT images (figure 2) show that samples receiving the menstrual hormone sequence to the cartilage or bone actually improved in bone mass as compared to untreated controls, particularly at the osteochondral junction. The osteochondral junction is the terminal location of endochondral ossification, where calcified cartilage is found and near which chondrocytes have a hypertrophic phenotype.

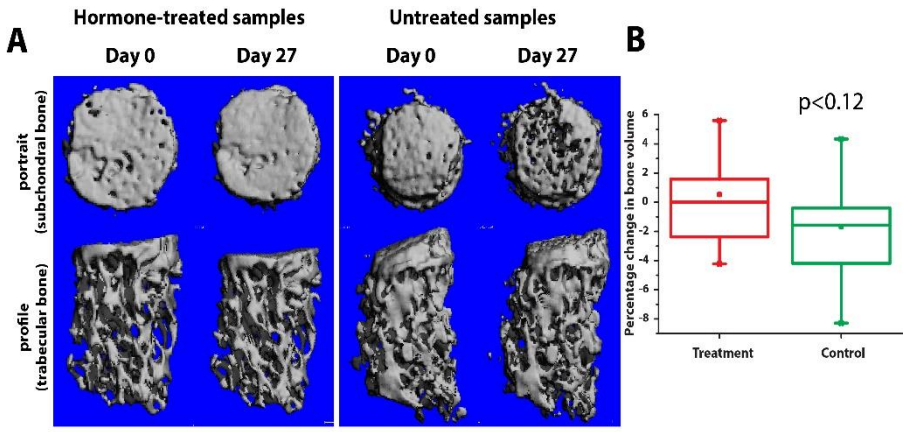

Figure 2: Preservation of bone volume and the OC junction in OC plugs exposed to estrogen/progesterone (E2/P4) menstrual sequence. (A) µCT (top and side views) of representative OC plugs at days 0 and 27, showing changes in bone volume, most markedly at the bone/cartilage interface. (B) Quantitative analysis of bone volume changes between days 1 and 27 in E2/P4

treated and controls OC plugs. $p<0.12$.

Moreover, we achieved an accurate analysis on engineered construct to assess the cellular response of the chondral or osseous component to direct hormone stimulation, and to identify any response induced in the adjacent unstimulated tissue by tissue-tissue communication (indirect stimulation) (figure 3).

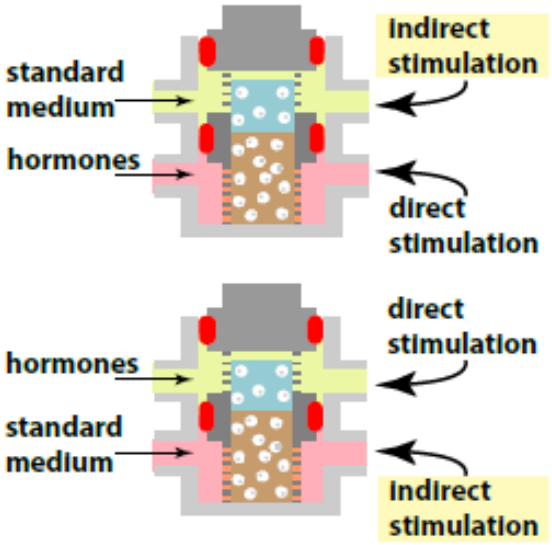

Figure 3: Schematic representation of the experimental set-up adopted to study the cellular response to a direct or indirect hormone stimulation

Finally, we analyzed by qRT-PCR the gene expression of anabolic (aggrecan) and catabolic (IHH, Gli1) gene after direct and indirect hormone stimulation (figure 4) for each week of the menstrual cycle.

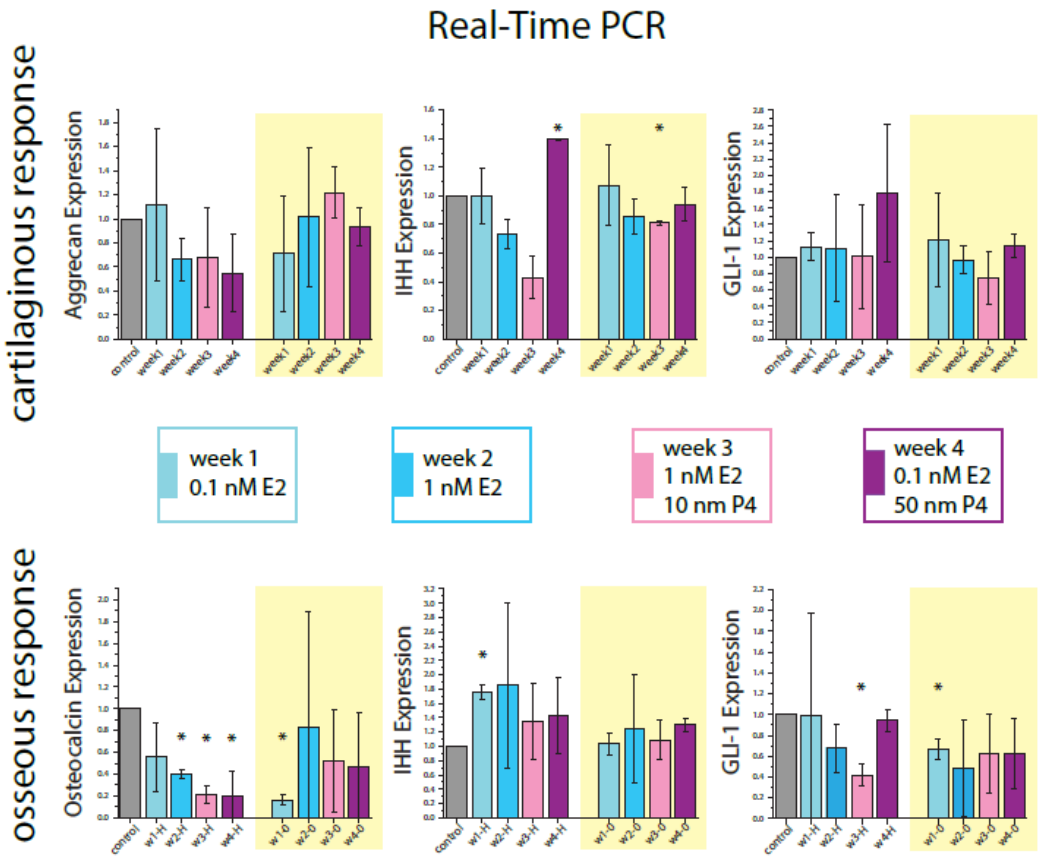

Figure 4: qRT-PCR analyses. The yellow square represent the indirect stimulation pattern.

4 Conclusions

Our results show that menstrual cycle hormonal exposure induces a slight decrease in chondrocytes/osteoblasts anabolism. In addition, direct progesterone/estrogen exposure causes increased IHH expression in chondrocytes/osteoblasts respectively, whereas indirect progesterone/estrogen exposure causes decreased IHH expression in chondrocytes/osteoblasts, respectively. Our results on native tissue also suggest that a hormonal sequence mimicking the restoration of the menstrual cycle might have a protective, pro-homeostatic effect on subchondral bone and a chondroprotective effect.