

Scaffold-Free Cartilage Spheroids Model: Application to Investigate Chondrocytes Regenerative Capacity

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Introduction

Rheumatoid arthritis and osteoarthritis are the most common cartilage diseases: a plethora of causes can contribute to accelerate cartilage degradation and these mechanisms are not fully understood. Due to an aberrant pro-inflammatory stimulus, the immune system attacks the synovial membrane of the joint capsule, leading to inflammation and swelling until joints destruction. VitroScreen ORA™ CARTILAGE spheroids are engineered scaffold-free spheroids designed for mimicking the complexity of human cartilage tissue in microscale 3D culture conditions mirroring the natural chondrocytes microenvironment, able to preserve their physiological profile and re-differentiate in a mature cartilage tissue. The CARTILAGE model is developed as predictive preclinical model to assess the biological response of chondrocytes when they came in contact with locally dispersed molecules (for example, after local infiltration with drug or Medical Device).

Experimental design

Primary human chondrocytes at early passage were cultured by hanging drop technique to produce CARTILAGE scaffold free spheroids as miniaturized micro-physiological cartilage system. After 7 days of culture, chondrocytes spheroids reached a full differentiation, expressing key biomarkers of natural cartilage profile. The metabolic activity was evaluated by ATP quantification. After 48h of exposure to two Medical Devices with different Jaluronic acid of different MW gene expression of hyaluronate synthase (HAS1) gene was quantified by qRT-PCR. Collagen type II and S100 expression and localization were investigated by IF on 3D whole mount samples. The 3D Z-stack acquisitions were performed by Leica Thunder DMI8.

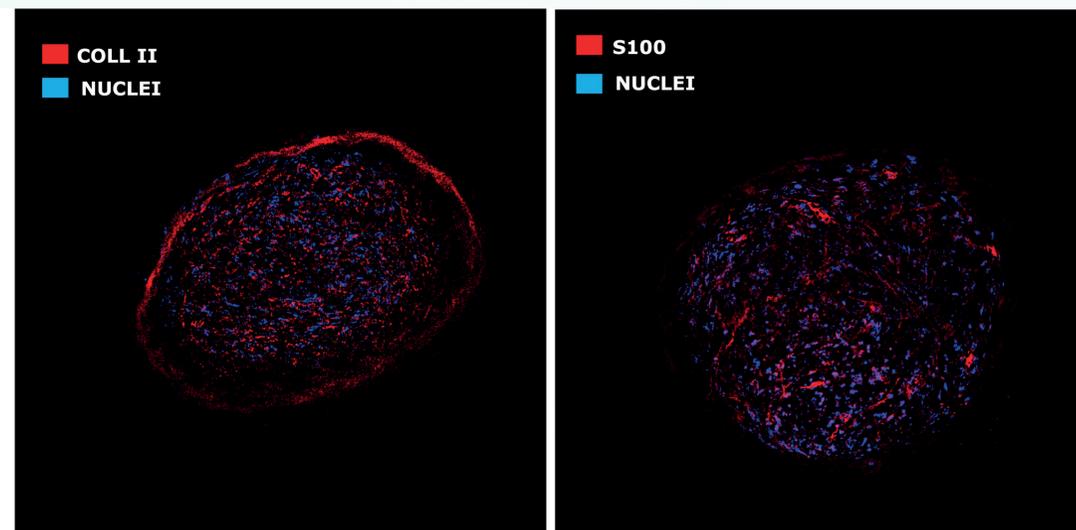


Figure 1. Expression of key biomarkers of cartilage differentiation and functionality activity after long term culture (7 days). Mag.20X.

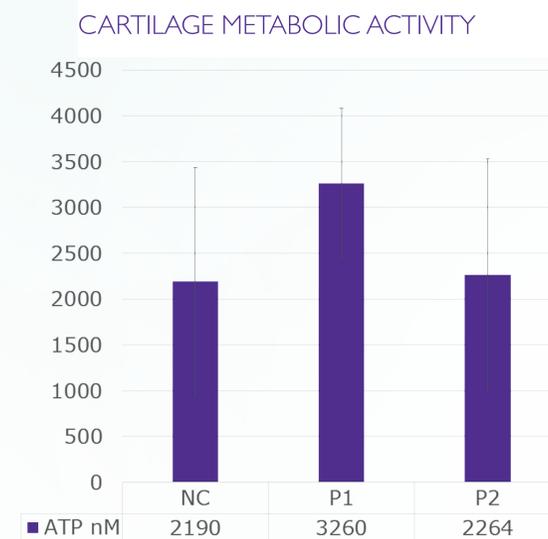


Figure 2. Metabolic activity by ATP measurement of ORA™ CARTILAGE MPS after 7 days of differentiation. Exposure to two cartilage stimulators (P1 and P2) compared to untreated samples (NC).



Figure 3. Differential modulation of HAS1 gene expression after exposure to two different cartilage stimulators, added for 48h.

Results

According to interfaces mechanics, the stable and regular geometry of spheroids minimizes deformations induced by contractile forces, supporting the natural dynamic change of tissue over time. ORA™ CARTILAGE, as floating unit, exhibits preserved mechanical properties, stable tissue tensile strength and low geometric deformation during remodelling as the tensile forces induced by exogenous substrates or biomaterials are absent, reaching the highest cell-cell and cell-matrix interactions. Clearing histological technique allows the evaluation of ORA™ CARTILAGE MPS in their 3D configuration according to an innovative histological approach, avoiding paraffin-embedding procedures. The 3D physiological geometrical guidance allows the generation of a functional and responsive tissue, close to the native human cartilage, as confirmed by expression of Collagen II, mature cartilage biomarker (fig.1).

The expression of collagen type II suggests also a complete chondrocytes differentiation and the deposition of a functional endogenous extracellular matrix, thanks to the topographical instructions driven by the micro-physiological niche. In addition, the expression of S100, a critical molecule of cartilage activation indicates that the model has preserved its re-regenerative potentiality. The treatment on fully re-differentiated CARTILAGE spheroids with two different formulations of Jaluronic acid, known for its activity on cartilage metabolism, has determined compared to untreated series

- a preserved, and in case of P1, higher metabolic activity (fig.2)
- an overexpression of hyaluronate synthase gene expression induced by P1

Conclusion

Engineered ORA™ CARTILAGE provides a miniaturized not conventional tissue platform based on a preserved natural niche where pre-chondrocytes are able to self-assembly according to their natural phenotype, generating an endogenous micro-environment for high-fidelity human cartilage tissue, providing natural regenerative properties laying the groundwork for pre-clinical application: ingredients screening and mechanism of action, synergies and personalized therapies.