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

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Rheumatoid arthritis (RA) and osteoarthritis (OA) 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 [1]. VitroScreenORA™ CARTILAGE spheroids are engineered scaffold-free spheroids designed for mimicking the complex physiology of human cartilage tissue in 3D microscale culture conditions, mirroring the natural chondrocytes microenvironment and preserving their physiological profile during re-differentiation in a mature cartilage tissue. The CARTILAGE ORA™ is produced with healthy primary human cells: 3D geometry, structured endogenous ECM, metabolic activity and self-renewing are high fidelity mirrored, reproducing mature cartilage tissue in homeostatic and inflammatory conditions (donor dependent or induced) and within different experimental windows mirroring: chondrocytes differentiation (1-7 days) and ECM remodelling in a fully differentiated cartilage (7-14 days).

EXPERIMENTAL DESIGN

Primary Human Chondrocytes (HC) were seeded to the desired concentration (10000 cells/spheroids) in the hanging drop plates (Akura® plate by InSphero) to allow cellular aggregation. After 3 days in hanging drops, cells appeared aggregated in round shaped spheroids, then tissues were transferred in the Akura® 96 V2 plates (InSphero) and cultured for additional 10 days in standard conditions to allow the physiological cells re-differentiation in a mature cartilage profile.
After complete differentiation, spheroids were treated with product (botanical extract) and a HMW HA (high molecular weight hyaluronic acid)-based reference for 7 days and were compared to untreated negative control series. S100 expression and localization were investigated by IF on 3D whole mount samples. The 3D Z-stack acquisitions were performed by Leica Thunder DMi8.

HOMEOSTATIC MODEL

S100 SIGNAL QUANTIFICATION

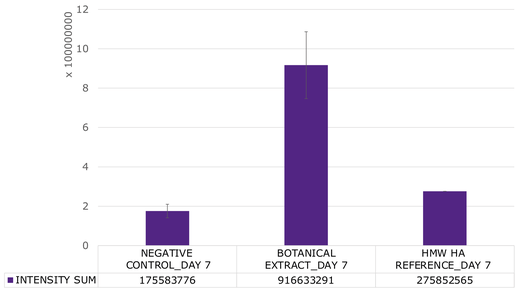

Figure 1. Maximum projection of day 7 signal intensity normalized to spheroids area

- In basal conditions of untreated series, S100-positive chondrocytes were observed within the spheroids in a differentiated architecture [2].
- The exposure to the Botanical Extract for 7 days led to a positive effect on chondrocytes metabolism with a significant increase of S100 expression suggesting a strong cellular response of cartilage model in terms of regenerative properties and activation of ECM remodelling [3].
- Treatment with HMW HA-based reference showed a positive modulation of S100 expression [4], with higher values compared to untreated series (negative control), but lower than Botanical Extract series.

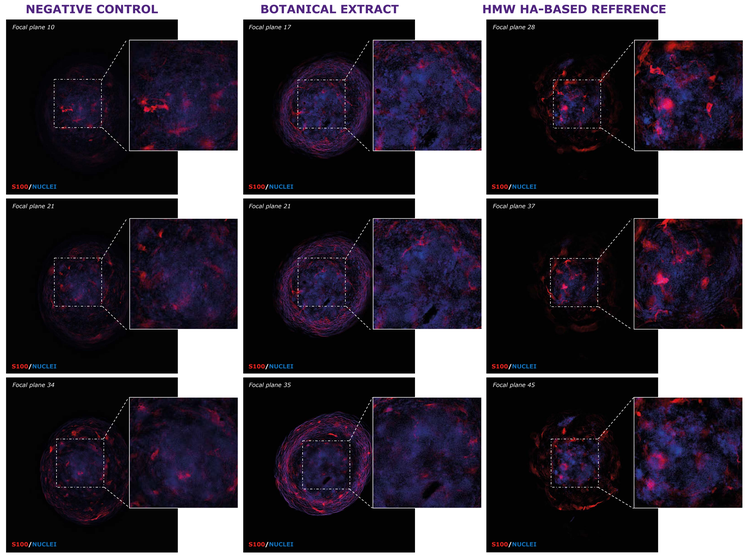

Figure 2. S100 expression by IF in whole mount after long term culture (7 days). Mag.20X.

References

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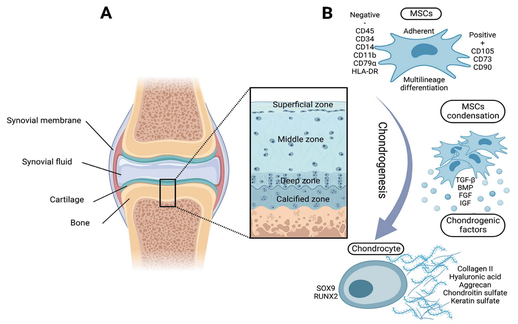
Mechanisms of transport in an avascular system



Since cartilage doesn't have its own blood supply like other tissues, it relies on nearby blood vessels to provide nutrients and remove waste products [5]. Here's a simplified explanation of how nutrients diffusion works:

1. **Active and passive diffusion through matrix:** being the extracellular matrix a gel-like substance composed of water, collagen fibers and proteoglycans, nutrients diffuse through this matrix to reach the chondrocytes.
2. **Chondrocytes uptake:** chondrocytes take up nutrients and release waste products into surrounding fluids to be carried away.

Figure 3. (**A**) A schematic illustration of the anatomical structure of articular cartilage (**B**) Chondrocytes of articular cartilage differentiate from mesenchymal stem cells (MSCs). The process of chondrogenesis involves MSC condensation and environmental chondrogenic factors that cause the activation of transcription factors SOX9 and RUNX2. Differentiated chondrocytes then produce the cartilage-specific ECM.

ENDOGENOUS ECM AS a DRIVER: The Extra Cellular Matrix (ECM) plays a crucial role in cartilage tissue. Being avascular, the ECM provides mechanical support, entraps nutrients and makes them available to cells, ensuring a homogeneous diffusion and spatial distribution within the tissue, mimicking the *in vivo* anatomy and providing guidance for cells orientation, polarization, and extra cellular matrix synthesis [6].

Thanks to homeostatic gradients, endogenous ECM binds substances and allows a uniform nutrient supply to the 3D spheroids. Endogenous **ECM** provides cells with fundamental information on molecular composition of pericellular environment that finally regulates cell responses, organ physiology and metabolism: in this physiological state a relative long term cultivation of the test system has been possible.

INFLAMMATORY MODEL

An inflammatory response was induced by IL-1β (PC) treatment (24h) in the medium and then, after complete removal of inflammatory stress, treatments were performed for 24h (D1) and 72h (D3) with HMW hyaluronic acid (0,25%) compared to untreated negative control (NC).
- IL-1β amount dosed in the culture media was quantified by ELISA assay;
- TNFα was quantified by qRT-PCR.

Despite its key role in mechanical stress support, HMW hyaluronic acid does not exhibit an anti-inflammatory activity: it has not reduced IL-1β release and gene expression confirming a physical-mechanical mechanism of action.

IL-1β RELEASE			
	NC	IL-1β	IL-1β + HMW HA
D1	1,24	71,93	146,46
D3	0,31	72,56	125,86

Table 1. IL-1β quantification on cartilage ORA® spheroids media after 24h and 72h treatment with products of inflammed cartilage.

GENE: TNFα	NC	IL-1β+HMW hyaluronic acid	IL-1β
	RQ VALUES		
D1	1,00	4,76	4,76
D3	1,00	2,07	1,61

Table 2. Results of TNFα gene expression.