Application Note

Mass Density as Non-Invasive Biomarker for Organoid Maturation

In the field of regenerative medicine, organoid development and maturation play a key role. This application note outlines an innovative approach to monitor organoid maturation through the non-invasive biomarker of mass density provided by W8. Based on the correlation between mass density and the expression of key biomarkers - pan-cytokeratins, cadherins, fibronectin, collagen and vimentin - this methodology offers a window into the structural and functional integrity of developing organoids. By establishing a protocol that exploits these correlations, mass density measurement emerges as a powerful tool for real-time monitoring of organoid maturation. This approach not only improves our understanding of organoid development, but also facilitates the application of organoids in tissue engineering and regenerative therapies by providing a reliable metric to assess their functional readiness and capacity. Through detailed analysis and correlation of biomolecular expressions with biophysical properties, this study supports the adoption of Mass Density as a standard biomarker in the field of organoids, promising to simplify research and application in this area of biomedical science.

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Characterization of Perinatal Stem Cell Spheroids for the Development of Cell Therapy Strategy, Paris et All, Bioengineering 2023

MATERIAL AND METHODS

Culture Conditions

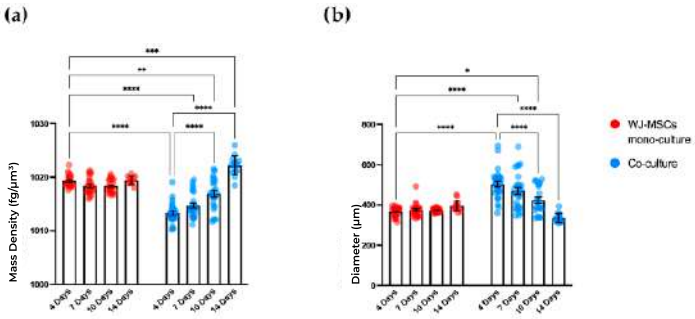
Organoids were cultured under controlled conditions to simulate the cellular microenvironment and support their growth and maturation. The culture conditions were optimized for both monoculture and co-culture systems, focusing on Wharton's jelly mesenchymal stromal cells (WJ-MSCs), and their co-cultures with amniotic epithelial cells (AECs) to study the synergistic effects on organoid formation and maturation. The cultures were maintained in a humidified incubator at 37°C with 5% CO2, with medium changes every 2-3 days to ensure optimal growth conditions.

Tests and Analyses Conducted

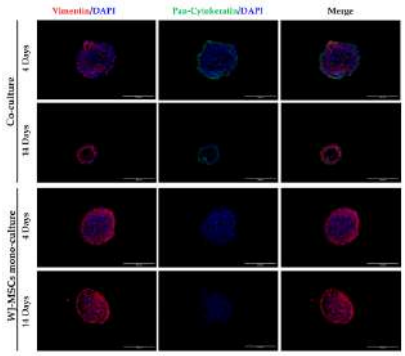
To validate the effectiveness of mass density as a biomarker for organoid maturation, we conducted a series of tests and analyses focused on both the biophysical properties and biomolecular characteristics of the organoids:

1. Mass Density Measurement: Utilizing a precision flow cytometry-based system, we quantitatively assessed the mass density of organoids over time. This non-invasive method allowed for the continuous monitoring of organoid compactness and structural integrity, reflecting their developmental progression.
2. Immunofluorescence Staining: To correlate mass density with biomolecular markers, we performed immunofluorescence staining for pan-cytokeratins, cadherins, fibronectin, collagen, and vimentin. This analysis provided insights into the cellular composition and extracellular matrix of the organoids, elucidating their differentiation status and functional potential.

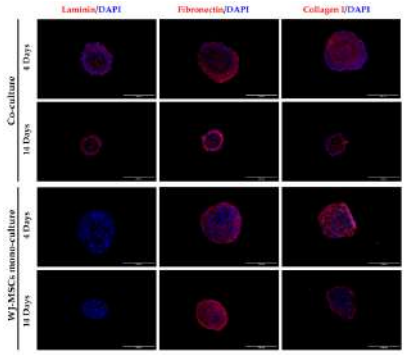
DISCUSSION



Measurement of mass density and diameter of mono-culture WJ-MSCs spheroids (red dots) and co-culture (WJ-MSCs + AECs) spheroids (blue dots). (a) Analysis of spheroid density (fg/m^3) at four time points: 4, 7, 10, and 14 days for both mono- and co-culture spheroids. The mas density of WJ-MSC spheroids remains stable over time while co-culture spheroids increase their density. (b) Analysis of spheroid diameter (m) at four time points: 4, 7, 10, and 14 days for both mono- and co-culture spheroids. The diameter of WJ-MSC spheroids remains stable over time similarly to the mass density while co-culture diameter decreases with the increase in the mas density. N = 4 independent experiments. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.



Representative images of WJ-MSC mono-culture and co-culture spheroids at early (4 days) and late (14 days) time points stained with Pan-Ck (green), vimentin (red), and DAPI for nuclei staining (blue). Cells were observed using a Nikon Inverted Microscope (Nikon Instruments, Tokyo, Japan), and images were acquired with a Digital Sight camera DS-03 using the imaging software NIS-Elements 4.1 (Nikon Corporation, Tokyo, Japan). Scale bar = 100 m.



Representative images of WJ-MSC mono-culture and co-culture spheroids at early (4 days) and late (14 days) time points stained to evaluate the presence of ECM proteins with laminin, fibronectin, and collagen I (red) and DAPI for nuclei staining (blue). Cells were observed using a Nikon Inverted Microscope (Nikon Instruments, Tokyo, Japan), and images were acquired with a Digital Sight camera DS-03 using the imaging software NIS-Elements 4.1(Nikon Corporation, Tokyo, Japan). Scale bar = 100 m. Magnification = 20.

DISCUSSION

In this investigation, the interplay between mass density, diameter variation over time, and the expression of key biomolecular markers, including vimentin, pan-cadherin, fibronectin, collagen, and laminin, was meticulously analyzed to elucidate their collective impact on organoid maturation and development. The discussion of these findings sheds light on the nuanced mechanisms governing organoid growth and differentiation, offering insights into the utility of these parameters as indicators of organoid readiness for applications in regenerative medicine and tissue engineering.

Mass Density and Diameter Variation

The correlation between mass density and diameter variation of organoids over the course of the study period emerged as a critical observation. An increase in mass density was noted alongside a decrease in diameter, suggesting a compaction and maturation process within the organoids. This densification is indicative of increased cellular organization and extracellular matrix (ECM) deposition, factors that are essential for the functional integrity of organoids. The variation in diameter and mass density over time provides a quantifiable measure of organoid development, underscoring the relevance of biophysical properties in assessing organoid maturity.

Vimentin and Pan-Cadherin Expression

The expression profiles of vimentin and pan-cadherin further contribute to our understanding of organoid maturation. Vimentin, a marker of mesenchymal cells, and pan-cadherin, indicative of cell-cell adhesion, offer insights into the cellular dynamics within the organoids. The presence of vimentin suggests a degree of mesenchymal characteristics, potentially reflecting a transition or differentiation state in certain cell populations. Conversely, pan-cadherin expression underscores the critical role of cell-cell interactions in maintaining the structural cohesion of the organoids. Together, these markers highlight the balance between mesenchymal and epithelial properties crucial for organoid integrity and function.

Fibronectin, Collagen, and Laminin

The ECM components, fibronectin, collagen, and laminin, play pivotal roles in organoid development, serving as the scaffold for cellular attachment and signaling. Their expression within the organoids was found to correlate with both mass density and the aforementioned biomolecular markers. Fibronectin and collagen, in particular, are instrumental in ECM deposition and organoid compaction, contributing to the increased mass density observed. Laminin, essential for basement membrane formation, further supports the structural maturation of the organoids. The coordinated expression of these ECM proteins not only reinforces the organoid structure but also influences cellular differentiation pathways, highlighting their significance in the maturation process.

CONCLUSION

The findings of our study reveal a strong correlation between biophysical properties, notably mass density, and biomolecular markers, establishing mass density as a viable non-invasive biomarker for monitoring organoid maturation. By defining a clear protocol and threshold levels, mass density can effectively gauge the maturity of organoids, bridging structural characteristics and molecular dynamics. This method offers a practical, non-disruptive approach to assess organoid readiness for applications in regenerative medicine and tissue engineering. Consequently, mass density stands out as a crucial tool in the advancement of organoid research, enabling precise and timely utilization in therapeutic contexts.