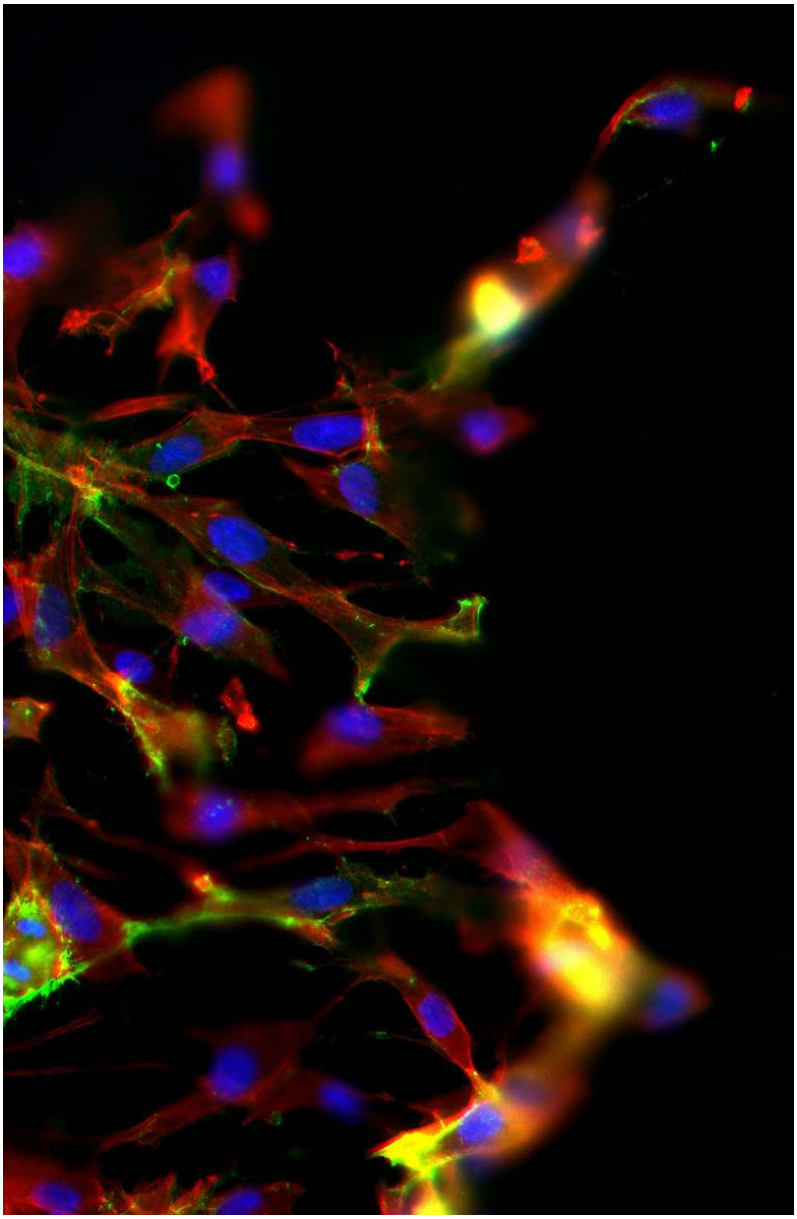
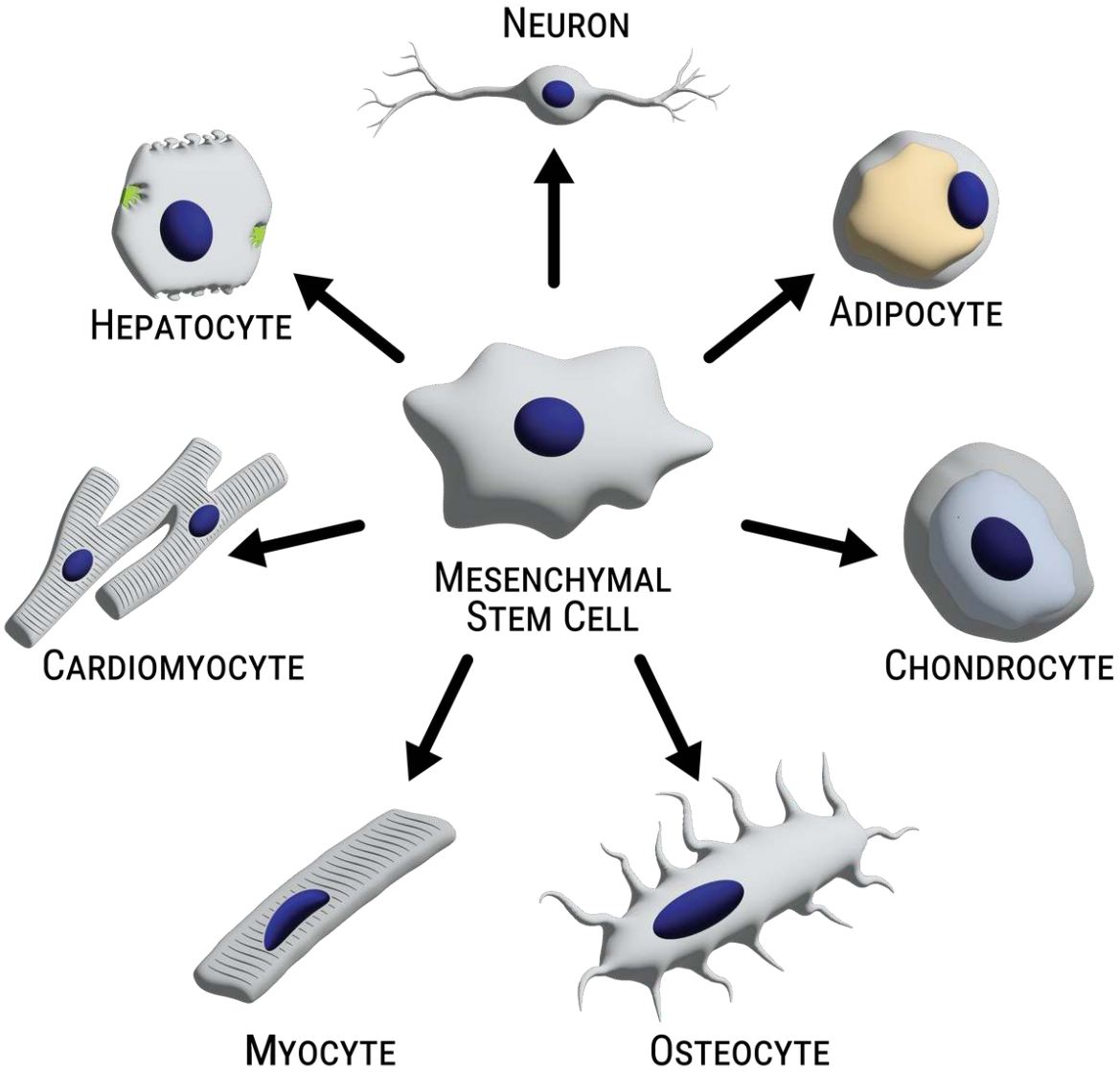
Ronawk, Inc.

Bio-Blocks Enable Improved Retention of Stem-like Surface Markers for Mesenchymal Stem Cells



Introduction

Mesenchymal stem cells (MSCs) have the ability to differentiate into various cell types, making them a promising candidate for regenerative medicine. However, traditional 2D culture methods for MSCs have limitations — required subculturing contributes to the loss of stemness and reduced differentiation potential as the number of passages increases.

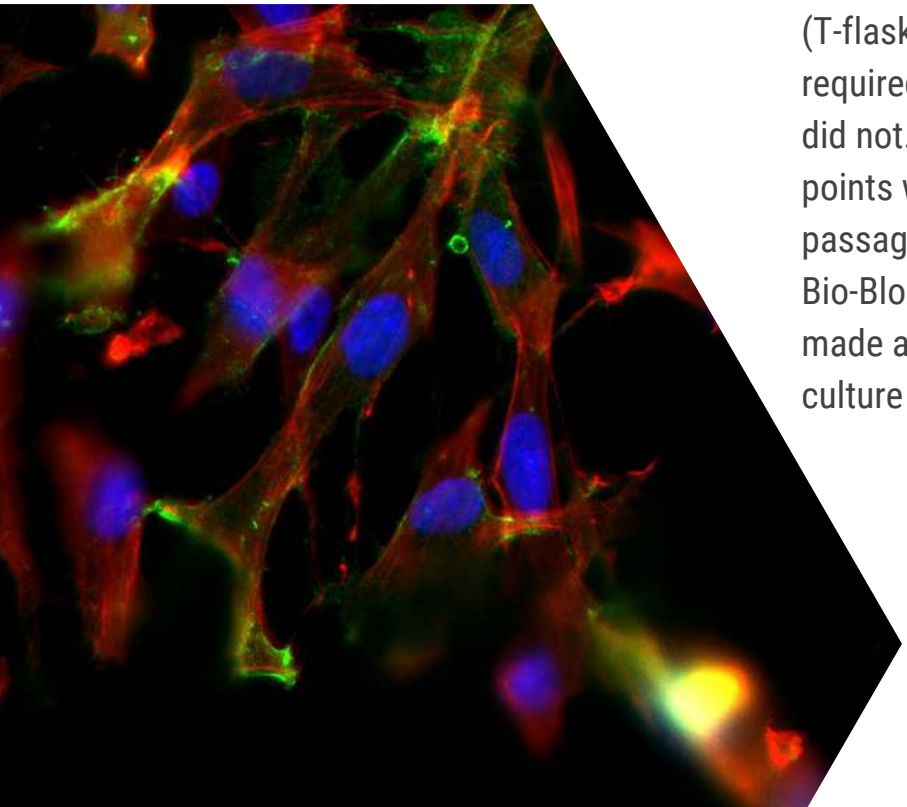


An illustration of a central mesenchymal stem cell with seven associated radial arrows. The top center is a neuron, which is followed in a clockwise fashion by an adipocyte, chondrocyte, osteocyte, myocyte, cardiomyocyte, and lastly a hepatocyte. These are among the types of cells into which a mesenchymal stem cell can differentiate.

Imagine if stem cells could be cultured for longer, greatly expanding the size of the usable population in culture, while still retaining their ability to differentiate into a range of cell types - how could this help strengthen your current stem cell culture practice and research?

Ronawk's Bio-BlocksTM are a promising, emerging culture system for MSCs. When MSCs are cultured in a tissue-mimetic environment using Ronawk's proprietary Bio-Blocks, stemness markers (i.e. CD73, CD90, and 105) (Hass et al, 2011) are retained at higher levels relative to conventional 2D culture methods when assessed using immunolabeling techniques.

Cell growth environments: 3D and 2D Cell Culture



Adipose-derived MSCs were cultured in both the 3D Bio-Block and traditional 2D culture (T-flask). The 2D culture environment required subculturing while the Bio-Blocks did not. As a result, passage-equivalent time points were utilized as an alternative to passage numbers. Comparisons between the Bio-Blocks and 2D culture environments were made at P2, P6, and P10 equivalents in 2D culture (Hodge et al, 2022).

Stemness Markers: Bio-Blocks vs. 2D Traditional Culture

CD73, CD90, and CD105 are three stem-like surface markers expressed on the surface of MSCs. These three markers were individually, immunohistochemically labeled; imaged using fluorescent microscopy; and then graphed and statistically analyzed (Hodge et al, 2022).

Greater percentages of MSCs cultured using Bio-Blocks retained surface markers of stemness relative to MSCs cultured in traditional 2D flasks. Specifically, stemness marker retention was statistically significantly higher in 8 of 9 passage-equivalent comparisons:

Passage Equivalent Timepoint	Stem-like Surface Markers of MSCs (average % positive)								
	CD73 (% positive)			CD90 (% positive)			CD105 (% positive)		
	2D Flask	Bio-Block	Difference (Bio-Block % — 2D flask %)	2D Flask	Bio-Block	Difference (Bio-Block % — 2D flask %)	2D Flask	Bio-Block	Difference (Bio-Block % — 2D flask %)
P2	21.5	37.1	15.7	45.2	67.2	21.9	64.4	69.3	4.9
P6	22.6	39.6	17.1	27.0	49.3	22.3	25.5	44.8	19.3
P10	6.6	23.6	16.9	19.3	36.1	16.8	21.7	34.0	12.3

Green shading indicates statistically significant differences between the 2D flask and Bio-Block culture environments for a given stem-like surface marker at a specific passage equivalent time point. A comprehensive statistical analysis is presented by Hodge et al (2022).

Conclusion

These results demonstrate that Bio-Blocks better maintain the stem-like surface markers of MSCs compared to traditional 2D culture systems over a greater number of passages. This has significant implications for using Bio-Blocks in regenerative medicine, as maintaining the stemness of MSCs is crucial for their successful application in tissue engineering and cell-based therapies.

Importance of Bio-Blocks

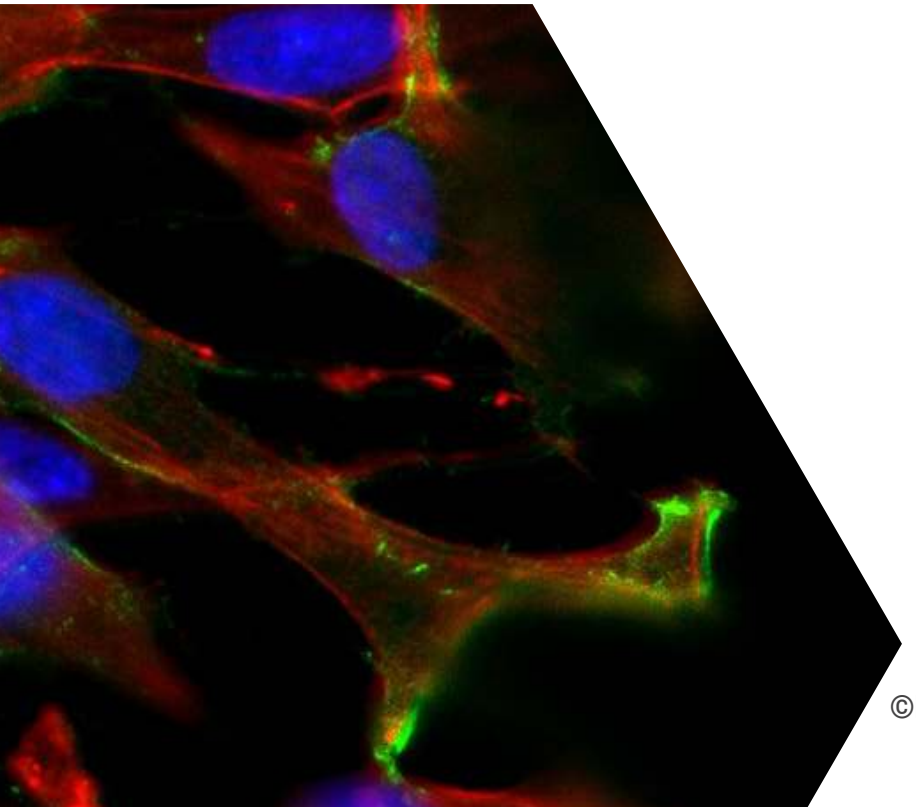
Ronawk's Bio-Blocks offer a unique tissue-mimetic microenvironment; when MSC culture is combined with the consistency and reproducibility of Ronawk's Bio-Block platform researchers can quickly and efficiently evaluate the potential of various conditions and treatments on MSCs. Collecting and analyzing quality data from the Bio-Block platform can lead to improved understanding and development of MSC-based therapies.

Highlights

- Mesenchymal stem cell (MSC) populations are highly adaptable progenitor cell populations that dynamically respond to physical, environmental, chemical, and biological cues.

- To date, most commercially available in vitro expansion systems are 2D in nature, forcing MSCs to grow in a 2D monolayer. These rigid 2D systems are unphysiological and result in loss of MSC stemness.

- Ronawk's Bio-Blocks are a tissue-mimetic culture system, which allows MSCs to retain markers of stemness at a higher level for a longer number of passage equivalents relative to traditional 2D systems



Contact Ronawk

How can Bio-Blocks' ability to retain stemness in cultured stem cell populations help get the most out of your cultured stem cell populations?

Reach out and schedule a time to discuss and learn more:

Ronawk.com
info@Ronawk.com
Schedule A Meeting

References

Ronawk, Inc. **Ronawk's Bio-Block Technology**. https://ronawk.com/mimetic-culture-technology/ (accessed February 23, 2023).

Hass R, Kasper C, Bohm S, Jacobs R. **Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC.** Cell Commun. Signal. 9, 12 (2011).

Jacob G Hodge, Jennifer L Robinson & Adam J Mellott. **Novel hydrogel system eliminates subculturing and improves retention of nonsenescent mesenchymal stem cell populations.** Regen Med. 2022 Oct;17(7):641-654. doi: 10.2217/rme-2022-0140.

About Ronawk

Ronawk's Bio-Block Universe™ is the first expandable Bio-Factory designed to accelerate the development of biotechnology applications, processes, and technologies. By leveraging advanced mimetic-culture technology, Ronawk's Bio-Block Universe™ streamlines cell and tissue production, ultimately expediting research for next-generation therapies.

The Bio-Block Universe™ simplifies the once-tedious process of mimetic-culture workflows by minimizing labor, consumables, and space. Bio-Block™ technology employs biomimicry of soft tissues to optimize the growth of cells outside the body in a way that closely mirrors their natural growth within the body. This approach not only increases biological opportunities but also ensures cell viability, preservation of key characteristics, and secretion of therapeutic biologics. The process also lowers senescence and risks of contamination by removing subculturing from the process.

Ronawk's Bio-Block™ platform is customizable, offering consistent, repeatable, and scalable bio-mimetic microenvironment production that accelerates research and paves the way for innovative regenerative therapies. By harnessing the power of mimetic culture technology Ronawk is committed to transforming the field of biotechnology and advancing the development of life-changing treatments for patients in need.

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Tissue Engineering Parts A, B and C

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