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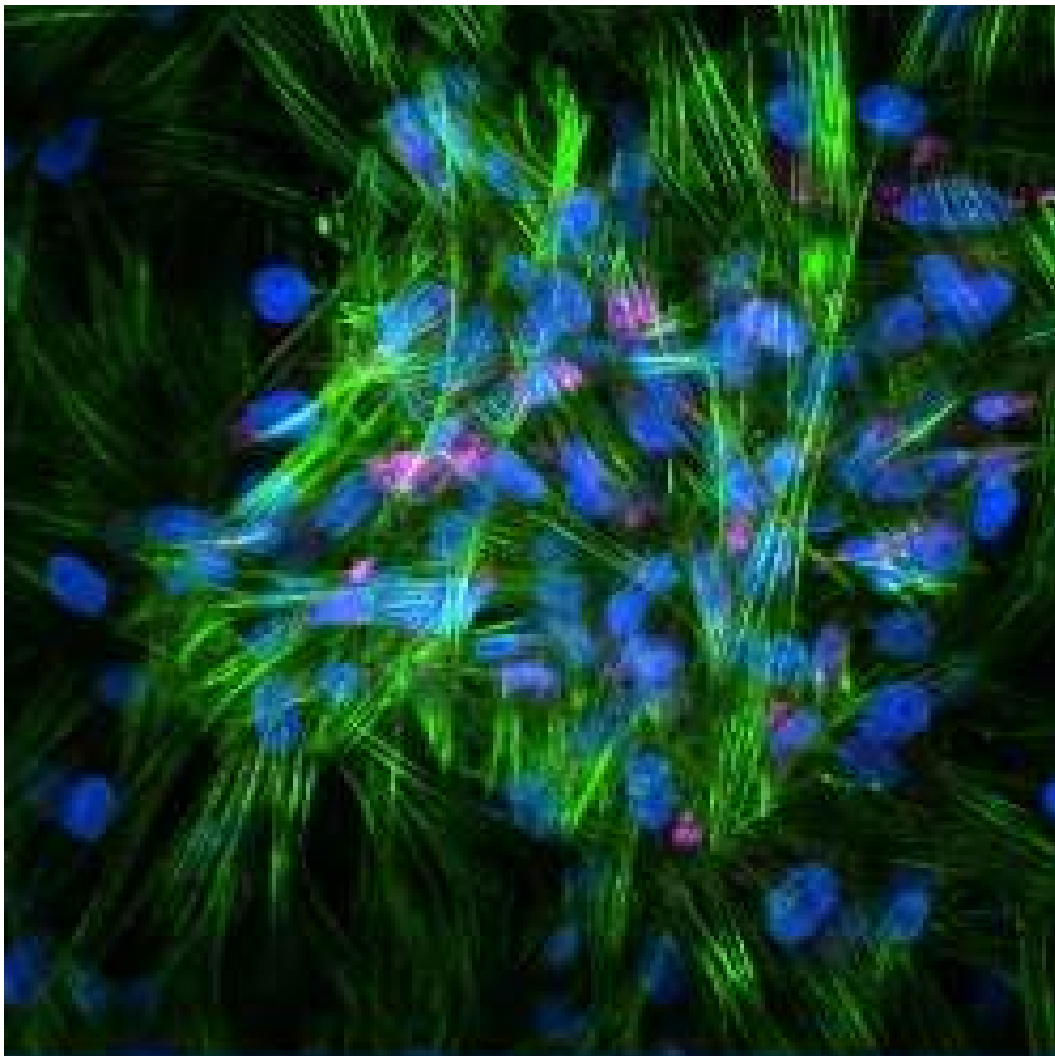
Bio-Blocks™ Massively Reduce Senescence in Adipose-Derived Mesenchymal Stem Cells



Introduction

Mesenchymal stem cells (MSCs) have enormous potential for tissue engineering and regenerative medicine applications, but traditional culture methods present some challenges when working with this cell population. One such challenge limiting the research and therapeutic potential of these cells is senescence – stem cells lose the ability to proliferate and differentiate over time in culture. The future success of these approaches relies on better understanding and optimizing the culture microenvironment for stem cells.

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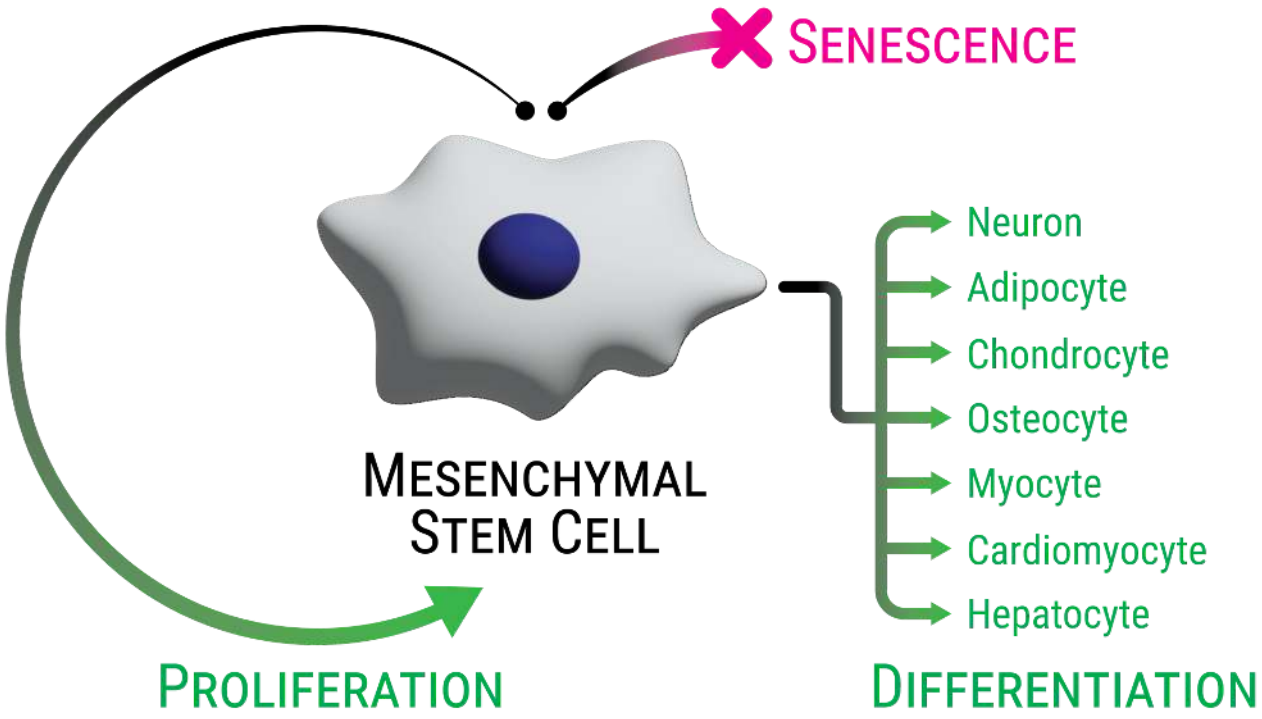
Traditional 2D culture systems and most 3D culture systems are not bio-mimetic – meaning they do not mimic in vivo conditions. However, Ronawk’s Bio-Block culture system in combination with the cultured cells results in a bio-mimetic microenvironment that mimics in vivo conditions. The bio-mimetic environment is beneficial because it contributes to reduced stem cell senescence compared to 2D, which results in a more robust culture. In this white paper, we present data collected from adipose-derived mesenchymal stem cells (ASCs) cultured in 2D flasks and Ronawk’s Bio-Blocks to explore this concept further.

“The structure and material of Ronawk’s Bio-Block culture system in combination with the cultured cells result in a bio-mimetic microenvironment that mimics in vivo conditions”

Bio-Blocks™ Massively Reduce Senescence in Adipose-Derived Mesenchymal Stem Cells



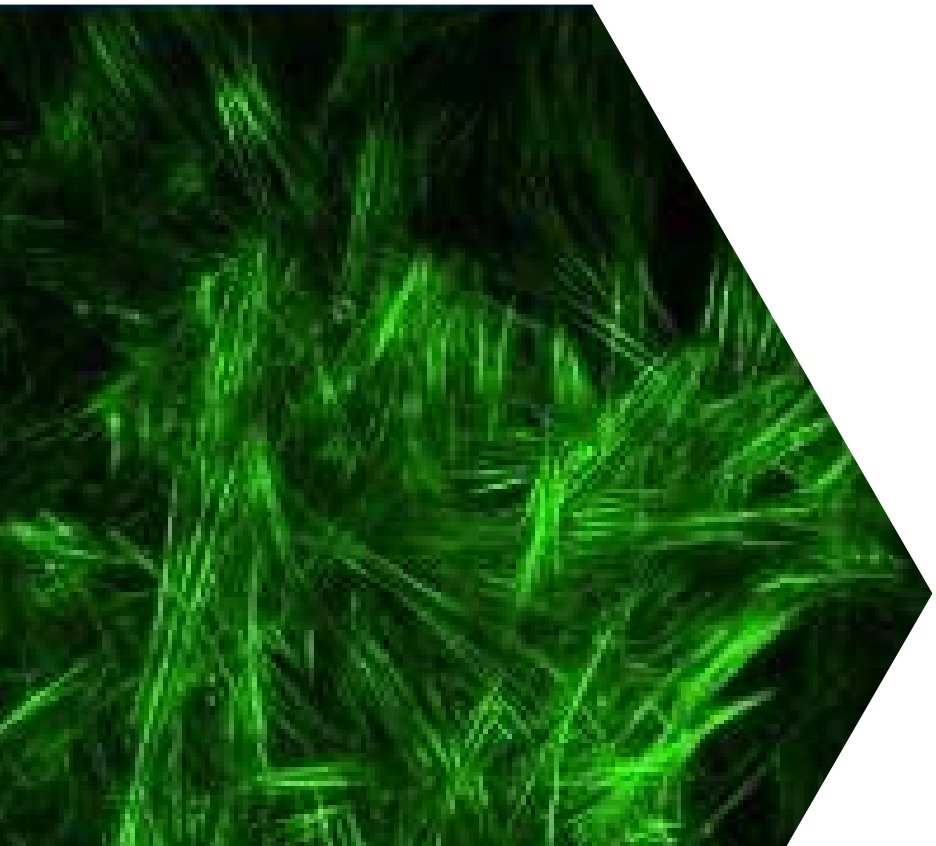
A mesenchymal stem cell (MSC) is shown in the center of the figure with three arrows extending from it to indicate cell behaviors - proliferation, differentiation, and senescence. The first arrow (black to green gradient) starts at the top center of the cell and completes a closed loop on the left side, connecting back to the cell at the bottom center, which is labeled proliferation. The second arrow (black to green gradient) starts at the right center of the MSC and splits into 7 different endpoints, each of which is individually named with possible differentiated cell types, and the group is labeled differentiation. The third arrow (black to magenta gradient) emerges from the top center and terminates a short distance to the right in an "X" shape, and is labeled senescence.

Stem Cells & Culture Environments

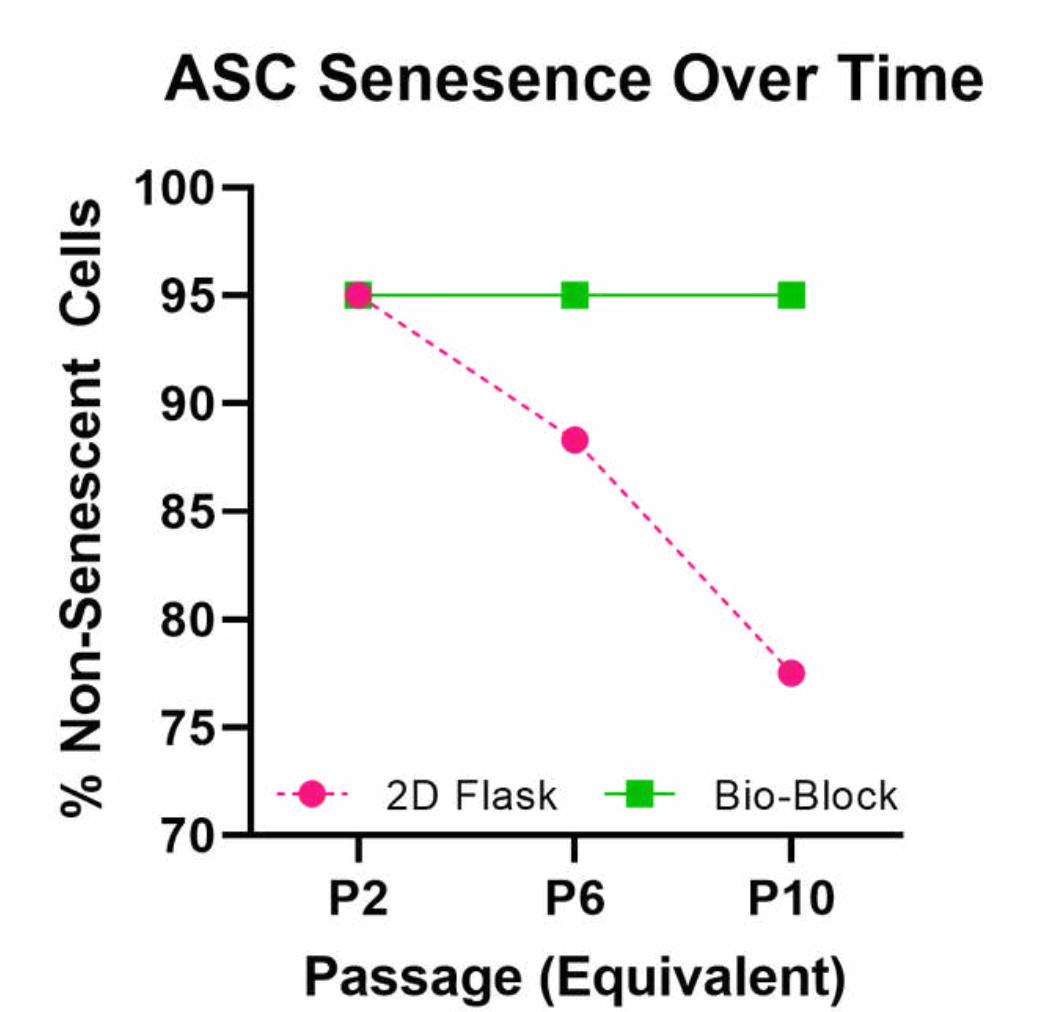
ASCs were continuously cultured in a 2D system and assessed using β-galactosidase staining (commonly used as a marker of senescence, (Ronald & Kindell, 2005)) at passages 2, 6, and 10. ASCs were also cultured in Ronawk's Bio-Blocks, which do not require passaging, and assessed using the same stain at the passage equivalent time points.

Senescence Marker Quantification: Bio-Blocks vs. 2D Traditional Culture

Quantification of the β-galactosidase staining revealed stable, high numbers of non-senescent cells cultured in Ronawk's Bio-Blocks – ~95% non-senescent cells at P2, P6, and P10 equivalents. In 2D culture, the number of non-senescent cells roughly decreased from ~95%, 88.3%, and 77.5% at P2, P6, and P10, respectively. 2.



Senescence Marker Quantification: Bio-Blocks vs. 2D Traditional Culture



A line graph showing the % non-senescent cells in the two culture conditions that were analyzed (Bio-Blocks and traditional 2D culture) at three experimental timepoint equivalents (passages 2, 6 and 10). Data collected from cells cultured in Bio-Blocks are shown using green square symbols paired with a solid green line. Data collected from cell grown in 2D flasks are shown using magenta-colored circles paired with a dashed line. The relative percent of non-senescent cells cultured in Bio-Blocks remained stable, while the relative percent of non-senescent cells cultured in 2D flasks decreased over time.

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Conclusion

These results suggest that the Bio-Block culture system meaningfully reduces the rate of stem cell senescence relative to traditional 2D culture methods. Bio-Blocks provide a promising approach for maintaining stem cell functionality over extended periods of time, which has important implications for tissue engineering and regenerative medicine applications.

Highlights

- The lack of senescence in the Bio-Block suggests that the bio-mimetic culture environment of the block is more conducive to maintaining stem cell functionality over time.

- The difference in the rate of senescence is attributed to the dynamic interaction between the unique and customizable microenvironment of the Bio-Blocks + the cells being cultured.

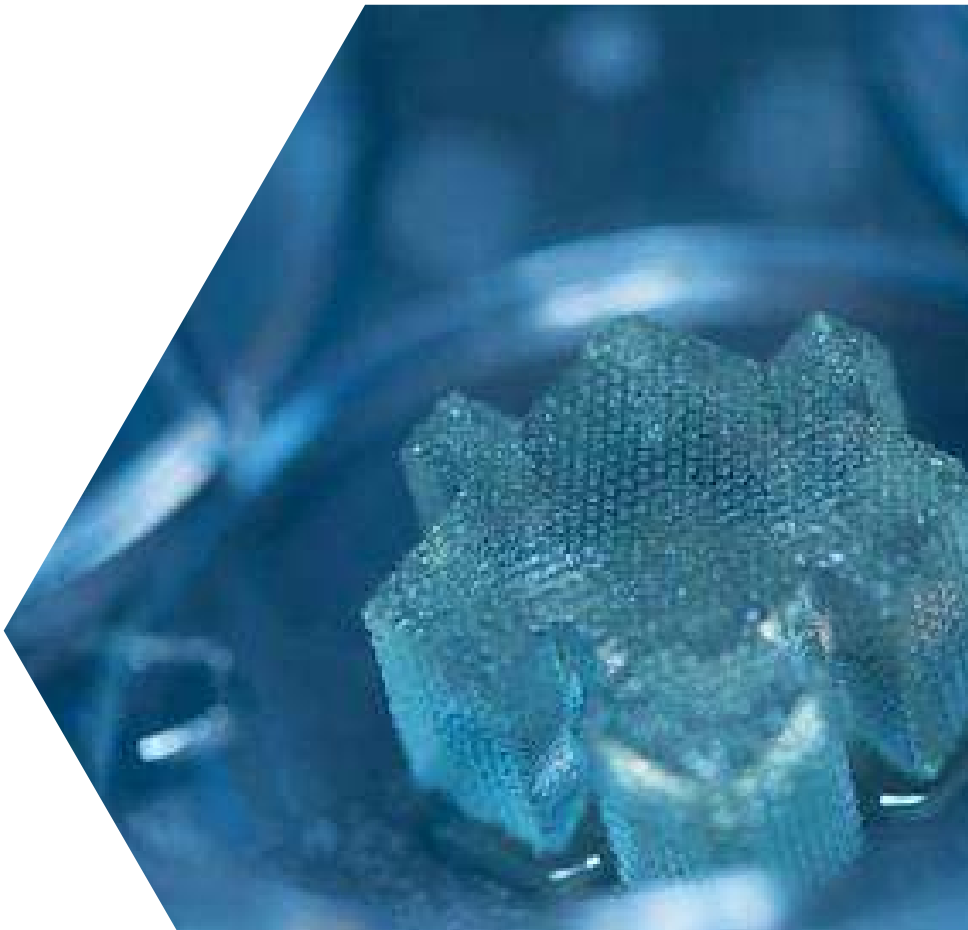
- Ronawk's Bio-Block provides an ideal platform for investigating the effects of bio-mimetic microenvironment on stem cell behavior, as they offer reproducible and customizable formulations that can be tailored to specific research needs.

References

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

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.

Ronald K. Gary, Susan M. Kindell**. Quantitative assay of senescence-associated β-galactosidase activity in mammalian cell extracts.** Analytical Biochemistry. 2005 Aug; 343(2): 329-334. doi.org/10.1016/j.ab.2005.06.003.



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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 contamination risks 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.

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

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Tissue Engineering Parts A, B and C

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