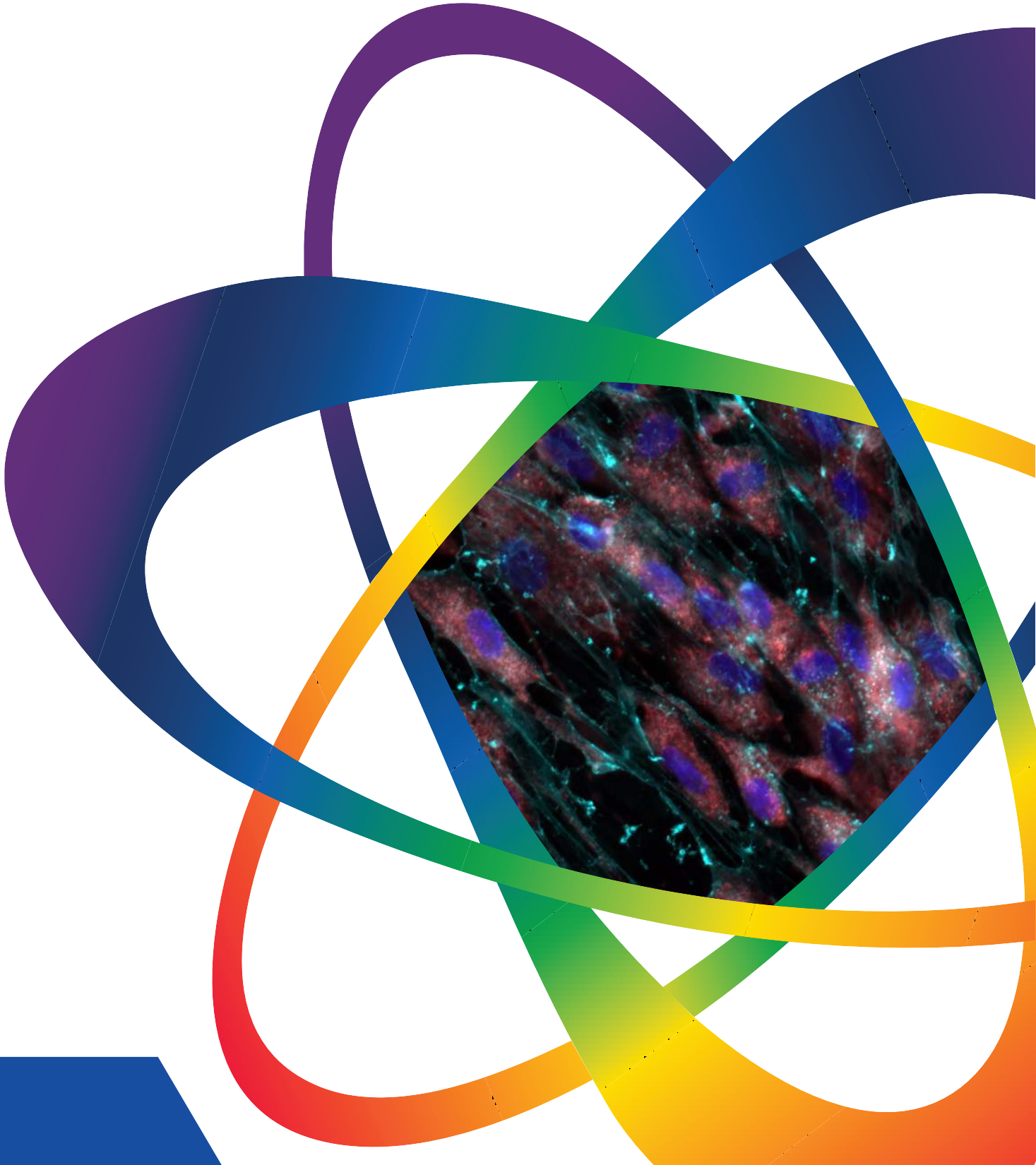
Senescence-Associated Gene Expression Improvements for Cells Cultured in Bio-Blocks™

Ronawk, Inc.

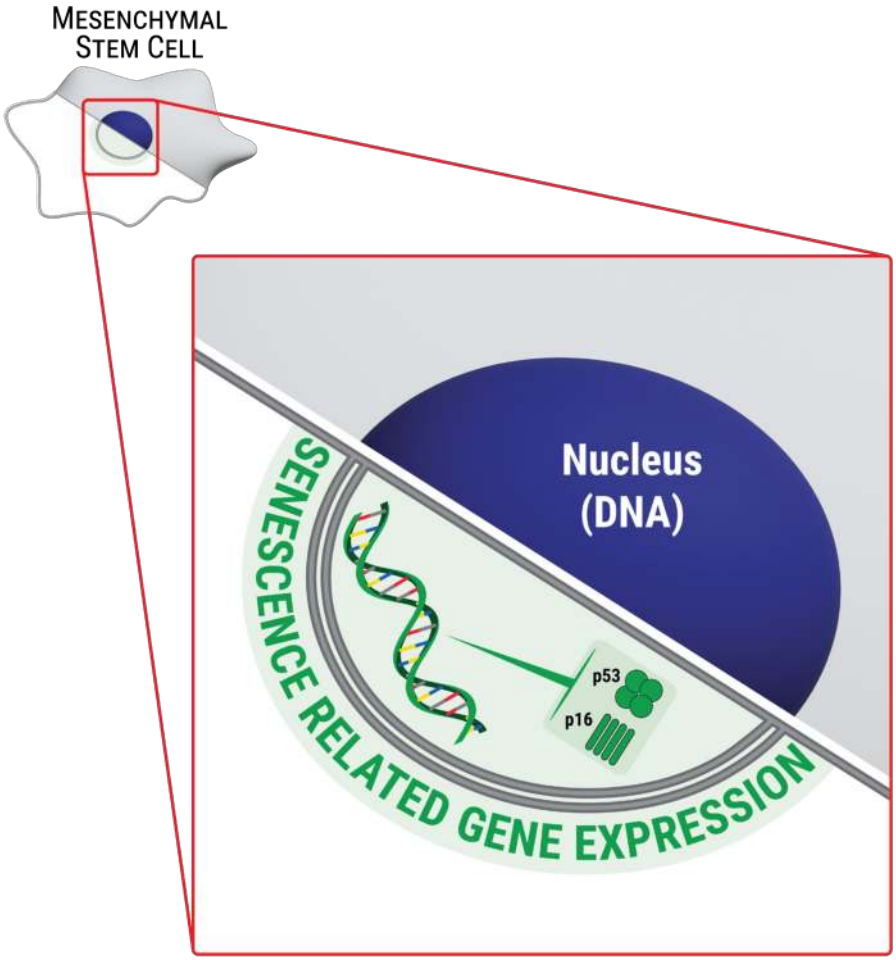


Introduction

When mesenchymal stem cells (MSCs) are grown in culture they will become senescent over time. Senescence is a progressive mix of negative changes that impact processes like growth, division, and differentiation. It is like aging for other organisms. Senescence directly impacts individual cells, and also indirectly impacts the culture environment. When cells become senescent the secreted factors made by the cells change (e.g. extracellular vesicles, proteins, etc…). This change has a negative, ripple effect in the culture environment. It is similar to how yawns are contagious - when one person in a room yawns many others do too. These direct and indirect effects of senescence present a challenge when working with MSCs; it limits how useful these cells and their secreted products can be in therapeutic applications.

A cell culture platform that maintains greater numbers of non-senescent cells for longer periods of time would address this limitation. We've previously shown data from fluorescent protein staining for senescence markers. These data suggest that MSCs grown in the Bio-Blocks remain non-senescent for longer compared to traditional, 2D culture (Ronawk Inc., 2023). However, looking at proteins only tells part of the story, and it's like skipping ahead to the ending. Proteins are made by following instructions from genes. P16 and P53 are two key genes involved in senescence regulation. Gene expression data for P16 and P53 tells a more complete story about how Ronawk's Bio-Blocks reduce senescence and improve MSC culture.

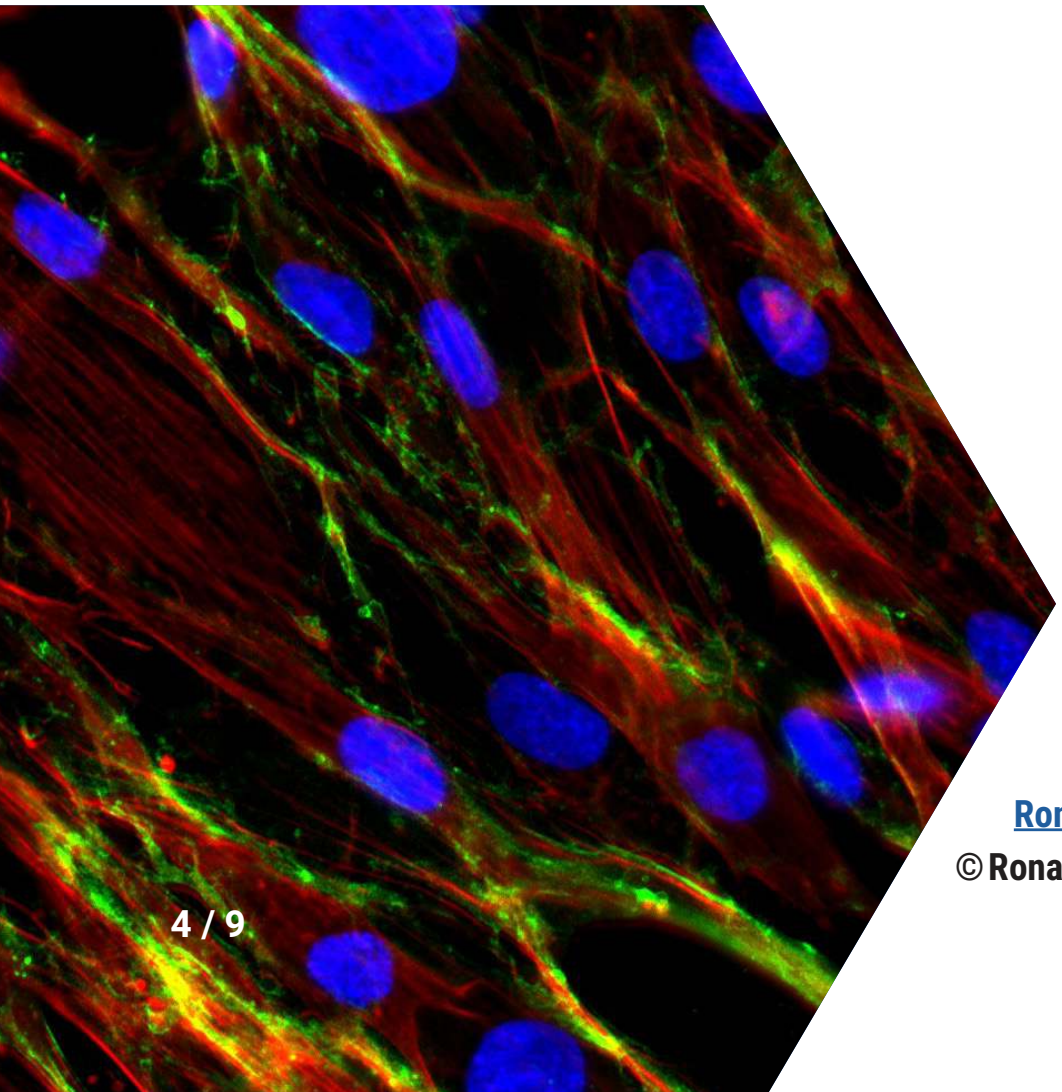
Senescence Gene Expression



In the upper left corner of the image is a small mesenchymal stem cell (MSC) that is divided in half by a diagonal line. A small red square outlines the area around the nucleus. The small red is connected to a large red square in the lower right part of the illustration that shows an enlarged image of the area around the cell's nucleus. The upper right half of the cell is shown with 3D shading. The upper right half of the nucleus is labeled "Nucleus (DNA)". The lower left half of the cell is a simplified cutaway image that focuses on the nuclear membrane and space just outside the nucleus. The nucleus has a green DNA double helix. It is on a background that is a light green circle that extends slightly beyond the borders of the nucleus. There is bold green text along the outer edge of the nucleus that says "senescence related gene expression". A bold, green line extends from the nucleus to the two proteins that are the products of the expressed genes.

Cell growth environments: Bio-Blocks and 2D flasks

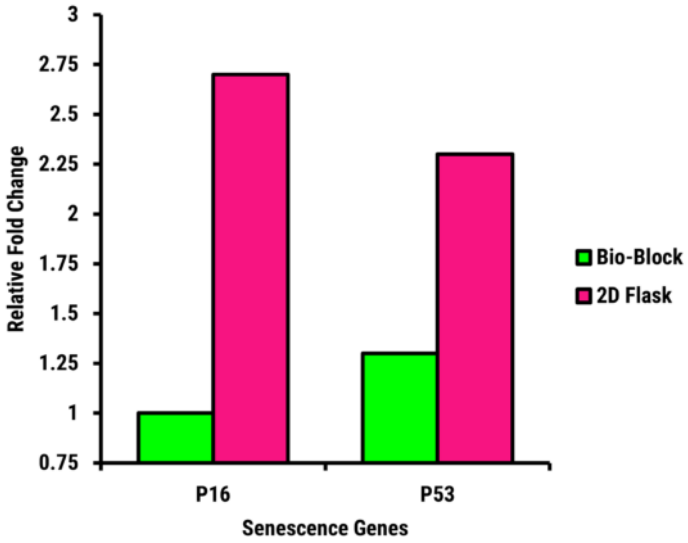
Adipose-derived MSCs (ASCs) were cultured in both the 3D Bio-Block and traditional 2D T-flasks . The 2D culture environment required subculturing while the Bio-Blocks did not. As a result, passage-equivalent time points were utilized as an alternative to passage numbers.



Gene Expression Analysis

Two specific genes, P16 and P53, were chosen because of their roles in senescence. Changes in gene expression from baseline were individually measured for both genes. Higher levels of gene expression indicate more senescence in the cell culture. Both genes were expressed at higher levels in cells cultured in 2D flasks compared to cells cultured in bio-blocks. A value of 1 indicated no change from baseline. Both genes were expressed at near baseline levels in cells cultured in the Bio-Blocks. This suggests minimal senescence in the bio-block culture environment. Complete results and statistical analysis can be found in (Hodge et. al. 2023).

Gene Expression Analysis



A clustered bar graph with two pairs of bars showing gene expression related to senescence. For each pair of bars the Bio-Blocks data are represented by green filled bars on the left and 2D flask data are represented by magenta bars on the right. The x-axis has two segments and is labeled "Senescence Genes" – p16 is in the left segment and P53 is in the right segment. The y-axis is labeled "relative fold change". There is also a dotted, reference line at 1 on the y-axis. This line represents no change in gene expression. For both genes the Bio-Blocks are close to 1, indicating no to minimal change.

Conclusion

For both P16 and P53, two senescence related genes, increased gene expression suggests higher numbers of senescent cells in culture. Both genes were expressed at higher levels in cells cultured in traditional 2D flasks relative to the bio-mimetic microenvironment of Ronawk's Bio-Blocks. In contrast, gene expression levels at or near 1 suggest minimal to no change and fewer senescent cells in culture.

The Bio-Blocks are able to maintain cell populations that have minimal to no change in expression of senescence genes over time. The gene expression data for both genes aligns with and further supports the findings in the previously discussed senescence protein staining (Ronawk Inc., 2023).

Reducing the amount of senescence in culture directly benefits the individual cells in the culture environment. Non-senescent cells have normal, healthy cell processes. This means they can grow, divide, and produce secreted biological products (e.g. extracellular vesicles, proteins etc…) without the negative impacts of senescence. Reducing senescence also indirectly benefits the entire cell population in culture. Senescent cells produce different secreted products than non-senescent cells. The profile of secreted products associated with senescence can negatively impact the whole cell culture, which includes the non-senescent cells in that culture environment.

Bio-Blocks' ability to reduce senescence can help get the most out of your cell culture. Reach out and schedule a time to discuss and learn more: Connect With The Ronawk Team.

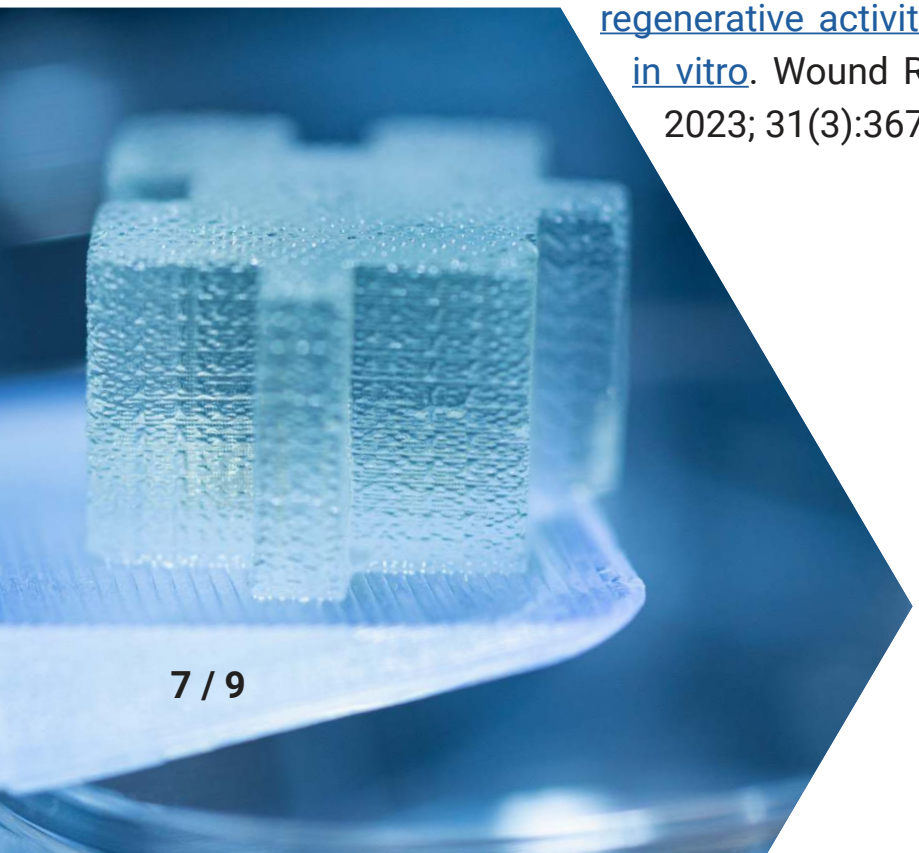
Highlights

- **P16 and P53 gene expression indicate reduced senescence in cells cultured in Bio-Blocks compared to cells grown in traditional 2D flasks.**
  - **This further supports protein staining data (Ronawk 2023) that shows Bio-Blocks reduce senescence in cultured cells.**
- **Reduced senescence gives more flexibility in cell culture based experiments, since it allows for longer time points.**
- **The material of the Bio-Blocks could be coated (e.g. collagen, fibrin etc..) to further augment the benefits of the bio-mimetic culture environment.**

References

Ronawk, Inc. (2023). "Bio-Blocks Enable Improved Retention of Stem-like Surface Markers for Mesenchymal Stem Cells". https://ronawk.com/category/white-papers/. (accessed July 31, 2023).

Jacob G Hodge, Heather E. Decker, Jennifer L Robinson & Adam J Mellott. Tissue-mimetic culture enhances mesenchymal stem cell secretome capacity to improve regenerative activity of keratinocytes and fibroblasts in vitro. Wound Repair and Regeneration. February 2023; 31(3):367-383. doi: 10.1111/wrr.13076.



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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MLA

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Tissue Engineering Parts A, B and C

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