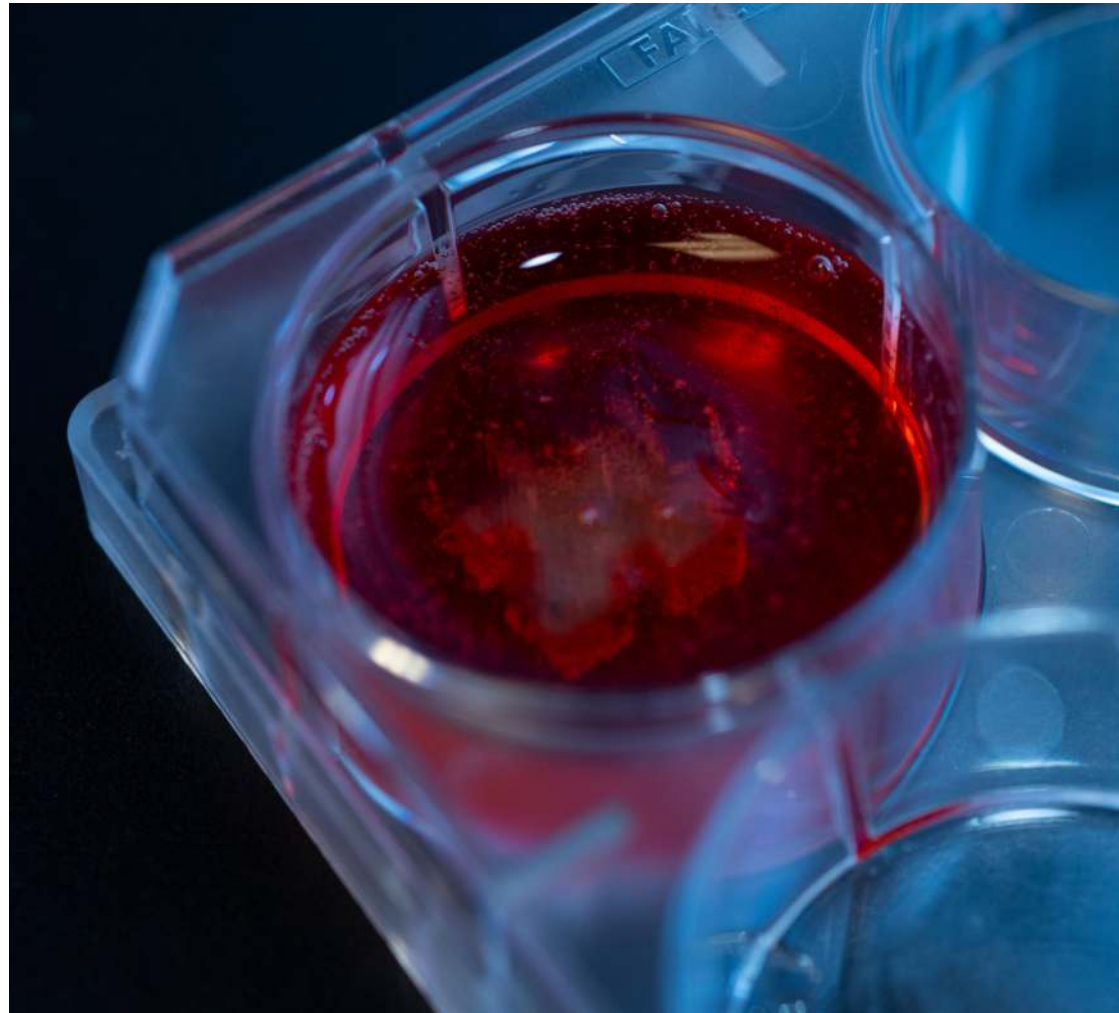


Ronawk, Inc.



## **Bio-Blocks & Conditioned Media: Improved Retention of Wound Healing Capacity when Treating Cells**





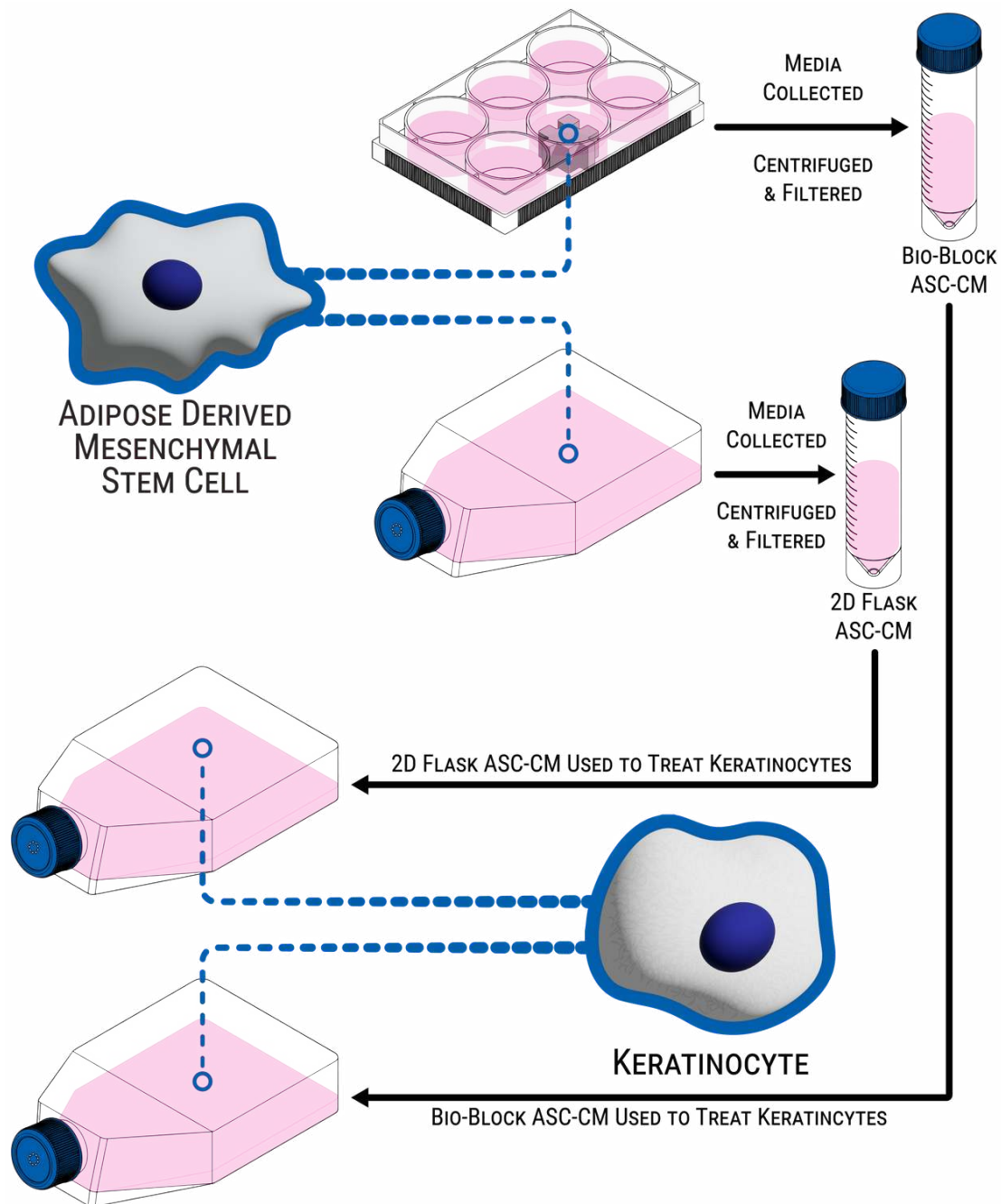
## Introduction

Wound healing processes restore tissue structure and function following injury, and a range of treatments are available to facilitate the process – antibiotics, dressings, and surgery. Unfortunately, some wounds do not respond to available treatments, and alternative therapies are needed in these situations

Adipose-derived mesenchymal stem cells (ASCs) have the ability to secrete bioactive molecules that can stimulate and modulate tissue repair and regeneration. Conditioned media (CM) collected from culturing these cells is emerging as a promising, cell-free therapy for wound healing. In a lab setting, ASC-derived CM (ASC-CM) can treat other cell populations (i.e. keratinocytes). Treatment of keratinocytes with ASC-CM modulates wound-healing activity (migration, metabolic activity, and proliferation).

Previous white papers have shown that the bio-mimetic environment in Roanwk's Bio-Blocks results in a more robust stem cell population with increased maintenance of stemness (Ronawk, Inc.(2023a)) and delayed senescence (Ronawk, Inc. (2023b)). If the stem cells cultured in the Bio-Blocks are of higher quality due to the bio-mimetic culture microenvironment, then will the Bio-Block's advantages also extend to improving the quality of conditioned media?





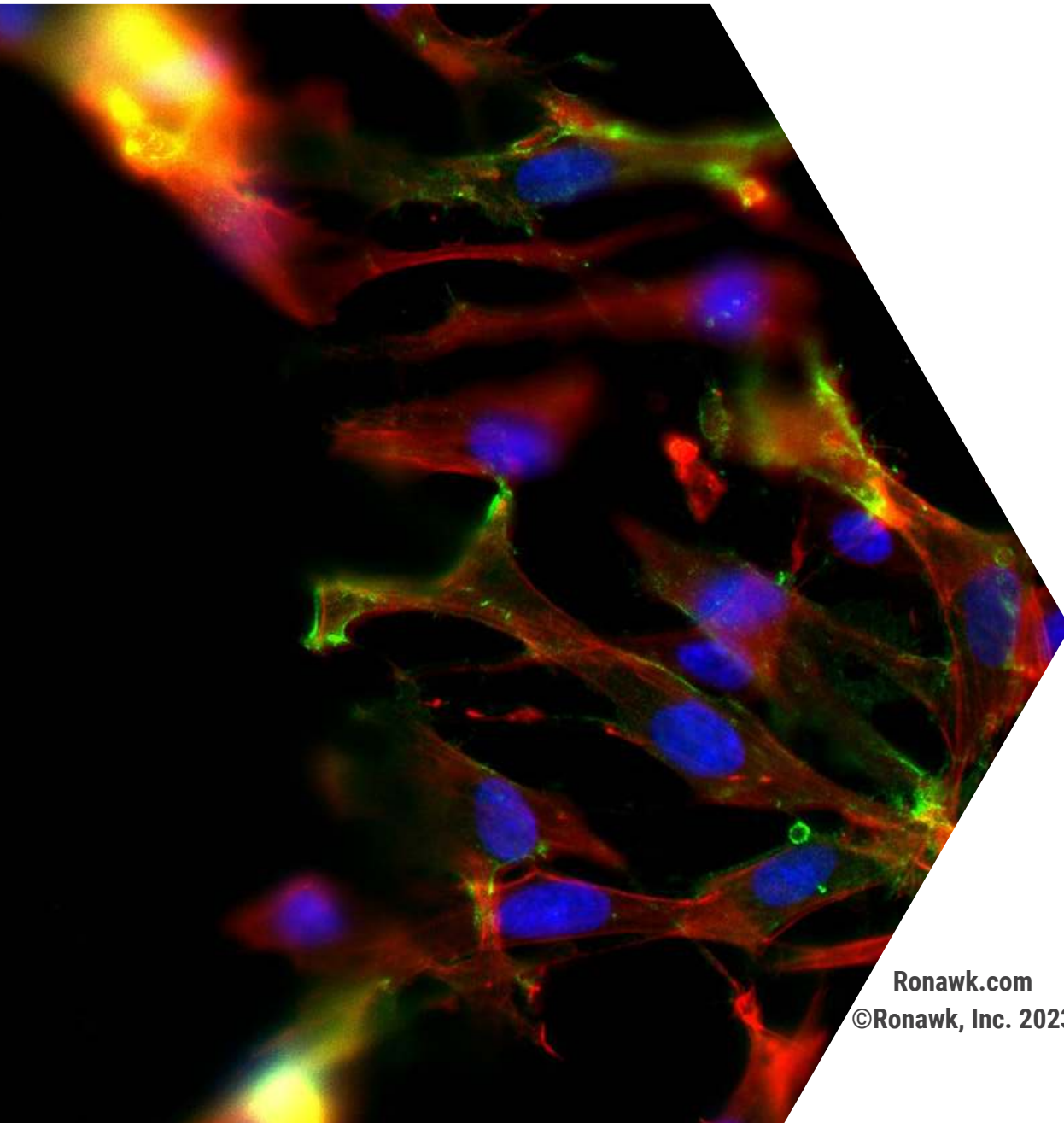
The figure illustrates two parallel workflows for collecting adipose-derived mesenchymal stem cell conditioned media (ASC-CM), which is then used to treat keratinocytes grown in 2D flasks. The workflow begins in the top, left corner and proceeds clockwise around the image. The workflow starts with an ASC connected to culture environments (Bio-Block in a 6-well plate or 2D flask) to the right by a dashed blue line. A black line with an arrowhead on the right connects the culture environment to a centrifuge tube filled with pink-conditioned media. The black line is labeled with “media collected, centrifuged, & filtered”. Another black line with an arrowhead on the right proceeds downward and to the right, connecting the centrifuge tubes with conditioned media to 2D culture flasks containing keratinocytes. The black line is labeled with “[source of the conditioned media, Bio-Block ASC-CM or 2D flask ASC-CM] used to treat keratinocytes”. In between these two parallel lines, a keratinocyte (outlined in blue) is shown and connected to the 2D culture flasks by a dashed blue line.



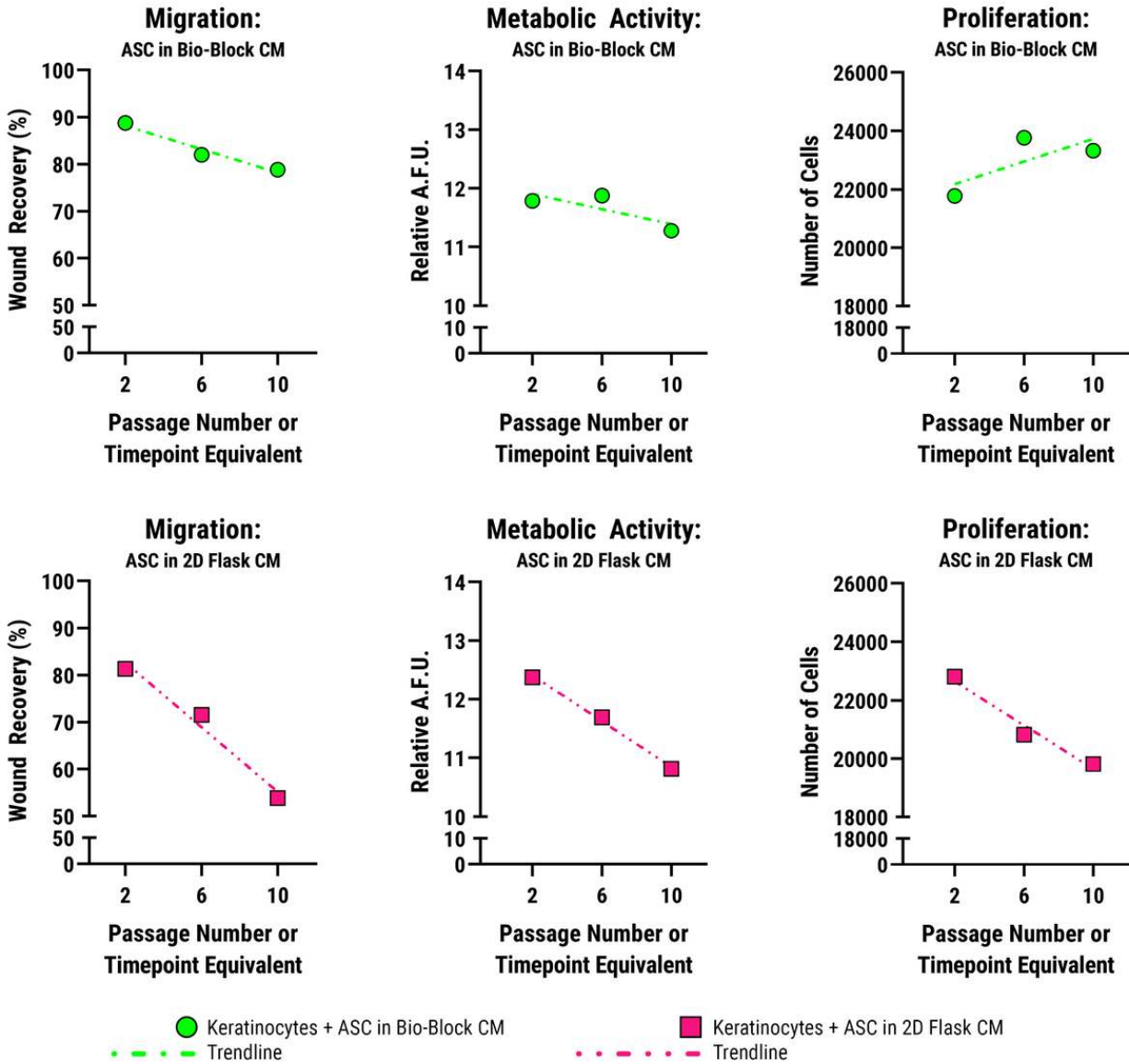


## Cells & Culture Conditions

ASCs were cultured in 2D flasks and in the Bio-Block, and the ASC-CM was separately collected from both culture environments at passages 2, 6, and 10 or time-point equivalents since Bio-Blocks do not require passaging. Keratinocytes were then treated with the ASC-CM, and their migratory, metabolic, and proliferative activity were assessed.







A two-row, three-column grid of graphs showing data collected from assays where keratinocytes were treated with CM derived from different sources. The top row shows data from assays that used CM derived from ASCs in Bio-Blocks (green circles with black outlines), and the bottom row shows data from assays that used CM derived from ASCs grown in 2D flasks (magenta squares with black outlines). A trend line is shown on each graph to generally indicate the general performance of the treated keratinocytes changes over time (repeating pattern of a dot followed by a dash (• -) in green for Bio-Block CM or a repeating pattern of two dots and a dash (• • -) in magenta for 2D flask). The left column displays migration assay data, the center column displays metabolic activity assay data, and the right column displays proliferation assay data. The x-axes on all graphs are for “passage number or timepoint equivalent” and the y-axes on graphs are assay specific – migration uses “wound recovery (%)”, metabolic activity uses “relative A.F.U.”, and proliferation uses “number of cells”. A legend is shown at the bottom of the figure.





## Conclusion

Keratinocytes treated with CM derived from ASCs cultured in 2D flasks showed similar results in the migration, metabolic activity, and proliferation assays – the trend was toward generally decreasing performance as the number of passages increased (magenta trendlines). These same trends were not observed in keratinocytes treated with CM derived from ASCs cultured in Bio-Blocks – the trend observed was a comparatively stable performance as the number of passage timepoint equivalents increased (green trendlines). Comprehensive statistical analysis can be found in Hodge et. al 2022.

We believe the data shown in this white paper further illustrates a strength of the bio-mimetic culture environment of the Bio-Block culture system - improved quality of conditioned media as a result of more robust stem cell culture relative to traditional, 2D flask-based culture systems. The Bio-Blocks allow MSC populations to retain more of their overall regenerative and wound healing capacity over time, and these benefits appear to extend to collected conditioned media as well. In future white papers, we will further explore these core ideas as they relate to wound healing, and how Bio-Blocks can be customized to tailor the conditioned media.

**"The Bio-Blocks allow MSC populations to retain more of their overall regenerative and wound healing capacity over time, and these benefits appear to extend to collected conditioned media as well."**





# Highlights

- Higher quality CM is produced by cells cultured in Bio-Block bio-mimetic culture system compared to traditional 2D culture flasks, as evidenced by the performance of treated keratinocytes on migration, metabolism, and proliferation assays.
- Customization of surface coatings could be used to further enhance the quality and potential applications of CM derived from cells cultured in Ronawk's Bio-Blocks.



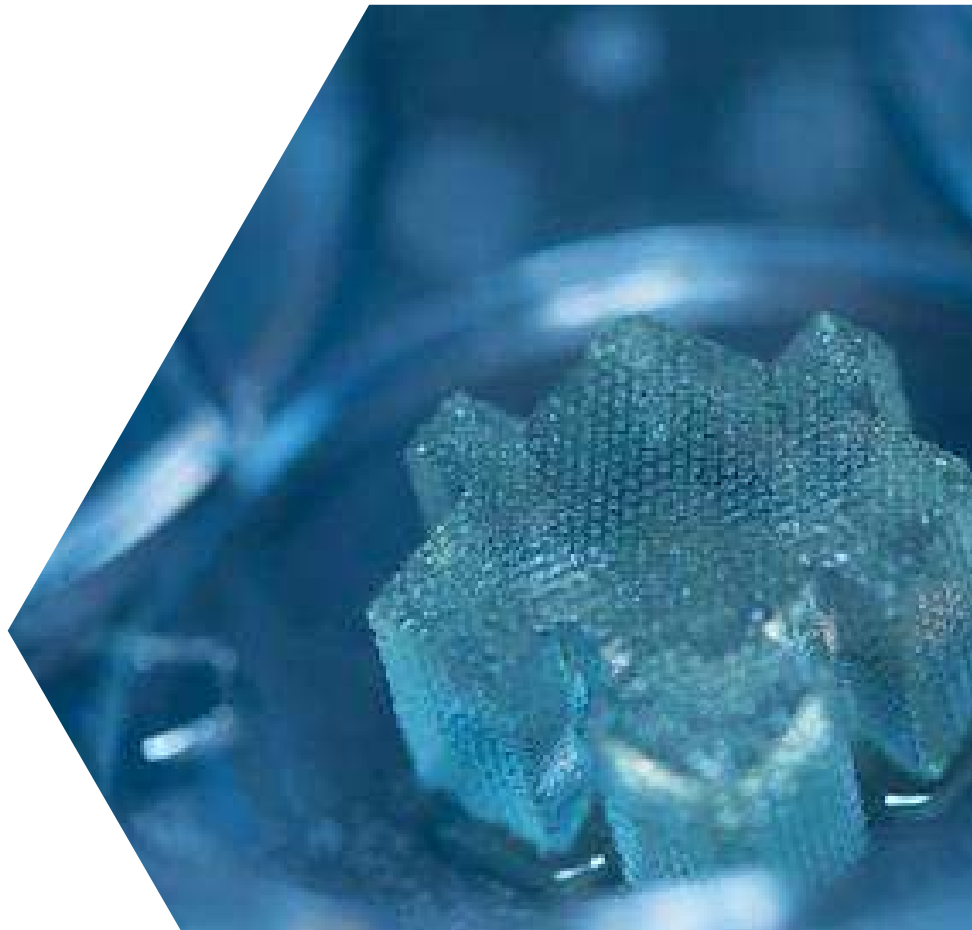


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Ronawk, Inc. (2023a). **“Bio-Blocks Enable Improved Retention of Stem-like Surface Markers for Mesenchymal Stem Cells”**. <https://ronawk.com/category/white-papers/>. (July 2023).

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Jacob G Hodge, Jennifer L Robinson & Adam J Mellott. **Novel hydrogel system eliminates subculturing and improves retention of non-senescent 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 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 improve the retention of wound healing capacity?

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

[Ronawk.com](https://ronawk.com)

[info@Ronawk.com](mailto:info@ronawk.com)

[Schedule A Meeting](#)



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## APA

Ronawk, Inc. (2023). Bio-Blocks & Conditioned Media: Improved Retention of Wound Healing Capacity when Treating Cells [White Paper]. Ronawk.com/bio-blocks-conditioned-media-improved-retention-of-wound-healing-capacity-when-treating-cells

## MLA

Ronawk, Inc. "Bio-Blocks & Conditioned Media: Improved Retention of Wound Healing Capacity when Treating Cells." <https://ronawk.com/category/white-papers/>. Date of online publication. **DATE OF ACCESS**.

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## Tissue Engineering Parts A, B and C

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