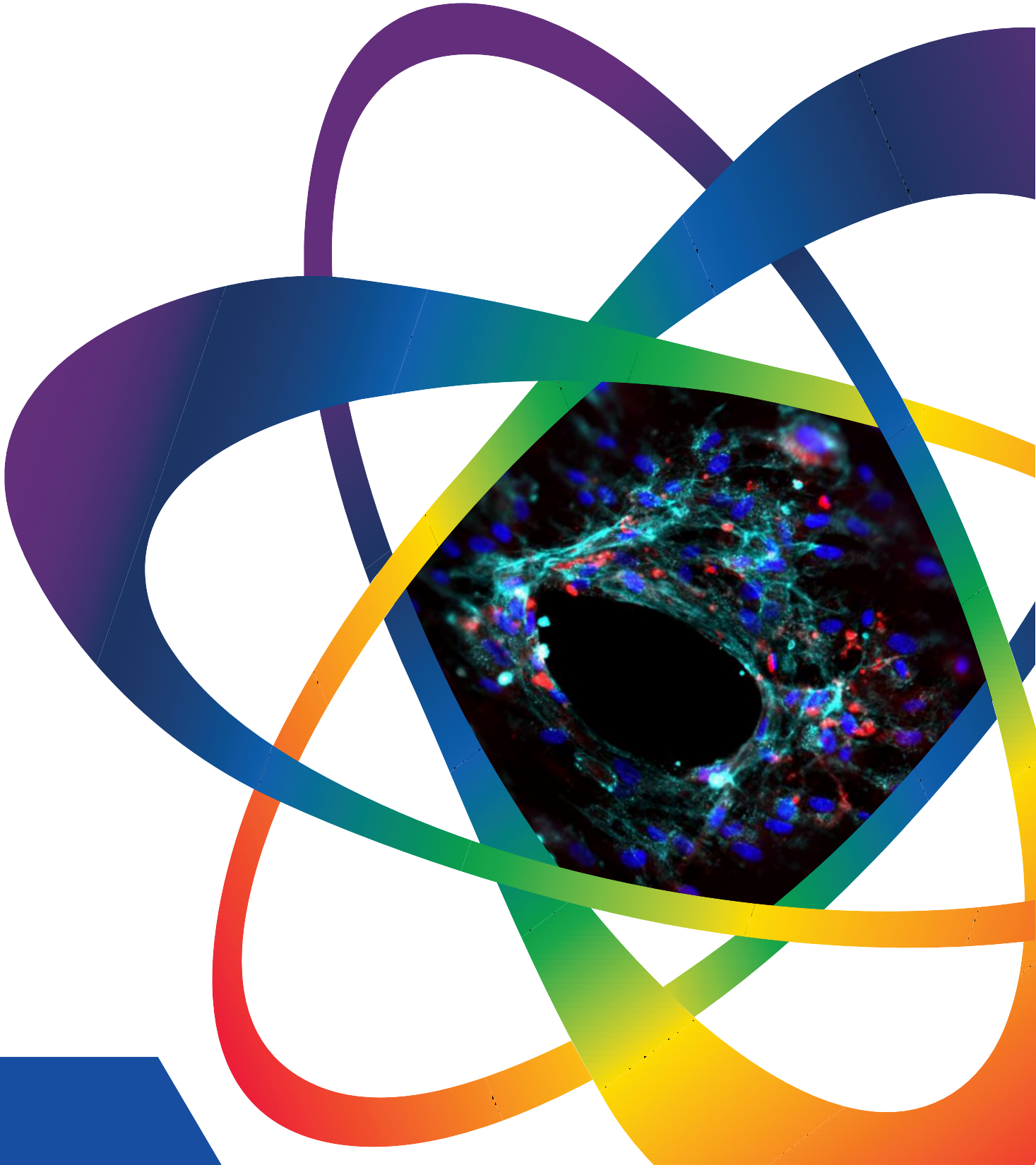
Extracellular Vesicle Production Increases in Cells Cultured in Bio-Blocks vs. 2D Flasks

Ronawk, Inc.

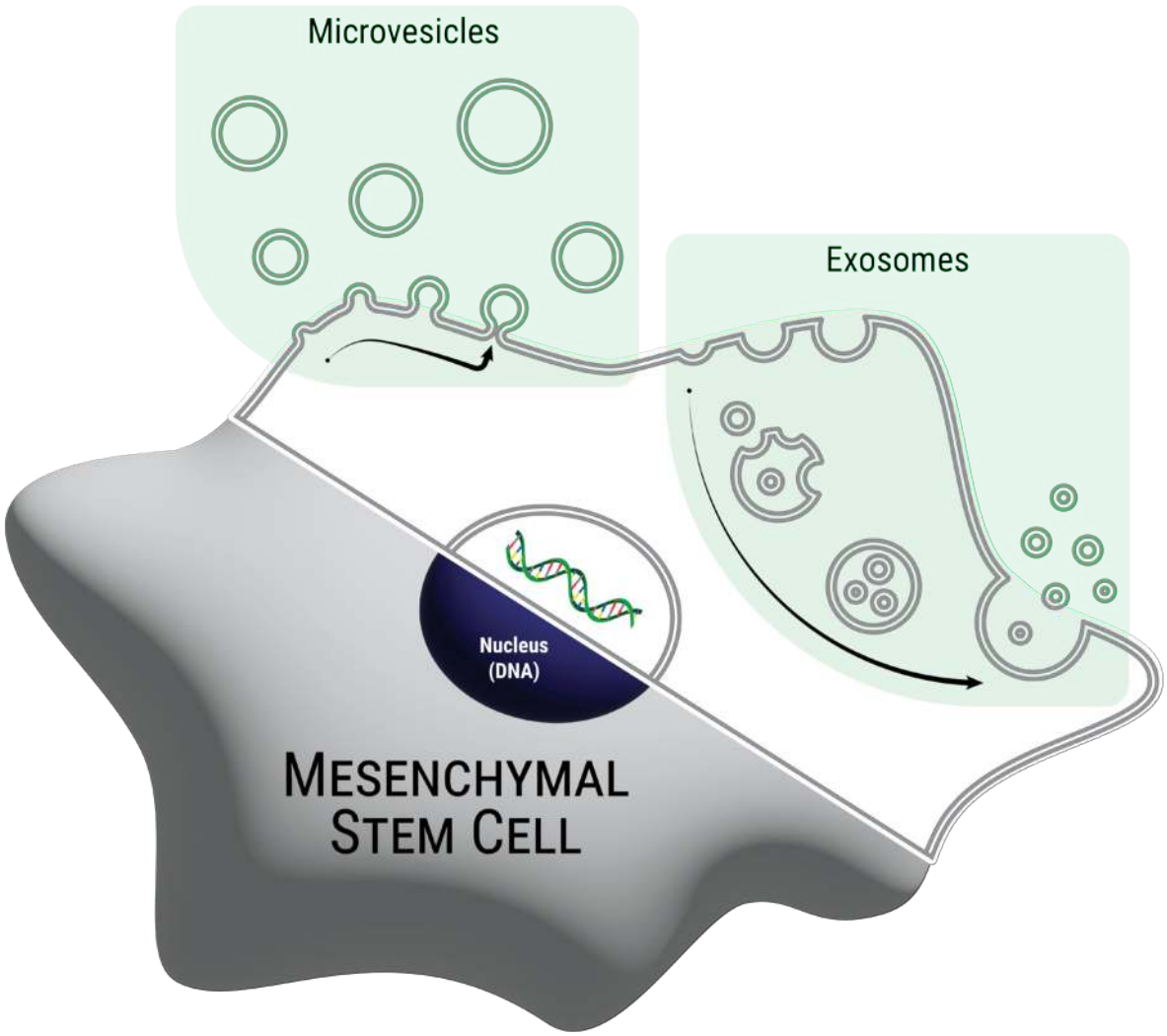


Introduction

Cells make and secrete extracellular vesicles (EVs) such as microvesicles and exosomes. EVs are small, membrane-bound structures that can contain a range of biological products. They function like microscopic cargo containers, transporting their contents around the microenvironment. EVs make up an important part of the secretome. These secreted products have a wide range of potential medical and research applications.

The quality of the EVs produced is related to the health of the cells. Cells cultured in Bio-Blocks are healthier as a result of the bio-mimetic culture environment - reduced senescence (Ronawk, Inc. (2023a), maintained stemness (Ronawk, Inc. (2023b). Conditioned media (CM) generated using these cells also benefits treated cell populations (Ronawk, Inc. (2023c). This suggests that the EVs produced by cells grown in Bio-Blocks are generally of high quality. Further exploration of these CM components will allow us to better understand the benefits provided by the Bio-Blocks. How many EVs are produced, and how large are they?

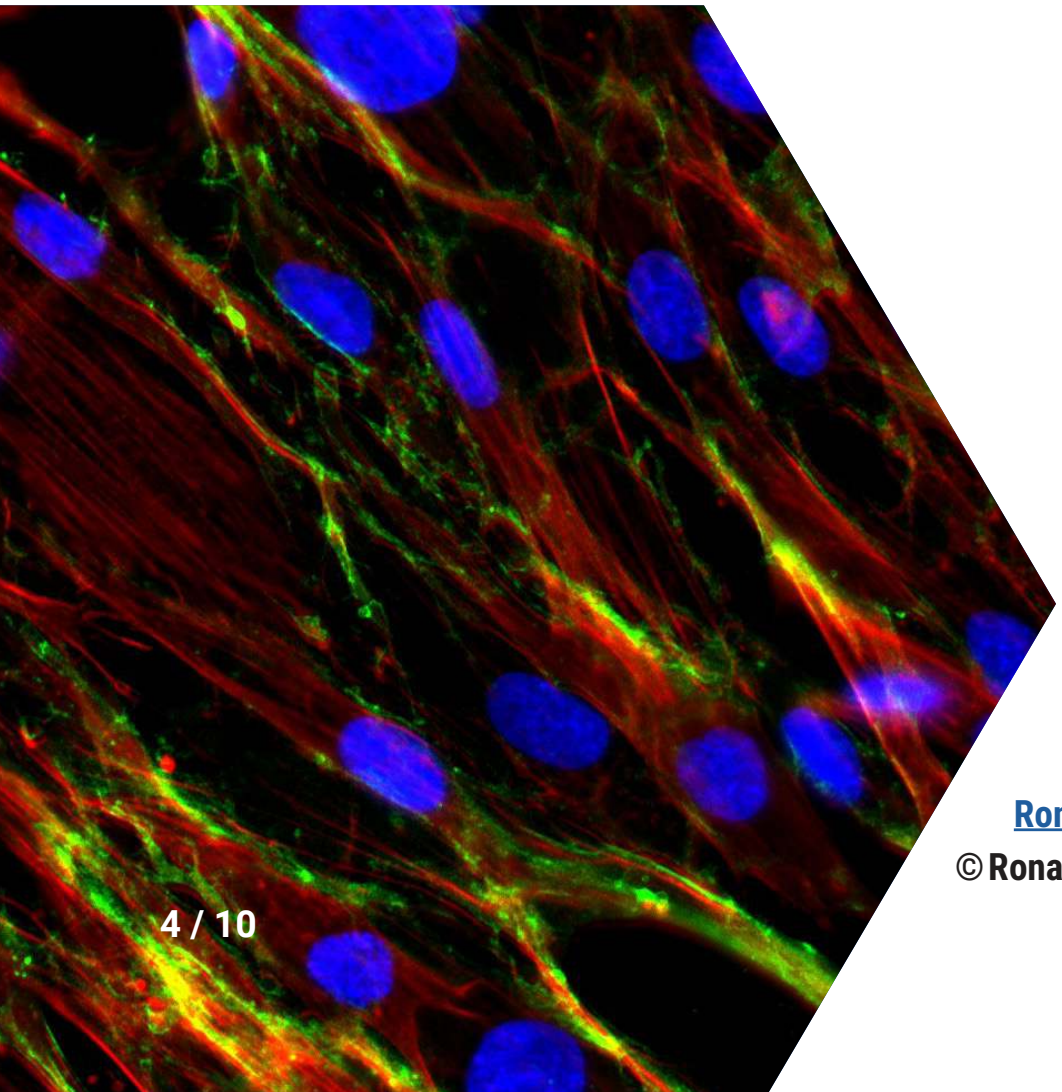
Extracellular Vesicles



A mesenchymal stem cell is divided in half by a diagonal line passing from the upper left through the central nucleus and down to the lower right. The lower left half of the cell is shown with 3D shading. It is labeled "mesenchymal stem cell" and the lower left half of the nucleus is labeled "Nucleus (DNA)". The upper right half of the cell is a simplified cutaway image that focuses on the cell membrane and extracellular vesicle production. Microvesicle production is shown on the left side of the cut-away, and exosome production is shown on the right side of the cutaway.

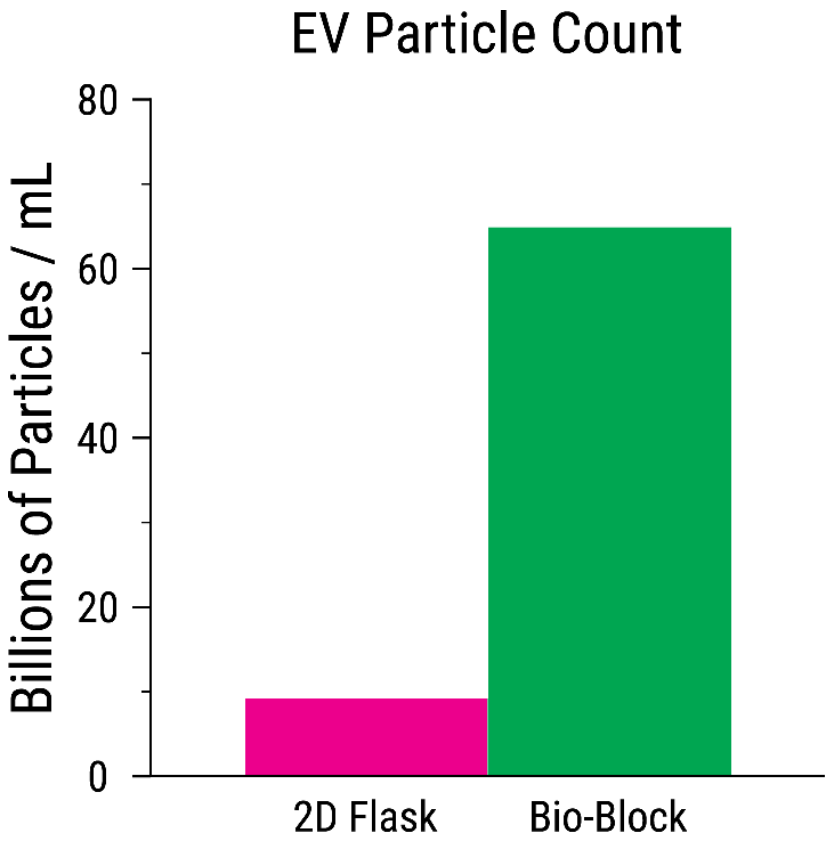
Cell growth environments: Bio-Blocks and 2D flasks

Adipose-derived stem cells (ASC) were cultured in 2D flasks and in the Bio-Block to create the CM. The CM was separately collected for protein analysis from both culture environments at passage 3 or the time-point equivalent since Bio-Blocks do not require passaging. Individual cultures of Keratinocytes and Fibroblasts were then individually treated with the ASC-conditioned media.



EV Particle Analysis

A protein array was used to analyze the amount of specific proteins present in the CM. The highlights of the results are shown in the bar graph (below). Full statistical analysis can be found in (Hodge et. al. 2023). Growth factors and cytokines were the proteins that were appreciably enhanced in CM collected from cells cultured in Bio-Blocks. These two classes of proteins are instrumental in biological processes like wound healing.



A bar graph comparing the number of EV particles produced by cells cultured in a 2D flask environment and the Bio-Block's bio-mimetic microenvironment. The bars representing the number of EVs from the 2D flask and Bio-Blocks are on the left (solid magenta) and right (solid green), respectively. The empty boxes with dashed, magenta outlines immediately above the solid magenta bar represent the same number of EVs as the solid magenta bar. There are 6 of these empty bars in total. When the solid and empty magenta bars are combined there are a total of 7, which are almost the same height as the single green bar. The cells cultured in Bio-Blocks produce ~7X more EVs than cells grown in 2D flasks.

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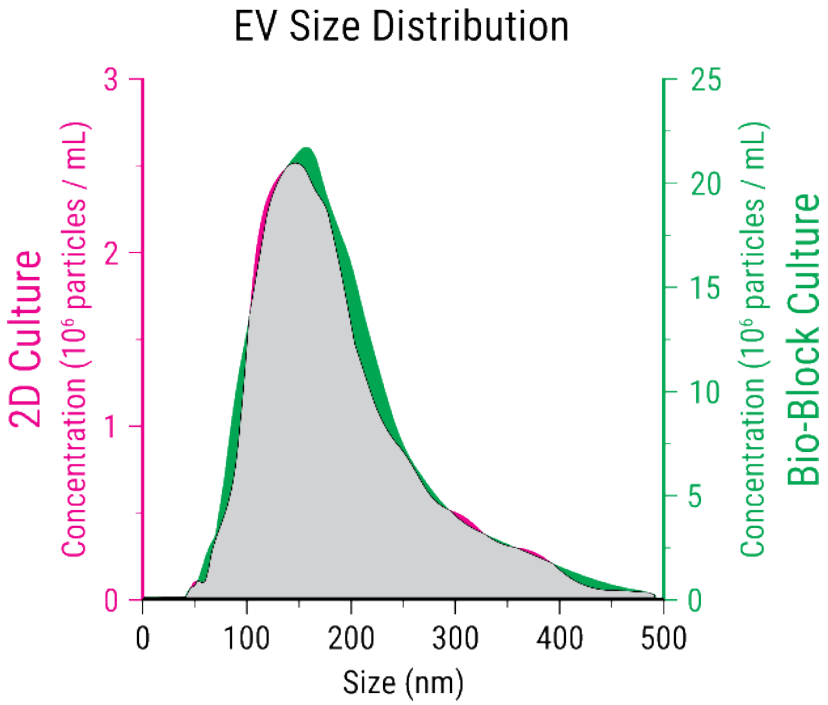
The graph of EV particle counts (previous page) shows a large difference between the Bio-Block and 2D-flask culture environments. Cells grown in the Bio-Blocks produce more EVs. The size of EVs is an additional important factor, but this graph does not show size distribution. These same count data were graphed as a function of size (graph, below):



A shaded line graph with two overlapping curves where each curve represents the EV size distribution for its respective culture condition. EVs grown in 2D flasks are shown in magenta (scale on left y-axis). EVs grown in Bio-Blocks are shown in green (scale on right y-axis). Light gray is used to indicate overlap between the two conditions. The majority of the graph is gray, indicating a highly similar size distribution.

In the size distribution graph graph (above) areas of overlapping size distribution are indicated by gray shading. Small areas of either magenta (2D flask) or green (Bio-Blocks) are visible around the edges of the gray, but it's a comparatively minor variance. Gray is the most abundant color of shading visible. It suggests that EV production in both environments results in EVs of highly similar sizes.

Conclusion

The bio-mimetic microenvironment of the Bio-Blocks greatly enhances EV production compared to traditional, 2D flask-based culture. The sizes of the EVs produced in both the Bio-Block and 2D flask environments are highly similar. The similarity in size distribution suggests the Bio-Block microenvironment primarily changes the number of EVs produced.

The power of the Bio-Block platform for EV production comes from its ease of use. Swapping a 2D flask for a Bio-Block, but keeping the cells cultured and media the same, produces more EVs. Bio-blocks also do not require passaging. It's simpler to grow the cells over time while continuing to collect media for EV isolation.

Bio-Blocks' ability to enhance EV production can help get the most out of your cultured cell populations. Reach out and schedule a time to discuss and learn more: Connect With The Ronawk Team.

Highlights

- **7X increase in extracellular vesicle production in a Bio-Block culture environment vs. 2D flask**
- **Highly similar EV size distribution for the Bio-Block and 2D Flask culture environments**
- **Potential for bioactive coatings on the Bio-Blocks to further modulate EV production**

References

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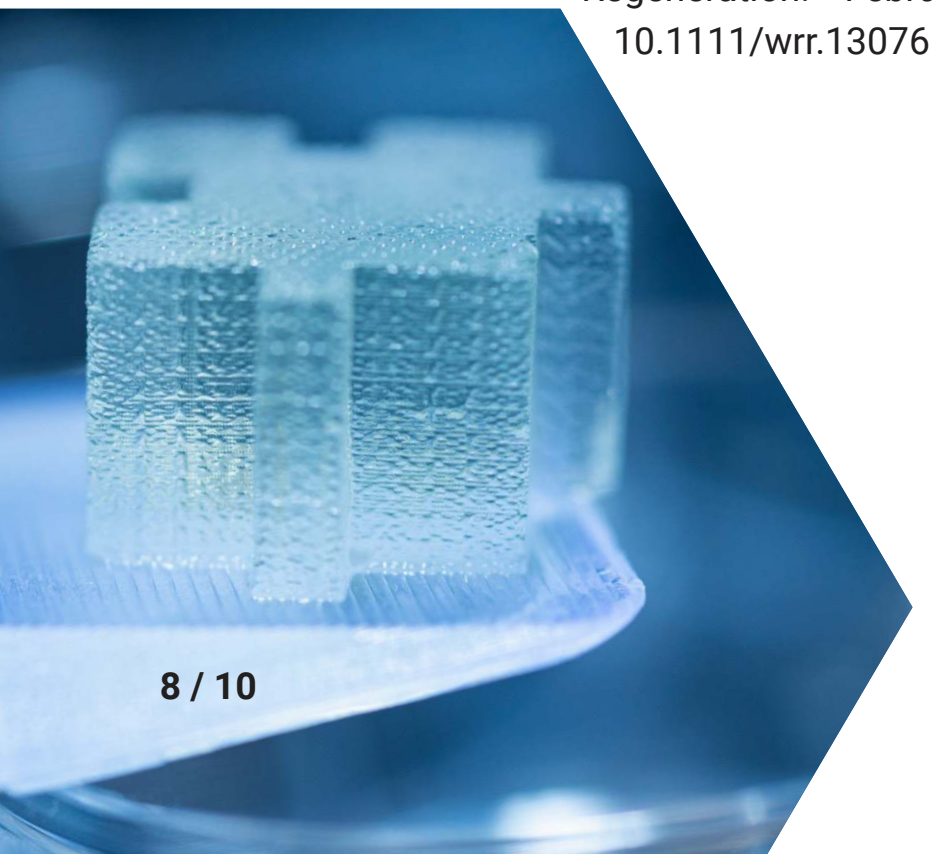

About Ronawk

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 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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Ronawk, Inc.. (2023). Extracellular Vesicle Production Increases in Cells Cultured in Bio-Blocks vs. 2D Flasks [White Paper]. URL for final, published PDF on website.

MLA

Ronawk, Inc.. "Extracellular Vesicle Production Increases in Cells Cultured in Bio-Blocks vs. 2D Flasks." https://ronawk.com/category/white-papers/. Date of online publication. DATE OF ACCESS.

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Tissue Engineering Parts A, B and C

Ronawk, Inc. Extracellular Vesicle Production Increases in Cells Cultured in Bio-Blocks vs. 2D Flasks [Internet]. 2023 [cited date YEAR MONTH DAY]. Available from: URL for final, published PDF on website.

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