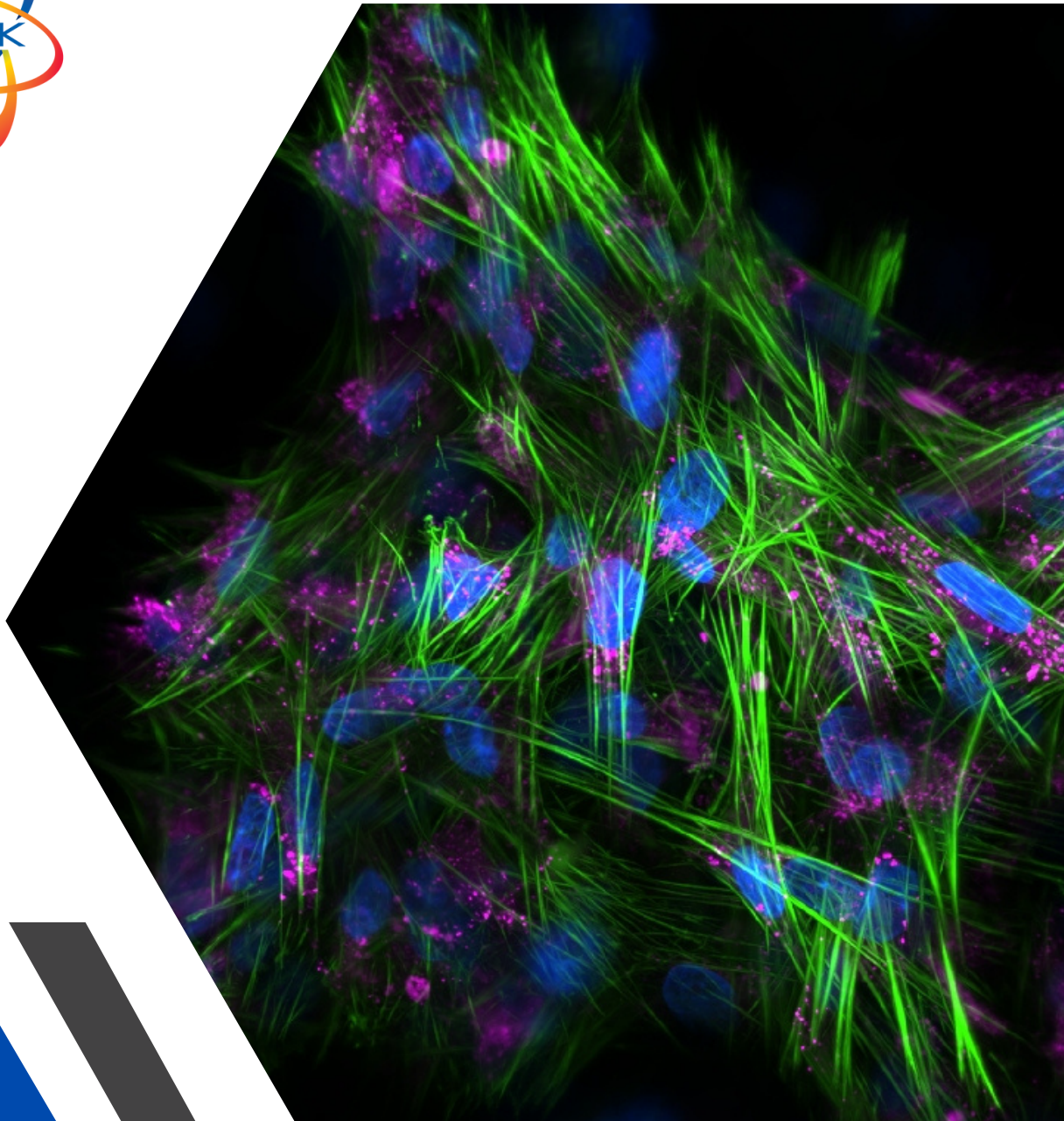


Ronawk, Inc.



# The Bio-Block™: A Game-Changer in Tissue Culture



## Introduction

Adipose-derived stem cells (ASCs) hold immense potential in regenerative medicine, thanks to their ability to secrete a diverse array of bioactive compounds that promote wound healing and tissue regeneration. However, traditional 2D culture systems have limitations that lead to the loss of 'stem-like' properties and reduced secretion of bioactive compounds. This is where Bio-Blocks, a novel 3D tissue-mimetic porous hydrogel system, offers a more effective solution to overcome these challenges.

## Advantages of the Bio-Block System

The Bio-Block system has demonstrated a range of advantages over 2D culture systems. Firstly, ASCs cultivated in the Bio-Block showed significantly higher viability (80.2%) compared to ASCs cultured in 2D (48.9%). Additionally, the Bio-Block enhanced the maintenance of ASC 'stem-like' properties, as evidenced by increased expression of stem cell markers CD44, CD73, CD90, and CD105. The Bio-Block system also reduced cellular senescence, as shown by the lower expression of senescence marker p16INK4a.

## Impact on ASC Secretome

When comparing the secretomes of ASCs cultured in 2D and 3D systems, the Bio-Block system demonstrated a significant increase in the total protein concentration of conditioned media (ASC-CM). ASC-CM from the 3D Bio-Block exhibited higher levels of antioxidant capacity, which is crucial for wound healing. Furthermore, the 3D system resulted in a significant increase in extracellular vesicle (EV) production, including exosomes and microvesicles.

### **These enhanced secretome components translated into improved wound healing and tissue regeneration capabilities.**

Both keratinocytes (KCs) and fibroblasts (FBs) showed increased proliferation and metabolic activity when treated with ASC-CM from the 3D Bio-Block. The recovery rate of scratch area for KCs and FBs was also accelerated when treated with 3D Bio-Block ASC-CM compared to traditional 2D ASC-CM.



# Potential Mechanisms of Action

The controlled secretion of EVs and exosomes is one possible mechanism through which the ASC secretome modulates wound healing activity. In vitro tracking assays showed that KCs and FBs endocytosed ASC-derived EVs, which can modulate cellular signaling and alter cellular morphology. Further research into the composition of EVs and exosomes, as well as the signaling dynamics in cells treated with ASC-CM, will provide valuable insights into the mechanisms behind the improved wound healing capabilities of the 3D Bio-Block system.

# Limitations and Future Studies

This study used a single ASC donor source, and future research should include multiple donor sources to ensure that the observed differences are not donor-specific. Additionally, the proteome array used in this study tested for only 55 secreted proteins, limiting the overall view of the ASC secretome. Future studies should employ mass spectroscopy analysis for a more comprehensive examination of the ASC-CM proteome in 2D and 3D cultures. Comparative analysis with other 3D culture systems will also help elucidate the relative benefits of the Bio-Block system.



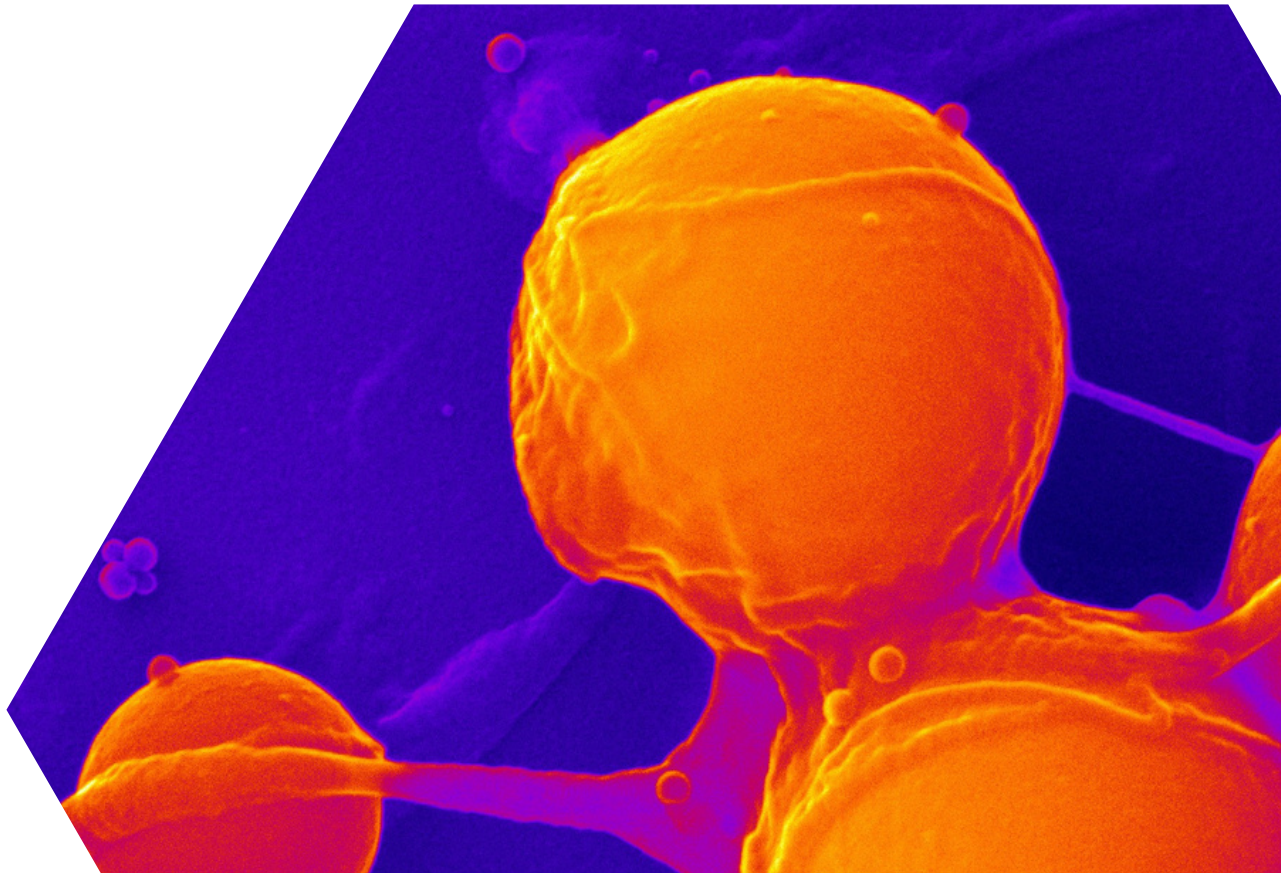


## Conclusion

The Bio-Block system represents a promising advancement in tissue-mimetic culture for ASCs, enabling the production of a more potent ASC secretome with improved wound healing capacity and regenerative capabilities. Further research into the Bio-Block system, its impact on ASC secretome components, and potential clinical applications will undoubtedly contribute to the future of regenerative medicine.

## Highlights

- Maintain their stem cell characteristics & properties
- Function more effectively
- Significant reduction in senescence
- No longer need to undergo subculturing
- Reduce the potential for cell damage and contamination
- Enable more consistent and reliable results

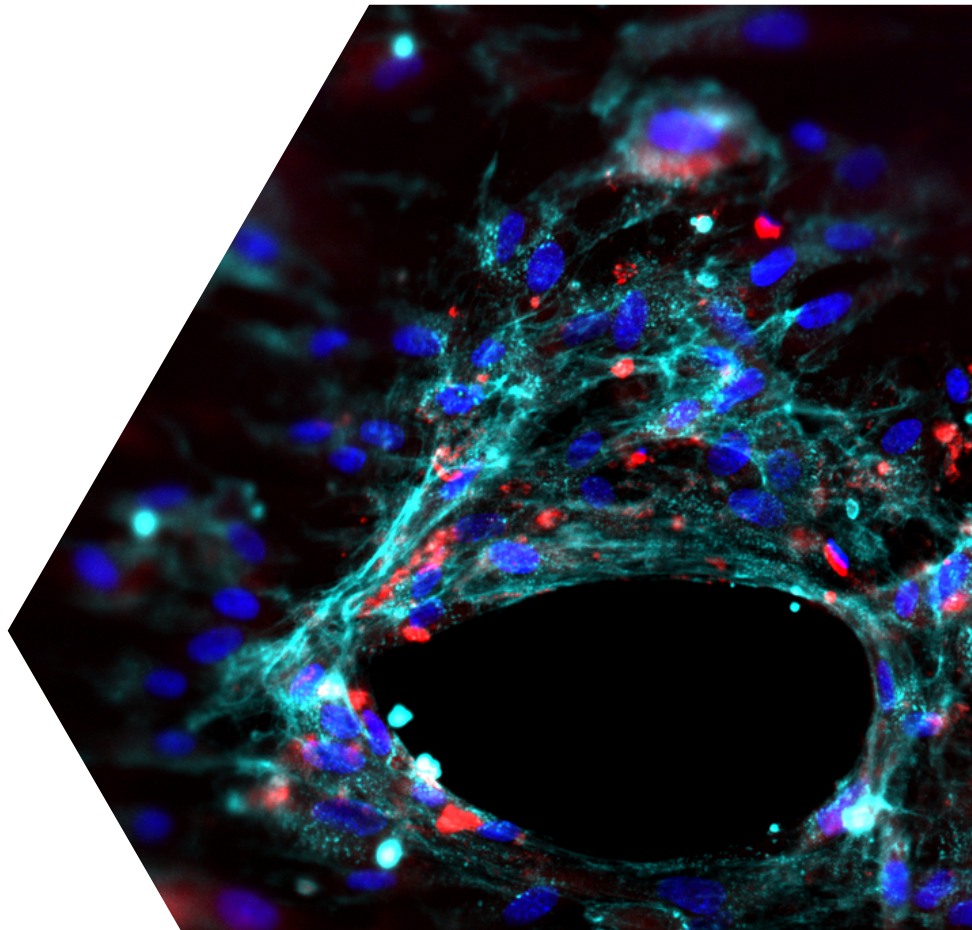




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## About Ronawk

Ronawk's Bio-Block Universe™ is the first expandable Bio-Factory designed to accelerate 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.

## Contact Ronawk

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