Beneficially Altered Secretory Activity of Cells Cultured in Bio-Blocks vs. Traditional Culture Flasks: Protein Production

Ronawk, Inc.

INTRODUCTION

Conditioned media (CM) is media collected from culturing cells. It contains the biological products that the cells secrete during their growth, but does not contain the cells themselves. This collection of products is called the 'secretome'. The secretome has a range of potential applications. Wound healing is a promising application, especially when wounds may not respond to traditional treatments like antibiotics, dressings, and surgery.

Bio-BlocksTM are an effective culture platform for generating high-quality CM. This CM has been shown to improve wound healing-related activity (migration, metabolic activity, and proliferation) in the population of treated cells (Ronawk Inc. 2023c). What types of changes in the CM may be responsible for these beneficial effects? What beneficial changes in secreted products (i.e. proteins) occur because of the Bio-Block culture environment?









CONDITIONED MEDIA



A simplified example of an experimental workflow for analyzing proteins in conditioned media. The cell type being cultured (ASCs) is shown on the far left and outlined in blue. This cell is connected to the two culture environments – Bio-Blocks in a 6-well plate (top) and a 2D flask (bottom). To the right of both culture environments are the centrifuge tubes filled with the collected conditioned media. On the far right of the image is an example of a protein array (top) and the paired example graph (bottom).





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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.





CM PROTEIN 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 showing the relative increases in 8 different proteins in CM collected from Bio-Block cultured cells. The six, left-most bars are growth factor-related proteins, and the two, right-most bars are cytokine-related proteins.





CONCLUSION

Culturing cells in the Bio-Blocks results in healthier populations of cells (Ronawk Inc. 2023a, b). The advantages of a healthy cell culture extend to the biological products the cells make (Ronawk Inc. 2023c). This initial protein array analysis has further revealed beneficial changes in key groups of proteins (growth factors, cytokines). The increases in these classes of proteins may begin to explain why some of the beneficial effects are observed. These promising findings also set the stage to explore how coated (e.g. collagen, fibrin, etc.) could further impact changes in the secretome.

Ultimately the Bio-Block cell culture platform can help you get the most from your cells and the biological products (e.g. proteins) they secrete. Culturing different populations of cells and/or using coated Bio-Blocks could allow for the tailoring of the secretome to suit the individual needs for experimental design.

Bio-Blocks' ability to beneficially alter proteins secreted by cells 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

- · Observed increases in specific proteins (growth factors, cytokines) could help to explain the beneficial effects of the CM
- Potential for customizable coatings on the Bio-Blocks to induce different changes in protein production





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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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FORMATTED REFERENCES

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Tissue Engineering Parts A, B and C

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