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3D Bio-Tissues
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Application note

CytoBoost™ for increased proliferation of muscle and fat cells in serum-free cultures

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Introduction

Currently, recombinant growth factors account for about 60-80% of the manufacturing costs of animal-free media used for bioprocessing applications, including cultivated meat production, with most current formulations ranging between €60-100 per liter. While this is expected to be considerably reduced as we achieve economies of scale, innovation in culture media formulation incorporating new technologies like CytoBoost™ from 3D Bio-Tissues and enhanced growth factors from Qkine will be necessary to produce cost-efficiency and overcome the technical challenges of working with sensitive cell-based systems.

In this collaborative application note CytoBoost™ formulations, Perform and Maximise are tested on the proliferation of myoblasts (muscle cells) and adipocytes (fat cells) in serum-free conditions with Qkine high quality growth factors IGF-1 (Qk047), HGF NK1 (Qk061) and thermostable FGF-2, FGF2-G3 (Qk052).

How CytoBoost™ can improve proliferation in animal cells

3D Bio-Tissues Ltd has developed CytoBoost™, the first range of medium additives especially designed for cell-care (Figure 1). CytoBoost™ achieves this by using biocompatible macromolecules that reproduce the very crowded conditions within in vivo biosystems, thus creating culture environments more amenable to cells. CytoBoost™ is broadly compatible with primary, secondary, immortalized, and induced pluripotent stem cells, versatile, and easy to use in both 2D and 3D cultures.

Figure 1:
The CytoBoost™ product range: ready-to-use additives for bestowing cell-boosting crowding effects to any culture medium.

Illustrative Case Studies

The effect of two of our most versatile CytoBoost™ formulations, Perform and Maximise (Figure 1) on the proliferation of myoblasts (muscle cells) and adipocytes (fat cells) were tested in serum-free conditions. Removal of serum and other animal-derived ingredients from culture media is a prerequisite for many bioprocessing applications due to accuracy, consistency, and/or ethical concerns.

Methods

Serum-free media comprising DMEM/F12 base supplemented with 1% (v/v) ITS-X, 2.5 mM L-alanyl-L-glutamine, 1.25 $g.L^{-1}$ recombinant albumin, 25 $\mu g.L^{-1}$ IGF-1 (Qk047), 2.5 $\mu g.L^{-1}$ FGF2-G3 (Qk052), and 1.25 $\mu g.L^{-1}$ HGF NK1 (Qk061) was used to grow porcine immortalized myoblasts (CB1-M cells) and secondary adipocytes (CPF1-A cells) with CytoBoost™ Perform and CytoBoost™ Maximise additives at 4% (v/v), or without the additives (control). Live cells cultured for 7 days in six independent experiments (n = 6) were counted, and proliferation expressed as average cell number ± standard deviation (SD) relative to control.

Results

The addition of 4% (v/v) of CytoBoost™ Perform or CytoBoost™ Maximise to a chemically-defined, serum-free medium significantly enhanced the proliferation of immortalized myoblasts compared with control cultures (Figure 2, bar chart). Specifically, myoblast cultures grown with Perform or Maximise for 7 days (green bars) had 77% and 59% more cells than those without CytoBoost™ addition (light grey bar). This increase in cell proliferation did not affect cell morphology meaningfully (Figure 2, micrographs).

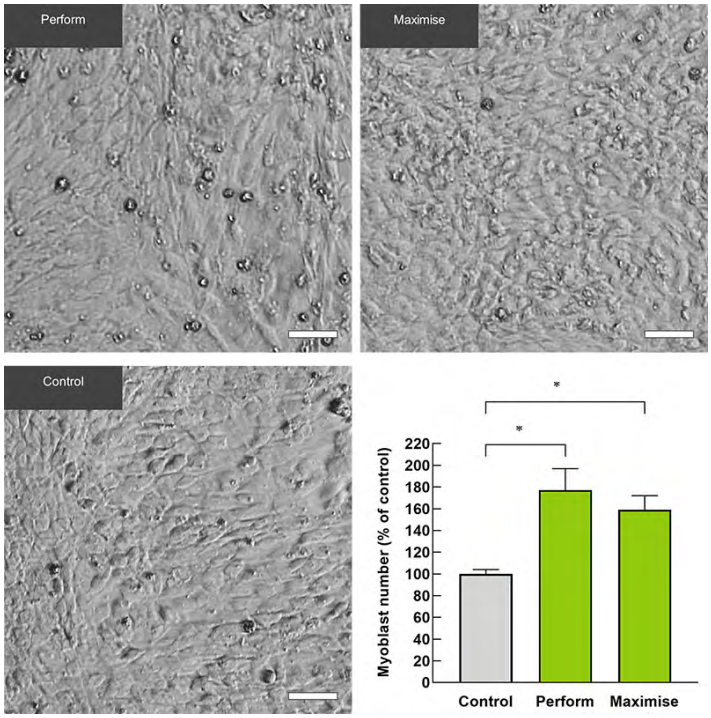

Figure 2:
Effect of CytoBoost™ Perform and Maximise on myoblast growth. Scale bars = 0.1 mm. Cell number expressed as average ± SD of control average (n = 6; * corresponds to $p < 0.001$).

Furthermore, the addition of CytoBoost™ Maximise to serum-free medium significantly enhanced the proliferation of secondary adipocytes compared with control cultures; however, a similar increase was not evident for CytoBoost™ Perform (Figure 3). Specifically, adipocyte cultures grown with Maximise for 7 days had 33% more cells than those without the additive, whereas cultures with Perform showed only a 5% increase in cell number (Figure 3, bar chart). Moreover, cultures grown with CytoBoost™ Maximise showed greater prevalence of cells with large accumulation of lipid vesicles, in contrast with remaining conditions (Figure 3, micrographs).

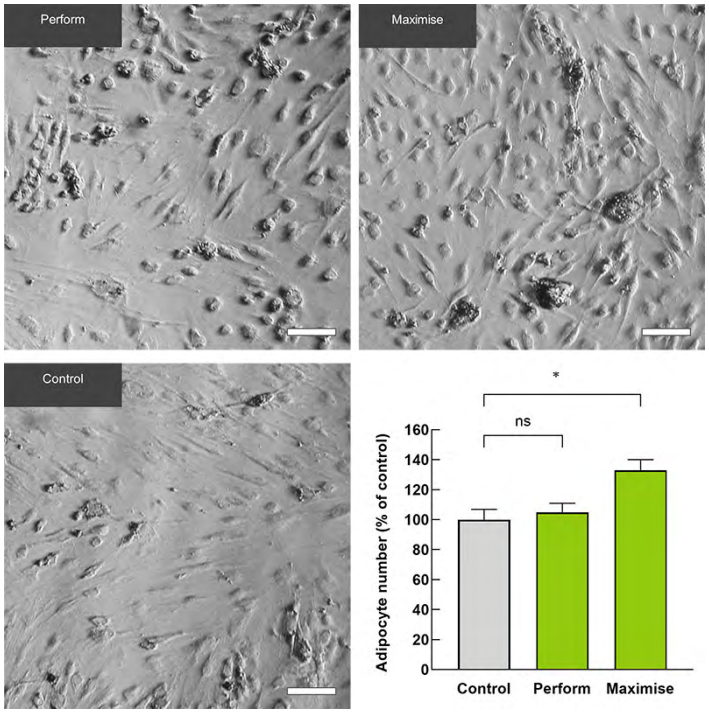

Figure 3:
Effect of CytoBoost™ Perform and Maximise on adipocyte growth. Scale bars = 0.1 mm. Cell number expressed as average ± SD of control average (n = 6; * corresponds to $p < 0.001$).

Conclusions

Macromolecular crowding is a fundamental aspect of cellular environments within organisms that significantly influences cell behavior. By mimicking crowding in vitro, CytoBoost™ can enhance muscle and fat cell proliferation, improve culture performance, and increase bioprocessing yields.

How Macromolecular Crowding Influences Animal Cell Proliferation

Macromolecular crowding provides a complex and dynamic environment that can significantly influence cell proliferation via various processes:

1. Excluded Volume Effects

Increased Effective Concentration: By occupying space, crowding agents increase the effective concentration of macromolecules like growth factors and their receptors, promoting ligand-receptor interactions and downstream signaling.
Macromolecular Compartmentalization: Crowding can lead to the formation of microdomains with locally high concentrations of signaling molecules, enhancing signaling efficiency and potentially lowering the threshold for cell proliferation.

2. Diffusion

Reduced Diffusion: Crowding hinders the diffusion of molecules, including growth factors. This can prolong their residence time near receptors, increasing the likelihood of binding and signaling.
Directed Transport: In some cases, crowding might facilitate directed transport of signaling molecules along cytoskeletal elements or within membranous compartments, enhancing signaling efficiency.

3. Protein Conformation, Stability and Interaction

Favored Complex Formation: Crowding promotes protein-protein interactions by increasing the likelihood of collisions and stabilizing complexes. This can enhance the formation of signaling complexes, leading to sustained signaling and proliferation.
Modulation of Signaling Networks: Crowding can alter the balance between different signaling pathways by differentially affecting the stability and activity of signaling complexes, potentially favoring pro-proliferative pathways.

Shifts in Conformational Equilibria

Crowding can shift the conformational equilibria of proteins towards more compact and stable states. This can enhance the stability and activity of growth factors and their receptors, promoting signaling and proliferation.

Other potential applications of CytoBoost™ in cell culture and bioprocessing

Stem Cell Expansion and Differentiation: Crowding could be leveraged to control stem cell fate and direct their differentiation into specific cell types.

Protein Production and Stability: Crowding could enhance protein production and stability in bioreactors, improving biopharmaceutical manufacturing.

Media Optimization: Crowding could be exploited to reduce the concentration of essential growth factors in media, including animal sera and albumins, thus reducing costs and addressing ethical and sustainability concerns.

Biosensing and Drug Discovery: Crowding can make biosensors and cellular and tissue models of disease more sensitive and accurate by better reproducing the original biological systems.

Considerations for Implementation of CytoBoost™

Choice of CytoBoost™ formulation: This product includes different formulations specifically designed for a range of applications. Trial kits are available for initial screenings.

Cell Type and Culture Conditions: The effects of CytoBoost™ can vary depending on the cell type and culture conditions, so optimization is crucial. If in doubt, use the QR code to contact our Product Specialists.

Potential Side Effects: Crowding influences many molecular mechanisms within in vitro systems, so careful planning and monitoring are necessary.

Key Message

CytoBoost™ offers a promising avenue for enhancing cell proliferation, especially in conditions where performance is impacted by absence of serum or other animal-derived ingredients. By harnessing the effects of molecular crowding, we can develop more efficient, more reliant, and more sustainable bioprocesses.

For more information

Please contact our team: **customerservice@qkine.com** if you would like to discuss commercial or academic collaborations, supply agreements or any aspects of growth factor optimization and other products.

Please visit: **qkine.com**

To explore our high-purity, animal-free bioactive proteins visit qkine.com

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