Plant Molecular Farming of Growth Factors: A Scalable Solution for Cellular Agriculture

Introduction

The cost-effective production of recombinant proteins remains a central challenge in biotechnology, particularly for applications requiring large-scale quantities such as cultivated meat. Growth factors (GFs), essential for cell proliferation and differentiation [1,2], can account for up to 70% of production costs in cultivated meat systems [3]. Current manufacturing platforms, microbial fermentation and mammalian cell culture, are often limited by high capital expenditure (CAPEX), costly raw materials, scalability constraints, and the need for sterile, tightly controlled environments

Molecular farming offers a promising alternative. This technology uses genetically engineered plants to express recombinant proteins and can be deployed in open-field agricultural settings. Unlike fermentation-based systems that rely on bioreactors and specialized infrastructure [4–7], molecular farming allows scale-up by expanding planted area, minimizing operational complexity, and reducing CAPEX [8–11]. Plant-based systems can also perform relevant post-translational modifications and are free from animal-derived inputs, offering additional advantages for regulatory compliance and product safety.

BioBetter utilizes *Nicotiana tabacum*, cultivated in open fields, to produce growth factors fused to a cellulose-binding domain (CBD). This design enables purification on inexpensive cellulose-based matrices. The CBD remains attached to the protein and, in some applications, is further complexed with cellulose nanocrystals (CNC) to allow for modular delivery formats or extended activity. This pipeline bypasses chromatography and other intensive downstream steps, supporting large-scale, low-cost production.

While BioBetter's platform is applicable across various areas of recombinant protein manufacturing, including biologics, industrial enzymes, and veterinary products, we focus here on growth factors relevant to cellular agriculture. Cultivated meat represents a compelling test case for this technology, where the cost and scalability of inputs remain key hurdles to commercial adoption. Demonstrating functional equivalence to commercial proteins in this context provides strong proof-of-concept for broader applications.

This paper presents biological validation data for plant-produced FGF2, SCI (single-chain insulin), and IGF1. Experiments were conducted both in-house and by third-party laboratories, including academic and commercial collaborators. Across multiple cell models, these proteins were shown to support proliferation, differentiation, and lineage-specific activity, demonstrating their suitability for cultivated meat and beyond.

Results

FGF2

FGF2 is a mitogenic factor that plays a critical role in supporting the proliferation and maintenance of undifferentiated cells in culture [12,13]. To validate the activity of

BioBetter's FGF2-CBD and FGF2-CBD-CNC, a series of functional assays was conducted in collaboration with external laboratories using relevant mammalian and avian cell models.

Dose-dependent response in mouse embryonic fibroblasts

To assess the biological activity of plant-derived FGF2-CBD, dose–response assays were performed by SBH Sciences using NIH 3T3 fibroblasts. In this experiment, FGF2-CBD was pre-bound to cellulose nanocrystal (CNC) particles prior to application (FGF2-CNC, **Figure 1**). The CNC-bound format is designed to improve protein half-life by reducing degradation and diffusion losses, thereby supporting sustained signaling in cell culture.

In a dose–response proliferation assay conducted by SBH Sciences using NIH 3T3 fibroblasts, FGF2-CNC promoted cell growth in a concentration-dependent manner. The dose–response curve showed that FGF2-CNC supported proliferation with similar potency and efficacy to commercial FGF2, confirming functional equivalence in this standard fibroblast model.

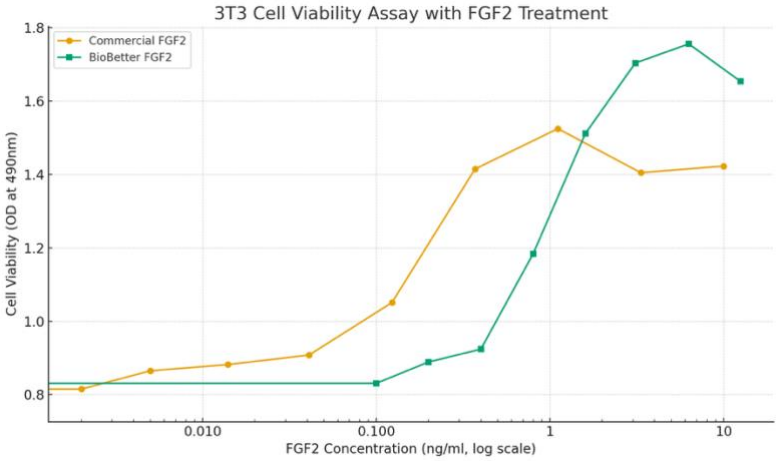

Figure 1: 3T3 Cell Viability Assay with FGF2 Treatment. *3T3 fibroblasts were treated with increasing concentrations of either BioBetter FGF2-CNC or Commercial FGF2. Cell viability was assessed by measuring optical density (OD) at 490 nm. Values represent a single representative experiment plotted on a logarithmic scale. BioBetter FGF2 is shown in green and Commercial FGF2 in orange.*

Activity in 3D Stem Cell Aggregates

To further assess activity in a more physiologically relevant context, a second experiment was conducted by a cultivated meat company using chicken embryonic stem cells (ESCs) cultured in aggregate format. These aggregates represent a 3D cell culture system, providing spatial cell–cell interactions that better mimic *in vivo* conditions. Cells were grown in 6-well plates on a shaker and sampled after 3 or 5 days. Treatment groups received either commercial FGF2 or BioBetter FGF2-CNC at concentrations of 50, 75, or 100 ng/mL. Cell viability and the percentage of cells in S-M phase (as an indicator of cell cycle progression) were quantified at both time points using FACS analysis.

As shown in Figure 2A, FGF2-CNC supported robust cell viability across all concentrations and time points, with performance comparable to or exceeding that of the commercial control. Importantly, FGF2-CNC treatment maintained or enhanced S-M phase progression by day 5, especially at higher doses, suggesting sustained mitogenic activity over time (Figure 2B).

Control experiments, where cells were treated with CNC particles alone, were conducted to rule out the possibility that the observed effects were attributable to the CNC particles rather than the growth factor. These controls showed no enhancement in viability or S-M phase entry, indicating that CNC alone does not account for the proliferative effects of FGF2-CNC. Additionally, since S-M phase percentages were determined by FACS analysis, we evaluated whether CNC particles interfered with the readout. Cells treated with CNC alone showed S-M phase values comparable to those treated with commercial FGF2, confirming that the particles do not artificially elevate the measurement.

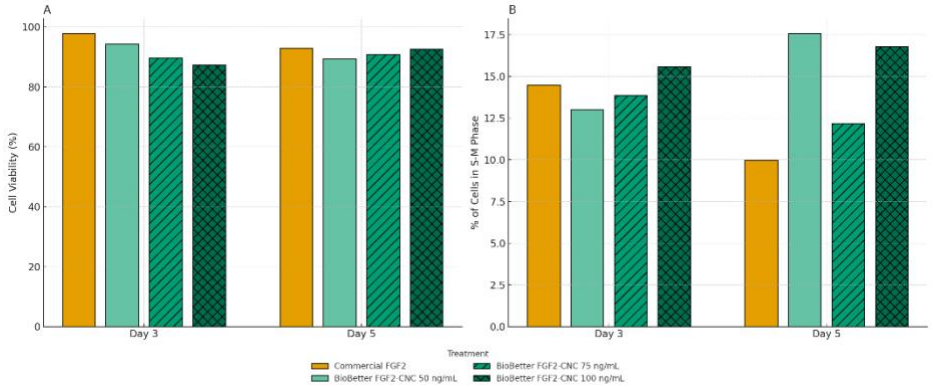

Figure 2: Effect of BioBetter FGF2-CNC on Chicken Embryonic Stem Cell Viability and Cell Cycle Progression. (A) *Cell viability was measured on days 3 and 5 in chicken embryonic stem cells (ESCs) cultured as aggregates and treated with either commercial FGF2 or BioBetter FGF2-CNC at concentrations of 50, 75, or 100 ng/mL.*
(B) *Percentage of cells in S-M phase, indicating cell cycle progression, under the same treatment conditions and time points. All experiments were performed by a cultivated meat company.*

Together, these results validate the biological activity of BioBetter's plant-produced FGF2 and support its functional equivalence to commercial FGF2 in both 2D and 3D cell culture models. The use of CNC binding may offer enhanced protein stability and signaling persistence, which could reduce media costs and improve performance in industrial-scale cell culture systems.

Insulin

Recombinant insulin is a key component in most mammalian cell culture media, supporting glucose uptake, metabolism, and anabolic signaling pathways [13]. BioBetter produces a CBD-tagged insulin analog, referred to as SCI (single-chain insulin), in *N. tabacum*. The biological activity of SCI-CBD was evaluated in two functional assays conducted by the Schlesinger laboratory at the Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem.

Adipogenic differentiation was assessed in bovine adipose-derived stromal cells (bASC, also referred to as fibro-adipogenic progenitors, FAPs), at passage 5. Cells were seeded on standard, uncoated 12-well plates at a density of 80,000 cells per well. After a 24-hour attachment period (day –1), differentiation was initiated on day 0 and continued for 10 days in adipogenic induction medium supplemented with either SCI-CBD or commercial insulin. Lipid accumulation was then visualized using Oil Red O staining. As shown in Figure 3A and 3B, both SCI-CBD and commercial insulin induced robust lipid droplet formation, indicating that SCI-CBD retains adipogenic functionality comparable to the commercial control.

Proliferative activity was measured in NIH 3T3 fibroblasts cultured in insulin-free medium supplemented with increasing concentrations of SCI-CBD or commercial insulin. In this assay, SCI-CBD was complexed with microcrystalline cellulose (MCC) prior to treatment. Cell viability was assessed by resazurin reduction after 72 hours. SCI-CBD promoted cell proliferation in a concentration-dependent manner, demonstrating a comparable dose–response and maximal effect to the reference insulin. Notably, treatment with 1.81 µg/mL of SCI-CBD bound to Microcrystalline cellulose (SCI-MCC) induced a 120% increase in viability relative to the positive controls, outperforming 30 µg/mL of commercial insulin ($p < 0.001$) (Figure 3C). These results suggest that cellulose binding enhances the stability and bioactivity of the recombinant protein.

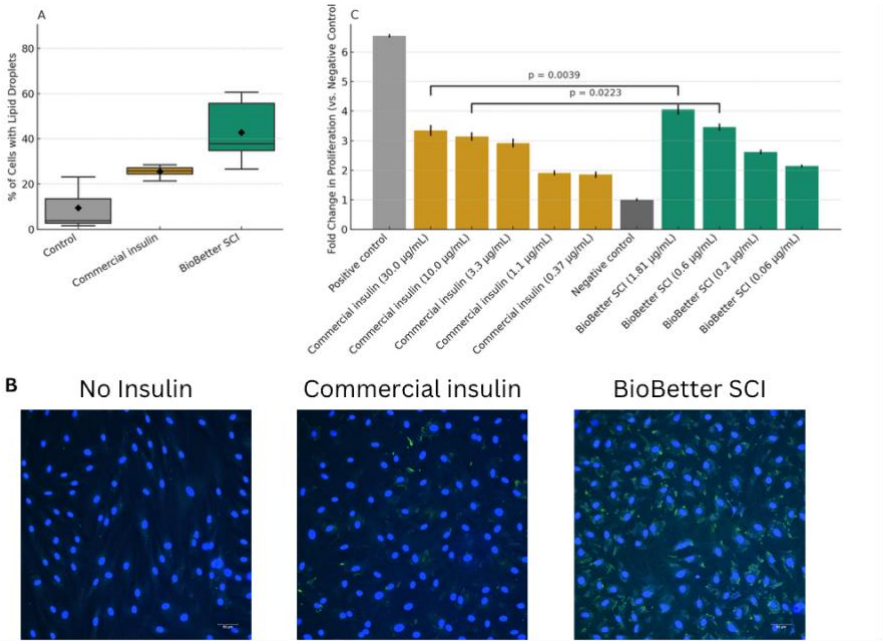

Figure 3: ***BioBetter single-chain insulin (SCI) promotes adipogenic differentiation and cell proliferation.***
(A) *Quantification of lipid droplet formation in bovine adipose-derived stromal cells (bASC; passage 5) cultured in 2D on uncoated 12-well plates. Cells (80,000/well) were seeded on day -1 and induced to differentiate for 10 days starting on day 0 with either BioBetter SCI-CBD or commercial insulin. Lipid accumulation was assessed by Oil Red O staining, and lipid-positive cells were quantified as a percentage of total cells. Data are presented as boxplots showing medians and standard deviation.*
(B) *Representative microscopy images illustrating lipid droplet accumulation corresponding to the conditions shown in (A).*
(C) *Proliferation of NIH 3T3 fibroblasts cultured in insulin-free medium and treated with increasing concentrations of SCI-MCC (SCI-CBD bound to microcrystalline cellulose, MCC) or commercial insulin. Cell viability was measured by resazurin reduction. SCI-MCC at 1.81 µg/mL achieved 120% of the proliferative activity observed with 30 µg/mL of commercial SCI ($p < 0.001$), suggesting enhanced functional stability conferred by cellulose binding. Error bars represent standard deviation*

Together, these findings demonstrate that BioBetter's SCI-CBD, particularly when bound to cellulose, retains full biological function across adipogenic and proliferative assays and may exhibit enhanced activity and stability compared to commercial insulin preparations.

IGF-1

Insulin-like growth factor 1 (IGF1) is a key regulator of muscle development, promoting myoblast proliferation, survival, and terminal differentiation[14]. While

structurally related to insulin, IGF1 acts through the IGF1 receptor (IGF1R) to initiate distinct signaling cascades that drive muscle cell fusion and hypertrophy[15]. In the context of cultivated meat, IGF1 is considered one of the most important mitogens for myogenic progenitor expansion and differentiation, supporting both proliferation and terminal fusion into mature muscle fibers[13].

To evaluate the biological activity of BioBetter's plant-derived IGF1-CBD complexed with cellulose nanocrystals (IGF-CNC), a myogenic differentiation assay was conducted in hTERT-immortalized human bovine mesenchymal stem cells expressing mouse MyoD (hTERT-MyoD). The experiments were performed by the Schlesinger laboratory at the Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem.

Cells were seeded on gelatin-coated plates and induced to differentiate over a period of 10 days using 1 µg/mL doxycycline. All treatment groups received 2% serum. One group was treated with serum alone (negative control), while the others received a defined myogenic differentiation cocktail supplemented either with no IGF, with commercial IGF, or with BioBetter's IGF-CNC.

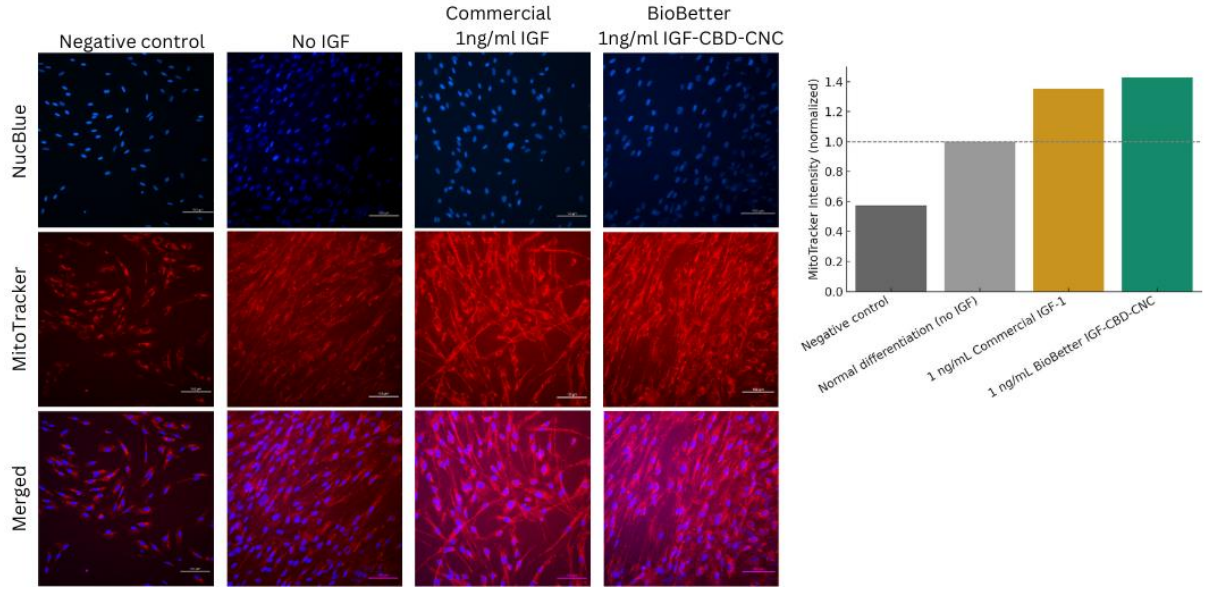

Figure 4: ***BioBetter IGF-CBD-CNC induces myogenic differentiation of hTERT-MyoD cells****. hTERT-immortalized bovine mesenchymal stem cells expressing mouse MyoD (hTERT-MyoD) were seeded on 0.2% gelatin-coated 96-well plates and induced to differentiate for 10 days with 1 µg/mL doxycycline (Dox). Cells were treated with either 2% serum (negative control), differentiation medium without IGF, commercial IGF, or BioBetter IGF-CNC. Results are normalized to medium without IGF.*
Left panels: *Representative fluorescence images showing NucBlue-stained nuclei (top), MitoTracker Red (middle), and merged channels (bottom). MitoTracker staining was used to visualize and track living cells during differentiation.*
Right panel: *Quantification of MitoTracker intensity per well. Bars represent mean intensity.*

Differentiation was assessed using MitoTracker Red to visualize viable, metabolically active cells and NucBlue to stain nuclei. Fluorescence imaging showed robust differentiation in cultures treated with either commercial IGF or IGF-CNC, while minimal MitoTracker staining was observed in the serum-only and no-IGF conditions (Figure 4, left). Quantification of MitoTracker intensity, normalized to the no-IGF condition, confirmed that IGF1-CNC supported differentiation at levels comparable to commercial IGF and significantly above the negative controls (Figure 4, right).

These results demonstrate that IGF-CNC, produced in *N. tabacum* using BioBetter's molecular farming platform, retains full biological functionality and effectively promotes myogenic differentiation *in vitro*.

Conclusion

Together, these results demonstrate that growth factors produced using BioBetter's open-field molecular farming platform—including FGF2, SCI (insulin), and IGF1—retain full biological activity *in vitro* and perform comparably to commercial recombinant proteins. Functional validation in multiple mammalian cell models relevant to cultivated meat confirms their capacity to support proliferation, adipogenic differentiation, and myogenic maturation.

The FGF2 results, particularly in three-dimensional embryonic stem cell aggregates, show a marked advantage in promoting sustained cell cycle activity over time. This suggests enhanced stability and prolonged mitogenic signaling compared to commercial FGF2, a key requirement for scalable cell culture systems in cultivated meat. Similarly, the insulin studies demonstrate that SCI-CBD retains potent adipogenic and proliferative functionality even when bound to cellulose, reducing the required protein dose significantly and potentially offering substantial cost savings.

Beyond biological performance, BioBetter's growth factors offer key production advantages in scalability, cost-efficiency, and infrastructure requirements. The platform uses cultivation of *Nicotiana tabacum*, circumventing the need for bioreactors, sterile facilities, and expensive fermentation infrastructure. This dramatically reduces capital expenditure (CAPEX), simplifies scale-up, and enables manufacturing using existing agricultural resources, directly addressing one of the major cost barriers in cultivated meat production, where growth factors can account for up to 70% of total production costs.

The inclusion of CBD enables efficient and low-cost purification using cellulose matrices. When complexed with cellulose nanocrystals (CNC), these growth factors may also display extended bioactivity in culture, reducing the frequency and amount of dosing required. This modular approach to protein presentation introduces new possibilities for optimizing culture performance in cost-sensitive applications.

While cultivated meat represents a timely and impactful initial use case, the flexibility of BioBetter's molecular farming system supports broader adaptation across the recombinant protein landscape. The platform is well-positioned for the expression of other structurally complex or glycosylated proteins with applications in regenerative medicine, vaccine development, and industrial biomanufacturing, offering a sustainable and economically viable alternative to traditional production platforms.

References

1. Swartz E, Ravi A, Bio L, et al. Anticipated growth factor and recombinant protein costs and volumes necessary for cost-competitive cultivated meat. Published online 2023.
2. Humbird D. Scale-up economics for cultured meat. *Biotechnol Bioeng*. 2021;118(8):3239-3250. doi:10.1002/BIT.27848

3. Specht L. *An Analysis of Culture Medium Costs and Production Volumes for Cultivated Meat*.; 2020.
4. Eidenberger L, Kogelmann B, Steinkellner H. Plant-based biopharmaceutical engineering. *Nature Reviews Bioengineering 2023 1:6*. 2023;1(6):426-439. doi:10.1038/s44222-023-00044-6
5. Chung YH, Church D, Koellhoffer EC, et al. Integrating plant molecular farming and materials research for next-generation vaccines. *Nature Reviews Materials 2021 7:5*. 2021;7(5):372-388. doi:10.1038/s41578-021-00399-5
6. Kulshreshtha A, Sharma S, Padilla CS, Mandadi KK. Plant-based expression platforms to produce high-value metabolites and proteins. *Front Plant Sci*. 2022;13:1043478. doi:10.3389/FPLS.2022.1043478/BIBTEX
7. Tripathi NK, Shrivastava A. Recent Developments in Bioprocessing of Recombinant Proteins: Expression Hosts and Process Development. *Front Bioeng Biotechnol*. 2019;7:496566. doi:10.3389/FBIOE.2019.00420/BIBTEX
8. Mir-Artigues P, Twyman RM, Alvarez D, et al. A simplified techno-economic model for the molecular pharming of antibodies. *Biotechnol Bioeng*. 2019;116(10):2526-2539. doi:10.1002/BIT.27093
9. Fischer R, Emans N. Molecular farming of pharmaceutical proteins. *Transgenic Res*. 2000;9(4-5):279-299. doi:10.1023/A:1008975123362
10. Tschofen M, Knopp D, Hood E, Stöger E. Plant Molecular Farming: Much More than Medicines. *Annual Review of Analytical Chemistry*. 2016;9(Volume 9, 2016):271-294. doi:10.1146/ANNUREV-ANCHEM-071015-041706/CITE/REFWORKS
11. Park KY, Wi SJ. Potential of plants to produce recombinant protein products. *Journal of Plant Biology*. 2016;59(6):559-568. doi:10.1007/S12374-016-0482-9/METRICS
12. Habenicht A, ed. *Growth Factors, Differentiation Factors, and Cytokines*. Springer Berlin Heidelberg; 1990. doi:10.1007/978-3-642-74856-1
13. Ahmad SS, Chun HJ, Ahmad K, et al. The roles of growth factors and hormones in the regulation of muscle satellite cells for cultured meat production. *J Anim Sci Technol*. 2023;65(1):16-31. doi:10.5187/jast.2022.e114
14. Duan C, Ren H, Gao S. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: Roles in skeletal muscle growth and differentiation. *Gen Comp Endocrinol*. 2010;167(3):344-351. doi:10.1016/J.YGCEN.2010.04.009
15. Cai W, Sakaguchi M, Kleinridders A, et al. Domain-dependent effects of insulin and IGF-1 receptors on signalling and gene expression. *Nature Communications 2017 8:1*. 2017;8(1):1-14. doi:10.1038/ncomms14892