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Simplifying Progress

The Critical Role of Growth Factors and Cytokines in Advancing Research & Development of Biotherapeutics

Cytokines play a crucial role in life science research due to their diverse functions in the activation, proliferation and differentiation of immune and stem cells. Their inclusion in cell culture systems, especially in advanced three-dimensional (3D) cell culture-based models enables researchers to mimic physiological and pathological conditions, ensuring the consistency and reliability of experimental outcomes. They are indispensable reagents in the R&D of biomedicines for various diseases, including cancers, autoimmune disorders, and infectious diseases.

Sartorius offers a range of high-quality Research Use Only (RUO) Growth Factors and Cytokines that are animal-derived component free and produced using recombinant human DNA technology. These growth factors are of high-purity, low endotoxicity, and can be used for the long-term maintenance or differentiation of organoid cultures in a non-perturbing and reproducible manner.

Key Applications Areas of Cytokines in Biotherapeutics R&D

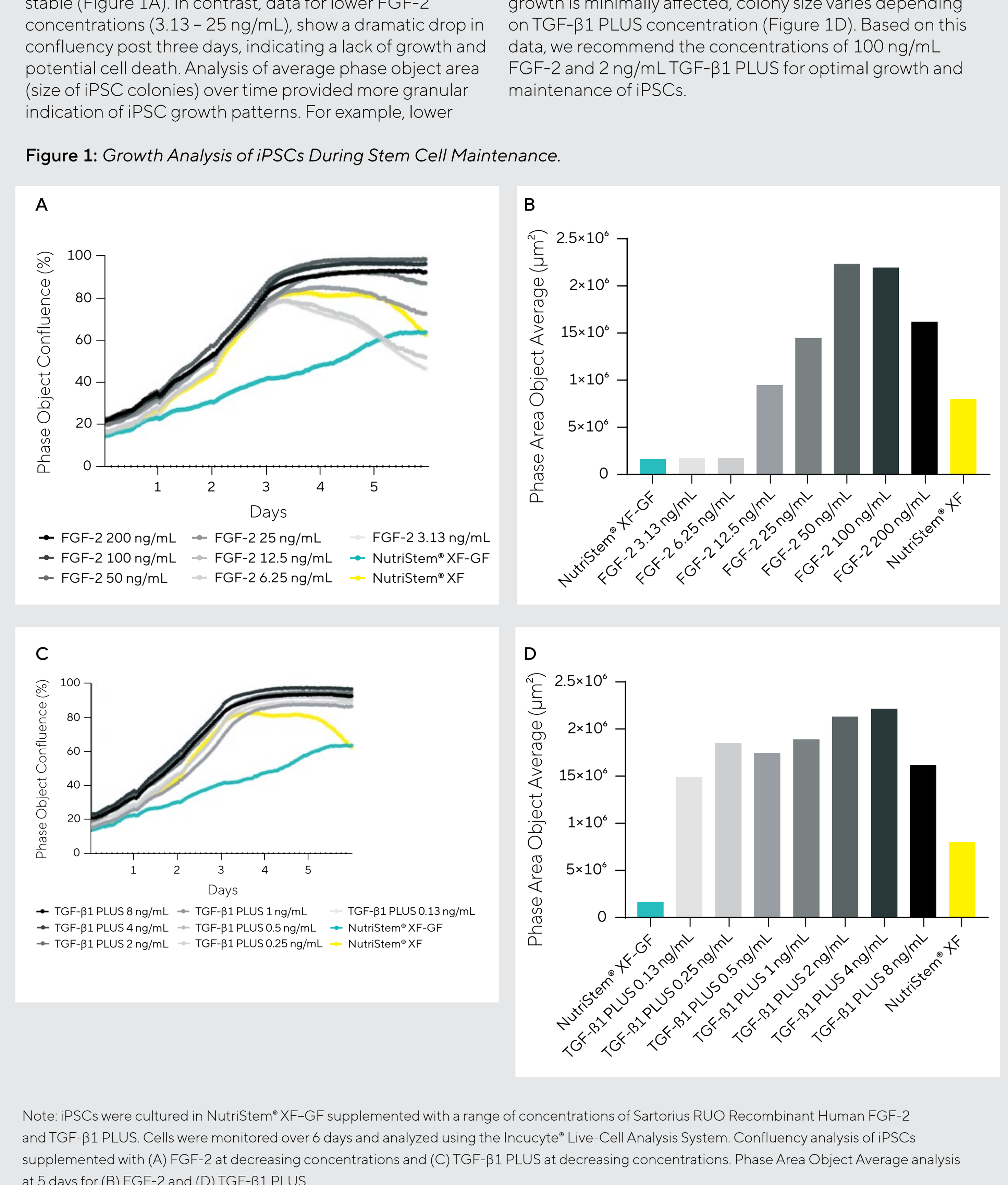
Challenges and Solutions

Challenges	Solutions
Determining the optimal concentration of cytokines	As each cell type will have different requirements, the optimal growth factor or cytokine concentration must be empirically determined for each culture system. Use preliminary experiments to observe the concentration and time dependent responses of cells to growth factors and select the most appropriate concentration for your application. **Example:** Generating Concentration Response Curves of iPSCs supplemented with FGF-2 and TGF-β1 PLUS **Our solutions for cytokine quantitation:** ▪ Cell Proliferation Assays for Live-Cell Analysis ▪ Label-Free Detection
Maintain stability over extended culture periods These proteins can be unstable and degrade over time, which can affect the consistency and reproducibility of experimental results	Using recombinant cytokines engineered for enhanced stability and activity. Regularly testing batches of cytokines for activity and stability to ensure consistent performance in cell culture applications. Lyophilization improves their shelf-life and stability during storage and transport, reconstituting them just before use. **Tip for reconstitution:** Please refer to the product data sheet for the resuspension solution recommendation for your growth factor or cytokine and centrifuge briefly before reconstituting to make sure that all of the powder is at the bottom of the vial. Add the reconstitution solution to give a protein concentration of 0.5-1mg/ml. Leave to stand for 1 minute before gently agitating – do not vortex as this will make the solution foam which must be avoided. You can prepare stock aliquots or single use vials from the master mix. **Example:** Use of Thermostable FGF-G3 Facilitates iPSC Culture Without Daily Feeding

Optimization of iPSC Culture Protocol Using High Quality Growth Factors and Cytokines

Growth Rate and Confluency of iPSCs Supplemented With FGF-2 and TGF-β1 PLUS is Concentration Dependent
The acquisition of kinetic data on the Incucyte® Live Cell Analysis System enabled easy quantification of iPSC growth via confluency and phase object area analysis (Figure 1). Here we showed the difference in iPSC growth when cultured with varying concentrations of FGF-2 and TGF-β1 PLUS (Figure 1). The data shows that in higher concentrations of FGF-2 (50 – 200 ng/mL), iPSC growth is similar with a plateau at 3 days, which remains stable (Figure 1A). In contrast, data for lower FGF-2 concentrations (3.13 – 25 ng/mL), show a dramatic drop in confluency post three days, indicating a lack of growth and potential cell death. Analysis of average phase object area (size of iPSC colonies) over time provided more granular indication of iPSC growth patterns. For example, lower concentrations of FGF-2 (3.13–25 ng/mL) produced iPSCs that form smaller colonies after 5 days, compared to higher concentrations of supplemented FGF-2 (25–200 ng/mL) (Figure 3B). TGF-β1 PLUS supplementation at a range of concentrations (Figure 1C), had minimal impact on the growth of iPSCs, indicating this protein is not as critical as FGF-2 for iPSC growth at this concentration range. Analysis of average phase object area, however, revealed that with decreasing concentrations of TGF-β1 PLUS, iPSCs form smaller colonies over time. Although growth is minimally affected, colony size varies depending on TGF-β1 PLUS concentration (Figure 1D). Based on this data, we recommend the concentrations of 100 ng/mL FGF-2 and 2 ng/mL TGF-β1 PLUS for optimal growth and maintenance of iPSCs.

Figure 1: *Growth Analysis of iPSCs During Stem Cell Maintenance.*



Note: iPSCs were cultured in NutriStem® XF–GF supplemented with a range of concentrations of Sartorius RUO Recombinant Human FGF-2 and TGF-β1 PLUS. Cells were monitored over 6 days and analyzed using the Incucyte® Live-Cell Analysis System. Confluency analysis of iPSCs supplemented with (A) FGF-2 at decreasing concentrations and (C) TGF-β1 PLUS at decreasing concentrations. Phase Area Object Average analysis at 5 days for (B) FGF-2 and (D) TGF-β1 PLUS.

Use of Thermostable FGF2-G3 Facilitates iPSC Culture Without Daily Feeding
iPSCs are high maintenance and require daily media refreshing to maintain a pluripotent phenotype. Thermostable growth factors enable reduced media change frequency due to increased compound stability, which eliminates the need for inconvenient weekend media changes. To test this, we grew iPSCs in NutriStem® hPSC XF GF-free with supplementation (2 ng/mL TGF-β1 PLUS, varying concentrations of FGF2-G3) for 5 days without media changes and analyzed marker expression on the iQue® Platform at day 6. The Sartorius RUO FGF2-G3 is an enhanced, thermostable version of FGF-2 designed to enable longer duration between feeds.

Pluripotency marker expression was higher in all cells treated with FGF2-G3 compared to all other treatment types, with a consistent level across the concentration range (~70%) outperforming an alternative commercial media (Figure 2A). Comparisons of SSEA-1 expression show very low levels in iPSCs cultured with FGF2-G3 from 50–200 ng/mL, lower than NutriStem® hPSC XF GF-free and NutriStem hPSC XF (Figure 2B). Control cells grown in RPMI, or THP-1 cell controls, presented high expression of SSEA-1 and low levels of pluripotency marker expression as seen previously. This data indicates that thermostable Sartorius RUO FGF2-G3 maintains function in culture for longer periods, mitigating the need for daily media changes.

Figure 2: *Long Term iPSC Culture Without Media Change.*

Note: iPSCs were cultured in NutriStem® XF–GF supplemented with thermostable FGF2-G3 at a range of concentrations for 5 days without media changes and pluripotency markers were analyzed on the iQue® HTS Cytometer on day 6. (A) Pluripotent (SSEA-1, SSEA-4 +, TRA-1-60 +) and (B) Non-pluripotent (SSEA-1 +) marker expression profile. Stabilized CM (Stabilized Commercial Media enabling extended duration between feeds).Representative data from one of 3 experiments. Data presented as mean ± SEM, n = 3.

Challenges and Solutions

Challenges	Solutions
Maintaining supply chain consistency	Sartorius offers assured, consistent supply of quality raw materials with global freight and delivery network including flexible shipping options and global harmonization of standards across manufacturing sites.
Achieving reliable and reproducible results	Our products are tested in relevant biological models to demonstrate activity and application with the documentation showing how the reagents can be used alongside example data. All information is available on Sartorius.com which is regularly updated with data as it becomes available. Optimizing cytokine concentrations in cell culture is crucial for achieving reliable and reproducible results. Our **iQue® HTS Cytometry, Cell Proliferation Assays for Live-Cell Analysis** and **Octet BLI® platforms** offer reliable and fast methods for cytokine quantitation.

Related Applications

Gain insights into morphological characteristics, growth and development of organoids with Incucyte® Live-Cell Analysis System

Read eBook

References

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Resources

- Flyer: Research Use Only (RUO) Growth Factors & Cytokines for Cell-Based Applications
- Application Note: Quantifying Organoid Growth Using Live-Cell Analysis and RUO Growth Factors and Cytokines
- More information can be found on our website

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