APPLICATION NOTE

Animal-free, defined culture system preserving chondrogenic phenotype: FRS™ Pioneer and animal origin-free TGF-β1

A collaborative study between Media City Scientific and Qkine

Introduction

Primary chondrocytes are notoriously challenging to maintain in culture. In the absence of appropriate biochemical signals, chondrocytes rapidly dedifferentiate toward a fibroblastic phenotype during monolayer expansion, a process characterized by the loss of rounded morphology, reduced collagen type II and aggrecan expression, and the acquisition of fibroblast-like gene signatures. This phenotypic drift is a critical problem for cartilage tissue engineering, osteoarthritis research, and cell-based therapies where chondrogenic identity must be maintained across expansion passages [1].

Transforming growth factor beta 1 (TGF-β1) is a central regulator of chondrogenic lineage identity and a well-established component of chondrocyte expansion and differentiation protocols [2, 3]. Qkine's TGF-β1 PLUS™ is the only commercially available animal-origin-free (AOF) recombinant TGF-β1. Qkine are a specialist recombinant protein manufacturer producing completely AOF, highly bioactive growth factors and cytokines, with guaranteed lot-to-lot consistency.

FRS™ Pioneer (Media City Scientific) is a fully chemically defined, AOF serum replacement formulated for broad cell type compatibility and lot-to-lot consistency. While FRS Pioneer is standardly used for 100% replacement of FBS in the culture of immortalized cell lines, here we evaluate FRS™ Pioneer supplemented with Qkine's TGF-β1 PLUS™ and FGF-2 as a serum replacement for primary chondrocyte culture. We demonstrate that a fully defined system can match FBS-supplemented expansion while maintaining chondrogenic morphology.

Methods

Culture conditions

Primary chondrocytes were thawed into FBS-containing medium. After 24 hours, cells were directly adapted into DMEM/F12 supplemented with one of five conditions: (1) no supplement; (2) 10% FRS™ Pioneer; (3) 9% FRS™ Pioneer + 1% FBS; (4) 10% FBS; or (5) 10% FRS™ Pioneer + 10 ng/ml FGF-2 (Qk025) + 1 ng/ml TGF-β1 PLUS™ (Qk010). Cells were maintained across four passages over 15 days.

Adhesion strategy

For serum-free and low-serum conditions, 50% conditioned medium from the preceding passage was retained and combined with 50% fresh medium at each re-seed. Chondrocytes produce endogenous ECM components including collagen type II and aggrecan; conditioned medium recycling exploits this to maintain surface conditioning without exogenous coating or serum-derived adhesion proteins.

Alternatively, results were equivalent when standard TC-treated plates were coated with GECKO adhesion proteins (Media City Scientific) at 1.25 µg/cm^2 for 2h at 37°C prior to receiving cells.

Primary cells are inherently variable between donors and isolates; the conditions described here represent a validated starting point, and optimization of growth factor concentrations or adhesion coating strategy may be beneficial for specific cell sources.

Results

Fully defined medium matches FBS expansion and preserves chondrogenic morphology

Primary chondrocytes cultured in FRS™ Pioneer supplemented with FGF-2 and TGF-β1 PLUS™ achieved expansion and morphology comparable to the 10% FBS-supplemented control over 15 days and four passages. Cumulative population doublings were equivalent between the defined and FBS-supplemented conditions. The 9% FRS™ Pioneer / 1% FBS hybrid condition also performed equivalently to 10% FBS, providing a practical transition pathway for laboratories not yet ready to eliminate serum entirely.

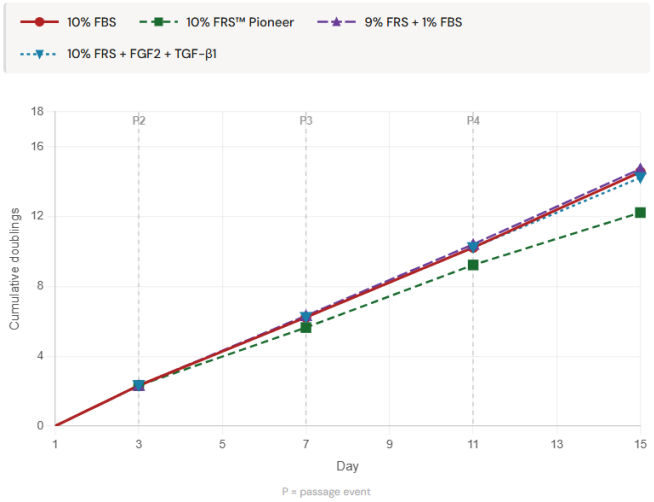

Figure 1. Cumulative population doublings of primary chondrocytes over 15 days. Defined medium (FRS™ Pioneer + FGF-2 + TGF-β1 PLUS™) achieved expansion equivalent to the 10% FBS positive control across four passages. Basal media alone failed to support cell proliferation beyond 72h. Dashed vertical lines indicate passage events. Points indicate measured values; lines represent interpolated growth between passage events. GFs: 10 ng/ml FGF-2 + 1 ng/ml TGF-β1.

TGF-β1 is required to maintain chondrogenic identity during expansion

Chondrocytes cultured in FRS™ Pioneer without TGF-β1 supplementation showed clear morphological drift toward a fibroblastic phenotype, adopting elongated, spindle-like morphology inconsistent with chondrogenic identity. This transition was not observed in cultures supplemented with TGF-β1 PLUS™, where cells maintained a compact, rounded morphology consistent with chondrogenic character. This finding underscores the role of TGF-β1 as a critical lineage-maintenance signal during chondrocyte monolayer culture [3].

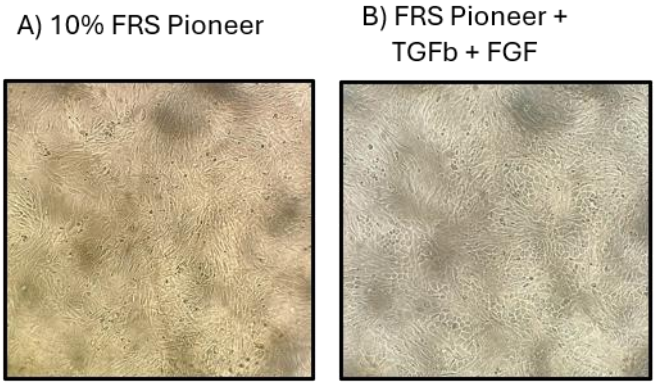

Figure 2. Representative morphology of primary chondrocytes under defined conditions. (A) FRS™ Pioneer without TGF-β1 showed morphological drift toward a fibroblastic phenotype. (B) FRS™ Pioneer + TGF-β1 PLUS™ + FGF-2 maintained chondrogenic morphology.

AOF TGF-β1: The only commercially available animal origin-free option

Qkine's TGF-β1 PLUS™ is the only commercially available animal-origin-free recombinant TGF-β1. When combined with FRS™ Pioneer — itself fully chemically defined and animal-origin-free — the complete culture system described here contains no animal-derived inputs. This represents a credible path toward manufacturing compliance for cartilage engineering and chondrocyte-based therapeutic applications.

Conclusion

FRS™ Pioneer supplemented with TGF-β1 PLUS™ and FGF-2 supports primary chondrocyte expansion equivalent to FBS over 15 days while maintaining chondrogenic morphology. TGF-β1 supplementation was required to prevent phenotypic drift: without it, chondrocytes adopted a fibroblastic morphology. The fully AOF system described provides a regulatory-compliant path for chondrocyte expansion with direct applications in cartilage tissue engineering and cell-based therapies.

References

[1] Chu Y, Hikita A, Asawa Y, et al. Advancements in chondrocyte 3-dimensional embedded culture: Implications for tissue engineering and regenerative medicine. Biomedical Journal. 2025; 48:2: 100786. doi.org/10.1016/j.bj.2024.100786.

[2] Murphy MK, Huey DJ, Hu JC, et. al. TGF-β1, GDF-5, and BMP-2 stimulation induces chondrogenesis in expanded human articular chondrocytes and marrow-derived stromal cells. Stem Cells. 2015; 33:3: 762-73. doi.org/10.1002/stem.1890.

[3] Tekari A, Luginbuehl R, Hofstetter W, et. al. Transforming Growth Factor Beta Signaling Is Essential for the Autonomous Formation of Cartilage-Like Tissue by Expanded Chondrocytes. Plos One. 2025; 10:3: e0120857. doi.org/10.1371/journal.pone.0120

Media City Scientific

Media City Scientific manufactures FRS™ Pioneer, a fully chemically defined, animal-origin-free serum replacement engineered for broad primary cell type compatibility and lot-to-lot consistency. FRS™ Pioneer is available directly and through Qkine.

https://www.mediacityscientific.com

Qkine

Qkine are committed to raising the standards of growth factors, cytokines and related proteins to better support long-term and complex stem cell culture.

https://www.qkine.com

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