Application note

Closed vial fill efficiency for iPSC cell banking

Abstract

Evaluative research of Larkem® V40 automated, closed fill system in comparison with traditional manual pipetting to produce induced pluripotent stem cell (iPSC) cell bank. Key analysis includes process efficiency, vial fill accuracy, output cell viability, concentration, iPSC pluripotency, post-thaw recovery, and reproducibility. Biological equivalence was observed for iPSC vials obtained via either fill process, demonstrating the feasibility for translation into Larkem for cell banking applications. Research was undertaken at the Cell and Gene Therapy Catapult (CGT Catapult) Edinburgh laboratories.

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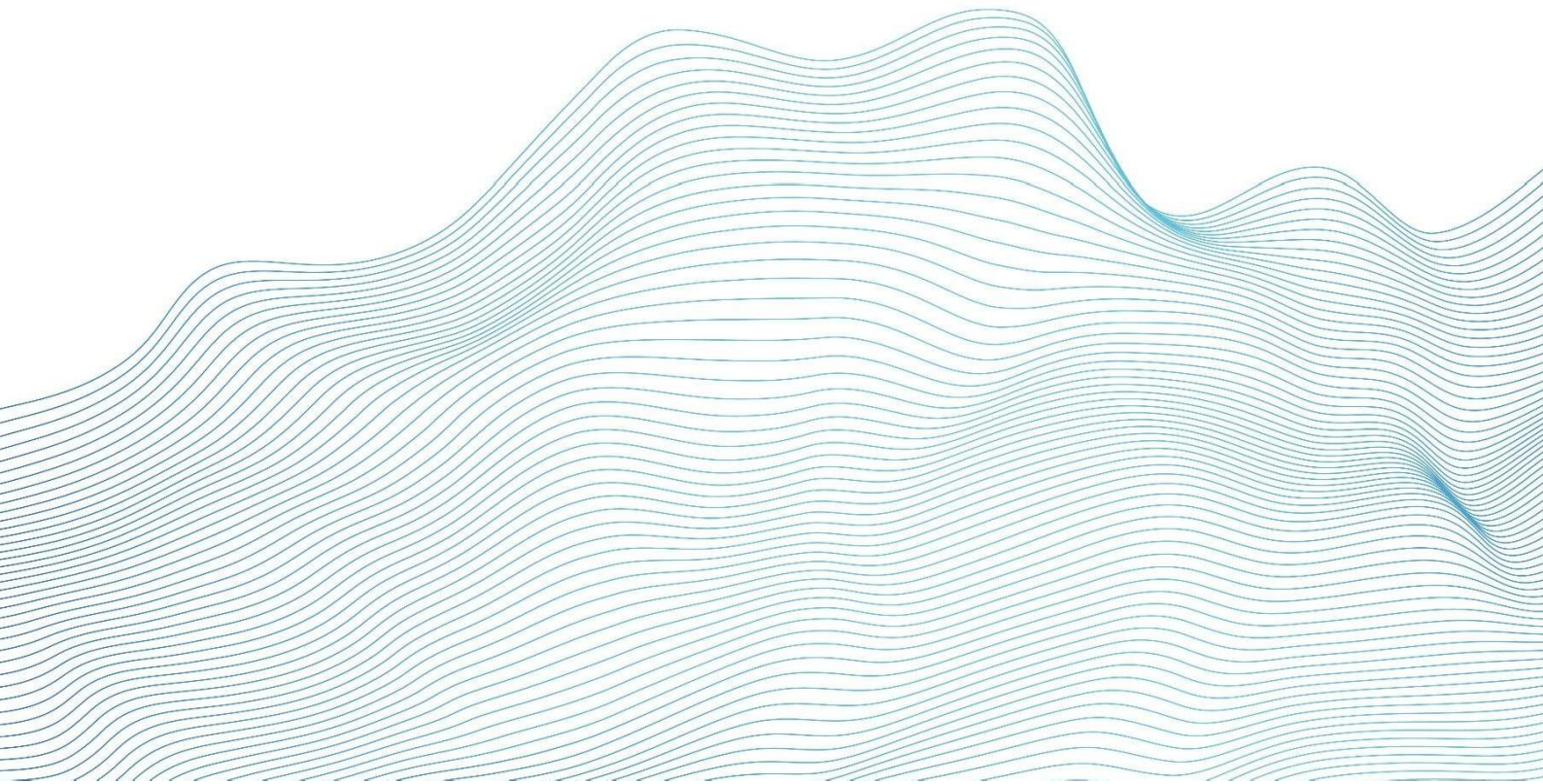

Introduction

iPSC-derived therapies offer unique advantages for scaling patient access as an allogeneic platform through unlimited expansion potential, high suitability to genetic engineering and their capability for multi-lineage differentiation giving rise to diverse somatic cell types. As such, iPSC-derived therapeutics are gaining traction as next-generation treatments for a wide range of diseases spanning type I diabetes, neurodegenerative indications, oncology and regenerative medicine.

The quality and health of the starting material, the cell bank, is a critical determinant of the therapeutic success, with efficient and reproducible vial filling vital in cell bank production. Currently, laborious manual operations remain industry standard. Manual vial filling creates several challenges including the ability to scale production, variability in vial-to-vial and bank-to-bank consistency prolonged processing times that can intensify dimethyl sulfoxide (DMSO)-related cytotoxicity, sterility risks due to open processing, and a high demand on operator time and cleanroom resources. Automated, closed-system approaches have the potential to improve process consistency and operational efficiency in cell therapy manufacturing.

Materials

Larkem V40

Celleo's Larkem V40 is a closed system for automated GMP vial filling in Grade C environments. To aseptically fill up to 40 vials in parallel, Larkem evacuates air from the closed Single-Use Set (SUS) incorporating the vials, before displacing the vacuum with fluid. The manifolded vials are automatically sealed and separated, providing industry-standard vials for subsequent use. CGT Catapult used a Tech Demonstrator (TD) version of the Larkem V40 for this study.

Methods

iPSC expansion, harvest and formulation

iPSCs were maintained and expanded in a defined, xeno-free culture system using Essential 8 medium on vitronectin-coated culture vessels. Cells were routinely passaged as single-cells using enzymatic dissociation.

For cryopreservation, iPSCs were processed according to established CGT Catapult proprietary procedures. Briefly, cells were harvested, formulated at a target cell density in a cryopreservation medium, and aliquoted prior to controlled centrifugation and resuspension to a concentration of 1 × 10^6 cells/mL.). Five input bags were filled via syringe with 45mL each and iPSC stock solution was retained and used to create manual control vials.

Fill-finish on Larkem V40 TD and manual controls, and cryopreservation

Each input bag was sequentially filled into 40x vials using Larkem V40 fill system and the 2.0mL internal thread single use consumable (SUS), illustrated in Figure 1. Vials were filled to a target volume of 1.0mL/vial, mixing speed 20rpm and fill speed of 20mL/min. Each run took 8 minutes. Vials were labelled post-fill, approximately 5 minutes for 40x vials. Manually, 12 vials were filled into Nunc 1.8mL internal thread cryovials using a 1000µL pipette at room temperature during each SUS fill. Under non-GMP controlled conditions, manual processing was approximately half as efficient as Larkem (12x vials filled in 4 minutes on average). Vials were labelled and set aside until all vials were ready to be placed into the controlled rate freezer. Cryopreserved vials were stored at −150°C for ~3 weeks prior to post-thaw analysis.

Analytics

Larkem-filled (n=15) and manual-filled vials (n=10) were thawed for viability and cell count assessment. Cell counts were performed using Nucleocounter NC-202, individual counts per vial. Subsets from each cell bank group were

pooled and analysed via flow cytometry for canonical surface pluripotency markers (CD30, SSEA3, SSEA4 and TRA-1-60), gated off live cell populations. Cell recovery was assessed by seeding iPSC onto vitronectin coated wells, tracking adhesion via microscopy and confluency over 24 and 48 hours. Statistical differences were assessed throughout.

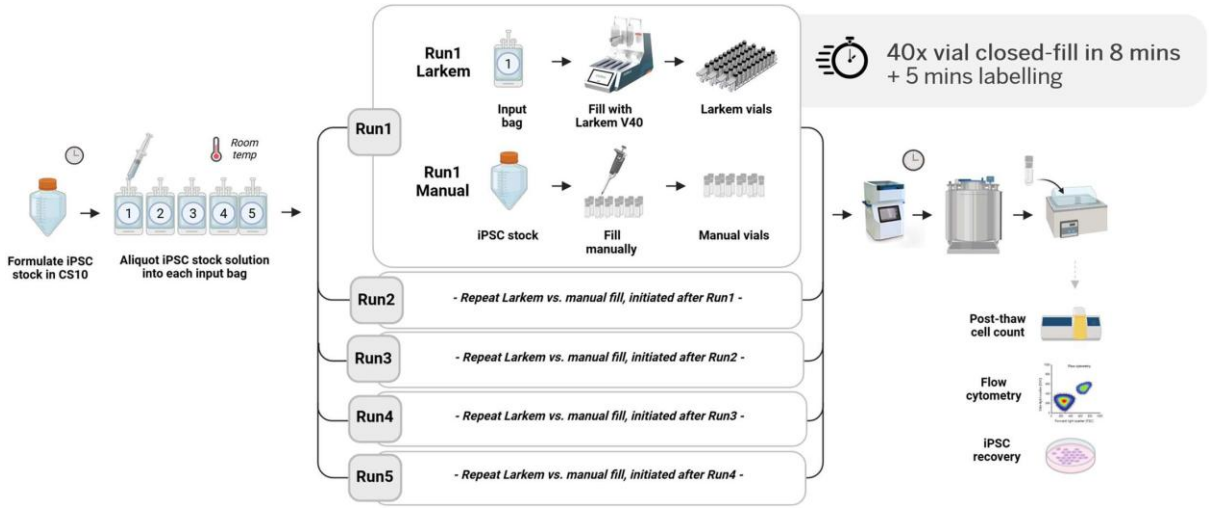

Figure 1: iPSC cell bank experiment workflow

Results and discussion

Equivalent viability and cell concentration

Larkem delivers equivalent viability and cell concentration output with manual pipetting, assessed over 5x replicate runs. This demonstrates that Larkem serves as a simple aliquoting system, ensuring that the inherent exposure to tubing and heat-seal-separate mechanisms to fill vials on Larkem do not affect cell health or concentration in vials.

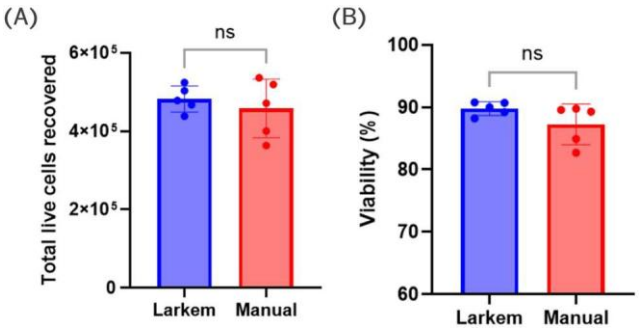

Figure 2: *(A) Total live cells recovered per vial and (B) viability following thaw of iPSC vials, filled using either Larkem V40 or manually via pipette is displayed as mean ± standard deviation (n=5). Each data point represents the average for all vials counted within each replicate run. A paired t-test was used to determine significant differences.*

Consistency run-to-run

A critical feature for cell banking is assurance of reproducibility across multiple runs. Figure 3 highlights no significant difference between each run on Larkem vs. manual fill, and consistency between each of the 5 runs. Furthermore, in Figure 2, the coefficient of variation for viability on Larkem is 1.2% and manual is 3.7%; the coefficient of variation for live cells/mL on Larkem is 6.9% and 16.3% for manual (Note: metrics were derived from a single feasibility study).

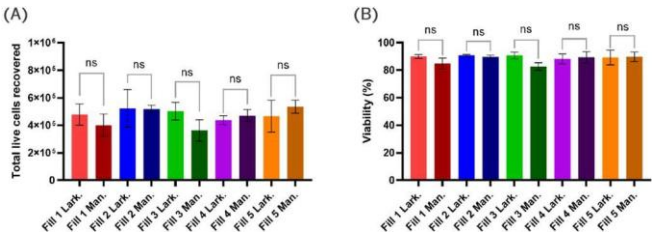

Figure 3: *(A) Total live cells recovered and (B) viability following thaw of iPSC vials, prior filled using either Larkem V40 or a manual fill. Mean ± standard deviation is displayed, cell counts were performed on NC-202, n=3 for Larkem filled vials and n=2 for manual controls. A paired t-test was used to determine significance levels between Larkem and manual filled vials per run. In addition, an ordinary one-way ANOVA was performed on both datasets across all fills, assuming normal distribution returned no significant differences.*

Pluripotency markers retained

Phenotypical expression of iPSC markers CD30, SSEA3, SSEA4 and TRA-1-60 are unaffected by Larkem or manual fill processing.

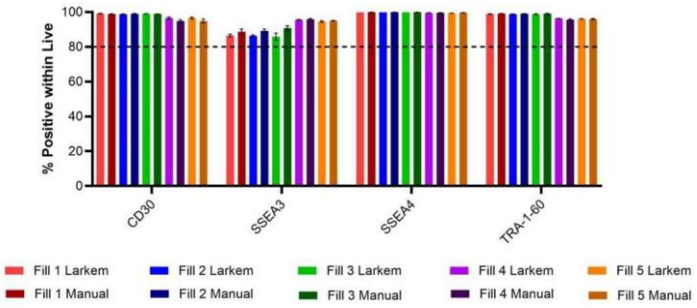

Figure 4: *Flow cytometric analysis of iPSC markers CD30, SSEA3, SSEA4 and TRA-1-60 are displayed for each group, Data was acquired on a Attune NxT (4 Laser) with analysis was performed using FlowJo v.10.10.0, where proportions of positively stained cells were gated off live. Mean ± standard deviation is displayed, (n=3 technical replicates via pooled vials from each group).*

iPSC adhesion and post-thaw recovery unaffected

Larkem or manual fill processing did not impact iPSC adhesion and post-thaw recovery, shown in Figure 5. Whilst viability and cell concentration are often good correlative signals for downstream culture recovery, this data visualises normal and equivalent iPSC morphology and recovery rates, supporting equivalent downstream functional performance to allow transition from manual pipetting workflows.

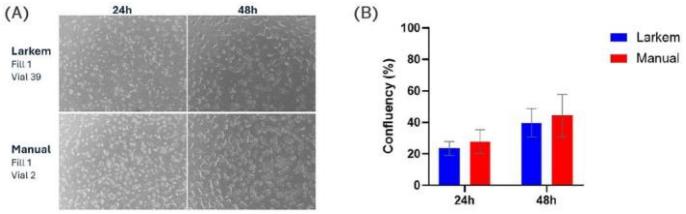

Figure 5: *(A) Representative morphology of iPSC cultures taken at 24 hours (left column) and 48 hours (right column) following thaw, seed and expansion on vitronectin coated plates. Top row displays images of a recovered Larkem filled vial and the bottom a manually filled vial. All images were captured on an EVOS M5000 microscope at x4 magnification. (B) Confluency measurements taken using CGT Catapult's confluency too. Mean ± standard deviation is displayed (n=15), where a single replicate represents a single well originating from a single vial.*

Conclusion

The evaluative research highlighted operational efficiencies using an automated, closed fill system, including reproducible performance and biological equivalence when compared to manual operations. This technology assessment demonstrates the application of Larkem V40 to streamline the workflow for manufacture of high-quality cell banks.

About CGT Catapult

Cell and Gene Therapy Catapult (CGT Catapult) is an independent innovation and technology organisation committed to the advancement of the cell and gene therapy industry. CGT Catapult's vision for the UK Industry is: globally-leading companies delivering transformative medicine to the world. Its mission is to create a pathway to accelerate breakthrough research in advanced therapies to commercialisation and scale. CGT Catapult works with Innovate UK. For more information, please visit ct.catapult.org.uk.

About Celleo

Celleo delivers intelligent production systems that enable efficient, scalable cell and gene therapy manufacture, with the vision of advancing transformative therapies toward population-level health impact. Celleo systems support leading biopharmaceuticals, CDMOs and developers of novel therapeutics across key workflows, including cell banking, drug product, QC samples, critical reagent preparation, and other emerging applications. To learn more, visit celleo.com.