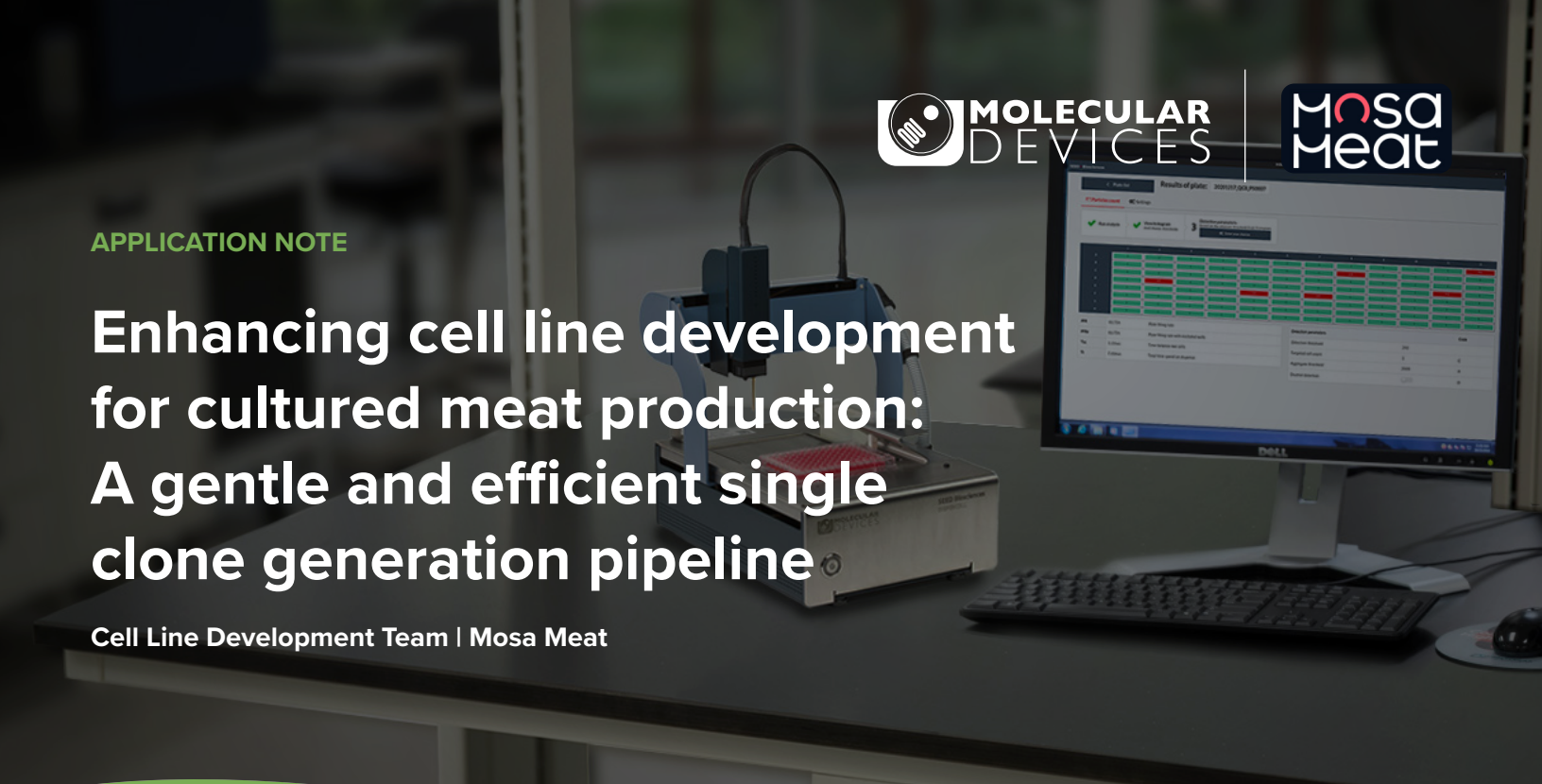

APPLICATION NOTE

Enhancing cell line development for cultured meat production: A gentle and efficient single clone generation pipeline

Cell Line Development Team | Mosa Meat

Abstract

Cultured meat (CM) is an emerging technology, which is crucial to supply the increasing meat demand while being more environmentally and animal friendly than conventional meat production. Central to this technology are muscle and fat tissues as well as their progenitor cells, satellite cells for muscle and fibro-adipogenic precursors for fat, for which Mosa Meat has developed culture media.[1,2,3,4] As part of our cell line development we generate cell lines to support the R&D at Mosa Meat, for which cell line clonality is important. Current workflows to generate single cell clones employ limiting dilution,[5] which inherently is rather inefficient, or flow cytometry-based cell sorting,[6] which is stressful especially for primary cells. As such we aimed to establish a gentle and efficient single clone generation pipeline. Here, we compared the clone generation with the DispenCell in terms of clonality, efficiency and survival as well as proliferation with a flow-based system.

Benefits

- Our gentle and efficient single clone generation pipeline ensures higher clonality rates, enabling more precise and consistent cell line development for cultured meat production.
- By utilizing the DispenCell system, improve the efficiency of cell line generation when compared to traditional methods, saving time and resources.
- Minimize cell stress and optimize culture conditions to ensure robust and sustainable cell lines.

Methods

Two bovine skeletal muscle-derived cell lines (Line 1 and Line 2) were single-cell sorted into 96-wp by either the DispenCell (DC) or the BD FACSAria Fusion (FX). For the DispenCell we set the sorting threshold to 250 ohm and the aggregate threshold to 2300 ohm. After 3 days, 96-wp were manually scored using phase contrast imaging for the presence of clones, and cells were passaged to 48-wp when they reached at least 50 % confluency.

Results

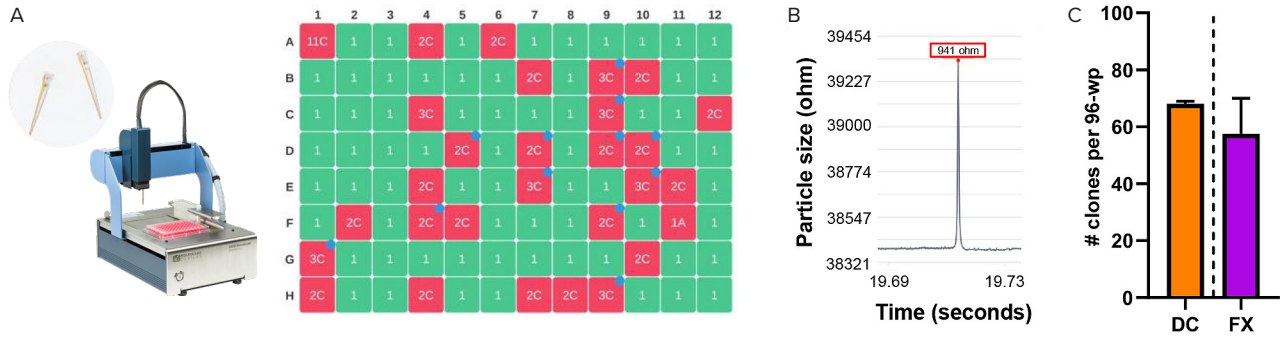

Figure 1. *Single-cell efficiency of the DispenCell. (A) The proof of clonality report shows 70 % of the cells contained a single cell and with an average sorting time of 1.8 s per well, the complete sorting of the plate took 5.5 min. A1 was intentionally seeded with more cells to ease focus finding when using the microscope. (B) Single cell event passing through the DC tip when sorting Line 2. (C) Two 96-wp (Lines 1 and 2) were sorted by DC or FX. DC efficiency was determined by impedance reading as well as phase contrast imaging, while FX efficiency was determined only by the latter.*

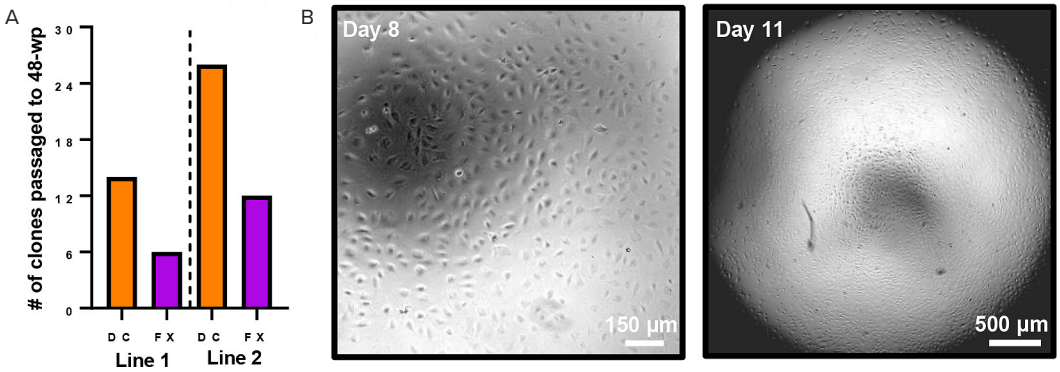

Figure 2. *Survival and proliferation of sorted lines. (A) Number of clones that were split from one 96-wp to 48-wp when the cells were at least 50 % confluent. (B) Phase contrast images of one clone on days 8 and 11 post sorting by DC in 96-wp.*

Conclusion

In terms of single-cell dispensing the DispenCell was as efficient as the FACSAria Fusion, while offering impedance-based clonality reports (Fig 1) and outperformed the flow machine in regarding survival and proliferation (Fig 2), indicating that the dispensing is gentler to the cells. Thus, for our purpose the DispenCell offers an attractive benchtop and low-footprint solution for the generation of clonal cell lines.

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