Traceable Single Cell Cloning Using A New Pipetting Robot

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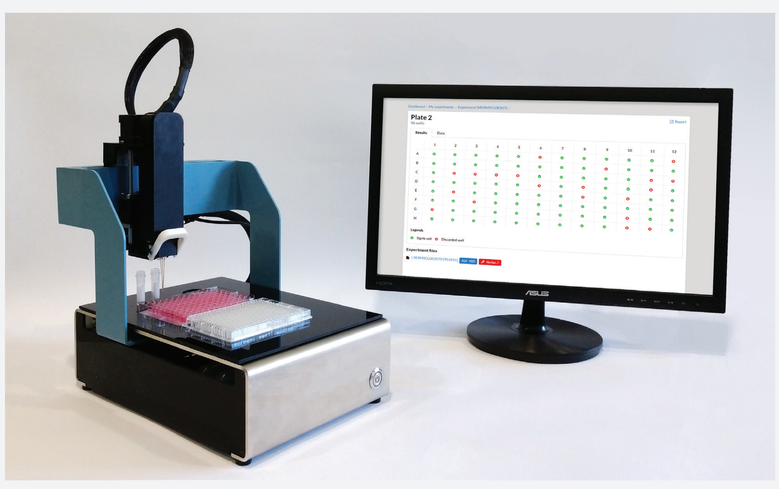
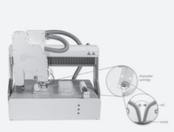
CONTEXT AND MOTIVATIONS

To ensure drug homogeneity, biotech companies should demonstrate that each new cell line has been cloned from a single progenitor cell. Because methods do not provide fully traceable cells as yet, companies may waste up to 50 weeks in validation. Here, we tested DispenCell, a new impedance-based pipetting robot allowing for traceable cloning of single cells. It is used with a disposable tip to avoid contamination and a software for single-cell quality control.

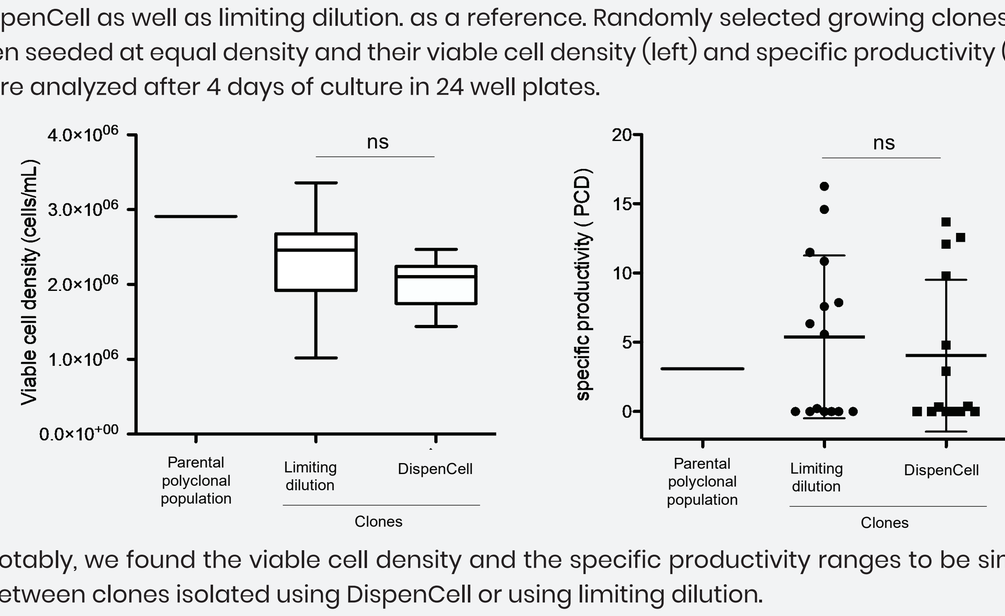


	 dilution	 cell picking	 flow cytometry	 new microfluidics
preserve cell functionality	+	+	-	?
reject doublet or aggregate	-	+	+	?
user-friendly	+	-	-	+
sterile	+	+	+	+
cost-effective	+	-	-	-
record a proof of clonality *	-	-	-	+

(*) as required by the World Health Organization (WHO, 2014); (+) met; (-) unmet; (?) lack of supportive data

IMPEDANCE -BASED SENSING TIP

The robot hosts an impedance analyzer connected to a computer and a micro-pump, coupled with a sterile disposable sensing tip that acts as a Coulter counter. The sensing tip features an external and an internal electrode. A 30 µm-diameter aperture in its center seals the lower end of the tip.

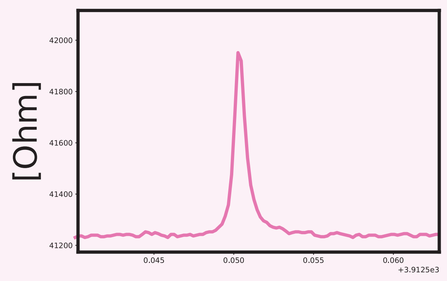


Altogether the device senses and records every single particle or cell that flows through the aperture of the tip

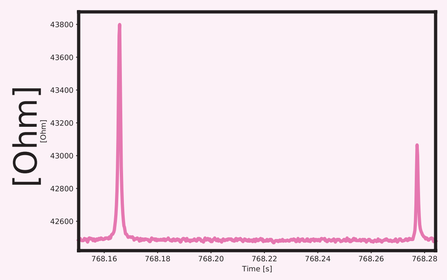
SYSTEM PERFORMANCE VALIDATION

At the end of the experiment, each impedance profile is magnified and examined in detail. . A single and sharp peak is the signature of a single cell whereas multiple peaks result from doublets, multiple cells, or doublets.

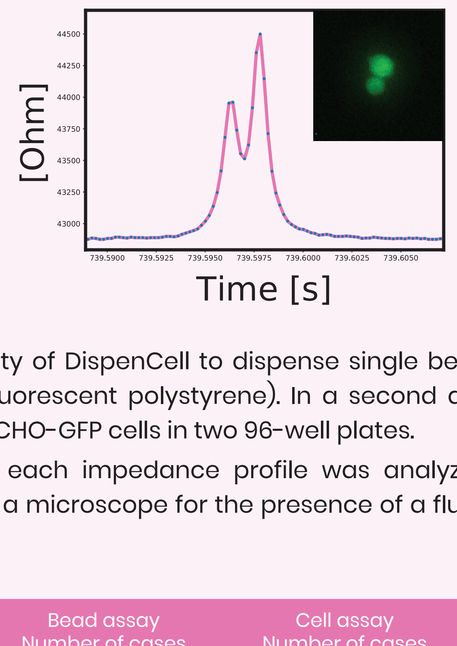
A single peak is the signature of a single cell.



Multiple peaks results from multiple cells.



Close peaks results from doublets.



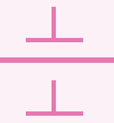
Antibody-production validation assay

Clones were isolated from an antibody-producing CHO polyclonal population using DispenCell as well as limiting dilution. as a reference. Randomly selected growing clones were then seeded at equal density and their viable cell density (left) and specific productivity (right) were analyzed after 4 days of culture in 24 well plates.

Notably, we found the viable cell density and the specific productivity ranges to be similar between clones isolated using DispenCell or using limiting dilution.

In a first assay, we tested the capability of DispenCell to dispense single beads in a 384-well plate (10 µm-beads, green fluorescent polystyrene). In a second assay, we tested its capability to dispense single CHO-GFP cells in two 96-well plates.

Single particles were dispensed and each impedance profile was analyzed. Each postive well was then examined under a microscope for the presence of a fluorescent bead..

		Bead assay Number of cases	Cell assay Number of cases
	Not checked	72	42
		303 (97%)	127 (84%)
	Empty well	5 (1.7%)	23 (16%)
		4 (1.3%)	0

This experiment demonstrated the practicality of the solution and the robustness of the impedance profile for quality control.

CONCLUSION

All of the clones isolated with DispenCell showed high cell density and viability, and they produced the protein within a similar range as the cells isolated by limiting dilution. Most importantly, this experiment showed that the robot may provide an immediate proof of single cell isolation that can be used to clone cells used for biopharmaceutical production in one single round.

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Reference: Expert Comittee on Biological Standardization, WHO 2014