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High-precision isolation of single spheroids using Dispen3D

Spheroids are an emerging trend in cell culture science, offering immense potential in areas such as drug discovery, personalized medicine and regenerative medicine. In this application note, we present a workflow for the isolation of single spheroids using the Dispen3D system, and show that it provides faster, more efficient and traceable isolation of single spheroids than is possible with limiting dilution.

Summary

Spheroids are monoclonal 3D cell cultures that self-assemble into spherical formations during cell proliferation, and have attracted much interest in a number of research fields. This interest stems from the fact that they demonstrate certain characteristics of other medically relevant cell aggregates, including:

- Extensive molecular signaling both between individual cells and with the extracellular matrix
- The formation of nutrient and oxygen gradients
- Differential drug penetration because of the presence of permeability barriers.

These characteristics mean that they can be used to model complex tissues and tumor environments better than conventional monoclonal '2D' cell cultures. However, obtaining consistent outcomes from spheroid assays requires the ability to pre-select them based on their size, and then to isolate single spheroids in various plate formats.

Until recently, this has not been straightforward, because standard methods conventionally used in industry for handling spheroids rely on slow and error-prone manual operations such as manual pipetting. Faster and more reliable methods are available, but these are typically highly complex and specialized, and are too expensive for many smaller labs and academic researchers.

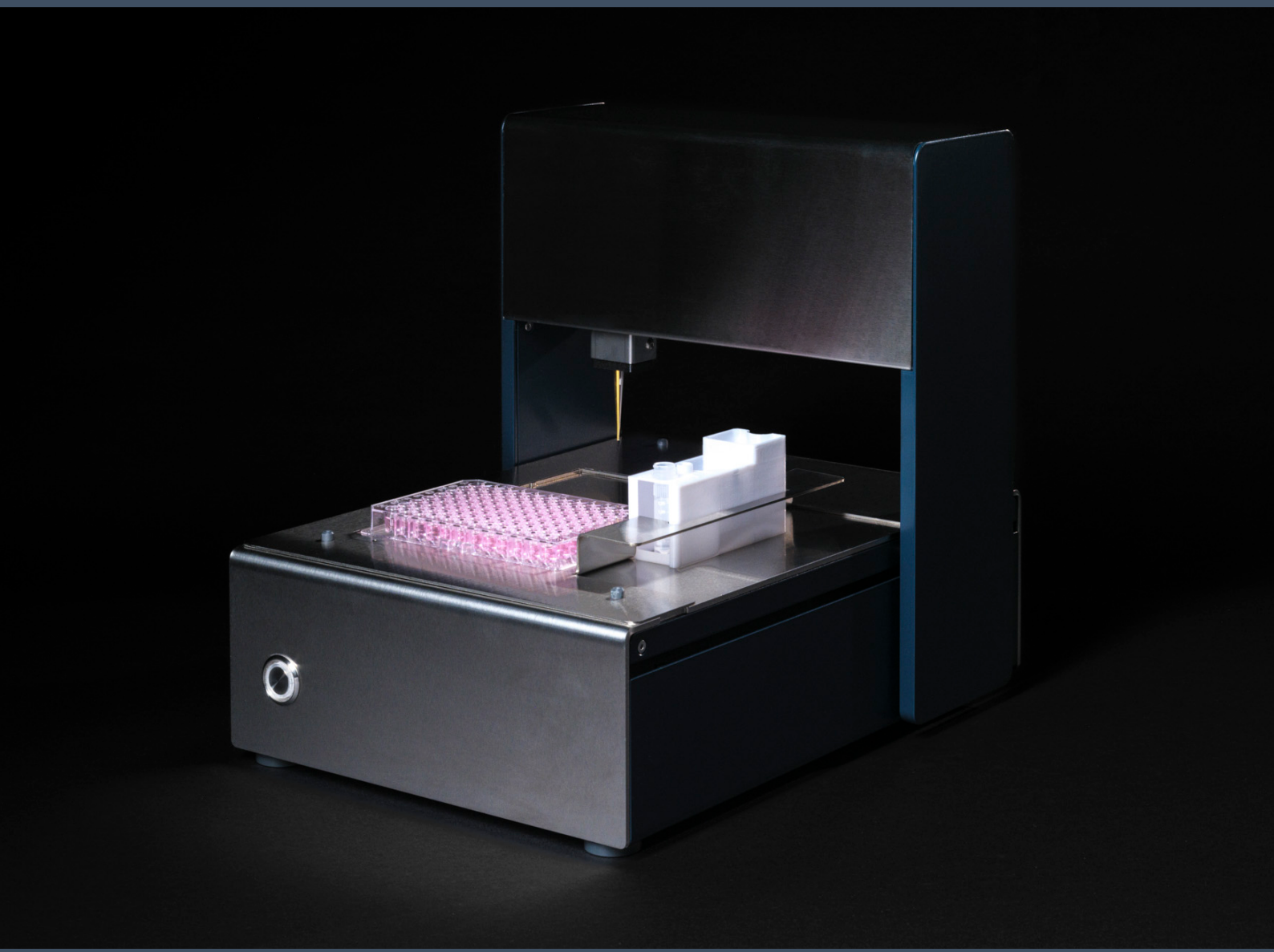
In this application note, we describe the use of Dispen3D, an affordable technology for spheroid handling that uses well-established impedance-based technology to gently dispense single spheroids into individual wells. We test this for the case of spheroids derived from a human colonic carcinoma cell line, and show that dispensing of single spheroids can be achieved more efficiently and more quickly than with conventional limiting dilution, while also ensuring their structural integrity.

Dispen3D: Impedance-based technology for gentle spheroid handling

Released in June 2024, Dispen3D is a compact and affordable system for the automated, fast and gentle isolation of cell aggregates, complementing SEED Biosciences' existing systems for single-cell dispensing.

Dispen3D uses impedance-based dispensing technology and data analysis software to precisely select and isolate spheroids and organoids ranging from 80–250 µm, in a traceable manner. With its user-friendly design and automation capabilities, Dispen3D democratizes access to advanced cell culture. This is in contrast to existing techniques of similar capability, which have limited accessibility because of the complex equipment and the expertise needed.

With a footprint of just 35 × 25 × 30 cm, Dispen3D fits into any laminar flow hood and is easy to move around. Along with its user-friendly design, automation capabilities, and the performance features described in this application note, this makes Dispen3D a convenient and powerful tool for characterizing and isolating spheroids. As a result, Dispen3D marks a transition away from conventional animal models in drug screening and other applications, and towards a future where 3D biology techniques are accessible to a broader range of researchers and laboratories.



Materials and methods

Spheroid formation

HCT-116 human colonic carcinoma cells (ECACC 91091005) were seeded at a concentration of either 48,000 or 60,000 cells/mL in AggreWell™ 400 microwell culture plates (STEMCELL Technologies), yielding spheroids with diameters of 110–175 µm after three days. These were harvested, pooled and counted, and 300 spheroids were diluted in 700 µL of DispenMe, ready for the next stage.

Spheroid isolation and growth

Spheroid dilutions were transferred to a round-bottom 96-well plate using either a limiting dilution method (with a nominal final dilution of 1 spheroid/well) or Dispen3D (Figure 1), and the results evaluated using microscopy. Spheroids were then grown for 11 days, and their radii were measured at days 0, 1, 4, 7 and 11.

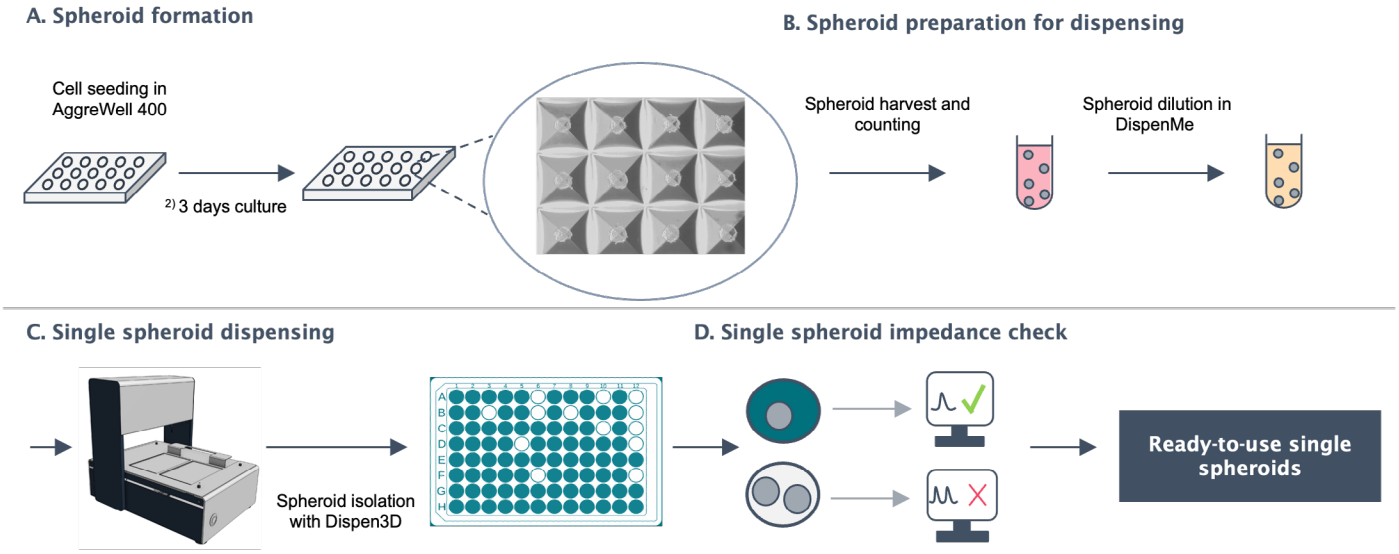


Figure 1: Workflow for spheroid preparation, dispensing and growth using Dispen3D.

Results and discussion

Reliably dispensing single spheroids

Unlike traditional methods like limiting dilution, which may be labor-intensive and prone to inconsistencies, Dispen3D offers precise and automated dispensing of cellular aggregates. This level of automation not only increases efficiency but also reduces the potential for human error, ensuring reproducibility and reliability in experimental results.

To assess the performance of Dispen3D, we first used microscopy to determine the results of dispensing spheroids into a 96-well plate using limiting dilution. This resulted in 24 wells that were empty and 44 wells with more than one spheroid, leaving just 28 wells containing one spheroid. Using Dispen3D, microscopy showed instead that 72 wells contained one spheroid, representing a 2.5-fold improvement (Figure 2). Using Dispen3D was also considerably faster, with spheroid isolation being achieved in just 6 min compared to around 30 min with limiting dilution (accounting for calculations, sample prep and dilutions steps).

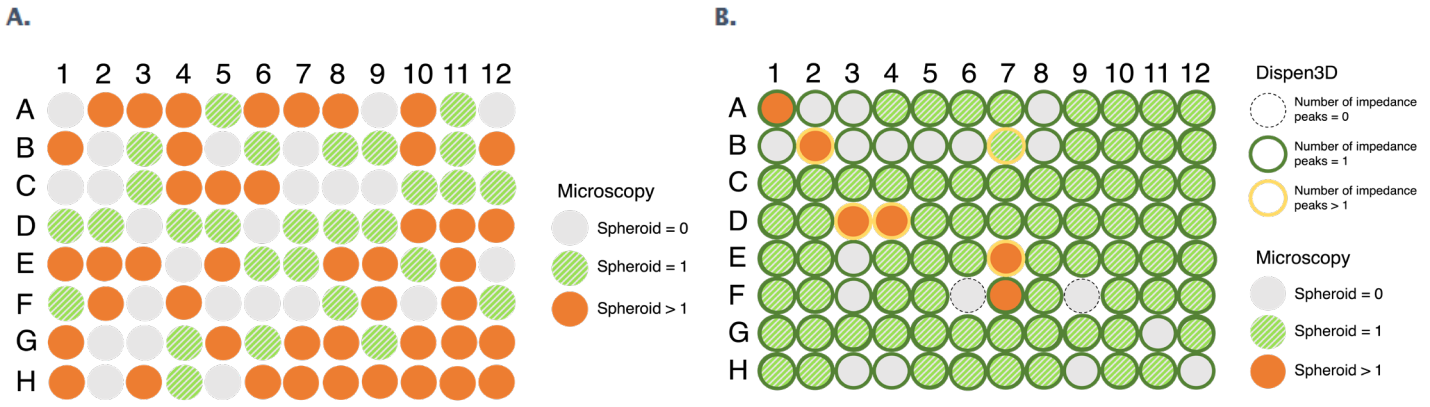


Figure 2: Results of spheroid isolation, using (A) limiting dilution and (B) Dispen3D, showing the considerable enhancement in single-spheroid dispensing (green symbols) with Dispen3D.

In real-world applications of Dispen3D, microscopy is not required, because the instrument uses a sensing tip to detect the electrical impedance as each spheroid is dispensed. This data is available within the instrument's software, with a single peak indicating that a single spheroid has been dispensed. In this study, cross-checking this data against microscopy observations showed that the impedance signal incorrectly assigns a single spheroid as being >1 spheroid on just two occasions out of 89, giving a success rate of >97%.

Achieving optimized outgrowth of spheroids

The actual magnitude of the impedance signal can also be used to provide information on the size of the spheroids. Correlating individual impedance signals obtained by Dispen3D with the area of the spheroid measured using microscopy showed a positive correlation of 0.87 (Figure 3), showing how the dispensing process can be used to discriminate spheroids based on their size.

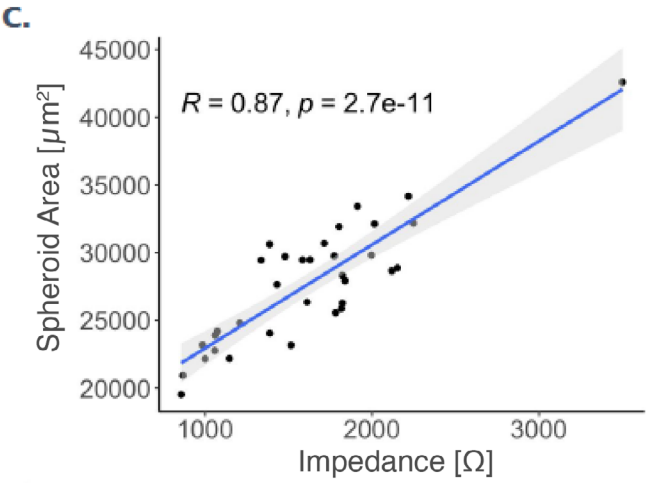


Figure 3: Analysis of the impedance values obtained during spheroid isolation with Dispen3D and the spheroid areas measured using microscopy, showing a strong correlation.

Ensuring gentle spheroid handling for consistent growth

Because spheroids are cell aggregates, they are often delicate, meaning that mechanical stress (such as that generated by pipetting or centrifugation) can easily cause aggregate fragmentation or damage to the individual cells, giving rise to inconsistent cell–cell and cell–matrix interactions. Dispen3D addresses this challenge by dispensing spheroids in an inherently gentle fashion, helping to maintain structural integrity and ensure consistency between experiments.

To assess this, the spheroids produced using limiting dilution and by Dispen3D in the previous stage were each cultured for 11 days, with microscopy observations taken at intervals. Visually, the absence of satellite cells on day 0 and the conserved circularity across the 11-day period suggest that the spheroids were undamaged by the Dispen3D process (Figure 4). This is reflected in consistent growth that is similar or even slightly better than that obtained with limiting dilution – with Dispen3D, an average 4.7-fold increase in radius is observed 11 days after isolation, compared to an average 4.4-fold increase using limiting dilution (Figure 5).

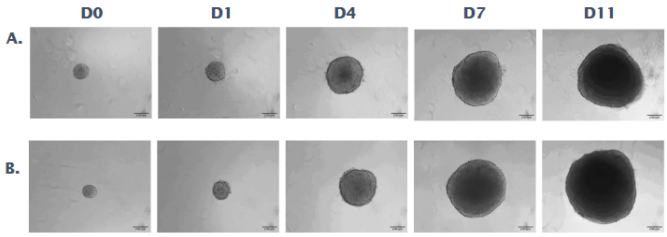


Figure 4: Microscopy evaluation of spheroid growth over an 11-day period following isolation using (A) limiting dilution and (B) Dispen3D, showing absence of satellite cells and conserved circularity.

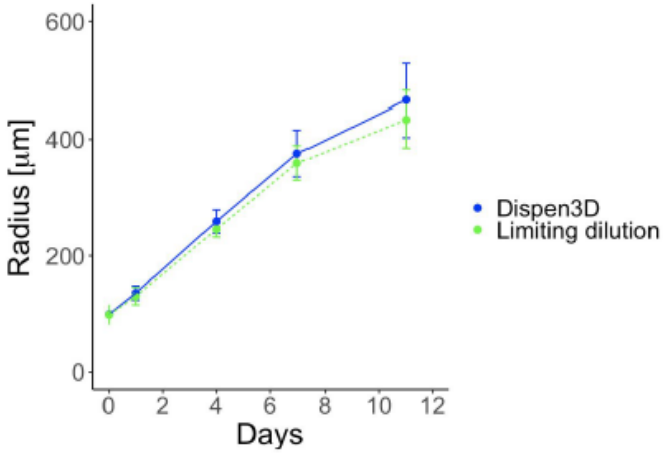


Figure 5: Microscopy-determined radii of spheroids over an 11-day period following isolation using limiting dilution (n = 15) and Dispen3D (n = 29), showing consistent growth.

Conclusions

This study has demonstrated that Dispen3D:

- Is over twice as successful at dispensing single spheroids into a 96-well plate compared to limiting dilution
- Provides confirmation of the position of wells containing single spheroids, simplifying onward processing, with an integrated analysis software.
- Is gentle enough to maintain the structural integrity of delicate spheroids, ensuring that growth, viability and function are not negatively affected
- Can be used to discriminate spheroids based on their size, reducing the need for a separate microscopy step.

These advantages, combined with its ease-of-use and compactness, make Dispen3D a valuable tool for research and medical applications where spheroids (or other organoids) are needed to mimic the 3D architecture of tissues. It is therefore envisaged that Dispen3D will find numerous applications where access to uniformly-sized and viable single spheroids is critical – such as drug development/ screening, tissue engineering, personalized medicine and immunotherapy.

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