High-throughput endometrial organoid plating in Matrigel using SPT Labtech's firefly®

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Introduction

Organoids are biologically complex three-dimensional structures that provide physiologically relevant models for studying multifaceted phenomena, such as disease progression, embryonic development, and drug response. The use of organoids has been steadily increasing and triggers the demand for reliable and scalable workflows for their generation and culture. Organoids typically require a supportive matrix that facilitates the formation of three-dimensional structures and sustains their proliferation.

Matrigel, a reconstituted basement membrane preparation derived from the Engelbreth-Holm-Swarm mouse sarcoma, is the most popular matrix used for 3D culture of endometrial organoids today. It includes a high content of extracellular matrix proteins to provide structural support and essential growth factors, to mimic the native environment and promote growth and self-organization of endometrial organoids.

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Challenges of handling Matrigel

- **Temperature sensitivity:** At temperatures above 10°C, Matrigel solidifies and cannot be easily pipetted. Therefore, precise temperature and timing control is required to prevent this from occurring. In practice, Matrigel typically needs to be kept on ice during transfer onto plates and cannot be handled at room temperature.
- **Viscosity:** Matrigel is relatively viscous, especially at higher concentrations. High viscosity reagents are challenging to pipette accurately using traditional air displacement pipetting technology and they tend to adhere to tips and vessels.
- **Throughput challenges:** Due to the properties listed above, it is challenging to use Matrigel in high-throughput applications. Increasing throughput typically requires either (a) a high number of original plates, or (b) higher density plates with smaller wells and hence smaller transfer volumes. In both cases, the careful temperature and timing control required to maintain Matrigel's liquid state becomes very complicated.
- **Strict quality requirements:** As precision-dependent readouts (such as automated microscopy) are typically used for organoid assessment, plated Matrigel domes must meet the following requirements to be considered high-quality:
  - Precise positioning
  - Accurate and uniform size
  - Spherical, without a tail
  - Sustains organoid viability and growth

Method

Endometrial organoids were thawed, propagated to p2, and suspended in 100% Matrigel. The suspension was used for plating 5 µL/well domes on a 96-well Corning Costar plate. 24 wells (B2 – G5) were plated manually and 24 wells (B8 – G11) were plated using the firefly dispense head. Using the same organoid suspension and the same plate ensured uniform conditions and enabled direct comparison between manual and automated plating. The plate was incubated for seven days and automatically imaged once a day in Incucyte SX5.

Manual and firefly-automated plating was compared using the following parameters:

1. Dome centrality
2. Organoid growth rate
3. Consistency across wells
4. Hands-on time
5. Dome morphology

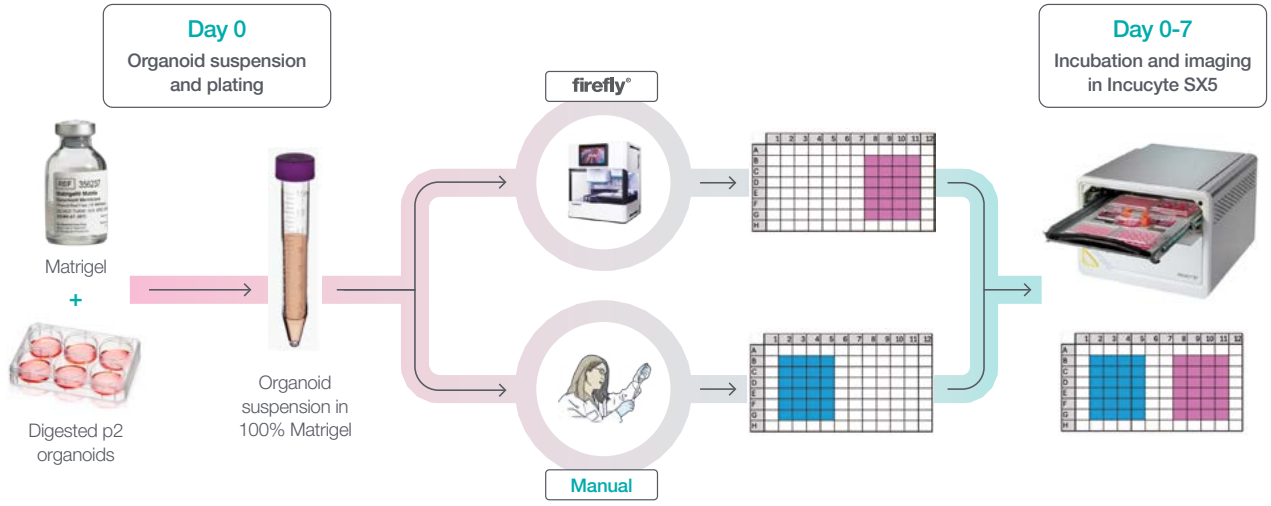

Figure 1. Experimental setup to assess organoid plating in Matrigel on firefly.

Results

The positional accuracy of the firefly dispense head yielded spherical domes that showed a high level of consistency and central placement across all 24 wells (Figure 2). Even for an experienced scientist, it would take a significant amount of time and concentration to match this performance and uniformity by hand.

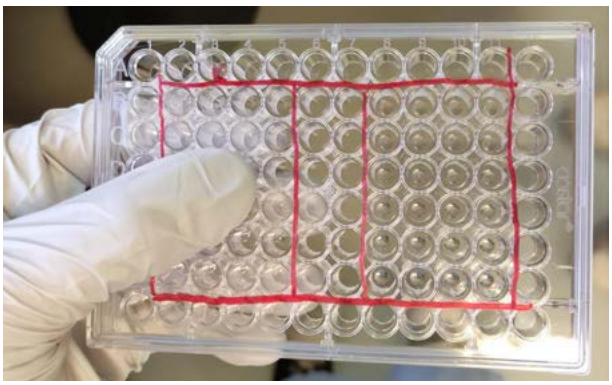

Figure 2. Uniform Matrigel domes were dispensed with high accuracy and precise positioning on firefly

Organoids plated by firefly exhibit good cell viability

The wells plated using firefly showed an increase in organoid numbers and organoid area over the 7-day incubation period, indicating that automated dispensing has no negative impact on cell viability and proliferative potential (Figure 3). The morphological features of the organoids plated by firefly are indicative of a physiologically healthy state, showing expected size, density, and appearance as hollow structures with relatively thin membranes (Figure 5).

Comparable organoid performance for manual and firefly plating

The growth rates of organoid number and area are comparable between wells plated manual and on firefly (Figure 3), indicating comparable physiological states of organoids in both conditions. Additionally, the morphology and eccentricity (measure of 'roundness') of organoids plated by firefly are comparable to those of organoids plated manually (Figure 5, Figure 3)

Organoid plating on firefly improves consistency across wells

Organoids plated by firefly showed less variability in number and growth area across wells (comparison of shaded areas in the graphs in Figure 3). Most notable was the difference in variability in organoid number observed at the point of plating. While the average numbers of organoids plated manually and on firefly were similar on day 0, the variability was significantly higher for wells seeded manually (Figure 4). Consistent plating is advantageous in organoid workflows as it improves reliability in assay setup, making results easier to interpret with less noise and uncertainty introduced at the seeding stage.

Additionally, organoids plated on firefly showed less variability in morphology and eccentricity (Figure 5 and Figure 3), most notably at the point of plating. Improved 'roundness' (lower eccentricity scores for organoids plated on firefly compared to manually-plated ones) may be indicative of more organoids showing good physiological health, thanks to firefly's gentle dispensing capability.

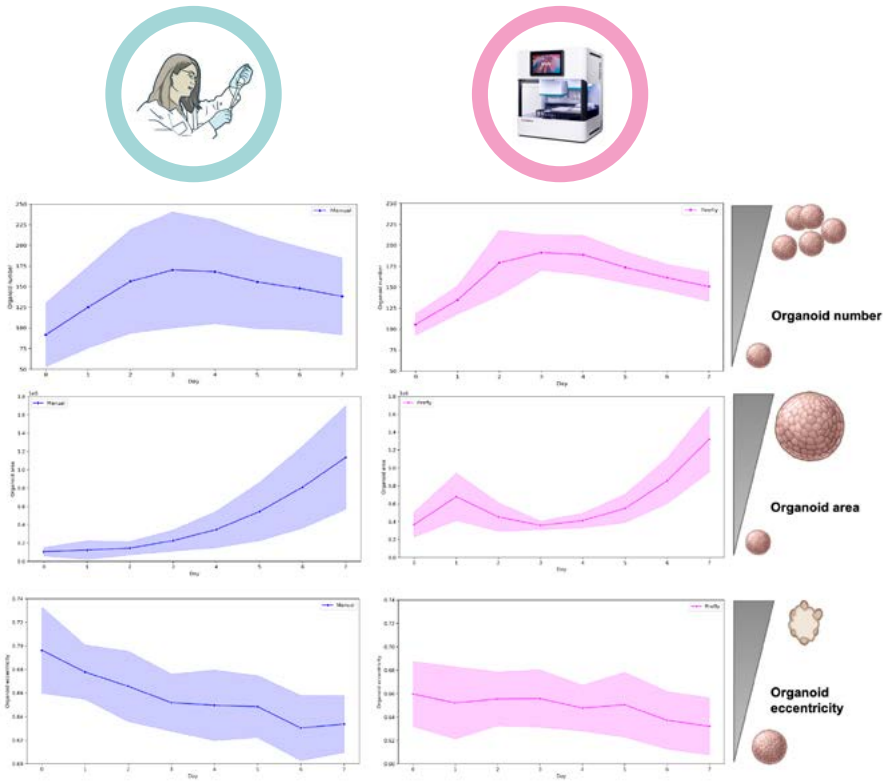

Figure 3. Dispensing organoid suspensions in Matrigel on firefly delivers excellent performance, confirmed by comparable organoid numbers, area, and eccentricity to manual plating over seven days of incubation. Each point corresponds to the mean value across 24 wells and the shading shows the variability between wells (standard deviation).

Number of seeded organoids day 0

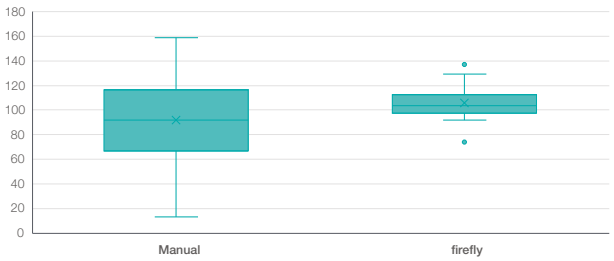

Figure 4. The uniformity of organoid numbers seeded by firefly across the wells at the start of the experiment is better than that of manual plating, which will support consistent assay setup.

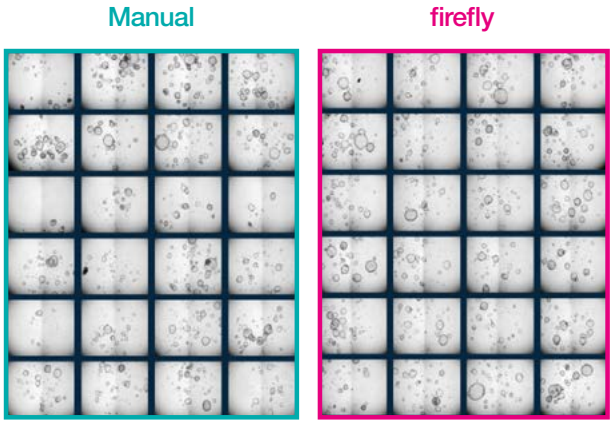

Figure 5. Organoid images of all 24 wells seeded manually and on firefly after seven days of growth.

How does firefly improve organoid plating?

- The non-contact dispense head leverages positive displacement technology to ensure precision and accuracy for even highly viscous materials, such as 100% Matrigel, while preserving organoid viability and proliferative potential.
- firefly delivers significant time savings through its fast dispense capability, taking less than 1 second per well (compared to approx. 25 seconds when performed manually by an experienced scientist).
- Reagent reservoirs can be temperature-controlled so that Matrigel is kept at a sufficiently low temperature to prevent gelation and ensure precise, accurate dispensing. The recently introduced thermal adaptors for reservoirs provide close contact and optimal temperature transfer from thermal module to the Matrigel. This promotes stable, low-temperature handling of Matrigel throughout the plating process.
- A single syringe can be used to dispense organoid suspensions across an entire plate, or multiple plates, significantly reducing plastics usage and variability.
- firefly offers scalability to support higher-throughput applications:
  - Users can choose up to 6 syringes in parallel to maximize dispense speed
  - The 16 available deck positions can accommodate sufficient labware to run multiple plates in parallel

Conclusion

firefly offers a more efficient and scalable method of dispensing 100% Matrigel-embedded endometrial organoids compared to manual pipetting. These results demonstrate improved consistency across wells in organoid plating with firefly compared to the manual process, along with comparable organoid growth and morphology over seven days of incubation. Automating organoid plating with firefly is especially advantageous for high-throughput applications, where precise, accurate deposition of small Matrigel volumes into high-density plates is required, and manual processing is too labour-intensive and variable.

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