A New Organoid Culture Platform for Forskolin-induced Swelling Assays and Drug Screening Applications

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INTRODUCTION

Organoid cultures are emerging as pivotal systems for studying human organ development, disease modeling, drug efficacy and toxicity screening, and are showing promise in the development of personalized medicine. Organoids offer higher biological complexity than traditional 2D cultures by supporting more physiologically relevant cellular diversity, spatial organization, and mass transfer dynamics. Traditional organoid culture methods rely on the seeding of cells within an oblate spherical volume of matrix conventionally referred to as a "dome". Dome culture methods routinely produce inconsistent culture geometries which add systematic variance to the biological complexity of the culture. This results in high heterogeneity that confounds downstream analysis during functional or drug screening applications.

The Organoid Culture Plate reduces systematic variation in organoid cultures by controlling the geometry and positioning of cell-containing matrices. In particular, the depth of the formed volume is maintained constant, reducing the occurrence of mass transfer differences throughout the culture and increasing well-to-well consistency. A second feature allows for the formation of a second matrix-based culture of equal volume and controlled depth above the first volume. Advantages of the Organoid Culture Plate include a standardized well position for manual and automated seeding, improved imaging, optimal matrix depth, and compatibility with a wider range of other extracellular matrices (ECMs). This novel culture plate geometry simplifies organoid culture and reduces heterogeneity to create a more robust screening platform.

METHODS

Evaporation

24- and 96-well Organoid Culture Plates were filled with 500 µL and 200 µL of distilled water coloured with red food coloring dye, respectively. Evaporation was quantified over 7 days through daily absorbance measurements taken at 504 nm. The concentration of red dye was correlated with absorbance according to the Beer-Lambert Law. Wells were categorized as "corner", "edge", and "inner" to determine any differences in edge effects due to increased evaporation in wells located at the edges of a plate.

Organoid Culture and Characterization

Hepatic organoids were maintained in HepatiCult™ and cultured in 24-well Organoid Culture Plates or traditional dome cultures for 5 passages. Intestinal organoids were differentiated in IntestiCult™-ODM either in 24-well Organoid Culture Plates or traditional dome cultures. SOX9, HNF4α, MUC2, KRT20, and CHGA expression was measured by immunocytochemistry (ICC).

Drug Response Assays

The impact of culture geometry on organoid drug response was tested in two different assays comparing organoids generated by Organoid Culture Plates with traditional dome culture organoids. Normal and cystic fibrosis (CF) donor-derived human intestinal organoids were cultured in IntestiCult-SF™ Organoid Growth Medium (Human). Organoid swelling was measured following pretreatment with 3 µM of Orkambi® and the addition of 5 µM of forskolin. In a toxicity assay, normal intestinal organoids were treated with 0, 1, or 10 µM of gefitinib, and the resulting IC_{50} values were compared.

RESULTS

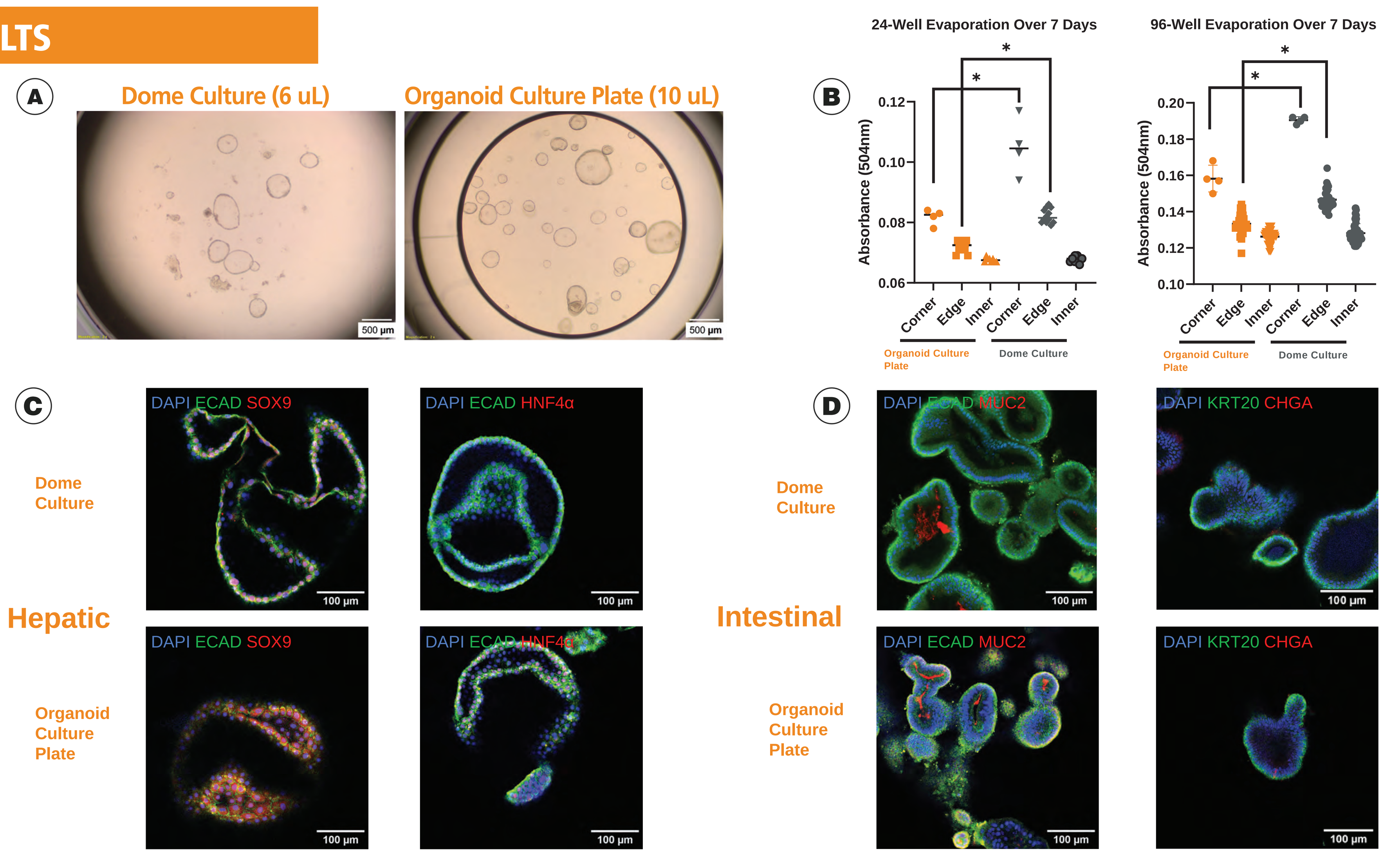

FIGURE 1. Benefits of Using the Organoid Culture Plate.

(A) A standard 96-well dome culture (left) is usually limited to 6 µL to avoid dome formation failure.The Organoid Culture Plate 96-well format contains 10 µL of organoid culture, allowing a larger surface area to support more organoids for analysis while maintaining a consistent centralized culture area. **(B)** The Organoid Culture Plate has a reduced evaporation rate and edge effects for better long-term cultures (data shown as mean ± SD; 24-well Organoid Culture Plate: 0.082 ± 0.002; 24-well dome culture: 0.106 ± 0.009; 96-well Organoid Culture Plate: 0.168 ± 0.007; 96-well dome culture: 0.19 ± 0.002; $p < 0.0001$; n = 4). **(C)** Human hepatic organoids maintained stem cell phenotypes (SOX9+ and HNF4α+) when cultured in the Organoid Culture Plate for 5 passages. **(D)** Human intestinal organoids differentiated in IntestiCult™-ODM and cultured in the Organoid Culture Plate displayed gobletcell (MUC2+), enterocyte (KRT20+), and endocrine secreting cell (CHGA+) characteristics.

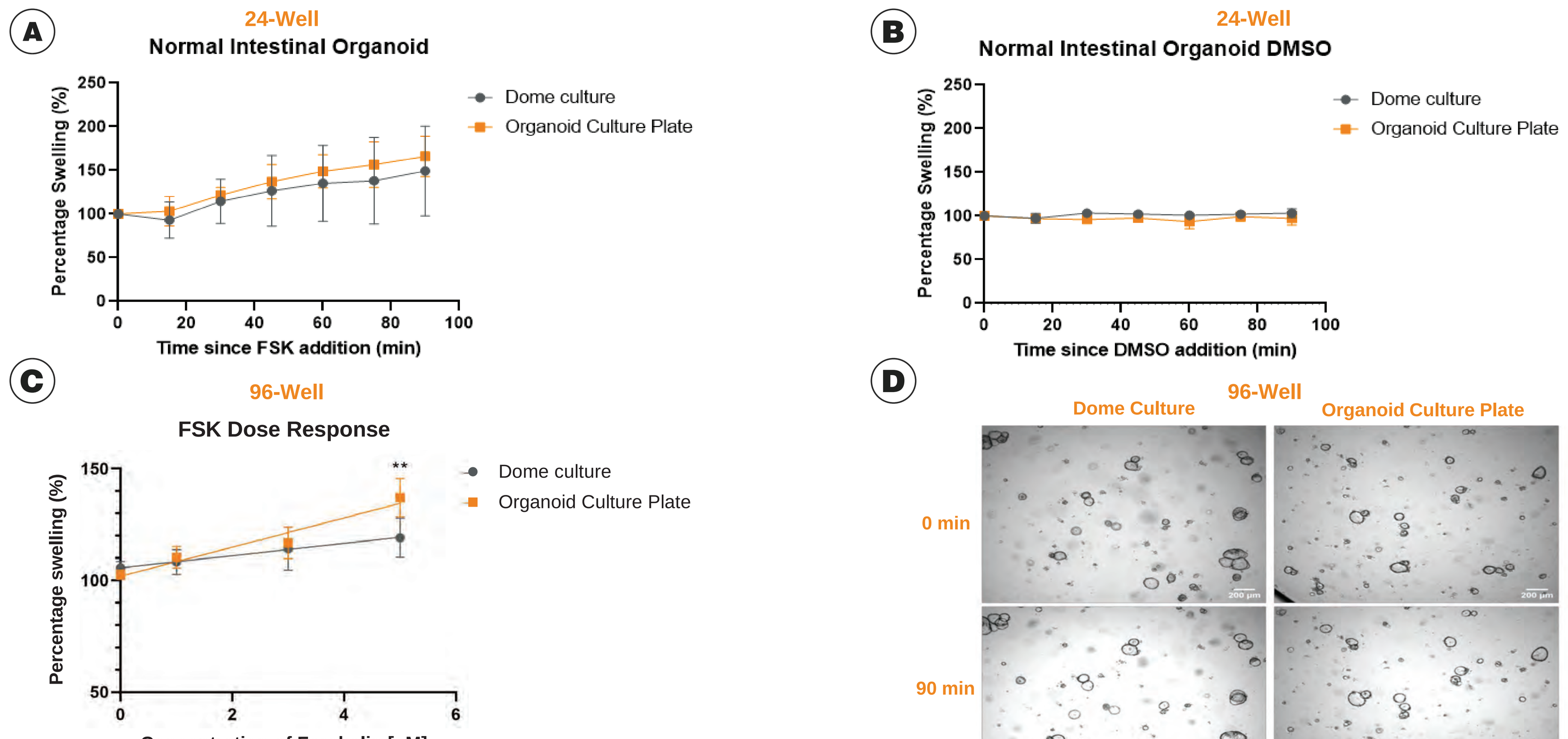

FIGURE 2. Normal Human Intestinal Organoids Cultured in the Organoid Culture Plate Displayed a More Robust Response to Forskolin-induced Swelling Assay.

(A) Normal human intestinal organoids demonstrated swelling in response to 5 µM forskolin (FSK), increasing in size by 66 ± 22% (mean ± SD) in the 24-well Organoid Culture Plate (n = 4, $p \leq 0.05$) versus 50 ± 50% increase in traditional 24-well dome cultures (n = 4, $p \leq 0.05$). **(B)** Normal human intestinal organoids showed no response to DMSO treatment in either 24-well plate (n = 4). **(C)** Normal human intestinal organoids cultured in 96-well Organoid Culture Plates showed a more robust response to increasing forskolin concentration compared to 96-well dome cultures (data shown as mean ± SD, 137% ± 8.5% for Organoid Culture Plate, and 119% ± 8.8% for dome culture; n = 3, $p \leq 0.01$. Two-way ANOVA). **(D)** Representative images showing comparable swelling of normal human intestinal organoids after 90 minutes in response to the addition of 5 µM forskolin in either Organoid Culture Plates or dome cultures.

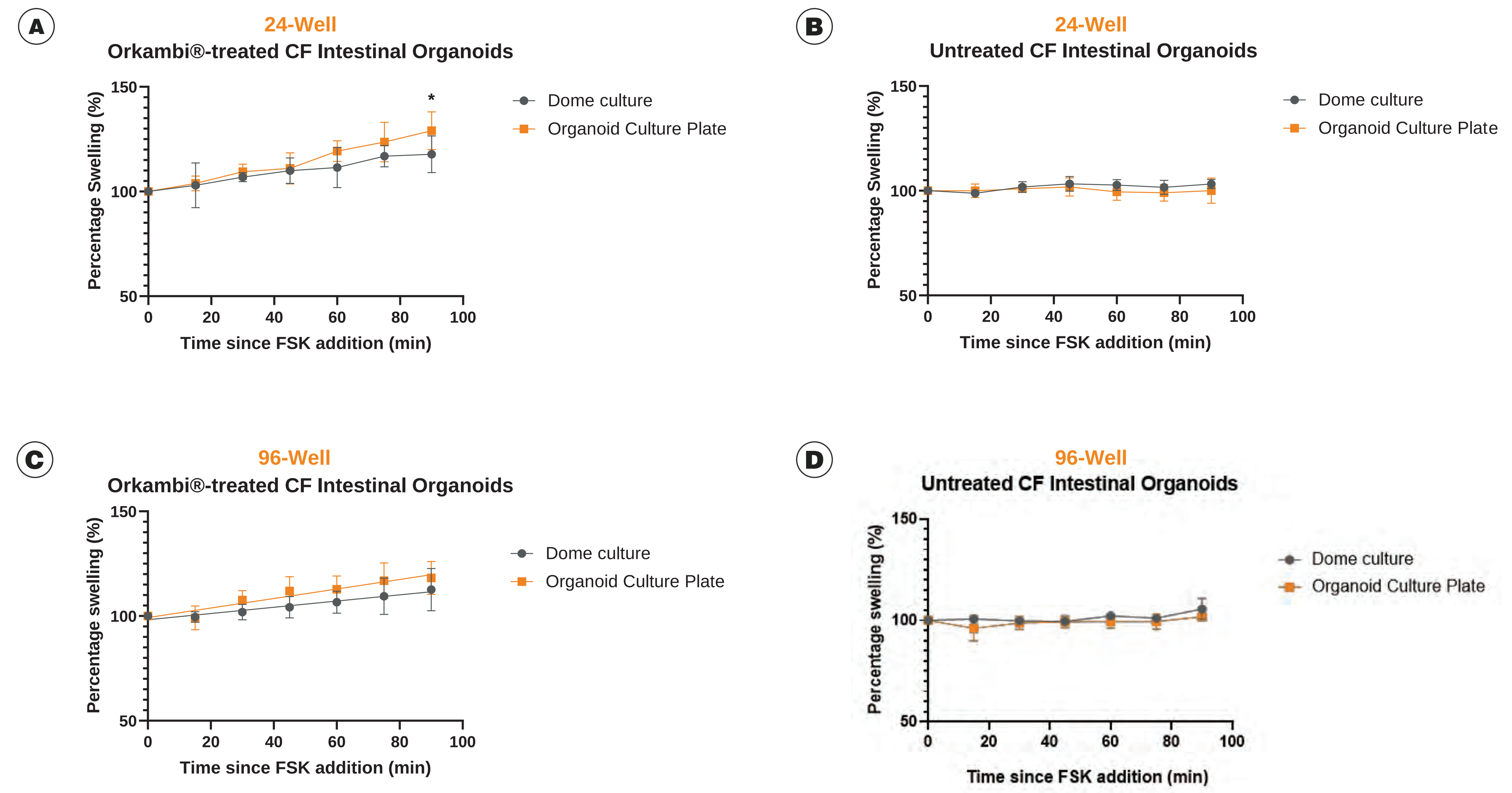

FIGURE 3. Organoid Culture Plates Provided Equal or Greater Response of Targeted Biological Readouts to Forskolin-induced Swelling Assay on Orkambi®-treated CF Intestinal Organoids.

After treatment with 3 µM Orkambi®, CF organoids partially restored CF transmembrane conductance regulator (CFTR) function, as observed by restored swelling response. **(A)** Organoids cultured in 24-well Organoid Culture Plates demonstrated a more robust swelling response, increasing in size by 29 ± 9% (mean ± SD), while 24-well dome cultures increased by 18 ± 9% (n = 4, $p \leq 0.05$). **(B)** Untreated CF human intestinal organoids failed to respond to forskolin in either culture condition. **(C)** Organoids increased in size in both the 96-well Organoid Culture Plate (16 ± 9%, n = 3, $p \leq 0.05$) and in 96-well dome cultures (11 ± 10%, n = 3, $p \leq 0.05$). **(D)** Untreated CF human intestinal organoids failed to respond to forskolin in 96-well plates (n = 3).

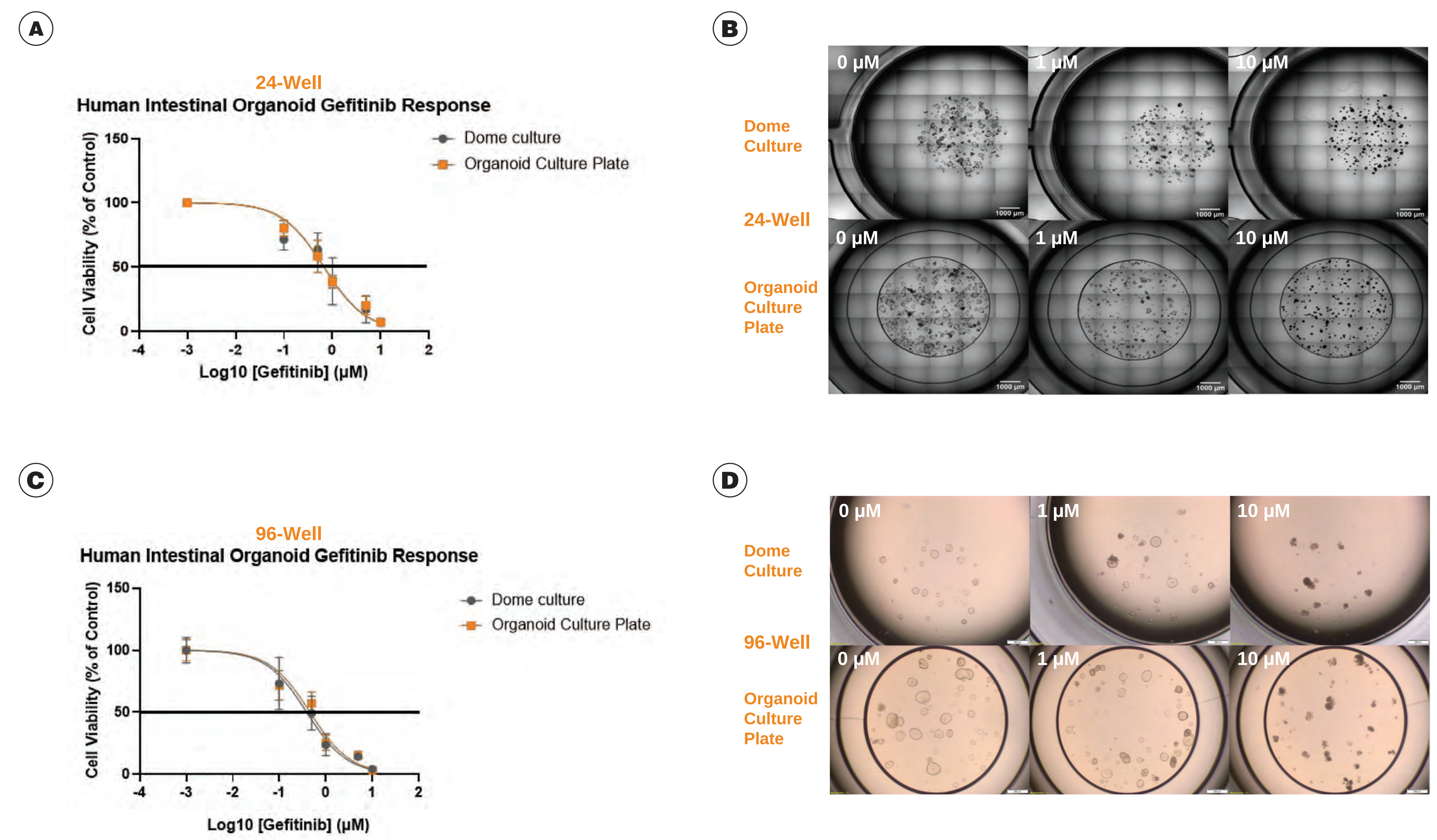

FIGURE 4. Organoid Culture Plates Displayed Higher Reproducibility for Gefitinib Toxicity Response Assay.

(A) Organoid Culture Plate (24-well) cultures produced equivalent IC_{50} values to dome cultures after gefitinib treatment, but with lower experimental variation (0.67 ± 0.079 µM and 0.67 ± 0.12 µM, respectively; $p \leq 0.05$, n = 3). **(B)** Representative images of human intestinal organoids responding to gefitinib treatment in 24-well plates. **(C)** Organoid Culture Plate (96-well) cultures produced similar IC_{50} values to dome cultures after gefitinib treatment, but with lower experimental variation (0.46 ± 0.08 µM and 0.39 ± 0.09 µM, respectively; $p \leq 0.05$, n = 3). **(D)** Representative images of human intestinal organoids responding to gefitinib treatment in 96-well plates.

Summary

- The Organoid Culture Plate is easy-to-use and can support both long-term maintenance culture and high-throughput screening, including forskolin-induced swelling and gefitinib toxicity assays.
- This system controls the geometry of 3D matrix-based culture volumes to reduce technical variability and minimize edge and corner well effects due to evaporation in whole plate cultures.
- In the work shown here, 24- and 96-well Organoid Culture Plates match or exceed the performance of traditional dome cultures in all applications tested, and consistently show lower replicate standard deviation than traditional methods.
- True demonstration of reduced variability and increased statistical certainty in biologically heterogeneous organoids cultures requires performance of experiments with extremely large numbers or replicates. Work currently in progress will evaluate this using automated seeding and handling procedures to compare multiple plates of each culture type.