APPLICATION NOTE

GelControl™ Inserts for Hydrogel Dome Workflows

Stabilizing hydrogel dome constructs to improve shape and positioning consistency, simplify handling, improve imaging accessibility, and maintain cellular health.

Summary

GelControl™ inserts are designed to complement soft hydrogel dome workflows (e.g Matrigel domes) to simplify and enhance the reproducibility of fragile 3D cell culture constructs. Experiments were conducted using NIH 3T3 fibroblasts suspended within Matrigel to assess the effects of initial hydrogel concentration and cell seeding density on cellular metabolic activity. GelControl™-supported cultures were compared with conventional dome cultures to identify compatible Matrigel concentrations and evaluate the effect of the inserts on cultures. The results revealed GelControl™ inserts improved shape consistency and enabled faster lifting and transfer with minimal sample disruption for simplified handling. The inserts also facilitated clear brightfield and fluorescence imaging with improved accessibility to the samples and did not reduce cellular metabolic activity. The experiments revealed two surprising findings: 1) the inserts enabled increased metabolic activity at higher cell densities ($2 \cdot 10^6$ cells/mL) and Matrigel concentrations (4.5 and 6.0 mg/mL) and 2) The inserts enabled stable and consistent workflows at lower Matrigel density of 3.0 mg/mL.

Key takeaways

- GelControl™ inserts stabilized Matrigel dome geometry and improved well-to-well positioning consistency.
- The inserts supported routine media exchange, lifting, and transfer with reduced deformation compared with unsupported domes.
- Supported domes remained compatible with brightfield monitoring, endpoint fluorescence imaging, and metabolic activity readouts.
- GelControl™-supported cultures maintained relative metabolic activity and increased metabolic activity at higher cell densities and gel concentrations.
- The inserts enabled stable handling of lower-concentration 3.0 mg/mL Matrigel domes that were not possible to maintain as conventional unsupported domes.

Background

Organoids are increasingly used as 3D models of development and disease because they can be expanded, cryopreserved, and experimentally manipulated using workflows adapted from 2D culture (1,2). Organoid cultures are often formed with basement membrane extract (BME), such as Matrigel domes (3,4), but BME composition, matrix concentration, dome formation, and handling conditions introduce substantial variability (5). Although synthetic ECMs offer more defined and tunable alternatives, reproducibility challenges associated with 3D construct geometry, manipulation, and handling commonly persist across matrix systems (5). Yet, the long-standing reproducibility challenges of 3D cell cultures thus remain a bottleneck, limiting the effectiveness of predictive models and translational 3D cultures. In larger 3D constructs, inconsistent position and geometry affect growth-factor and nutrient diffusion and may contribute to necrotic-core formation (6). In-fact the dome shape is not designed for organoids, but an artifact of the available materials and technologies (7). Fragile 3D constructs can also be disrupted by routine workflows such as media exchange, scraping, dissociation, embedding, and transfer to other containers (8). Automation and higher-throughput workflows could reduce some sources of variability such as shape and position. But, changing the entire volume of medium without disrupting fragile domes cannot be easily solved solely by liquid handlers, and organoid cultures often rely on limited patient-derived or iPSC-derived cells and costly, low-volume ECMs. Practical tools that simplify BME dome handling, reduce manual variability, and lower the expertise required for consistent execution could significantly improve experimental reproducibility of

organoid cultures. This application note introduces GelControl™ well-plate inserts as a workflow tool for stabilizing hydrogel domes, simplifying routine handling, and improving consistency across 3D culture experiments.

Introduction

GelControl™ inserts provide a support structure to compliment the workflow of soft hydrogel dome constructs. The inserts arrive mounted on the bottom of well plates and sterilized, readily integrating in cell culture workflows (Figure 1). GelControl™ streamlines soft gel workflows by providing a mold to ensure consistent shape and positioning with less pipetting dexterity and efficient handling of constructs with a dedicated insert handle. The molding action of the inserts also introduces a more flat dome structure due to the capillary action of the inner ring. Importantly, the inserts promoted higher cellular metabolic activity in higher cell density cultures (> $1.0 \cdot 10^6$ cells/mL) and higher Matrigel concentrations (> 3.0 mg/mL), potentially increasing organoid throughput by soft gel workflows. GelControl inserts are composed of an inner ring, a thin microfiber grid, and wings that engage the edges of a well (Figure 2). The inner ring provides a mold forming consistent construct geometries, the fine grid beneath the hydrogel permits lifting and transfer of fragile cultures with minimal deformation, and the wings facilitate a centralized sample location that mitigates the edge effect and ensures even diffusion of growth factors and nutrients.

This application note evaluates GelControl inserts with NIH 3T3 fibroblasts cultures prepared with standard Corning Matrigel. Two experimental variables were evaluated: initial cell density and Matrigel concentration. Construct geometry, handling and transfer, construct imaging, and metabolic activity were compared between GelControl-supported constructs and conventional hydrogel dome controls.

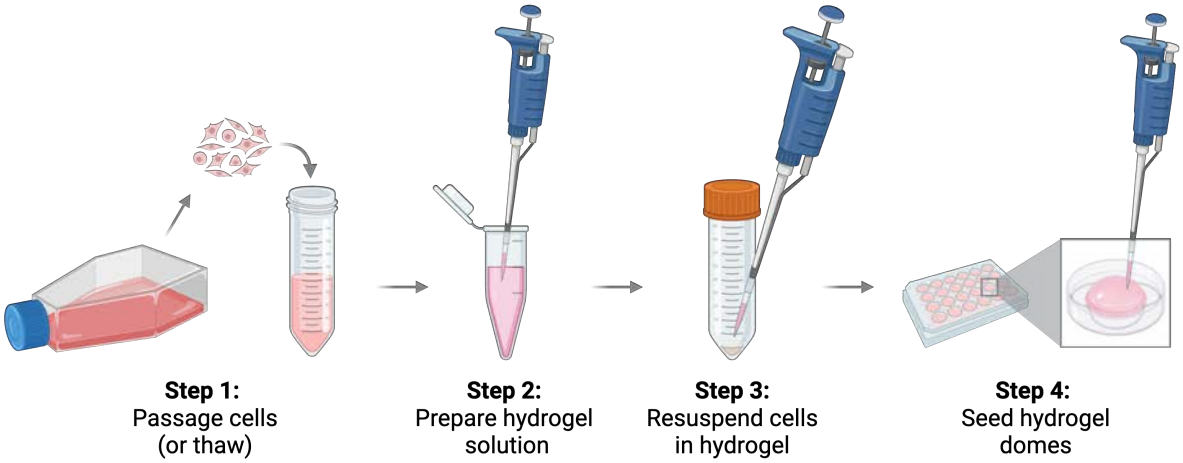

Figure 1: Hydrogel dome workflow with GelControl inserts. GelControl inserts provide a defined structural support for soft hydrogel domes, enabling more consistent construct placement and geometry.

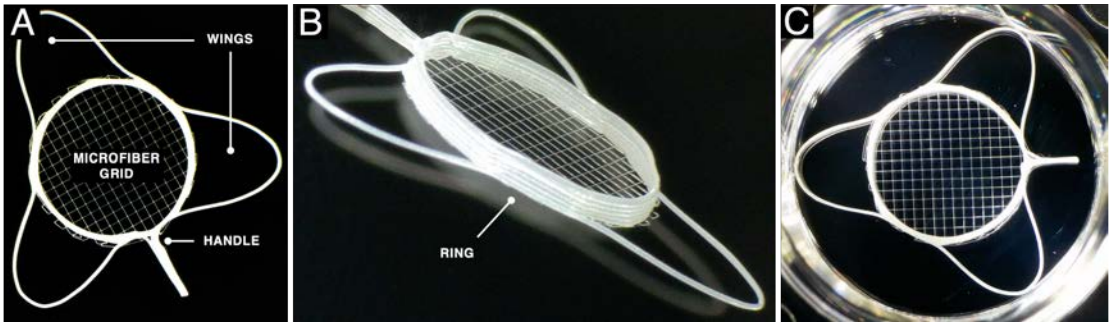

Figure 2: Detailed view of GelControl Inserts sized for 24 well plate. A) Top view of inserts highlighting the wing and handle portions. B) Isometric view of insert highlighting the inner ring portion that molds soft hydrogel shape. C) Top view of insert mounted inside a well plate. The wings press against the walls to keep hydrogel constructs centered within the well.

Design rationale: stabilizing hydrogel domes

GelControl™ inserts are intended compliment soft hydrogel workflows, such as Matrigel domes used in organoid cultures, to reduce sample loss and improve reproducibility. The inserts are not a replacement ECM. This application note evaluates the contribution of the inserts into measurable and qualitative benefits:

- **Construct geometry:** area and circularity were measured to determine whether GelControl inserts improve shape consistency.
- **Handling:** lifting and transfer were evaluated to determine whether constructs could be moved without inducing deformation.
- **Imaging accessibility:** brightfield and fluorescence images were used to assess whether flatter, more consistently positioned constructs are easier to image during culture and at endpoint.
- **Cellular metabolic activity:** relative metabolic activity was measured using alamarBlue to evaluate the impact of the inserts on cellular health.

Experimental design

NIH 3T3 fibroblasts were embedded in Matrigel and cultured either as conventional hydrogel domes or as GelControl-supported domes. Two experiments were performed to evaluate the effect of GelControl inserts on Matrigel dome workflow across cell seeding density and Matrigel concentration, two independent variables that are crucial for organoid cultures.

Experiment	Variable tested	Experimental Groups			Fixed parameter	Outcomes
1	Matrigel Concentration	3.0 mg/mL	4.5 mg/mL	6.0 mg/mL	$2.0 \cdot 10^6$ cells/mL	Area, circularity, brightfield images, fluorescence imaging, qualitative transfer tests, and alamarBlue metabolic activity
2	Cell seeding density	$0.5 \cdot 10^6$ cells/mL	$1.0 \cdot 10^6$ cells/mL	$2.0 \cdot 10^6$ cells/mL	4.5 mg/mL	

Table 1. Overview of the two experiments assessing the effect of GelControl Inserts on Matrigel dome workflow.

Experimental Workflow

The workflow began with cell expansion and preparation of GelControl™ inserts before Matrigel dome seeding. All experiments detailed in this document were carried using 24 well-sized inserts. NIH 3T3 fibroblasts were expanded under standard culture conditions and used within passage 3-5 for the study. The media consisted of DMEM with 10% FBS and 1% penicillin and streptomycin; reagent sources and catalog numbers are listed in the Appendix: Materials and Reagents. Before seeding, GelControl inserts were prepared for culture by rinsing with PBS and sterilizing using ultraviolet exposure for 20 minutes and submersion in 70% ethanol for 30 minutes, followed by washing sterile PBS three times and allowing the inserts to dry inside a sterile BSC.

Matrigel dome cultures were prepared under equivalent seeding and gelation conditions for GelControl-supported and conventional dome formats. Matrigel was diluted to the target concentrations indicated in the experimental design and combined with fibroblasts to generate single-cell suspensions at the specified seeding densities. For GelControl-supported samples, 60 μL of the cell–Matrigel suspension was pipetted into the inner ring of each insert to define the hydrogel footprint. Matched conventional dome controls were prepared using the same cell–Matrigel formulations and dome volume, but without insert support. All constructs were incubated for 20 minutes to allow gelation before addition of 1000 μL of media.

During culture, the workflow focused on comparing construct stability, imaging accessibility, and shape reproducibility between supported and unsupported domes. Constructs were maintained for 3 days in 1000 μL of complete culture medium per well. Brightfield imaging using an Echo Revolve (Echo, CA, USA) was used to monitor hydrogel position and accessibility to the cells, construct morphology, and compare GelControl-supported and conventional domes. Media exchange was performed after 48 hours in culture. Construct area and circularity

were measured directly after polymerization from whole-construct images to compare shape reproducibility between supported and unsupported domes.

Endpoint assays were used to determine whether GelControl inserts affected cellular metabolic activity or compatibility with immunofluorescence imaging. Relative metabolic activity was assessed on day 3 using alamarBlue. For each sample, 300 μL of assay solution was incubated with the samples for 1 hour, and fluorescence values were averaged from multiple technical replicates before normalization. For endpoint fluorescence imaging, constructs were fixed in 4% paraformaldehyde for 30 minutes, washed with PBS, permeabilized with 0.1% Triton X-100, and stained with Hoechst and phalloidin for 1 hour to visualize nuclei and F-actin. Fixed constructs were imaged in the same 24-well format using a Leica THUNDER widefield microscope with 10X and 20X objectives.

Recommended workflow parameters from this study

Parameter	Value used in this application note (24-well)
Dome volume	60 μL cell–Matrigel suspension per dome
Culture medium volume	1000 μL complete culture medium per well
Gelation time	20 minutes before media addition
Culture duration evaluated	3 days
Tested Matrigel concentrations	3.0, 4.5, and 6.0 mg/mL
Tested cell densities	0.5, 1.0, and 2.0 × 10^6 cells/mL
Imaging readout	Brightfield monitoring during culture and fixed-sample immunofluorescence imaging
Biochemical readout	alamarBlue assay with 300 μL and multiple technical replicates per sample

Results

1. GelControl™ inserts improved construct shape consistency

Across the tested conditions, GelControl™-supported constructs showed a more consistent area fraction of their well surface area and more repeatable circularity compared with conventional Matrigel dome controls (Figure 3). Qualitatively, the dome formation process was easier for technical execution with a mold structure and less pipetting accuracy and steadiness required (Figure 1A-B). The inserts also confine the dome geometry to a region centralized within the well (Figure 2B). These findings are supported by quantitative data. Both the area and circularity shape metrics revealed significant differences between experiment 1 and 2 in conventional domes, but no differences across experiments in GelControl-supported domes (Figure 3C-D). The area fraction was also different across the dome groups for both experiments (Figure 3C), and the circularity was different across the dome groups only in experiment 2 (Figure 3D). The data also showed reduced standard deviation (SD) and coefficient of variation (CV) in GelControl domes compared with conventional domes (Table 2). Thus, GelControl inserts improved the consistency of Matrigel dome shape and positioning.

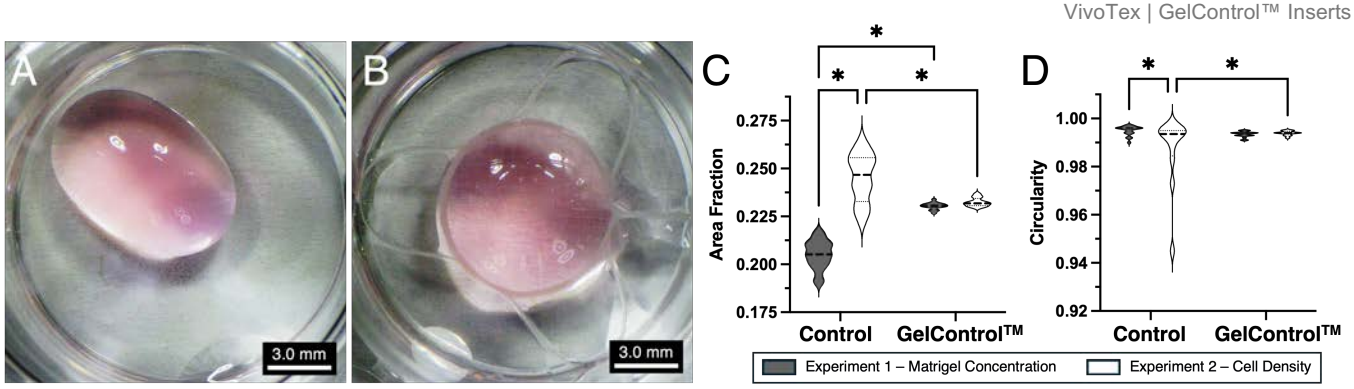

Figure 3. Shape comparison of constructs formed using conventional domes and GelControl™ inserts immediately after gelation. A) Optical image of conventional Matrigel dome with an irregular shape positioned off-center. The image represents an example of the potential for irregular shapes. B) Optical image of Matrigel dome formed using GelControl inserts. The dome assumes the area of the inner-ring and remains centrally located within the well. C) Area fraction of Matrigel dome within a 24 well plate comparing conventional domes to GelControl domes. Statistical analysis of the data revealed significant differences between experiments within conventional domes, and across the dome groups for both experiments. D) Circularity of the perimeter shape of Matrigel domes revealed significant differences between experiments within conventional domes, and experiment 2 had differences dome groups. Statistical analyses determined using 2-way ANOVA with a Tukey post hoc and significance determined at $p<0.05$.

Experiment	Group	Area mean ± SD	Area CV (%)	Circularity mean ± SD	Circularity CV (%)
1	Control Dome	0.205 ± 0.007	3.624	0.995 ± 0.002	0.198
	GelControl™ Insert	0.230 ± 0.002	0.764	0.993 ± 0.001	0.121
2	Control Dome	0.245 ± 0.013	5.204	0.987 ± 0.015	1.519
	GelControl™ Insert	0.232 ± 0.002	0.965	0.994 ± 0.001	0.091

Table 2. Summary table for construct geometry statistics

2. GelControl™ inserts facilitated reduced disruption during media exchange and enabled lifting and transferring constructs

A key workflow advantage of GelControl™ inserts is the ability to lift and transfer constructs without insulting the fragile ECM. The supporting evidence of this advantage are qualitative in this application note. The inserts facilitated more consistent and extensive media exchange. Dispensing of liquid against the walls of a well directed the flow away from the centralized domes while the inserts added stability to soft constructs and prevent pipetting-induced deformation (Figure 3B). Aspirating media was more streamlined as the domes were easier to locate by visual inspection and the added stability afforded by the inserts further prevented movement and deformation. This enabled near-100% removal of media by tipping the plates that was not possible for conventional domes.

Lifting of domes was simplified with GelControl inserts due to the sparse microfiber grid below the domes (Figure 4, Figure 2A). Lifting and transfer of organoid cultures are often required for imaging, histological sectioning, and passaging. The inserts provided a minimally invasive mold with distributed contact points along the microfiber grid that permits lifting and transfer of fragile hydrogel domes (Figure 4B,C). By comparison, lifting and transfer of conventional domes induced substantial deformation, slower in execution, and required higher levels of dexterity (Figure 4A). The inserts also facilitated consistent positioning after transfer to a well of the same size, as the wings extend against the walls (Figure 4D). This handling benefit remained for duration of culture. Even in the case where the domes undergo cell-mediated contraction, the domes remained anchored to the microfiber grid and the bottom of the well (Figure 5A).

The inserts enabled consistent construct formation and handling and streamlined workflows at lower Matrigel concentration than recommended by the manufacturer. The conditions of each experimental group augmented the viscosity and density of the solution and impacted the ability to handle conventional domes. Domes formed with higher Matrigel concentration (4.5 and 6.0 mg/mL) and higher cell densities ($2.0{\cdot}10^6$ cells/mL) were noticeably firmer and did not incur as much pipetting-induced deformation. By contrast, the lower Matrigel Concentration of 3.0

mg/mL did not polymerize to a robust hydrogel. The constructs underwent significant movement and deformation with the addition of media, and all conventional constructs floated within 24 hours in culture. This made media changes more challenging and time consuming and ultimately led to a larger variation in sample-to-sample metabolic activity (Figure 7) and phenotypic appearance in immunofluorescence imaging (data not shown). However, GelControl inserts anchored all domes to the microfiber grids (Figure 5A), streamlining the handling of all samples. Thus, the inserts allowed for the use of Matrigel concentrations below commonly reported ranges, expanding the window of concentrations that can be used in culture.

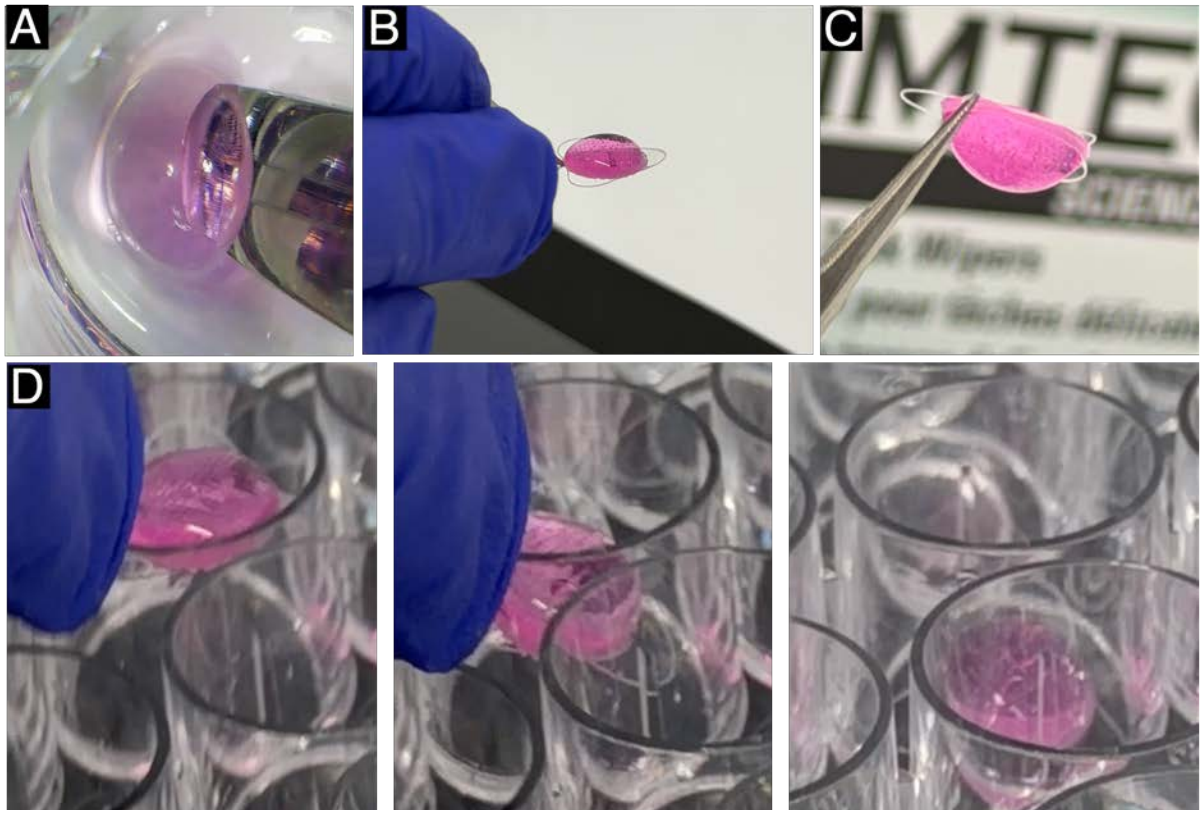

Figure 4. Handling and transfer workflow of Matrigel domes with GelControl inserts. A) Attempted lifting of a conventional Matrigel dome using a spatula. The spatula was difficult to place underneath the construct to effectively lift it and caused significant deformation. B) Snapshot of a supported dome lifted with forceps grasping the handle of the inserts and the dome structure maintained in transit. C) Matrigel dome supported by a GelControl insert held upside down. The dome remains anchored to the insert. D) Transfer of a GelControl supported dome to a new well after lifting. The dome was also centrally located in the new well as the wings pressed against the wall. Images shown in a 24 well plate.

3. GelControl™ improved imaging accessibility during culture and after fixation

GelControl™-supported constructs were easier to monitor during culture by brightfield imaging (Figures 5 below). The inserts promoted attachment to the fine microfiber grid, anchoring constructs to the bottom of the well, and inducing a flatter dome geometry compared to conventional domes that were more spherical (Figure 5A-B). This improved the imaging accessibility, as constructs had reduced thickness variability and consistent positioning with less movement. This resulted in efficient brightfield imaging through the light path of the microfiber grid with the cells clearly in focus (Figure 5C). In contrast, locating regions with the cells in focus for conventional domes was more challenging (Figure 5D). The most focused images were found around the periphery of the domes and had poor visibility to the cells.

Matrigel domes formed with GelControl inserts also exhibited improved immunofluorescence imaging after fixation (Figure 6 below). The domes were fixed with 4% PFA and stained with Hoechst and phalloidin to visualize the cell nuclei and f-actin of their cytoskeleton. The fixation and imaging occurred within the same plastic well plates the GelControl inserts arrived and used during culture. Mosaic images using a 10X objective were acquired to visualize the entire constructs (Figure 6A-B). GelControl constructs had narrower focal plane variation and less displacement during stage movement that led to better quality images. The movement of the constructs introduced imaging

artifacts in most unsupported Matrigel domes (Figure 6B). The inserts also promoted a flatter dome geometry that retained more area compared to conventional domes (Figure 6A-B). Similarly to the brightfield images (Figure 5C-D), higher magnification 20X immunofluorescence images demonstrated GelControl constructs had higher contrast (Figure 6C-D). The sparse microfiber grid of the inserts was present in the images. Yet, most of the focal depth of the constructs resided above the microfiber grid that is 14.0 μm thick (Figure 6C).

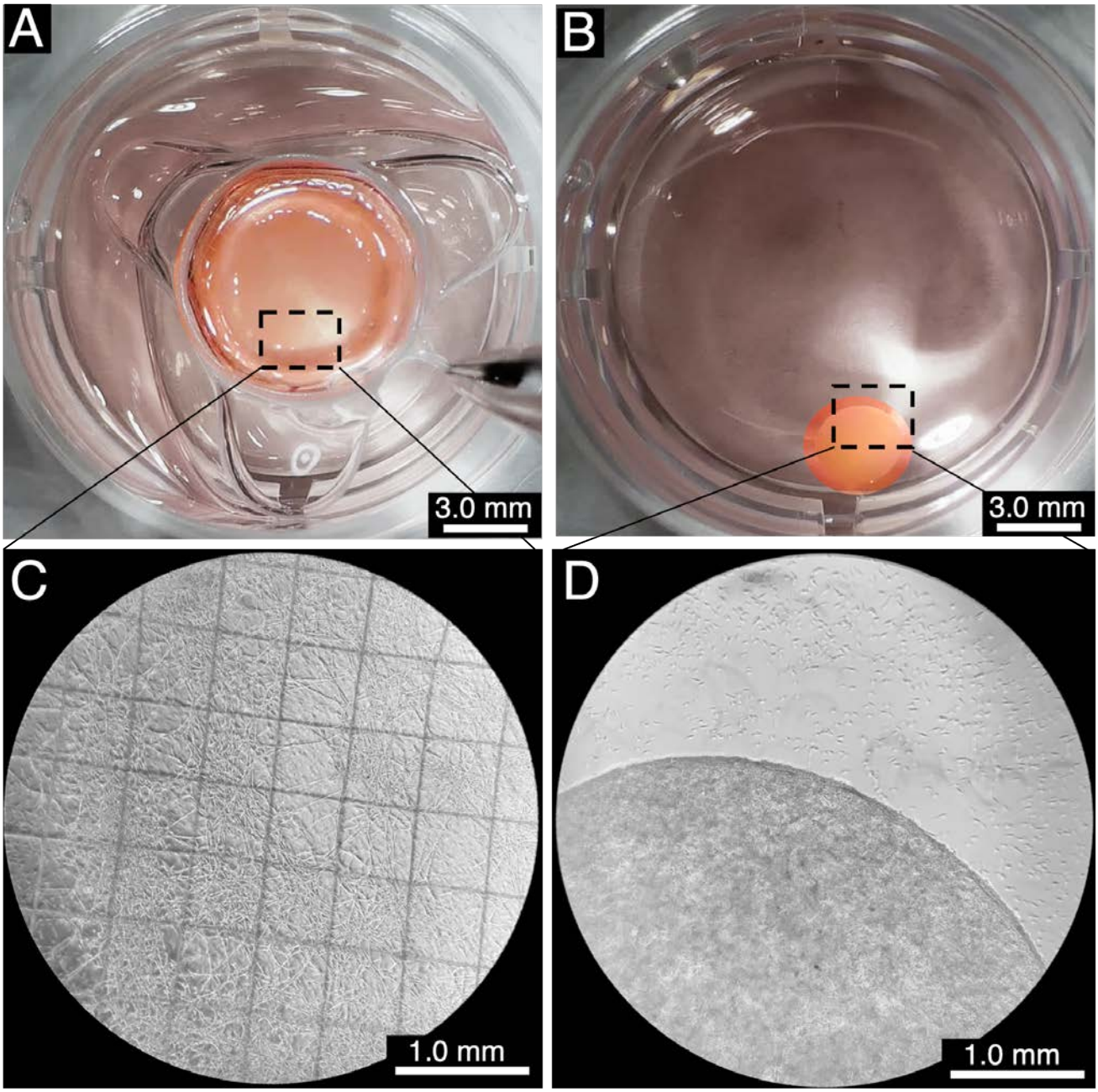

Figure 5. GelControl™ inserts improved the imaging accessibility of Matrigel domes via brightfield during culture. A) Optical image of a low concentration dome at day 3 of culture, lifted with forceps clasping the insert handle. B) Optical image of conventional dome on day 3 of culture. The rounded morphology and location of the construct challenged the monitoring of the cells with reduced imaging accessibility. C) Brightfield image acquired with an inverted microscope with a 4X objective below the sample assessing a GelControl supported dome. Cellular structures were located quickly and easily due to the narrower focal range and the construct position at the bottom of the well, while the microfiber grid was visible. D) Brightfield image of a conventional dome culture with the edge of the dome visible and poor contrast of cellular structures.

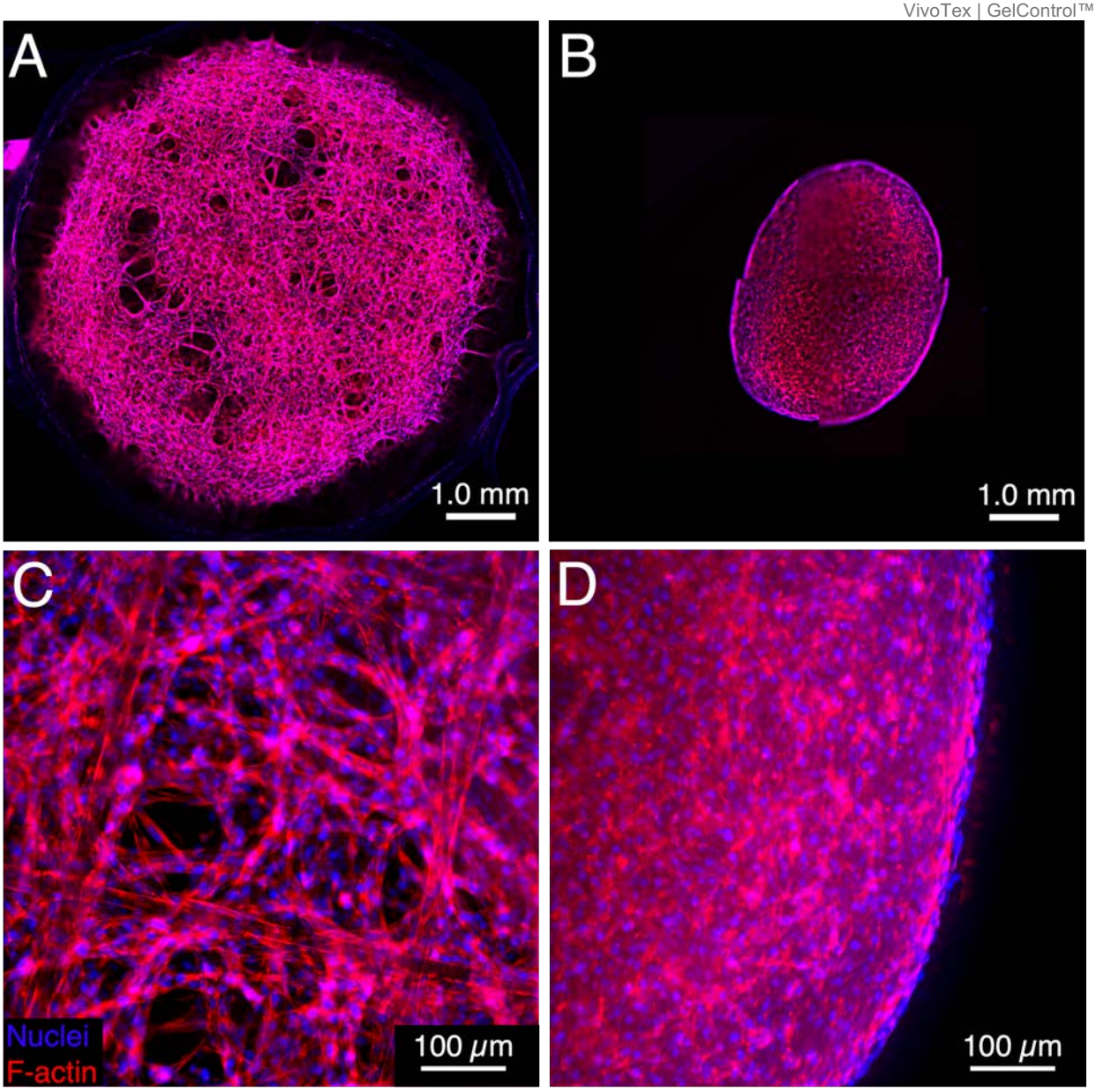

Figure 6. GelControl™ inserts improved the imaging accessibility of Matrigel domes via immunofluorescence imaging. A-B) Mosaic images acquired with a 10X objective on a widefield fluorescence microscope of constructs fixed after 3 days in culture. Domes grown on GelControl (A) remained anchored to the inserts and facilitated faster and clearer imaging with reduced focal plane variation. Conventional domes (B) were more difficult to image with the cells in focus. Scale bar = 1.0 mm. C-D) Higher magnification 20X images of the Matrigel domes. GelControl-supported constructs had clearer images with higher contrast and better focus on the cellular structures. Images are a projection of a Z-stack composed of 15 slices 10.0 μm apart. Both samples exhibited in this figure had Matrigel concentration of 4.5 mg/mL and cell seeding density of $2.0 \cdot 10^6$ cells/mL. Scale bar = 100.0 μm. F-actin represented by red, nuclei represented by blue.

4. GelControl™ inserts did not reduce cellular metabolic activity and led to increased metabolic activity at higher densities

Evaluation of the effects of GelControl inserts on the cells inside Matrigel domes showed no reduction in metabolic activity. The impact of the inserts on cellular health was assessed via the relative metabolic activity measured using alamarBlue assay across two experiments with varying Matrigel concentration (Experiment 1) and cell seeding density (Experiment 2). GelControl-supported Matrigel dome cultures did not have a decrease in metabolic activity comparable to matched conditions at all tested groups, while improving cellular activity at higher cell densities and gel concentrations.

The effect of Matrigel concentration (Experiment 1) on cellular health revealed no negative impacts of the inserts, and increased total cell activity at higher gel concentrations (Figure 7A). The experiments were conducted with a constant cell seeding density of $2.0 \cdot 10^6$ cells/mL, and all samples of a concentration group were prepared at the same time and seeded together on the same batch of plates. The fluorescence data from the alamarBlue assay was normalized to the conventional Matrigel domes prepared with a concentration of 6.0 mg/mL. GelControl-supported Matrigel cultures had significantly increased relative metabolic activity at higher Matrigel concentration groups of 4.5 and 6.0 mg/mL (Figure 7A). The lower concentration group did not display a difference in cellular activity between conventional and GelControl supported domes.

The effect of cell seeding density (Experiment 2) on cellular health also revealed no negative impacts of the inserts, and increased total cell activity at higher cell densities (Figure 7B). The experiment was conducted with a constant Matrigel concentration of 4.5 mg/mL, and all samples of each density group were prepared at the same time and seeded together on the same batch of 24 well plates. The fluorescence data from the alamarBlue assay was normalized to the conventional Matrigel domes prepared at cell seeding density of $0.5 \cdot 10^6$ cells/mL. GelControl supported cultures had significantly increased relative metabolic activity at the higher density of $2.0 \cdot 10^6$ cells/mL compared with the matched Matrigel dome controls. The insert-supported cultures were significantly different from each other across each density group, while conventional cultures only differed between the 0.5 and 2.0 $\cdot 10^6$ cells/mL groups (Figure 7B). The lower density group did not display a difference in cellular activity between conventional and GelControl supported cultures.

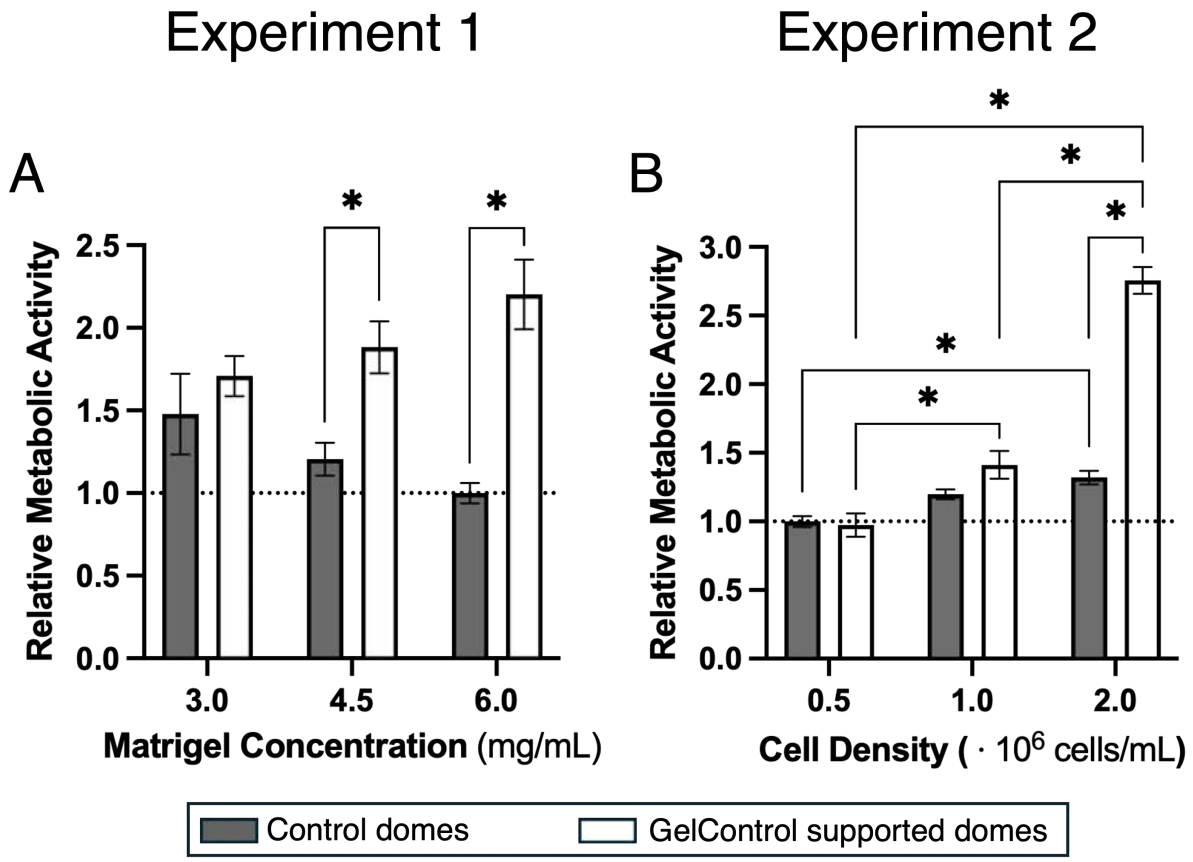

Figure 7. The effect of GelControl inserts on cellular health inside Matrigel dome cultures assessed via alamarBlue assay after 3 days of culutre. A) Evaluation of Matrigel concentration on cellular activity. GelControl inserts did not have detrimental effect on cellular activity in any concentration groups tested, and led to significant increase in metabolic activity at higher concentration gel groups of 4.5 and 6.0 mg/mL. B) Assessment of cell seeding density on cellular activity. GelControl inserts did not have detrimental effect on cellular activity in any cell density tested, and led to significant increase in metabolic activity at the high density of $2.0 \cdot 10^6$ cells/mL. Statistical analyses determined using 2-way ANOVA with a Tukey post hoc and significance determined at $p<0.05$.

Discussion

This application note evaluated GelControl™ inserts as a structural support for Matrigel dome workflows using NIH 3T3 fibroblasts across two experimentally relevant variables: Matrigel concentration and initial cell seeding density. Across these conditions, GelControl-supported constructs exhibited more reproducible dome geometry, improved positional consistency within the well, and improved handling during media exchange, lifting, and transfer. These workflow advantages were achieved without a measurable loss of cellular metabolic activity. Further, GelControl inserts improved the imaging quality of Matrigel dome cultures as the flatter construct geometry enhanced the imaging accessibility, and the microfiber grid anchored constructs to the well bottom. Two results were particularly notable. First, GelControl-supported cultures showed increased relative metabolic activity at higher Matrigel concentrations of 4.5 and 6.0 mg/mL and at the highest tested cell density of 2.0×10^6 cells/mL, suggesting that the inserts do not limit and may improve cellular viability. This may be due to the flatter, more planar geometry of constructs that improved nutrient access compared with conventional domes that are known to suffer from necrotic cores (6). Second, the inserts enabled stable culture and manipulation of low-concentration 3.0 mg/mL Matrigel domes, whereas conventional domes at this concentration were prone to deformation and detachment during routine culture handling. Together, these results indicate that GelControl inserts improve the shape reproducibility, mechanical robustness, and imaging accessibility of soft hydrogel dome cultures while preserving cellular function. They also indicate that GelControl inserts expand the scope of hydrogels that can be reliably used in cultures and a higher potential of cellular health.

The current study was intentionally scoped as a workflow and feasibility evaluation rather than a comprehensive validation study. NIH 3T3 fibroblasts were used as a robust model cell type to assess construct formation, handling, imaging, and metabolic activity, but the results should not be assumed to generalize directly to organoid systems without model-specific verification. The experiments assessed a limited range of Matrigel concentrations, cell seeding densities, culture durations, and endpoint assays, and the handling and transfer advantages were primarily supported by qualitative observations rather than force, throughput, or operator-to-operator variability measurements. However, the range of Matrigel concentrations and cell seeding densities was representative of substantial scope of tissue engineering and drug development experiments. The alamarBlue assay provides a useful readout of relative metabolic activity, but it does not distinguish between changes in cell number, proliferation, apoptosis, necrosis, metabolic state, oxygen or nutrient gradients, or matrix-mediated differences in cell behavior, and is thus used as a general indicator of cellular health. The outcomes provide an indication there was no negative influence of the inserts on cellular health. The insert itself also imposes practical boundaries on the workflow: the microfiber grid is visible in transmitted-light and fluorescence images, the insert geometry defines the dome area and may constrain contraction or remodeling, and compatibility may vary with dome volume, organoid type, and downstream processing method. Therefore, the present data support the inserts as a workflow-enabling tool for Matrigel dome culture, but additional validation is needed for each intended organoid model and assay endpoint.

Future development of GelControl-supported dome workflows should focus on exploiting the insert as both a mechanical support and a spatial reference frame for higher-throughput organoid culture. Because the insert centralizes and stabilizes the hydrogel footprint, it may reduce operator-dependent variability during dome formation and improve compatibility with automated liquid handling, plate-based imaging, and parallelized assay formats. The sparse microfiber grid also provides a fixed coordinate system that could enable automated live tracking of organoids during growth, remodeling, drug exposure, or co-culture experiments, particularly if image-analysis algorithms are trained to register organoid position relative to the grid and distinguish biological structures. A second opportunity is to streamline interaction assays by stacking GelControl-supported organoid domes of different types, allowing defined physical proximity between separate hydrogel constructs. This configuration could support controlled studies of paracrine signaling, stromal-epithelial interactions, immune-organoid interactions, or sequential exposure workflows without requiring complete dissociation or re-embedding of fragile cultures. More broadly, GelControl inserts may provide a scalable bridge between conventional Matrigel dome culture and more automated, reproducible organoid assay platforms.

Workflow tips and common pitfalls

Common pitfall	Recommended practice
Premature Matrigel gelation during preparation	Keep Matrigel, tubes, and pipette tips cold until loading, and minimize handling time before dome placement.
Air bubbles or uneven dome filling	Dispense directly into the inner ring and avoid introducing bubbles while mixing or pipetting the cell–Matrigel suspension.
Dome disruption during media addition or exchange	Confirm gelation before adding media, then add or aspirate liquid gently along the well wall rather than directly onto the construct.
Low-concentration gels deforming or detaching	Verify matrix concentration, gelation conditions, and culture handling for the specific model; GelControl support can improve handling but does not replace model-specific optimization.
Microfiber grid visible during imaging	Optimize focal plane, exposure, and image-analysis settings to distinguish grid features from biological structures.
Microfiber grid deforming	The microfiber grid is quite thin and fragile, and is not designed to be contacted by solids.
Assay performance differing by organoid model or hydrogel formulation	Validate each organoid type, hydrogel formulation, dome volume, and downstream assay independently before adopting the workflow for routine studies.

Appendix: Materials and Reagents

Material/reagent	Description	Corporation	Catalog Number
GelControl Inserts	Well plate inserts for soft gels	VivoTex	VGC.0001.24.24.S
NIH 3T3 fibroblasts	Cell line	MilliporeSigma	93061524-1VL
Matrigel	Basement membrane gel	Corning	356234
24 Wellplate	Non-treated well plate	VWR	10861-558
DMEM	Culture Medium	ThermoFisher	11995065
Fetal Bovine Serum	Media Supplement	FisherScientific	FB12999102
Penicillin/streptomycin	Media Supplement	ThermoFisher	15140122
Paraformaldehyde	Cell fixation agent	Electron Microscopy Sciences	15710
Phalloidin	F-actin Stain	ThermoFisher	A12380
Hoechst	Nuclear stain	ThermoFisher	62249
alamarBlue	Cellular health indicator	ThermoFisher	DAL1025

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