Spheroid Culture of HEK-293T using PDMS MicroBubble™ Arrays

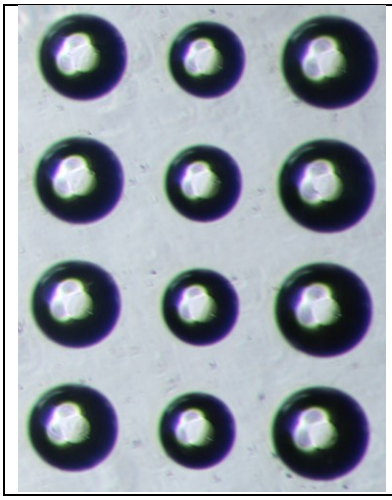
Overview:

This experiment demonstrates the biocompatibility and spheroid culture capability of PDMS MicroBubble™ Arrays using a representative mammalian cell line.

Methods:

SiMPore 50 µm opening MicroBubble™ Arrays (SiMPore Inc MB50-4W-001, See detailed cavity properties in figure 1 below), were used as received from the manufacturer. Wells were prefilled first with 0.2 µm filtered IPA via house vacuum to completely void air from the bubble cavities. Excess IPA was decanted, and immediately replaced with 1.0 mL / well of complete DMEM (Corning 10-017-CV supplemented with 10% FBS (Gibco 26140079), Sodium Pyruvate, L-Glutamine, and antibiotic cocktail (Penicillin, Streptomycin, Amphotericin B (Lonza 17-745E)). After DMEM addition, devices were incubated for 1 hour at 37°C to enable complete dissociation of IPA from the bubble cavities.

Large T antigen transformed Human Embryonic Kidney cells (Q401, Genhunter Corporation) were used at passage 4, approximately 60% confluence using standard T-25 culture flasks. A $1x10^6$ cells/mL suspension was prepared after hemocytometer counting to verify viability (>96%) in freshly prepared and sterile filtered complete DMEM. Finally, cells were seeded at $1x10^4$ cells/cm^2 and allowed to settle to the PDMS bubble surface for 10 minutes at room temperature, after which excess cells were washed from the PDMS surface via Hanks Buffered Saline Solution (HBSS). Devices were incubated at 37°C with fresh complete DMEM for 96 hours with periodic evaluation via microscopy (Nikon TS100 Inverted Microscope) of cell proliferation at regular intervals.



Average Cavity Diameter	**145.3 ± 9.9 µm**
Cavity Opening	50 ± 5.1 µm
Diameter Range	134 – 158 µm
Average Cavity Volume	1.5 ± 0.1 nL
Cavity Spacing	1:4

Figure 1. MicroBubble™ Arrays overview. A representative image of the 50 µm opening MicroBubble™ Array used for this experiment (left) and a summary of the cavity properties (right)

Results:

Following the initial seeding and localization of cells within wells, the washing process appeared to remove all surface-localized cells. This process resulted in a seeding density of approximately 4 cells per cavity (range of 0 – 6 cells/ cavity were observed across three representative high-powered fields per well).

Culture under optimal growth conditions (5% CO_2, 100% RH, 37°C) and occasional observation via light microscopy (~12hr intervals) indicated ~94% spheroid formation in cavities containing at least one cell, likely due to the intrinsically high seeding density. Moreover, spheroid size reached a maximum confined by the cavity geometry at essentially the same rate, indicating uniform and robust exchange of cellular metabolic waste with no observable variation across neighboring cavities or replicate wells. A representative image of spheroid formation after 72 hours of culture post seeding is shown in Figure 2.

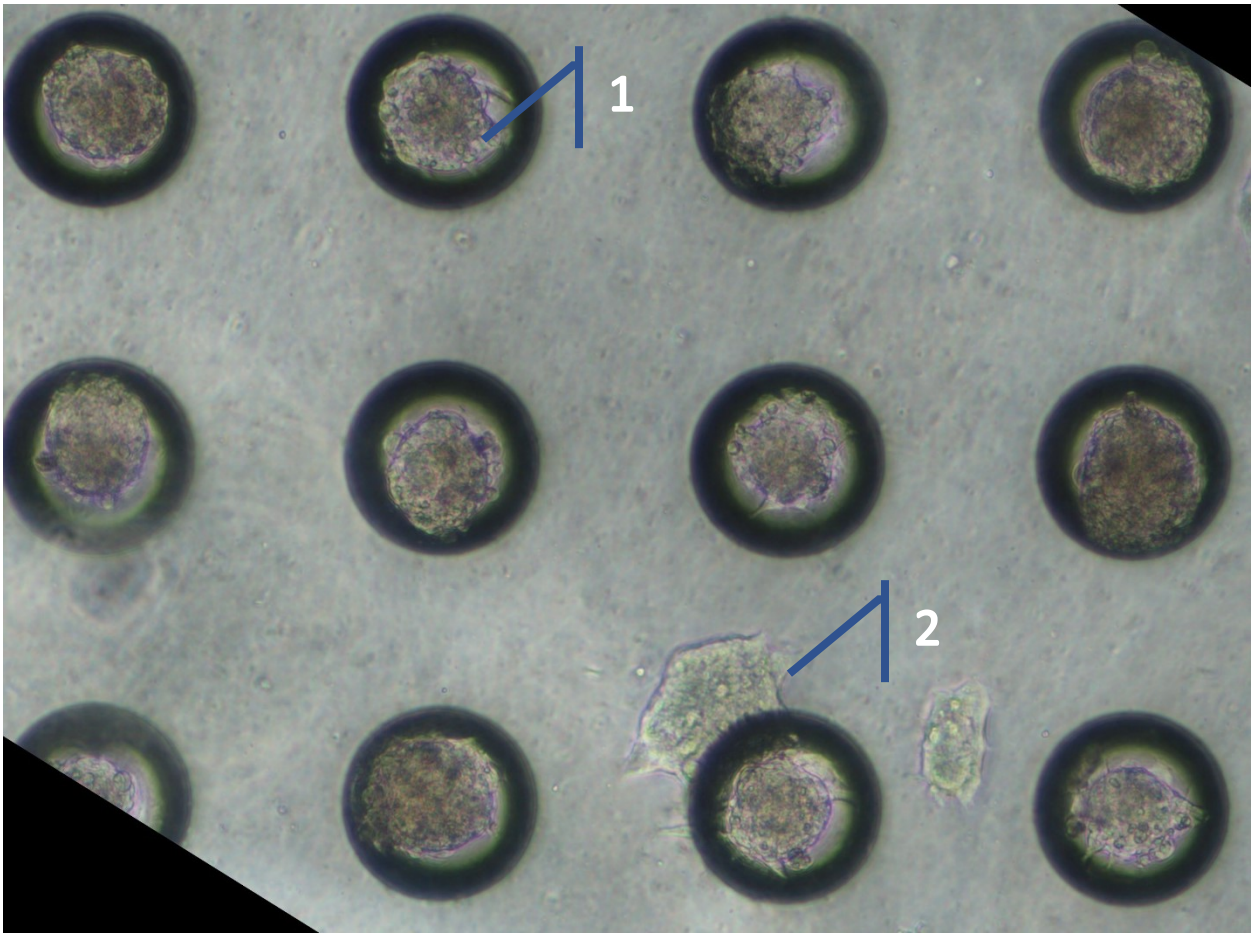


Figure 2. HEK293-T Spheroids localized within individual MicroBubble™ cavities (1) imaged at 10X via phase microscopy. While surface-deposited cells were removed during the seeding process, some isolated instances of proliferation out of the MB cavity were observed after prolonged culture (72hrs) (2)

Conclusions:

These data demonstrate the preliminary utility of Human Embryonic Kidney spheroids after 72hrs of culture using a standard complete media environment. Further exploration of more difficult to culture cell lines, co-culture of multi-cell types, and single-cell isolation and expression may further demonstrate the wide utility of MicroBubble™ Arrays for a variety of spheroid, organoid, and tumoroid tissue culture assays.

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