A 3D fluid-dynamic tumor microenvironment-on-chip to model tumor-stroma-vascular interactions in esophageal adenocarcinoma

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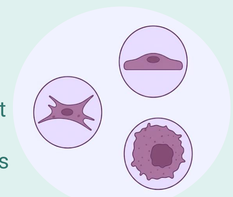
REACT4LIFE
mirroring human complexity

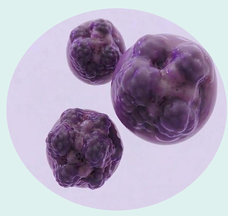
BACKGROUND

- Esophageal adenocarcinoma (EAC) is a highly lethal cancer with a rapidly rising incidence in western countries with low overall 5-year survival despite multimodal treatment.
- Tumor progression and therapeutic resistance in EAC are orchestrated by a complex tumor microenvironment (TME) composed of malignant epithelial cells, stromal fibroblasts, endothelial cells, immune cells, and extracellular matrix, together shaping disease evolution and treatment response.
- Currently available preclinical models of EAC are largely limited to 2D monocultures and simplistic xenografts, which insufficiently recapitulate the human TME and thus fail to predict clinical responses reliably.
- To overcome the reductionist nature of conventional 2D cultures and better recapitulate this complexity, we established a 3D, fluid-dynamic EAC model on the MIVO® (multi-invitro organ) milli-fluidic platform, developed to support physiologically relevant perfusion of 3D tissues and more predictive pharmacological testing.

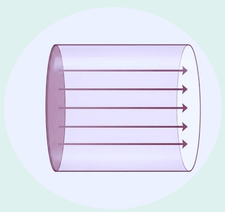
MULTICELLULARITY
Cancer cells, endothelial cells, and fibroblasts interact with each other and drive tumor growth and metastasis



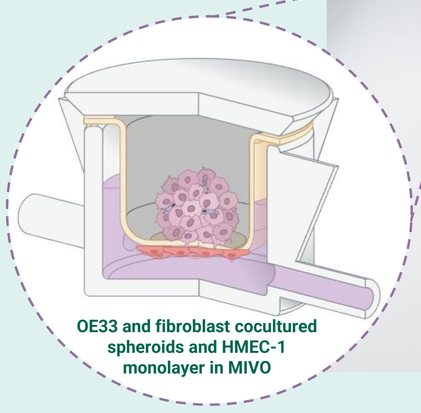
3-DIMENSIONAL CULTURES
Compared to 2D monolayers, spheroids are better representatives of the tumor tissue



FLUID DYNAMICS
Flow condition replicates fluidic pressure and nutrient and drug diffusion in a realistic manner

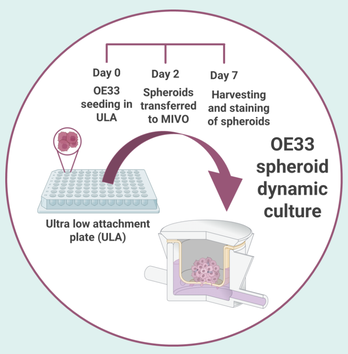


MIVO® based model of the tumor microenvironment

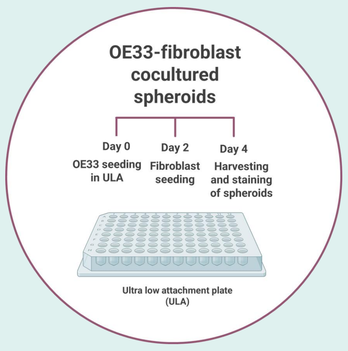


OE33 and fibroblast cocultured spheroids and HMEC-1 monolayer in MIVO

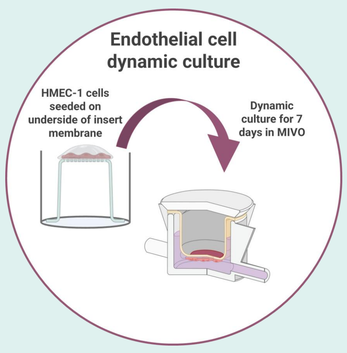
METHODOLOGY



OE33 cells (EAC cell line) in different seeding densities were seeded in the ULA plates and incubated for two days. The 24 well insert membranes were coated with 0.5% alginate to avoid spheroid attachment. The spheroids were placed in these inserts and transferred to the MIVO chambers. The spheroids were monitored for 2 and 5 days under the dynamic condition with flow rate of 0.07 mL/min.



OE33 cells (1000 cells/well) were seeded in ULA plates and cultured for 2 days to form spheroids. Fibroblasts (1000 cells/well) were then added sequentially onto the OE33 spheroids and co-cultured for an additional 2 days. In parallel, OE33 cells and CCD-1113Sk dermal fibroblasts were seeded as monocultures at 1000 cells each per well and cultured for 4 days.



HMEC-1 endothelial cells (1×10^5 cells) were seeded on the underside of the insert membrane and allowed to attach for 2 hours. The inserts were then transferred to MIVO chambers and cultured under dynamic conditions (0.07 mL/min) for 7 days.

RESULTS

Optimization of OE33 spheroids under dynamic condition

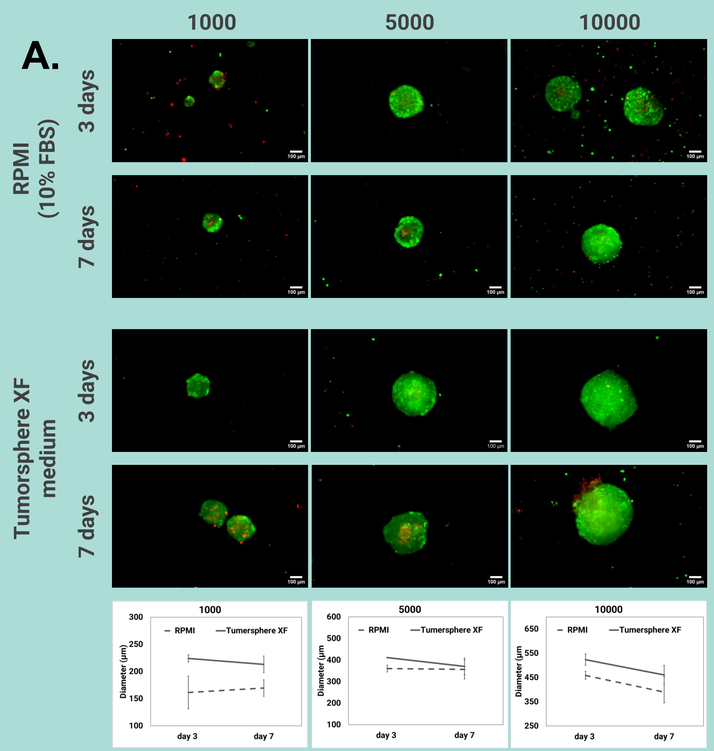


Figure 1A: RPMI (10% FBS) and PromoCell Tumorsphere XF medium were assessed for OE33 spheroid formation at seeding densities of 1,000, 5,000, and 10,000 cells per well under static conditions over 7 days. Live/dead staining using calcein AM (green) and propidium iodide (red) was performed on days 3 and 7. Spheroids remained viable throughout the 7-day culture period, with those grown in Tumorsphere medium displaying larger sizes across all seeding densities.

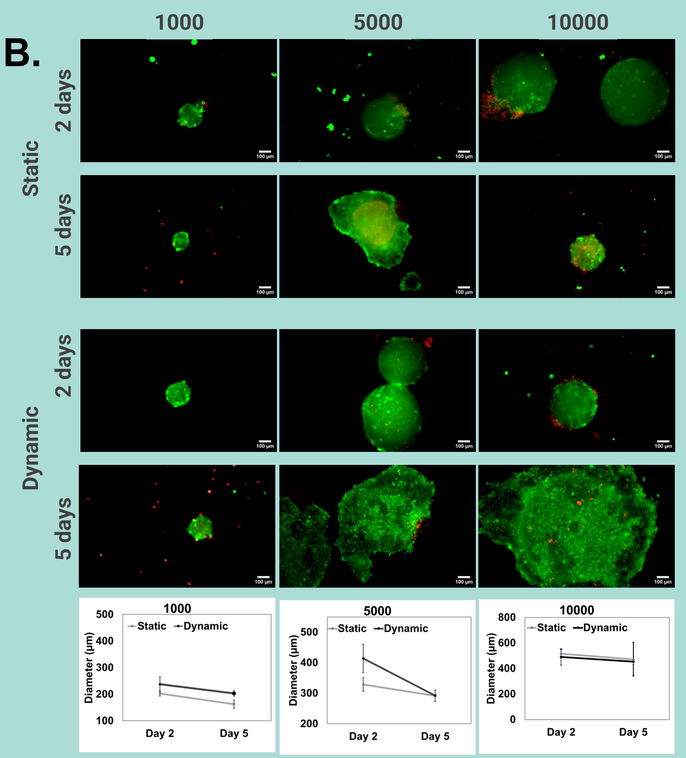


Figure 1B: OE33 spheroids were cultured under static and dynamic conditions (0.07 mL/min) in the MIVO chamber for up to 5 days. Viability was assessed on days 2 and 5 using calcein AM (green) and propidium iodide (red) staining. Smaller spheroids (<400 µm) remained viable under dynamic conditions and exhibited increased size compared to their static counterparts.

OE33-fibroblast coculture

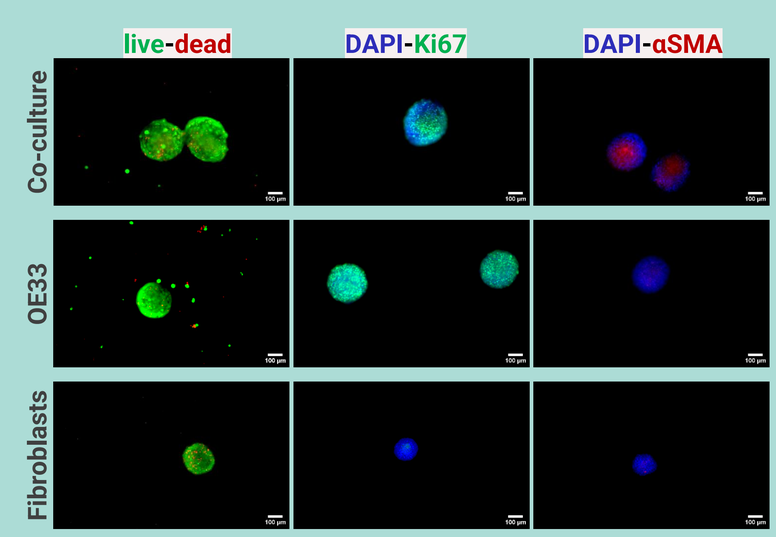


Figure 2: OE33 cells and dermal fibroblasts CCD-1113Sk were sequentially seeded in 1:1 ratio and cultured under static conditions for 2 days in co-culture or 4 days as monocultures. Spheroids were stained with calcein AM and propidium iodide, followed by immunostaining to assess Ki67 and αSMA expression. The αSMA signal was elevated in the co-culture condition compared to either monoculture.

Endothelial cells under dynamic condition

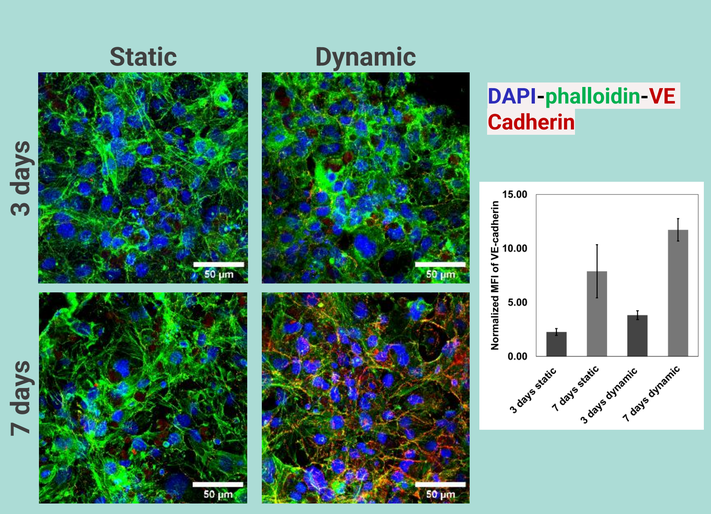


Figure 3: HMEC-1 cells were cultured as monolayers under static and dynamic conditions (0.07 mL/min) in the MIVO chamber for up to 7 days. Cells were then stained with phalloidin to assess cytoskeletal organization and for VE-cadherin expression. VE-cadherin expression was higher under dynamic conditions at both day 3 and day 7.

CONCLUSIONS

- OE33 spheroids formed viably across seeding densities (1000–10000 cells/well) over 7 days in both media, with PromoCell Tumorsphere XF yielding larger sizes than RPMI. Smaller spheroids (<400 µm) thrived under dynamic flow (0.07 mL/min) for 5 days, showing enhanced growth vs. static conditions.
- OE33–CCD-1113Sk co-culture spheroids maintained high viability and proliferation (Ki67+ve). αSMA expression was strongly upregulated in co-culture compared to monocultures, confirming fibroblast activation toward a CAF-like phenotype in 3D tumor context.
- HMEC-1 cells formed stable monolayers with organized actin cytoskeleton under dynamic conditions for 7 days. Dynamic flow (0.07 mL/min) enhanced VE-cadherin expression vs. static culture, promoting endothelial junctional maturation and barrier phenotype.
- The MIVO® milli-fluidic platform successfully enables physiologically relevant 3D EAC modeling. Future work will integrate all components (tumor–stroma–vascular) and validate the system for mechanistic studies of therapy resistance and pharmacological screening.

Acknowledgement
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References
- *Glabman RA, Choyke PL, Sato N. Cancers (Basel). 2022;14(16):3906. doi:10.3390/cancers14163906*
- *Marzagalli M, Pelizzoni G, Fedi A, et al. Front Bioeng Biotechnol. 2022;10. doi:10.3389/fbioe.2022.945149*
- *E. W. Lin, et al.Oncogene. 2016, doi: 10.1038/onc.2016.34*

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