Unlocking 3D: TumorMACS Media as a dual-purpose solution for primary 2D and 3D tumor organoid culture

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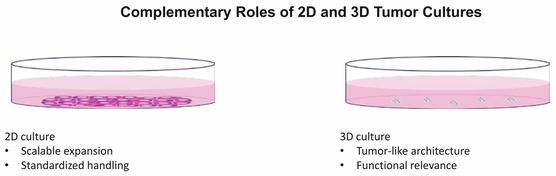
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Miltenyi Biotec B.V. & Co KG

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Introduction

1 Connecting 2D expansion with biologically relevant 3D tumor models

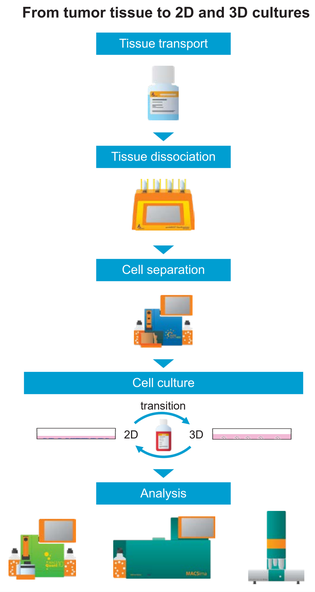
Figure 1



- Tumor research requires both scalable 2D expansion and physiologically relevant 3D models
- Current workflows require media switching between formats
- This introduces variability and complexity
- We evaluate TumorMACS media as a dual-purpose solution

Experimental Workflow

Figure 2



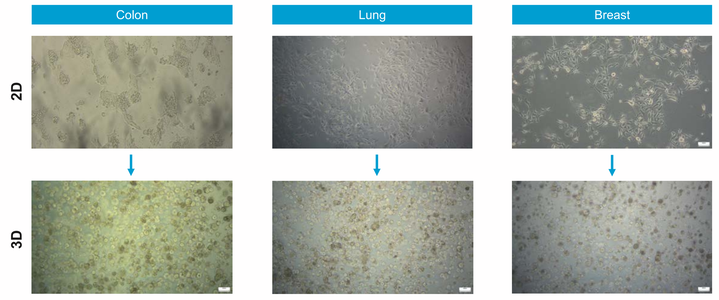
Primary tumor cell lines were established and expanded in 2D using their entity specific TumorMACS media and transitioned into 3D cultures using the same medium.

This experimental design enables direct comparison of phenotypic changes between 2D and 3D conditions without introducing media-related variability.

Results

2 Efficient transition from 2D expansion to 3D tumoroid formation

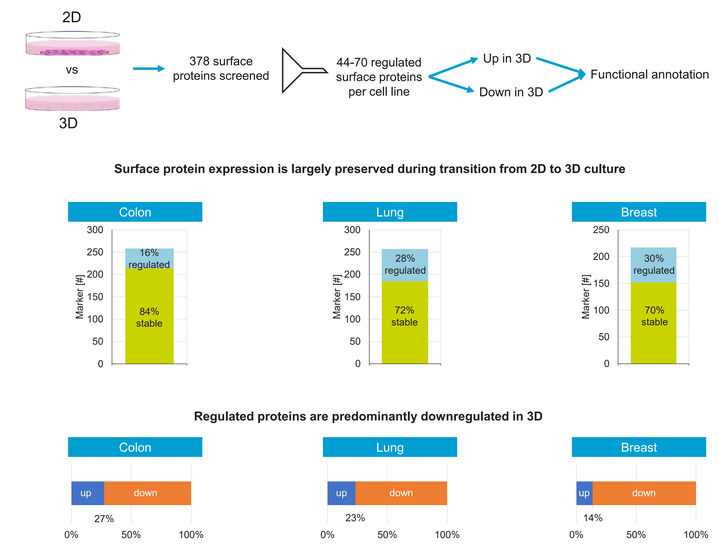
Figure 3



Representative images of tumor-derived cell lines cultured in 2D and transitioned into 3D conditions using entity-specific media. For each model, identical media formulations were used across 2D and 3D formats, enabling a seamless transition. Across colon, lung, and breast models, cells efficiently form tumoroids in 3D while maintaining robust growth.

3 Majority of surface markers remain stable between 2D and 3D

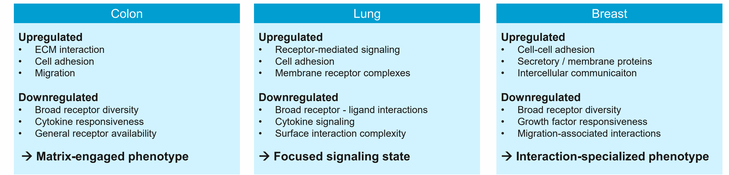
Figure 4



High-dimensional surface marker profiling of tumor cells cultured in 2D and 3D using identical media conditions within each model reveals that the majority of markers remain stable, while only a limited subset (16% - 30%) is differentially regulated. Across all tumor entities, regulated markers are predominantly downregulated in 3D, indicating a reduction in broadly expressed surface programs rather than global phenotypic disruption.

4 3D culture induces selective phenotypic reprogramming

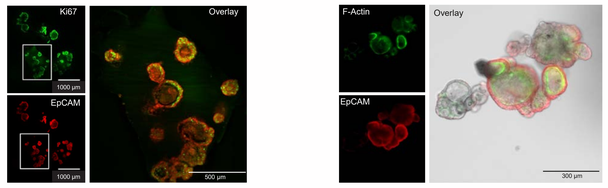
Figure 5



Selective regulation of surface markers in 3D culture translates into distinct, model-specific functional phenotypes. Colon-derived cells adopt a matrix-engaged phenotype characterized by increased adhesion and ECM interaction. Lung-derived cells exhibit a more focused signaling state with enrichment of receptor-associated signaling components. Breast-derived cells display an interaction-specialized phenotype with enhanced cell–cell communication. As identical media conditions were maintained within each model, these differences reflect adaptation to 3D architecture rather than media-driven effects.

5 3D culture enables formation of structurally organized tumoroids with localized proliferative activity

Figure 6



Light sheet microscopy (left) and fluorescence microscopy (right) of different 3D colon tumoroids stained for epithelial marker EpCAM (red), proliferation marker Ki67 (green), and F-actin (green). The left panel reveals organized epithelial structures with spatially heterogeneous proliferation, as indicated by region-specific Ki67 expression. The white box highlights representative tumoroids shown at higher magnification in the overlay. The right panel demonstrates cytoskeletal organization within tumoroids, with F-actin highlighting cortical actin structures and enrichment at inner surfaces. Together, these images illustrate the formation of structurally organized and functionally active tumor models in 3D culture.

Conclusion

Defined culture conditions enable robust transition from 2D expansion to 3D tumoroid formation while preserving core surface phenotypes. Selective remodeling of surface marker expression reflects architecture-driven, model-specific functional adaptation rather than media-induced effects. In parallel, 3D cultures form structurally organized tumor assemblies with coordinated epithelial and cytoskeletal organization and spatially heterogeneous proliferation, supporting their relevance as physiologically meaningful *in vitro* models.

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