A novel organoid-based model for early *in vitro* investigation of resistance development during the validation of new drugs

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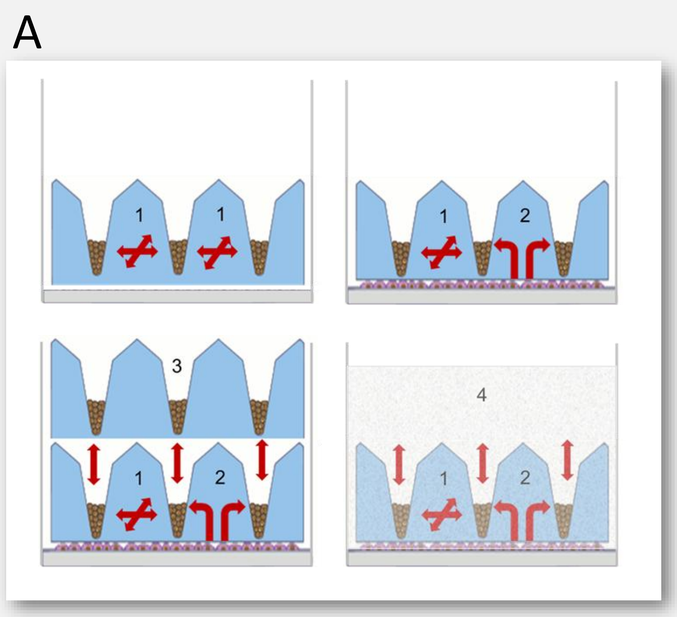
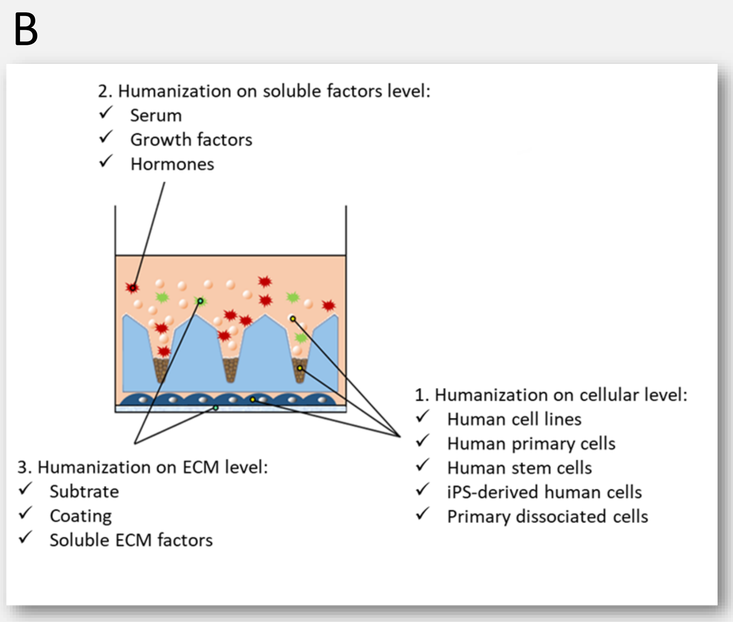
Introduction

The development of resistance to anticancer therapies represents one of the major challenges in oncology, as it often leads to treatment failure and disease relapse. Tumor cells can adapt to targeted drugs, chemotherapy, or immunotherapies through a variety of mechanisms, ultimately reducing their effectiveness. Identifying and understanding the emergence of treatment resistance is therefore crucial for improving patient outcomes and guiding the design of more effective treatment strategies. In this context, the development of a novel, reliable in vitro system capable of modeling and detecting resistance development is essential. Such systems not only allow for early identification of resistant phenotypes but also provide valuable insights into the underlying biological processes, enabling the exploration of alternative therapeutic approaches before resistance becomes clinically evident. Here we present how the innovative 3D CoSeedis multi-organoid™ models enable the detection of tumor resistance phenomena in a 3D in-vitro system.

The foundation: The 3D CoSeedis *in chip* communication technology™



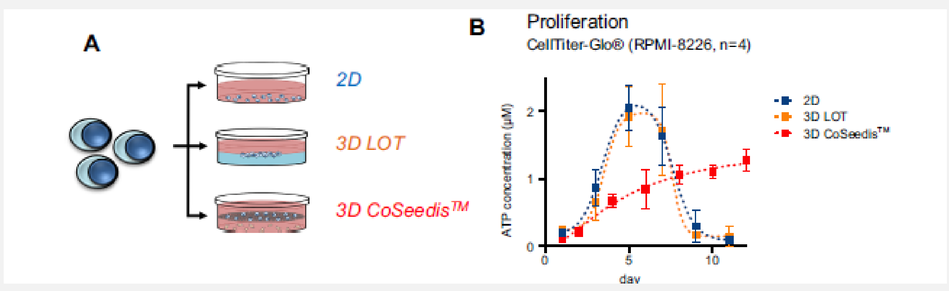


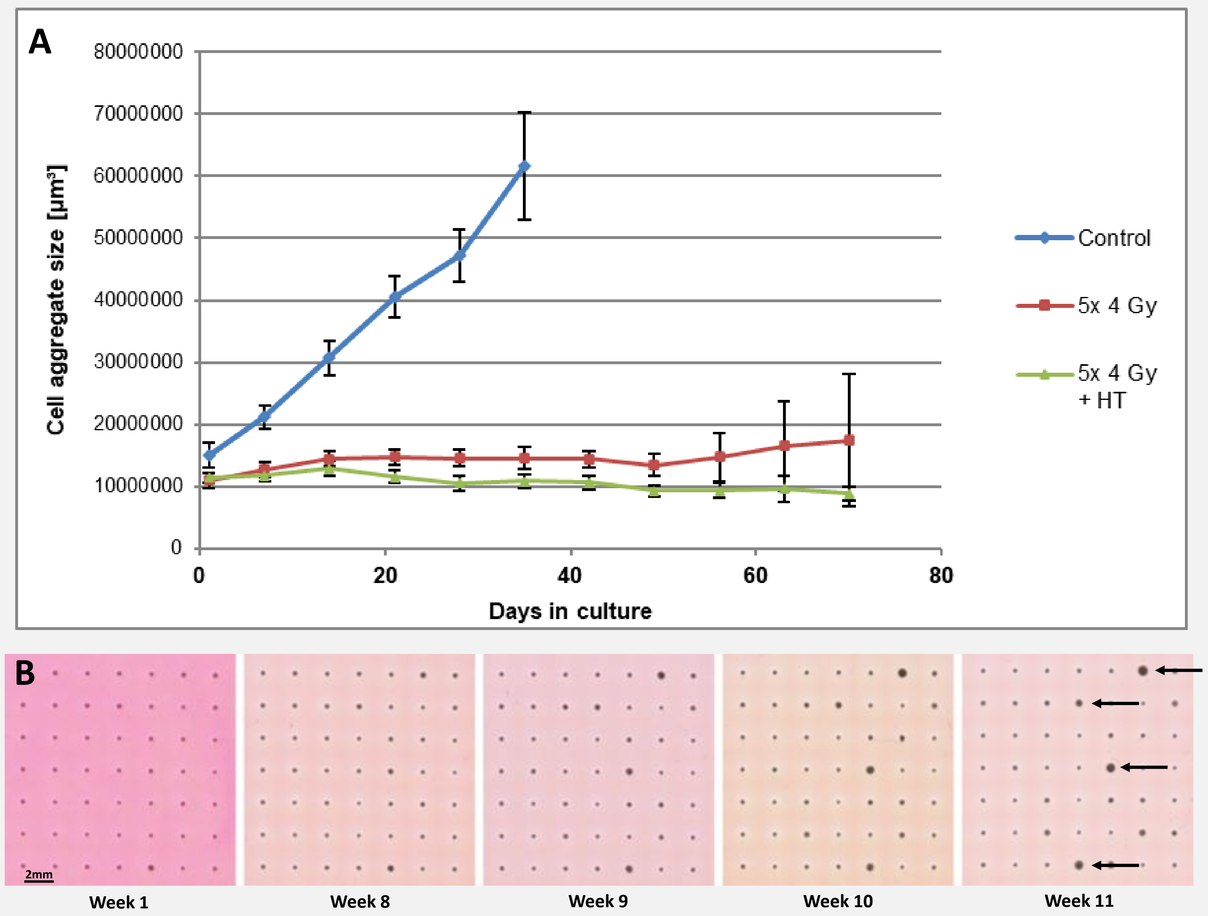
Modeling and monitoring tumor relapse—or drug resistance more broadly—*in vitro* models require fulfilling several stringent criteria. First, the model must exhibit sufficient biological and structural complexity to emulate physiologically relevant conditions *ex vivo*, thereby ensuring meaningful drug-responses. Second, because drug resistance emerges as a stochastic phenomenon, the platform must support large-scale biological replication to enable robust and reliable detection of rare resistance events. Finally, the model must permit longitudinal tracking of drug responses over extended periods to capture the dynamics of resistance formation with high fidelity.

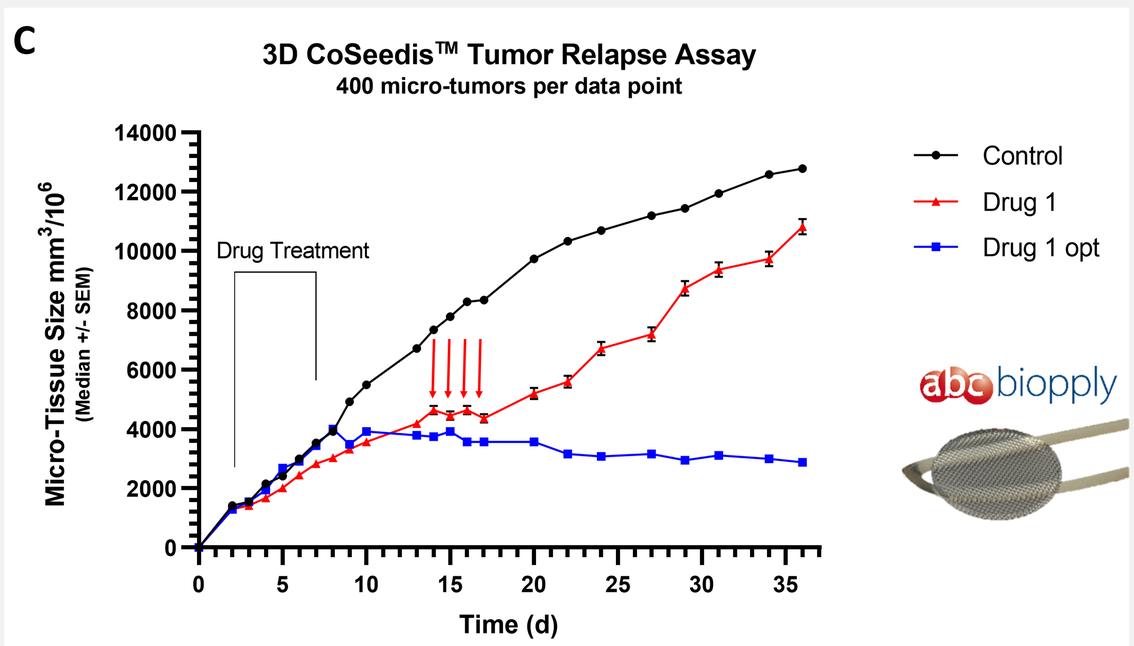
The 3D CoSeedis *in chip* communication technology™ addresses these critical challenges by providing a physiologically relevant ex vivo system capable of generating humanized disease models at scale, with unprecedented reproducibility and homogeneity. Its unique design enables the parallel formation of hundreds of microtissues under uniform growth, maintenance, and treatment conditions, ensuring consistency across individual microtissues within the system. Moreover, the fully humanized composition of the technology not only allows detailed investigation of treatment resistance in complex disease models (see panel A) but also opens new avenues for tackling unresolved questions surrounding sex-specific treatment responses and resistance formation (see panel B).

The biological manifestation: A physiologically accurate model for Multiple Myeloma[1]

Culturing Multiple Myeloma cells *in vitro*, particularly within 3D systems, remains notoriously challenging. While these cells initially exhibit rapid proliferation, they typically undergo a sharp decline, with viability peaking around day 5 before substantial cell death ensues (panel A, 2D and 3D liquid overlay [LOT] conditions). In contrast, when cultured with the 3D CoSeedis *in chip* communication technology™, Multiple Myeloma cells demonstrate sustained growth over extended periods, maintaining viability without detectable signs of cell death even after 12 days. This growth pattern aligns with a classic sigmoidal curve and represents a physiologically relevant advance, enabling prolonged maintenance of viable cell populations. Such stability not only provides a robust platform for capturing accurate drug-response dynamics but also constitutes a critical step toward uncovering mechanisms of resistance formation.







***The 3D CoSeedis Drug Resistance Assay*: *ex vivo* detection of tumor relapse**

Using abc biopply's 3D CoSeedis *in chip* communication technology™, a novel *ex vivo* assay has been established to monitor the emergence of drug resistance with high accuracy and reproducibility across diverse disease models and treatment regimens. One application of this technology is demonstrated in the optimization of combinatorial hyperthermia and radiation therapy (panel A[2]). In addition, high-resolution 3D CoSeedis tumoroid scans reveal the presence of individual resistant tumor clones within the culture system (panel B[2]), highlighting its ability to capture clonal dynamics. Finally, comparative drug testing on tumoroids (panel C) illustrates differential resistance profiles of antibody-drug conjugates (ADCs), with Drug 1 displaying a characteristic resistance plateau (red arrows) followed by renewed tumor outgrowth.

Conclusion

- The 3D CoSeedis *in chip* communication technology™ provides a physiologically relevant and scalable tool to identify, track, and quantify resistance formation in complex therapeutic contexts.
- The 3D CoSeedis Drug Resistance Assay provides the first reliable and physiologically relevant *ex vivo* assay optimized for drug development.

Outlook

- Use sex-specific preclinical models to investigate differences in the development of drug resistances in male and female disease models.
- Differential clonal analysis of resistant vs. susceptible microtissues allows the isolation and analysis of resistance formation in the drug development setup.

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[1] J. M. Waldschmidt et al., JCRCO, Volume 148, pages 1045–1055, (2022); [2] A. R. Thomsen et. al., Lab chip, 2018, 18, 179