

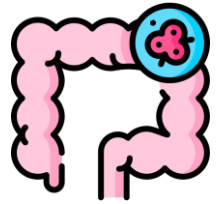
# Development of innovative 3D colorectal cancer models as preclinical tools for drug screening

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# Colorectal Cancer

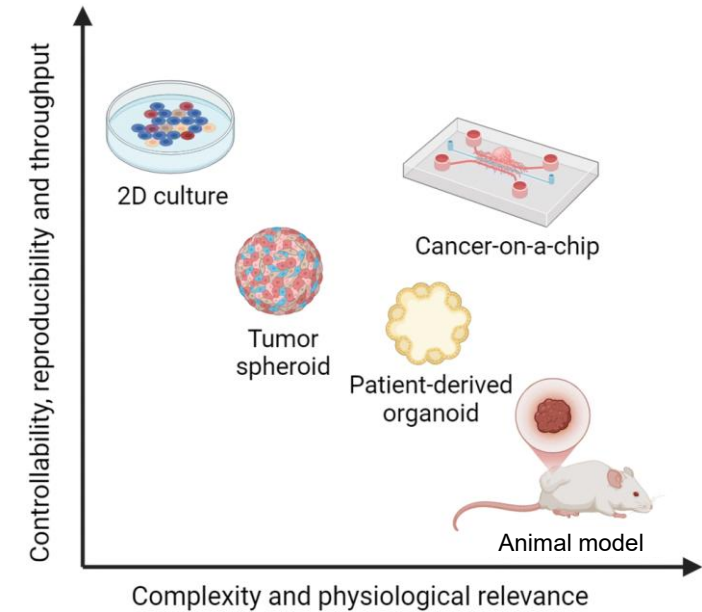


**3<sup>rd</sup>** Most commonly diagnosed

**2<sup>nd</sup>** Most fatal cancer globally

**URGENT**

Need of novel drug products with enhanced outcomes



Establish a 3D CRC-colon *in vitro* model to study the efficacy of drugs and nanomedicines

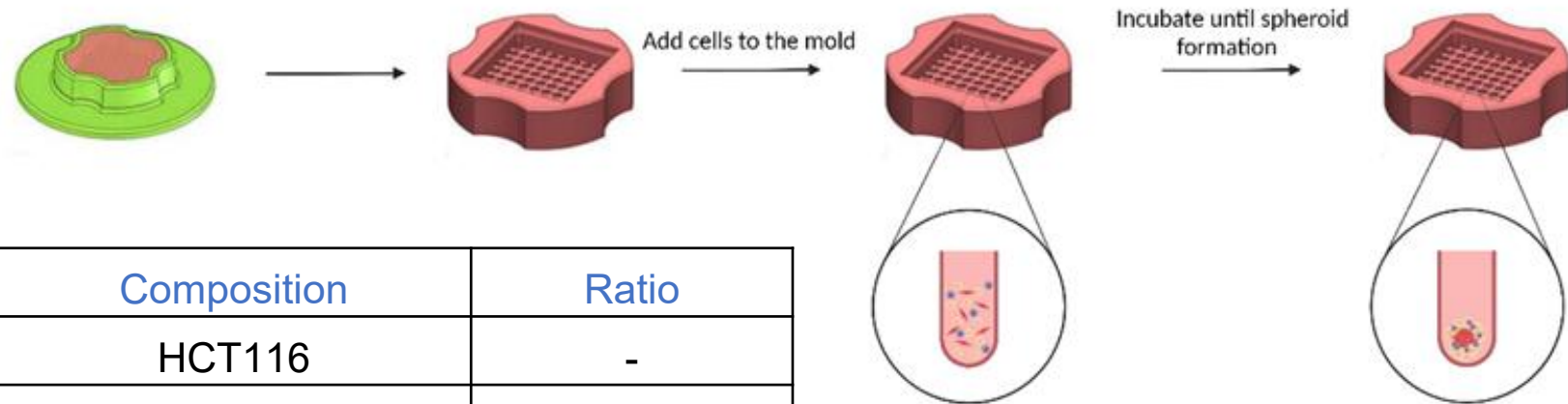


Liu L, et al. Lab Chip, 2025,25, 3314-3347

# CRC Multicellular tumor spheroids

5000 cells/MCTS

MicroTissues® 3D Petri Dish®

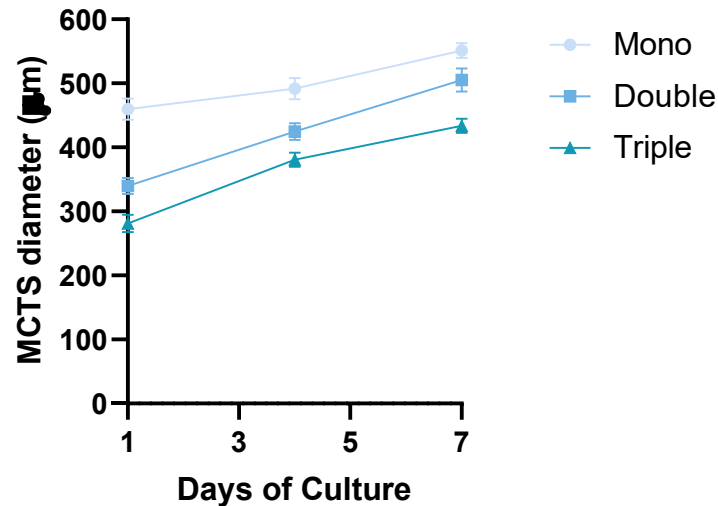


| Spheroid       | Composition          | Ratio |
|----------------|----------------------|-------|
| Monoculture    | HCT116               | -     |
| Double culture | HCT116:HIF           | 1:4   |
| Triple culture | HCT116:HIF:Monocytes | 1:4:4 |

Bauleth-Ramos T, et al. J Control Release. 2020;323:398-411

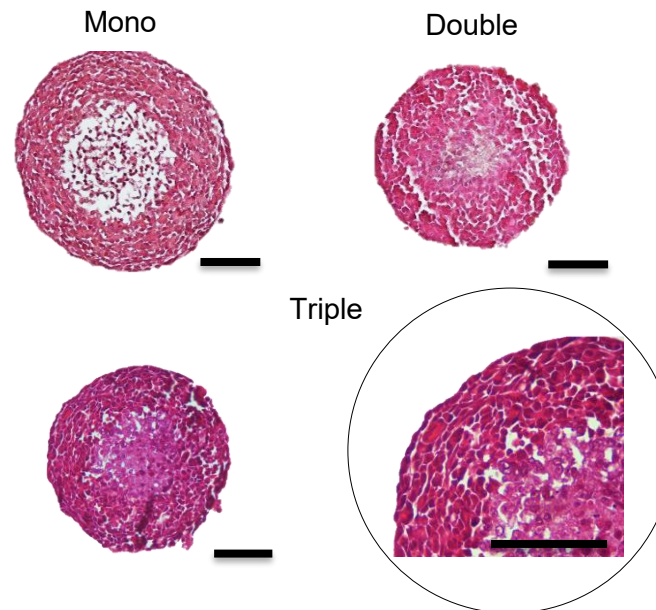
# CRC spheroid characterization

Size



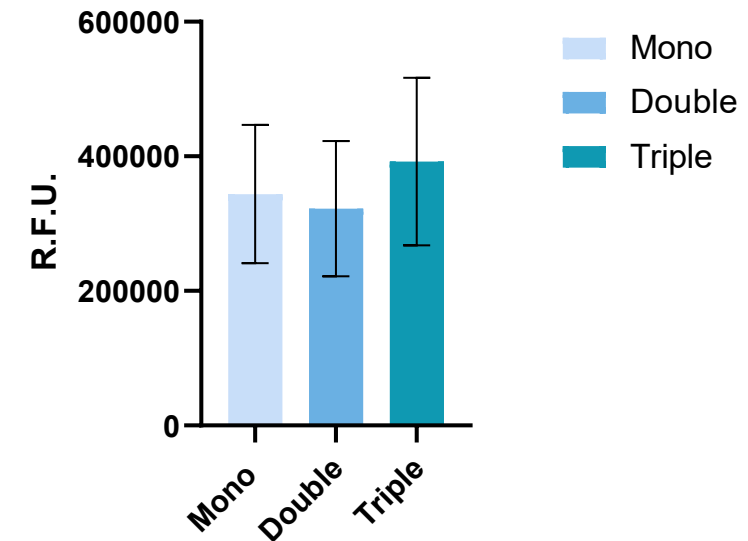
Size increase through time

Histology



Necrotic core

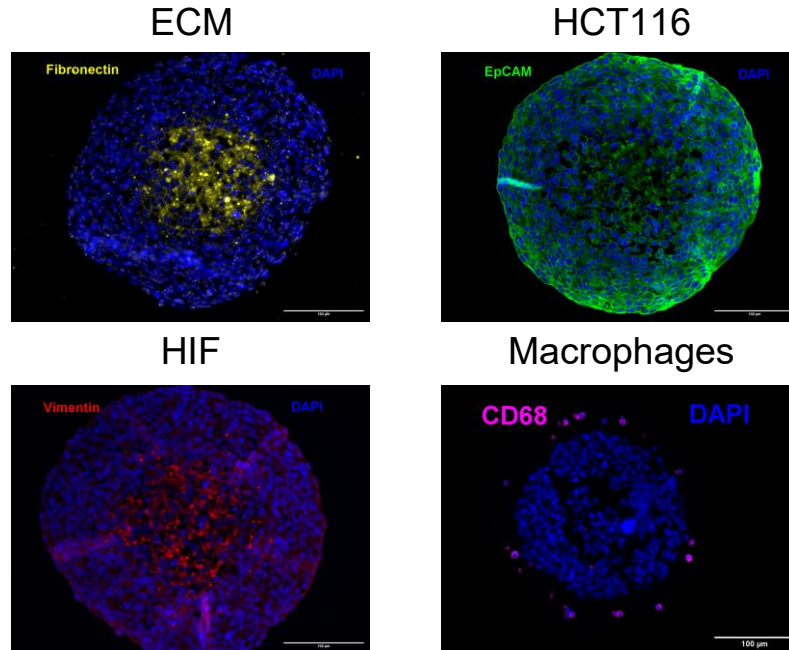
Metabolic Activity



Similar metabolic activities

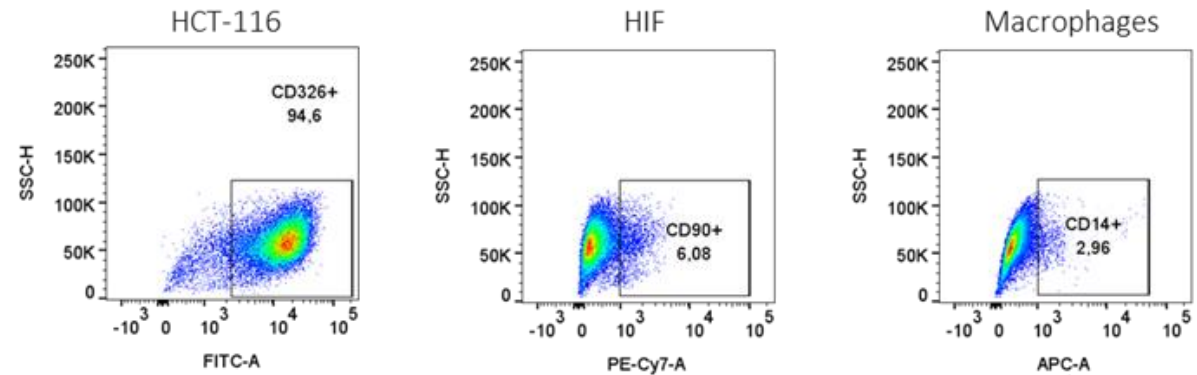
# CRC spheroid characterization

## Immunofluorescence



Production of ECM  
Different cell distribution within the spheroid

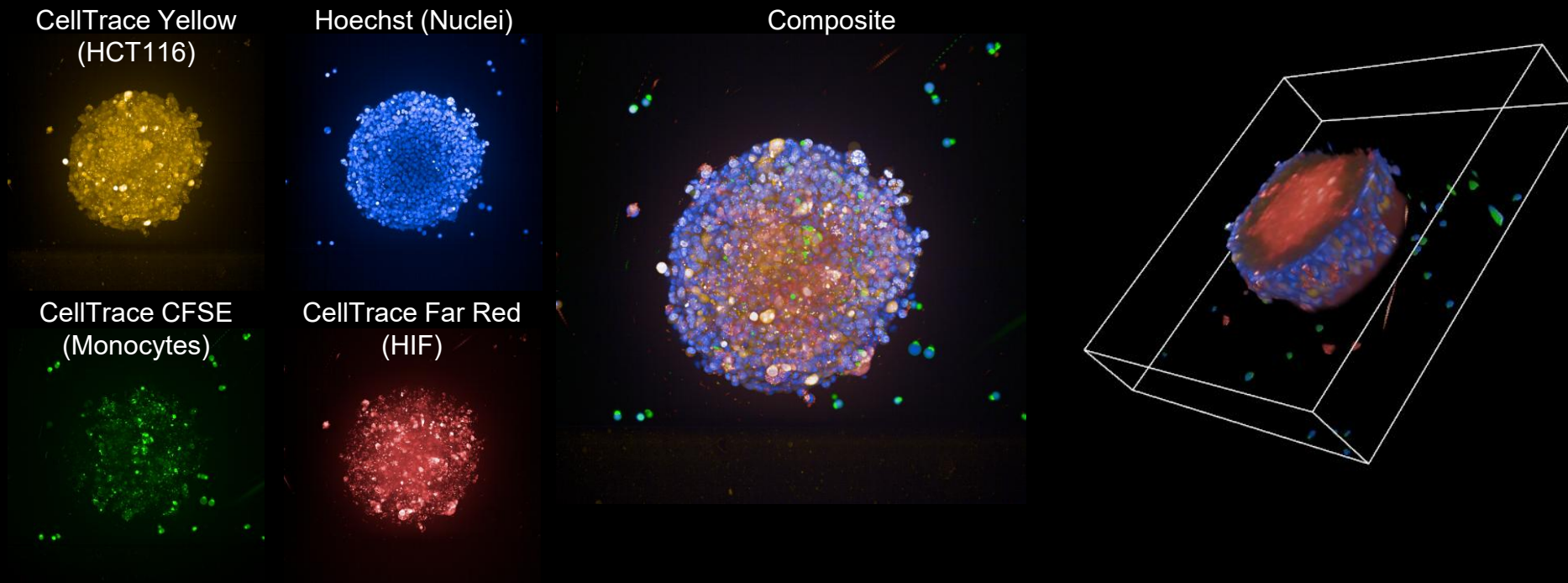
## MCTS cellular constitution



≈ 90% tumor cells  
≈ 5% fibroblasts  
≈ 3% macrophages

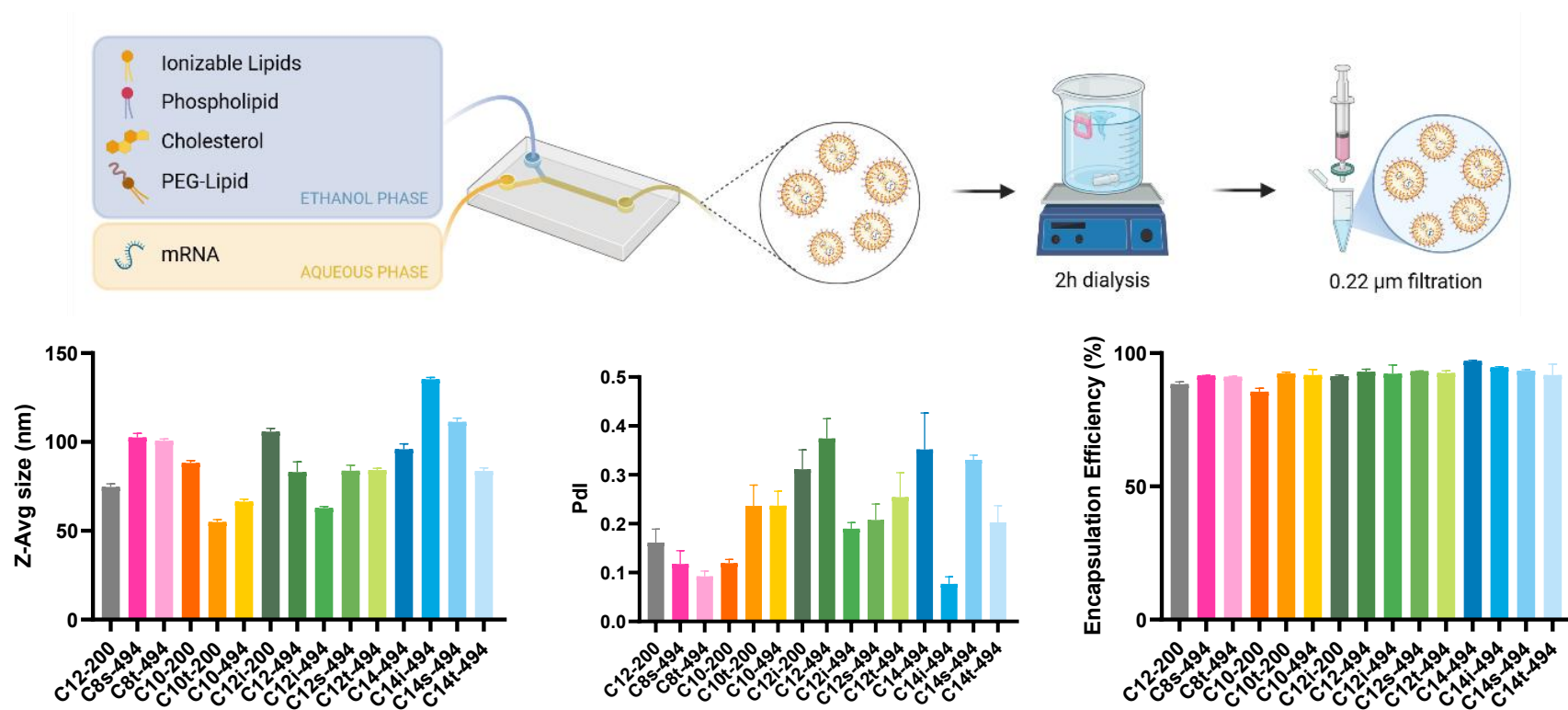
# CRC spheroid characterization

## MCTS cell distribution



Unpublished data

# Lipid nanoparticles production and characterization



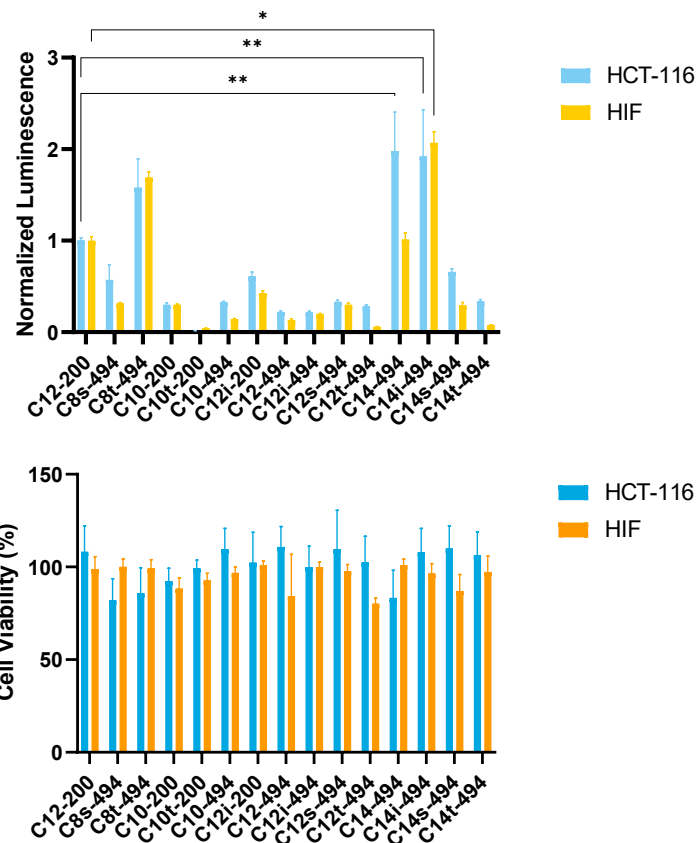
- Uniform LNP formulation
- mRNA encapsulation efficiencies  $\approx 90\%$

Molar composition: 35% Ionizable lipid/16% DOPE/46.5% Cholesterol/2.5% PEG

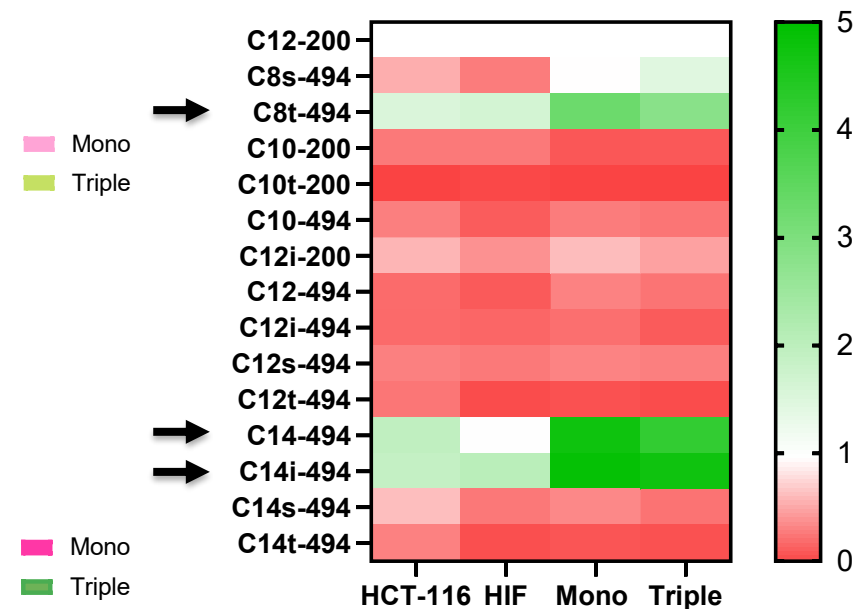
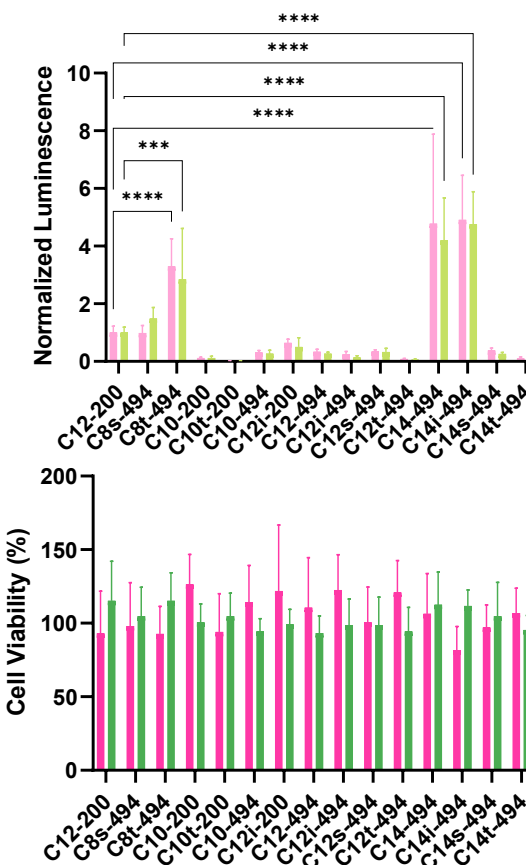
Unpublished data

# LNP transfection and cytotoxicity

## 2D monolayers



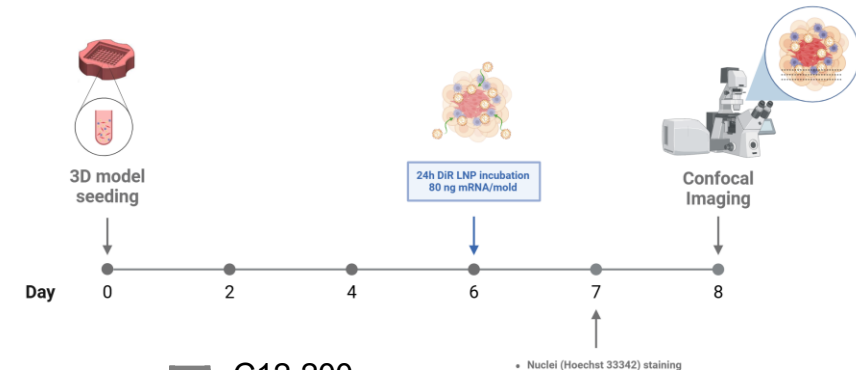
## 3D spheroids



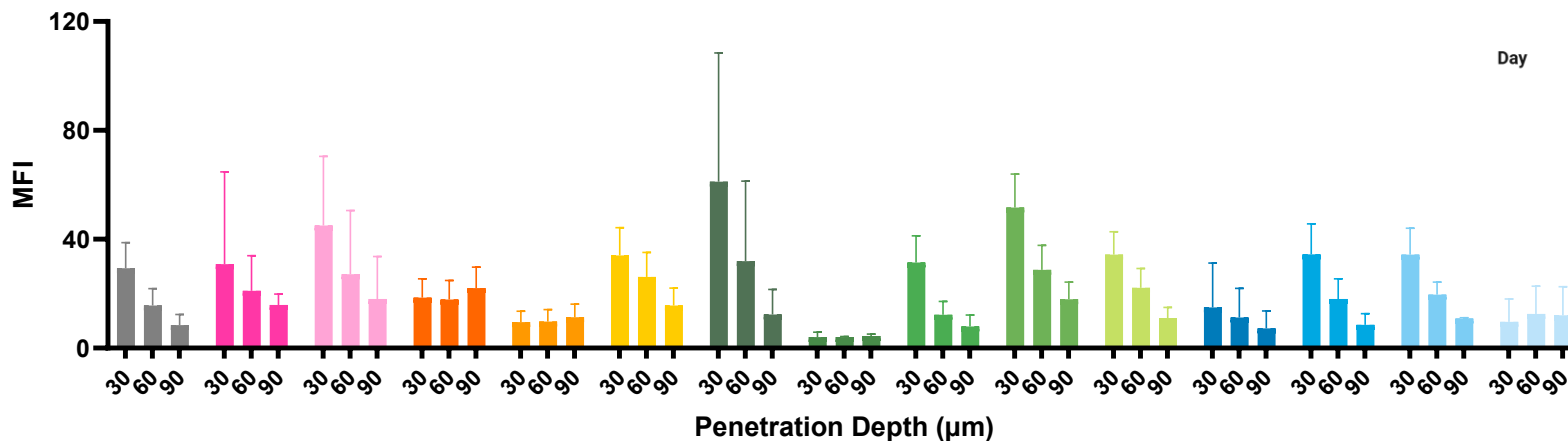
No cytotoxicity  
Top performers ionizable lipids:  
C8t-494, C14-494 and C14i-494

Unpublished data

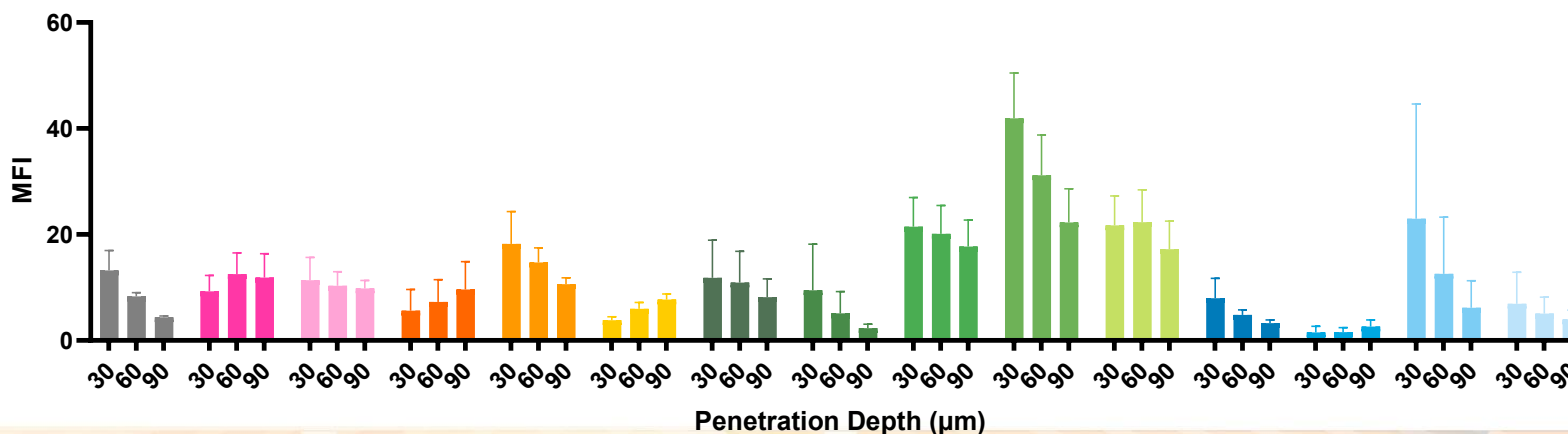
# LNP penetration studies



## Mono MCTS



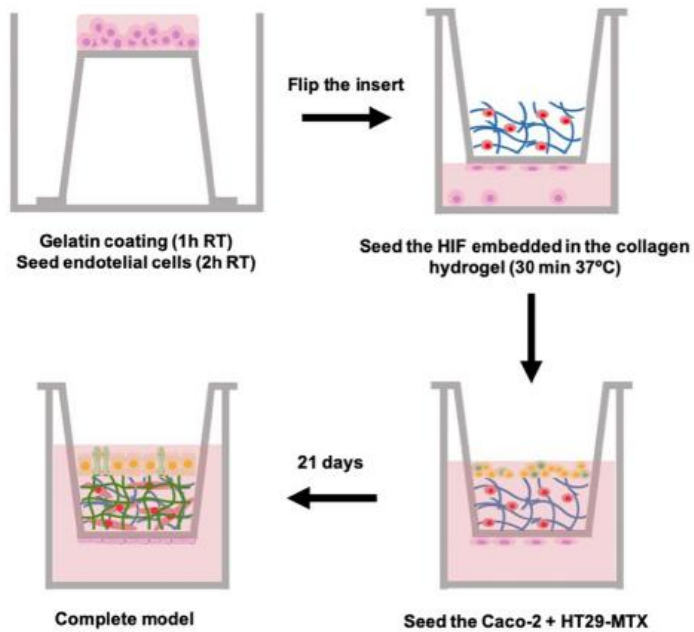
## Triple MCTS



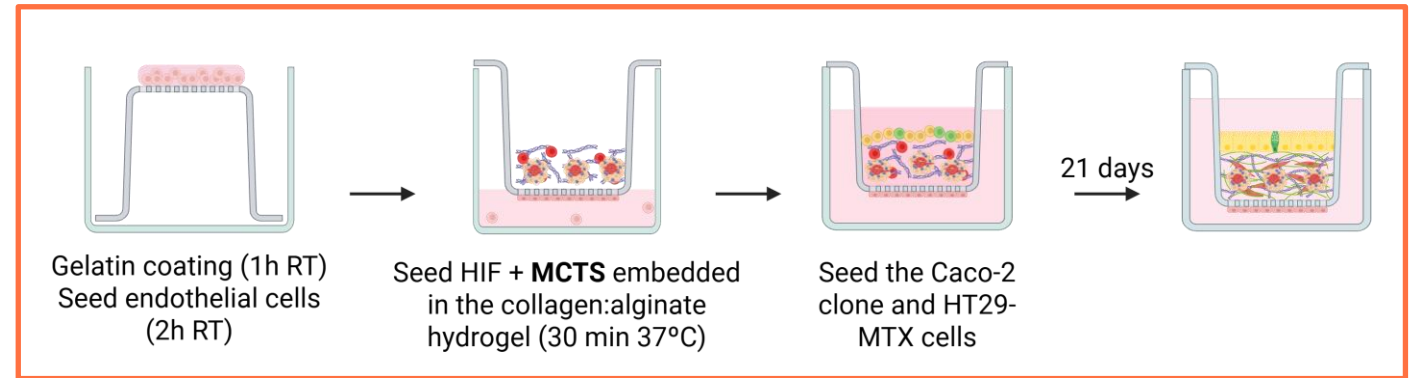
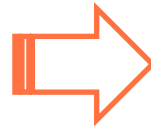
Higher penetrability in the monoculture spheroids

Unpublished data

# Model Combination



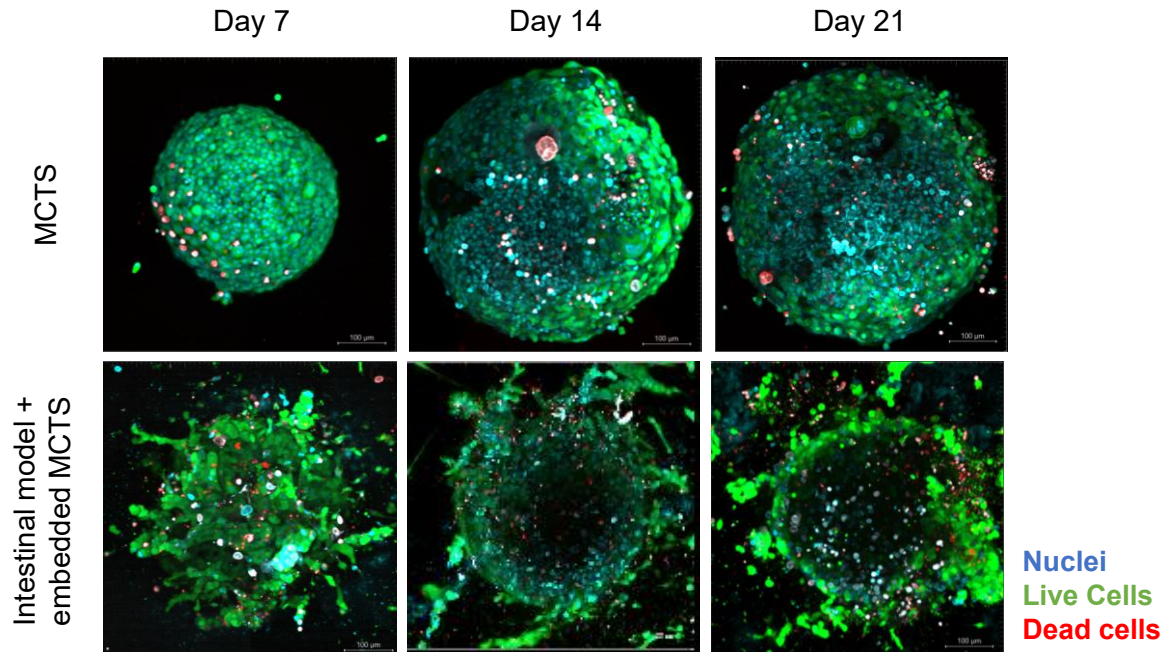
3D intestinal model



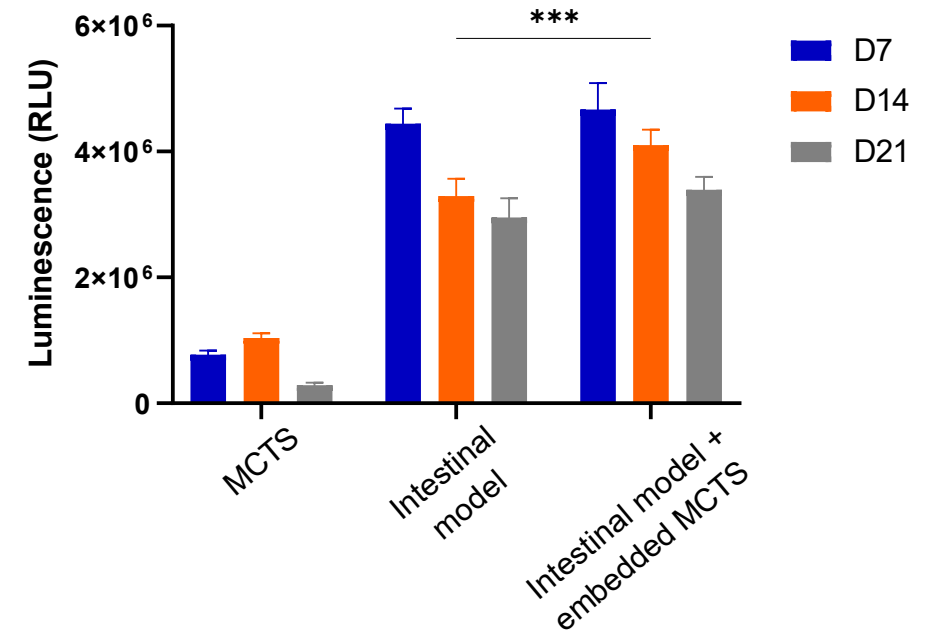
+ MCTS = Spheroid-laden 3D intestinal model

Unpublished data

# Spheroid-laden 3D intestinal model characterization



Spheroid remained viable within the hydrogel  
Invasion of the surrounding hydrogel



Spheroid remained metabolically active within the hydrogel

Unpublished data

# Conclusions

- MCTS presented **improved physiological relevance**
  - MCTS mimics different tumor features such as **ECM production, cell spatial organization and formation of a necrotic core.**
- The embedding of the MCTS didn't impact its morphology over time, as well as its metabolic activity and viability, presenting an invasive behavior within the hydrogel.

# Future Work

- Further characterization of both 3D models combined (cell-cell interaction; matrix remodeling)
- Drug and functionalized nanoparticles efficacy screening using the combined models

# Acknowledgments

NTDD Group



Professor Bruno Sarmento (supervisor)  
Dr. Catarina Leite Pereira (co-supervisor)  
All lab members

Mitchell Lab



Professor Michael J. Mitchell  
Dr. Marshall S. Padilla  
All lab members

**Thank you for your attention!**

**U. PORTO**



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