Advancing Colorectal Cancer Research

3D-Printed "Cancer-on-a-Chip" Model for Studying Angiogenesis

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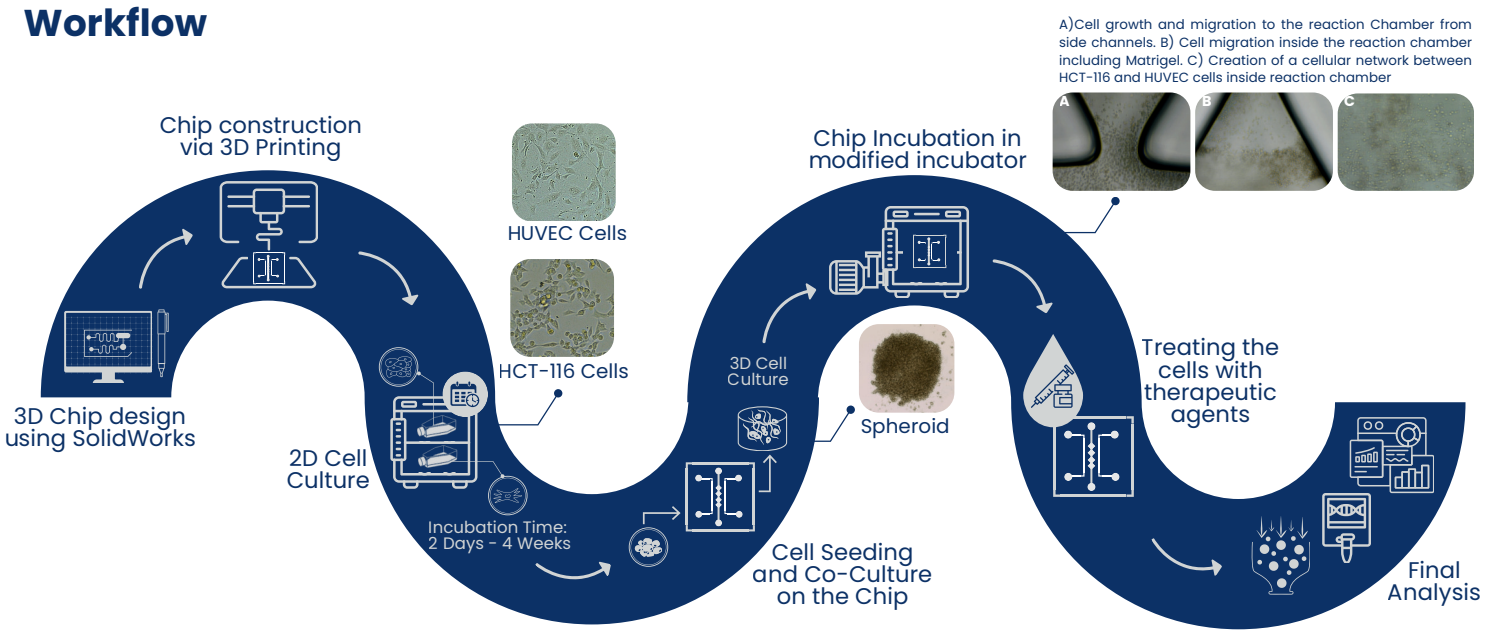
Introduction

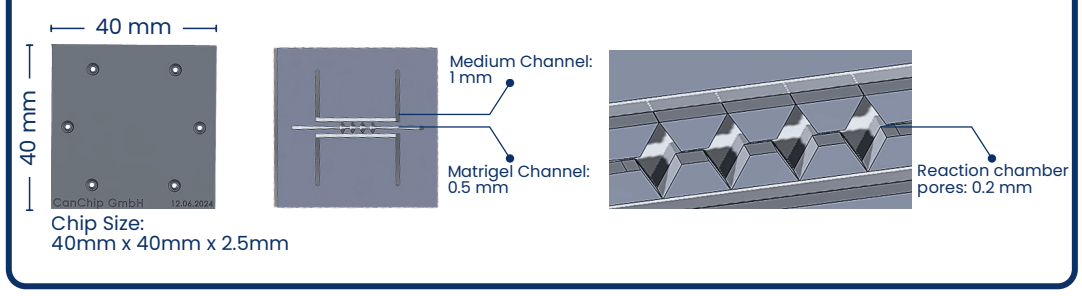
Tumor angiogenesis is central to CRC progression and metastasis, yet conventional 2D models cannot replicate the spatial and physiological complexity of the tumor microenvironment. This limits insights into cell–cell interactions and vascularization.

To overcome these gaps, we developed a 3D-printed "CRC-on-a-chip" system co-culturing HCT116 cells with HUVECs to mimic angiogenic interactions. This platform offers a more realistic model for studying CRC angiogenesis and provides a reliable foundation for drug testing and molecular research.

Workflow

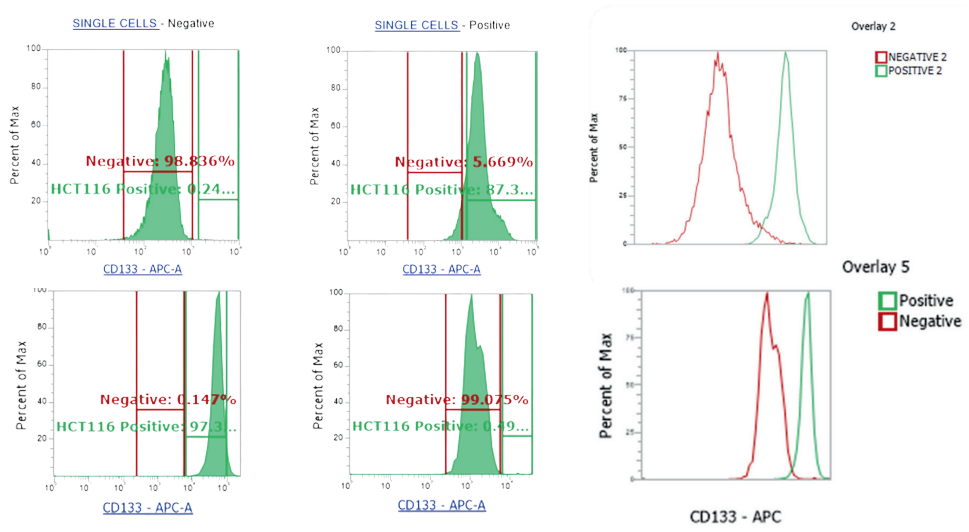


Chip Design



Chip Size:
40mm x 40mm x 2.5mm

Flow cytometry Analysis

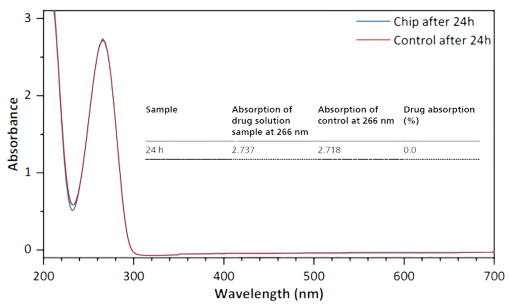


Flow cytometry of HCT-116 cells stained with APC-CD133 on the Attune cytometer shows single-cell gating for CD133-positive (green) and CD133-negative (red) populations. The plots display percentage distributions and overlay histograms comparing fluorescence intensities between positive and negative groups.

Drug Absorption Test

Testing drug absorption on 3D-printed microfluidic devices is key to evaluating material–drug interactions. Assessing how 5-Fluorouracil (5FU) behaves helps determine device compatibility and efficiency for delivery, testing, and screening.

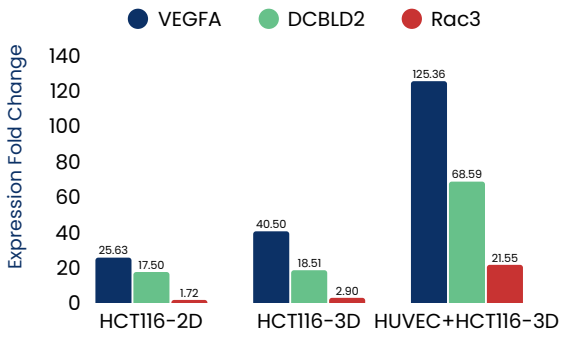
The lack of significant absorption indicates minimal interaction with 5FU, suggesting the devices are material-neutral and suitable for drug screening applications.



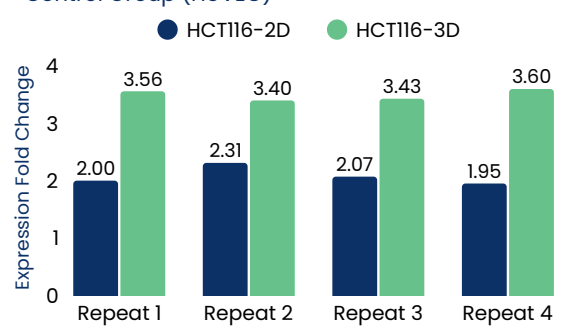
UV-Vis spectrum for drug solution pipetted out of chips after 24 hours. Control was a stock drug solution in 200 µL-microtubes.

Gene Expression Analysis

Comparison of Gene Expression Level Across Different Sample Types (Expression fold changes have been resulted as data normalization to HUVEC, as control group)



Rac3 Expression Fold Change Normalized to Control Group (HUVEC)



Genes used for qPCR

Rac3 – Promotes gene expression linked to cancer stemness and CRC progression.
DCBLD2 – Drives tumor growth, metastasis, and 5-FU resistance through EMT and angiogenesis.
VEGFA – Key regulator of angiogenesis; stimulates endothelial proliferation, migration, and vessel permeability.

In the HUVEC–HCT116 3D co-culture, VEGFA, DCBLD2, and Rac3 expression was significantly higher than in HCT116 3D monoculture or 2D systems, highlighting the role of cell–cell interactions in driving complex biological processes.
Chip performance was validated for reproducibility through Rac3 expression assays in four repeats over four months.

Experimental Results

Gene expression analysis and flow cytometry have revealed the role of the Rac3 gene in CRC angiogenesis. Quantitative PCR data showed a 2.54-fold increase in Rac3 expression in HCT116 cells compared to HUVEC controls. Further experiments with spheroids demonstrated a 1.36-fold increase in Rac3 expression compared to monolayer cultures, highlighting the importance of 3D tumor structures in modulating gene activity.

Conclusion

The 'CRC-on-a-chip' model presents a transformative leap in colorectal cancer research, offering an ethical, cost-effective, and scalable alternative to animal testing. By accurately mimicking the tumor microenvironment, this platform provides new insights into CRC biology, angiogenesis, and drug response, paving the way for innovative treatments and therapies.

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CRC-on-a-Chip: A Predictive Platform for Colorectal Cancer Drug Testing

A 5-Fluorouracil (5-FU) Case Study

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The Challenge in CRC Drug Development

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality worldwide. Despite intense research, **over 90% of oncology drug candidates fail in clinical trials**, largely because traditional preclinical models lack predictive power:

- **2D cell cultures** oversimplify the tumor microenvironment.
- **Animal models** are costly, time-consuming, and poorly mimic human tumor–vascular interactions.

Pharma companies need **clinically relevant in vitro systems** that bridge the gap between discovery and the clinic.

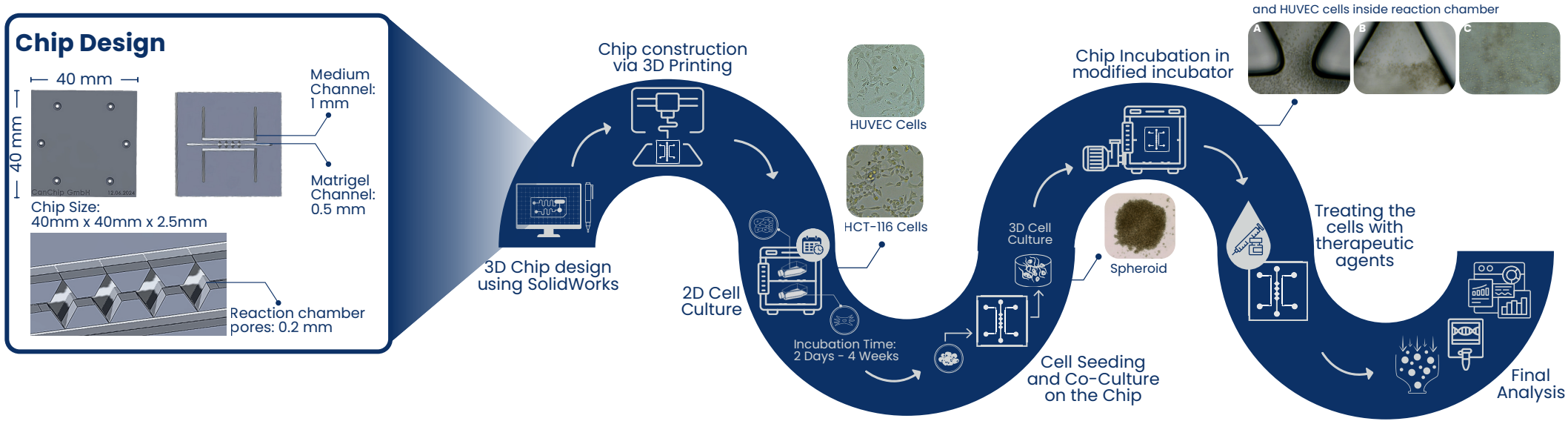
Our Solution: CRC-on-a-Chip

CanChip has developed a **3D microfluidic co-culture platform** that integrates HCT116 colorectal tumor spheroids with HUVEC endothelial cells under **continuous perfusion flow**.

- Preserves tumor–endothelial crosstalk.
- Mimics **in vivo-like pharmacokinetics**.
- Generates **mechanistic readouts** (gene expression, viability, morphology).
- Ensures reproducibility through **ISO 9001–aligned QA systems**.

This approach delivers **translationally relevant insights** for drug screening, mechanism studies, and resistance profiling.

Workflow

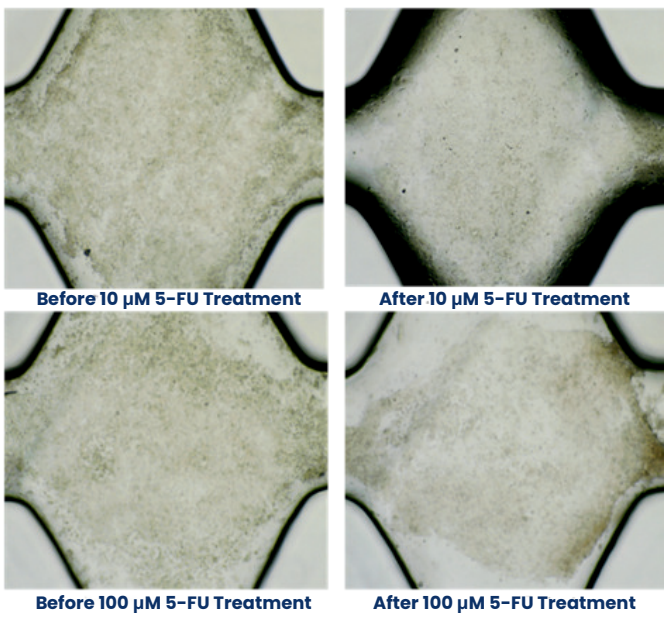


Case Study: 5-Fluorouracil (5-FU)

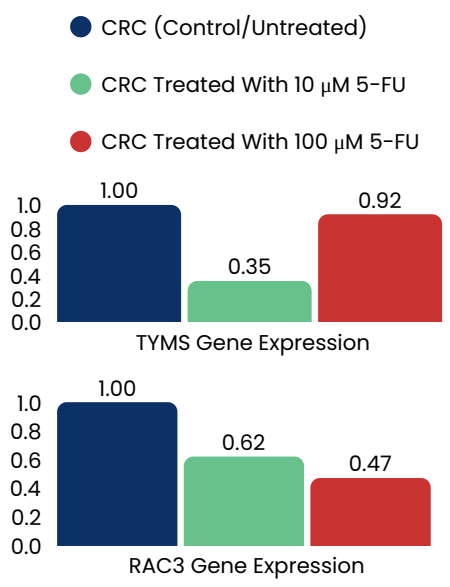
We evaluated 5-FU, the clinical standard for CRC therapy, at 10 µM (clinically relevant) and 100 µM (supra-physiological) for 48 hours.

Result

- **Morphology:** Dose-dependent shrinkage, 3D cell disintegration, and debris accumulation.
- **Viability (MTT):** HCT116 tumor cells highly sensitive at low micromolar concentrations, endothelial cells more resistant.
- **Gene expression (qRT-PCR):**
  - **RAC3:** Dose-dependent downregulation (0.69× at 10 µM; 0.47× at 100 µM vs. control).
  - **TYMS:** Maximal suppression at 10 µM (0.35× vs. control), less reduction at 100 µM (0.92×).



Microscopic images from the CRC chips before and after treatment.



Relative RAC3 and TYMS gene expression at 0, 10, and 100 µM of 5-FU.

Implications for Pharma R&D

- **Clinically aligned efficacy:** Matches patient plasma levels of 5-FU (~10 µM).
- **Mechanistic clarity:** Distinguishes between target engagement (TYMS) and broader cancer signaling (RAC3).
- **Predictive advantage:** Captures drug–vascular interactions missed in static assays.
- **Applications:** Candidate screening, biomarker validation, resistance studies, combination therapies.

Partner with CanChip

Our CRC-on-a-Chip platform demonstrates **scientific rigor and translational impact**. Partnering with **CanChip** enables pharma to:

- **Reduce** attrition in oncology pipelines.
- **Accelerate** preclinical decision-making.
- **De-risk** early clinical trials with mechanistically validated models.

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A Microfluidic Prostate-on-a-Chip Platform

For Dose-Dependent Cytotoxicity and Molecular Response Studies

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CanChip GmbH

The Challenge in Prostate Cancer Drug Development

Prostate cancer is a leading malignancy in **men**. Although AR-targeting therapies like **enzalutamide** work initially, most patients develop resistance. Traditional models fall short:

- **2D cultures** lack tumor–stroma and vascular interactions
- **Animal models** don't mimic human AR signaling
- **Static assays** miss dynamic drug distribution and shear stress

Pharma needs predictive, human-relevant models to evaluate AR-targeting drugs in realistic conditions.

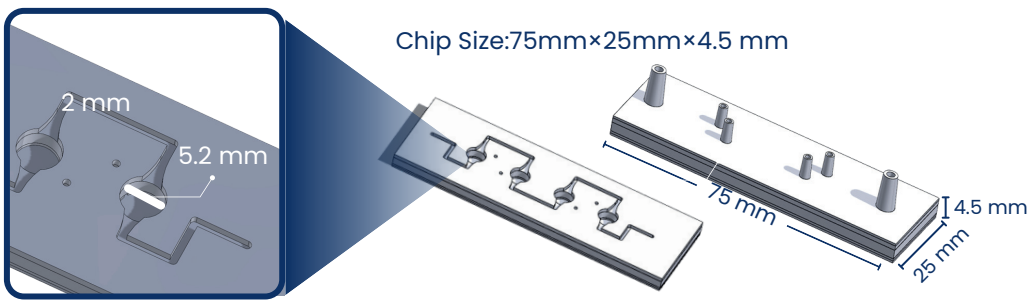
Our Solution: CRC-on-a-Chip

CanChip developed a **3D microfluidic co-culture system** integrating **LNCaP cells** with **HUVECs** under continuous perfusion. It:

- Maintains AR-regulated behavior
- Recreates tumor–endothelial crosstalk
- Enables quantitative readouts (viability, PSA, gene expression)
- Uses **ISO 9001**–aligned workflows for reproducibility

This dynamic model bridges the gap between static assays and **in vivo conditions** for anti-androgen drug testing.

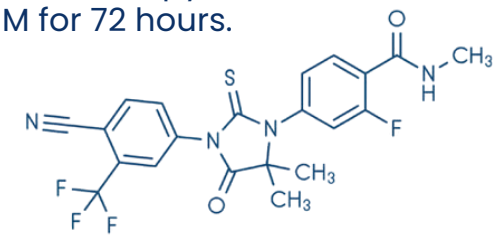
Chip Design



Case Study: Enzalutamide

We evaluated enzalutamide, a clinically approved AR antagonist for prostate cancer therapy, at 30, 50, and a supra-physiological dose of 100 µM for 72 hours.

enzalutamide



Results

Morphology:

- 30 µM: minor LNCaP density loss
- 50 µM: partial detachment and reduced cell–cell junctions
- 100 µM: extensive apoptosis and endothelial disruption

Viability (MTT):

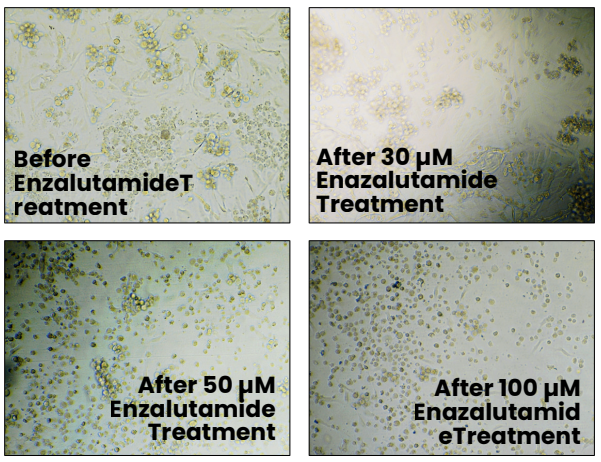
- LNCaP IC_{50} ≈ 22.95 µM
- HUVEC IC_{50} ≈ 80.77 µM
- Co-culture IC_{50} ≈ 36.24 µM

PSA (KLK3) Secretion (ELISA):

- Control: 546 pg/10^5 cells
- 30 µM: 307 pg/10^5 cells (-44%)
- 50 µM: 187 pg/10^5 cells (-66%)

Gene Expression (qPCR):

- **PSA:** Dose-dependent suppression (1.01× → 0.77× → 0.33×)
- **PSMA:** upregulation (1.71× → 2.25× → 0.30×), reflecting AR pathway inhibition and cytotoxic effects at high doses.



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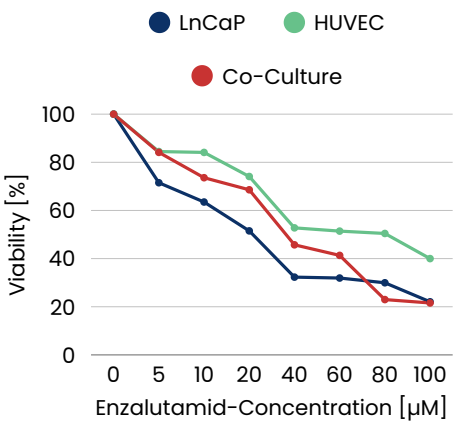
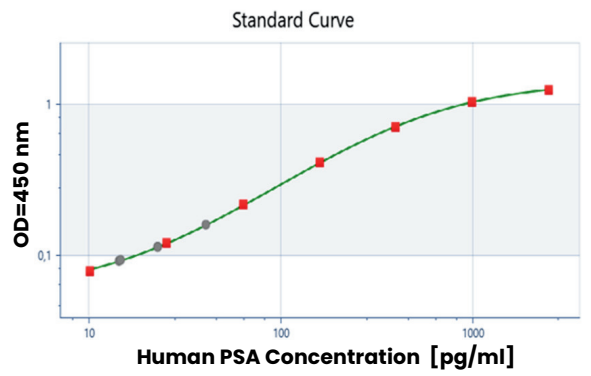
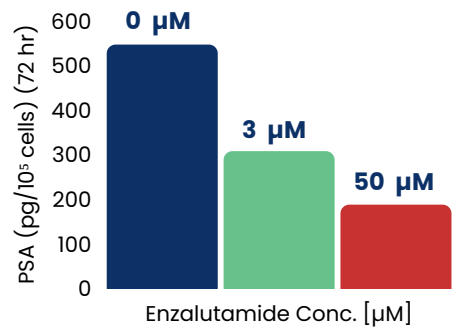


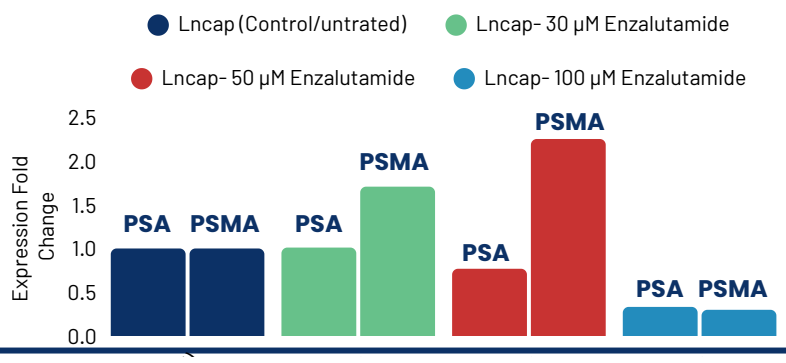
Enzalutamide sensitivity of LNCaP prostate cancer-on-chip cultures



PSA secretion in the LNCaP-on-Chip model.



Effect of Enzalutamide on PSA secretion in LNCaP cells.



PSA & PSMA Expression Fold Change Normalized to Control Group (Untreated)

Partner with CanChip

The **Prostate-on-a-Chip model** offers a robust, human-relevant platform for evaluating AR-targeted therapies. Partnering with **CanChip helps pharma companies:**

- Reduce attrition in oncology pipelines
- Strengthen preclinical decisions
- Accelerate the development of next-generation anti-androgen drugs

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