Advancing Colorectal Cancer Research

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

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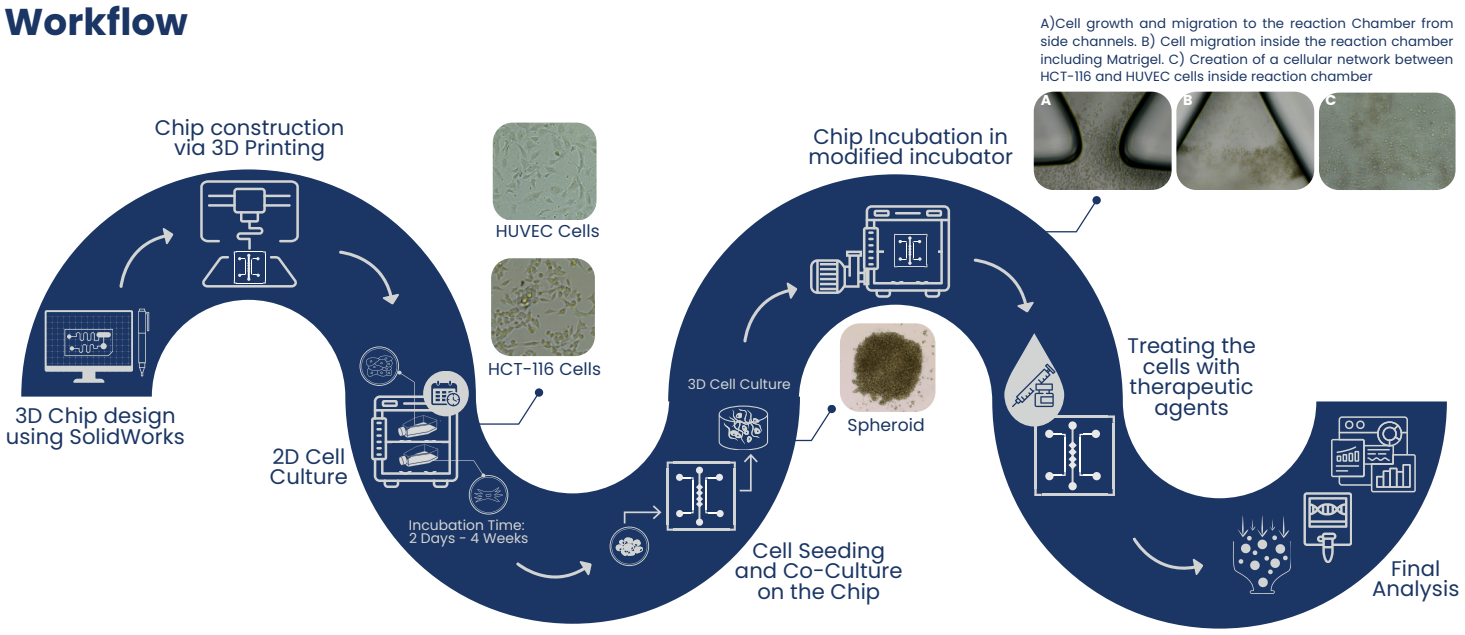
Introduction

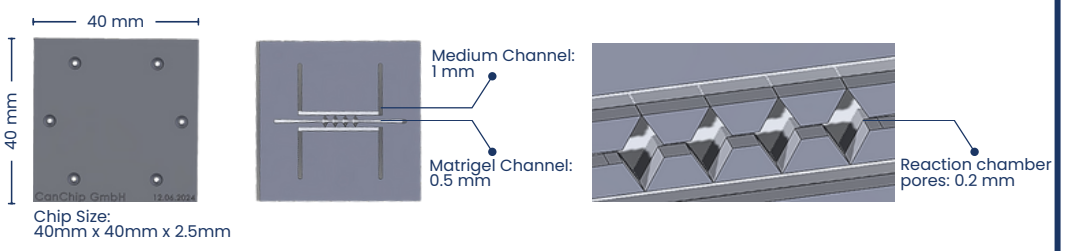
Tumor angiogenesis plays a crucial role in CRC progression and metastasis. Studying these processes in vitro, however, requires sophisticated models that can replicate the tumor microenvironment. Conventional 2D cultures fail to provide the spatial and physiological context of a 3D environment, thus limiting insights into cellular interactions and vascularization processes.

To address these limitations, we designed and developed a 3D-printed "CRC-on-a-chip" system that allows the co-culturing of HCT116 colorectal cancer cells and HUVECs to simulate angiogenic interactions. This innovative approach provides a more physiologically relevant setting for investigating CRC angiogenesis and offers a robust platform for future drug testing and molecular studies.

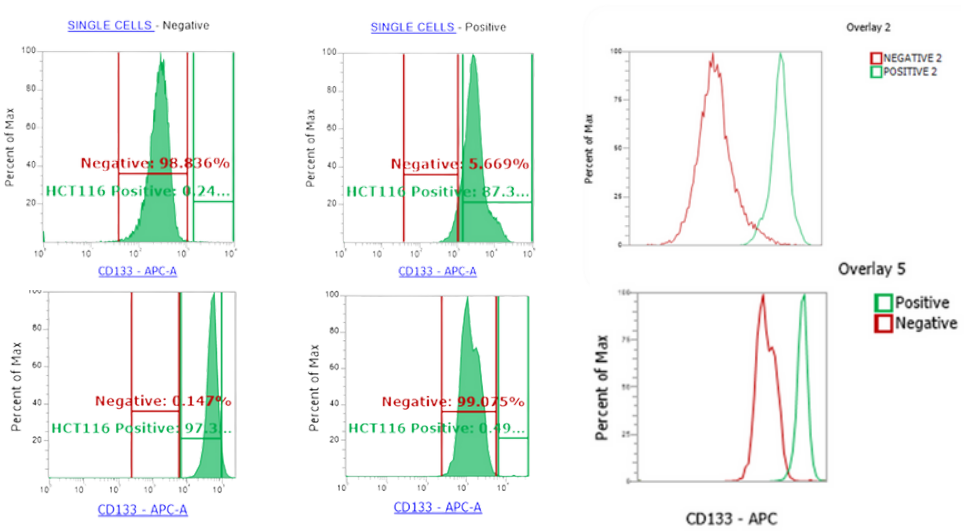
Workflow



Chip Design



Flow cytometry Analysis



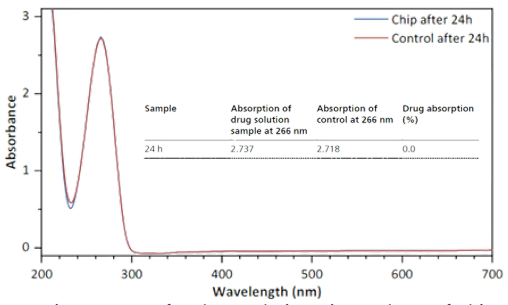
Flow cytometry analysis of HCT-116 cells stained with APC-conjugated CD133 using the Attune cytometer. The plots illustrate single-cell gating for negative and positive populations, showing percentage distributions of CD133-positive (green) and CD133-negative (red) cells across different samples. Overlay histograms further compare the fluorescence intensity of CD133-positive and -negative populations.

Drug Absorption Test

The test to determine drug absorption on 3D-printed microfluidic devices is essential for evaluating the material properties of these devices and their interaction with pharmaceutical compounds.

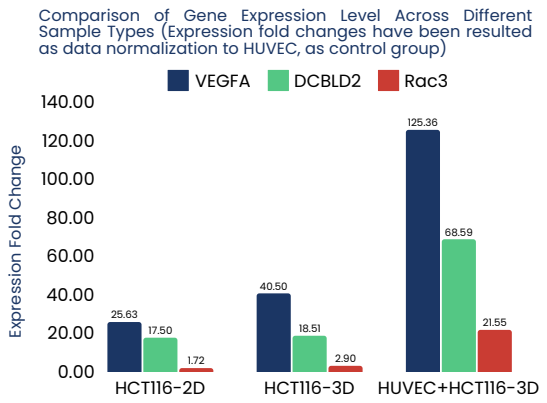
By understanding how a drug like 5-Fluorouracil (5FU) interacts with the device material, we can assess the compatibility and efficiency of these devices for drug delivery, testing, and screening applications.

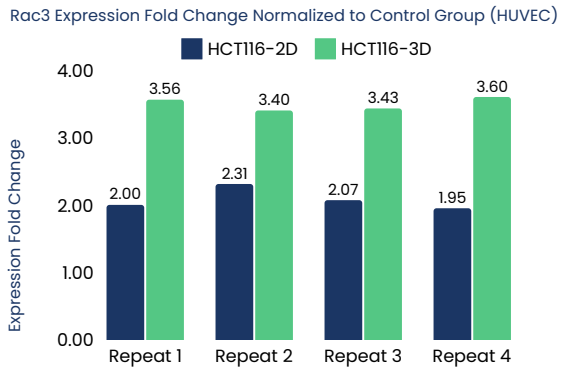
As the results showed lack of significant absorption, it suggests the 3D-printed microfluidic devices did not interact strongly with 5FU, indicating that these devices may be well-suited for applications where material neutrality with the drug is required.



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





Genes used for qPCR

Rac3: It drives an increase in the expression of genes that may contribute to cancer stemness and colorectal cancer development

DCBLD2: Its overexpression promotes tumor occurrence, development, and metastasis. Affects the Development of Colorectal Cancer via EMT and Angiogenesis and Modulates 5-FU Drug Resistance.

VEGFA: Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels.

In the co-culture of HUVEC and HCT116-3D, the expression of key genes such as VEGFA, DCBLD2, and Rac3 was markedly higher compared to HCT116 cells cultured in 3D alone or in 2D systems. These results demonstrate the critical influence of cell-cell interactions in driving complex biological processes.

In addition, the reproducibility of chip performance has been checked and validated with Rac3 Expression assays in 4 repeats within 4 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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