White Paper

A Dynamic Colorectal-Cancer-on-a-Chip Model Enabled by the VitroFlow System

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Abstract

The tumour microenvironment plays an important role in colorectal cancer progression and therapy response. However, commonly used in vitro models, particularly two-dimensional (2D) cell culture, lack key physiological features such as continuous perfusion, shear stress, and controlled nutrient supply. As a result, these systems often fail to fully reflect realistic cell behaviour. While more advanced three-dimensional models exist (e.g. spheroids, organoids), they frequently lack dynamic flow conditions and precise environmental control.

In this white paper, we present a proof-of-concept for a dynamic colorectal cancer-on-a-chip (CCoC) model using the VitroFlow microfluidic system. Human colorectal cancer cells (LoVo) were cultured in a microfluidic chip under continuous perfusion, enabling controlled flow conditions as well as stable temperature and gas supply. The aim was to explore whether such a system can influence cell morphology and drug response compared to standard 2D culture.

Cells cultured in the chip showed clear morphological differences compared to 2D conditions, adopting a more compact and tissue-like appearance. This was supported by a significant increase in cell circularity. In addition, enhanced expression of the marker CD44 was observed in the chip-based system, indicating increased cell–cell and cell–matrix interactions. Treatment with 5-fluorouracil (5-FU) led to reduced proliferation in both systems, while an upregulation of RRM2 confirmed drug activity.

Overall, these results demonstrate that even a simple dynamic culture setup can influence cancer cell behaviour and better approximate tissue-like characteristics compared to static 2D culture. While this model represents an early-stage proof of concept and does not yet include additional components of the tumour microenvironment, such as immune or stromal cells, it provides a promising basis for further development. With additional complexity, this approach could contribute to more physiologically relevant in vitro models for drug testing and cancer research.

Keywords: Microfluidics, Organ-on-a-Chip, 3D cell culture, Organoids-on-Chips, Lab-on-a-Chip

1. Introduction

1.1.The Tumour Microenvironment in Colorectal Cancer

Tumour development, progression, and therapeutic responses are strongly influenced by the tumour microenvironment (TME) [1]. The TME comprises extracellular matrix components, soluble signalling molecules, nutrient and oxygen gradients, and mechanical forces such as shear stress [2]. These factors collectively regulate cancer cell behaviour, including proliferation, migration, metabolism, and response to therapies.

In colorectal cancer, the dynamic interplay between tumour cells and their surrounding microenvironment critically determines disease progression and treatment outcome [3]. Mechanical forces arising from interstitial and vascular flow, as well as spatial gradients of nutrients and other substances, play a decisive role in shaping tumour physiology. However, these parameters are often not sufficiently represented in conventional static cell culture systems.

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1.2. Limitations of Conventional Cancer Models

Two-dimensional in vitro cancer models are widely used due to their simplicity, low cost, and high reproducibility. Nevertheless, these models lack essential features of the in vivo environment, including dynamic perfusion, mechanical stimulation, and realistic tissue architecture. As a result, cancer cells grown in 2D culture often exhibit artificial morphologies, altered gene expression patterns, and drug responses that poorly correlate with clinical outcomes [4].

Three-dimensional culture systems, such as spheroids and organoids, represent an improvement by partially recapitulating tissue architecture [5][6]. However, these models typically lack controlled perfusion, defined shear stress, and precise spatial compartmentalisation, which limits their ability to fully mimic physiological conditions. Another common downside of oncological organoid models is that due to the strong proliferation, typical for cancer cells, can quickly lead to necrotic organoid cores, due to limited diffusion and lack of perfusion and vascularisation. Animal models, while more complex, frequently fail to predict human-specific drug responses due to species differences. Thus the lack of predictive and human-relevant preclinical models remains a major bottleneck in cancer drug development.

1.3.Organ-on-a-Chip Technology

Organ-on-a-Chip (OoC) creates a dynamic culture with a relevant physiological microenvironment with an artificial blood flow by constantly perfusing cell culture media, creating capillary shear forces by using microfluidics with physiological geometries, as well as respiration, i.e. oxygen supply and body temperature as is done in 2D cell culture [7].

The centre piece of the technology is a microfluidic chip. The term microfluidics refers to the ability to precisely control liquids on a microliter scale through channels commonly ranging from ten to a few hundred micrometres in size located in a chip. The chip is a flow chamber that has different structures depending on the organ model. These chips enable the controllable arrangement of different cell types and thus realistic compartmentalisation of the respective organ-specific cells, which is crucial for its interaction and reaction. These chips also enable combined with perfusion, the precise control of chemical and physical reactions, controllable volumes and significantly lowers reagent consumption. The arrangement and type of cells vary depending on the organ and model and can be very different depending on the intended use. After seeding of the cells, the chip is then connected to a control unit that provides a physiological microenvironment. Key parameters here are shear stress, nutrient content, oxygenation and temperature [8], which are all regulated by the control unit VitroFlow of the company VitrofluidiX GmbH which is comprised of several units, fully integrated in one stand-alone device.

1) The simulation of a circulatory system

The first component simulates a circulatory system through pumps, in which the interaction between pressure and flow velocity gives rise to shear forces acting on the cells. Secondly, the circulatory simulation enables the continuous exchange and distribution of nutrients that are essential for cell survival [9]. This dynamic environment leads to the activation of cell surface molecules and signalling cascades, while maintaining a stable balance of nutrients and other biochemical factors. Altogether, these processes closely resemble physiological conditions found in the human body [10].

2) Incubation

Incubation is used for oxygen supply in both normal cell culture and OoC. A normoxic gas mixture of 95 % (v/v) ambient air (approx. 21 % (v/v) O2) and 5 % (v/v) CO2 is usually used for this purpose [11]. The oxygen serves to simulate the so-called oxygenation or enrichment of the blood with oxygen and is essential for the cellular functions and the behaviour of the cells in the chip and thus for the organ simulation [12].

3) Temperature

Temperature is also an important parameter for the survival of cells and the creation of a physiologically correct environment. The body temperature of a healthy person is approx. 37°C.

The physiological microenvironment in conjunction with the controllable compartmentalization of the cells has several advantages [13]. It makes it possible to mimic extrinsic properties of organs not only with the basic parameters mentioned, but also with other factors such as the circulating immune

system or microbiota. In addition, perfusion generates shear stresses that lead to a tissue-like structure of the cells. In general, culturing cells in a dynamic culture results in cells that behave similarly to the in vivo state, for example with a more realistic proliferation rate, drug response and general physiology. OoC thus mirrors structural and functional characteristics of human organs, allowing the study of responses to environmental influences, drugs and diseases [14]. The following figure shows the key advantage of OoC. The technology combines the reproducibility and controllability of standard cell culture systems with the complexity and physiological relevance of animal models [15]. In some cases, organ-on-a-chip systems have demonstrated superior predictive performance compared to animal testing. For example, a human liver-on-a-chip model identified 87% of drugs causing liver toxicity in patients - despite these compounds previously passing animal testing - while achieving a specificity of 100%. These findings highlight the potential of organ-on-a-chip systems for more reliable and human-relevant preclinical evaluation [16].

1.4.Aim of the Study

The aim of this study was to establish a mono-culture colorectal cancer-on-a-chip for drug testing under physiologically relevant conditions. The specific objectives were:

- to cultivate human colorectal cancer cells in a dynamic microfluidic environment,
- to compare cancer cell morphology and proliferation in the chip system versus conventional 2D culture,
- to evaluate the response of colorectal cancer cells to the chemotherapeutic agent 5-fluorouracil (5-FU), and
- to assess molecular markers associated with drug response and three-dimensional cell architecture.

To achieve this, LoVo cells cultured under conventional 2D conditions were directly compared with cells grown in the colorectal cancer-on-a-chip (CCoC) system under dynamic perfusion. Morphological differences were quantitatively assessed by analysing cell circularity using ImageJ software based on microscopic images acquired during the experiments. This approach enables an objective comparison of cell shape across culture conditions. In 2D culture, cells are expected to exhibit low circularity due to the formation of pseudopodia and their spread, flattened morphology. In contrast, cells in three-dimensional or dynamically perfused environments typically show increased circularity, reflecting a more tissue-like organisation, although they do not become perfectly round due to ongoing cell–cell interactions and structural

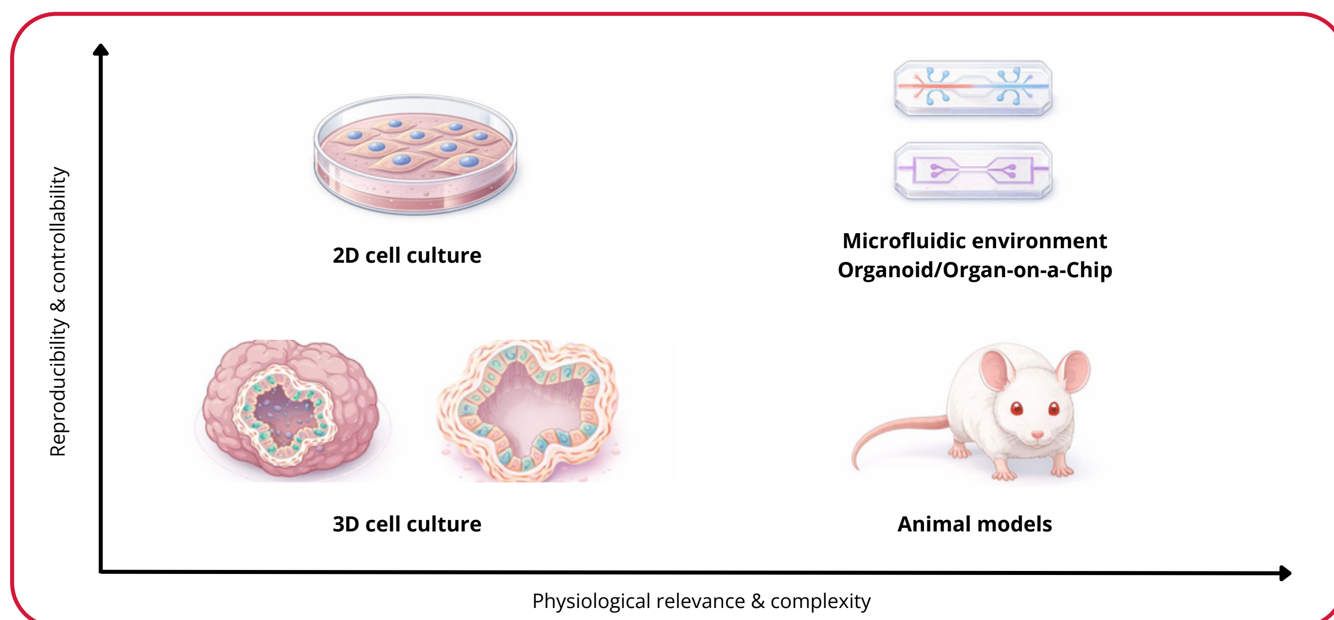


Figure 1: OoC technology establishes a close link between animal models and traditional in vitro models. It has the physiological relevance and complexity of animal experiments as well as the reproducibility of traditional cell cultures [17]

constraints within the tissue. Importantly, completely round cells would not represent a physiological state, as this can indicate cellular stress, hypotonic conditions, or cell death. To exclude such artefacts, nuclear integrity and cell viability were monitored by DAPI staining.

In addition to morphological analysis, molecular markers were evaluated to characterise drug response and three-dimensional cell architecture. Ribonucleotide reductase M2 (RRM2) was used as a marker for drug-induced effects. These enzymes catalyse the conversion of ribonucleotides into deoxyribonucleotides, thereby maintaining the intracellular dNTP pools required for DNA biosynthesis, repair and replication. RRM2 plays a critical role in cancer cell proliferation and has been reported to be upregulated in response to 5-FU treatment, making it a suitable indicator of drug activity. CD44 was analysed as a marker associated with three-dimensional growth. CD44 is a cell surface adhesion protein that mediates cell–cell and cell–extracellular matrix interactions and binds components such as hyaluronic acid, heparan sulphate, collagen, and fibronectin. Its expression is known to be upregulated in three-dimensional culture systems compared to conventional 2D conditions. Based on these characteristics and the observed morphological differences, CD44 was selected to assess whether the chip-based culture system induces molecular features characteristic of tissue-like organisation.

The resulting model serves as a foundational colorectal cancer-on-a-chip platform that can be further expanded for advanced cancer research and preclinical drug testing.

2.Materials and methods

2.1. Cell culture

2.1.1. Thawing the cell lines

The cell line "LoVo CLL-229" (short LoVo) by "ATCC" was stored permanently in a tank filled with liquid nitrogen. For thawing, a cryotube with cells was heated for 1–2 min in a 37 °C water bath. Then 1 ml RPMI 1640 cell culture media with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin was added, the cell suspension was transferred to a reaction tube and centrifuged at 1000 rpm for 5 min. The supernatant was aspirated and the cell pellet was resuspended in the cell culture medium. The cell suspension was then transferred to a T25 adherent culture flask and cultivated at 37 °C and 5% CO_2.

2.1.2. 2D Cell cultivation

After thawing, the LoVo cells were cultured in T25 adherent culture flasks in RPMI 1640 cell culture media with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin until they reached 80% confluence. Once the cells had reached 80% confluence, they were passaged. The medium was first removed, the cells were washed with DPBS and incubated at 37 °C for 3–5 min after adding 0.5 ml TrypLE™ Express. The cells were then detached with 4.5 ml RPMI 1640 medium and placed in a Falcon tube. In the next step, 0.5 ml of the cell suspension was transferred to a new T25 adherent culture flask with 4.5 ml RPMI 1640 medium and cultivated at 37 °C and 5% CO_2.

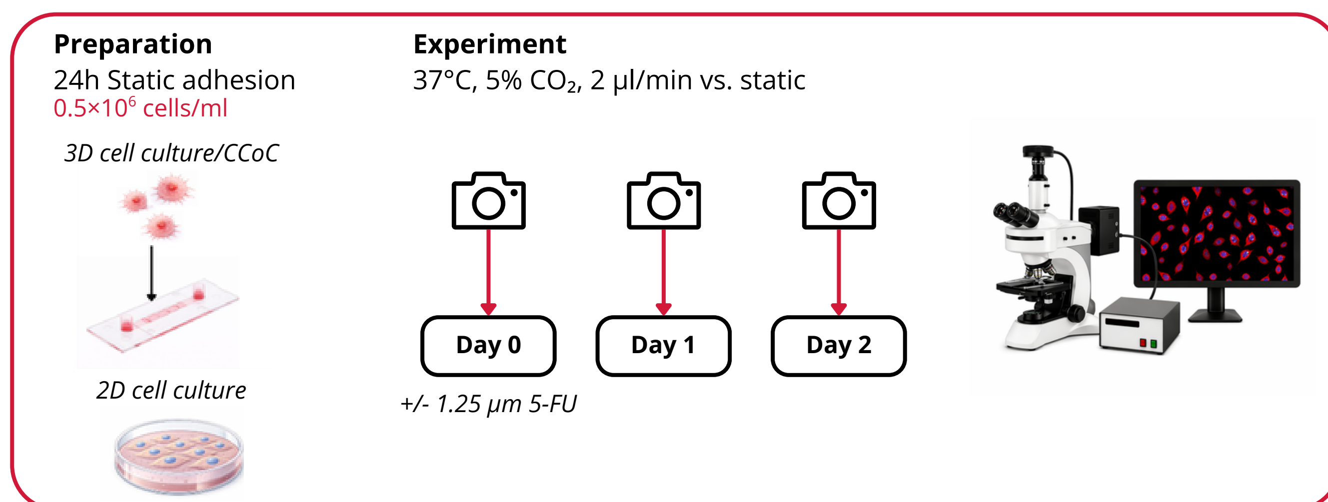


Figure 2: Schematic representation of the experiments, the chip used for the experiment is shown on the left [18].

2.2. Chip assembly

The chip from Ibidi (Cat. No. 87176) with the name µ-Slide I Luer 3D was used. IbidiTreat was chosen as the coating. This is a prefabricated coating of the chips, which makes the surface hydrophilic and adhesive for almost all cell types [19]. The LoVo cancer cells were seeded into the every chamber and allowed to adhere for 24 hours. The cells were seeded at a concentration of 0.5×10^6 cells/ml. One well of the chip has a volume of 16 µl. This means that 8,000 cells were seeded per well at a concentration of 0.5×10^6 cells/ml. According to the manufacturer Ibidi, the chip was closed with a plastic film shortly before connection. The chip was then connected to the VitroFlow device and perfusion was started with 2 µl/min at 5% CO_2 and 37°C.

2.3. Immunohistochemical methods

2.3.1. PFA fixation

The cells were washed with DPBS and then incubated for 10 min in 4% PFA at room temperature. After removal of the PFA, the cells were washed again twice with DPBS. The fixed cells were either used immediately for immunohistological staining or stored at 4 °C in DPBS. For the Colorectal cancer-on-a-chip, all steps were conducted directly in the chip.

2.3.2. Immunohistochemical staining of cells

For fluorescence staining, the fixed cells were first permeabilised with 4% paraformaldehyde in DPBS for a total of 10 min and then blocked for 10 min with 5% (v/v) donkey serum in DPBS. The cells were then incubated for one hour with the respective primary antibody (RRM2 Rabbit IgG, CD44 Rat IgG2b) diluted in 5% (v/v) goat serum in DPBS, subsequently washed twice with DPBS and then incubated for one hour with the corresponding secondary antibody (Goat anti-Rabbit IgG) which was diluted in a DAPI solution (1 µg/ml) for nuclear staining. Finally, the cells were washed again three times with DPBS. The plates were then covered with mounting medium.

2.4. Controls

To ensure appropriate benchmarking of the experimental system, two types of controls were implemented. Conventional two-dimensional (2D) cell culture was used as a reference condition to enable direct comparison with the dynamic organ-on-a-chip (OoC) environment, allowing assessment of the impact of perfusion, shear stress, and three-dimensional growth conditions on cellular morphology, proliferation, and drug response. In addition, untreated conditions served as negative controls in both 2D and OoC systems. In these controls, cells were exposed to the ammonium hydroxide instead of 5-fluorouracil (5-FU), ensuring that observed effects could be attributed specifically to the drug rather than to experimental handling or solvent-related influences. Together, these conditions enabled a systematic evaluation of both culture format - dependent and drug-specific effects.

2.5. Data analysis

Image analysis for the determination of cell circularity was performed using ImageJ (version v1.53t. Cell outlines were manually traced using the “Freehand Selection” tool. Based on these regions of interest, the perimeter (px) and area (px^2) of each cell were calculated. Circularity was then determined using the standard formula

$$4\pi \cdot \frac{area}{perimeter^2}$$

yielding values between 0 and 1, where a value of 1 corresponds to a perfect circle and values approaching 0 indicate increasingly elongated or irregular shapes.

3.Results

3.1. 2D cancer cell culture

LoVo cancer cells were cultured under conventional two-dimensional (2D) conditions to primarily assess the impact of a physiologically relevant, dynamic microenvironment - as further recapitulated in organ-on-a-chip-based systems - on cellular phenotype, and to enable direct comparison with cells cultured in static 2D conditions; drug response to 5-fluorouracil (5-FU) was also evaluated. The colorectal cancer cell line used for these experiments was LoVo, an epithelial cell line originally isolated in 1971 from a metastatic tumour node in the left supraclavicular region of a 56-year-old Caucasian male patient diagnosed with adenocarcinoma of the colon.

$4\pi \cdot$

LoVo cells are characterised by mutations in the adenomatous polyposis coli (APC) gene, Kirsten rat sarcoma viral oncogene homolog (KRAS), and tumour protein p53 (TP53), and are widely used for anti-cancer drug screening [20]. Cells were seeded at a concentration of 0.5×10^6 cells/ml in 6-well and 24-well plates. One day after seeding, 5-FU was added at a concentration of 1.25 µM (see Figure 2 for steps). Figure 3 shows the images on day 0. Comparison of treated and untreated cells revealed clear differences. Cells exposed to 5-FU showed a markedly reduced cell density and, consequently, lower proliferation compared to control cells (see Figure 4 E, F compared to G, H; and Figure 4 M, N compared to O, P). This effect was particularly evident when comparing cell density on day 0 with that observed on day 2 (see Figure 3 A, E compared to Figure 4 G, H & O, P).

3.2. Colorectal cancer-on-a-chip (CCoC)

As described above, the LoVo cell type was used for each colorectal cancer-on-a-chip (CCoC) model. The experiments started with the preparation of the chip by seeding LoVo cancer cells at a concentration of 0.5×10^6 cells/ml, corresponding to 8,000 cells per chip chamber, in RPMI 1640 medium supplemented with 10% FCS and 1% penicillin/streptomycin. After seeding, the cells were cultured on the chip under static conditions for 24 hours in a humidified incubator to allow proper cell adhesion prior to perfusion. Following the adhesion phase, the chip was connected to the VitroFlow organ-on-a-chip device, and perfusion was initiated under physiological conditions (37 °C, 5% CO_2) at a flow rate of 2 µl/min. Cells were cultured under continuous perfusion for one day to allow adaptation to the dynamic microenvironment. After one day of perfusion, drug treatment was initiated by adding 5-fluorouracil (5-FU) at a concentration of 1.25 µM to the circulating medium. In control conditions, ammonium hydroxide was added instead of 5-FU. Drug exposure was maintained under continuous perfusion for a total of 48 hours. At the end of the experiment, the cells were fixed directly on the chip using 4% paraformaldehyde (PFA) for subsequent immunofluorescence staining and analysis. Figure 3 provides images of the cells at the start of perfusion and on day 0 (see Figure 2 for steps).

Figure 4 shows representative microscopic images of LoVo cells cultured in the colorectal cancer-on-a-chip system at different time points throughout the experiment (day 1 - 2).

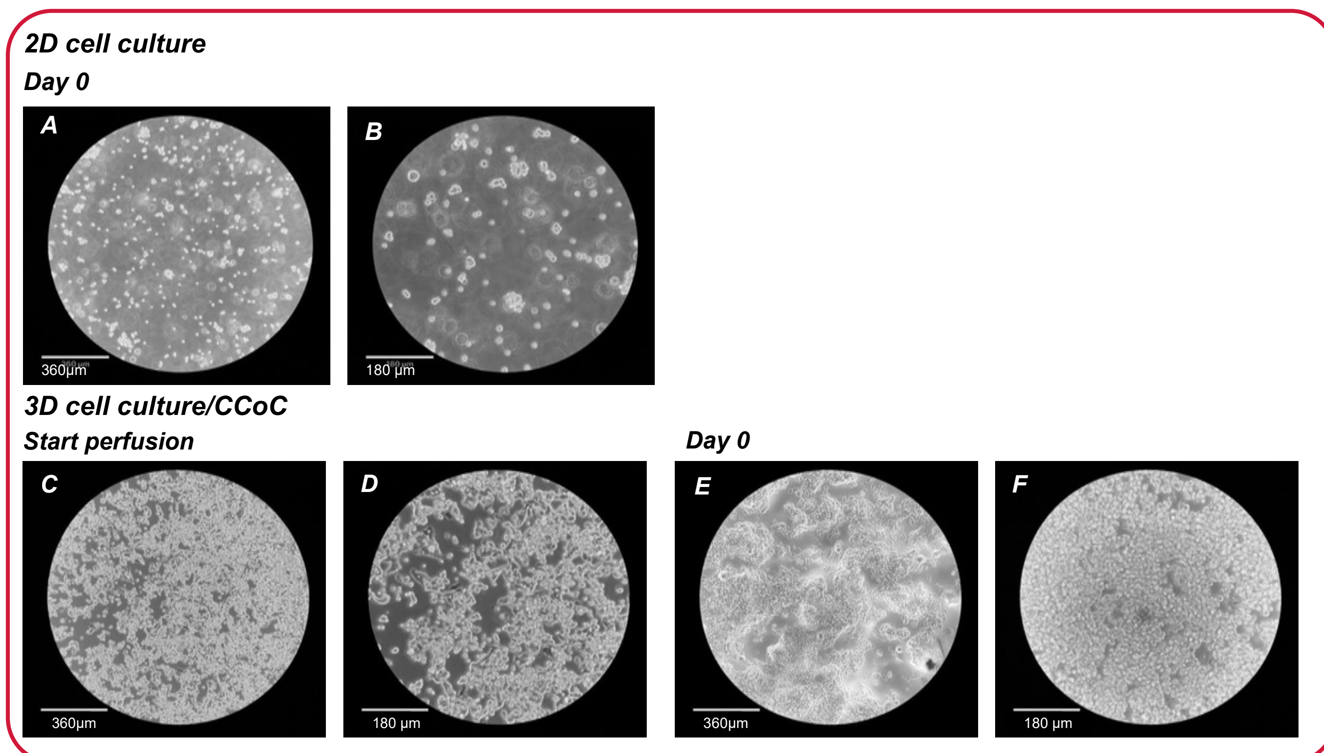


Figure 3: Representative microscopic images of LoVo cells cultured under static 2D conditions and in the colorectal cancer-on-a-chip (CCoC) model. **(A, B)** 2D cell culture at day 0. **(C, D)** CCoC at the start of perfusion. **(E, F)** CCoC at day 0 after initiation of perfusion. Scale bars: **(A, C, E)** 360 µm; **(B, D, F)** 180 µm.

Firstly, a fundamental difference in cellular morphology was observed between LoVo cells cultured in the colorectal cancer-on-a-chip (CCoC) model and those maintained under conventional two-dimensional (2D) conditions, independent of control or drug-treated status. While LoVo cells cultured in static 2D conditions exhibited an elongated and flattened morphology, cells cultured in the CCoC adopted a rounder, more three-dimensional shape. This morphological difference was evident in both control and 5-FU-treated conditions (see Figure 4 I - P). The difference in cell structure became particularly apparent when comparing cells at the start of perfusion (Figure 3 A–B) with images acquired at later time points (Figure 4 M - P). Notably, a clear morphological divergence was already detectable after 24 hours of perfusion (Figure 3, E, F), indicating a rapid cellular response to the dynamic micro-environment within the chip.

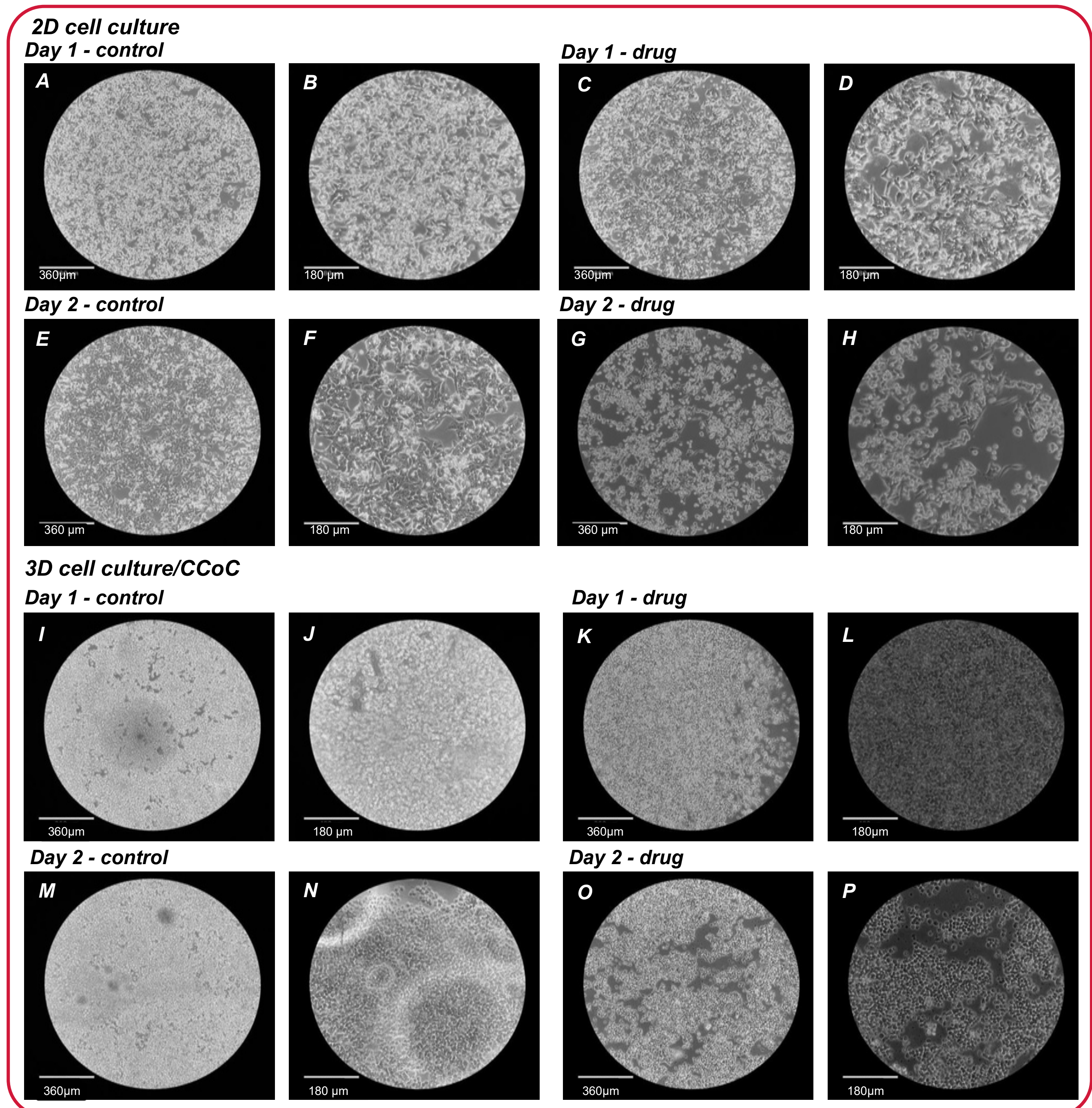


Figure 4: Representative microscopic images of LoVo cells cultured under static 2D conditions and in the colorectal cancer-on-a-chip (CCoC) model with and without 5-FU treatment. **(A–H)** 2D cell culture; **(A, B)** day 1 control, **(C, D)** day 1 drug, **(E, F)** day 2 control, **(G, H)** day 2 drug; **(I - P)** 3D cell culture/CCoC; **(I, J)** day 1 control, **(K, L)** day 1 drug, **(M, N)** day 2 control, **(O, P)** day 2 drug; Scale bars: **(A, C, E, G, I, K, M, O)** 360 µm; **(B, D, F, H, J, L, N, P)** 180 µm.

To quantitatively assess these morphological changes, cell circularity was analysed using ImageJ software. Circularity values of LoVo cells cultured in the CCoC were compared with those obtained from conventional 2D culture as well as with reference models. For this purpose, microscopic images of LoVo cells in 2D culture and cells cultured in the CCoC on day 2 of drug treatment were analysed. As reference systems, a published colon-on-a-chip model reported by Sontheimer-Phelps et al. (2020) [21] was included, in which human colon epithelium isolated from colon resections or endoscopic biopsy specimens was used. In addition, circularity values were determined from histological sections of human colorectal carcinoma reported by Zhou et al. (2022) [22]. According to the authors, these sections predominantly contained cancer cells, although no further distinction between cell types was performed and only cell roundness was analysed. In all cases, images from the respective control conditions were used for comparison (see Figure 4 A–D). The analysis revealed an increase in cell roundness of approximately 25%, from an average circularity of Ø ≈ 0.396 in 2D culture (n = 25) to Ø ≈ 0.728 in the CCoC model (n = 25). Comparable circularity values were obtained for cells from histological sections of human colorectal carcinoma (Ø ≈ 0.798, n = 25) and for the colon-on-a-chip model described by Sontheimer-Phelps et al. (Ø ≈ 0.793, n = 25) (see Figure 5 C and E). Statistical analysis demonstrated a highly significant difference in cell circularity between LoVo cells cultured in the CCoC and those maintained under 2D conditions ($p < 0.001$; see Figure 5 B and E), indicating favourable cultivation conditions within the chip system. In contrast, no significant difference in circularity was detected between the CCoC model and either the reference colon-on-a-chip model or the histological data from human colorectal carcinoma tissue (see Figure 5 D and E).

In addition to analysing cell behaviour under dynamic culture conditions, the effects of 5-fluorouracil (5-FU) on LoVo cells cultured in the colorectal cancer-on-a-chip (CCoC) model were investigated (see Figure 4). On day 1 of drug exposure, no pronounced differences were observed between control samples (Figure 4 E–F) and 5-FU-treated cells (Figure 4 G–H).

To further assess drug efficacy and to characterise the cellular phenotype at the molecular level, immunohistochemical staining was performed following completion of the experiment. RRM2 was used as a marker for drug efficiency, while CD44 was analysed as a marker associated with functional three-dimensional cell culture systems [25].

Figure 6 shows representative immuno-fluorescence images of LoVo cells cultured in the colorectal cancer-on-a-chip model under control and 5-FU-treated conditions on day 2. Double staining with anti-CD44 and anti-RRM2 antibodies was performed to simultaneously evaluate tissue-like phenotype and drug response. A comparison of the RRM2 expression of the two conditions shows a clear difference. The cells treated with 5-FU show a stronger immunofluorescence signal (see Figure 6 ; D – F) than the cells in the control condition (see Figure 6; A – C). In order to also compare the CD44 expression of the cells in the chip with that of the cells in 2D cell culture, the LoVo cells described in section 4.2 were also stained with anti-CD44 and anti-RRM2 antibodies on day 2 of drug treatment. However, the antibodies were used individually.

The figure 7 shows the results of the staining of the LoVo 2D cell culture of the control condition (Figure 7, A - F) and the cells treated with 5-FU (Figure 7, G - L). On the one hand, the control 2D culture (Figure7, A - F) and the cells in the control chip model (see Figure 6; A - C) show hardly any fluorescence signal of the RRM2 antibody compared to the drug condition (see Figure 6, F; Figure 7, G - L). A comparison of the strength of the CD44 fluorescence signal in the CCoC model with the signal in the 2D cell culture (Figure 7 E, K) shows a clear difference in fluorescence intensity. In the CCoC model, a strong fluorescence signal could be detected in both control and drug conditions (see Figure 6), whereas the CD44 signal in the 2D cell culture was visibly weaker.

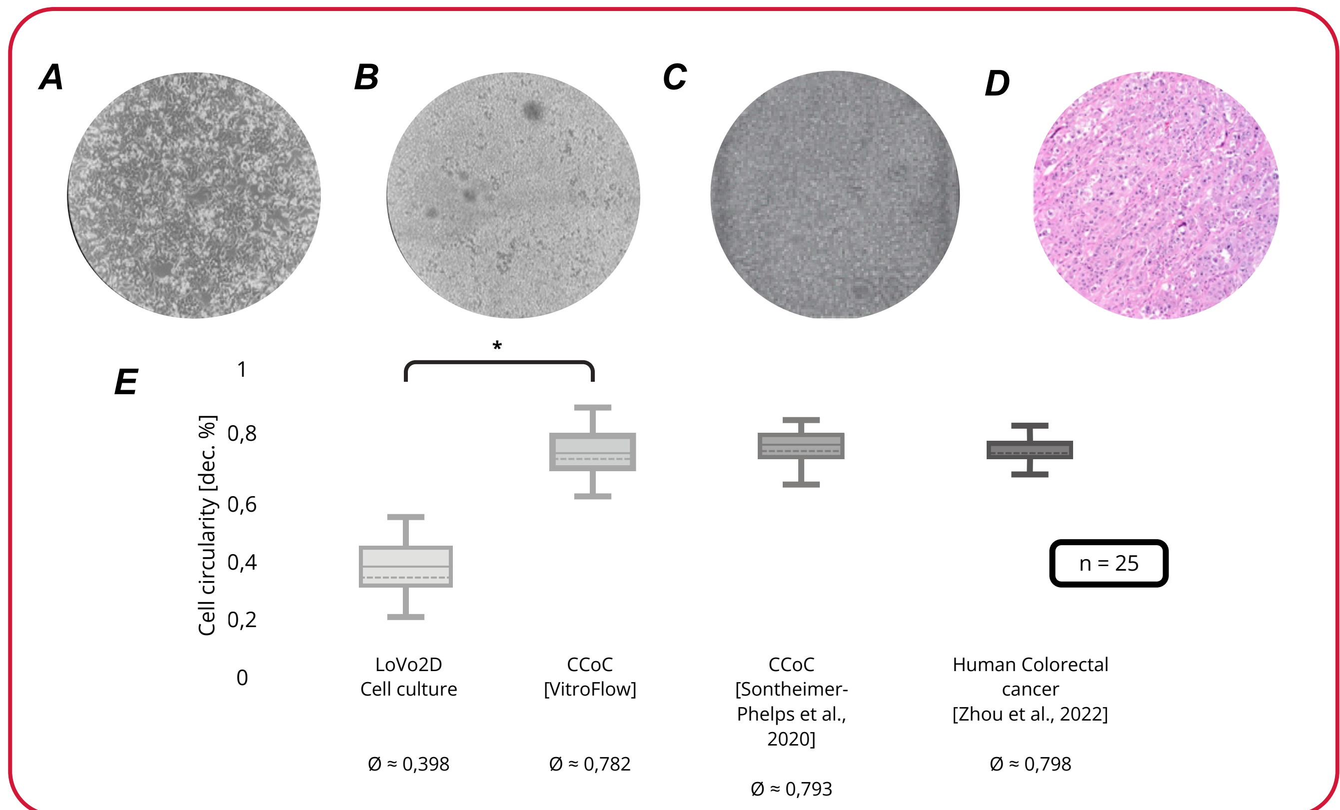


Figure 5: Evaluation of the cell circularity of the colorectal-Cancer-on-a-chip (CCoC) model, comparison with LoVo 2D cell culture, the Sontheimer-Phelps et al. cancer-on-a-chip model (2020) [23] and a histological section of a colorectal cancer by Zhou et al. (2022), **A** LoVo 2D cell culture **B** Colorectal-Cancer-on-a-chip (CCoC) **C** Colon-on-a-Chip model [A. Sontheimer-Phelps, Ingber et.al 2020] [24] **D** Histological cut of a human colorectal cancer [P. Zhou et.al 2022] **E** Boxplot of cell circularity of LoVo 2D cell culture, different C/CoC modells and human colorectal cancer

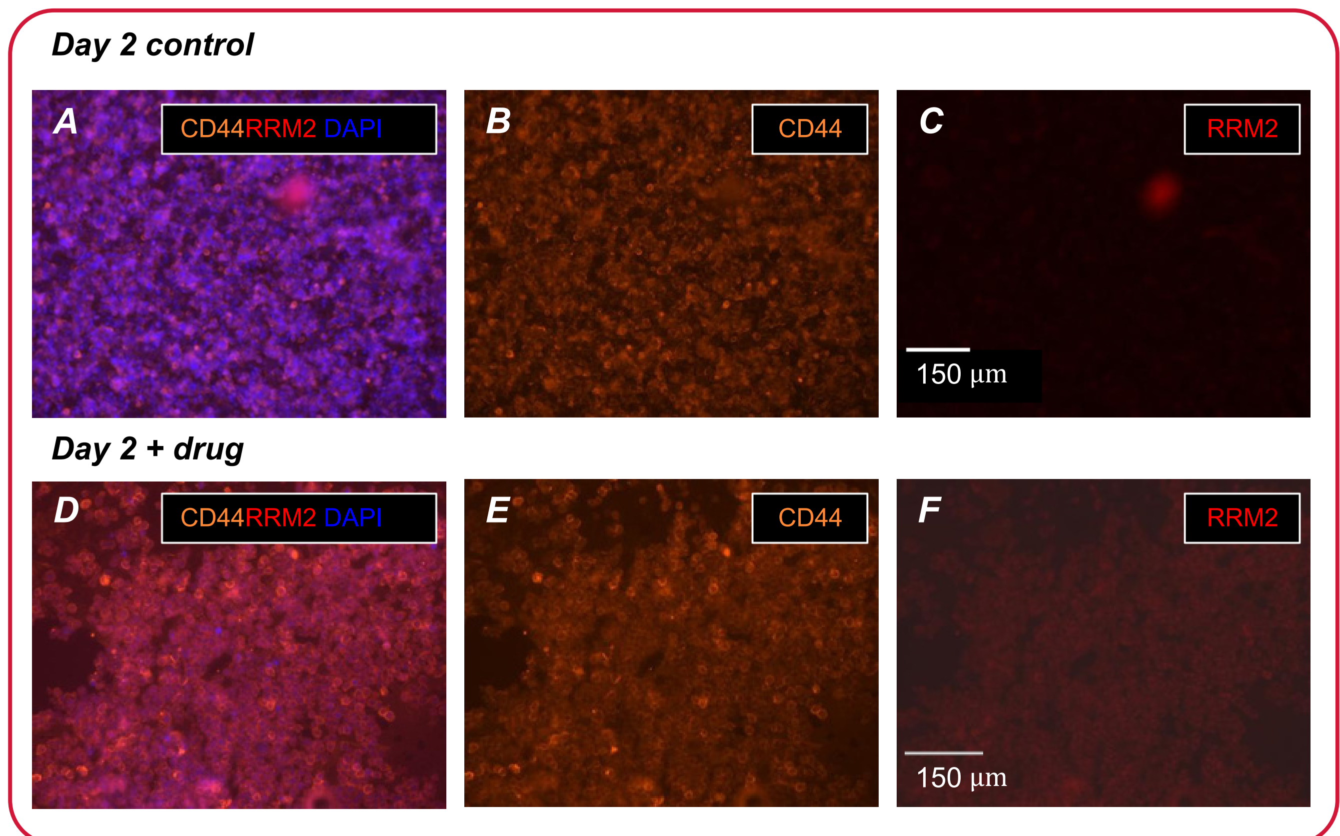
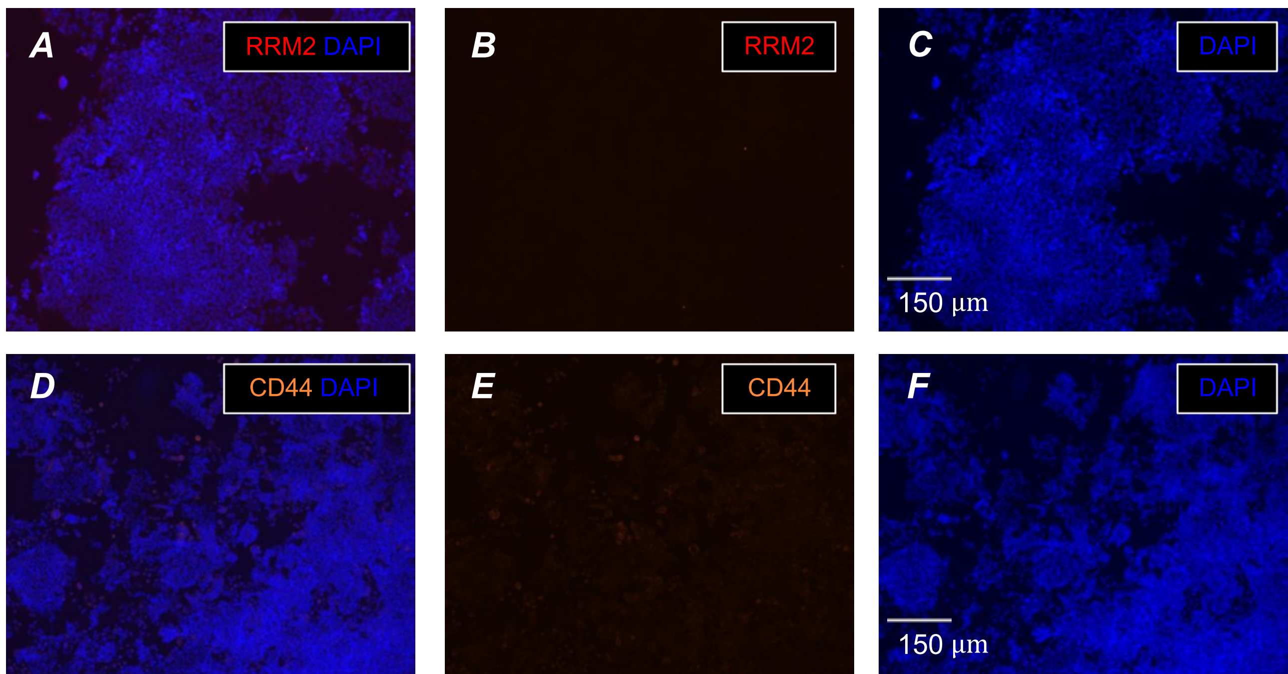


Figure 6: Immunohistochemical staining of the mono-colorectal-cancer-on-a-chip model. **(A–C)** Day 2 control; **(D–F)** Day 2 + drug. CD44 (orange), RRM2 (red), and nuclei (DAPI, blue). Scale bar = 150 µm.

Day 2 – control, 2D cell culture



Day 2 + drug, 2D cell culture

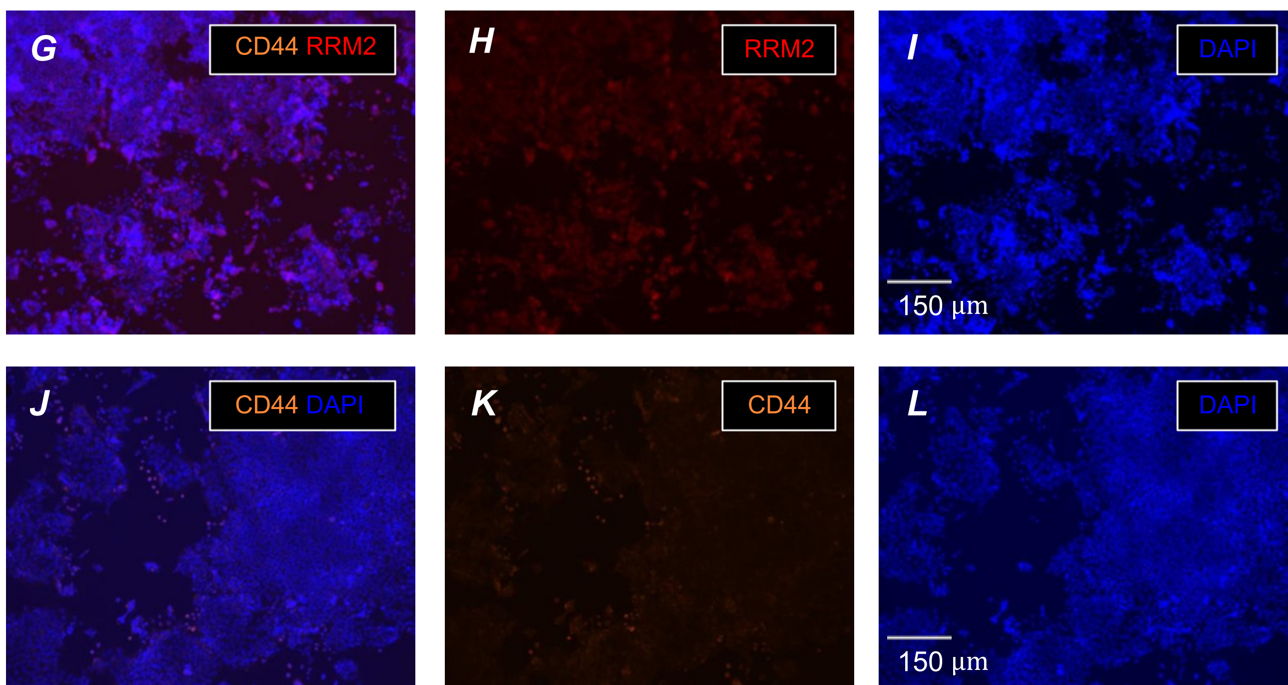


Figure 7: Immunohistochemical staining of the LoVo cells in 2D cell culture, **(A - F)** day 2 control, **(G - L)** day 2 +drug; scale bar = 150 µm; blue = DAPI, orange = CD44, red = RRM2

4.Discussion

The colorectal cancer-on-a-chip model established in this study successfully captures selected physical aspects such as perfusion and shear stress and provides a more dynamic culture environment. The environment is actively regulated by the VitroFlow system, enabling a stable and reproducible dynamic culture that more closely reflects in vivo conditions than conventional static models.
Compared to standard two-dimensional (2D) culture, cancer cells cultured in the chip exhibited pronounced differences in morphology, proliferation behaviour, and molecular expression [26]. The increased cell circularity observed under dynamic conditions indicates a shift towards a more tissue-like architecture [27]. In contrast to 2D cultures, where cells adopt a flattened morphology with pronounced pseudopodia, the microfluidic environment promotes a more compact and physiologically relevant organisation [28]. Importantly, the observed morphology does not reflect fully rounded cells, which would be indicative of non-physiological conditions such as cellular stress or loss of viability. Instead, the intermediate increase in circularity suggests intact cell–cell interactions and structural organisation within the tissue-like environment.
These morphological findings are supported by molecular data. The increased expression of CD44 in the chip model compared to 2D culture indicates enhanced cell–cell and cell–matrix interactions, which are characteristic of three-dimensional tissue organisation [29]. At the same time, the upregulation of RRM2 in response to 5-fluorouracil (5-FU) treatment confirms the activation of drug-induced molecular pathways associated with DNA synthesis and repair [30]. Together, these results demonstrate that the model not only reproduces structural features of tumour tissue but also captures functionally relevant biological responses.
With regard to drug response, 5-FU treatment led to a reduction in cell proliferation in both 2D and chip-based cultures. However, the delayed response observed in the colorectal cancer-on-a-chip model may indicate altered drug response dynamics, which are commonly associated with more physiologically relevant tumour architectures. This highlights a key advantage of organ-on-a-chip systems: they can reveal drug effects and resistance patterns that remain undetected in conventional 2D assays, thereby improving the predictive value of preclinical testing [31].
Overall, the mono-colorectal cancer-on-a-chip platform, in combination with the VitroFlow control system, demonstrates high reproducibility, precise environmental control, and strong physiological relevance. This makes it a powerful tool for investigating cancer biology and drug responses under human-relevant conditions.
As a next step, the integration of additional components of the tumour microenvironment, such as immune cells, as well as benchmarking against standard incubation systems, will further enhance the biological relevance and translational potential of the model.

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