Chapter 7

Integrated cell culture platform for real-time monitoring of adherent cell cultures

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Abstract

The concept of automation and integration of experiments in order to make them more efficient is well recognized in pharmaceutical industry, though this can be a costly undertaking in case if non-standard procedures are involved. While in research laboratories this laboratory equipment automation concept rarely appears. Therefore, we decided to firstly investigate a possibility of contribution to laboratory automation idea and afterwards we developed the Integrated Cell Culture Platform, where the concept of laboratory equipment miniaturization and automation in biomedical research settings is brought to the reality, and as a result increase experimental capacity of the research laboratories.

We designed, engineered and tested an Integrated Cell Culture Platform for microfluidic devices, which proves to make experiments with microfluidic cell culture chips more robust, reproducible and user-friendly. The created tool can help researchers, interested in biomedical experiments, in stepping closer to in vivo conditions by application the naturally occurring physical cues in the organism (in our case it was a shear stress). Additionally, by application of developed platform it is possible to increase the experimental capacity of regular research laboratory, by not occupying excessive number of lab equipment and automation of experiments.

Due to the design and capabilities included in the Integrated Cell Culture Platform can be transformed into a commercial product, which can be adapted by every biomedical lab interested in carrying microfluidic experiments on a daily basis. Presented equipment does not require long training and everybody familiar with cell culture research can easily adapt this device in their research. Due to dedicated software operating the ICCP, the setup is programmable and easy to modify on demand, depending on the carried experiment.

The integrated cell culture platform was applied for combined microfluidic-nanophotonic devices to monitor cellular micromotion in confluent endothelial cell layers under a flow. This report shows capabilities of miniaturized platform for cell cultures and presents initial results from experiment where cellular micromotion was monitored under static and flow conditions.

Keywords: Cell cultures, Micromotion, Integrated Cell Cultivation Platform (ICCP), Human Umbilical Vein Endothelial Cells (HUVEC)
7.1. Introduction

Currently, automation of most types of laboratory settings is limited and often means nothing more than application of pipetting robots for liquid dispensing into well-plates with reagents. However, this process still requires a researcher’s presence and manual preparation of the solutions used. Especially in cell culture experiments, automation of media refreshment and sample collection steps would be a significant help for scientists, as it would reduce human errors in pipetting and manpower necessary to perform the experiment.

Microfluidics has been applied to the miniaturization, integration, and automation of laboratory equipment in chemistry, biology, medicine, and physics since its introduction [1], [2]. The field has greatly contributed to a large number of discoveries in the biomedical in vitro research in the past decade, due to the delivery of new tools for carrying out experiments in laboratory settings and beyond [3]–[6]. However, most of the effort in the development of new microfluidic devices for biomedical research is dedicated to adaptation of already developed assays for DNA, RNA, and proteins to the microfluidic format [7]–[10]. The application of microfluidic devices in cell cultures is rapidly developing, and it has been shown that microfluidic setups can provide an improved resemblance of physiological conditions for cultured cells compared to well plates [11], [12], [13]. In addition, advances have been made in terms of engineering in vitro cell culture devices to achieve physiological status of cultivated cells by geometry optimization of the device or implementation of 3D scaffoldings for cell growth [14], [15].

This is made possible by the application of flow and/or optimization of the geometries that cells encounter in confined, sub-microliter channels and chambers. Application of flow is recognized as an approach to engineering more physiologically relevant in vitro cell and tissue models [9], [13], [16]–[18]. Additionally, technologically advanced monitoring of biological processes is possible with microfluidic systems using integrated electrodes, optical detectors, or miniaturized on-line assays [17], [19], [20]. Therefore, for many researchers in the biomedical field, it is becoming crucial to have access to laboratory equipment which is compatible with their research microfluidic devices. This goal is often difficult to achieve without development of dedicated experimental setups capable of carrying out a specific experiment. The reasons for this are the small dimensions and fragility of the microfluidic devices, which make them difficult to operate without a mechanical aid (e.g. clamping system, holder). However, the required peripheral instrumentation for experimentation (e.g. pumps, microscopes, other analytical components) is often bulky and cannot be placed in the incubator with the device. As a result, the workbench can be crowded with wires and tubes leading to and from the incubator, which makes it difficult to operate the experimental setup and can lead to mistakes in an experimental procedure or device breakage. Moreover, microfluidic cell cultures generally need to be intermittently removed from the incubator for visual inspection, which creates an additional opportunity for introducing error to a measurement. In this situation, a potential end-user might be discouraged from the implementation of complex microfluidic devices in his/her research, because of a number of switches, valves, chambers, and, more importantly, pieces of standard lab equipment necessary to perform a single experiment. In such a situation, integration of different experimental functions into a single platform would be a great help in a researcher’s daily routine. We felt important to develop a research instrument for users of microfluidic devices for “plug-and-play” cell culture experiments. Additionally, the developed setup would fill a gap between microfluidic research, which is performed on the small scale, and high-throughput cell analysis using microfluidics [21].

We have observed a growing interest in miniaturization and automation of cell cultures with simultaneous visual inspection and cell analysis, but at the same time we could not find a
commercially available generic setup suitable for supporting different formats of microfluidic chips. The closest systems to our interests are incubated microscopes, but usually they are designed according to end-users specifications and consist of a microscope possibly with motorized stage, thermostat, and CO₂ controller. To the best of our knowledge, there are no setups integrating pumps and sensors, which can deliver extra information about cultured cells besides the microscope images. On the other hand, microfluidic chips become more functional by integrating multiple assays on one device with cultured cells, but to perform a readout it is necessary to use different laboratory equipment, not integrated into the microfluidic chip [16], [18], [22].

The features we identified as worth implementing in our generic platform are also summarized in the review by Mark et al [18]. It is noteworthy, that many of the recent developments in cell culture platforms emphasize the benefits of integrating all components and functions into a chip. Essentially, this involves integrating sensors and flow control normally relegated to peripheral modules. Ultimately, however, there remains a basic requirement to interface even highly integrated chips with standard-sized readout and electronic equipment plus cell culture control (temperature and gas composition). Therefore, we decided to prepare a generic Integrated Cell Cultivation Platform (ICCP) for microfluidic perfusion culture with broadly applicable features for cell culture control, thus for use with devices that have specialized readout function. The developed system allows control and maintenance of selected culture parameters including temperature, flow rate, and CO₂ concentration. Additionally, the ICCP is capable of simultaneous and automated cell culture microscope imaging without moving a device from an incubator to a microscope stage. Last but not least, the setup footprint is that of a standard microscope (approximately 50 x 50 x 50 cm), thus not lab-space consuming.

In order to demonstrate the platform capabilities, the ICCP was employed for monitoring human umbilical vein endothelial cell (HUVEC) micromotion with a combined microfluidic-biophotonic device. Cellular micromotion is a manifestation of cell membrane fluctuation caused by the constant rearrangement of the cytoskeleton [23]. Impaired micromotion might be an indicator of pathophysiological states like inflammation, which are observed in the onset of cardiovascular diseases (CVDs) [24]. Endothelial cell micromotion should be characterized under well-defined conditions in order to better understand how this parameter changes under pro-inflammatory conditions.

7.2. Materials and Methods

7.2.1. The design of the Integrated Cell Cultivation Platform

The ICCP prototype (Cellix Ltd., Ireland) shown in Figure 1 and Figure 2 was designed with the purpose of facilitating simultaneous cell culture and monitoring of cellular behavior in perfused microfluidic systems with minimal required manual operation and manipulation by a user. For full functionality, the ICCP requires a power socket (220 V), a desktop computer with USB port, and a gas supply to pressurize the system and induce medium flow (usually CO₂). The ICCP has dimensions 40 x 35 x 45 cm which correspond to the standard microscope size commonly used in cell culture laboratories. Thus, both the required peripheral power and gas supplies, as well as lab space for the ICCP, are available in every cell culture lab. This limits adaptation of the space for the new device to a minimum. A description of technical details is presented below.
Figure 1. (A) A cutaway view of the ICCP. (B) View of the built-in microscope for real-time cell monitoring. Additional images of the ICCP are in the ESI 1. Supplementary images of the ICCP.

Figure 2. (A) Working prototype of the ICCP platform including microscope, camera and pumping system without casing, and with the optical chip mounted onto the microscope stage. (B) Prototype of the ICCP casing.

**Microscope:** The stand-alone phase-contrast microscopy setup consists of an X-axis positioning stage, Z-axis controlling the objective focus, 20x microscope objective with phase contrast ring holder from Zeiss (Carl Zeiss AG, Germany), and LED illumination to achieve phase contrast observation of endothelial cells (Figure 1). For visualization purposes a Hamamatsu ORCA-Flash-OEM camera (Hamamatsu Photonics K.K., Japan) was installed in the system. The microscope stage has a magnetic
clamp with a footprint of 5 cm x 3 cm to firmly fix installed microfluidic devices to the stage. The ICCP thus can be used for cell culture experiments with most microfluidic devices fitting this versatile microscope stage. The ICCP microscope was designed and built by Cellix Ltd (Ireland).

![Figure 3](image)

**Figure 3.** (A) The ICCP on a lab bench ready for use. (B) A bottle used as a medium container in experiments with the ICCP. The red arrow indicates a gas supply (95% air, 5% CO\(_2\)), and the blue arrow indicates a medium supply connection to the cell culture. The valve (green arrow) allows addition of different chemicals to the medium during the time course of experiments.

**Pump:** A Unigo™ (Cellix Ltd., Ireland) pressure-driven pump was installed in the ICCP to continuously perfuse the channels of a microfluidic chip installed on the microscope stage. The pump works in a unidirectional fashion in a flow-rate range from 1 µL/min to 1 mL/min. The applied flow is controlled by a flow sensor (Cellix Ltd., Ireland) connected to the Unigo™ pump. The flow sensor allows monitoring of the flow rate and active PID (Proportional, Integral, Differential) feedback control of the current pump flow rate. The ICCP pumping bottle is pressurized by a carbon dioxide (CO\(_2\)) external supply. The required gas composition for the cell culture is achieved by pressurizing (1 Bar) a bottle with cell medium, which is afterwards delivered to the microfluidic device through connected tubing. It is important to note that the bottle containing medium is not warmed up; the cell medium is pre-warmed before contact with cell culture in the proximity of the microfluidic device in the area inside the casing (orange box) (Figure 3 A). Due to decreased gas solubility at 37°C compared to 20°C (Room Temperature, RT), an air bubble can appear in the system and be introduced to the microfluidic device, resulting in the destruction of the cell culture. Therefore, an air bubble trap was introduced into the tubing guiding cell medium to microfluidic chip. The air bubble trap is a Millex-GP syringe filter unit (0.22 µm) from Merck Millipore (USA) connected in an opposite direction than it would be when attached to a syringe for filtration. All the system components are connected together with Teflon tubing. The bottle for cell medium has a cap adapted for the system and contains three sealable inlets, namely gas connection, cell medium outlet and an additional connection if a compound has to be added to the medium (e. g. drug) (Figure 3). Connectors to the Cellix
multichannel devices were customized by Cellix Ltd. to ensure a leakage-proof tubing connection to the chip with a cell culture (Figure 4).

**Figure 4.** Examples of microfluidic connectors used for connection of the microfluidic chip to the ICCP. For introduction of media to multichannel Cellix cell culture devices tubing with stream splitters were used. Therefore, a simple stream of medium can be split over multiple inlets.

**Internal Control Unit:** A control Board (PCB) (Cellix Ltd., Ireland) was developed to operate X and Z motorized axes of microscope and Unigo pump. The control unit communicates with a desktop computer via a USB interface. The parameters controlled by the PCB are presented in Table 1.

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<th>Table 1. Specifications of motorized stage</th>
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<td>Minimal step (Repeatability)</td>
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**Temperature control unit:** An indium tin oxide glass heater (ITO glass) allows precise temperature control of the microfluidic chip with resolution of 0.1°C degree. The chip is covered from the top with this heater.

**Customized control software:** Software was specifically developed by Cellix for the ICCP and programmed in C++ programing language for Windows (MFC library was used for the programming of software). The developed software allows automatic positioning of the stage, time-lapse imaging of monitored cell culture, flow adjustment, and recording of the forward light scattered optical signal (required for presented in this work application). The latter function was incorporated specially for this work.

**7.2.2. Chip design**

The material used for making chips is an important factor in miniaturization and automation of cell culture experiments. Often, the material of choice for microfluidic cell cultures is poly(dimethylosiloxane) (PDMS) [7], [25], because of its biocompatibility, formability and transparency. However, this is not suitable for mass production, and, as a consequence for usage in automated processes. Therefore, most of the commercially available microfluidic devices are
fabricated in glass and polymers like poly(methyl methacrylate) (PMMA), polyethylene (PE) or its chemically modified version, cyclic olefin copolymer (COC) [9], [18]. The optical chips used in this work consist of fused silica with patterned waveguides (silicon nitride layers, Si$_3$N$_4$), which guide light to and from a measurement site. The light waveguiding layer is sandwiched between two layers of fused silica. The measurement sites were etched through the top silica and silicon nitride layers and form small recesses or cuvettes (nanocuvettes) with dimensions of 100 x 100 µm, such that a cell layer at the bottom of the recess is at the height of the waveguide (Figure 5A) (more detail in Chapter 5). An adapted Vena8 (Cellix Ltd., Ireland) fluidic chip consisting of four channels with dimensions of 28 x 0.8 x 0.12 mm made in cyclic olefin copolymer (COC) was sealed to the fused silica chip (Figure 5B and 5C). Before bonding of both chip parts with an adhesive tape (Cellix Ltd., Ireland), The alignment of the channels was etched to ensure, that nanocuvettes are positioned within the fluidic channel boundaries.

![Diagram](image)

**Figure 5.** (A) A schematic diagram of the nanocuvette (not to scale). The nanocuvette has been etched down about 100 nm below the waveguide layer to facilitate coupling of the light into the thin periphery of endothelial cells. Single-mode light (638 nm) is introduced into the cell layer from the left (waveguide in red). (B) The photo shows the nanocuvette chip with integrated VENA8 chip (Cellix Ltd); the black block is the Fiber Array Unit which facilitates light input and collection from the optical chip. (C) Expanded view of the optical chip with fluidic connectors.

### 7.2.3. Cell culture

#### 7.2.3.1. Sterilization of the optical chip used in cell culture experiment

Optical chips were sterilized with 70% ethanol (Sigma-Aldrich Co Ltd, UK) for 5 min., followed by three consequent rinses with sterile phosphate buffer saline (PBS) (Sigma-Aldrich Co Ltd, UK) to ensure complete removal of ethanol.

#### 7.2.3.2. Coating of optical chip with 1% gelatin

Porcine gelatin (1%) (Sigma-Aldrich; G9382) solution in sterile PBS (Sigma-Aldrich Co. Ltd, UK) was injected into the microchannels and incubated at room temperature (RT) for 45 min. The gelatin acts as a substitute for the extracellular matrix (ECM), which encourages HUVEC (or potentially other endothelial cell types) to adhere to the surface. Cross-linking of the gelatin was then performed
by injection of 0.5% glutaraldehyde (25% glutaraldehyde stock solution; Polysciences Europe GmbH, Eppelheim, Germany) solution in sterile PBS into the microchannels. The reaction was carried out at RT for 15 min. Coated microchannels were rinsed twice with sterile PBS with 10 min incubation at RT in between consecutive rinses. The last step in optical chip preparation was conditioning with endothelial cell medium (composition below) in the cell incubator (Thermo, model 3111, USA) for 1h before HUVEC seeding.

7.2.3.3. Culture conditions

Cells were cultured in cell medium (the composition of which is described below) in an incubator under 95% air and 5 % CO$_2$ at 37°C (Thermo, model 3111, USA) prior to seeding in the optical chip and installment on the ICCP.

7.2.3.4. Cell medium

Lonza EGM-2 MV medium (CC-3202) (Lonza Group Ltd., Switzerland) supplemented with BulletKit from Lonza (CC-3156 & CC-4147) (Lonza Group Ltd., Switzerland) was used for HUVEC culture.

7.2.3.5. Cells

Lonza HUVEC (CC-2519) (Lonza Group Ltd., Switzerland) were cultured for confluence in T25 flasks for at least 2 passages before being seeded in the microchannels of the optical chip. For experiments presented in this work, only HUVEC from passages between 2 and 5 were used. HUVEC were harvested from T25 bottles by prior trypsinization (0.05% trypsin solution in sterile PBS; 20 µL/cm$^2$ of trypsin solution), counted and resuspended in fresh cell medium at a concentration of 5000 cells/µL. After cell collection, HUVEC were kept on ice; before introduction to the optical chip, they were gently shaken in the Eppendorf tube to ensure even cell distribution in the cell suspension.

7.2.3.6. Cell seeding and culture in the optical chip with microfluidic interface

HUVEC were injected into conditioned and pre-warmed microchannels at a concentration of 5000 cells/µL in a volume of 30 µL per microchannel. After cell injection, chips were placed in the incubator for 1h to allow HUVEC to attach to the bottom of the microchannels. Unattached HUVEC were then removed by rinsing with fresh cell medium, and chips were placed in the incubator for another hour (1h). HUVEC cultured in the optical chip with integrated microchannels were seeded at confluency to facilitate flow application right after cell attachment to the chip surface. Approximately 2 hours after cell seeding the Unigo® pump of the ICCP (Cellix Ltd.) was connected to the chip, and perfusion with medium was commenced according to the described program (Medium perfusion program). After experiments, the optical chips were cleaned according to the cleaning protocol (Section 2.3.7 of this chapter). As a positive control, cell culture in standard 96-well-plates was carried out in parallel with the optical chip.

7.2.3.7. The cleaning protocol for the optical chip

After the culture period, microchannels were rinsed with a pre-warmed to 37°C 0.05% trypsin solution in sterile PBS. A volume of 20 µL/cm$^2$ of trypsin solution was used in order to clean the chip and reuse it for subsequent experiments. The trypsinization process was carried out for a period of one hour (1h) at 37°C and followed by three consequent rinses with PBS with 5 min incubation at 37°C in between rinses. The washed optical chips were dried and stored at room temperature in an insulated box in order to prevent accidental fiber detachment from the fused silica substrate.
7.2.4. Demonstration of ICCP with the optical sensor

A preliminary demonstration of the ICCP capabilities was performed with the optical chip which employs a new approach for real-time, non-invasive, label-free monitoring of cellular micromotion in HUVEC cultures. Briefly, HUVEC are cultivated in a confluent layer on a microchannel surface containing a nanocuvette, as described above. Red light (638 nm) is directly coupled into the few cells in the nanocuvette from an integrated waveguide, and the forward-scattered portion of that light is recorded as a measure of cellular micromotion (Figure 5) (Method described in Chapter 5).

7.2.4.1. Medium perfusion program

The Unigo® pump of the ICCP (Cellix Ltd.) was programmed with dedicated software to perfuse the cell culture in the optical chip with flow resulting in a shear stress of 1 dyn/cm² for a period of time defined in the experiment. In the presented experiments, HUVEC were exposed to 1 dyn/cm² for a period of three hours (3h). The duration of the initial flow period was dictated by the status of HUVEC culture in the optical chip after cell seeding. Cells should be firmly attached to the surface before application of a shear stress of 5 dyn/cm², as they otherwise will be removed from the surface and flushed away from the optical chip. After the initial period of flow, the flow was increased and resulted with a shear stress of 5 dyn/cm² (flow rate: 76.8 µL/min) until the experiments were finished.

7.2.4.2. Optical sensing setup

The optical sensing setup was composed of a light source, detectors, and the optical fibers employed for measurements of micromotion in HUVEC culture. As a light source, the NovaPro Laser Diode (638 nm, continuous wavelength (CW), max. power output 75 mW) (RGB-Lasersystems GmbH, Germany) was used with an initial power output of 5 mW in the experiments presented here. An APC/UPC connective optical fiber was installed on the laser by connection to a light coupler, and light was guided to an FC/UPC fiber attached to the optical chip. The FC/UPC optic fibers were irreversibly attached to the optical chip. Light is coupled to the chip with an optical fiber with core diameter of Ø 4 µm, and then divided evenly on the chip to be guided to four independent nanocuvettes. Light from each cuvette is collected separately with an optical fiber with core diameter of Ø 50 µm. All fibers are aligned and fixed in a plastic block structure (Fiber Array Unit, FAU) to facilitate proper light coupling into the optical chip and light collection from the waveguide structures on the optical chip. The light-collecting fibers were connected to PM100USB power meters (400-1100 nm, working range: 1 nW - 20 mW, resolution: 100 pW) (Thorlabs, Germany) for signal acquisition. In the experiments presented, data were acquired for a period of 1h at a frequency of 1 Hz (1 measurement point per second). A detailed description of the experimental setup can be found in Chapter 5.

7.2.4.3. Forward Light Scattering measurement

Optical measurements of HUVEC micromotion in the optical chip mounted on the ICCP were performed for 3h and 24h cell culture under a shear stress of 1 dyn/cm² and 5 dyn/cm², respectively. Simultaneously with the optical measurement, the ICCP was programmed to acquire images at 2 min intervals over the one hour (1h) period of the light-scattering measurement.

7.2.4.5. Time-lapse imaging

Images of cultured HUVEC in the optical chip were automatically acquired with the ICCP at 1 or 2 minute intervals from predefined spots on the optical chip. Afterwards, series of images were stitched in Windows Movie Maker (Microsoft Inc. Seattle) and displayed as a time-lapse movie to show HUVEC micromotion under different flow conditions. Stitched images are displayed at a rate of 1 frame per second (1 fps).
7.3. Results and discussion

7.3.1. Monitoring of HUVEC culture in microfluidic channels under static and flow conditions with the ICCP

Initial experiments in which HUVEC micromotion was monitored with the ICCP were performed under static conditions. The ICCP was programmed to keep the temperature at 37°C, and no medium flow was applied for the hour after HUVEC injection into the microchannels in order to allow cell attachment to the chip surface. After completion of the cell seeding procedure, a shear stress of 1 dyn/cm² (flow rate: 15.36 µL/min) was set in the ICCP operating program. The aim of this action was to remove unattached HUVEC and supply fresh medium to the microchannels, and in consequence prevent attached cells from dying because of nutrient depletion. The administered shear stress of 1 dyn/cm² during this initial period does not affect HUVEC behavior, and so this condition can be considered as “quasi-static” for the cells [26]–[28].

Approximately three hours (3h) after cell seeding in the microfluidic chip, the first series of images was acquired for a period of one hour (1h) and presented as a time-lapse movie (movie 1, Figure 6). HUVEC micromotion in the movie can be described as vigorous and stochastic [29]. Afterwards, the applied shear stress was adjusted to 5 dyn/cm², and cells were cultured for 24h to induce cellular response to the shear stress. To visualize induced changes, the ICCP was set to acquire another set of images of HUVEC cultured in microchannels (movie 2, Figure 6). Our observation indicated decreased HUVEC motion for cells cultured under this higher-flow condition in comparison with cells cultured under static conditions (this observation is based on the movies provided with this manuscript). This difference is caused by the cells adapting an optimal position according to the experienced flow, in contrast to the case of no flow/very low flow, when HUVEC can freely adjust their shape in a continuous fashion. Additionally, we noticed gap formation in static HUVEC culture (Figure 5), which is an undesirable effect in endothelial cell cultures (endothelial cells naturally form tight barriers in order to control molecule trafficking [30]). No gap formation was noted at a shear stress of 5 dyn/cm². Observation of gap formation has been regularly noted in previous experiments with HUVEC cultured in static conditions (data not shown). It appears that cells not exposed to a shear stress move a disorganized (stochastic) fashion in relation to neighboring cells, which leads to disruption of cell-cell connections and temporary gap formation. HUVEC exposed to shear stress show reduced intensity of micromotion and appear to behave in a more organized fashion, thus preventing gap formation.
Figure 6. ICCP images of the same HUVEC culture in a nanocuvette under static conditions (3h after cell seeding) and flow (5 dyn/cm², red arrow indicates flow direction in images) (15h after cell seeding) acquired over 45 min. HUVEC in the static culture appeared prone to cellular gap formation (red rim), whereas the HUVEC layer under flow remained intact. These images originate from movie 1 and movie 2, respectively. The nanocuvette is outlined in white in all images and has dimensions of 100 x 100 µm.

7.3.2. Monitoring of HUVEC micromotion under a flow in the microfluidic optical chip with the ICCP

After confirmation of the cell culture parameters, mechanical stability of microscope stage in the ICCP, we performed preliminary monitoring of HUVEC micromotion with the forward-light-scattering method (described in detail in Chapter 5) facilitated by the optical chip with the microfluidic interface. The experiment was carried out according to protocols presented in the Material and Methods section and optical measurements were made three hours (3h) and eighteen hours (18h) after cell seeding. After the first optical measurement, performed under “quasi-static” conditions (1 dyn/cm²), flow was adjusted to 5 dyn/cm² in order to stimulate the HUVEC to find an optimal position in the context of the cell culture.

In Figure 7, optical measurements obtained with the optical chip mounted on the ICCP are presented as scattered-light-intensity traces of the same HUVEC culture recorded under static and flow conditions. HUVEC cultured under a shear stress of 5 dyn/cm² showed decreased micromotion compared to static HUVEC cultures (Figure 7). Reduced cell micromotion is indicated as a trace with damped fluctuations in comparison to the trace for cells under static conditions (Figure 7B). This is a result of individual HUVEC optimal alignment in the current culture condition. In parallel to light-scattering experiments, images of HUVEC were acquired (every 2 min.) in order to compare the light scattering patterns with cell images. Unfortunately, during the first optical measurement the ICCP camera lost autofocus and in consequence no good-quality images were obtained. For the second measurement, this malfunction was fixed and we obtained a series of images which were stitched together in order to create a time-lapse movie (movie 3, Figure 8). In movie 3, we can observe a cell migrating from the nanocuvette to the higher region of the chip. This observation shows that HUVEC growing inside and outside of the nanocuvette remain in close contact and communicate with each other, thus forward light scattering data of a few measured cells in the cuvette does reveal the status of the whole HUVEC culture. By simultaneous measurement of a few cells status instead of the whole
culture, we can obtain more detailed information about the physiological status of sampled cells [7]. At the same time, this method has the advantage of data acquisition in the context of a cell culture over e.g. flow cytometry, where individual cells have to be harvested and labelled for single cell analysis in a flow cytometer [31].

Figure 7. (A) The plot presents power readouts acquired for HUVEC cultured under static conditions three hours (3h) after cell seeding into the microfluidic channels. These data indicate that HUVEC are constantly undergoing cytoskeletal rearrangement under static conditions, as also observed in the greater fluctuation of recorded power for this condition. (B) HUVEC under shear stress retain an optimal morphology for the applied flow. The blue arrow indicates spikes in registered power outputs caused by interruption of the experiment in order to refill the bottle with cell medium.

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Figure 8. The ICCP images of HUVEC morphology in the nanocuvette under conditions of flow (5 dyn/cm²) applied for a period of fifteen hours (15h). The red arrow indicates the flow direction. The blue arrow points at the light injection spot. These images originate from movie 3. The nanocuvette is outlined in white in all images and has dimensions of 100 x 100 µm.

7.4. Conclusions

The ICCP was successfully applied in our research laboratory in experiments with an optical chip, facilitating automation of experimental procedures in which human intervention was reduced to a minimum, as a result. The chances of introduction of infection to a device or accidental chip breakage were thus reduced. This is an important aspect of the work with the optical chip, as it contains fragile elements, which can be accidentally removed from the chip. By application of the ICCP, the optical chip manipulation was limited to only absolutely necessary steps like chip coating, cell seeding, flow attachment and cleaning steps, whereas the rest of actions was taken care of automatically by the platform.

Using the ICCP for our experiments, it became possible to obtain valuable insight into cellular motion behavior by combining visual inspection with a microscope and forward light scattering measurement. Without the ICCP, which provides camera with autofocus, the whole procedure would
have been tedious and difficult to perform, because of the manual operation of the optical chip and microscope at the same time. Additionally, manual image acquisition demands constant focusing, time control, and delicate operation with the microscope, because an accidental shift of the chip would result in the device shifting out of the set position and a resulting perturbation in the time-lapse movie production.

Although the ICCP has been a great help in carrying out microfluidic experiments, it is not perfect yet. Currently, the ICCP cannot operate in the Y-axis; we identify this as a first spot for improvement of our device. This problem can be easily resolved by the addition of this function to the hardware and a software upgrade. It would also be beneficial to enlarge the current microscope stage to allow a greater level of freedom in adaptation of microfluidic devices. The bigger microscope stage would result in the need to enlarge the casing, but after the described adjustments we would obtain a generic cell culture platform broadly applicable in microfluidic research.

This work presented a successful demonstration of the ICCP together with microfluidic and photonic insight into cellular micromotion with flow and no flow condition. The ICCP proved to be a useful tool with possibility of broad application in cell culture research by both experienced microfluidic experimenters and inexperienced researchers starting their microfluidic experimenting lines.

7.5. Acknowledgements

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7.6. References


PART IV
3D PRINTING