RESEARCH REPORT

Transparent polymeric cell culture chip with integrated temperature control and uniform media perfusion

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Modern microfabrication and microfluidic technologies offer new opportunities in the design and fabrication of miniaturized cell culture systems for online monitoring of living cells. We used laser micromachining and thermal bonding to fabricate an optically transparent, low-cost polymeric chip for long-term online cell culture observation under controlled conditions. The chip incorporated a microfluidic flow equalization system, assuring uniform perfusion of the cell culture media throughout the cell culture chamber. The integrated indium-tin-oxide heater and miniature temperature probe linked to an electronic feedback system created steady and spatially uniform thermal conditions with minimal interference to the optical transparency of the chip. The fluidic and thermal performance of the chip was verified by finite element modeling and by operation tests under fluctuating ambient temperature conditions. HeLa cells were cultured for up to 2 weeks within the cell culture chip and monitored using a time-lapse video recording microscopy setup. Cell attachment and spreading was observed during the first 10–20 h (lag phase). After approximately 20 h, cell growth gained exponential character with an estimated doubling time of about 32 h, which is identical to the observed doubling time of cells grown in standard cell culture flasks in a CO₂ incubator.

INTRODUCTION

Cell culturing is a core method in biological science and clinical research as well as in many biotechnological and biomedical engineering areas. Typically, mammalian cells are grown in a nutrient buffer in plastic culture flasks or multiwell plates, which are placed in a standard benchtop incubator. The incubator maintains the physiological conditions necessary for cell growth, such as sterility, proper temperature, pH, and osmotic pressure. Standard cell culturing is reliable and is used in the majority of cell culture-based experiments. However, it has limitations when dynamic processes of the cells have to be investigated. The cells can be taken out of the incubator for a brief observation and then returned to the incubator until the next observation, but this does not allow for continuous online monitoring of the cellular processes.

For the continuous real-time observation of cells, the incubator has to be: (i) enlarged to the size of a room to accommodate all observation instruments as well as the operator; (ii) modified to enclose the observation instrument and provide the instrument controls to the outside operator; or (iii) reduced to fit into the instrument; for example, on the stage of a microscope. The first option is usually chosen when running many parallel online experiments on a regular basis, but it requires a large investment and running costs. Furthermore, to allow the operator to breathe normally, the atmosphere is not enriched for CO₂, which requires special cell culture media and thus limits the range of cell types that can be investigated. Moreover, the operator working with the incubator might contaminate cell cultures. Enclosing the investigation instruments—typically a microscope—by the incubator is less costly and provides better sterility than a room incubator because it isolates the operator from the cell culture and the instrument. However, in this case, physical accessibility to the instrument is significantly reduced, and therefore it has to be equipped with remote controls and handling tools. Miniaturized cell culture incubators not only enable the growth and online observation of cells directly on the stage of an optical microscope, but they also provide the whole list of benefits intrinsic to microsystems, including low consumption of power and reagents as well as fast response due to the small mass and volume of the devices. In addition, this type of setup provides opportunities for parallel operation for high-throughput analysis and integration of multiple sensors for monitoring environmental parameters. Finally, the microsystems can be designed as single-use disposable devices due to the low costs, which are a consequence of the requirement for only a small amount of raw material and the potential for mass production using a batch-type fabrication (1).

The opportunities offered by microfabrication and microfluidic technologies in design and fabrication of miniaturized cell culture systems are being explored by researchers (2). One trend is the development of
extremely small cell culture chambers intended for the study of single/few cell-related biological phenomena (3–7). Alternatively, microchambers are designed to provide and/or investigate effects of unique physiological cell culture conditions in terms of culture media composition, pressure, shear stress, and chemical and geometrical microenvironments (8–17).

Commercially available miniaturized cell culture systems are primarily developed for live-cell microscopy, sometimes including options for mechanical cell micromanipulations and electrophysiology. Typically, they consist of two parallel glass cover-slips, defining the top and the bottom of the chamber, and a sealing spacer, providing the walls of the chamber (18). For long-term cultures, such systems contain inlets for continuous media perfusion, which provides fresh nutrients and removes metabolic wastes (19,20). The perfusion is typically driven by gravity or by peristaltic/syringe pumps. The temperature required for cell growth is maintained by a heated airstream directed to the chamber, by heated water flow in the channels surrounding the chamber, or by integrated electric heaters consisting of resistance wires, thin films, or thermoelectric (Peltier elements), all controlled via an electronic feedback loop (20,21).

Several modifications of these setups exist, such as open chamber systems containing a cleaned oil layer on top of the culture media instead of a glass coverslip, which prevents fluid evaporation without hindering direct access to the cells by manipulation or probing tools (22,23). Some devices do not contain an integrated cell chamber but accommodate a standard cell culture dish and interface it with media perfusion and temperature control systems (Open Perfusion Microincubators; Harvard Apparatus, Holliston, MA, USA).

The thin dimensions and material properties of the glass coverslips used in the described systems provide a very good optical setup for high-magnification microscopy. Unfortunately, commercial cell culture microincubators are expensive and available in a limited number of designs (24). Moreover, many commercial chambers provide uneven environmental conditions for cells and therefore might contribute to artifacts or irreproducibility in the experimental data recorded from cells (16,21). For example, cells experience different temperatures in the central part of the chamber compared with peripheral parts when the heat is induced by heaters that are designed as a ring surrounding the cell chamber. Moreover, the perfusion of the media in most commercial chambers is carried out via localized inlet apertures, which induces high flow velocities in the vicinity of the inlet or outlet apertures and low velocities in the remote chamber parts. This causes uneven shear stress and supply of the nutrients to the cells in different parts of the chamber. Although many of these problems can now be eliminated by using more advanced designs and technologies (10,21), only a few improvements have been implemented in commercially available cell culture chambers. The added sophistication significantly increases the cost of these devices.

Our approach is to utilize the advantages of modern microfabrication and microfluidics technologies in order to develop a novel disposable polymeric chip containing a fully transparent perfusion chamber that enables reliable, long-term cell culture and is compatible with all types of microscopy (upright/inverted, reflective/transmission).

**MATERIALS AND METHODS**

**Fabrication**

The cell culture chip was composed of five poly(methyl methacrylate) (PMMA) sheets (Figure 1A), which were separately cut and micromachined by laser ablation and assembled into a functional structure by a thermal bonding technique previously described by Klank et al. (25). Briefly, an infrared laser ablation system (50W Synrad F48 laser equipped with an FHIW 30-200 marking head; Synrad, Mukilteo, WA, USA) was run using 500–800 mm/s beam movement velocity, 28–32 W laser power, and 1000 dpi exposure resolution to induce chip contours, alignment holes, chamber and heater spacers, and microfluidic channels on 1.5- and 0.25-mm thickness PMMA sheets (Type 99530; Röhm GmbH & Co. KG, Darmstadt, Germany). Prior to the bonding processes, the micromachined PMMA parts were annealed at 80°C temperature for at least 30 min in order to avoid stress cracks in the structure. After annealing, the parts were wiped with 96% ethanol, aligned, and exposed to the 110°C heat treatment under mechanical pressure for 1 h, which

![Figure 1. Construction of the cell culture chip. (A) Structure of the chip consisting of five transparent PMMA sheets. The uppermost layer serves as the chamber lid. The second layer defines the cell culture chamber, microfluidic channels, inlets for perfusion, and cell seeding, as well as incorporates the temperature probe. The third layer separates the chamber from the heater, which consists of an ITO-coated PET film integrated in the fourth layer. The fifth layer contains alignment grooves for electrodes to the heater and protects the heater from the environment. Each layer contains four alignment holes. (B) Layout of the fabricated cell culture chip with the functional parts labeled. PMMA, poly(methyl methacrylate); ITO, indium tin oxide; PET, polyethylene terephthalate.](image-url)
caused an irreversible bonding of the structured PMMA parts.

The integrated heater was made from indium tin oxide (ITO)-coated polyethylene terephthalate (PET) film (Sigma-Aldrich, St. Louis, MO, USA) embedded in the chip below the cell culture chamber bottom. The electrodes were made from standard copper wires and attached to ITO film by conductive epoxy-based glue (CircuitWorks®, Chemtronics, Kennesaw, GA, USA). The resistance of the ITO film was used as a measure of the connection quality. Only chambers with a resistance lower than 17 Ω were used.

Inlets and outlets of the chip consisted of 18 gauge blunt-end stainless steel needles (Becton Dickinson, Drogheda, Ireland) that were abraded on the surface for better interlock with the polymer structure of the chip. The needles were fixed and sealed to the structure with epoxy glue (5-Min. Epoxy; R&G GmbH, Waldenburg, Germany).

Temperature Control

The temperature inside the cell culture chamber was controlled by a proportional-integral-derivative (PID) feedback loop that was implemented using LabView™ v7.0 software (National Instruments, Austin, TX, USA). The computer program controlled the voltage applied to the ITO film-based heater depending on the temperature readings from the model TS67-170 micro-thermistor (Oven Industries, Mechanicsburg, PA, USA) located within the cell culture chamber. A USB-based multifunction data input/output board (LabJack™ U12; LabJack, Lakewood, CO, USA) and two homemade electronic circuits (see Supplementary Figure S1) were used to connect the heater and the thermistor to the computer and the external power source (9 V, 500 mA, AC/DC converter type OTC 910801; Otron Electronics, Hong Kong). One electronic circuit controlled the electrical power provided to the heater, depending on the voltage level in the analogic output (AO) port of the LabJack board. The voltage applied to the heater followed the AO voltage but with much higher current supplied from the external power source. The second electronic circuit kept constant current (50 μA) passing through the thermistor, and therefore converted temperature-dependant resistance changes of the thermistor into voltage signals that were registered by the analogic input (AI) port of the LabJack board. The voltage signal was further converted into temperature values and logged into the computer memory by the same LabView code.

Physical Modeling

Two-dimensional (2-D) finite element modeling was performed to evaluate the fluidic and thermal properties of the chip. Modeling was made using fluid dynamics and heat transfer modules of FEMLAB™ v3.1b software (COMSOL™ A/S, Kgs. Lyngby, Denmark). The physical parameters of the materials used in simulations are listed in Table 1.

First, the incompressible Navier-Stokes equations were solved to simulate the steady-state velocity field of the culture media perfusion. No-slip boundary conditions were applied to the walls of the channels and the chamber, a parabolic velocity profile with an average speed corresponding to 0.1 mL/h perfusion rate was used at the inlet, and zero pressure conditions were set at the outlet.

In the second step, the obtained flow velocity field was used to compute heat transfer by convection and conduction of heat energy. It was assumed that the liquid, which is continuously entering the chip, is initially at room temperature. The heat from the heater was induced at the same power across the entire surface of the chip. Heat dissipation to the ambient at 20 W/(m²·K) was also taken into account in the model.

Cell Culture and Time-Lapse Microscopy

The human carcinoma cell line HeLa was maintained in 75 cm² culture flasks (EasyFlask; Nalge Nunc International, Rochester, NY, USA) in 25 mL RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 1000 U/mL penicillin, and 1 mg/mL streptomycin (all components from Sigma-Aldrich). The alkalinity of the media was inspected by a miniLab™ IQ125 pH meter (IQ Scientific Instruments, Carlsbad, CA, USA). Cells were routinely cultured in a benchtop incubator (Assab; Don Whitley Scientific Ltd., Shipley, West Yorkshire, UK) providing 37°C and a 5% CO₂-enriched atmosphere. Seeding of the cells was performed with a density of 6667 cells per cm² and the cells were subcultured every 3 to 4 days when approximately 90% confluence was reached. For cell growth doubling time estimation, two independent cell culture experiments, each lasting 4 days, were carried out. Cell counting was performed using microscopic images of the cell culture taken by temporarily removing the cell culture flasks from the CO₂ incubator and placing them on the stage of the inverted microscope. Three to four images were grabbed from each culture flask every day over a 4-day period and counting was performed (27 counts in total). For on-chip cell culture experi-

Table 1. Material Properties Used in 2-D Finite Element Modeling of Fluid and Heat Transfer Within the Cell Culture Chip

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA density</td>
<td>1800 kg/m³</td>
</tr>
<tr>
<td>PMMA heat capacity</td>
<td>1500 J/(kg·K)</td>
</tr>
<tr>
<td>PMMA thermal conductivity</td>
<td>0.18 W/(m·K)</td>
</tr>
<tr>
<td>Buffer density</td>
<td>1007 kg/m³</td>
</tr>
<tr>
<td>Buffer dynamic viscosity (37°C)</td>
<td>0.0007 N·s/m²</td>
</tr>
<tr>
<td>Buffer heat capacity (37°C)</td>
<td>4000 J/(kg·K)</td>
</tr>
<tr>
<td>Buffer thermal conductivity (37°C)</td>
<td>0.62 W/(m·K)</td>
</tr>
<tr>
<td>Heat dissipation to ambient</td>
<td>20 W/(m²·K)</td>
</tr>
</tbody>
</table>

2-D, two-dimensional; PMMA, poly(methyl methacrylate).
ments, the perfusion inlet of the chip was connected to a 20-mL sterile syringe (Becton Dickinson) by polytetrafluoroethylene (PTFE) tubing (inner diameter: 0.8 mm; Bohlender GmbH, Grünsfeld, Germany), which was attached by flexible polydimethylsiloxane (PDMS) connectors. The syringe was loaded with cell culture media that was preheated to 37°C to minimize bubble formation inside the syringe. Continuous media perfusion was done using a syringe pump (Model 22; Harvard Apparatus), generating a flow rate of 0.1 mL/h (1.67 μL/min).

The chip was mounted on a microscope stage of a standard phase-contrast microscope (Alphashot 2 YS2; Nikon, Tokyo, Japan). Injection of cells was performed through a 25-gauge needle attached to a 1-mL syringe. Cells were seeded at a density of 10–100 cells within the field of view of the ×10 magnification objective. Cell culture images were digitally recorded using a Coolpix 990 camera attached to the microscope via a Coolpix MDC lens and equipped with an MC-EUI remote control unit (all from Nikon) operating in time-lapse mode, typically with a 5-min period. The images were converted into AVI movie format and time-stamped using Scion Image v.4.0.2 freeware (Scion, Frederick, MD, USA) and home-written macros.

Cell growth doubling time in on-chip culture was estimated from two independent experiments that lasted 50 and 120 h. Cell counting was performed using time-lapse microscopy image sequence. Cell counts were made on the images taken every 10 h, making 20 counts in total.

RESULTS

The composite layers and the resulting complete fabricated chip are shown in Figure 1. The basic design of the chip incorporates a single 7.6 × 13.0 mm² cell culture chamber, which contains an inlet and outlet for media perfusion and an additional inlet for cell seeding. All channels have a Gaussian cross-section (25) that is 400 μm wide and 500 μm high. The

Figure 2. Modeling of the flow within the chamber. (A) FEMLAB-modeled flow when the perfusion media enter the chamber via the single aperture of the inlet and (B) results when the media enter via the multiple apertures of the equalization barriers. Velocity field intensity is indicated by a grayscale color code. The arrows visualize flow direction and intensity. (C) For comparison, the flow velocity profiles 2 mm away from the cell chamber entrances (indicated by the straight gray lines) are plotted.
microfluidic barriers, which consist of a series of microchannels on both sides of the cell chamber, establish uniform media flow during perfusion and cell entrapment during the seeding process. The microchannels are 1.5 mm long and are distributed with 0.6 mm pitch along the barriers. The channels have a Gaussian cross-section that is 300 μm wide and 350 μm high. The integrated thin-film heater entirely covers the bottom or top of the cell chamber (depending on whether an upright or inverted microscope is used) and part of the microfluidic channels, which helps to preheat the perfusion media before it enters the cell chamber. The temperature probe is incorporated directly into the chamber to ensure on-site readings of the temperature.

The homogeneity of the cell culture environment was significantly improved by flow equalization barriers and an integrated heater, which regulate media supply distribution, shear stress, and thermal conditions within the chamber. As can be seen from the two-dimensional numerical modeling results (Figure 2), the equalization barriers are predicted to cause a uniform flow velocity field over the majority of the cell chamber area: ±10% variation over 70% of the chamber area compared with ±90% variation in the same area with direct inlet/outlet configuration. The modeling was also performed on the chamber configuration containing three inlet and outlet channels (Supplementary Figure S2). Such configuration significantly improves flow distribution in the chamber without equalization barriers (±60% variation over 70% of the chamber area). However, in this case, the flow profile is more sensitive to clogging of one or more of the channels and is still less uniform than in the chamber containing microfluidic flow equalization barriers. In the chip itself, the effectiveness of the barriers was confirmed by observing dye flow in the chamber (see Supplementary Movies S1 and S2). A similar configuration was proven to be effective on a smaller scale by Hung et al. (10,11), while Focht (21) used “T” shaped grooves as inlet and outlet for laminar media perfusion.

Results of 2-D temperature distribution modeling are shown in Figure 3. Temperature variation along the cell culture chamber is predicted to be up to 3°C when a steady state of 37°C is achieved in the middle part of the chamber. Bottom-to-top variation is modeled to be less than 0.6°C. The temperature gradients are mainly caused by the infusion of room temperature cell culture media. In the functional device, the entering media has more time to be preheated in the inlet channels lying above the heater. Assuming that the media reaches 28°C in the inlet channels, we estimate the temperature variation within the chamber is less than 1.5°C.

To test the chip for thermal control stability over long time periods, temperature was recorded during operation for 1 week (Figure 4). Despite significant fluctuations in ambient temperature (18.0°C–24.3°C) caused by the day-night cycle, the temperature in the chamber was maintained unperturbed (37.00°C ± 0.26°C) by PC-controlled feedback, which continuously adapted the voltage amplitude applied to the heater (2.2–2.9 V).

No changes in the pH of the perfused media were observed, indicating that the enclosed perfusion system efficiently eliminated the need for CO₂ buffering of the media.

HeLa cell culture experiments indicated normal growth and morphology of the cells during the online observations lasting up to 2 weeks. Contamination of the cell culture was efficiently eliminated by dry sterilization (100°C, 1 h) of the chip and wet autoclaving (140°C, 15 min) of the connection tubing prior to media infusion and seeding of cells.

Time-lapse microscopy was used to evaluate cell attachment and growth kinetics inside the chamber of the cell culture chip. Cell attachment and spreading was observed during the first 10–20 h (lag phase). After approximately 4–6 h, the cells started to divide. After approximately 20 h, cell growth became exponential, with an estimated doubling time of 32 h with 6 h standard deviation (Figure 5). The doubling rate of the reference cells cultured in standard culture flasks in a benchtop incubator was 32 h with 1 h standard deviation. A movie of a 141 h culture experiment is available as Supplementary Movie S3.

DISCUSSION

The goal of this study was to develop a low-cost polymeric chip that provides optimal conditions for cell culturing and that is optically transparent for online microscopic observation of the cultured cells. By optimization of cell culture conditions, we aim to achieve long-term on-chip cell culturing with a homogeneous environment throughout the cell culture chamber and with expressed cell biological behavior,
identical to the cell cultures grown in benchtop incubators and standard cell culture flasks. This is important for collecting reproducible, high-quality data from real-time cell observations performed using the chip and for the ability to compare and relate these results with previous standard cell culture studies. The time-lapse microscopic observation of HeLa cells allowed us to follow cell attachment and spreading right after the seeding (Figure 5) as well as the growth kinetics of the culture for up to 2 weeks. This indicates that cells can be successfully cultured outside a benchtop incubator with on-chip thermoregulation and continuous flow of cell culture medium.

Basic physiological conditions necessary for cell growth were established by using an enclosed perfusion chamber design. The enclosed perfusion chamber prevents the evaporation and interaction of the culture media with atmospheric air and therefore eliminates the need for CO₂ buffering and humidification of surrounding atmosphere. Moreover, the enclosed system reduces the risks of infection or contamination of the culture. Unlike standard cell culturing, where the medium is replaced every third or fourth day, continuous perfusion of the chamber with fresh culture media provides a constant supply of nutrients and metabolic waste removal. Continuous perfusion eliminates the periodic changes in cell culture conditions, such as altered concentration of nutrients and metabolic products caused by media exchange procedures when using standard cell culture techniques (17). Continuous perfusion is not always advantageous; for example, maintaining cell cultures that are strongly dependent on signaling and conditioning metabolites excreted by the cells themselves requires perfusion involving periodic partial media exchange in the chamber instead of continuous flow (26).

However, cell culture chips with continuous perfusion can be a particularly valuable tool to identify such critical signaling factors because perfusion constantly eliminates cell-secreted factors within the chip, and external factors can be added in a controlled manner. The chip is therefore highly suitable for studying effects on cell-to-cell communication by excreted factors or determining effects of pharmaceutical drugs in a highly controlled manner. Controlled addition of desired stimulating drugs or solutions to the media in the chip enables online monitoring of the kinetics of the cellular responses to these stimuli either in terms of cell morphological or motility changes identifiable by optical microscopy or in terms of expression of fluorescently labeled proteins detectable by fluorescent microscopy.

Additionally, online observations of changes in cell-excreted factors due to changes in media formulation or addition/removal of selected growth factors might also be realized by perfusion of the outlet media through an antibody array if a labeled antibody is added to the inlet media. Indeed, autofluorescence measurements of the chip materials in Cy³ and Cy5 fluorescence channels using a microarray laser scanner (ScanArray® Lite; Packard Biochip Technologies, Billerica, MA, USA) showed that fluorescence studies of the cultured cells are completely possible in the Cy3 channel. However, the ITO-coated PET sheet was found to autofluoresce in the Cy5 channel (data not shown), which limits the range of fluorescence dyes that can be used for cell staining or protein/antibody labeling.

In this study, polymers have been chosen as a base material of the cell culture chip because they offer an attractive combination of functional and processing properties. First of all, polymers, especially polystyrene (PS), are known to be biocompatible with the majority of cell cultures (27). Additionally, many polymers are transparent to the light and therefore allow online microscopy of the cells and optical readout of integrated probes/sensors. From the fabrication point of view, polymers are inexpensive materials and have well-established techniques for large-scale microreplication and production (e.g., hot embossing, injection molding) (28). This provides the possibility of producing inexpensive, easy-to-use disposable biochips, ensuring a sterile environment for the cells and high-quality bioanalysis (1). The combination of polymer micro-machining techniques used in this study—computer-controlled laser microablation and thermal bonding of

Figure 4. Thermal performance of the cell culture chip during 1-week operation under 0.1 mL/h perfusion conditions.

Figure 5. Cell growth kinetics in the cell culture chip. The time-lapse sequence of phase-contrast images shows initial cell attachment and spreading on the chamber bottom during the first 4 h, and the plot demonstrates the growth of the cell population during 120 h of cell culturing in the chip.
polymers—allows rapid production of prototype microsystems, giving the opportunity for easy modification and optimization of the chip during the development phase (25,29). Laser micromachining combined with thermal bonding is a technique used to make general lab-on-a-chip systems (25), so the cell culture chamber of the chip can be integrated with microfluidic components such as pumps, valves, mixers, or with complete sample pretreatment and analysis microsystems. Microfluidic drug concentration gradients for high-throughput drug screening (10,11), optical sensors for metabolism analysis (30), and on-chip staining and flow cytometry (31) are a few integration options that we plan to add to the cell culture chip in the future. In comparison, such structures are complicated to do in glass or silicon because it requires a lot of specialized machinery and clean room facilities (10,11).

To ensure uniform heating, ITO-based heaters have been integrated in some commercial glass microincubators instead of using peripheral heaters (21). Being optically transparent and electrically conductive, ITO film does not obscure illumination of the chamber and, at the same time, can generate Joule heat when electrical power is applied to it. However, there are challenges in coating polymeric materials with ITO because standard thin-film vapor-deposition systems melt polymeric targets due to high temperatures in the vicinity of the vapor sources, and alternative sol-gel nanoparticle deposition methods (32,33) give relatively poor quality and stability of the ITO coatings (34,35). Therefore, specialized low-temperature sputter-deposition systems are typically required (36). Due to the lack of direct access to such equipment, the ITO heater was incorporated into the PMMA structure of the chip by using commercially available ITO-coated PET films. These films were laminated in between PMMA sheets during the thermal bonding procedure of chip fabrication (Figure 1). Because PET has a higher glass transition temperature than PMMA, it maintains the integrity of thin ITO coating during the bonding at 110°C under pressure. Such a configuration also increases the reliability of the ITO film during chip operation because the surrounding PMMA completely isolates the coating from culture media liquids and therefore protects the film from chemical and electrolytic erosion (Figure 1A).

Biocompatibility of the chip materials is an important issue for the long-term culture of healthy cells. Generally, PMMA is known as a biocompatible material and is widely used as implantable intraocular lenses due to its excellent optical properties (27). In our study, HeLa cells cultured within the PMMA chip showed identical morphology and doubling times (Figure 5) compared with cells grown in commercial culture flasks made of tissue culture-quality polystyrene (TC-PS). However, lower biocompatibility of osteosarcoma cells and endothelial cells (CPA-47) has been observed on untreated PMMA compared with TC-PS (37,38).

Interestingly, the biocompatibility of PMMA was significantly increased by radiofrequency plasma treatment, which is also a standard treatment of PS, in order to reach TC-PS quality (38). Changes in PMMA surface biocompatibility by plasma treatment allow the possibility of systematically modifying surface properties and, with the use of the chip, directly observing cell culture responses to these modifications. For demanding cell cultures, biocompatibility of the chip might be improved by using TC-PS instead of PMMA as chamber material. Unfortunately, due to its physical properties, PS cannot be ablated by infrared laser with the same resolution as PMMA, so PS can replace only those chamber parts that do not contain microfluidic channels or other microscale objects. Thus, in the presented design of the chip, the entire chamber lid and bottom, which basically are the only surfaces exposed for cell adhesion, can be replaced by TC-PS. The only difference in chip fabrication necessary for the combination of TC-PS and PMMA is that thermal bonding has to be performed at 95°C due to the lower glass transition temperature of PS.

In conclusion, we have developed a low-cost optically transparent polymeric chip with integrated perfusion microfluidics and thermoregulation for long-term cell culture under controlled conditions. The fabrication process, which is based on laser micromachining and thermal bonding, offers the flexible design of the chip, providing numerous lab-on-a-chip integration possibilities in the future.

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COMPETING INTERESTS

The authors declare no competing interests.

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