Accepted Manuscript

Evaluation of cell culture in microfluidic chips for application in monoclonal antibody production

A. Peña Herrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez

PII: S0167-9317(16)30186-1
DOI: doi: 10.1016/j.mee.2016.03.059
Reference: MEE 10242

To appear in:

Received date: 6 November 2015
Revised date: 29 March 2016
Accepted date: 31 March 2016

Please cite this article as: A. Peña Herrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez, Evaluation of cell culture in microfluidic chips for application in monoclonal antibody production, (2016), doi: 10.1016/j.mee.2016.03.059

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Microfluidics allows miniaturization of basic conventional biological or chemical laboratory operations. Lab-on-a-chip technology has been well accepted by biological and medical research communities as a promising tool for engineering microenvironments at cellular, tissue levels. In the early 1990s the first microfluidic devices for biochemical applications were developed, and since then the field has been rapidly expanding. These microfluidic chips have been used on a broad range of cell-oriented applications including monitoring cellular activity, cell-based assays to test drug sensitivity, cell-free protein synthesis or monoclonal antibodies production among others.

In contrast to conventional static approaches, microfluidic-based cell cultures are not only able to maintain well-defined culture conditions, but more importantly, allow to continuously provide cells with fresh media containing oxygen, carbon dioxide and nutrients while removing metabolic products at a controlled flow rate.

Alternatively to early glass microfluidic chips, today polymers have become the popular choice offering a wide range of chemical and mechanical properties as well as better biocompatibility. Commonly employed polymers are polydimethylsiloxane (PDMS) and poly(methylmethacrylate) (PMMA). Garza-Garcia and collaborators engineered a chip with PMMA body and PDMS cover plate to produce the monoclonal antibody Infliximab. Recombinant monoclonal antibodies are used for treatment grave diseases including autoimmune disorders and cancer, becoming one of the fastest growing areas in biopharmaceutical industry. Currently, monoclonal antibodies commercial production and other biotherapeutics are based on the synthesis in bioreactors with suspended mammalian cells with agitation operated in fed-batch or perfusion mode. The monoclonal antibodies production in stirred tanks faced challenges related to product quality and process such as demand for higher productivity, glycosylation control and reproducibility. Most of these challenges are related to large spatial and temporal variability of intrinsic fermenters conditions. One way to improve control is to reduce the scale of the system by miniaturization in the form of micro devices. A micro device provides several advantages, including shorter time response, a higher surface/volume ratio and a more homogeneous and controllable microenvironment.

---

**Key points**

- Microfluidics is useful for cell culture that allow to grow cells under highly controlled conditions.
- Microfluidic chips are used for production of therapeutic recombinant proteins.
- The optimal conditions for growth of cells amenable of recombinant protein expression in these devices were cultivated HEK-293T cells under different experimental conditions.
- The presence of poly-D-lysine improves the adhesion and viability of the cells in continuous or discontinuous flow.
- The optimal adhesion of cells was observed in the corners of the microchannels and wide channels possibly due to the flow rate in these areas.

**Abbreviations**

- HEK-293T: Human embryonic kidney cells
- PMMA: Polymethyl methacrylate
- PDMS: Polydimethylsiloxane

**Keywords**

- Lab on a chip
- Microfluidic
- Microchannel
- Cell culture
- HEK-293T cells

---

**Acknowledgements**

These authors contributed equally to this work.

---

**References**


---

**Corresponding author**

M.S. Pérez, University of Buenos Aires (UBA), Buenos Aires C1063ACV, Argentina.

E-mail addresses: gustavoh@ibyme.conicet.gov.ar (G. Helguera), max@fullgen.com (M.S. Pérez).

---

**CRediT**

- Data curation: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez
- Funding acquisition: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez
- Project administration: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez
- Visualization: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez
- Writing - original draft: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez
- Writing - review & editing: A. Peñaherrera, C. Payés, M. Sierra-Rodero, M. Vega, G. Rosero, B. Lerner, G. Helguera, M.S. Pérez

---

**Financial Support**

This work was supported by National Technological University (UTN), Buenos Aires 1076, Argentina and University of Buenos Aires (UBA), Buenos Aires C1063ACV, Argentina.
Moreover, PDMS devices offer surfaces that can be modified through oligopeptides, polysaccharides, proteins adsorption or via plasma processing to obtain specific surface features. Mimicking extracellular matrix is a challenge that has been addressed by texturing microchannels with fibronectin [6,7] and collagen [4,16] to achieve a better surface attachment of cells.

In this study, antibody production cells HEK-293T [17] were cultured in PMMA and PDMS microchannels in presence or absence of cell adhesion agent poly-o-lysine to assess their compatibility for mammalian cell culture and the effect of microchip geometry on cell growth. Coating with poly-o-lysine was chosen because it increases the number of positively-charged sites on chip surface, enhancing electrostatic interactions with the negatively charged groups on cell surface, therefore improving adsorption while preserving biological activity.

2. Materials and methods

2.1. Microfluidic devices design and fabrication

Two different microfluidic devices have been designed using Layout editor software (http://www.layouteditor.net). The first chip consists in three different microchannel shapes 40 μm height × 0.4 mm width (linear, zigzag and square waves), with an internal volume of 3.68 μL (Fig. 1A).

In the second microfluidic chip, channels of 40 μm height, with different serpentine shapes 100 μm wide and lengths between 12 and 80 mm, feed by a central channel (40 × 1.9 mm), and spacer channels (12 × 1.2 mm) between serpentine, were designed. The internal volume is 17.8 μL (Fig. 1B). These different microchannel shapes and widths have been designed and fabricated to test if cell adhesion and growth depend on geometry.

The microdevices were built in PDMS. To do this, a mold of the design in high relief was made by photolithography in a silicon wafer 700 μm thick (Virginia Semiconductor, Inc.), by using the negative resin SU-8 (MicroChem). The silicon substrate was cleaned by sonication in acetone and isopropyl alcohol, and substrate surface was dehydrated for 10 min at 200 °C. Then, SU-8 resist was dispensed on the substrate and spun in two cycles. The spinner was accelerated for 5 s at 100 rpm·s⁻¹ until 500 rpm, and held at 500 rpm for 5 s. In the spin cycle, a ramp of 300 rpm·s⁻¹ was applied until 2000 rpm, and held for 30 s. The resist was soft baked firstly at 65 °C for 20 min, and secondly at 95 °C for 50 min. The substrate was aligned and the resist was exposed to near UV at 650 mJ. The first step of a post-exposure bake consisted on 65 °C for 12 min, and the second step at 95 °C for 15 min. Finally, the resist was developed for 15 min under agitation.

PDMS chips fabrication steps are described in supplementary material. The first chip was also constructed from PMMA, which was manufactured using a Class 2 CO₂ laser etching system (Megalaser ML-609), operating at 60 W and 200 mm·s⁻¹ scan speed. Designs of the plano-convex lenses were created using Layout editor software, interfaced directly with the CO₂ laser. Finally, the inlet and outlet of the microdevice linked the microchannels with a syringe needle.

Two flow types have been used: continuous and discontinuous. Continuous flow experiments consisted in connecting microfluidic chips to a peristaltic pump (APEMA) and a bubble trap using PVC tubes in a recirculation mode. This set-up was inserted into an incubator at a constant temperature of 37 °C, whereas discontinuous flow studies were carried out by renewing culture medium each 24 h. Cells were cultured in an incubator (Cherry) that is commonly used to incubate eggs as it allows to control temperature and humidity (See supplementary material).

2.2. Cell culture and distribution

The microfluidic chips were disinfecting using NaOH 0.1 mol·L⁻¹ for 24 h, and then rinsed with sterile water. Before cell seeding, chip was treated with poly-o-lysine hydrobromide 0.1 mg·mL⁻¹ (Sigma) sterile solution to improve cells attachment. The microdevice was incubated with poly-o-lysine solution for one hour at 37 °C. The solution was then removed and let dry 24 h at 4 °C. HEK-293T cells (ATCC CRL-3216) were cultured in complete DMEM medium (Gibco), supplemented with fetal calf serum heat-inactivated (FBS) 10%(w/v) (Interneogencos SA), L-glutamine 2 mmol·L⁻¹ (Gibco), penicillin 100 units·mL⁻¹, streptomycin 100 μg·mL⁻¹ and fungizone 0.250 μg·mL⁻¹ (Gibco) at 37 °C in an incubator with 5% CO₂. Cells were resuspended with trypsin 0.50 mg·mL⁻¹ and EDTA-4Na 0.2 mg·mL⁻¹ (Gibco), and incubated at 37 °C for 3 min. Trypsin was inactivated with FBS and cells were washed with phosphate buffer solution (PBS) (NaH₂PO₄ 50 mmol·L⁻¹, NaCl 150 mmol·L⁻¹, pH = 7.6) and centrifuged at 1000 rpm for 15 min. Finally, cells were resuspended in the same complete DMEM medium, supplemented this time with 20% FBS at 10⁷ cells·mL⁻¹.

The microchannels and PVC tubes were filled with 15 mL complete DMEM medium, and the system was purged for 2 h. Next, HEK-293T cells in suspension were seeded into the syringe needle in the inlet of the microfluidic device. Cells were allowed to settle and microchannels were filled with 15 mL complete DMEM medium, and the system was purged for 2 h. Next, HEK-293T cells in suspension were seeded into the syringe needle in the inlet of the microfluidic device. Cells were allowed to settle and microfluidic device was incubated at 37 °C in incubator overnight. A flow rate of 5 μL·min⁻¹ was applied to the peristaltic pump to constantly refresh the DMEM medium, so each channel flow rate was 1.67 μL·min⁻¹. The microchannels were visualized using an inverted Olympus microscope CKX41. Brightfield images were taken with Olympus objectives LUCPlan FLN 40×/0.60; LCPlan N 20×/0.40; PlanC N 10×/0.25; and PlanCC N 4×/0.10 with an Olympus QColor 5; and processed with QCapture Pro 6.07 software. Cell density inside microchannels with linear, zigzag, or square wave (Fig. 1A) treated with poly-o-lysine was quantified. After two days incubation with peristaltic pump, cell density in each configuration was determined through quantification using Open CFU software [18] of 10× images acquired in triplicates. Bar graph analysis was performed with Excel and the signification was determined through t-test with the Statistica Package software.

The analysis of cell distribution over time in multiple configuration microdevices was determined through area quantification using Image J software of 4× images acquired in quadruplicates for wide channels and serpentine channel overtime, respectively. Bar graph and...
showed good adhesion to the bottom. In one of the chips, three different
port for cells to attach, but they did not grow further.
charge. It is possible that the mild texture of walls provides enough sup-
walls after the second day even though PDMS does not have intrinsic
the microchannel, as it can be seen in Fig. 2A (see videos in supplemen-
lysine). In the case of adhered cells, 5 μL·min⁻¹ continuous-flow culture
medium was provided by peristaltic pump.
Since presence of flow washed away cells in the chip without poly-o-
lysine we tested whether discontinuous flow allowed cell adhesion.
HEK-293T cells were seeded in a PDMS microdevice without poly-o-
lysine addition and in discontinuous flow with fresh media pulses
every 24 h (Fig. 2B). Although most of the cells were washed away by
the first discontinuous flow, after 24 h some cells attached to channel
walls instead of glass floor. Cells remained attached to the channel
walls after the second day even though PDMS does not have intrinsic
charge. It is possible that the mild texture of walls provides enough sup-
port for cells to attach, but they did not grow further.
In contrast, when poly-o-lysine was present in PDMS chips, cells
showed good adhesion to the bottom. In one of the chips, three different
microchannel geometries (linear, zigzag and square wave, Fig. 1A) were
assessed. Before starting flow rate, cells settled and the distribution was
homogeneous trough all microchannels. When flow rate started to run,
it was observed that zigzag and square waves configurations presented
cell accumulation at corners due to the lower local flow velocity and
vorticity field in these areas, facilitating cell deposition and attachment,
as previously described by Garza-Garcia et al. [6,7]. As expected, linear
channel showed less cell adhesion. Fig. 2C shows differences in cell den-
sity after two days incubation in different microchannel configurations.
As thought, the linear channel showed significantly lower cell density
compared to zigzag and square wave channels. This could be associated
to the fact that linear microchannel exhibit the fastest lineal speed
(1.1 mm·s⁻¹) in comparison with zigzag (0.57 mm·s⁻¹) and square
waves (0.47 mm·s⁻¹), which have the lowest lineal speed. We also
observed that there was a significant difference in density (p < 0.0001) between zigzag versus square waves microchannels.
Different serpentine designs separated by wide channels were test-
ed in a PDMS microdevice in a discontinuous flow rate. In this case,
after 16 h of cells deposition, the DMEM culture medium was renewed
each 24 h under 5% CO₂ and 37 °C (Fig. 3).
Under this condition, at seeding day (day 0) cells were not distribut-
ed uniformly through all microchannels (Fig. 3). As expected, it was ob-
served that cell localization is more frequent in the wide channels than
in the serpentine channels. In fact, at day 0 cells cover a significantly
larger area (1.5 higher) in wide channels than serpentine channels.
Two days after cells seeding, the most of cells in serpentine channels
were washed and the individual cells were adhered to the wide chan-
nels bottom. High fluid velocity of the narrow microchannels seems to
result in constant washing of cells, favoring their carrying and deposi-
tion to quieter areas (See supplementary material). Over time, cells
began to form clusters and to extend processes around the clusters. At
day five cells consolidate their growth in clusters on the floor of the wide
microchannels, with almost no cells in the serpentine channels
(Fig. 4), resulting an area covered by cells that is 29.5 fold higher in
wide channels compared to serpentine channels (See supplementary
material). At day eight, cells began to show signs of stress, with granu-
lations in the cytoplasm and the experiment was concluded.

4. Conclusions
HEK-293T cells have been cultured in microdevices with different
materials, microchannel geometries and experimental conditions. Use-
ffulness of poly-o-lysine as cell adhesion molecule in microfluidic chips
has been confirmed in both PMMA and PDMS materials. Besides, chan-
nel geometry significantly influences cells adhesion and growth,

---

**Fig. 2.** Cells incubation on microdevices with and without poly-o-lysine coating. Panel A shows inverted microscope optical images of HEK-293T cells, attached to PMMA microchannel bottom. HEK-293T cells were attached to microchannels bottom, coated with poly-o-lysine (0.1 mg·mL⁻¹), at 37 °C. Panel B shows HEK-293T cells incubated for two days on a PDMS chip without poly-o-lysine coating. HEK-293T cells were attached to walls but not to glass floor. Cells were cultured under the following conditions: discontinuous flow, atmosphere of 5% CO₂, 37 °C. Panel C shows a bar graph of the quantification of HEK-293T cell densities in linear, zigzag and square waves microchannels geometries in PDMS chips coated with poly-o-lysine. Error bars indicate standard deviation of triplicate determinations and significance in difference was determined by t-test for non-paired data, with two tails and unequal variances. Significant difference is represented by * p < 0.001.

---

**Fig. 3.** Distribution of cells along microchannels with multiple configurations. HEK-293T cells were not distributed uniformly through microchannels on day 0. Microchannels were coated with poly-o-lysine and cells were cultured in discontinuous flow at 5% CO₂ and 37 °C.

---

Please cite this article as: A. Peñaherrera, et al., Evaluation of cell culture in microfluidic chips for application in monoclonal antibody production, Microelectronic Engineering (2015), http://dx.doi.org/10.1016/j.mee.2016.03.059
showing that cells tend to grow in the microchannel corners, and consequently in the square waves channel. Moreover, in another chip with multiple configurations it has been observed that wide channels are more suitable for cell stabilization, providing adequate environment for cells to attach and grow. This seems to be associated to reduction in fluid speed in wide microchannels favoring cells retention, accumulation, and attachment.

This paper provides new insights in cells growth for monoclonal antibodies production, using microfluidics chips as potential bioreactors. These results show that HEK-293T cells can attach and grow in microfluidic devices preferably in areas with reduced flow for at least eight days, demonstrating the usefulness of microfluidics chips as bioreactors, which defines a direction for future work. Although we did not show antibody expression by the cultured cells, an extensive characterization of cell growth as well as antibody production study will be the subject of future publications.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mee.2016.03.059.

Acknowledgements

The authors thank the financial support from ANPCyT (PICT2013-0840, PICT-2014-3772), CONICET PIP (114-2011-01-00139) and Fundación René Barón. We would also like to thank C.L.A. Berli, C.A. Lasorsa, F. Sacco, J.L. Fernandez and P. Granell for general support and discussion.

References


Fig. 4. Incubation of cells on PDMS microdevices with poly-o-lysine coating. Images of HEK-293T cells cultured for eight days attached to the bottom of the wide microchannels coated with poly-o-lysine.