Микрофизиологические модели органов человека

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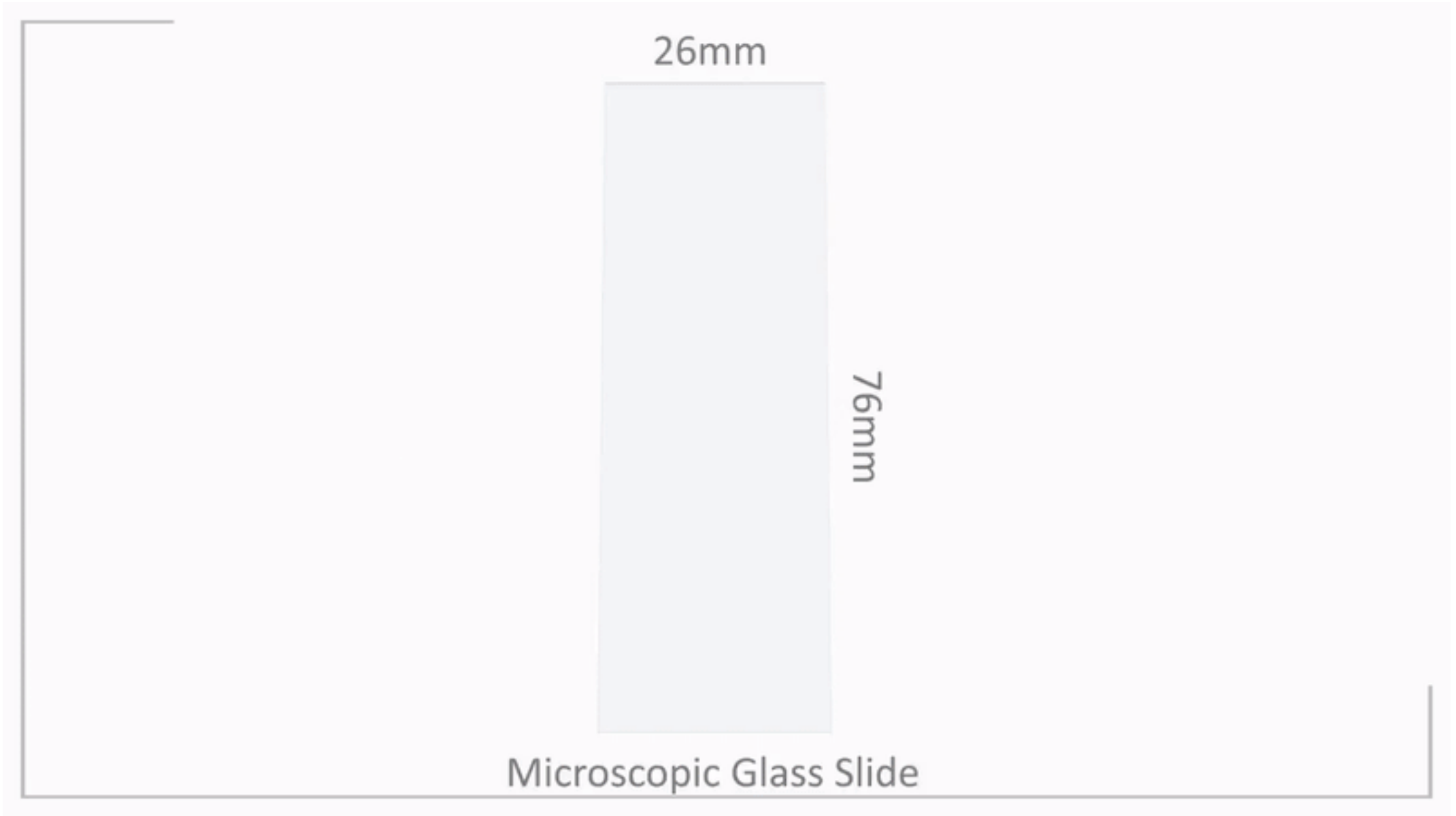
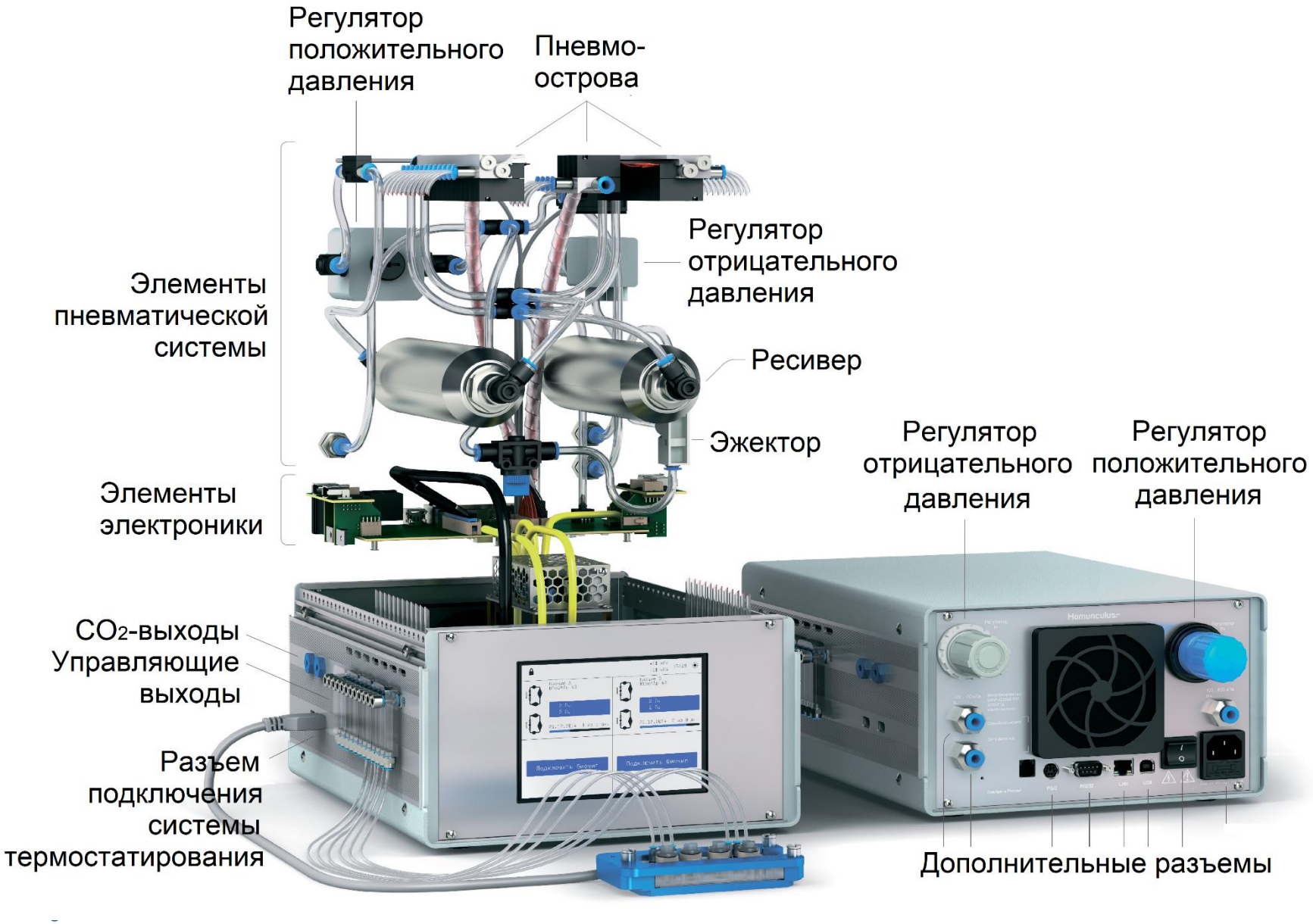
- Микрофлюидная платформа
- Клеточные модели
  - Печень на чипе
  - Кишечник на чипе
  - Метастаз на чипе
  - Плацента на чипе

1. Микрофлюидная платформа

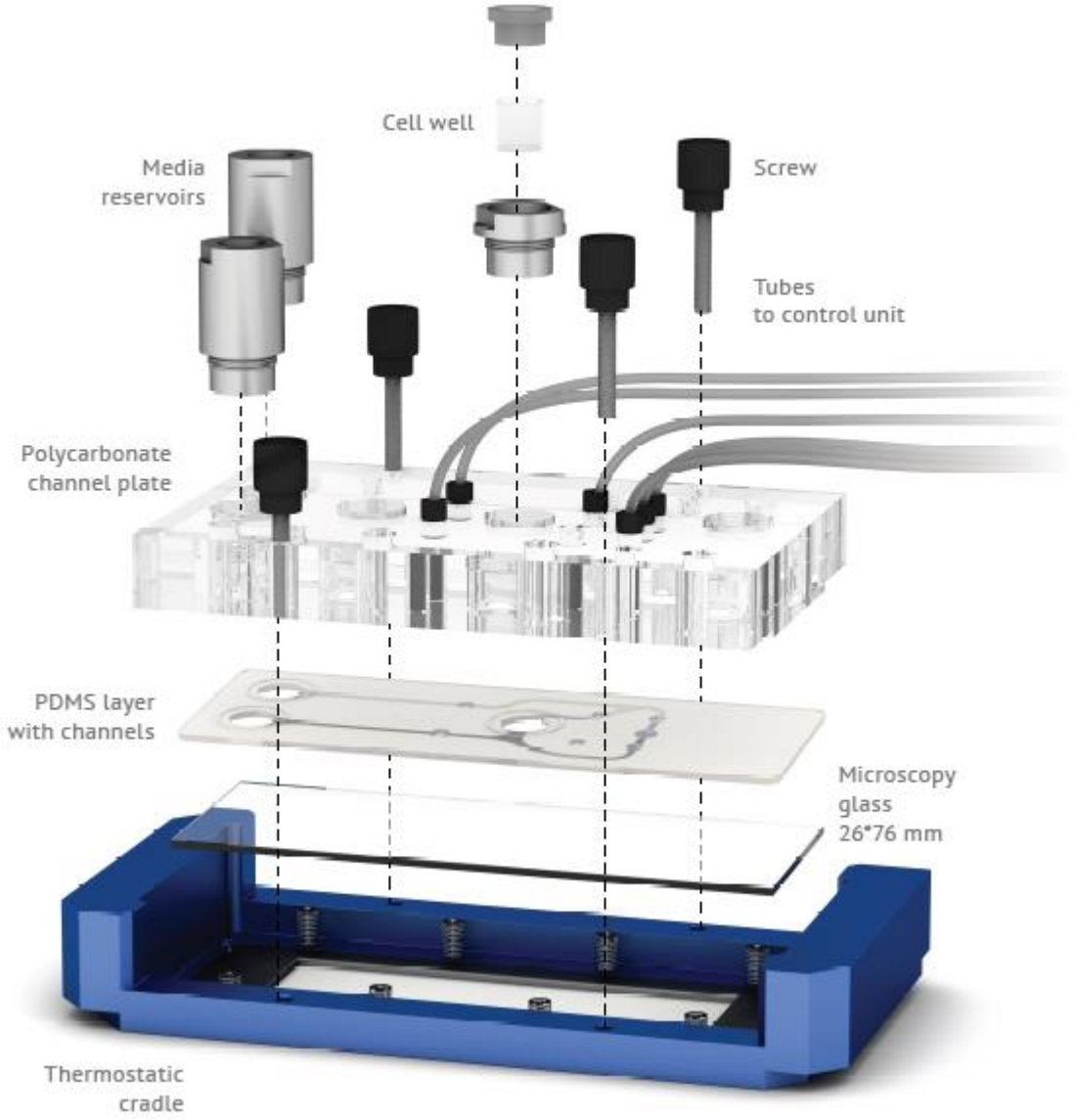

26mm
76mm
Microscopic Glass Slide

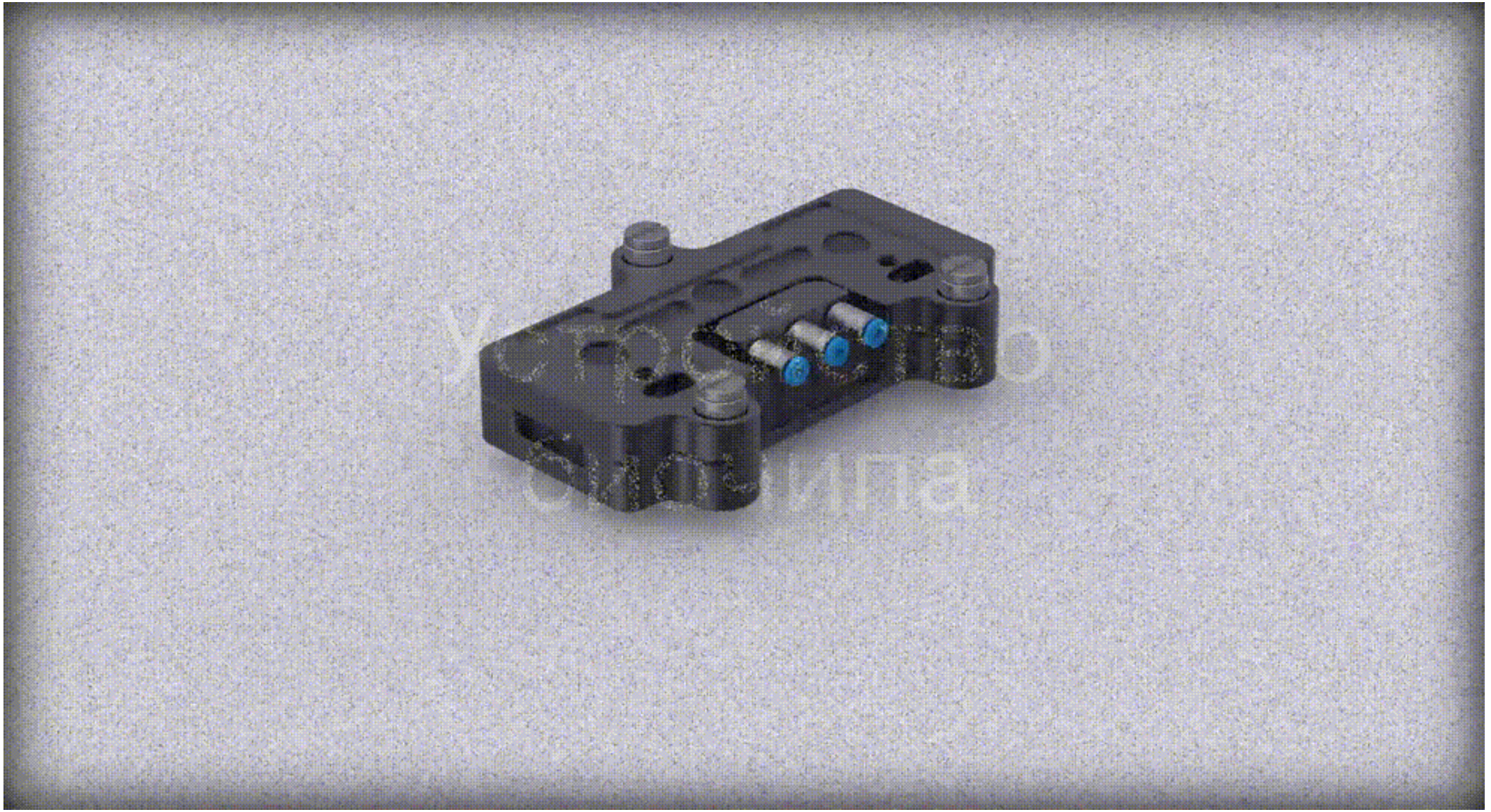
- Пневматические выходы с фильтрами
- 24 выхода на прибор (8 чипов)
- Независимое управление
- Регуляторы и датчики вакуума и давления

БЛОК УПРАВЛЕНИЯ



- Предметное стекло
- ПДМС слой
- ПК панель
- Фитинги и трубки
- 8 чипов на один блок управления



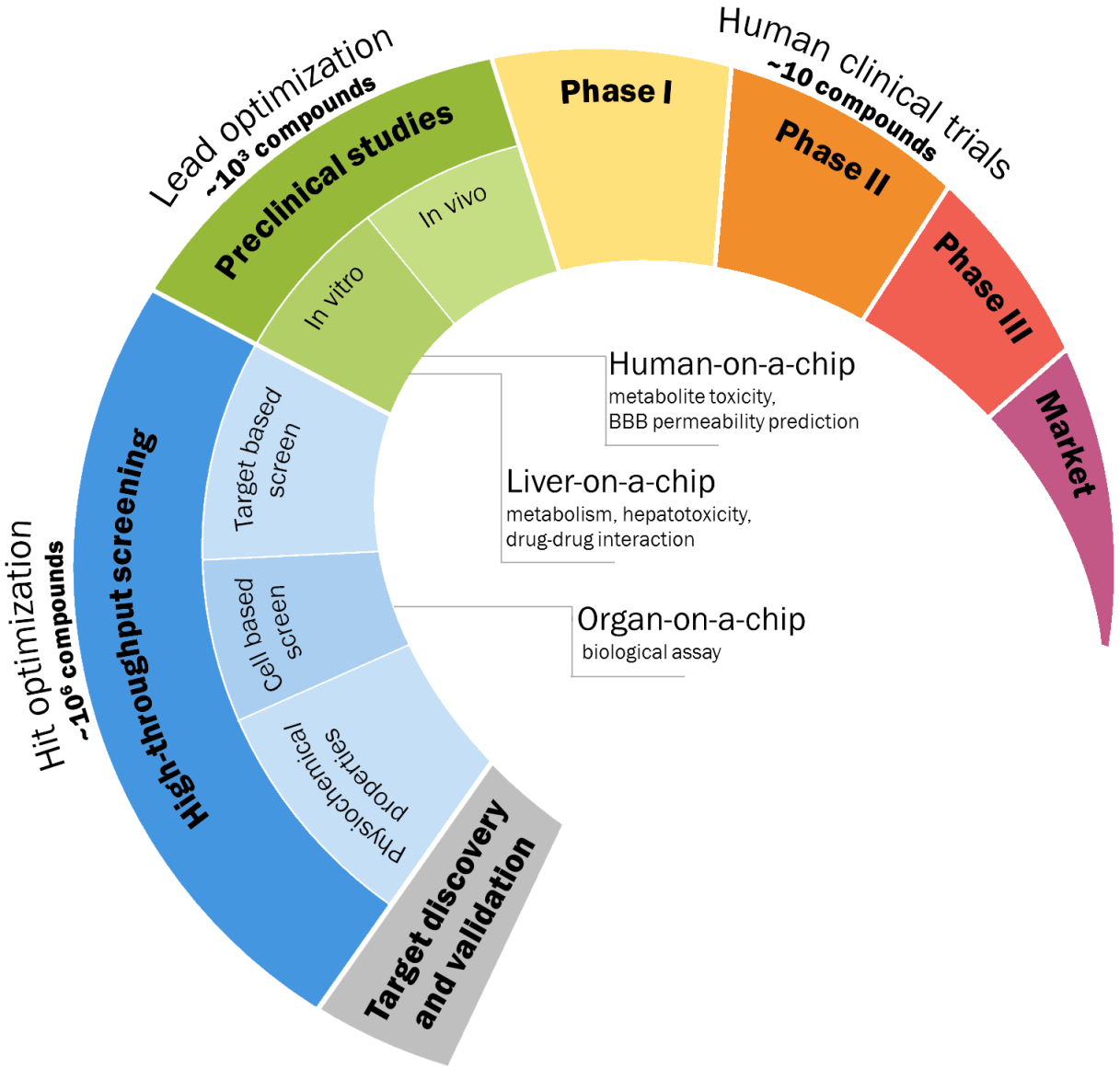


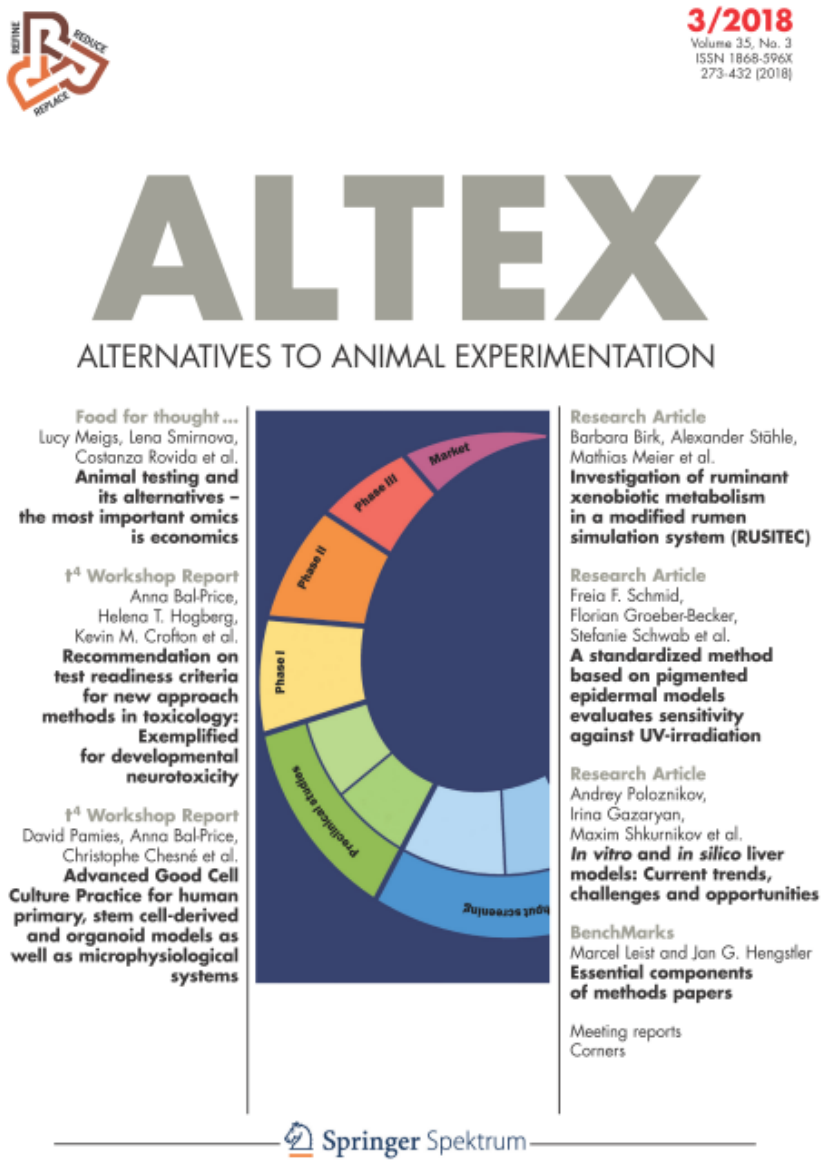

И

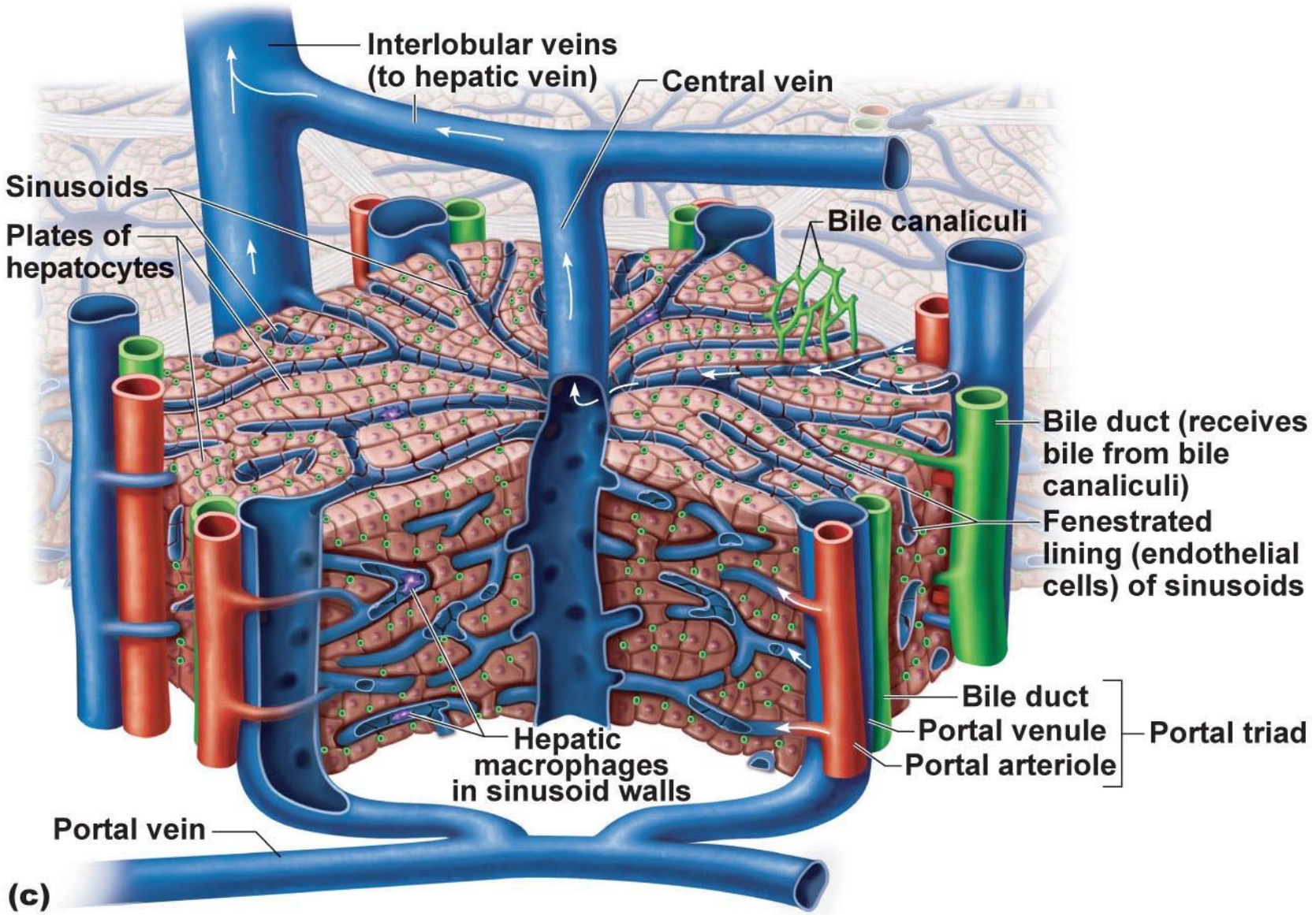
2.1 Клеточные модели

Печень на чипе

ГЛОБАЛЬНАЯ ПРОБЛЕМА ДОКЛИНИЧЕСКИХ ИСПЫТАНИЙ

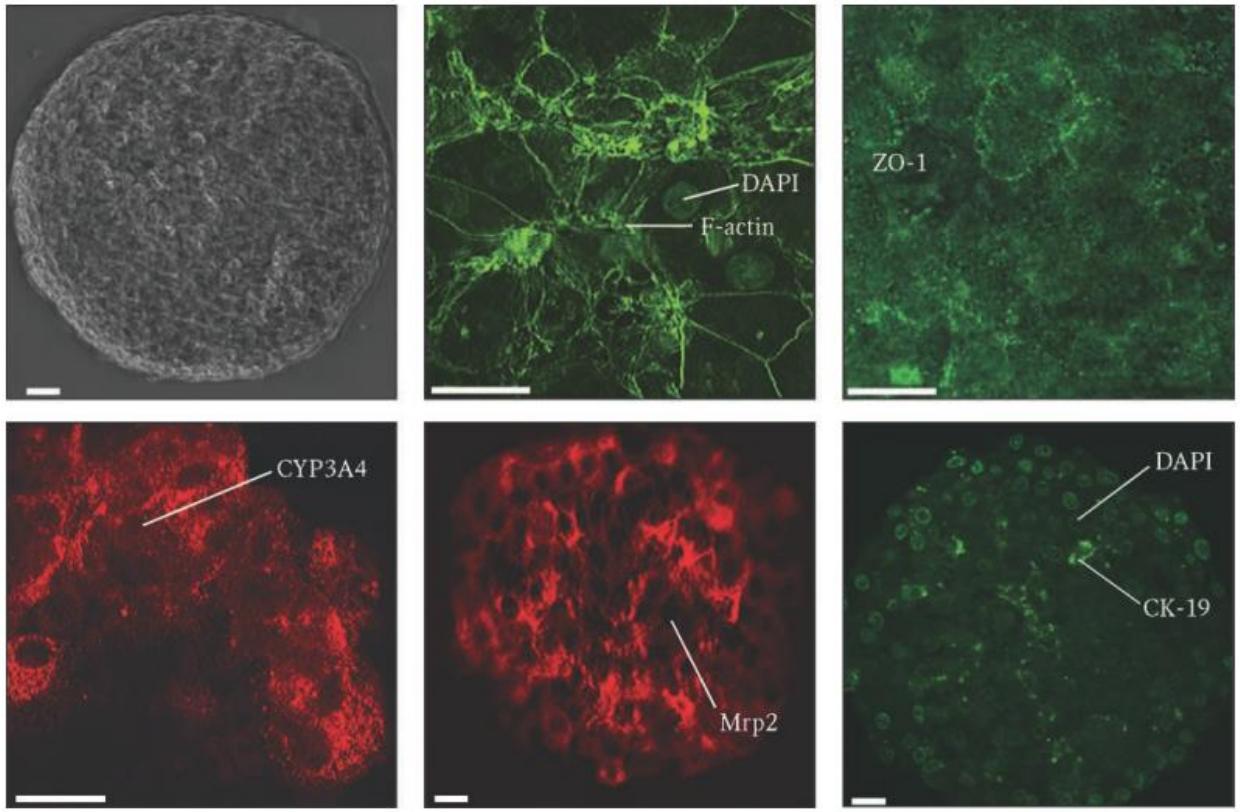






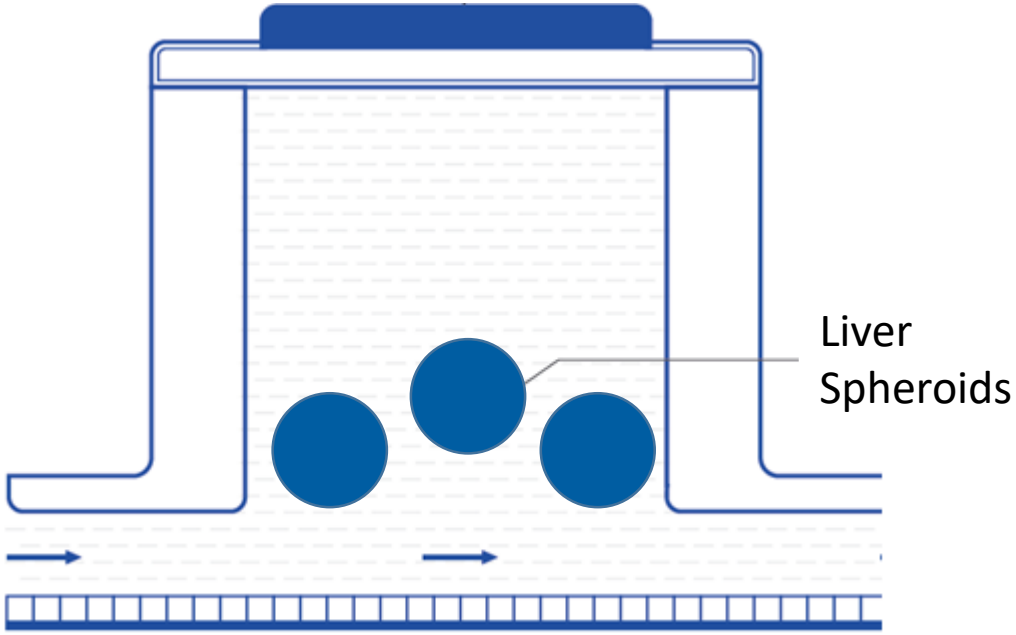
Сфероиды HepaRG:

- 2000 дифференцированных клеток на сфероид
- 6 дней инкубации
- 30 сфероидов на лунку
- 24 ч инкубации с субстратами

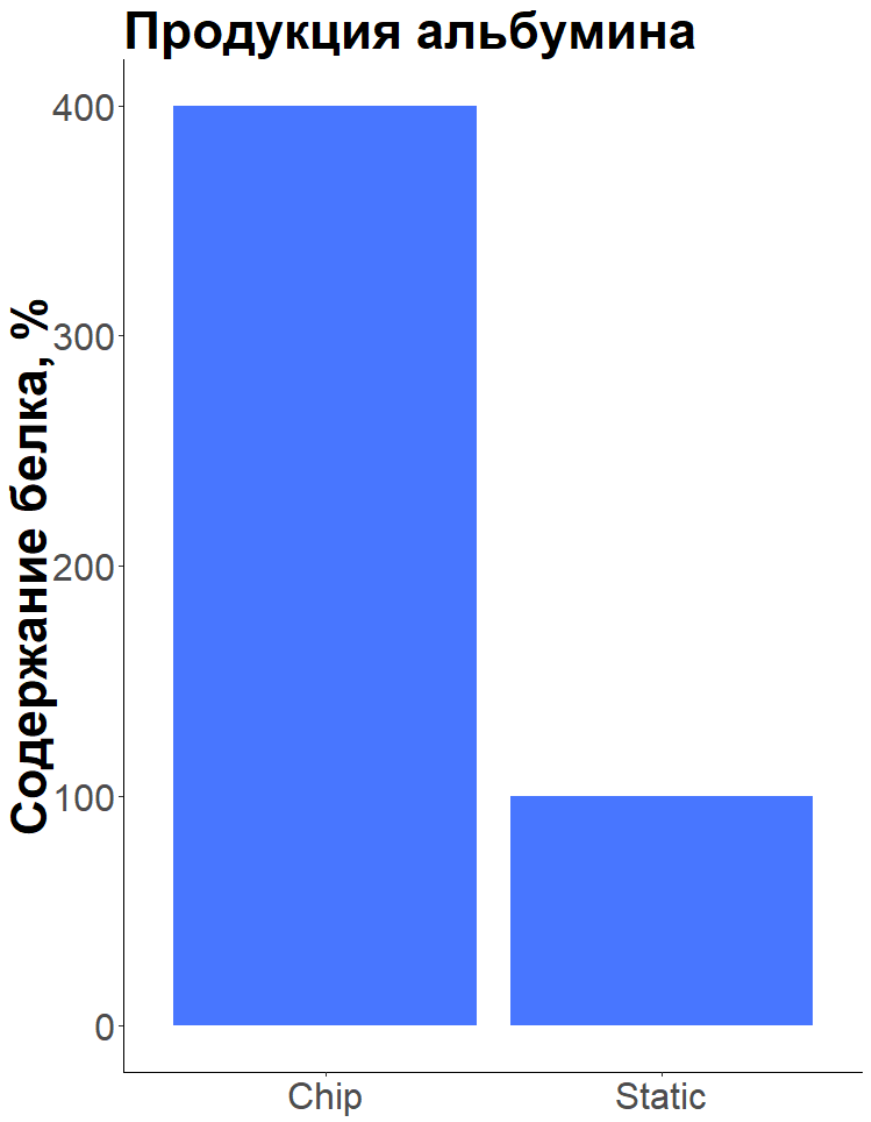


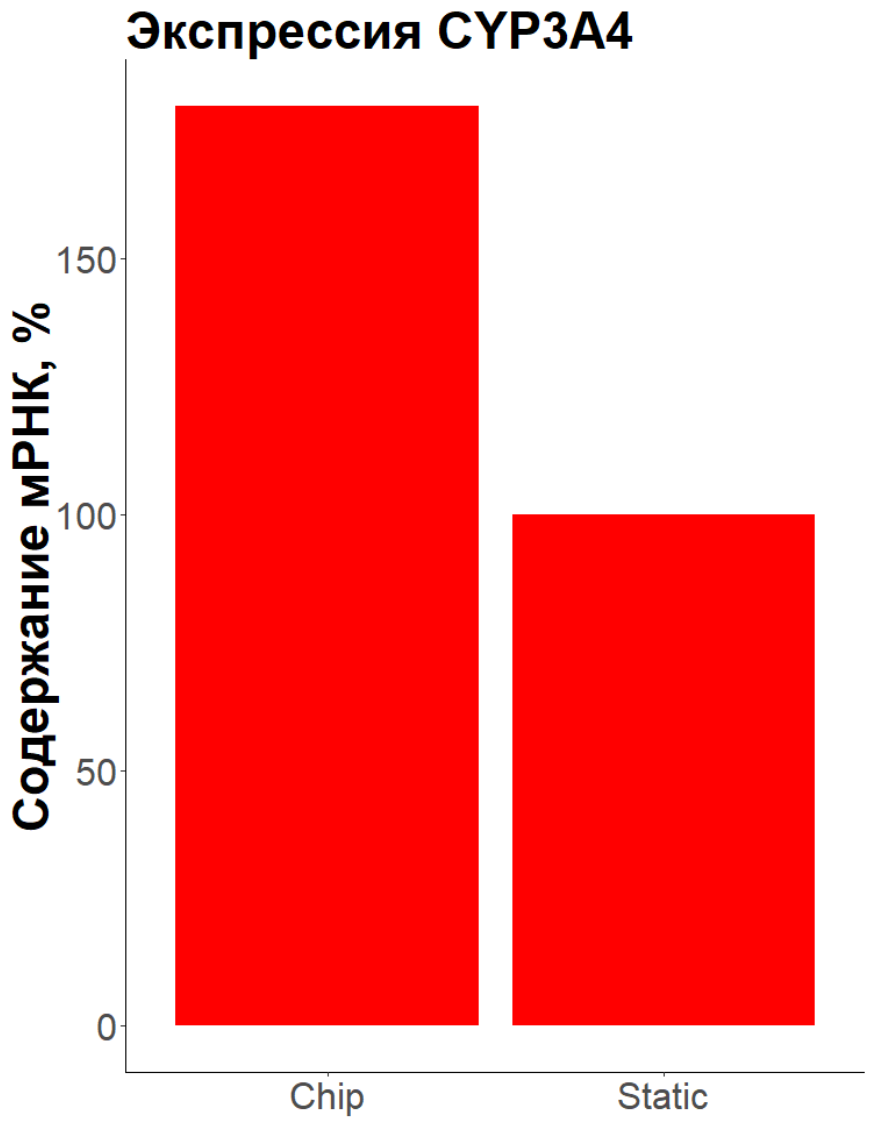
Изучение

- Токсичности
- Биотрансформации
- Межлекарственного взаимодействия

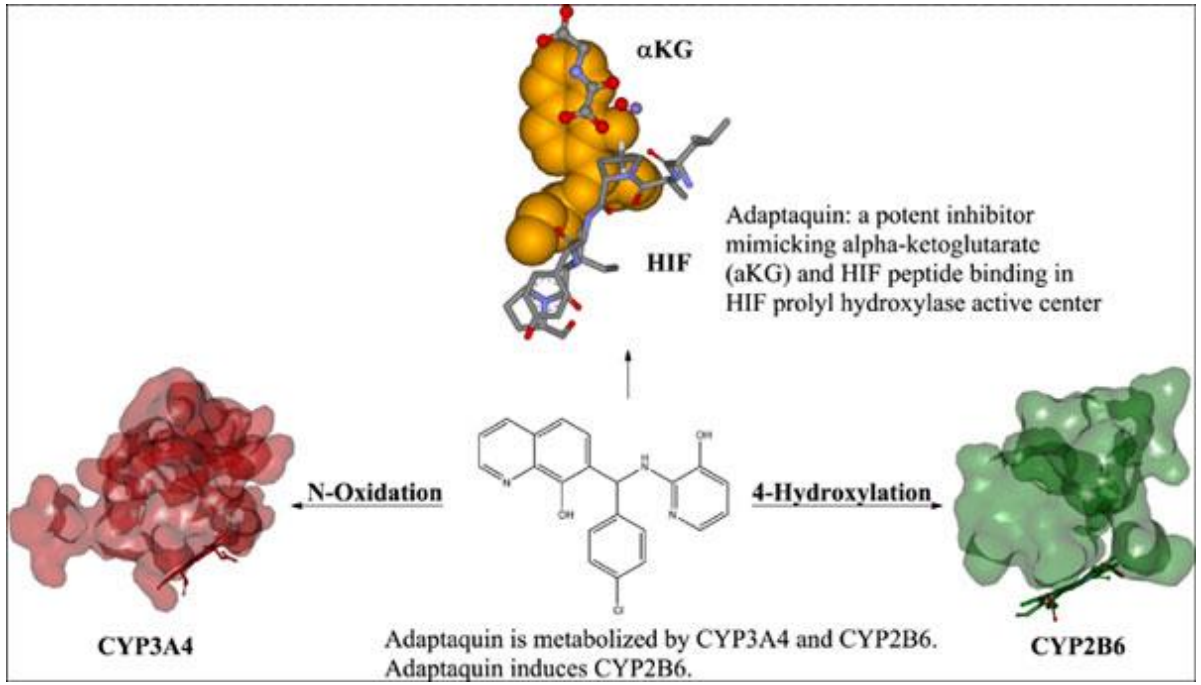


- Увеличение продукции альбумина в 4 раза
- Увеличение уровня экспрессии CYP3A4 в 1.8 раза





ОПРЕДЕЛЕНИЕ МЕТАБОЛИЗМА ПРОИЗВОДНЫХ ОКСИХИНОЛИНА В МОДЕЛИ ПЕЧЕНИ-НА-ЧИПЕ



Drug Metabolism Letters, 2019, *13*, 45-52

RESEARCH ARTICLE

BENTHAM SCIENCE

"Branched Tail" Oxyquinoline Inhibitors of HIF Prolyl Hydroxylase: Early Evaluation of Toxicity and Metabolism Using Liver-on-a-chip

Andrey A. Poloznikov[1,2,*], Sergey V. Nikulin[3], Arpenik A. Zakhariants[4], Anna Y. Khristichenko[1], Dmitry M. Hushpulian[1], Ildar N. Gazizov[5], Vladimir I. Tishkov[4,6,7] and Irina G. Gazaryan[1,4,8]

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ARTICLE HISTORY

Received: June 30, 2018
Revised: October 18, 2018
Accepted: November 07, 2018

DOI:
10.2174/1872312813666181129100950

Abstract: ***Background*:** "Branched tail" oxyquinolines, and adaptaquin in particular, are potent HIF prolyl hydroxylase inhibitors showing promising results in *in vivo* hemorrhagic stroke models. The further improvement of the potency resulted in identification of a number of adaptaquin analogs. Early evaluation of toxicity and metabolism is desired right at the step of lead selection.

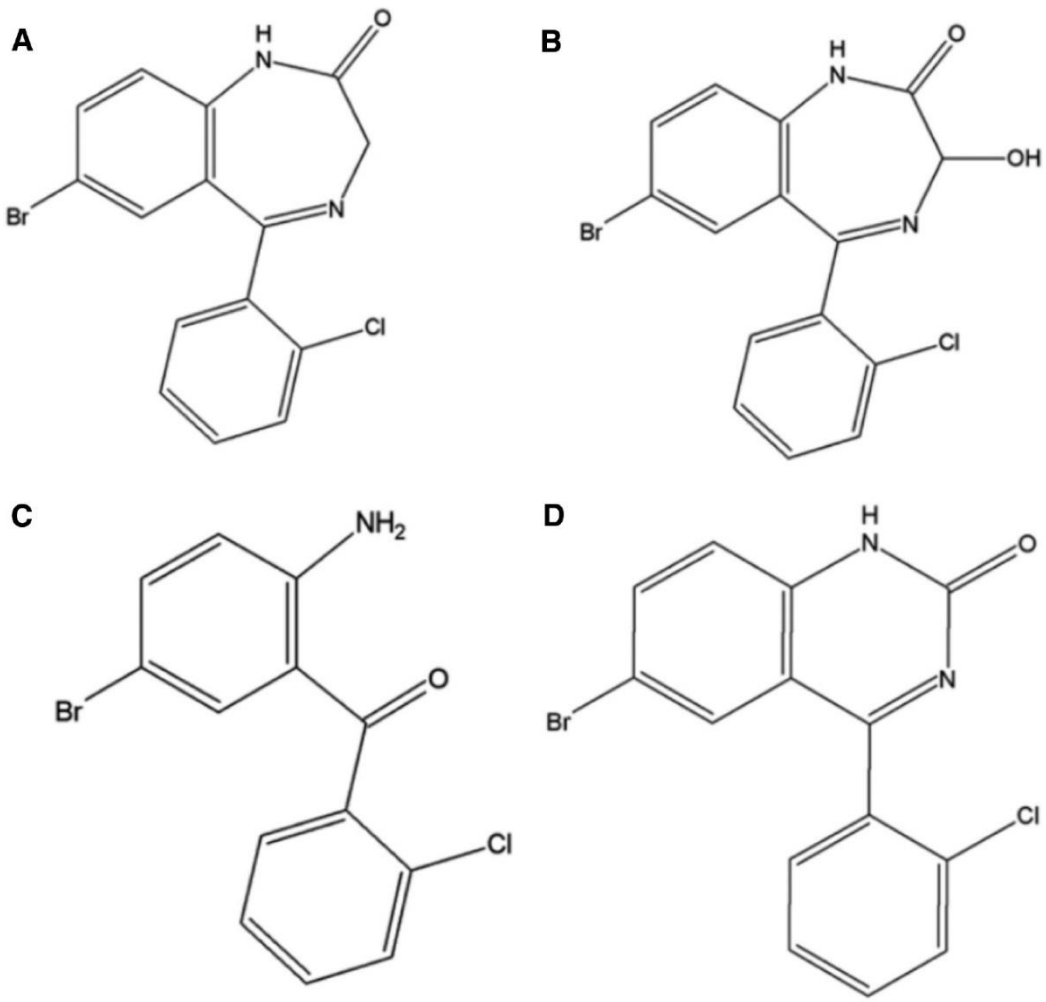
***Objective*:** The aim of the study is to characterize the toxicity and metabolism of adaptaquin and its new improved analogs.

***Method*:** Liver-on-a-chip technology with differentiated *HepaRG* cells followed by LC-MS detection of the studied compounds and metabolites of the P450 substrate-inhibitor panel for CYP2B6, CYP2C9, CYP2C19, and CYP3A4.

***Results*:** The optimized adaptaquin analogs show no toxicity up to a 100-fold increased range over EC_{50}. The drugs are metabolized by CYP3A4 and CYP2B6 as shown with the use of the cytochrome P450 substrate-inhibitor panel designed and optimized for preclinical evaluation of drugs' *in vitro* biotransformation on a 3D human histotypical cell model using "liver-on-a-chip" technology. Activation of CYP2B6 with the drugs tested has been observed. A scheme for adaptaquin oxidative conversion is proposed.

***Conclusion*:** The optimized adaptaquin analogs are suitable for further preclinical trials. Activation of CYP2B6 with adaptaquin and its variants points to a potential increase in Tylenol toxicity if administered together.

Keywords: CYP2B6 induction, early drug-discovery stage, *HepaRG* cells, HIF prolyl hydroxylase inhibitor, adaptaquin, oxyquinolines.



DE GRUYTER

Drug Metabol Pers Ther 2018; 33(2): 65–73

Dmitriy V. Ivashchenko*, Anastasia V. Rudik, Andrey A. Poloznikov, Sergey V. Nikulin, Valeriy V. Smirnov, Alexander G. Tonevitsky, Eugeniy A. Bryun and Dmitriy A. Sychev

Which cytochrome P450 metabolizes phenazepam? Step by step *in silico*, *in vitro*, and *in vivo* studies

https://doi.org/10.1515/dmpt-2017-0036
Received November 18, 2017; accepted February 3, 2018; previously published online May 4, 2018

Abstract

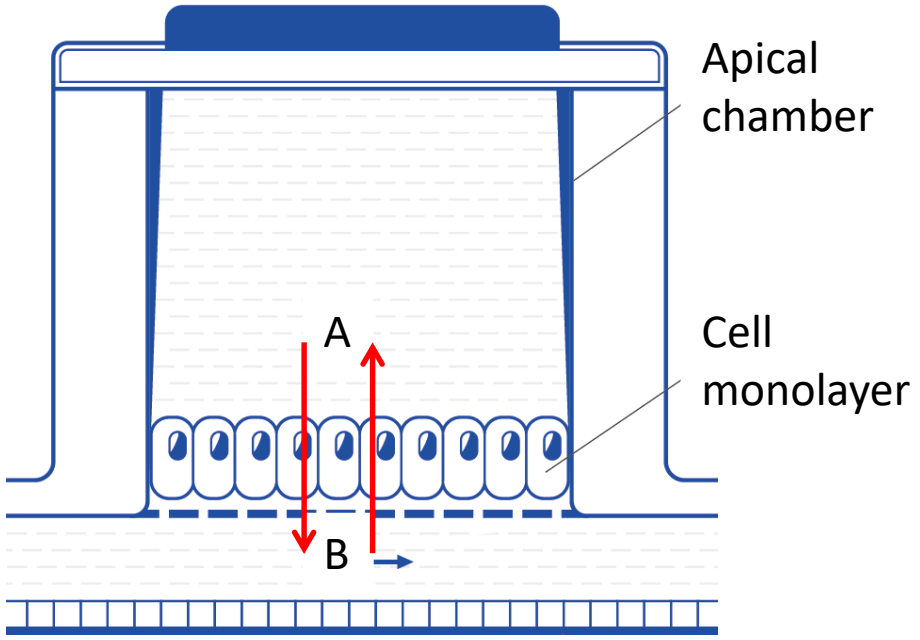
Background: Phenazepam (bromdihydrochlorphenylbenzodiazepine) is the original Russian benzodiazepine tranquilizer belonging to 1,4-benzodiazepines. There is still limited knowledge about phenazepam's metabolic liver pathways and other pharmacokinetic features.
Methods: To determine phenazepam's metabolic pathways, the study was divided into three stages: *in silico* modeling, *in vitro* experiment (cell culture study), and *in vivo* confirmation. *In silico* modeling was performed on the specialized software PASS and GUSAR to evaluate phenazepam molecule affinity to different cytochromes. The *in vitro* study was performed using a hepatocytes' cell culture, cultivated in a microbioreactor to produce cytochrome P450 isoenzymes. The culture medium contained specific cytochrome P450 isoforms inhibitors and substrates (for CYP2C9, CYP3A4, CYP2C19, and CYP2B6) to determine the cytochrome that was responsible for phenazepam's metabolism. We also measured CYP3A activity using the 6-betahydroxycortisol/cortisol ratio in patients.
Results: According to *in silico* and *in vitro* analysis results, the most probable metabolizer of phenazepam is CYP3A4. By the *in vivo* study results, CYP3A activity decreased sufficiently (from 3.8 [95% CI: 2.94–4.65] to 2.79 [95% CI: 2.02–3.55], $p = 0.017$) between the start and finish of treatment in patients who were prescribed just phenazepam.
Conclusions: Experimental *in silico* and *in vivo* studies confirmed that the original Russian benzodiazepine phenazepam was the substrate of CYP3A4 isoenzyme.

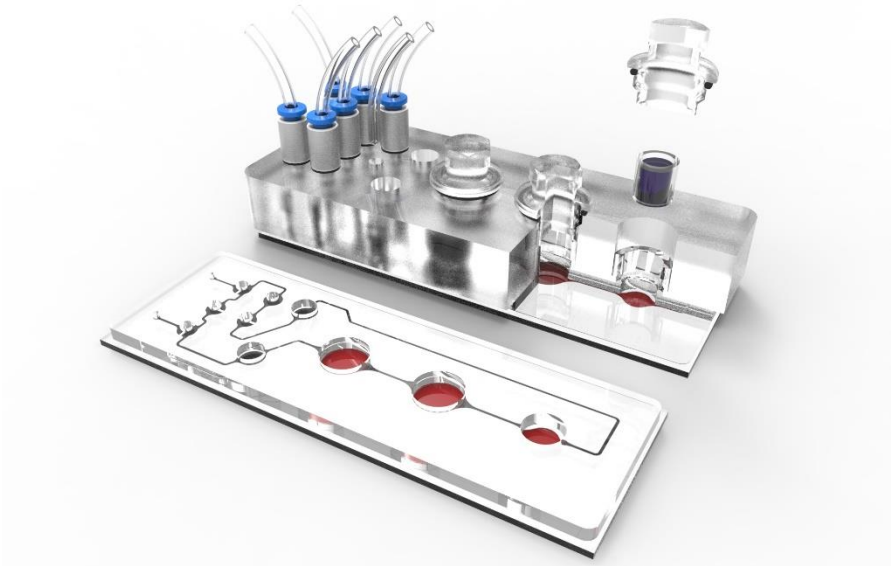
Keywords: benzodiazepine; cell culture; computer modeling; cytochrome P450; metabolic pathway; phenazepam.

2.2 Клеточные модели

Кишечник на чипе

- Монослой клеток Caco-2 на мембране
- Барьерная функция
- Адсорбция
- 20 000 – 30 000 клеток на трансвелл





BioChip J.
DOI 10.1007/s13206-016-0308-3

Original Article

Tumour-like Druggable Gene Expression Pattern of CaCo2 Cells in Microfluidic Chip

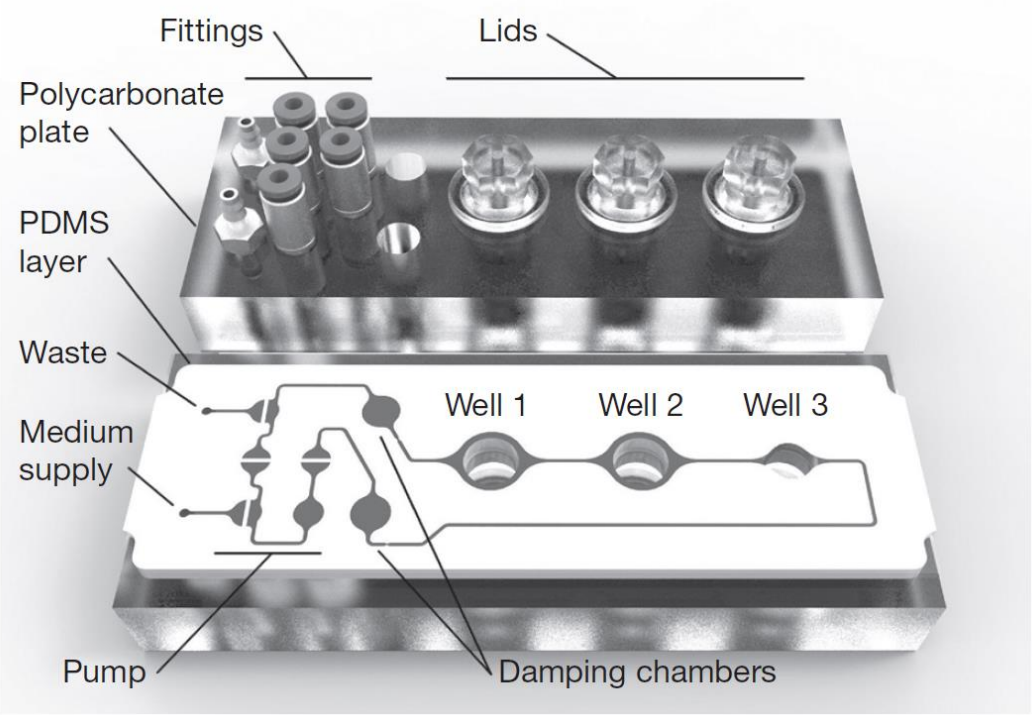
Timur R Samatov[1,2,*], Nadezhda V Senyavina[3], Vladimir V Galatenko[4], Eugene V Trushkin[1], Svetlana A Tonevitskaya[1,2], Dmitriy E Alexandrov[4], Galina P Shibukhova[3], Udo Schumacher[5] & Alexander G Tonevitsky[3,4,*]

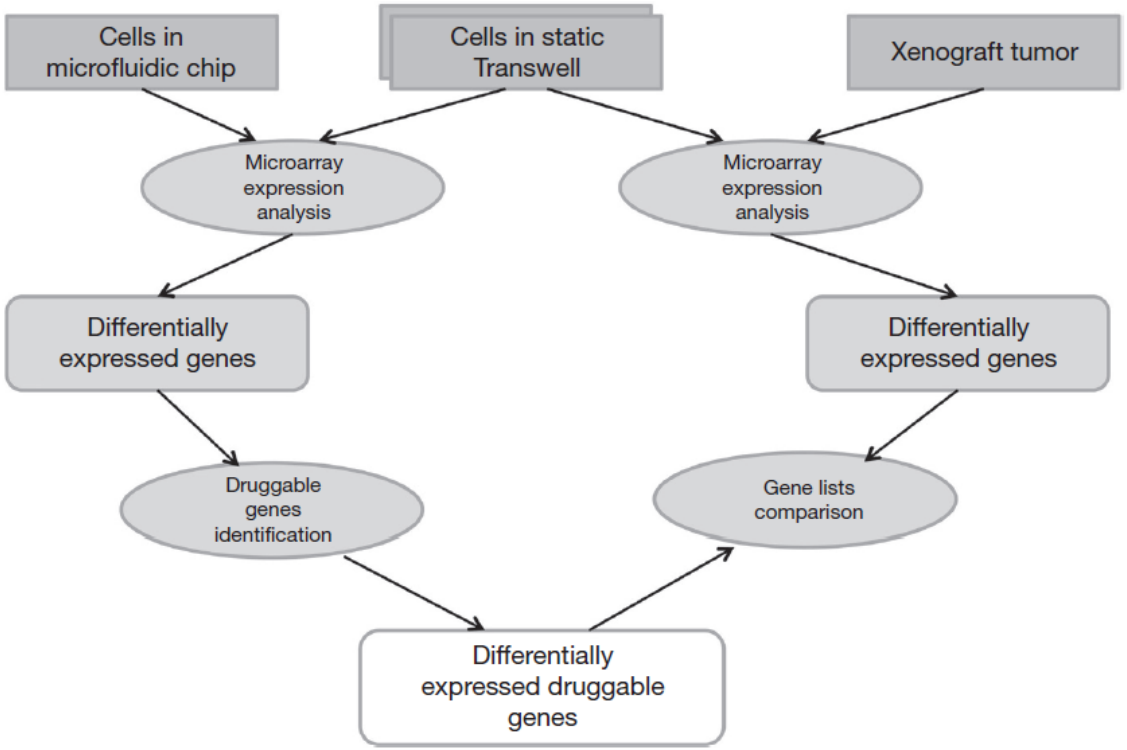
Received: 19 February, 2016 / Accepted: 23 May, 2016 / Published online: 29 July, 2016

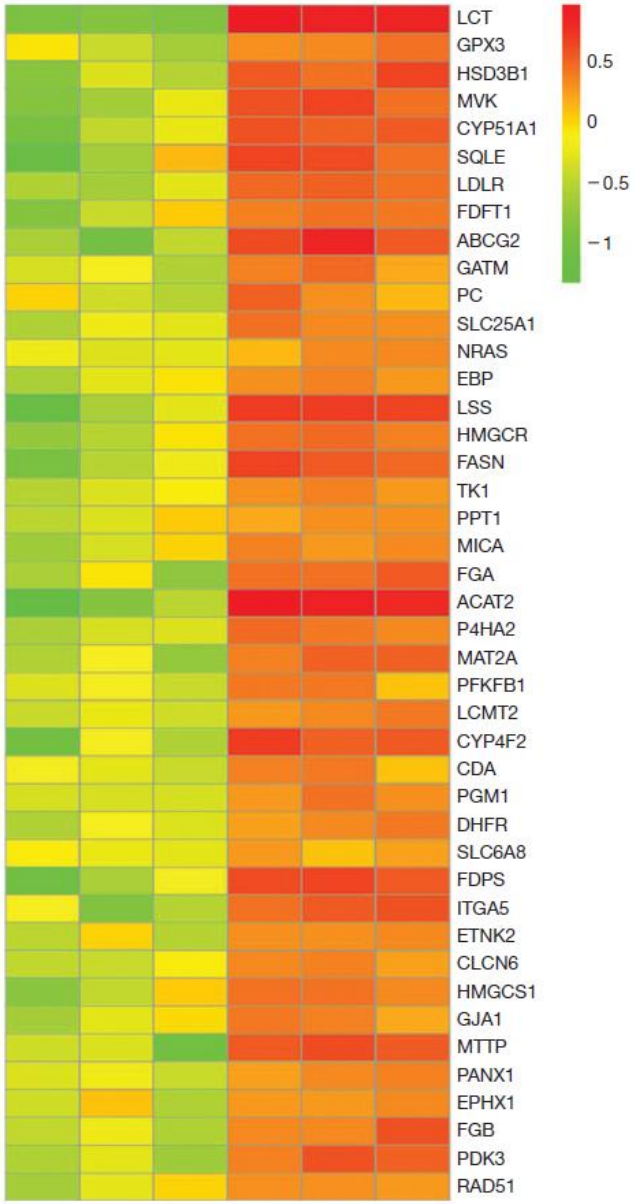
Abstract The human-on-chip technology provides an efficient basis for preclinical studies and has potentially a greater predictive power for human drug response than classical 2D cell culture. Here we report the expression profile of druggable genes in the human colon cancer cells CaCo2 in static culture and within a microfluidic chip. We identified gene expression pattern under flow to be closer to the one of CaCo2 primary xenograft tumours as compared to those cells grown without circulation. The obtained results indicate that a microenvironment connected to a circulation within a chip brings the cells closer to *in vivo* situation. Hence the human-on-chip technology is a more powerful tool for drug development than conventional 2D cell culture.

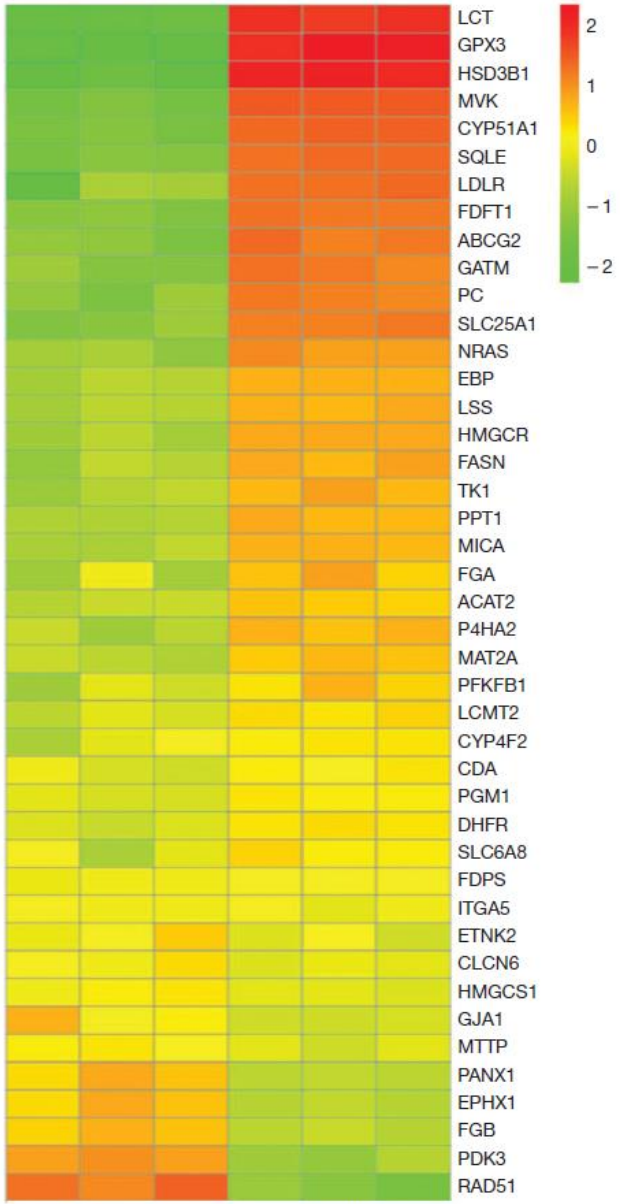
Introduction

Microfluidic organotypic chips are aimed to emulate human physiology on a small scale. These devices are capable of culturing the cells under continuous perfusion and physiological shear stress recapitulating functions of the cells in culture under specific tissue- and organ-level circulatory conditions[1]. PDMS (polydimethylsiloxane) is among the most popular materials used for microfluidic chip fabrication since it is compatible with living cells and provides for the flexibility in the design of the devices[2]. To date these systems are reported to be used to study cancer progression as they are able to model combinations of the interrelated metastatic steps, EMT plasticity and proliferation[3]. The




Cells in microfluidic chip
Cells in static Transwell
Xenograft tumor
Microarray expression analysis
Microarray expression analysis
Differentially expressed genes
Differentially expressed genes
Druggable genes identification
Gene lists comparison
Differentially expressed druggable genes


LCT
GPX3
HSD3B1
MVK
CYP51A1
SQLE
LDLR
FDFT1
ABCG2
GATM
PC
SLC25A1
NRAS
EBP
LSS
HMGCR
FASN
TK1
PPT1
MICA
FGA
ACAT2
P4HA2
MAT2A
PFKFB1
LCMT2
CYP4F2
CDA
PGM1
DHFR
SLC6A8
FDPS
ITGA5
ETNK2
CLCN6
HMGCS1
GJA1
MTTP
PANX1
EPHX1
FGB
PDK3
RAD51
0.5
0
−0.5
−1
Chip
Static


LCT
GPX3
HSD3B1
MVK
CYP51A1
SQLE
LDLR
FDFT1
ABCG2
GATM
PC
SLC25A1
NRAS
EBP
LSS
HMGCR
FASN
TK1
PPT1
MICA
FGA
ACAT2
P4HA2
MAT2A
PFKFB1
LCMT2
CYP4F2
CDA
PGM1
DHFR
SLC6A8
FDPS
ITGA5
ETNK2
CLCN6
HMGCS1
GJA1
MTTP
PANX1
EPHX1
FGB
PDK3
RAD51
2
1
0
−1
−2
Xenograft
Static

ИМПЕДАНС

Интерфейс передачи данных на ПК

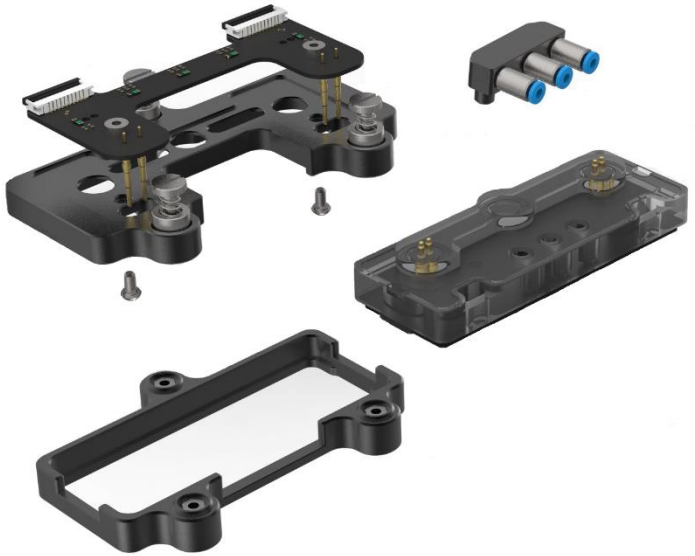
Интерфейс подключения к периферийным устройствам

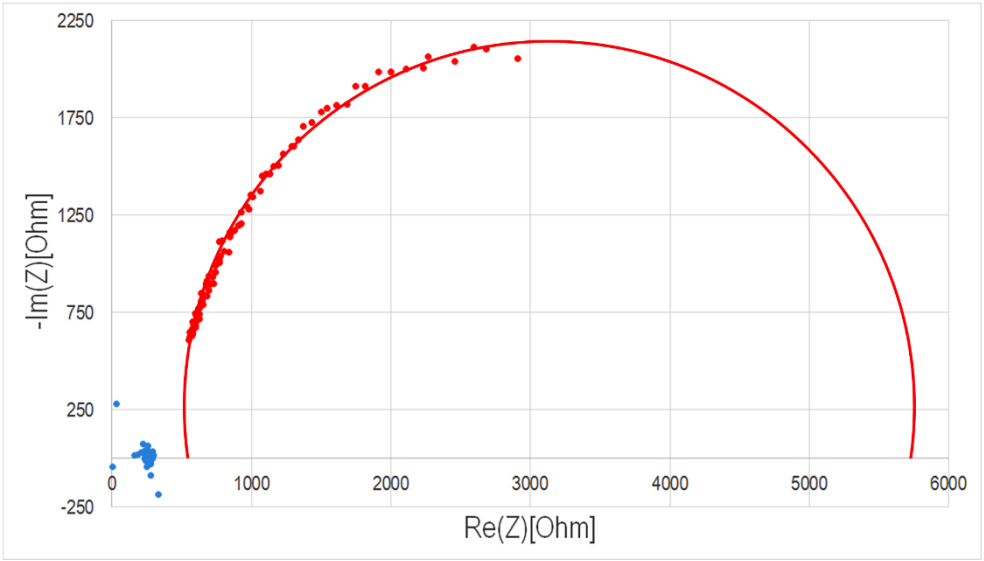
OLED дисплей отображения настроек прибора

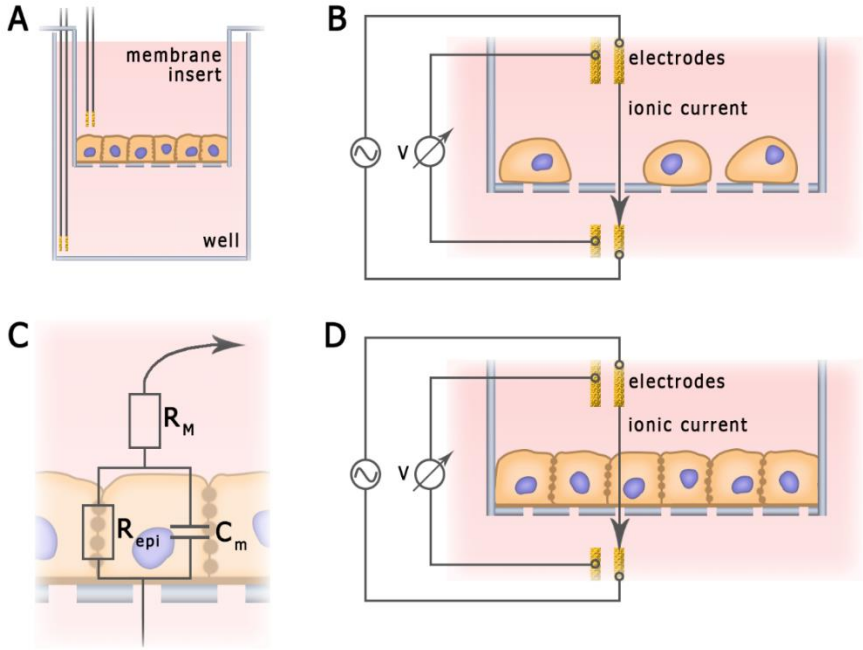
Интерфейс подключения измерительных систем

OLED дисплей отображения показаний прибора

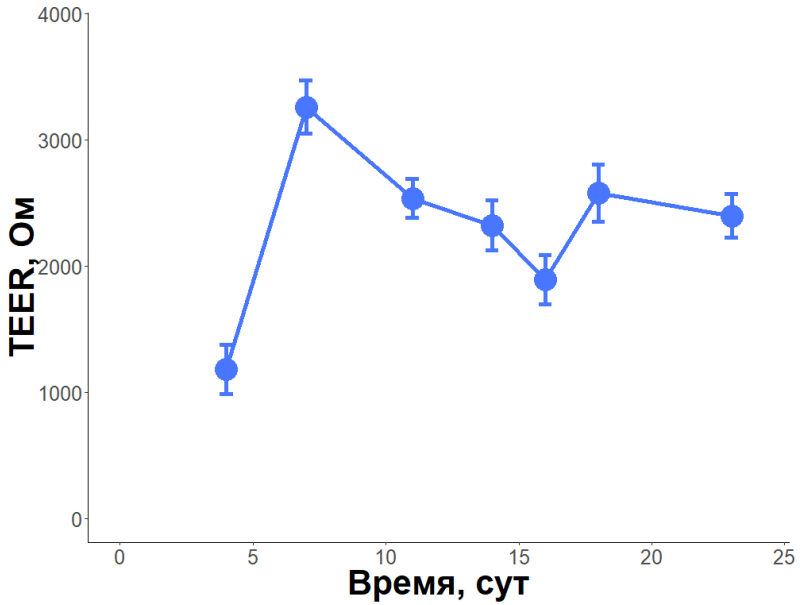
Элемент навигации

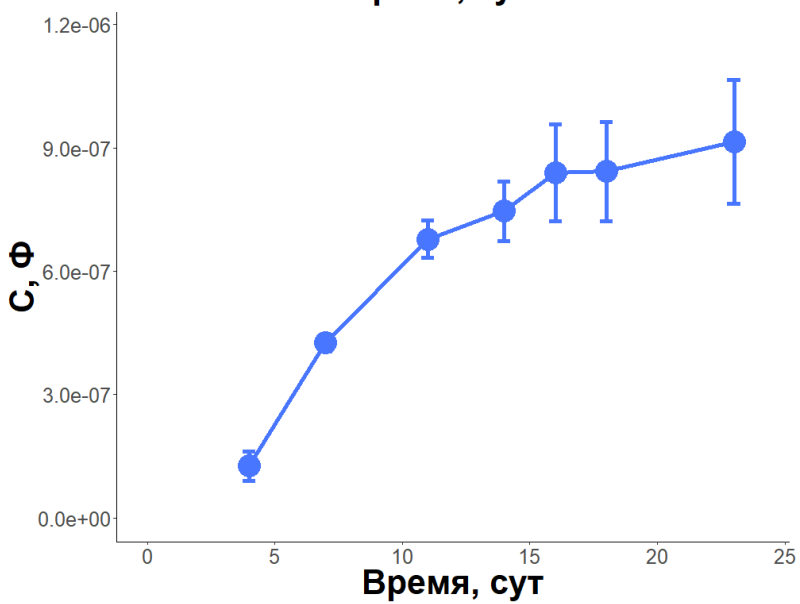


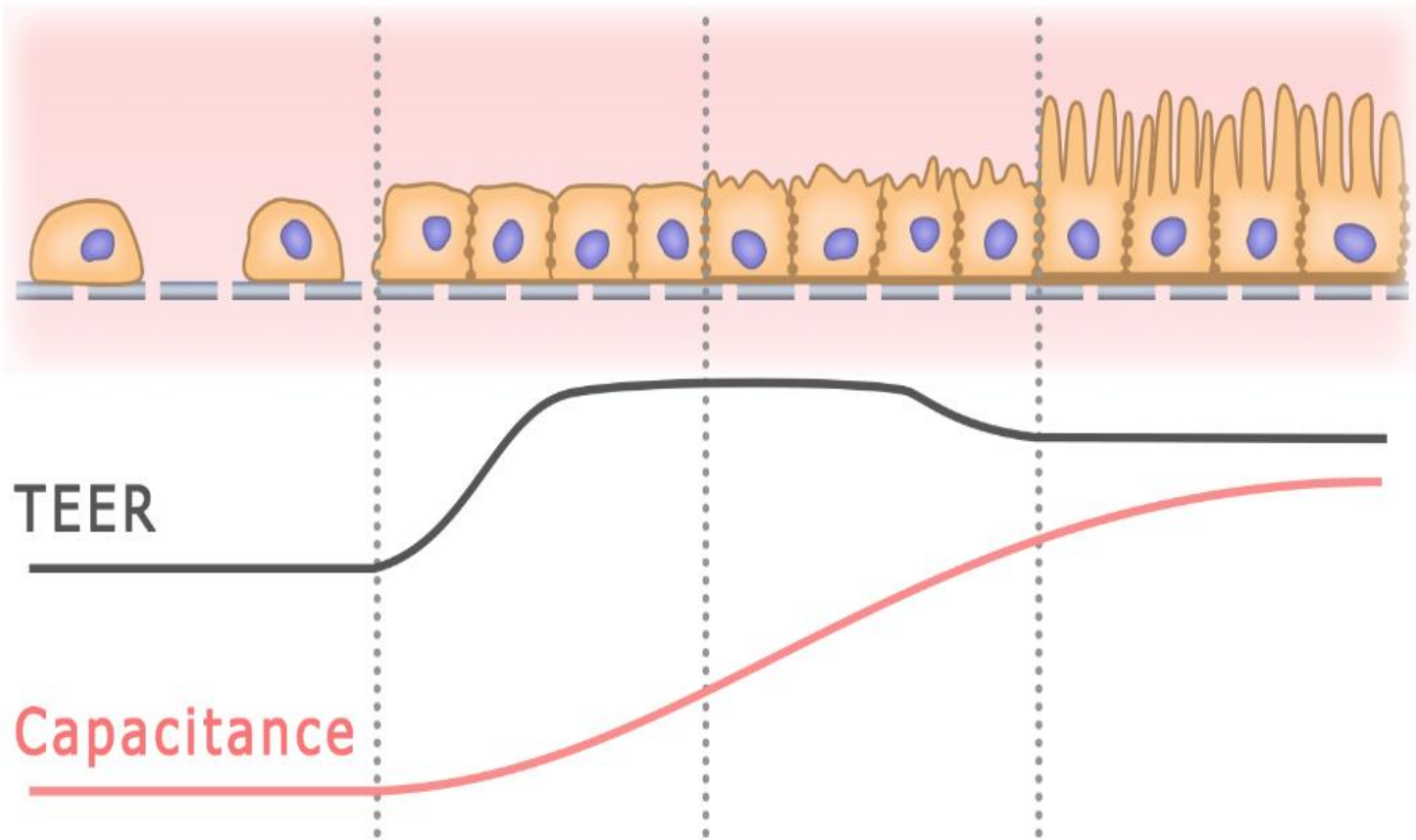




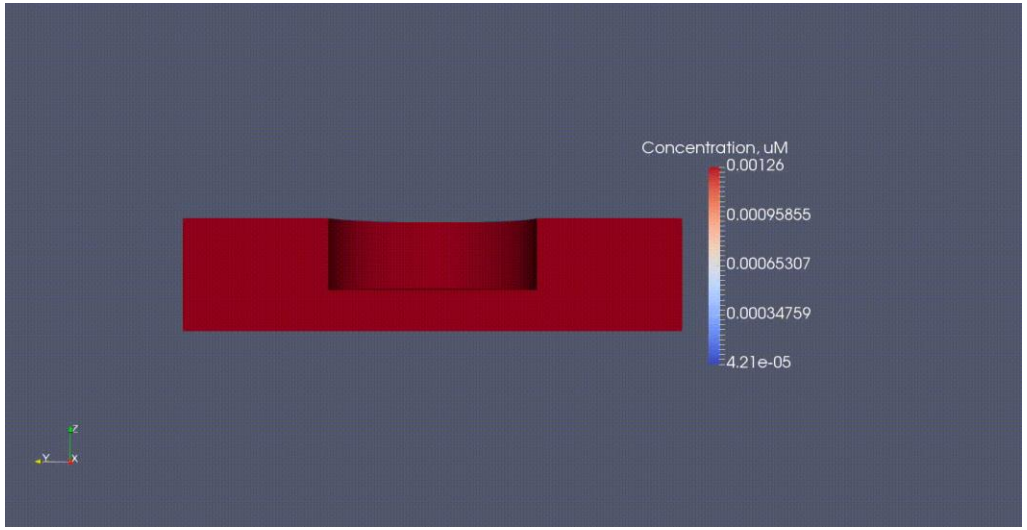
ИМПЕДАНС

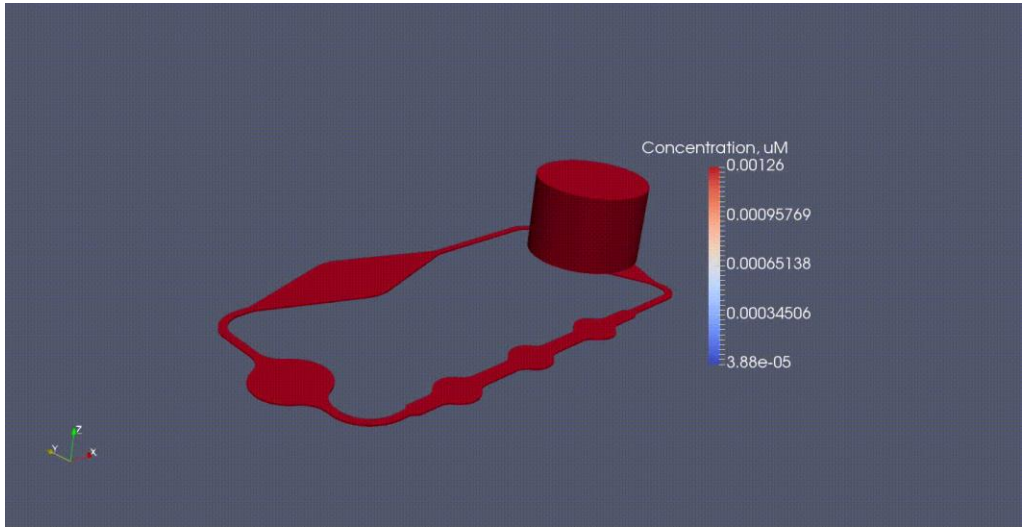

TEER, Ом
4000
3000
2000
1000
0
0
5
10
15
20
25
Время, сут


C, Ф
1.2e-06
9.0e-07
6.0e-07
3.0e-07
0.0e+00
0
5
10
15
20
25
Время, сут


TEER
Capacitance

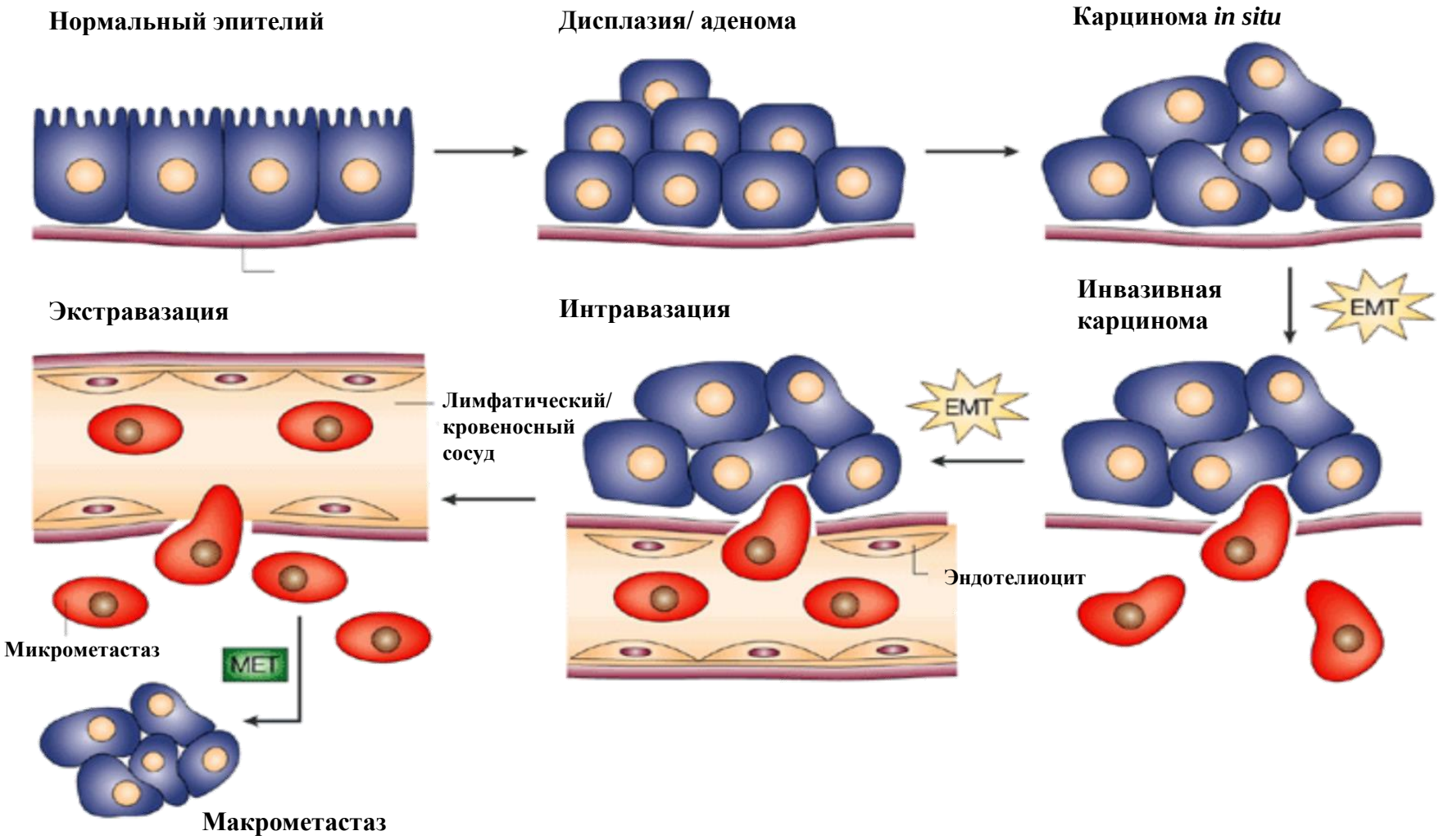
ПОГЛОЩЕНИЕ В СТАТИЧЕСКИХ И ДИНАМИЧЕСКИХ УСЛОВИЯХ

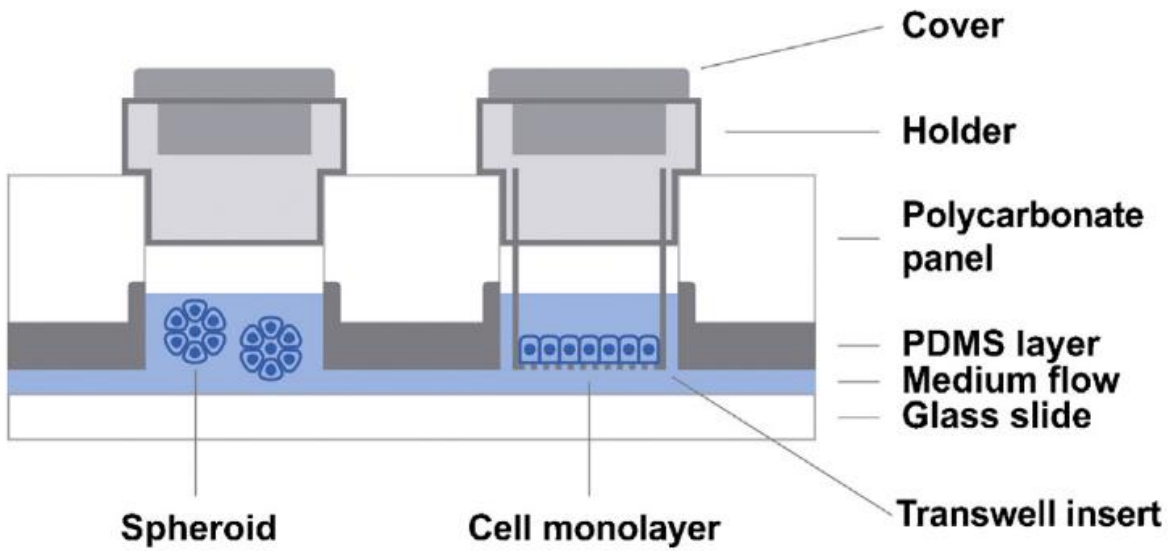




2.3 Клеточные модели

Метастаз на чипе


Нормальный эпителий
Дисплазия/ аденома
Карцинома *in situ*
Инвазивная карцинома
EMT
Интравазация
EMT
Эндотелиоцит
Лимфатический/ кровеносный сосуд
Экстравазация
Микрометастаз
MET
Макрометастаз



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Progress in Histochemistry and Cytochemistry 49 (2015) 21–29

PROGRESS IN HISTOCHEMISTRY AND CYTOCHEMISTRY

www.elsevier.de/proghi

Review

Modelling the metastatic cascade by in vitro microfluidic platforms

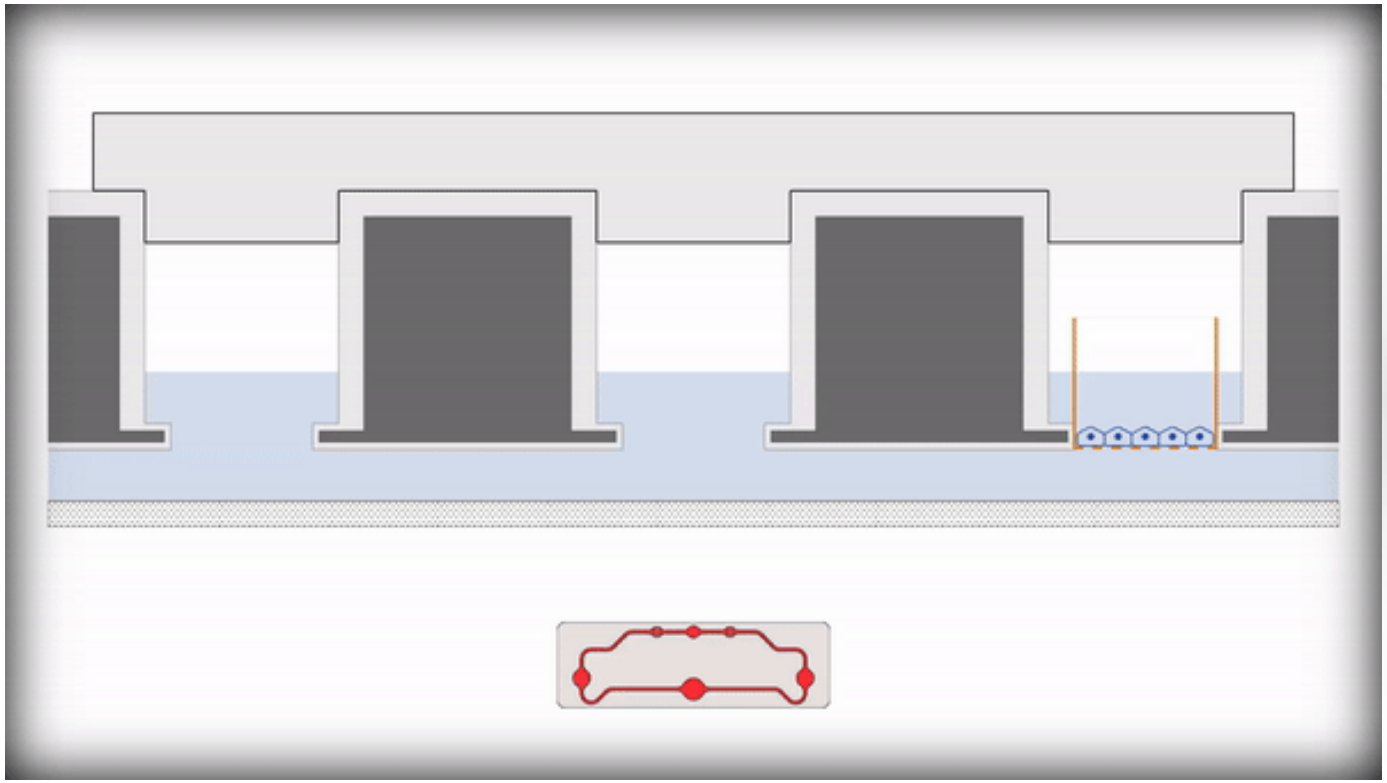
Timur R. Samatov [a,b,*], Maxim U. Shkurnikov [c], Svetlana A. Tonevitskaya [b], Alexander G. Tonevitsky [c,d,*]

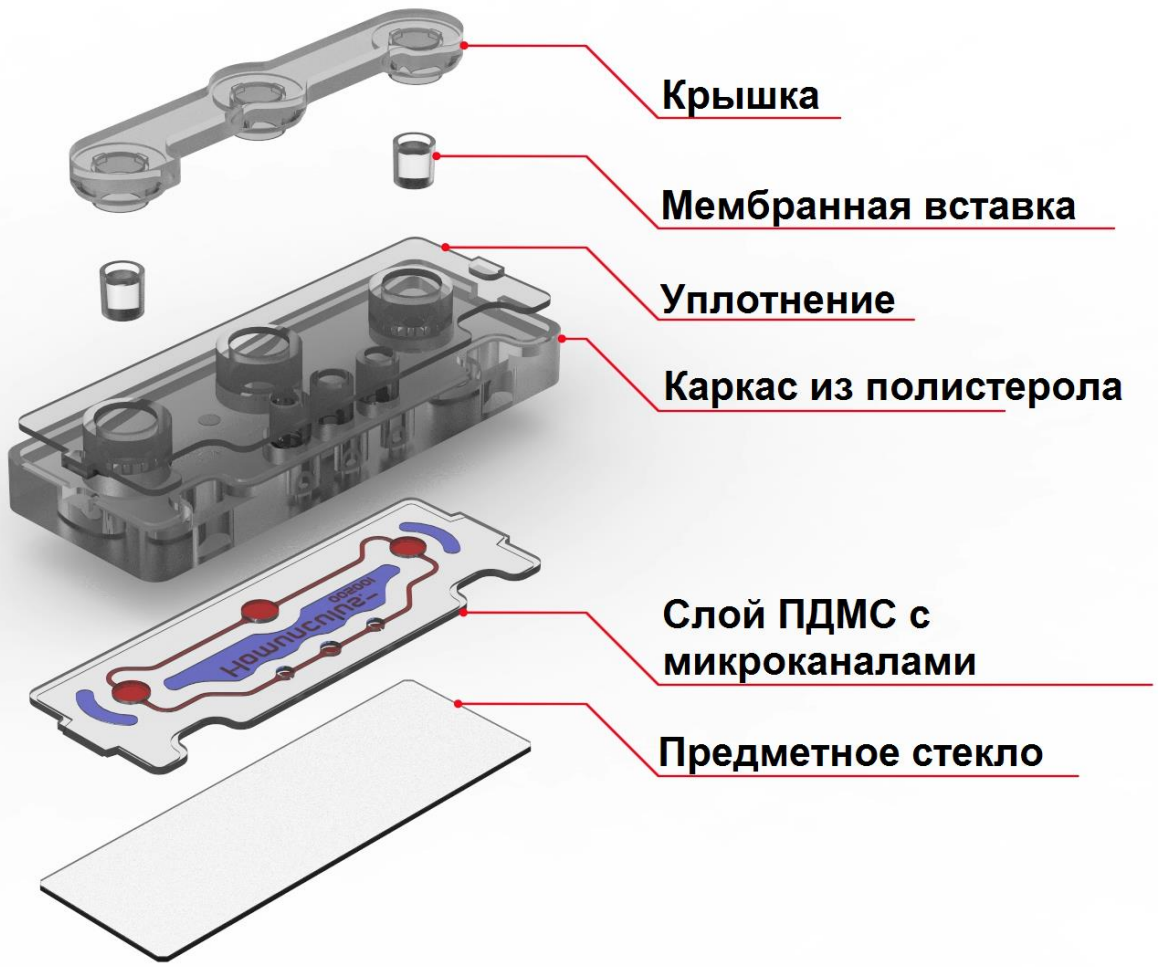
[a] *SRC Bioclinicum, Ugreshskaya str 2/85, 115088 Moscow, Russia*
[b] *Moscow State University of Mechanical Engineering, Bolshaya Semenovskaya str 38, 107023 Moscow, Russia*
[c] *Hertsen Federal Medical Research Centre of the Ministry of Health of the Russian Federation, Koroleva str 4, 249036 Obninsk, 5 Russia*
[d] *Moscow State University, Leninskie Gory, 119991 Moscow, Russia*

Received 22 January 2015; accepted 22 January 2015

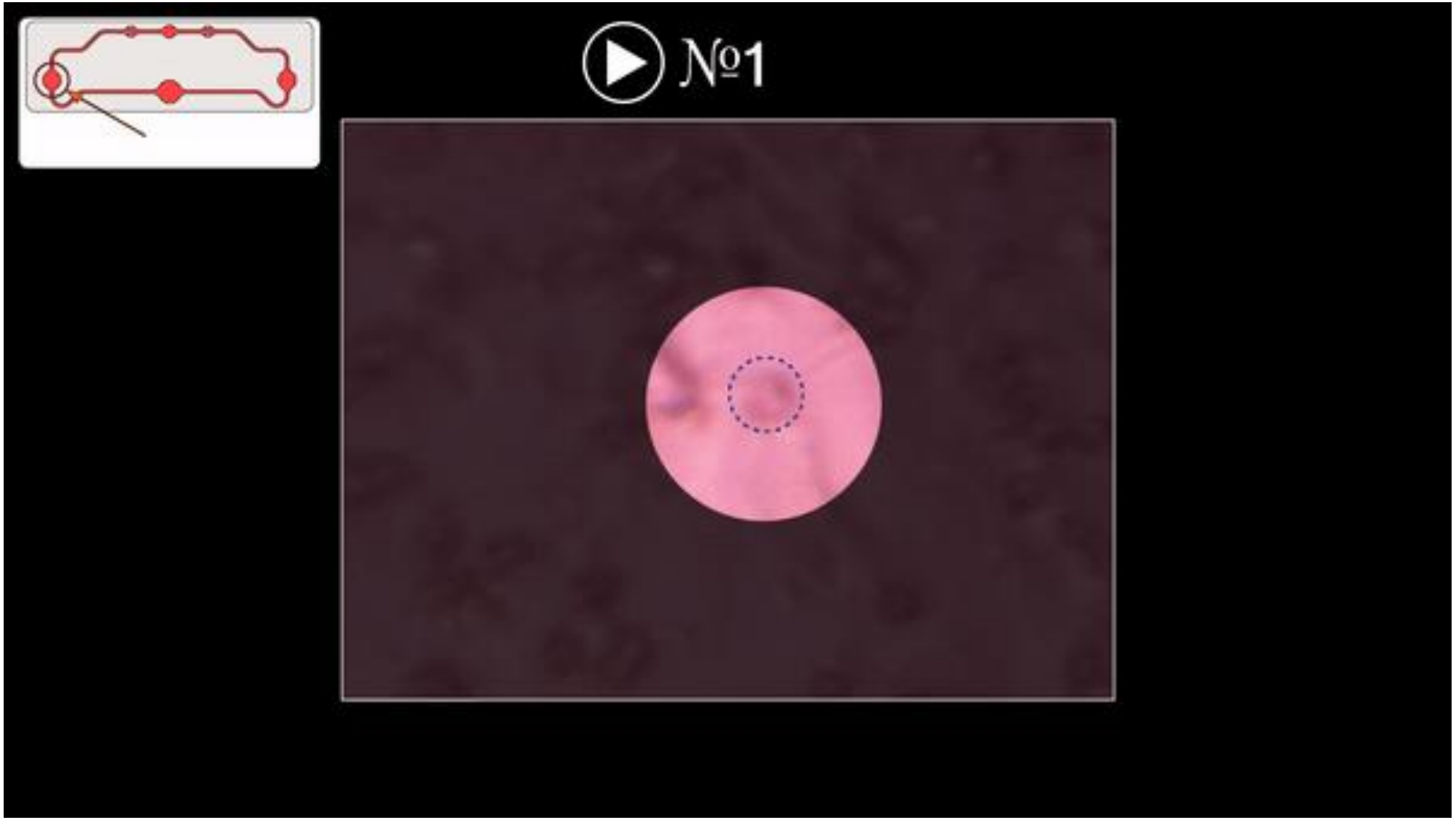
Abstract

The metastatic cascade comprises the following steps in sequential manner: the future metastatic cell has to leave the primary tumor mass, degrade the surrounding extracellular matrix, extravasate and circulate within in the bloodstream. Thereafter it has to attach to the endothelium of a target organ, intravasate into the connective tissue and has to proliferate to form a clinically detectable metastasis.

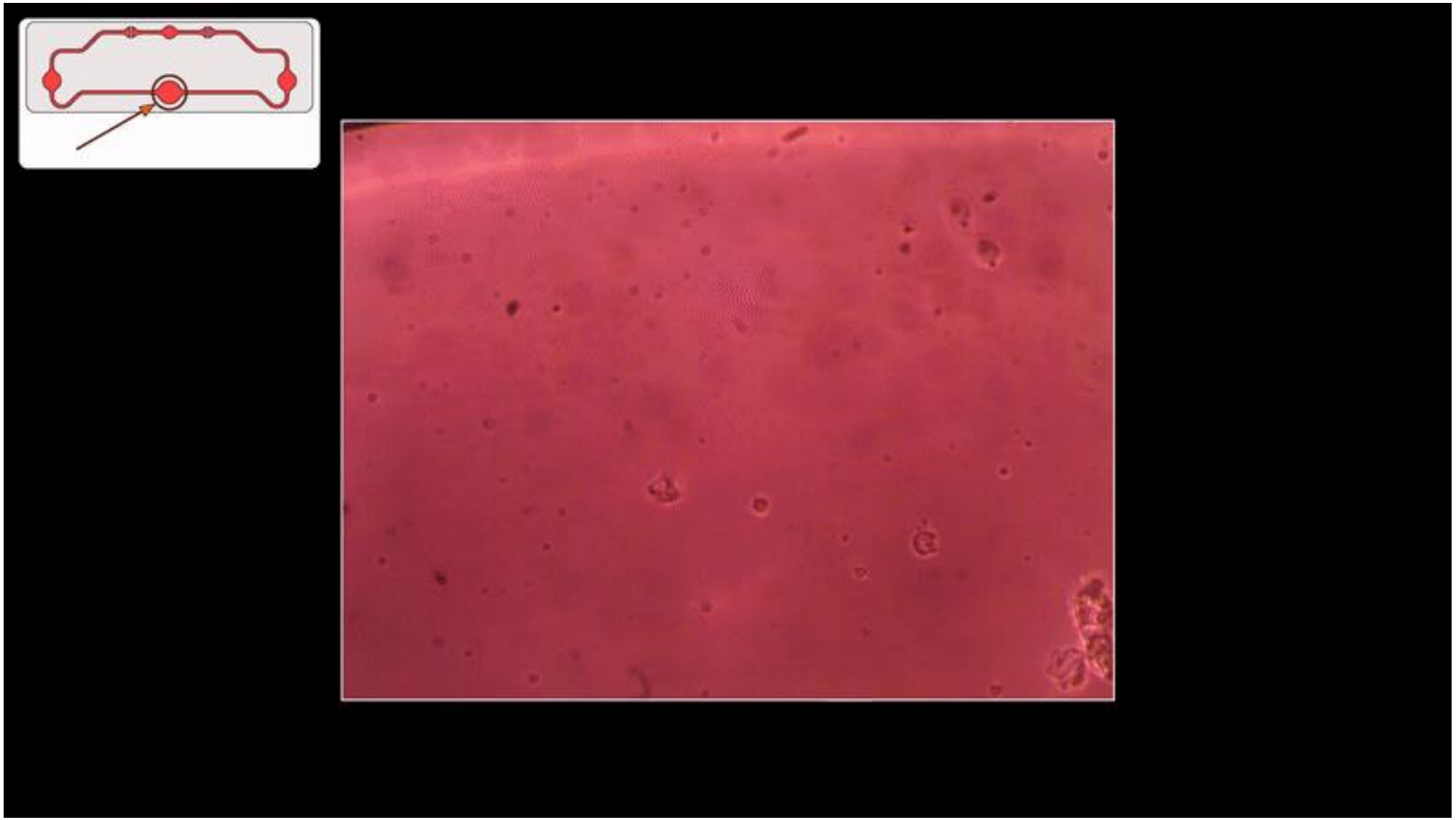



Крышка
Мембранная вставка
Уплотнение
Каркас из полистерола
Слой ПДМС с микроканалами
Предметное стекло

МЕТАСТАЗ НА ЧИПЕ (МЕМБРАНА)



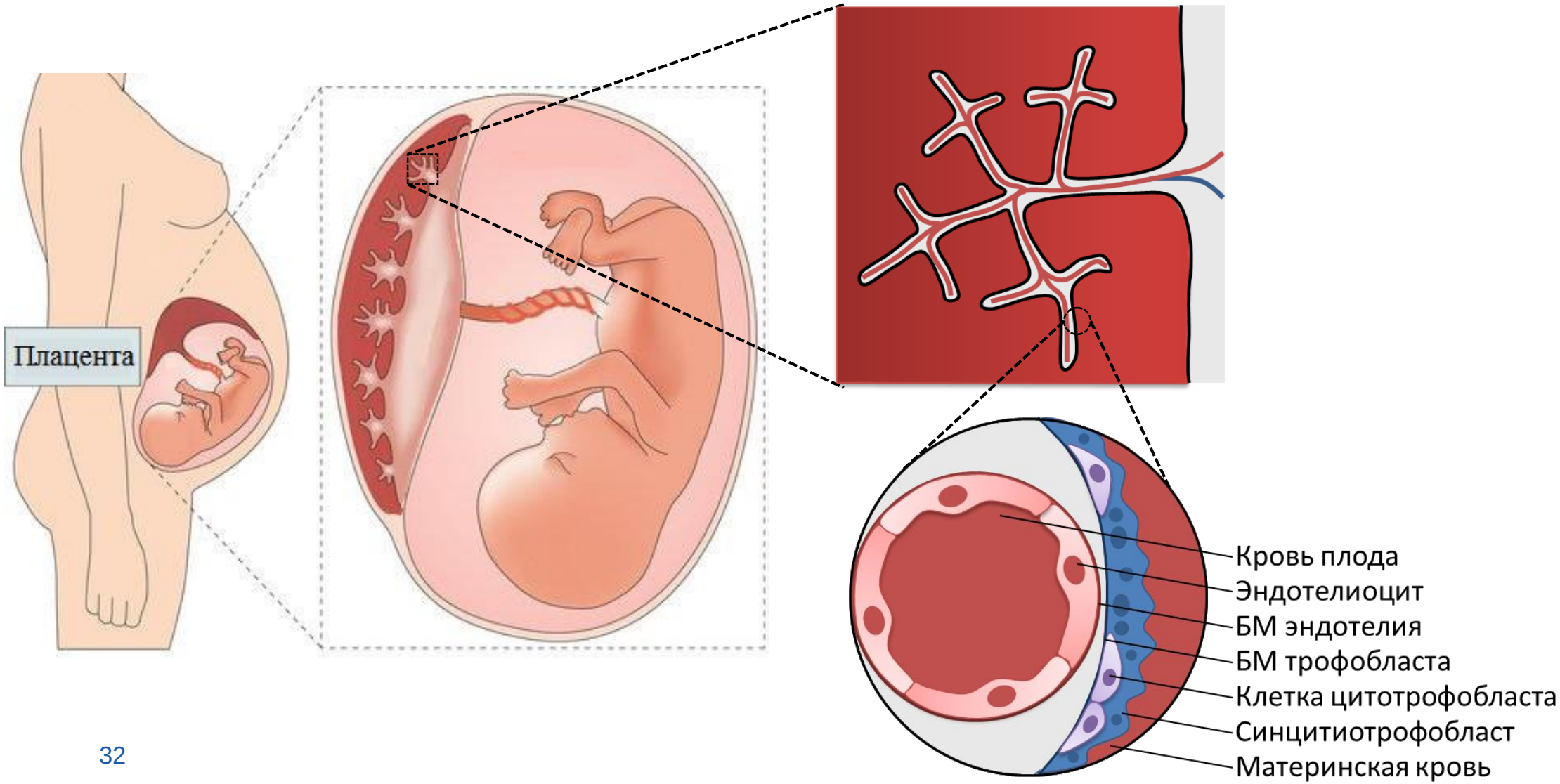
МЕТАСТАЗ НА ЧИПЕ (ЦЕНТРАЛЬНАЯ ЛУНКА)

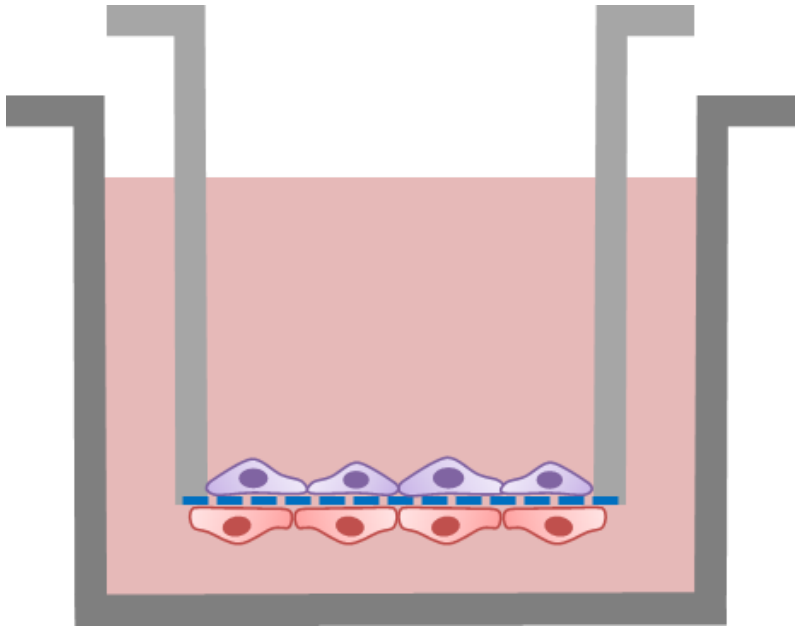


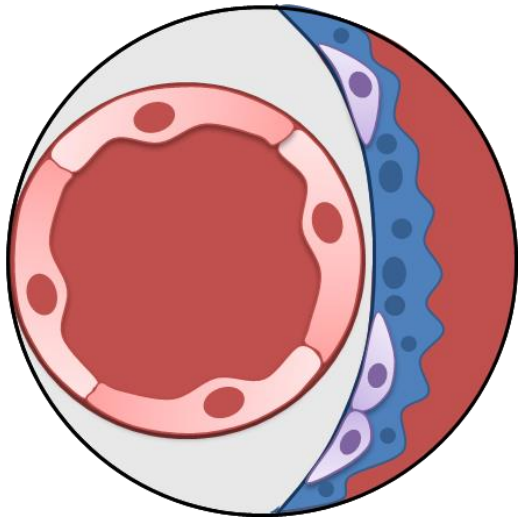
Homunculus-

2.4 Клеточные модели

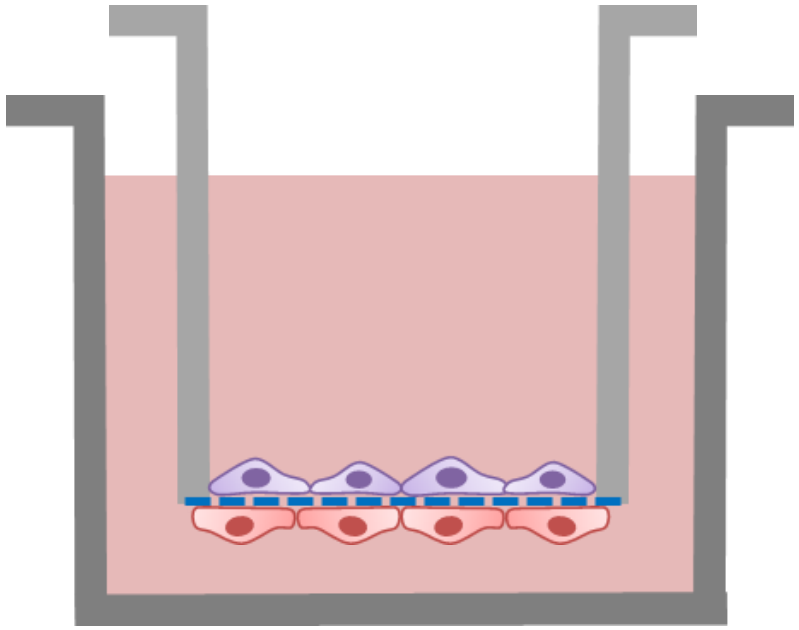
Плацента на чипе

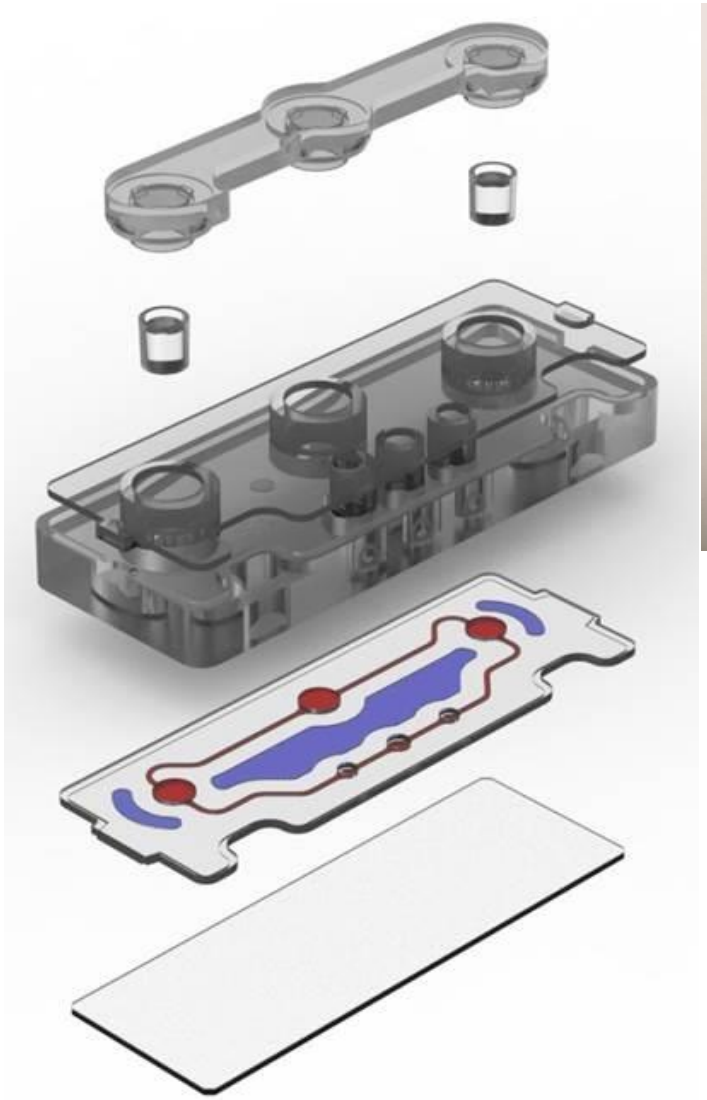

Плацента
Кровь плода
Эндотелиоцит
БМ эндотелия
БМ трофобласта
Клетка цитотрофобласта
Синцитиотрофобласт
Материнская кровь

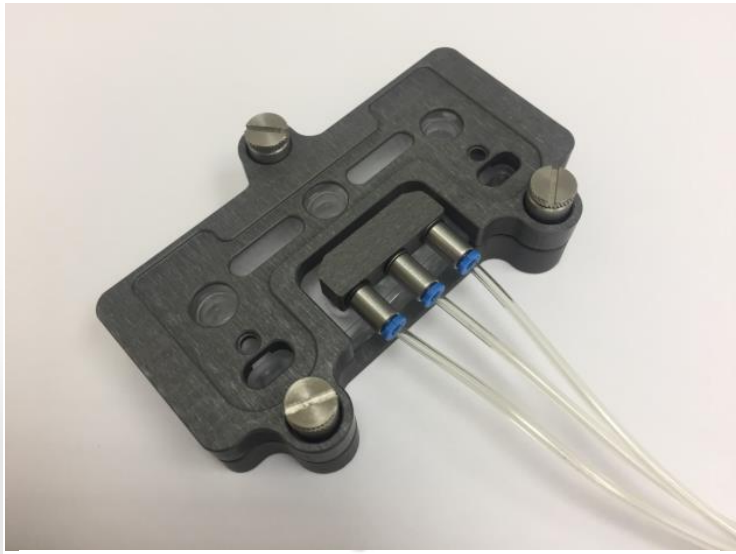


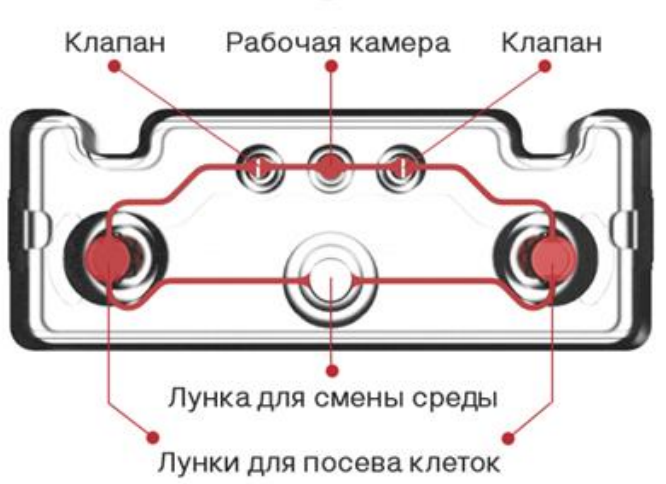


ПЛАЦЕНТА НА ЧИПЕ

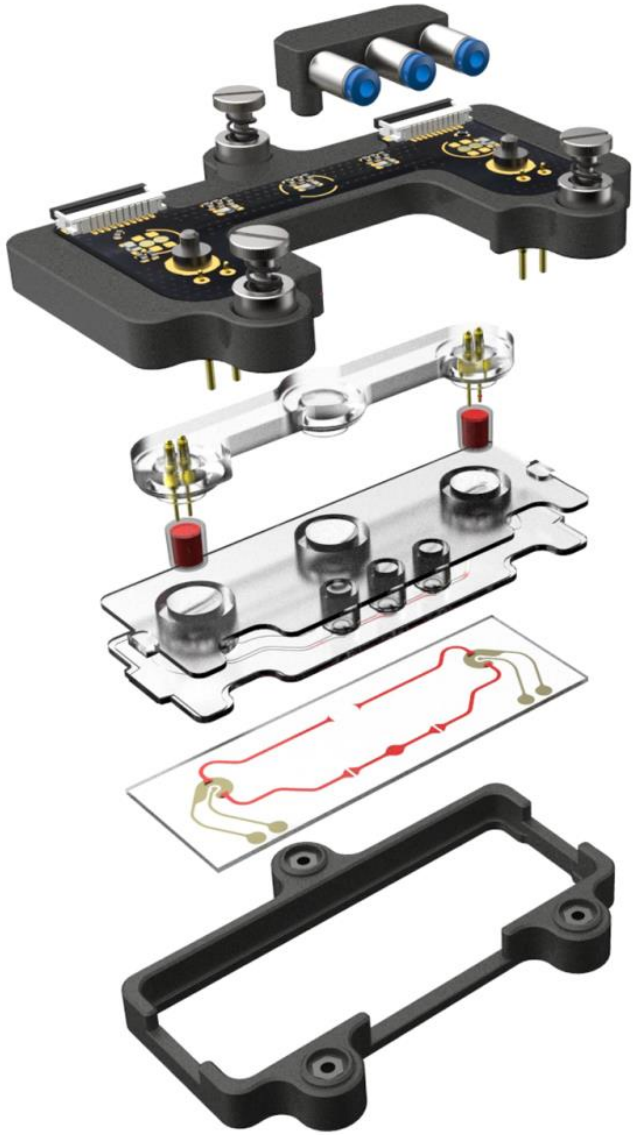


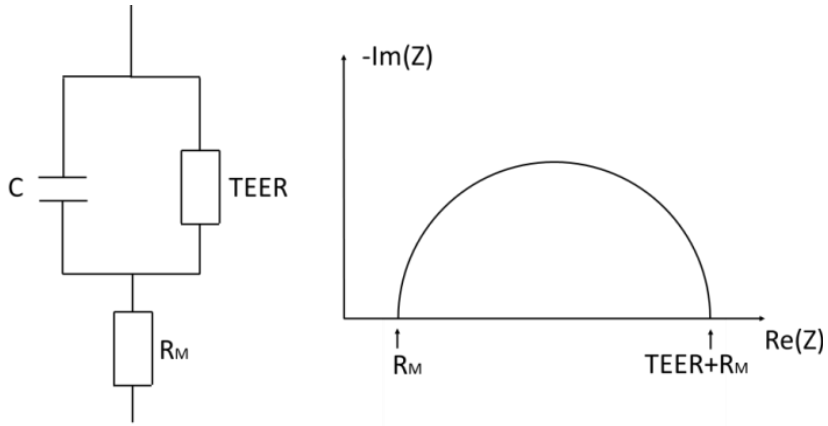


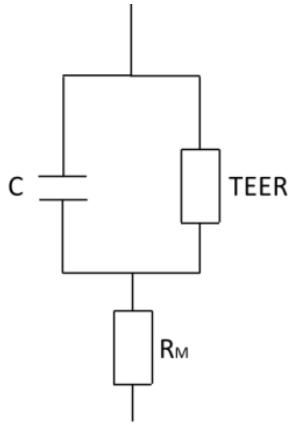


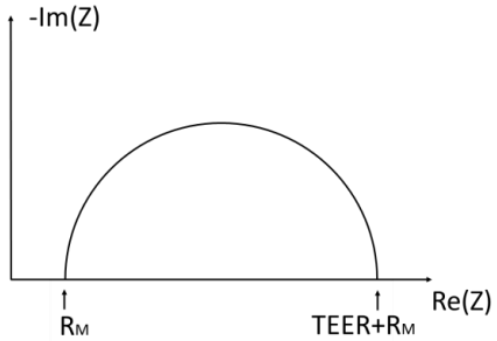


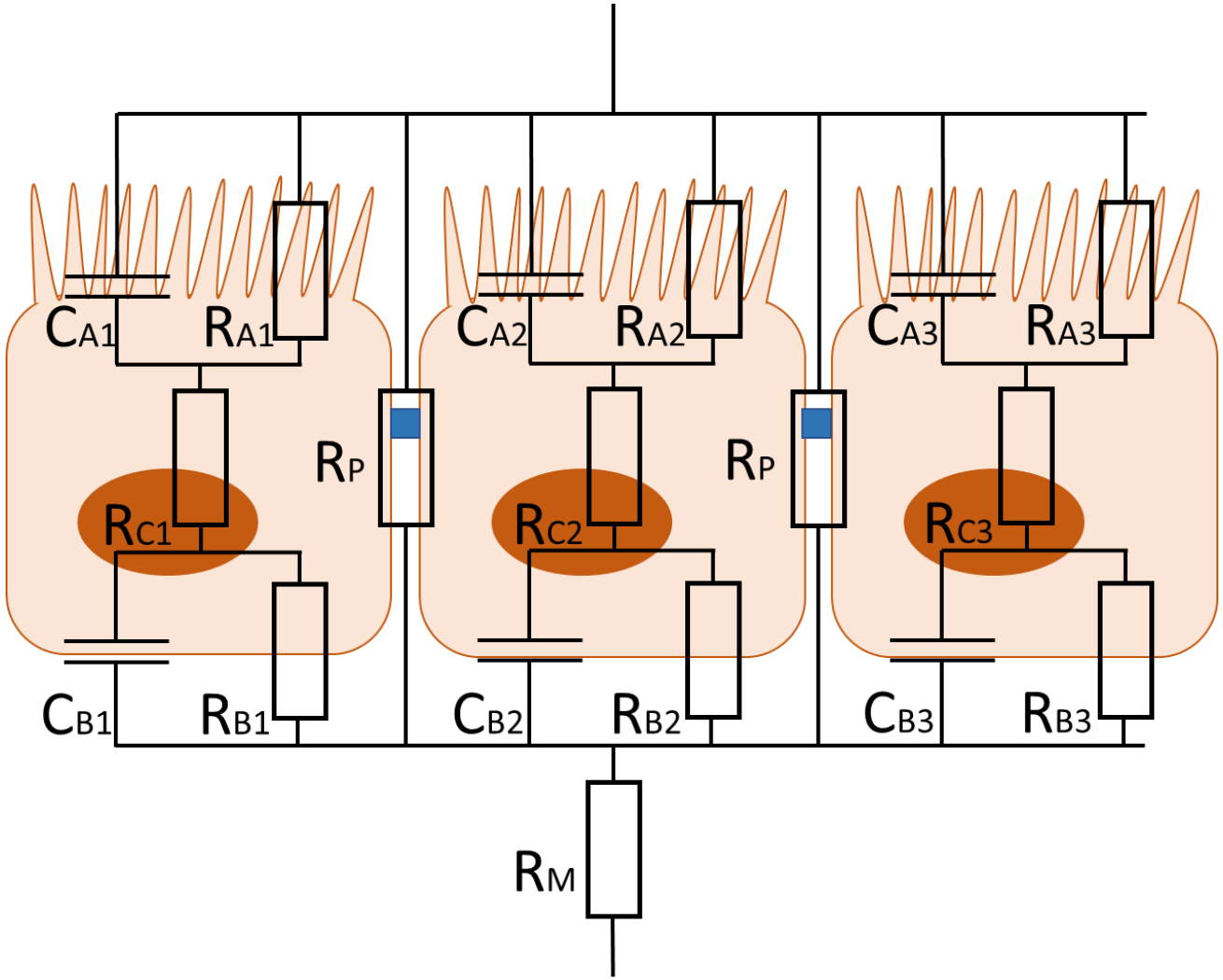
ИЗМЕРЕНИЕ ИМПЕДАНСА

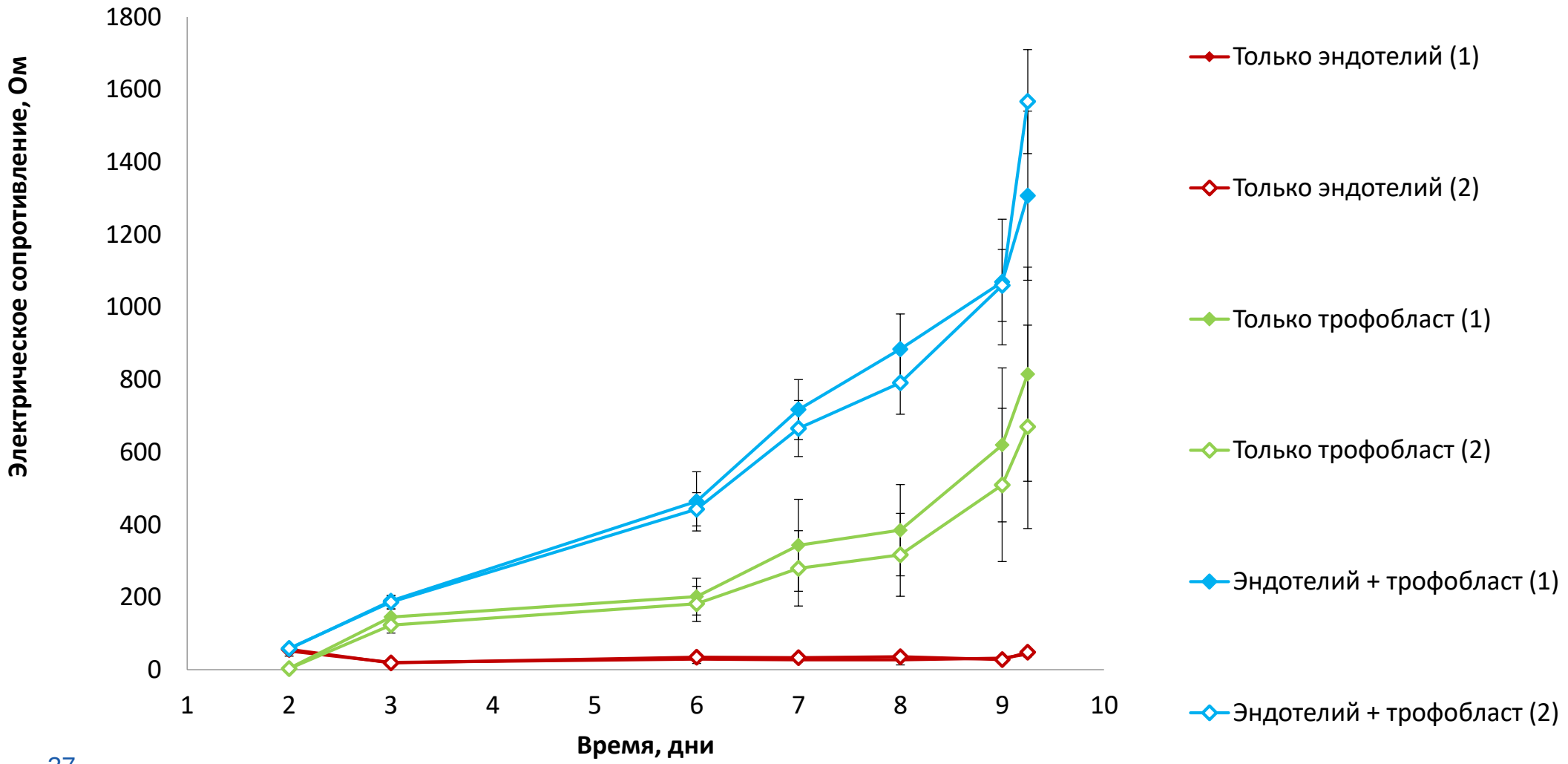


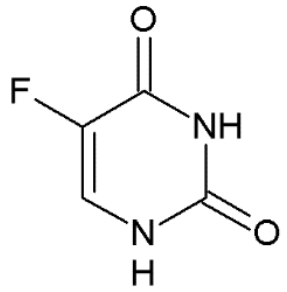



C
TEER
RM


-Im(Z)
Re(Z)
RM
TEER+RM

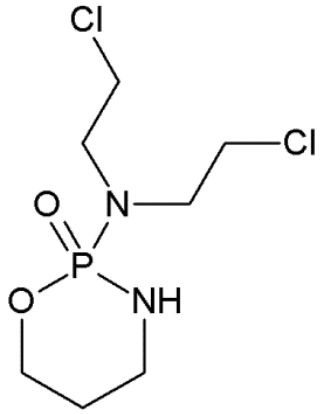

CA1
RA1
CA2
RA2
CA3
RA3
RP
RP
RC1
RC2
RC3
CB1
RB1
CB2
RB2
CB3
RB3
RM


Электрическое сопротивление, Ом
1800
1600
1400
1200
1000
800
600
400
200
0
1
2
3
4
5
6
7
8
9
10
Время, дни
Только эндотелий (1)
Только эндотелий (2)
Только трофобласт (1)
Только трофобласт (2)
Эндотелий + трофобласт (1)
Эндотелий + трофобласт (2)



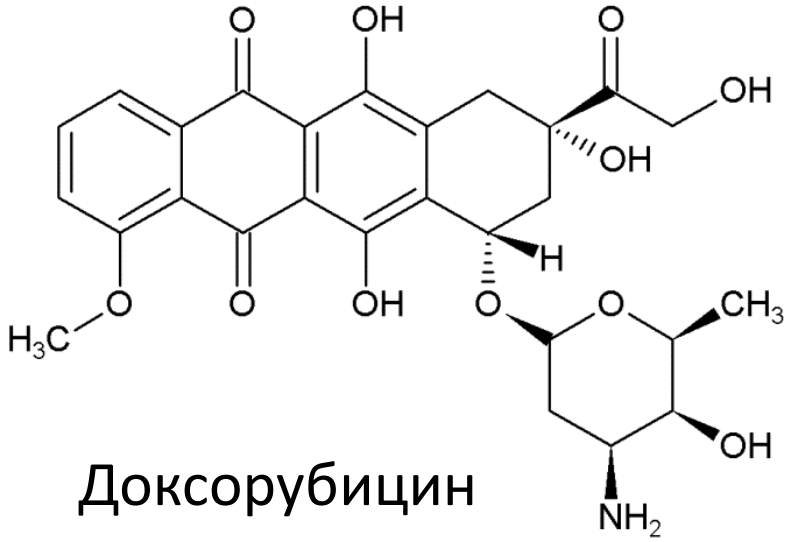
5-фторурацил

- Гидрофильная молекула
- M_r = 130,1 Да



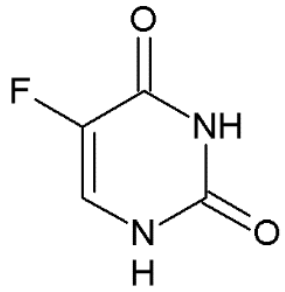
Циклофосфамид

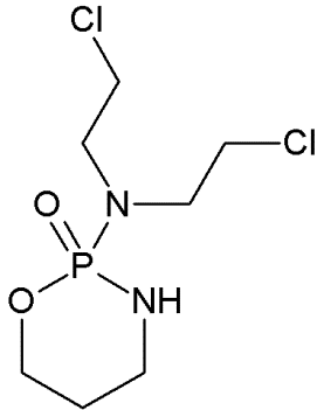
- Липофильная молекула
- Низкая степень связывания с белками
- M_r = 261,1 Да

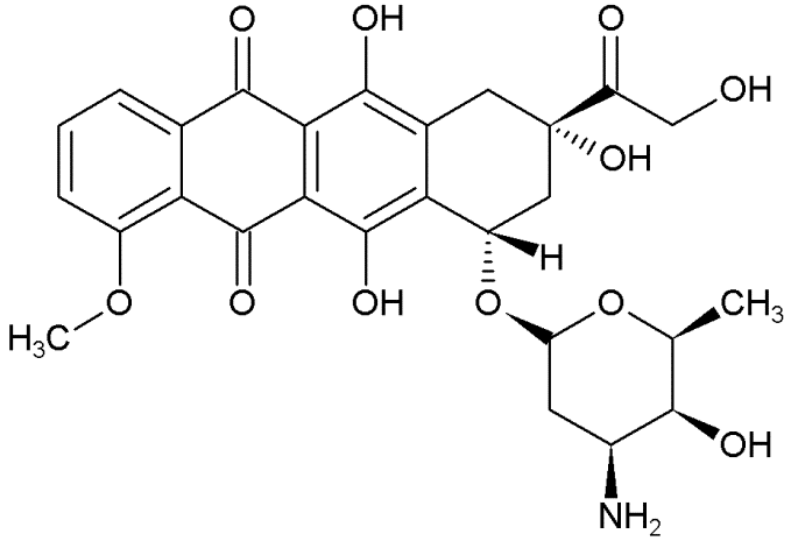


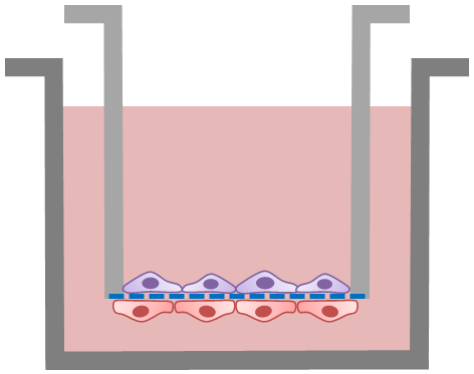
Доксорубицин

- Липофильная молекула
- Высокая степень связывания с белками и ДНК
- M_r = 543,5 Да

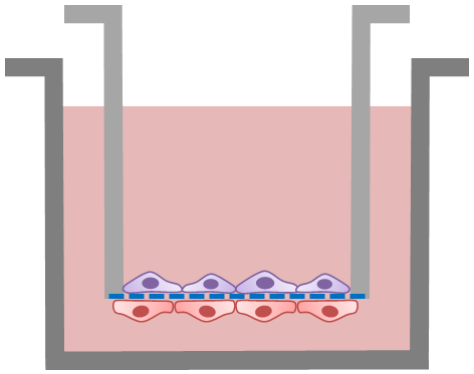




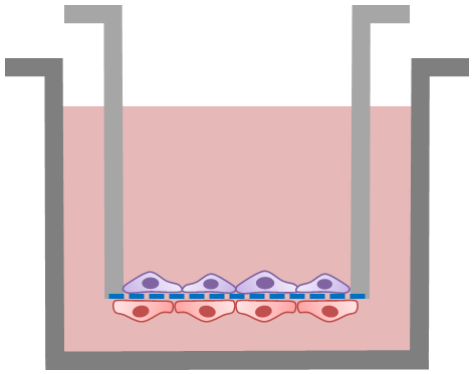




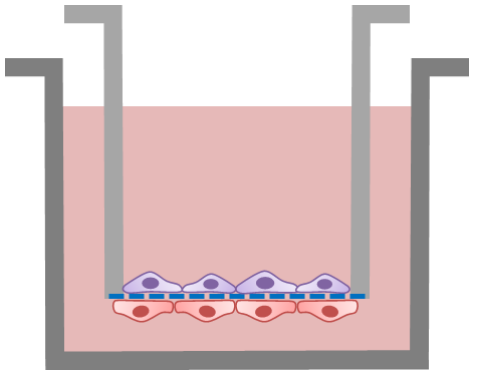
5-ФУ



ЦФ

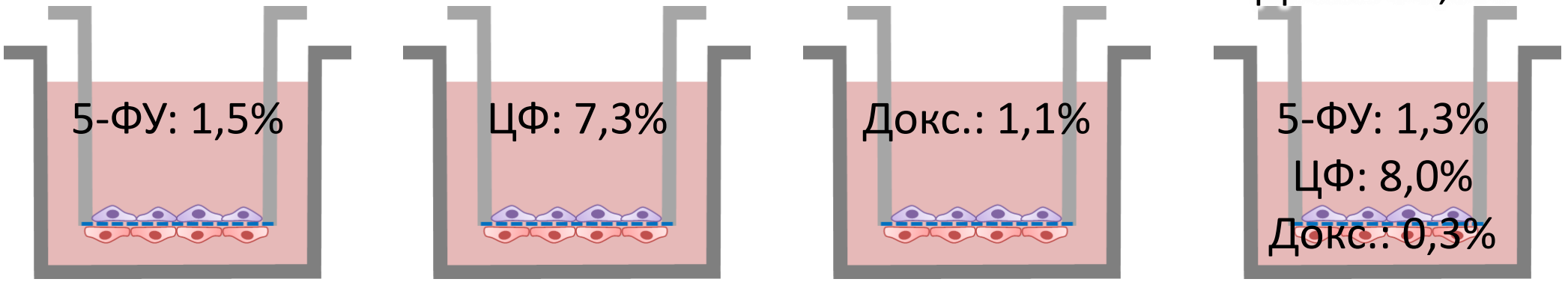


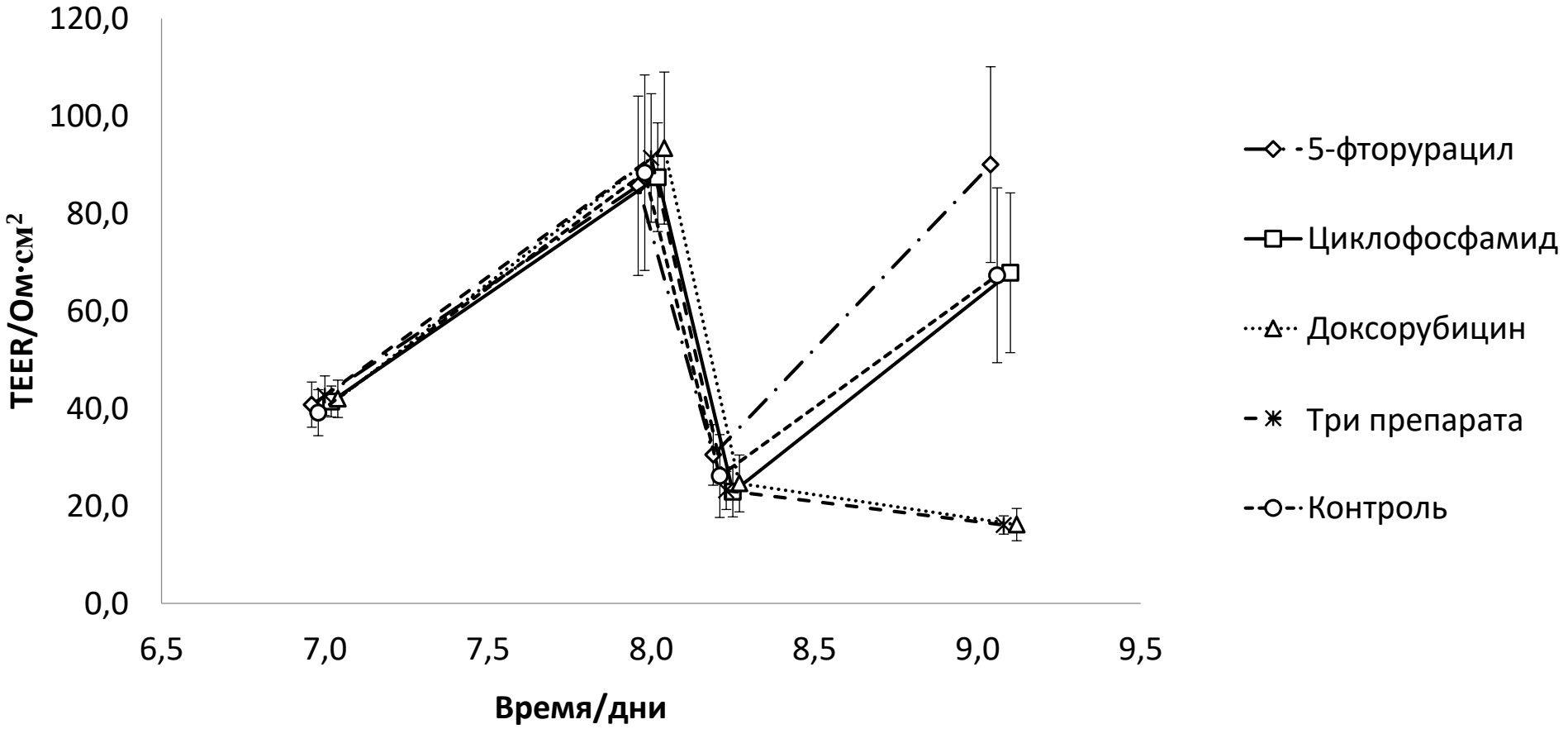
Докс.



Три препарата

5-ФУ	ЦФ	Докс.	Три препарата
5-ФУ: 90,3%	ЦФ: 85,7%	Докс.: 64,8%	5-ФУ: 77,6% ЦФ: 91,3% Докс.: 64,4%
5-ФУ: 8,2%	ЦФ: 7,0%	Докс.: 34,1%	5-ФУ: 21,2% ЦФ: 0,7% Докс.: 35,3%
5-ФУ: 1,5%	ЦФ: 7,3%	Докс.: 1,1%	5-ФУ: 1,3% ЦФ: 8,0% Докс.: 0,3%




120,0
100,0
80,0
60,0
40,0
20,0
0,0
TEER/Ом·см²
6,5
7,0
7,5
8,0
8,5
9,0
9,5
Время/дни
5-фторурацил
Циклофосфамид
Доксорубицин
Три препарата
Контроль

Спасибо за внимание!