An Interphase Microfluidic Culture System for the Study of Ex Vivo Intestinal Tissue

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Abstract: Ex vivo explant culture models offer unique properties to study complex mechanisms underlying tissue growth, renewal, and disease. A major weakness is the short viability depending on the biopsy origin and preparation protocol. We describe an interphase microfluidic culture system to cultivate full thickness murine colon explants which keeps morphological structures of the tissue up to 192 h. The system was composed of a central well on top of a porous membrane supported by a microchannel structure. The microfluidic perfusion allowed bathing the serosal side while preventing immersion of the villi. After eight days, up to 33% of the samples displayed no histological abnormalities. Numerical simulation of the transport of oxygen and glucose provided technical solutions to improve the functionality of the microdevice.

Keywords: organotypic tissue model; intestinal tract model; ex vivo microfluidic tissue culture; long term viability maintenance

1. Introduction

Organotypic tissue models, either explants or organoids, have a long history [1–4]. They complement the set of two-dimensional (2D) and three-dimensional (3D) in vitro culture techniques with the goal of offering, ex vivo, more advanced tissue functions than any current in vitro models without the difficulties inherent to in vitro studies (i.e., low throughput and high cost). Explanted tissues are likely to recapitulate the whole complexity of in vivo. They can be cultured in a controlled environment and many of them can be harvested from a single animal reducing the use of animals testing in agreement with the 3 Rs (replacement, reduction and refinement) principle. In addition,
several experiments can be performed with the same donor, increasing the robustness and reproducibility of the model. However, the maintenance of the viability is challenging due to limited diffusion of molecules when the size of the solid biopsy is greater than a few hundred micrometers. The extraction and interpretation of information also remains difficult as compared with simpler models of the organ [5]. Therefore, increasing their longevity is of great interest to the biomedical community.

Organotypic models of the intestine have been used to study the role of the microbiome in health and disease [6,7], to elucidate the origin of several pathologies [8] and to screen new therapeutic molecules [9]. Important advances have been made recently in their development and validation, although none have replaced human tests [10]. They include organoid culture [11], organ-on-a-chip [12], precision-cut slice [13], and tissue explant [14]. The introduction of microfluidics and microfabrication technologies in organotypic culture has the potential to provide more relevant culture conditions through accurate control of spatial and temporal distribution of fluids, transported molecules, and physical stimuli applied [15]. These miniaturization technologies have already largely diffused the field of three-dimensional (3D) engineered culture assays [16–19]. Indeed, microflow systems have been tested with solid biopsies of different organs [5,20–27] including the intestine [28–31]. Midwoud et al. studied the perfusion of precision-cut intestinal slices in a multilayer polydimethylsiloxane (PDMS) device and retained their in vivo metabolic rate up to 8 h [28]. Costa et al. developed a 3D printed device to study porcine gut and obtained viability up to 24 h [29]. Yissachar et al. adapted the air–liquid interface culture model to a microfluidic format preventing loss of architecture of the mucosa up to 40 h [30]. Dawson et al. reported the microfluidic culture of punched human intestinal fragment where both the luminal and serosal sides were perfused with a culture medium up to 72 h [31]. The design of the culture microsystem was inspired by the method of Browning and Trier consisting of culturing the explant at the air–liquid interface (ALI) [32]. ALI culture systems offer several advantages as compared with submerged culture systems since they keep the stratified architecture of the epithelium and provide a better oxygenation and nutrients delivery to the bottom part of the explant thanks to the porosity and permeability of the supporting membrane. However, to properly feed the tissue and ensure its long-term viability, the culture media needs to be changed periodically or continuously perfused. The technological challenge with dynamic perfusion is to select a membrane material with physical properties (i.e., porosity and permeability) that, on the one hand, allows a sufficient transport of nutrients and gases to the biopsy and, on the other hand, prevents its submersion. Our group has previously succeeded in maintaining a colonic static explant, mechanically supported by a nitrocellulose (NC) porous membrane, viable for 35 days [14]. In order to make the change of the culture medium easier, we designed a microfluidic system incorporating an ALI for the biopsy culture. Due to technological difficulties, it was not possible to use NC membrane and we chose to integrate a commercial polycarbonate (PC) membrane. To understand the effects of the materials’ properties of both NC and PC materials, we implemented a numerical model taking into account the mass transport and culture medium flow. The experimental result showed that our PC membrane was less efficient than our previous NC membrane to deliver enough oxygen and nutrients to the biopsies and this was confirmed by the numerical analysis.

2. Materials and Methods

The microfluidic device was composed of two polydimethylsiloxane (PDMS) layers separated by a PC microporous membrane (25 mm diameter and pores size 0.4 μm) (Figure 1a). The bottom layer was a replica obtained from a micromachined silicon mold. It formed a microchannel (150 μm thick and 1 cm wide) for the perfusion of the explant. The culture chamber was defined in the top layer (5 mm thick) using a punch biopsy needle (8 mm diameter) to generate a through hole. The sealing was performed by plasma activation, mechanical and thermal compression. Silicon tubing with an internal diameter of 500 μm was secured to the inlet and outlet holes by PDMS polymerization (Figure 1b). The microsystem was connected to a microfluidic flow control system (MFCS-350mb, Fluigent, Le Kremlin-Bicêtre, France) in order to bath the serosal side of the explant,
while avoiding immersion of the mucosa layer (Figure 1c). Four microfluidic devices were run in parallel (one triplicate and one empty control) at 37 °C and 5% CO₂ inside a humidified incubator at a volumetric flow rate of 10 μL/min for up to 8 days (Figure 1d). The colon explants were prepared and analyzed as previously described [14]. Three explants (9 in total) were placed in each incubation chamber. Animal protocols were approved on 3 May 2011 by the French regional ethical committee (approval number CEEA 112011). Detailed description of the device fabrication and operation is available in the appendix A.

Figure 1. (a) Schematic top view of the microfluidic system made of two polydimethylsiloxane (PDMS) layers and one polycarbonate (PC) membrane; (b) current assembled device; (c) schematic drawing of the A-A’ cross-section showing the location and dimensions of the culture chamber, PC membrane, and microchannel, three colon explants are placed in each chamber; (d) entire set-up introduced in an incubator at 37 °C and 5% CO₂ including 4 devices, 9 colon explants, 4 independent culture medium reservoirs and wastes.

To understand how the membrane properties modified the tissue viability, we implemented a computational model to calculate the transport by convection and diffusion of glucose and oxygen in the bioreactor and their consumption by the cells in the tissue explant. In order to quantify the efficiency of the microsystem to maintain the viability of the tissue, we estimated the reaction efficiency for glucose and oxygen, \( R_{\text{gl}} \) and \( R_{\text{ox}} \) respectively, as the average rates of glucose and oxygen consumption in the tissue divided by the maximal rate of glucose and oxygen consumption (for glucose and oxygen concentrations equal to the values set at the inlet of the microchannel).

Mass transport model: Transport of oxygen and glucose is assumed to be governed by the steady-state mass transport Equations (1) and (2) either for aqueous media or explant tissue, respectively.

\[ 0 = -u \nabla c_i + \beta D_i^{\text{aq}} \nabla^2 c_i \]  
\[ (1) \]

where \( c_i \) denotes the concentration (mol/m³) of the species \( i \), \( \nabla \) the nabla operator, \( D_i^{\text{aq}} \) the diffusion coefficient (m²/s) of the species \( i \) in the aqueous phase, \( u \) is the velocity of the aqueous media (m/s), and \( \beta \) is an empirical factor taking into account the retardation effect observed in the diffusion of molecules within the membrane (\( \beta = 1 \) in the culture medium and is defined in Table 1 for the PC and NC membranes).

\[ 0 = -R_i^{\text{t}} + D_i^{\text{t}} \nabla^2 c_i \]  
\[ (2) \]
where $c_i$ denotes the concentration (mol/m$^3$) of the species $i$, $\nabla$ the nabla operator, $D_i^a$ the diffusion coefficient (m$^2$/s) of the species $i$ in the explant tissue, and $R_i^t$ is the reaction rate (mol/m$^3$/s) of species $i$ in the explant tissue.

The reaction term is expressed with a Michaelis–Menten consumption kinetics:

$$R_i^t = R_i^{t,\text{max}} \frac{c_i}{c_i + c_i^{\text{max}/2}} \delta(c_i > c_{i\text{th}})$$

where $R_i^{t,\text{max}}$ is the maximum consumption rate of species $i$ in the explant tissue, $c_i^{\text{max}/2}$ the Michaelis–Menten constant corresponding to the concentration of species $i$ where consumption drops to 50% of its maximum, $c_{i\text{th}}$ is the threshold concentration of species $i$ below which the reaction term is cancelled, and $\delta()$ a step-down function to cease the consumption when $c_i$ is below $c_{i\text{th}}$.

Fluid dynamics model: The culture medium is an aqueous media, being considered as incompressible and flowing from the microchannel to the explant tissue through the porous membrane. We do not take into account the effect of the inlet velocity and liquid evaporation on the localization of the air–liquid interface. The Reynolds number ($R_e$) is small ($1 < R_e < 10$), and thus the conservation of linear momentum can be modeled by the Stoke equation:

$$\eta \nabla^2 u = \nabla p - F$$

where $F$ (kg/m$^2$/s$^2$) is a body force acting on the aqueous media, $\eta$ is the dynamic viscosity (Pa·s), $u$ is the aqueous media velocity (m/s), $p$ is the pressure (kg/m$^2$/s$^2$).

Dimensionless numbers: The Peclet number is defined as follows:

$$P_e = \frac{L u}{\beta D_i^a}$$

where $L$ (m) is the characteristic length of the microsystem (here the membrane thickness), $u$ is the average velocity of the culture medium through the membrane, $\beta$ is the empirical coefficient for diffusion retardation effect, and $D_i^a$ is the diffusion coefficient of species $i$ in the culture medium.

$R_{e\beta}$ number ($i$ standing either for oxygen or glucose) is defined as the actual rate of reactant consumption (averaged over a given volume/surface of the biological tissue) and divided by the reaction rate that is measured if the reactant concentration in the tissue is uniform and equal to the value set at the micro-channel inlet:

$$R_{e\beta} = \frac{(\sum_i^n c_i)/n}{\left(\sum_i^n c_i^\text{max}/2\right)}/\left(\frac{c_{i\text{th}}}{c_{i\text{th}} + c_i^\text{max}/2}\right)$$

where $i$ stands for either oxygen or glucose, $n$ ($n > 4000$) is the number of nodes in the bottom part of the explant domain (i.e., half of the thickness), and $c_{i\text{th}}$ is the maximal concentration of either oxygen or glucose entering at the inlet of the microfluidic channel (see Table 1). $C_{o,0} = 0.174$ mol/m$^3$ and $C_{g,0} = 25$ mol/m$^3$.

The analytical equation giving the permeability of fibrous materials as defined by Tomadakis is as follows:

$$\frac{k}{r^2} = \frac{\varepsilon}{8 + \ln^2(\varepsilon)} \frac{(\varepsilon - \varepsilon_p)^{(a+2)}}{(1 - \varepsilon_p)^2 \cdot ((a + 1) * \varepsilon - \varepsilon_p)^2}$$

where $k$ is the permeability (m$^2$), $r$ is the radius of the fiber (m), $\varepsilon$ is the porosity of the porous media, $a$ is a constant (0.785), and $\varepsilon_p$ is the percolation threshold (0.11).

Geometry and boundary conditions: The calculation of the distribution of glucose and oxygen have been implemented in a finite element solver (COMSOL Multiphysics, COMSOL, Genoble, France) by solving mass transport and fluid flow in two-dimensional (2D) for the different device geometries in steady-state regime. The microchannel height is $T_c = 150 \mu$m, the tissue explant thickness is $T_c = 300 \mu$m, the culture media height is $T_c = 250 \mu$m in the culture chamber, the diameter of the porous membrane is $L_m = 8000 \mu$m, the diameter of the explant tissue is $L_t = 3600 \mu$m, the length of the device is $L_d = 36,000 \mu$m. The finite element method (FEM) model is made of about 1 487 841
degrees of freedom for the PC geometry and 1275851 degrees of freedom for the NC geometry using the predefined “extra fine” mesh refinement. In the mass transport model, the following boundary conditions were implemented: zero normal mass flow at side walls, continuity between culture medium, porous membrane and tissue explant, and fixed concentration for culture medium in contact with exterior. In the fluid flow model, no slip was imposed to all surface corresponding to a solid–liquid interface, a fixed velocity (between 0.01 and 10 m/s) was used for the inlet and a fixed pressure (P = 0 Pa) for the outlet. It takes about 600 s and 9.7 Gb of memory to solve the equations on an Intel Core i7-7500U CPU cadenced at 2.7 GHz with 16 Go RAM.

Table 1. Physical properties for mass transport or consumption of oxygen and glucose in the culture medium, porous membrane, and tissue explant.

| Parameter                        | Unit  | Aqueous media | Porous membrane | Tissue explant | Reference |
|----------------------------------|-------|---------------|-----------------|----------------|-----------|
| Temperature                      | °K    | 310           | 310             | 310            | –         |
| Density                          | kg/m³ | 993           | N/A ¹           | N/A            | –         |
| Viscosity                        | Pa·s  | 0.7 × 10⁻³     | N/A ¹           | N/A            | –         |
| Porosity                         | %     | N/A ¹         | 79 ± 15 b       | N/A            | –         |
| Permeability                     | Darcy | N/A ¹         | 1 to 100        | N/A            | –         |
| Oxygen diffusivity               | m²/s  | 2.6 × 10⁻⁹     | 9.4 × 10⁻¹¹ b   | 2.0 × 10⁻⁹     | [33]     |
| Glucose diffusivity              | m²/s  | 0.7 × 10⁻⁹     | 0.3 × 10⁻¹¹ b   | 0.3 × 10⁻⁹     | [34]     |
| Retardation effect (β)           |       | 1             | 0.407 ± 0.036 b | 1              | [35]     |
| Max oxygen reaction rate         | mol/m³/s | N/A ¹    | N/A            | –0.034         | [36]     |
| Max glucose reaction rate        | mol/m³/s | N/A ¹    | N/A            | –0.028         | [37]     |
| Critical oxygen conc.            | mol/m³ | N/A ¹        | N/A            | 1 × 10⁻⁴       | [36]     |
| Critical glucose conc.           | mol/m³ | N/A ¹        | N/A            | 0.1            | [37]     |
| Initial oxygen conc.             | mol/m³ | 0.174        | 0.174          | 0.174          | [38]     |
| Initial glucose conc.            | mol/m³ | 25           | 25             | 25             | –         |
| M.M.¹ constant (oxygen)          | mol/m³ | N/A ¹        | N/A            | 1 × 10⁻³       | [36]     |
| M.M.¹ constant (glucose)         | mol/m³ | N/A ¹        | N/A            | 1 × 10⁻²       | [37]     |

¹ value for NC membrane, ² value for PC membrane, ³ not applicable, ⁴ Michaelis–Menten.

3. Results

To test the efficiency of the microfluidic culture chamber to maintain the interphase conditions, we studied the evolution of the flow rate as a function of the pressure difference between the inlet and outlet. The volume flow rate was found to increase linearly in the microchannel up to 100 mbars corresponding to a hydrodynamic resistance of 1.2 × 10⁻³ kg/m³/s. The working pressure drop was fixed at 20 mbars to robustly prevent flowing of the culture medium through the PC membrane. The histological analysis (Figure 2), showed the following: (i) three explants presented a preserved histological organization, (ii) four explants presented signs of stress, and (iii) two explants were necrosed.
We studied the influence of culture medium velocity and permeability of the membrane on $R_{dlI}$ and $R_{dfI}$. The channel height was fixed to 150 μm and the glucose concentration to 25 mol/m$^3$ according to the composition of the DMEM-F12 medium. Oxygen concentration was fixed by the operating conditions in the incubator (0.174 mol/m$^3$). In order to reduce the computing time, the phenomena responsible for mass transport and consumption were modeled in 2D. However, we simulated the laminar flow in a 3D model for a pressure drop of 20 mbars (Figure 3a) to apply the right flow boundary conditions at the inlet of the 2D model. The result shows that the average velocity of culture medium is 0.15 m/s in the widest section of the microfluidic channel (10 mm wide). Consequently, we studied the behavior of the device for inlet velocity between 0.010 and 10 m/s. The dimensions of the microsystem and boundary conditions are detailed in the 2D longitudinal cross-section in Figure 3b. The spatial distributions of glucose and oxygen were predicted by a convection-diffusion-reaction equation assuming Michaelis–Menten kinetics. The flow of the culture medium was calculated with the Stoke equation.
In order to synthesize the results of our study, we plotted (Figure 4) the variation of $R_{\text{eff}}$ as a function of the Peclet ($Pe$) dimensionless number, for different membrane permeability values (between 1 and 100 Darcy). To define the range of permeability, we used the analytical model of Tomadakis giving four orders of magnitude between PC and NC membranes permeability for a given radius of the fibers [39]. Knowing the range of culture medium velocities at the microdevice inlet, we limited our analysis to three permeabilities: 1, 10, and 100 Darcy (1 Darcy = 9.869233 × 10⁻¹³ m²), a medium with a permeability of 1 Darcy permits a fluid flow of $10^{-6}$ m³/s with a viscosity of $10^{-3}$ Pa·s under a pressure gradient of 10,132,500 Pascal/m acting across an area of $10^{-4}$ m². The delivery of oxygen and glucose is assumed to be achieved only through the culture medium. Thus, it depends on the inlet flow velocity and the transport properties of the membranes. The porosity values were provided by the manufacturers for both PC and NC membranes while permeability values were unknown. The combined effect of the inlet velocity and membrane permeability is represented in the Peclet number as defined above.
integrate distributions

to
difficult

keeping
tissue
morphology
up
to
33%
of
the
samples.
Previous
studies
combining
intestine
explant
cultures
and
microfluidics
reported
a
maximal
viability
of
72
h.
Many
factors
can
influence
solid
biopsy
viability
[40].
Among
them,
organism
size
[41],
donor
age
[42],
localization
of
the
fragment
in
the
organ
[42,43],
composition
of
the
culture
medium
[44],
format
of
the
culture
[45]
(either
static
or
dynamic,
fully
immersed
or
mechanically
supported
explant
at
the
air-culture
medium
interface),
and
duration
of
the
transfer
[46]
can
play
key
roles.
Therefore,
a
significant
variability
in
duration
of
their
viability
was
highlighted,
from
several
hours
in
Ussing
chamber
[47]
up
to
91
days
[48].

A
porous
membrane
provides
high
viability
for
3D
explant
culture
[49].
Polytetrafluoroethylene
(PTFE)
NC-
and
PC-based
membranes
are
classical
materials
found
in
commercial
inserts.
The
incorporation
of
membrane
functionalities
in
microfluidic
devices
is
a
relatively
new
research
area
[50].
Various
techniques
such
as
gluing
[51],
sandwiching
[52],
clamping
[53],
or
direct
in
situ
fabrication
[54] have
been
reported,
even
if
they
remain
technologically
challenging.
The
major
difficulties
are
(i)
the
sealing
step
due
to
incompatibility
between
sticking
properties
of
the
various
polymeric
materials
and
thickness
of
the
membrane
(typically
few
microns)
and
(ii)
the
reproducibility
in
the
fabrication
to
ensure
constant
flow
through
the
different
experiments.

As
a
continuation
of
previous
work
on
explant
culture
by
our
team
[14],
first,
we
tried
to
integrate
a
NC-based
membrane
(160
µm
thick)
in
the
microfluidic
device.
Although
this
technological
approach
has
been
reported
[55,56],
the
achievement
of
a
perfect
sealing
remains
very
difficult
to
obtain
[57]
and
we
did
not
succeed
in
preventing
the
immersion
of
the
explant.
Therefore,
we
introduced
a
PC
membrane
(20
µm
thick)
according
to
the
process
reported
by
Chueh
et
al.
and
it
was
very
effective
[58].
However,
NC
and
PC
materials
do
not
have
the
same
transport
properties.

Indeed,
one
can
observe
in
Figure
4
that
the
highest
permeability
has
the
highest
Peclet
number
for
a
given
inlet
velocity.
For
all
permeabilities,
the
reaction
efficiency
is
higher
for
NC
than
that
of
PC
membranes.
The
difference
in
reaction
efficiency
for
oxygen
between
NC
and
PC
membranes
decreases
when
permeability
increases.
Indeed,
at
high
permeability,
the
transport
of
molecules
through
the
membrane
is
performed
mainly
by
convection
reducing
the
effect
of
the
difference
between
the
diffusive
properties
of
NC
and
PC
membranes
on
the
reaction
efficiency.

Figure
6
give
the
2D
distributions
of
culture
medium
velocity,
as
well
as
oxygen
and

glucose
concentrations
for
the
two
extreme
cases
we
studied,
namely
a
device
with
a
PC
membrane
having
a
permeability
of
1
Da
and
an
inlet
flow
velocity
of
0.015
m/s
and
a
device
with
a
NC
membrane
having
a
permeability
of
100
Da
and
an
inlet
velocity
of
2
m/s.
Additionally,
the
corresponding
velocity
line
distributions
for
these
two
cases
are
given
in
Figures
B1
and
B2.
In
the
first
case,
the
flow
magnitude
around
the
explant
is
very
low
that
prevents
convective
transport
of
oxygen
and

glucose
in
the
explant.
In
this
configuration,
the
delivery
of
oxygen
and

glucose
at
the
heart
of
the
explant
depends
only
on
diffusion.
The
difference
between
oxygen
and

glucose
distribution
is
due
to
the
difference
of
gradients
(0.174
mol/m³
for
oxygen
and
25
mol/m³
for

glucose).
In
the
case
of
a
more
permeable
membrane (100 Da) and higher inlet flow rate (2 m/s), the delivery of oxygen and glucose to the explant is performed by both convection and diffusion. As a result, the amount of oxygen and glucose in the tissue is higher and the difference between the distribution of both species is lowered. For the value of the inlet flow velocity (i.e., 0.15 m/s represented by the dash vertical red line on each graph in Figure 4) used in the experiments, the reaction efficiency for the PC membrane is always lower than 0.5, while for the NC membrane it is always above 0.8. Even if the same trend is found for glucose delivery, one can see that the concentration of glucose remains quite high in the explant with a reaction efficiency always higher than 0.94 (results not shown). According to Figure 4, we conclude that it would be necessary to multiply the flow rate by almost 10 in future experiments to improve the oxygen delivery to the explants and allow a longer preservation of their morphology.
Figure 5. 2D distribution in the microfluidic devices containing a PC membrane for a 1 Da permeability of (a) the culture medium velocity; (b) oxygen; and (c) glucose with an inlet flow velocity of 0.015 m/s. The insert on the velocity figure (a) schematizes the main flow of the culture medium around the explant and its effect on oxygen and glucose transport (red arrows accounts only for diffusion process).
5. Conclusions

The majority of microfluidics models of human intestinal culture rely either on the culturing of an intestinal epithelial cell monolayer or on an organoid culture. Regardless of the importance of this type of culture, it remains limited when it comes to the in vivo microenvironment. We described an organotypic microfluidic mice colon culture model that kept the morphology of the intestinal tissues up to 192 h for 33% of the explant. Numerical simulation showed that the PC membrane is less adapted to oxygen delivery than the NC membrane and, consequently, it would be necessary, in the future, to multiply the flow rate by almost 10 to increase the rate of success with PC membrane.
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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Microfluidic device fabrication: The master mold is made of silicon (Siltronix Silicon Prime wafers CZ (diameter 76.2 ± 0.3 mm, thickness 380 ± 25 μm, orientation <100>, type doping P-Boron, resistivity 1 to 10 Ω cm). Selective silicon etching is obtained by protection of the silicon surface with a resist mask. AZ15nXT negative photoresist (MicroChemicals, Ulm, Germany) is spun-coated to obtain a 10 μm thick layer (speed = 1500 rpm, acceleration = 3000 rpm·s⁻¹, time 40 s). The substrate is soft baked for 3 min at 110 °C on a hotplate and AZ15nXT is exposed to UV radiation at λ = 365 nm for 15 s @ 10 mW. After this exposure step, a post-exposure is realized on a hotplate for 1 min at 120 °C. Exposed resist is removed by the MIF 326 developer (MicroChemicals, Germany) for 4 min and rinsed with water for 15 s. The substrate is etched with STS DRIE plasma equipment (SPTS, Newport, UK) by a deep reactive ion etching (DRIE) process (Bosch process), with C6F₅ passivation and SF₆ etching steps (C6F₅ flow rate = 100 sccm, passivation time = 2.2 s, RIE/ICP power = 20 W/1500 W, SF₆ flow rate = 450 sccm, etching time = 3 s, RIE/ICP power = 50 W/2200 W). The substrate chiller is cooled down to −10 °C in order to improve the thermal evacuation. The etching rate is 5.5 μm·min⁻¹ and the etching depth is 150 μm for the bottom PDMS replica. A thin layer of “Teflon-like” coating is deposited on the surface of the silicon substrate using a C6F₅ plasma to facilitate the peeling of PDMS. The liquid mixture containing the precursor and the curing agent (10:1 (v/v)) is poured on the silicon mold and polymerized in a furnace at 70 °C for 2 h. The resulting micro-structured elastomer is peeled off by hand. The top PDMS layer is fabricated using the same protocol but on a flat silicon substrate and punched to make an 8 mm diameter through hole. The sealing of the two PDMS layers and the PC membrane is performed in two steps. First, the PC membrane is bonded to the top PDMS layer. The PDMS layer and PC membrane are washed with isopropyl alcohol (IPA) and deionized water (DI) and dried with compressed air. The 5% v/v aqueous APTES solution is prepared by mixing APTES (99%) reagent with EDI and heating at 80 °C. The porous membrane is activated by corona treatment, then, immersed in the APTES solution for 20 min. The PC membrane and the activated PDMS are brought into contact and, subsequently, pressed together. Second, the PDMS/PC bilayer is sealed to the bottom PDMS layer using corona activation and manual assembly. The entire structure is placed in a furnace at 90 °C to improve adhesion between layers. Before starting the experiment, the microsystems are autoclaved at 120 °C.

Animals: The animals were kept in aseptic conditions in an isolator and were regularly inspected to assess microbial and parasitological infections (including Helicobacter spp.). Three SCID mice were administered with 4 mg/L of dexamethasone sodium phosphate (Dex) (Merck, Lyon, France) via drinking water, as previously described [14], two weeks before euthanasia by carbon dioxide inhalation for tissue culture experiments. Experiments were conducted in the animal facility (PLETHA Pasteur) at the “Institut Pasteur de Lille” (research accreditation number A59107). The animal protocols were approved on 3 May 2011 by the French regional ethics committee (approval number CEEA 112011).

Colon explant preparation: The colon recovered from the animals was dissected and cleaned of fecal contents with cold Hank’s Balanced Salt Solution (Sigma, St. Louis, MI, USA) supplemented with penicillin (100 U/mL, Thermofisher, Waltham, MA, USA), streptomycin (100 μg/mL, Thermofisher, USA), and metronidazole (50 μg/mL, Sigma, USA). The tissue was opened along its
length and cut into 8 mm² pieces. The explants (9 mm² in surface, 300 μm thick) were manually transferred into the device. Dulbecco’s modified Eagles medium (DMEM-F12, Sigma, USA) containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), insulin/transferrin/selenite mix (1:100, Thermofisher, USA), Albumax (1 mg/mL, Life Technologies, Carlsbad, CA, USA), hydrocortisone (1 μM, Sigma, USA), glucagon (14.3 nM, Sigma, USA), 3,3’,5’-triiodo-L-thyronine (1 nM, Sigma, USA), ascorbate-2-phosphate (200 μM, Sigma, USA), linoleic acid (20 μM, Sigma, USA), estradiol (10 nM, Sigma, USA), and keratinocyte growth factor (KGF) (50 ng/mL, Sigma, USA) were provided to the bottom microchannel of the four devices through the silicon tubing via the four-way MFCS equipment. The medium waste was collected in four independent tubes. The absence of bacterial contamination was screened by plating the medium onto culture media (Trypticase soy), for at least 72 h at 37 °C. Mouse colonic sections obtained immediately after dissection were used as controls for histology characterization.

Histological analysis: The cultured explants were stopped after 8 days of culture and, then, fixed in 10% formalin and embedded in paraffin. Sections of 5 μm were stained with hematoxylin/eosin and safranin (Leica Autostainer-XL, Rueil-Malmaison, France). Sections stained with hematoxylin/eosin and safranin were examined using a Leica DMRB microscope equipped with a Leica digital camera connected to an Imaging Research MCID analysis system (MCID software, Cambridge, United Kingdom).

**Appendix B**

![Figure B1](image1.png)

**Figure B1.** 2D distribution in the microfluidic devices containing a PC membrane with a 1 Da permeability for an inlet flow velocity of 0.015 m/s.

![Figure B2](image2.png)

**Figure B2.** 2D distribution in the microfluidic devices containing a NC membrane with a 100 Da permeability for an inlet flow velocity of 2 m/s.
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