

microchannels kit complete.handy.simple

USER GUIDE



Innovative cell culture systems.



MATERIAL NEEDED

Material included



Recommended material

Desiccator or centrifuge Oven (up to 80°C)



Fig. 1: 4Dcell epoxy mold

This user guide is made for 4 chips. You can also adapt it to your need, making 1 or more chips. The weight ratio is 10:1 and the volume ratio 12:1 for the PDMS precursors. The recommended materials are not indispensable but it allows to accelerate the process. The chip fabrication (part A) lasts about 2 hours.

Microchannels protocol

A. Molding of 4 chips



MANIPULATION

) Fill the graduated plastic cup with 12 mL of the 4Dcell PDMS elastomer.



Fig.2: Illustration of point A.2



Pipet 1mL of 4Dcell curing agent in the graduated plastic cup. You should have 13 mL in the container.



Fig.3: Illustration of point A.2



Homogenize the mix for 2 minutes using the glass spatula. NB: You should see bubbles forming in the mixture.



Fig.4: Illustration of point A.3





Remove trapped bubbles by degassing the mix with the centrifuge at 500 rpm for 2 minutes or the desiccator for about 10 minutes. You can also let the plastic cup at room temperature and wait at least one hour.



Fig.5: Illustration of point A.4



Pour the PDMS into the epoxy mold.



Fig.6: Illustration of point A.5



Degas once again until there is no bubbles left.



Fig.7: Illustration of point A.6





Place the mold in the oven at 80°C for 1 hour. If you don't have any oven, you can still let the mold at room temperature and wait for about 8 hours.



Fig.8: Illustration of point A.7



Once cooled down, gently unmold the chip in alignment with the microchannels. N.B: Do not touch the chip with your fingers (even with the gloves) on the microchannels side, oil will cause unattachment of the chip against the fluorodish.



Fig.9: Illustration of point A.8



Take the white adhesive tape and use it as a support. First, take off any dust on the PDMS chip. Then, using the sharpened punches round, cut two Ø3 mm holes at the entry and exit of the channels.



Fig.12: Illustration of point A.11





) In sterile condition, activate the chip on the microchannels side and the bottom of the fluorodish with the corona tool by approaching it at 1 cm of the surface.



Fig.11: Illustration of point A.10



Directly after activation, bond the PDMS device to the fluorodish and press it softly, avoiding pressure on the channel area. N.B: Make sure that the PDMS chip is well attached to the glass bottom.



Fig.12: Illustration of point A.11





B. Plating the cells

Fill the entry of the channels with 20 μ L of the surface coating solution, fibronectin. Incubate 30 minutes. N.B: The solution should infiltrate by capillary forces into the channels after simply placing a drop of solution at one of the ports.



Fig.15: Illustration of point B.1



After coating the fibronectin, extensively wash the device with PBS. Load with culture medium (3mL) and incubate the device at a temperature of 37 °C for 15 min. N.B: The PDMS absorbs molecules in the medium. So if you have fragile cells, an overnight incubation with PBS (3mL) at 37°C is recommanded before seeding the cells.



Fig.16 Illustration of point B.2

Remove the medium, and place a 20 μ L droplet of cell solution at one inlet of the device and tilt it to introduce the cells into the microfluidic channel. Incubate 20 minutes (37°C, 5% CO₂) to allow cell adherence. N.B: We advise a cell concentration ranging from 3 to 4.10⁶ cells/mM for classical cell line solution. Otherwise, concentration should be adjusted to have a confluency of 60-70% inside the hole.



Fig.17 Illustration of point B.3



Finally, add 3mL of culture medium in the fluorodish, close the lid, and incubate it into an appropriate incubator (classically 37°C, 5% CO₂ for mammalian cells). N.B: Incubate overnight before observing cells into the channels.



4DCell Epoxy mold specifications

Microchannels engraved in epoxy well (Ø 2.2 mm, height 2mm) allowing printing of 1 PDMS chip

a. Structure of the microchannels

Exemple of microchannels applications:

- Cell migration model
- Mimicking vascular network
- Angiogenesis and metastasis model
- Investigating the neuronal network
- Cell plasticity model



SMALL MICROCHANNELS



LARGE MICROCHANNELS



Included in the consumables box

FluoroDish FD35-100	Dish ø 35mm, Glass ø 23 mm, Glass thickness 0.17 mm
Number of Fluorodishes	100
Extracellular matrix protein, volume	Fibronectin, 1mL x 5 tubes, stored in +4°C
Silicon precursors, volume	PDMS Elastomer, 250 mL - Curing agent, 25 mL
Glass spatula	20 cm
Graduated cup	Plastic - 30 mL
Adhesive tape	10 cm x 10 cm
Sharpened punches	Round, 3 mm
Scalpel	Stainless steel blade

Order separately

Microchannels Mold	Ероху
Mold size	ø 4 cm, height 1 cm
Well size	ø 2.2 cm, height 2 mm
Number of molds	4
Microchannels geometry	Straight
Microchannels width	Small 3, 4, 5, 6, 7, 8 µm / Large 10, 12, 14, 16, 20 µm
Microchannels height	Small 3,5 µm / Large 10 µm
Corona charging tool	Laboratory Corona Treater, 230V, Electro-technic products



The link between biophysic and biology

Based on their laboratory experiments, 4DCell teams offer a product whose innovation lies in its flexibility of use. Our goal is to make the researcher's life easier providing affordable biophysical technologies, with a custom-made use. This without ever cutting back on quality, thanks to 4DCell selection of the best chemicals and materials on the market.



73 rue Alexandre Dumas 75020 Paris

+33(1) 84 25 16 14

contact@4dcell.com

www.4dcell.com



Terms and conditions

Returns Policy Damaged or Incorrect Goods

We hope that you will be pleased with your purchase. However, in the unlikely event that you should receive damaged or incorrect goods in your delivery, then please notify us within 7 business days of receipt. You will be offered the option of a refund or an exchange (provided the goods are in stock). You may be asked to return goods to us for inspection and we will refund the cost of return postage. Should the damaged or incorrect item be no longer available, you will be given the option of a refund. Please note that goods that become damaged or broken after 7 business days of receipt cannot be returned.

Unwanted items and cancellations If for any reason you do not wish to keep your purchase and would like a store credit, then please notify us within 7 business days of receipt. We cannot accept unwanted returns that have been opened, used or damaged by the customer. We will only issue a credit upon receipt of all returned goods. Please note that we are unable to refund your costs in returning unwanted goods or the delivery costs of sending the goods to you in the first place. If you wish to cancel your order please email us immediately on contact@4dcell.com.

Privacy Policy

Customer details remain private and confidential and will not be released to a third party unless required to do so by law. We use the information we collect about you to process shopping experience and, if you request, to notify you of products, special offers or other information that may be of interest to you. We do not sell or pass on any personal information to any other Companies or Organizations.

Payments & Procedures

Payment from private customers must be paid for in advance of shipment. Trade orders from registered companies or organizations can be invoiced. Payment is due strictly within 30 days of the invoice date.

Products & Prices

Please note that some goods may vary in style, color or detail from the image shown. We reserve the right to change prices at any time without notice. Important : read all Instructions, warnings and precautions prior to use.

Limitation of warranty 4DCELL explicitly disclaims all warranties, express or implied, for any period during which the goods are operated or stored not in accordance with the technical specifications. 4DCELL does not assume any liability arising out of any application or use of any product or circuit and specifically disclaims any and all liability, including without limitation conse-quential or incidental damages. All operating parameters, including without limitation recommended parameters, must be validated parameters, for each customer's applications by customer's technical experts. Recommended parameters can and do vary in different applications. 4DCELL reserves the right, without further notice, (i) to change the product specifications and/or the information in this document and (ii) to improve reliability, functions and docige (ii) to improve reliability, functions and design of this product. The foregoing warranty shall not apply to defects resulting from improper or inadequate maintenance by the Customer, Customer-supplied software or interfacing, unauthorized modification or misuse, operation outside of the environmental specifications for the product, or improper site preparation and maintenance.

Exclusive remedies

The remedies provided herein are the customer's sole and exclusive remedies. 4DCELL shall not be liable for any direct, indirect, special, incidental, or consequential damages, whether based on contract, tort, or any other legal theory. No other warranty is expressed or implied. 4DCELL specifically disclaims the implied warranties of merchantability and fitness for a particular purpose.

Assistance

4DCELL Sales and Service office for further information on 4DCELL full line of Support Programs. See: www.4dcell.com. The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. 4DCell assumes no liability for the customer's failure to comply with these requirements.

General

Caution for research use only: wear proper body protection during experience. Do not operate in an explosive environment. Do not operate in wet/damp conditions. Do not use this product as safety or emergency stop devices or in any other application where failure of the product could result in personal injury. The protective features of this product may be impaired if it is used in a manner not

specified in the operating instructions. Before installing, handling, using or servicing this product, please consult the data sheet and user guide. Failure to comply with these instructions could result in death or serious 4DCELL products for any unintended or unauthorized application, the buyer shall defend, indemnify and hold harmless 4DCELL and it officers employees cubridities and its officers, employees, subsidiaries, affiliates and distributors against all claims, costs, damages and expenses, and reasonable attorney fees arising out of, directly or indirectly, any claim of personal injury or death associ-ated with such unintended or unauthorized use, even if 4DCELL shall be allegedly negligent with respect to the design or the manufacture of the product.

ESD precautions

The inherent design of this component causes it to be sensitive to electrostatic discharge (ESD). To prevent ESDinduced damage and/or degradation, take customary and statutory ESD precautions when handling this product.

Environmental conditions

This instrument is intended for indoor use. It is designed to operate at a maximum relative humidity of 60% and at altitudes of up to 2000 meters. Operating temperature range is $+5^{\circ}$ C to 50°C. Do not operate in an explosive atmosphere. Do not operate the instrument in the presence of flammable gases or fumes. Before applying power, verify that the line voltage matches the product's input voltage requirements and the correct fuse is installed. Use only the specified line cord for this product and make sure the line cord is certified for the country of use.

Fuses Only fuses with the required rated current, voltage, and specified type (normal blow, time delay, etc.) should be used. Do not use repaired fuses or short circuited fuse holders. To do so could cause a shock or fire hazard. Keep away from live circuits Operating personnel must not remove instrument covers

Component replacement

Component replacement and internal adjust-ments must be made by qualified service personnel from 4DCELL company only, subject to the loss of the warranty. Do not replace components with power cable connected. Under certain conditions, dangerous voltages may exist even with the power cable removed. To avoid injuries, always disconnect power, discharge circuits and remove external voltage sources before touching components. Do not service or adjust alone. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not substitute parts or modify instrument. Because of the danger of introduc-ing additional hazards, do not install substitute parts or perform any unauthorized modifica-tion to the instrument. Return the instrument to an 4DCELL Office for service and repair to to an 4DCEL Office for service and repair to ensure that safety features are maintained. Instruments which appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.

CE compliance

This product meets the essential requirements of applicable European Directives, as amended for CE marking, as follows: Electromagnetic Compatibility COUNCIL DIRECTIVE 89/336/EEC of 3 May 1989. This directive has been amended by the following Council Directives: (i) 92/59/eec of 29 June 1992 (General Product Safety) ; (ii) 93/68/eec of 22 July 1993 (CE Marking directive); (iii) 99/5/ec: Directive of Radio Equipment & Telecommunications Terminal Equipment (R&TTE).

Delivery Delivery dates are indicative. 4DCELL shall not be liable for any delay in delivery. Claims arising from delivery delays will not lead to any financial compensation from 4DCELL company. In case of major delays in delivery dates, claims will be treated amicably.