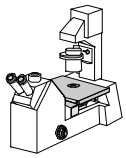


POC - Chamber - System

A Chamber - System for cells in vitro - for

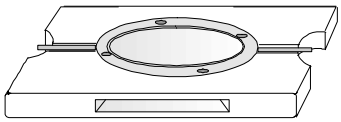
Perfusion, Open and Closed cultivation.

Improved microscopes, their objectives and optical techniques now allow higher standards in microscopic analysis of living cells. In addition, heated microscope stages and climate boxes are available which allow observation of cells under stabilized temperature. The climate box is capable to hold the correct temperature and the pH-value of the medium in which the cells are being cultured by regulation of the CO_2. (Carl Zeiss, Göttingen; Leica, Wetzlar). This is an important step in stabilizing specific parameters for the standardization of in vitro tests.

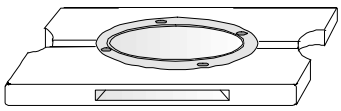
The POC-Chamber-System satifies conditions for the use of different microscopic methods for observation and analysis of living cells.

The POC-Chamber-System provides 5 different applications possibilities

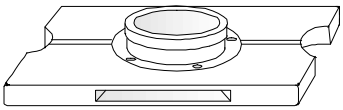
Closed Perfusion



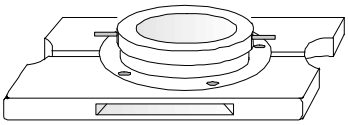
Closed Cultivation



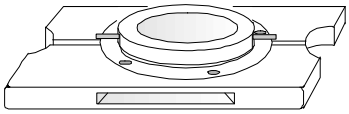
Open Cultivation



Open Perfusion A



Open Perfusion B



External Dimensions: Base plate 81 x 55.5 x 5.5 mm (Aluminium, black anodized)
Microscope: Special object holder for Microtestplates (Terasaki) or the Universal mounting holder M and K.

Cultivation area
- Cultivation on glass: Cover slip 0.17 mm (ideal refractive index) or foil (e.g. CultFoil, 0.025 mm, gas permeabel).

Observation area (30 mm ∅)
- High resolution oil immersion objectives can be used.

Variable height of the chamber
- Using the *closed* chamber version the distance between the upper and lower glass is variable (1or 2 mm). Cells can also be cultivated on both cover slips.

Perfusion
- In the case of perfusion in the *closed* version the inner height of the chamber is 0.7 mm or less.

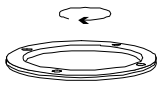
The chamber is alterable
- The chamber can be modified between open and closed cultivation, as well as the perfusion in the open and closed version of the same cells.
- The cells can be precultivated on cover slips (42 mm Ø) in Petri dishes (60 mm ∅).

Advantage
- easy to use, rapid to assemble
- produced of non-toxic materials
- sterilization of the mounted chamber

Accessories
- Heating Frame
- Heatable Cover for the Heating Frame
- Heating- and Cooling Frame
- Temperature regulator

"Closed" Cultivation System

Assembly



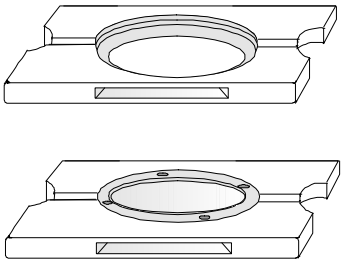
(mounting key)

screw ring

cover slip, 42 mm ∅

silicon gasket, 1 oder 2 mm

cover slip, 42 mm ∅



Sterilization: 165°C for 2 hrs. - After sterilization
the screw ring should again be tightened

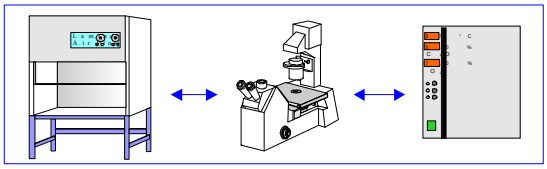
Dimensions: 81 x 55.5 x 5.5 mm
Observation area: 8 cm², 32 mm ∅

Volume: 1 mm gasket approx. 0.9 ml
2 mm gasket approx. 1.8 ml

Material: Base plate: Aluminium, black anodized
Screw ring: stainless steel
Gasket: Silicon
Cover slip: glass, 42.0 ∅ x 0.17 mm
Mounting key: Aluminium with stainless steel-pins

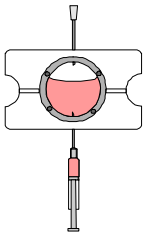
Characteristics: *Silicon:* gas permeable, dry sterilization, non-toxic and "closed" again when the needle is removed.
Aluminium is an extremely good heat conductor
Cover slip hydrolytic class 1, borosilicate glass, preferable for all microscopic procedures

Feature:
- Optimum conditions for the microscopic observation - plane surfaces - no meniscus.
- *The chamber can be turned:* Cultivation of cells on one or both cover slips.
- The cells can be observed on a "upright" microscope using a long-distance condenser.



changing of medium

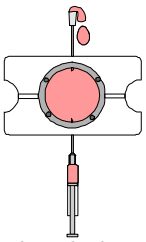
so



remove the medium and introduce fresh medium slowly (the better method)

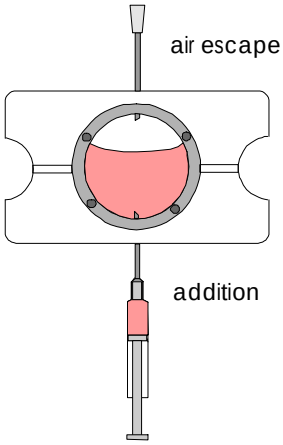
or

so



introduce fresh medium slowly, displacing the "old" medium

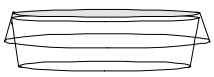
addition of nutrition medium + cells



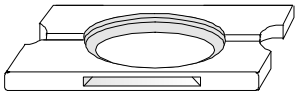
Recommendation *needles*
using 1 mm silicon : addition with 25 G (No. 18), exit 24 G (No. 17)
using 2 mm silicon : addition with 23 G (No. 14), exit 20 G (No. 1)

A precultivation of cells on cover slips in Petri dishes ("60er") is possible !

sterile cover slip



cover slip with cells

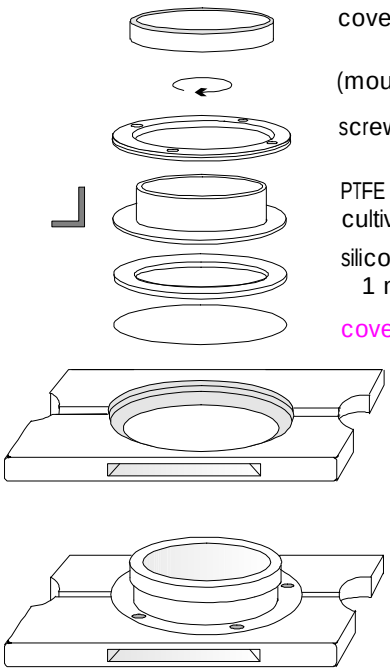


the different components of the POC-Chamber must be sterilized beforehand

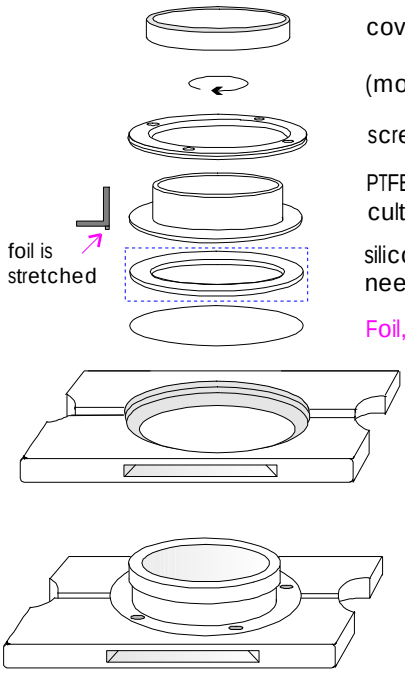
"Open" Cultivation System

Assembly

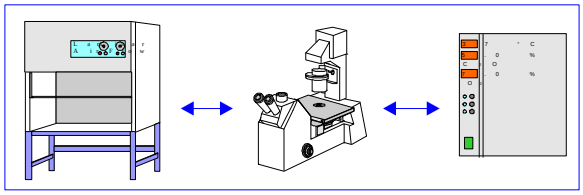
cell culture on glass



cell culture on foil



Sterilization of the mounted chamber with cover at +165°C approx. 2hrs.



Introduce or displace nutrition medium, cells etc. as a Petri dishes.

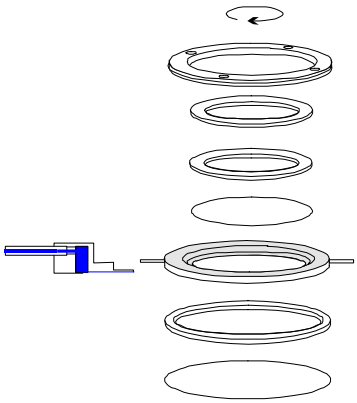
A precultivation of cells on cover slips in Petri dishes ("60") is possible!

the different components of the POC-Chamber must be sterilized beforehand

* Changing from "open" cultivation chamber (glass) to the "closed" perfusion system under the laminar air flow is possible using the 0.5 mm silicon gasket:
sterilize mounting key - remove the PTFE adapter - place the sterile perfusion adapter with the 32 mm Ø cover slip and the silicon gasket, fix this unit with the mounting key

Perfusion in the "closed" cultivation chamber

Assembly



(mounting key)

screw ring for PTFE adapter

PTFE ring

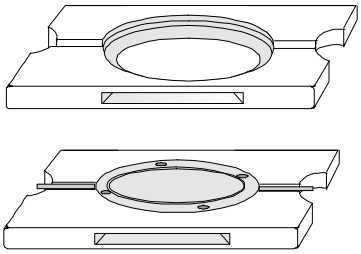
silicon gasket, 32 mm Ø,1 mm

cover slip, 32 mm Ø

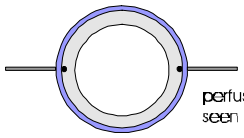
perfusion adapter

silicon gasket, 42 x 2 Ø x 0.5 mm

cover slip, 42 mm Ø

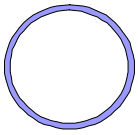


New !!!



perfusion adapter, seen from below

During perfusion the distribution of the liquid was optimized by the shift of the gasket onto the rim zone. Additional, perfusion can now be realized also with higher rate.

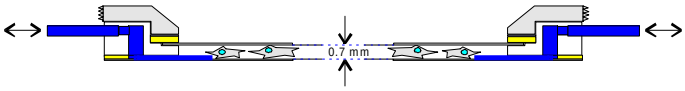


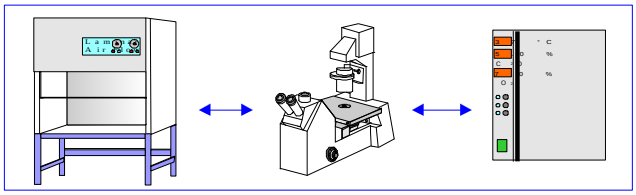
Now, the gasket (42 x 2 mm) has a narrower rim.
Two different gaskets are available:

* Silicon gasket, 0,5 mm thick,
  inner height of the chamber: 0.7 mm (# 0727.023)
* FEP gasket, 0.25 mm thick,
  inner height of the chamber: 0.45 mm (# 0727.014)

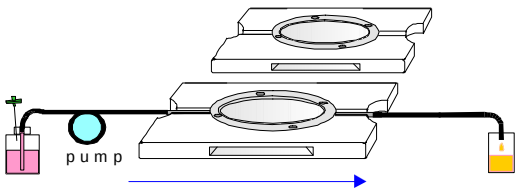
* Inner height of the chamber:
  Silicon gasket 0.5 mm = 0.7 mm
  FEP gasket 0.2 mm = 0.4 mm

Sterilization:
+165°C for 2 hrs. - After sterilization
the screw ring should again be tightened





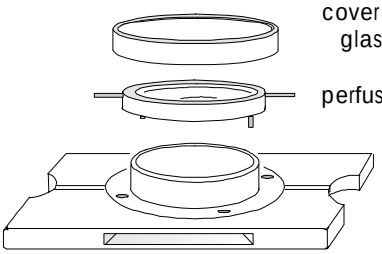
Example of a perfusion



* Draw the sterilized tubes (e.g. silicon tube, inner Ø 0.7-0.8 mm) over the canal tubes (stainless steel tube, inner Ø 0.6 mm) at the perfusion adapter..
* Normally, the perfusion is realized by pressure:
  syringe by hand
  automatic syringe pump
  peristaltic pump
* Flow-rate: For optimal physiological conditions in cell culture with a middle density a flow rate of 0.1 - 0.25 ml/hr. is used.

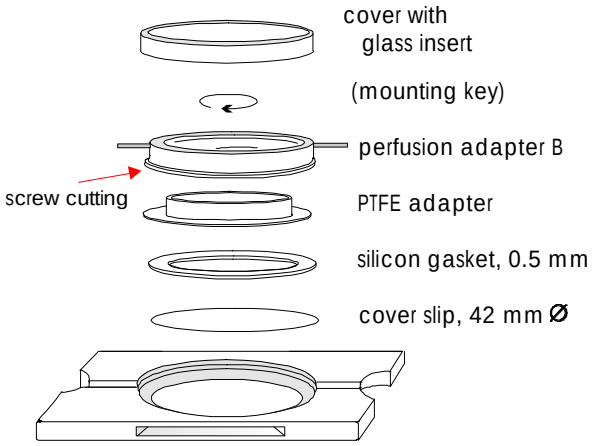
Perfusion in the "open" cell cultivation system

Perfusion adapter A
for PTFE adapter



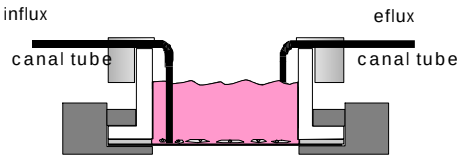
A: the distance between the growth surface
and the top of the adapter = 12 mm

Perfusion adapter B
screw-in, with a special flat PTFE adapter
(micromanipulation, injection of cells)



B: the distance between the growth surface
and the top of the adapter = 7.5 mm

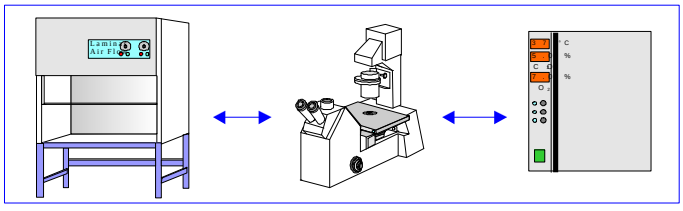
section through the perfusion adapter A



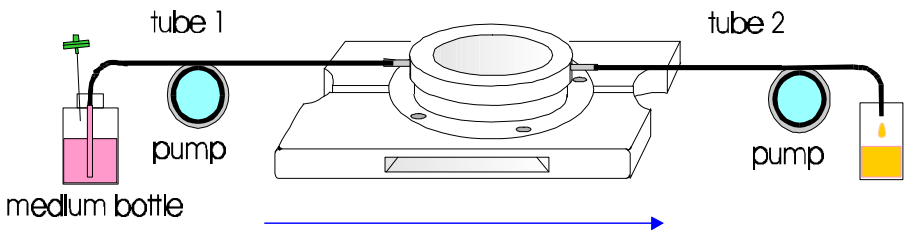
Sterilization: +165°C for 2 hrs.. - After sterilization
the screw ring should again be tightened

Advantage of the perfusion adapter, A + B:

- observation area: 29 mm
- open access to the cell culture (cover)
- the medium changes can be performed either slowly or rapidly using two canal tubes of different length
- the height of the tubes within the PTFE adapter can be varied for a distinct amount of medium



Example of a perfusion system



Peristaltic pump

If a multicanal peristaltic pump is used, the *influx* and *eflux* of the culture fluid can be performed by using the same pump. In this case the tube through which the fluid is removed has a s slightly greater diameter than that through which it is enters.

Syringe pump

Instead of a peristaltic pump, a syringe can be used to introduce medium into the chamber.

Medium bottle

A 25 mm diameter hole is bored into the top of the medium bottle lid. A 4 mm thick silicon plate is used for sealing. A needle, for example an injection needle (1.2 mm external dia., 15 mm long) is inserted through the silicon seal and bent sligthly. The needle is inserted not through the top, but through the buttom of the seal so that it will be immersed in the medium when the lid is put on the bottle. So that the pressure is equalized, a second needle is pierced through the seal. This needle is attached to a hydrophobic sterile filter. The unit without the filter must be sterilized.

Silicon tubing 1

Tubing with an *internal diameter of 0.7 mm* for medium influx.

Silicon tubing 2

Tubing with an *internal diameter of 1.0 mm* for medium eflux.
The outer diameter of the perfusion adapter tubes is 1.4 mm, the inner diameter is 1.0 mm.
Different types of tubing can be used;
e.g. silicon, Norprene®, Tygon®, Teflon®. *See Material Description!*
All components can be dry-heat sterilized (2-3 hrs. at +165°C) or autoclaved at +121°C

Perfusion Procedure

slow perfusion

Medium is passed from the medium reservoir bottle through the tubing via the pump into the POC-Chamber through the longer of the two adapter tubes. The medium is withdrawn through the shorter adapter tube.
The perfusion speed can be varied widely, but an optimal flow rate for a cell culture would be between 0.1 and 0.25 ml/hr.
For *long-time observations*, an inverse microscope with a constant temperature stage with the possibility for constant pH-values must be used. For these studies a *climate box*, in which the temperature and CO is controlled, is used in conjunction with the heated microscope stage (Carl Zeiss, D-07740 Jena; Leica, D-35530 Wetzlar). The medium container can then be outside of this box. The slow perfusion allows the medium to be warmed as well as the pH to be controlled via the gas permeable silicon tubing, before it reaches the cells.

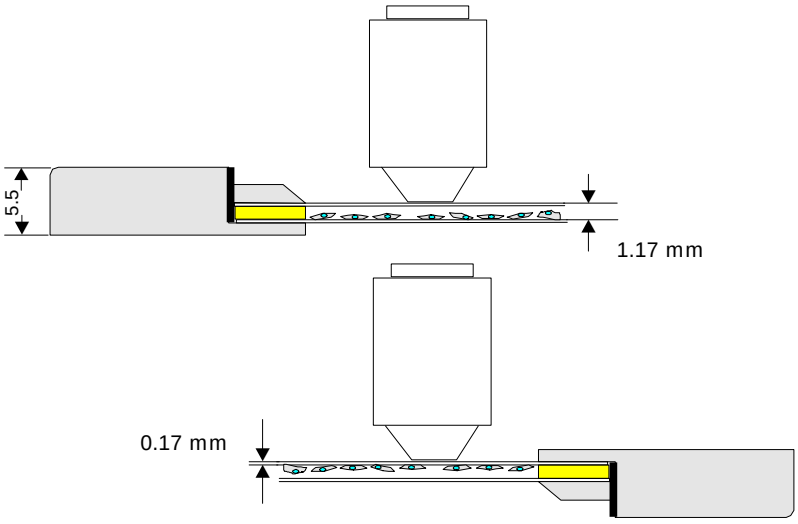
fast perfusion

To achieve a fast perfusion, the medium enters through the longer perfusion adapter tubes and exits via the shorter tube. A syringe can be used for this procedure. In this way, a medium change can occur in about *8 sec*: addition of 2.5 ml in *4 sec* and removal under vacuum in *4 sec*. In this case, silicon tubing with an internal diameter of 0.7 mm is used. Since the internal diameter of the tubes of the perfusion ring is 1.2 mm, this allows the flow rate to be increased with a reduction in time of between *2.5 and 3 sec*.

POC-Chamber-System on "upright" microscopes

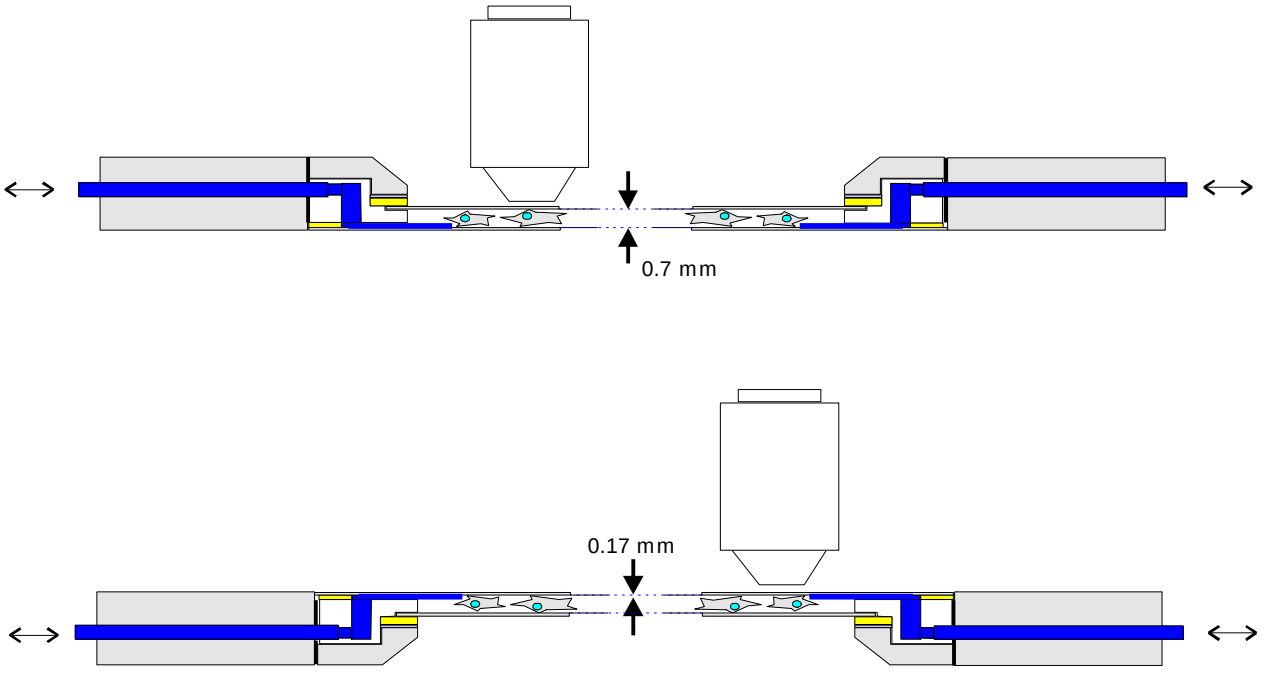
The POC-Chamber-System can also be used on "upright" microscopes.
In this application the working-distance of the objectives and condensers is important.

Closed cultivation



The POC-Chamber can be turned

Perfusion in the *closed* system



Use of foils for the preparation of a monolayer cell culture for electron microscopy

Beside the FEP foil, there is a special polyester foil, called "EM foil" which is especially suitable for the preparation of monolayer cell cultures for electron microscopy, since it can removed easily from the embedding resin. During sterilization, the 23 µm thick foil becomes a partially crystallization of amorphous material on the surface. But this does not have any effect of cell growth and can be removed using sterile medium.
If the foil is handled carefully, extra cleaning of the foil is not necessary. Dust can be removed with filtered air. Dirty foils can be cleaned using a detergent and pure ethanol and then washed in double distilled water.

Cell Culture

The 42 mm diameter foil is placed in the base plate of the POC-Chamber. The PTFE adapter (without the silicon seal) is depressed onto the foil by screwing the screw ring in the base plate using the mounting key. The EM foil is thereby stretched and simultaneously sealed. The cover is added and the POC-Chamber sterilized using dry heat for 2 h at +165°C or autoclaved at +121°C. When the chamber has cooled down, the cells with medium can be introduced into the chamber and incubated in a CO_2-incubator.

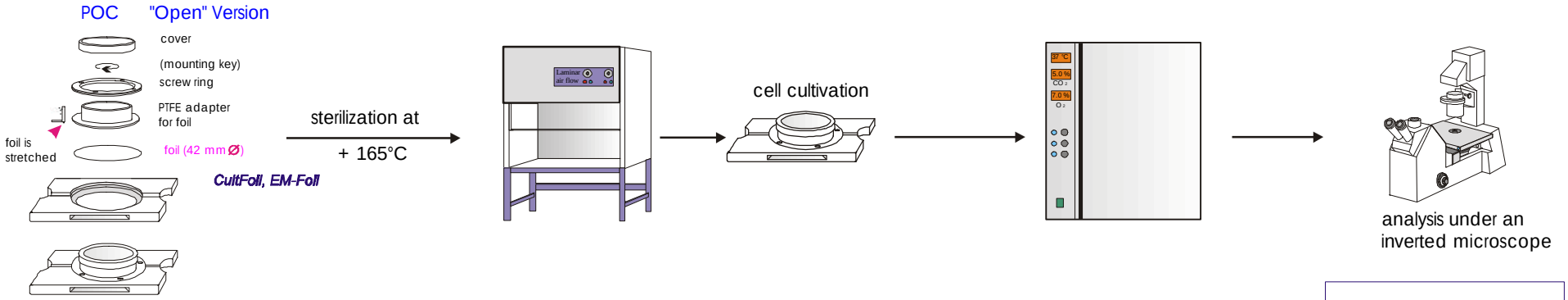
Preparation for electron microscopy

- Displace the cover from the PTFE-adapter of the POC and suck off the medium.
- Wash the cells twice with medium without serum, cautiously (temperature of the medium 37°C).
- Fix the cells with 2 % glutaraldehyde dissolved in 0.05 M Na-cacodylate buffer, pH 7.2, for 1.5 hrs at room temperature.
- Wash twice with Na-cacodylate buffer (0.05 M, pH 7.2).
- Postfix in 1 % osmium tetraoxide in 0.05 M Na-cacodylate buffer, pH 7.2 + 8 % sucrose for 1.5 hrs at room temperature.
- Dehydration in pure ethanol: 50 % for 2 x 7 min, 70 % over night, 90 % for 2 x 7 min, methacrylic acid-2-hydroxypropylester for 3 x 15 min.
- Embedding: mixture of methacrylic acid-2-hydroxypropylester with epoxy resin at times for 30 min - 2:1, 1:1, 1:2 at room temperature; epoxy resin trice for 1 h.
- Polymerize the resin at 65°C for 48 hrs.

Important:

- During polymerization the cover of the adapter should not be in place, since during this process, components of the resin could collect on the cover and flow down the side into the screw thread of the base plate or screw ring.

After polymerization, the screw ring is removed using the key and the PTFE-adapter with the resin and foil attached taken out of the base plate.
The foil is peeled off and the resin block removed from the PTFE-adapter.
The embedded cells can now be viewed under an inverted light microscope (phase contrast) and, if necessary, marked.
Vertical sections without the "sandwich" method (see Fig.: cells between two resin layers) would be disadvantageous because under the electron microscope, in most cases, the slices bend from the influence of the electron beam.



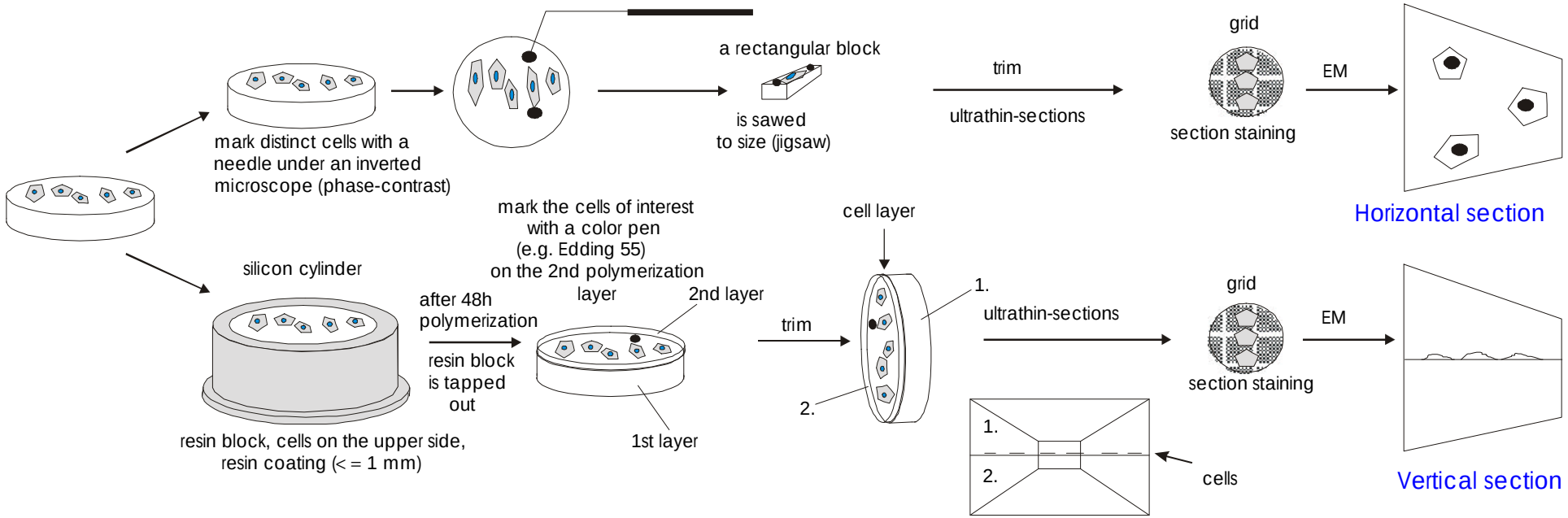
Preparation of cultivated anchorage-dependent cells in the POC-Chamber

-- wax embedding, staining of slices and light microscopy
-- preparation of cells for EM studies ⇒ ⇒

fixation
contrasting
dehydration
polymerization

after polymerization

PTFE-adapter is unscrewed, the foil peeled off, and the resin block is tapped out



Material Description

Aluminium: Base plate and mounting key

- Aluminium is an extremely good heat conductor. The surface of these parts are heat-treated and strengthened due to an anodized coating. However, this procedure does not compatible with strong alkali cleaning materials. For this reason, these parts should only be cleaned with distilled water or 70 % ethanol.
- The black anodized base plate should not be placed into a CO_2-incubator with copper trays since this will cause "electrolysis" and damage the surface of the chamber. In this case place the chamber on a glass, plastic or stainless steel base.

V2A-Steel: Perfusion adapter, Perfusion adapter **A**, Perfusion adapter **B**, screw ring, cover frame and and canal tube

- The Perfusion adapter for closed cultivation should be handled with care because the rim which supports the cover slip (0.2 mm, on the outer area, 0.1 mm) can easily be bent. The canal tube can be cleaned with 70 % ethanol or other cleaning fluids and well rinsed with distilled water.

PTFE und FEP: Registered name Teflon® (DuPont), Hostaflon® (Hoechst)

- *PTFE adapter:* This is a very inert material which is insensitive to most cleaning materials. It is best cleaned with 70 % ethanol or other laboratory cleaning fluids and then rinsed well with distilled water.
  temperature: PTFE more than 200°C
- *FEP-Foil (CultFoil, PeCon):* This foil has a treated (hydropholic) and smooth (hydrophobic) surface.
  The thickness of the foil is 25 µm.

To distinguish between the two surfaces

a.) The hydrophilic side demonstrates an adhesion to paper.
b.) It is not possible to write on the hydrophobic side using a felt tip pen

Characteristics and uses

- high gas permeability
- permeable to ionizing radiation even below 300 nm
- non-toxic
- inert to most cleaning materials

hydrophilic surface:

- excellent cell adhesion

hydrophobe surface:

- suspension culture
- colony formation in agar or methyl cellulose
- can be easily removed from resin after embedding for electron microscopy
- temperature: +170/180°C

Silicon: Gaskets: non-toxic, gas permeable with a hardness of 40 and 60 Shore

- After use, wash immediately under running water and rinse in distilled water. If necessary, boil for 10 min in distilled water or autoclave at +121°C.
  Do not use cleaning liquids, although ethanol can be employed.
  temperature: + 180°C

Tubing material for perfusion: non-toxic

- *Silicone* is very elastic, gas permeable, can be sterilized by autoclaving as well as by dry heat (165-180°C).
- *Norprene*® is much less gas permeable, but has a longer life than silicon in a peristasltic pump. It can also be autoclaved (121°C).
- *Tygon*® tubing is crystal clear, does not oxidized, has very smooth surfaces, is elastic and ideal for peristaltic pumps. It is not particularly gas permeable and can be autoclaved (121°C).
- *TeflonÒ* (PTFE, TFE, FEP) is extremely inert to almost all solvents: If thin tubing is used, it can be gas permeable but is not particularly elastic. It must be connected to steel tubes of the perfusion adapters by silicon tubing and cannot be used in peristaltic pumps. It can be sterilized by dry heat or autoclaving.

Glass: cover slips, 0.17 mm

- Hydrolytic class 1, borosilicate glass. Cleaning is not usually necessary. However, normal laboratory cleaning liquids can be used followed by rinsing in distilled water. Always use a pair of tweezers to handle the cover slips and grip them only on the rim.
- *Recommended cleaning in a laminar air-flow bench:* Dip the cover glass in ethanol and let the latter drip into a beaker. Then pass the cover slip through a flame to burn off rest of the ethanol. Keep the cover slip in a horizontal position until it has cooled.

Sterilization: Either the whole chamber or parts of the chamber can be sterilized.

- Dry sterilization at +165°C to 170°C for 2 h
- Autoclaving at +121°C

After sterilization of the perfusion or *"closed"* chamber, the screw ring should again be tightened.

Trouble-shooting

Observation	Possible cause	Correction
Glass breaks when putting chamber together or by sterilization	Bad quality glass	New or different glass
	"Closed" culture:	
	Too much pressure within the chamber during sterilization	Insert a needle trough the silicon walls to release pressure inside the chamber
	"Open" culture:	
	Instead of the PTFE adapter for "glass", the adapter for the FEP-Foil has been used	Change the adapter
Chamber leaks	Screw ring not tight enough	Tighten ring with key
	Silicon ring not properly cleaned, so that dust is present on the surfaces	Clean silicon ring again, if necessary with ethanol
	PTFE adapters: Deep scratches or indentations in the surface connecting with the silicon ring	Use a new adapter

References

Pentz, S. and Hörler, H. (1992) A variable cell culture chamber for "open" and "closed" cultivation, perfusion and high microscopic resolution of living cells. J. Micros. **167**, 97-103.

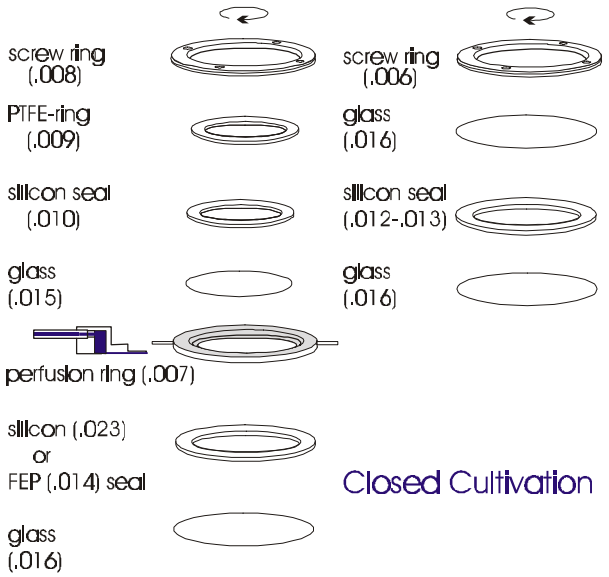
Pentz, S. and Hörler, H. (1990) Beating heart cells in vitro - a cells system for drugs and toxins. Eur. J. Cell Biol.m Suppl. **30**, 51.

Freimann, R. Pentz, S. and Hörler, H. (1997) Development of a standing-wave fluorescence microscope with high nodal plane flatness. J. Micros. 187, 193-200.

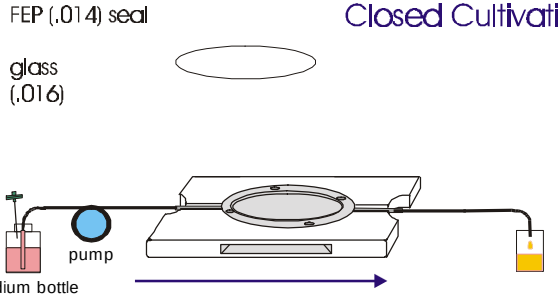
POC-Chamber-System

(Cat. No. 0727.xxx)

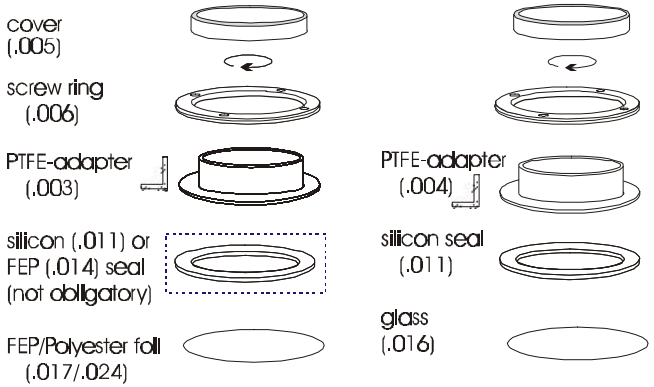
"Closed" Perfusion



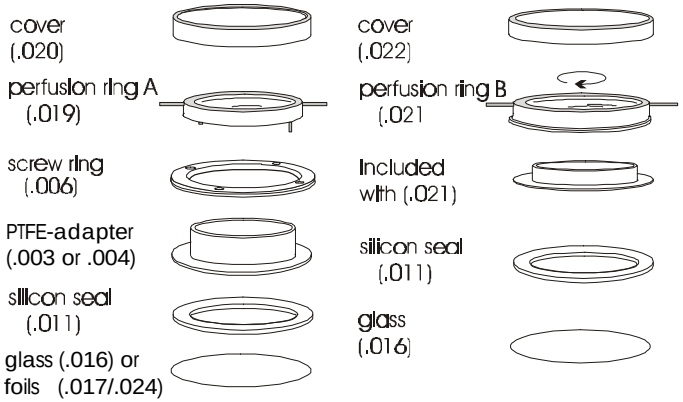
Closed Cultivation



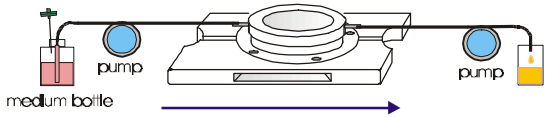
"Open" Perfusion A



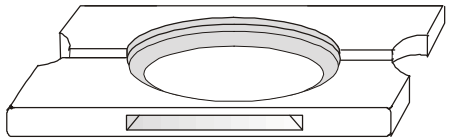
"Open" Perfusion B

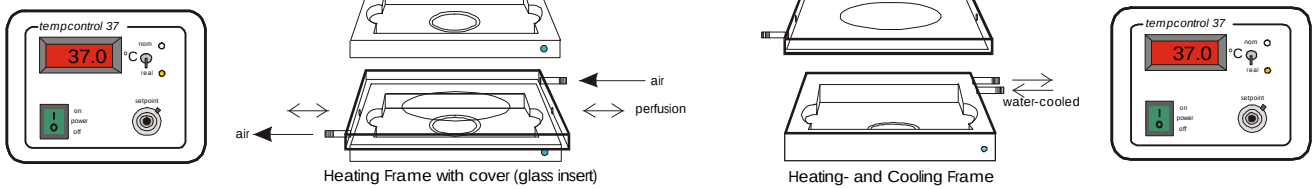


Open Cultivation

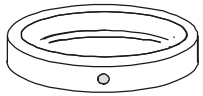


POC-Chamber base plate (0727.001)





ROC-Chamber base plate (instead of the POC base plate) (0728.001)



without "Closed" Perfusion

LaCon