Microchips

Andrei Leitão

Organs-on-a-chip: Example

KITChip system

10^7 cells in 1,156 microcontainers in a $2cm^2$ area.
Cultures may last days to weeks.

polymeric scaffolds with microcontainers (usually 300 × 300 × 300 µm w × l × h). Routinely, polycarbonate (PC), polystyrene (PS), and polymethylmethacrylate (PMMA)
are used as nonbiodegradable polymers, whereas polylactic acid (PLA) is used as a biodegradable
polymer for chip production.

45×10^3 to 2×10^6 pores per cm^2

Chip-Based Tissue Engineering in Microbioreactors

CHAPTER 5

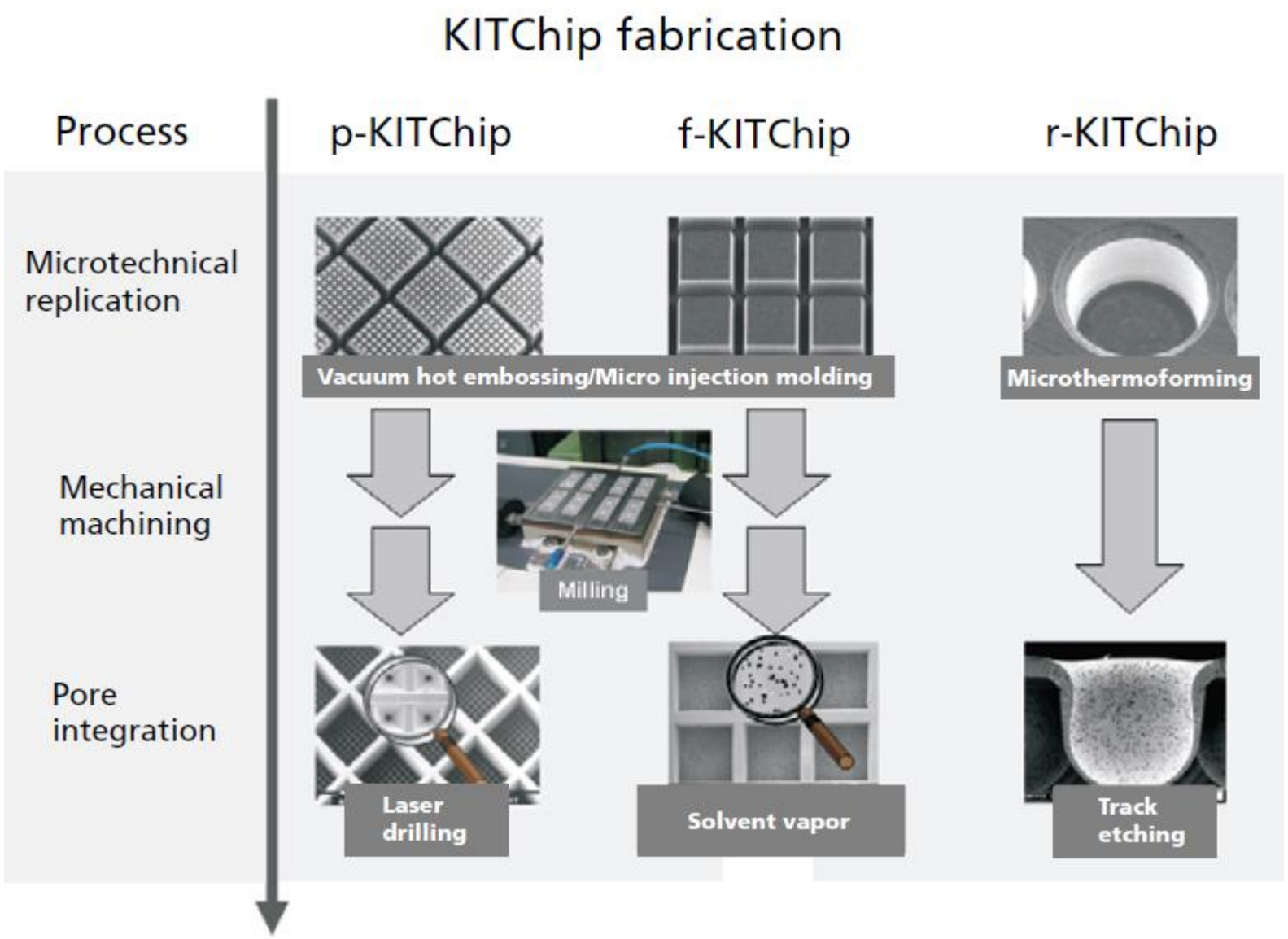
Brigitte Altmann,[1] Stefan Giselbrecht,[1] Matthias Rieke,[2] Alexander Welle,[1] Tim Scharnweber,[1] Karl-Friedrich Weibezahn,[1] and Eric Gottwald[1]

Methods in Bioengineering

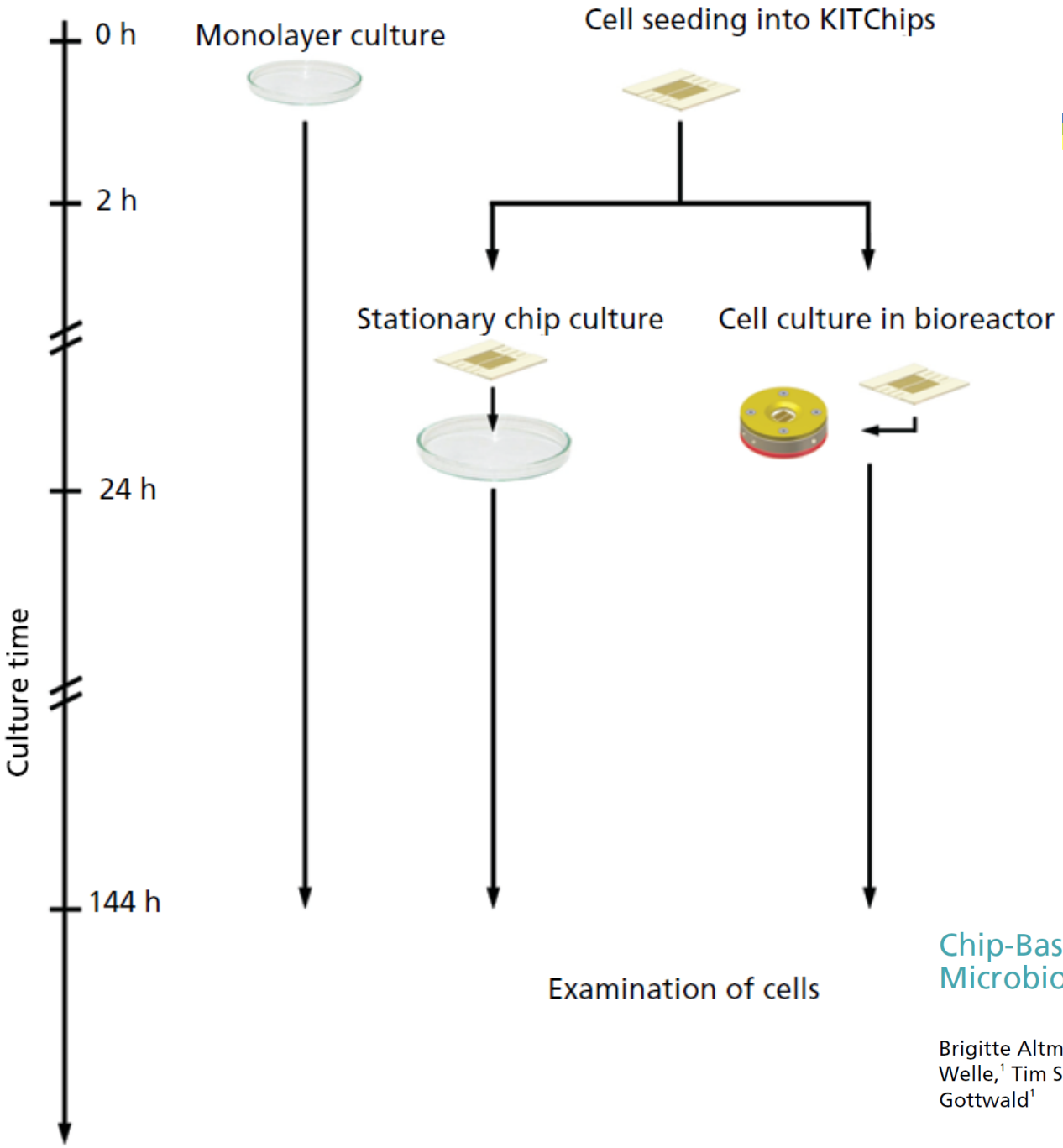
3D Tissue Engineering

François Berthiaume and Jeffrey Morgan, editors

Organs-on-a-chip



Setup

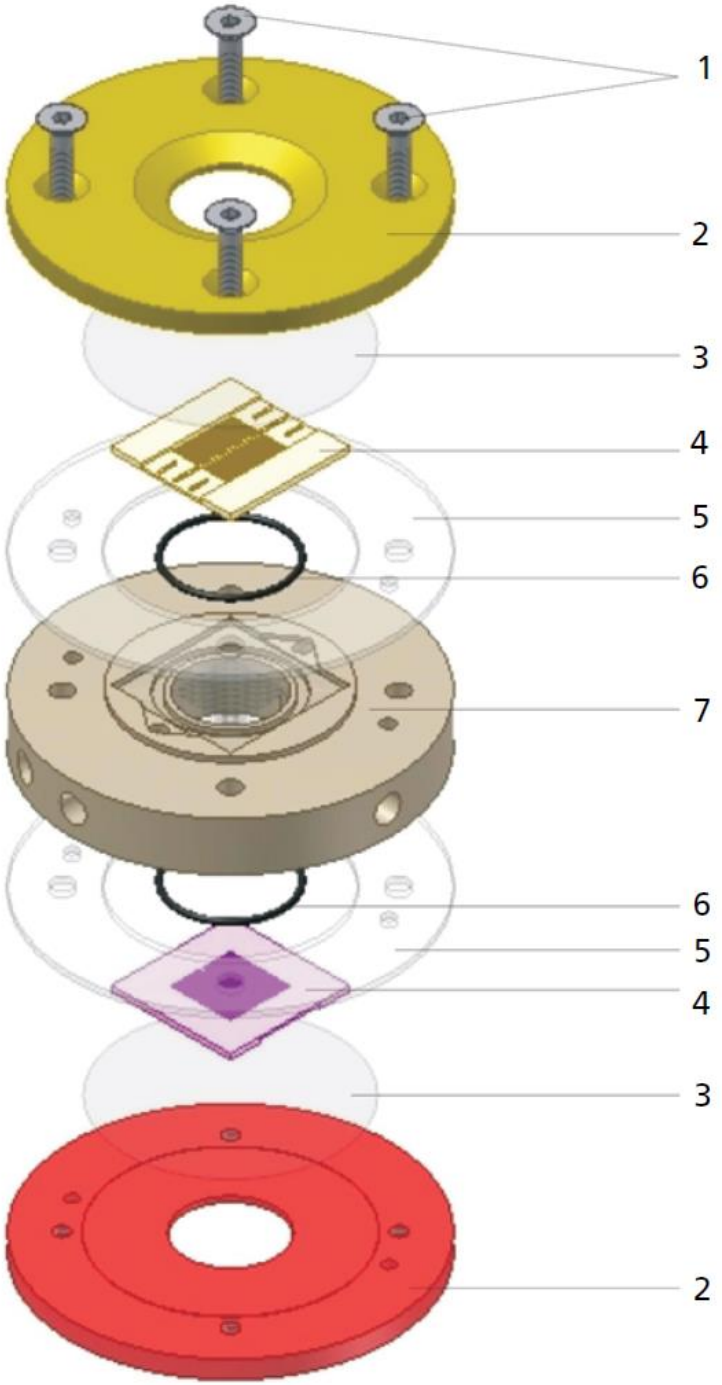


Renovation of 50% cell culture every 2-3 days

Chip-Based Tissue Engineering in Microbioreactors

Brigitte Altmann,[1] Stefan Giselbrecht,[1] Matthias Rieke,[2] Alexander Welle,[1] Tim Scharnweber,[1] Karl-Friedrich Weibezahn,[1] and Eric Gottwald[1]

Assembly of the bioreactor



1. Screws
2. Lids
3. Microscope cover slip
4. Chip
5. Outer gasket
6. Inner gasket
7. Bioreactor body

Chip-Based Tissue Engineering in Microbioreactors

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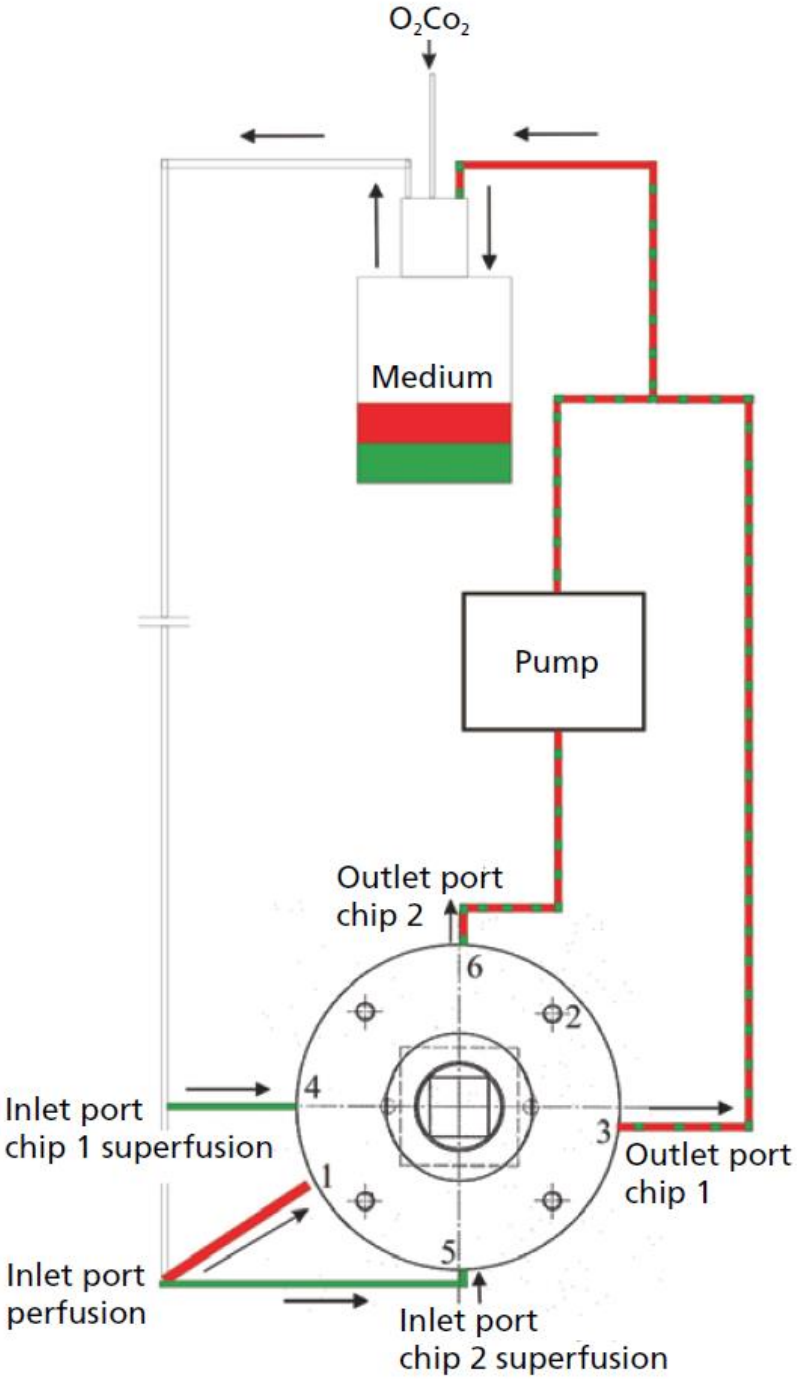
Feeding system



perfusion

superfusion

Chip-Based Tissue Engineering in Microbioreactors

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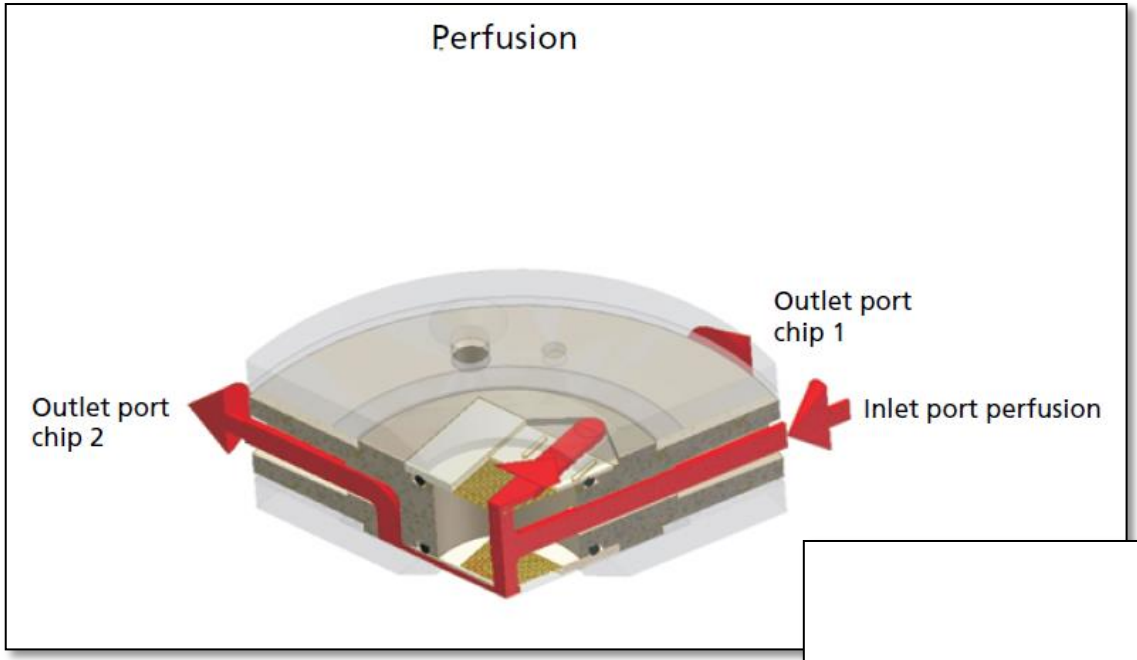
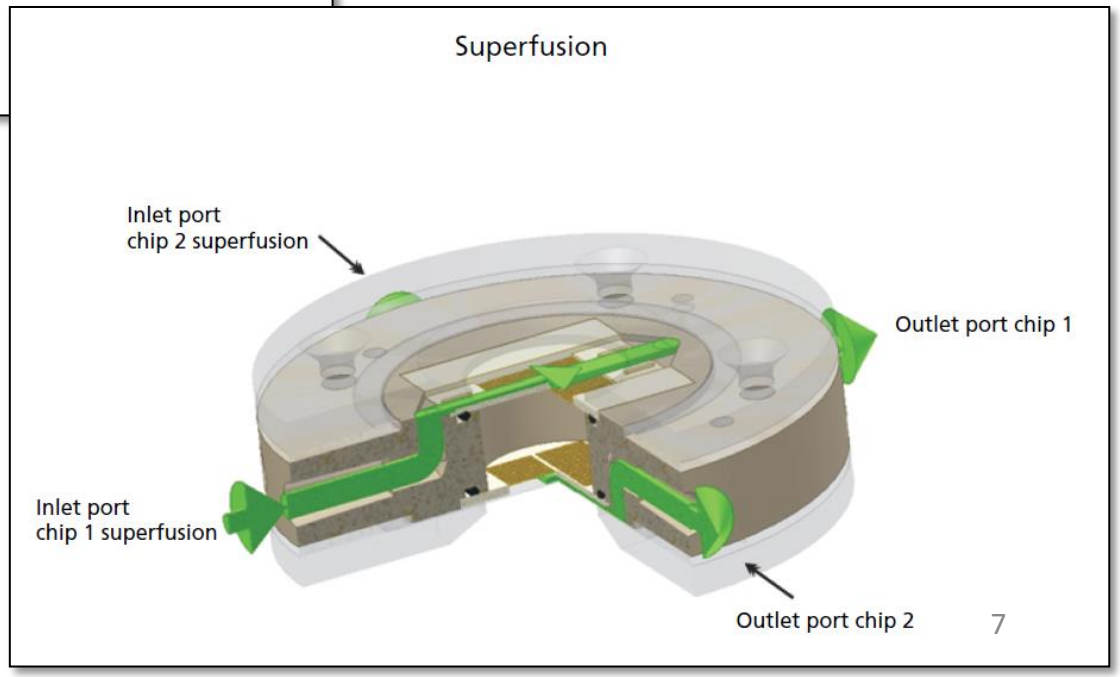
Details of the feeding system





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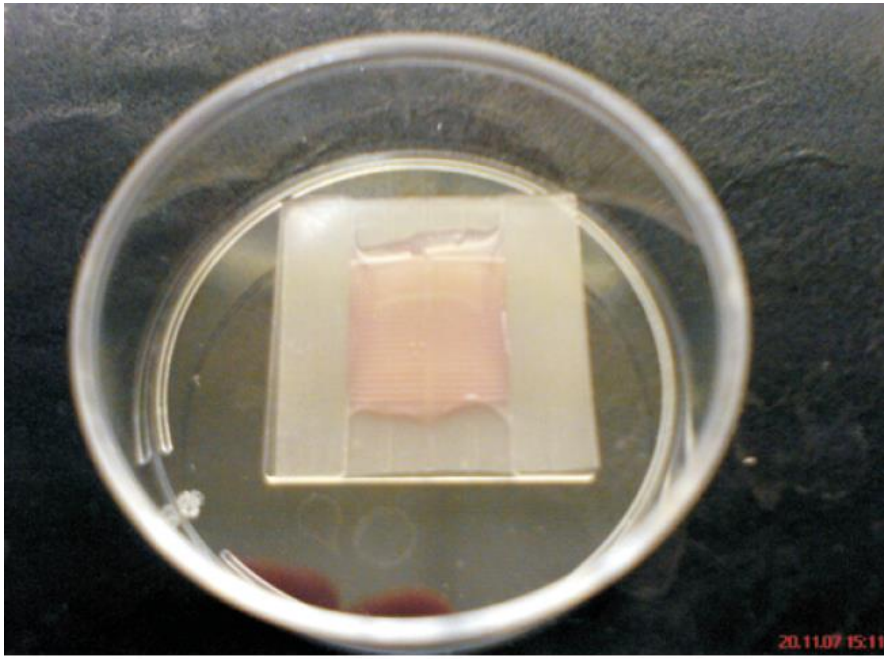
Organs-on-a-chip

Sterilization of the system: γ-irradiation or ethylene oxide

Multiple washes with isopropanol/water solutions from 100% to 0%

Add collagen type I for hepatocyte growth

Pipette 150 µL cell suspension and let it adhere (example) →



It can be adapted to 96 well plates

Chip-Based Tissue Engineering in Microbioreactors

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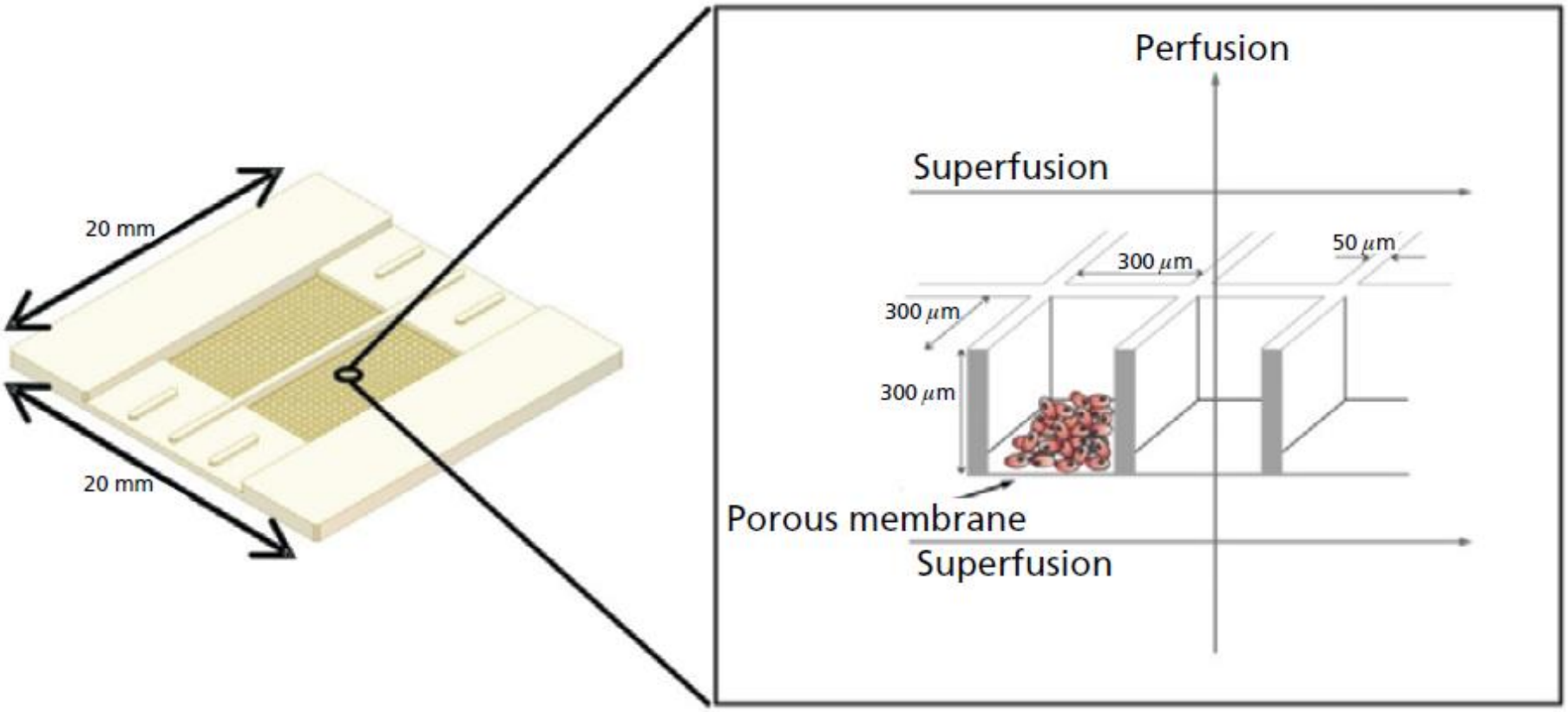
Final layout



Chip-Based Tissue Engineering in Microbioreactors

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Organs-on-a-chip

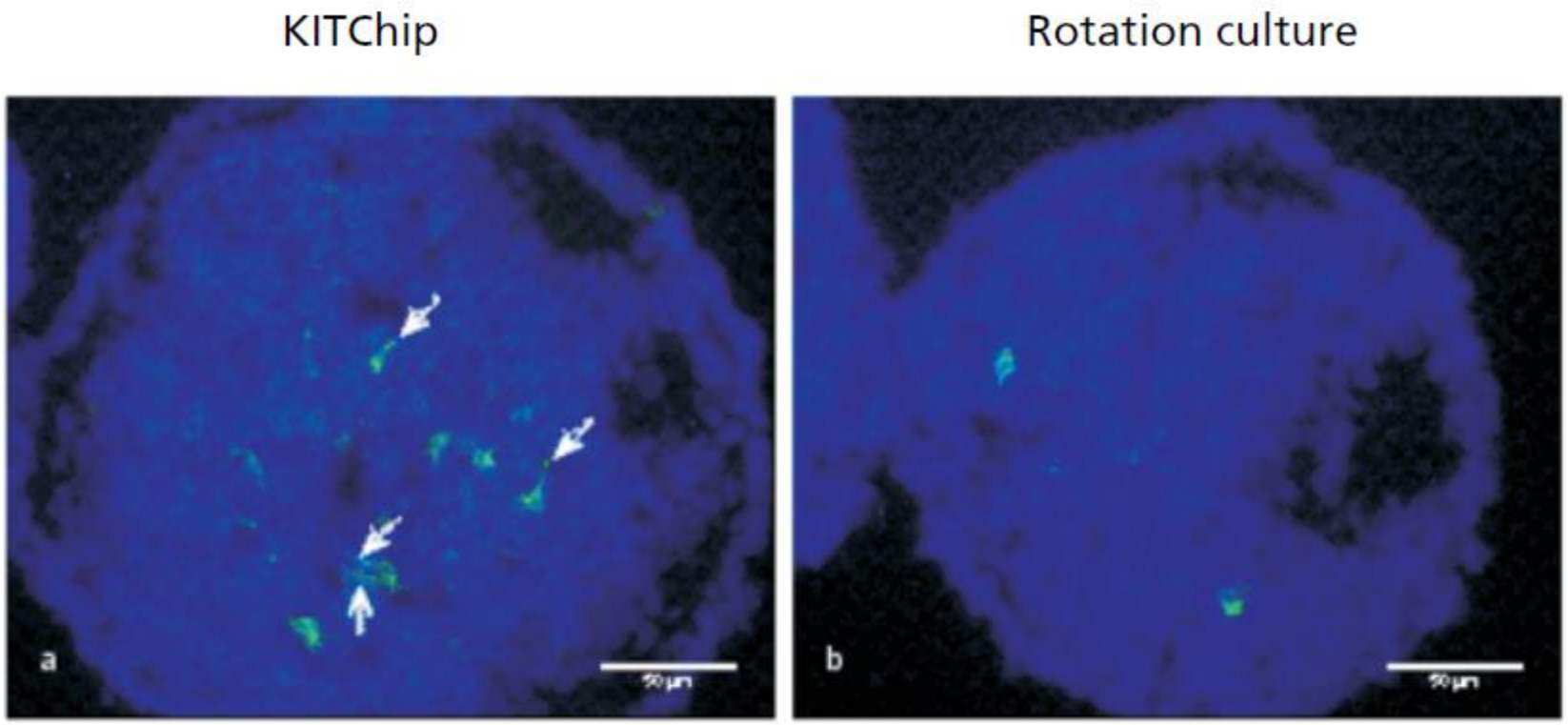

Figure 5.4 Differentiation of (a) photoreceptors (PR, green) in retinal reaggregates derived on the KITChip is further advanced, compared to (b) their counterparts cultured in classical rotation culture. Increased numbers of PR have been found in the KITChip reaggregates; note their histotypic morphology showing features like prolonged rhodopsin$^+$ processes (arrows) corresponding to developing outer segments.

How to begin?

Questions to be answered...

Material - Application of bioengeneering

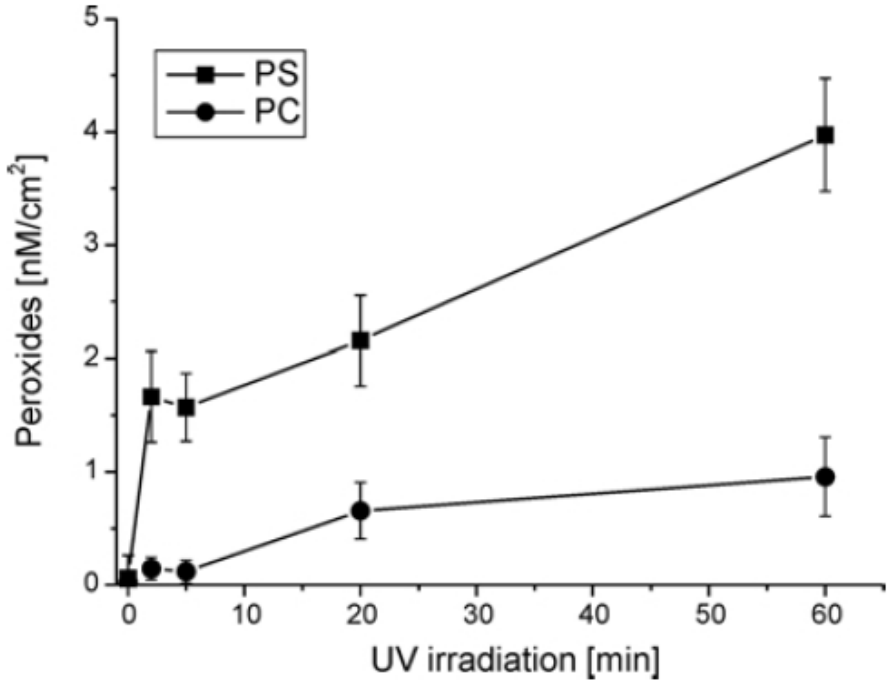

Fig. 1. *Surface peroxide density on polystyrene (squares) and polycarbonate (circles) after different UV treatment times.*

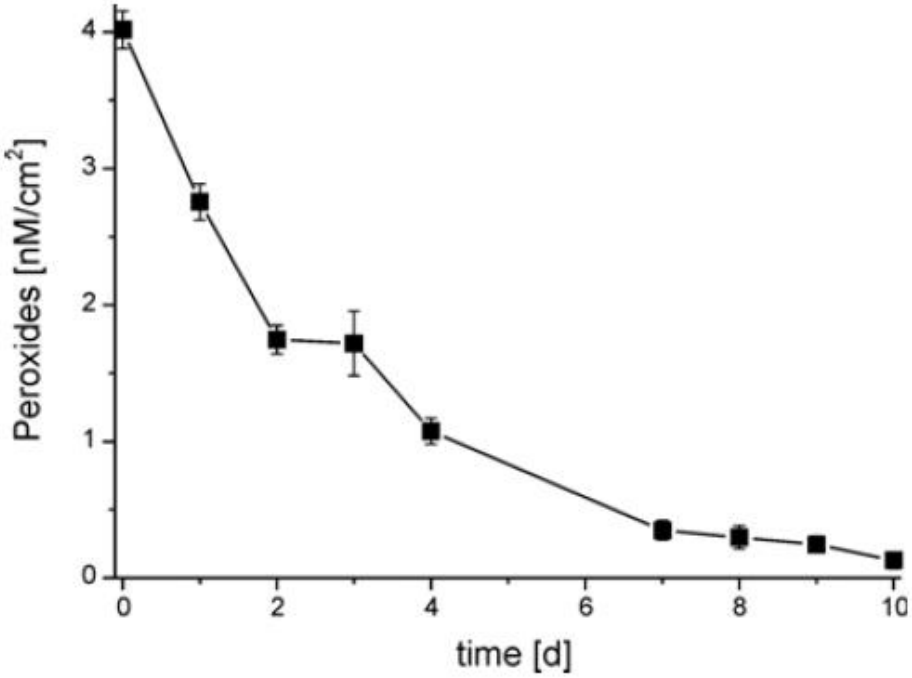

Fig. 2. *Surface peroxide density on UV irradiated (1 h) polystyrene after different storage times.*

A. Welle, E. Gottwald, Biomed Microdevices **2002**, *4*, 33-41

Material - Application of bioengeneering

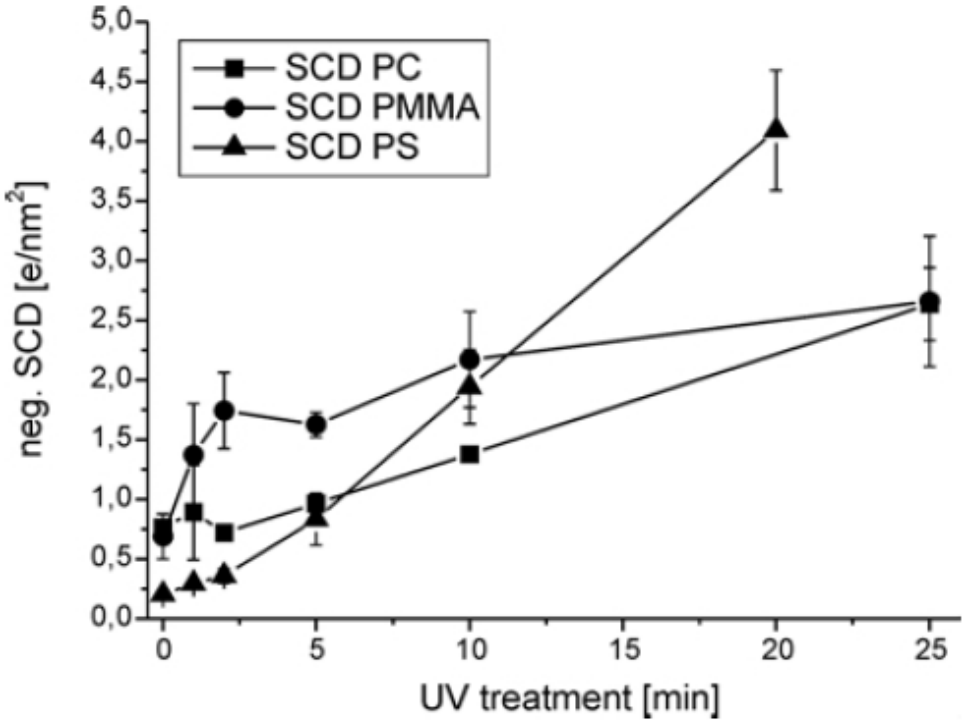

Fig. 5. *Negative surface charge densities on polycarbonate (squares), polymethylmethacrylate (circles) and polystyrene (triangles) against UV irradiation times.*

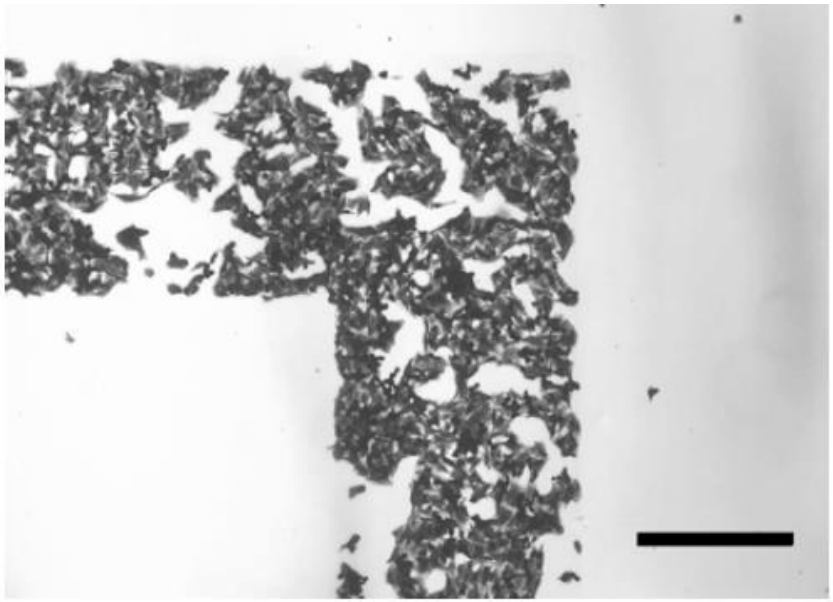

Fig. 7. *Microscopy of HepG2 cells on structured PS. Image was taken after 2 days cell culture, washing and crystal violet staining. Scale bar represents 200 µm. Note that almost no cells are adherent on non irradiated regions (lower left quarter and to the upper and right side of the image).*

Table 2. *Plating efficiency of L929 and HepG2 cells on polystyrene with different UV treatment times. Incubation time 4 hours*

UV irradiation time (min):	0	2	5	20	60
Plating efficiency L929	2%	89%	97%	98%	98%
Plating efficiency HepG2	6%	25%	77%	99%	99%

A. Welle, E. Gottwald, Biomed Microdevices **2002**, *4*, 33-41

Application of bioengeneering (2)

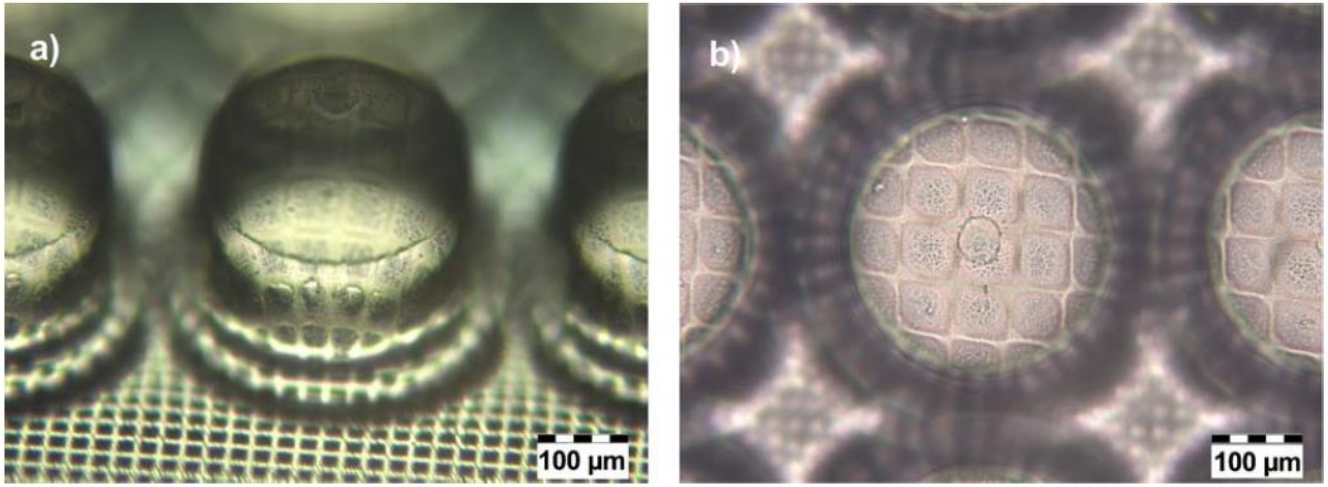

Fig. 1 Microthermoforming of PC films patterned with thin silver spots. The unstretched region in the foreground of the microcavities in the left picture (a) shows the original pattern of the 25 × 25 μm^2 sized silver spots

S. Giselbrecht, *et al* Biomed Microdevices **2006**, *8*, 191–199

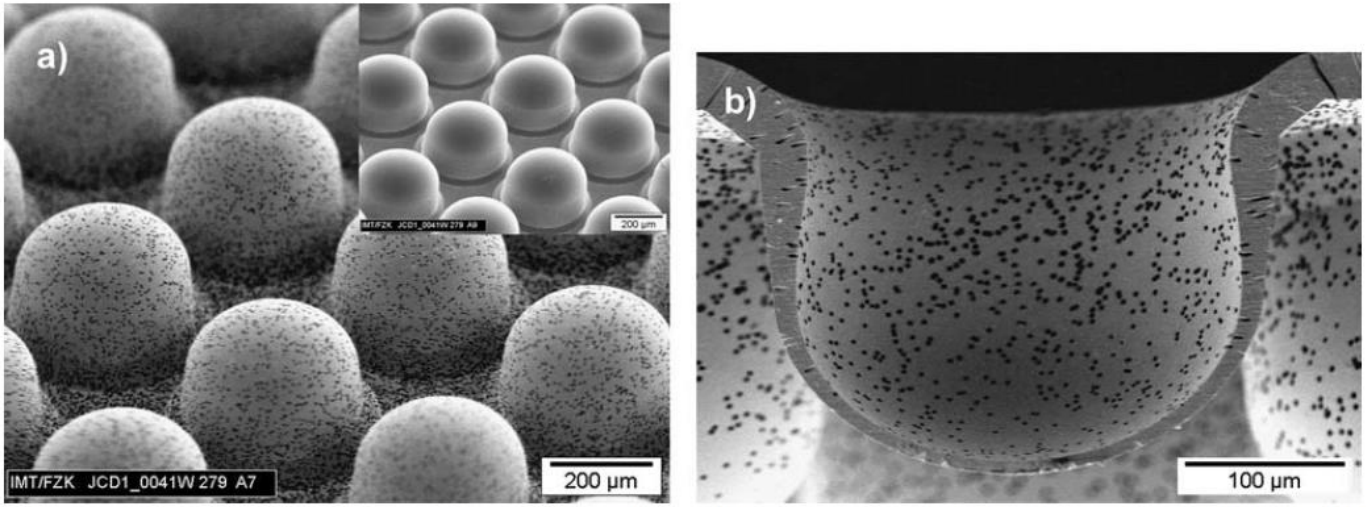

Fig. 3 Ion track technology combined with microthermoforming for the fabrication of highly porous, three dimensional microstructures. (a): PC films irradiated with Xe ions (1460 MeV, fluence 10^6 ions/cm^2) and microthermoformed before (inset, scale bar 200 μm) and after the etching process for 6 h at 50°C in 5 Mol/L NaOH/10% w/v MeOH. Note, that non-etched latent tracks are not visible by scanning electron microscopy. On the right (b): etched cross section of a single cavity

Application of bioengeneering (2)

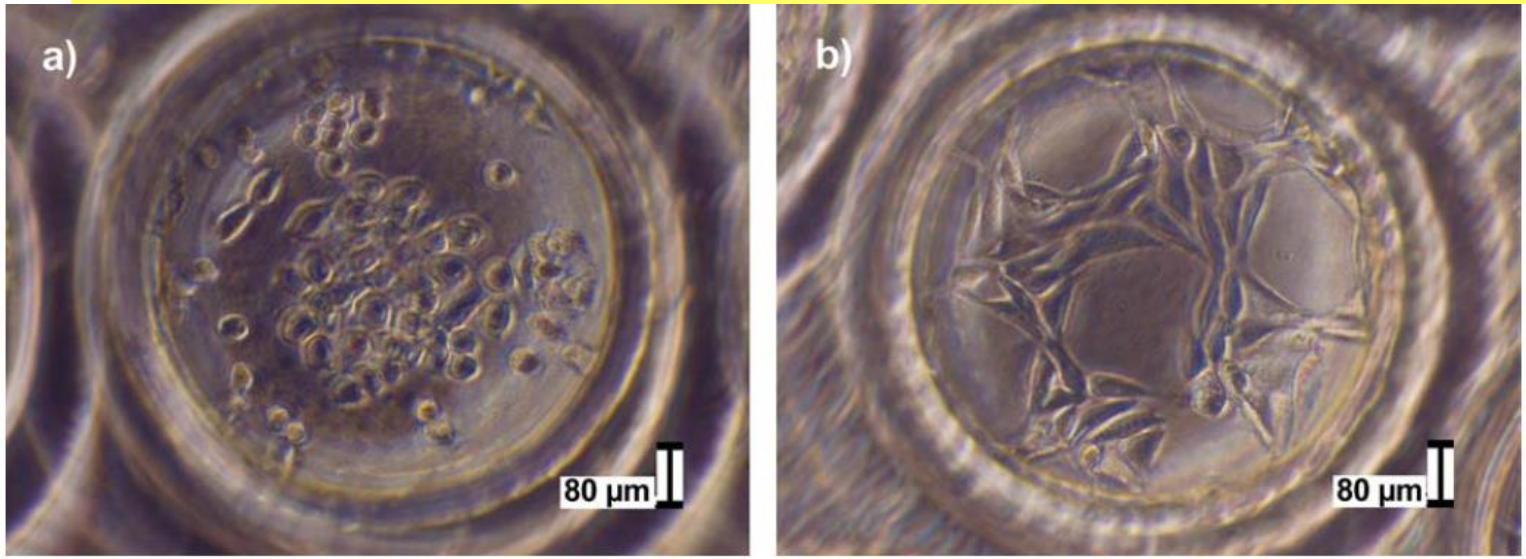

Fig. 4 SMART processed scaffold with a three dimensional cell adhesion pattern. Living fibroblasts in microcavities without (a) and with (b) a patterned surface (PS, day 3 of cultivation). Phase contrast microscopy from the rear side

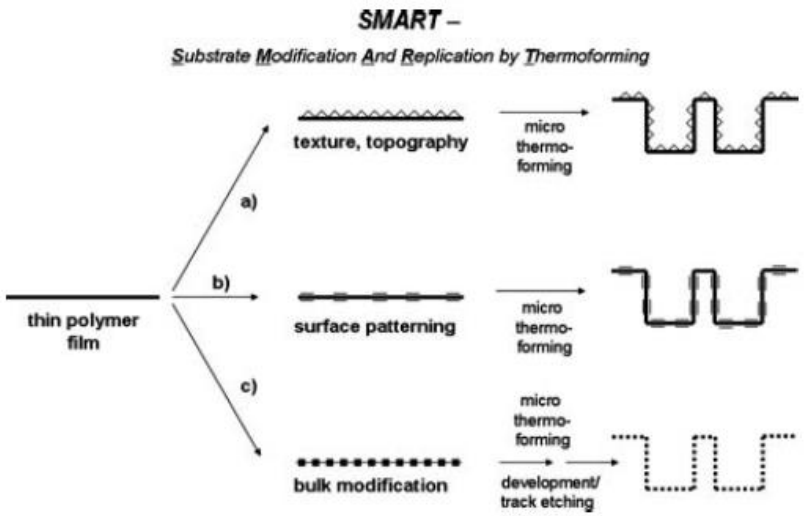

Fig. 7 Microthermoforming of pre-processed, modified polymer films (textured, surface and bulk patterned)

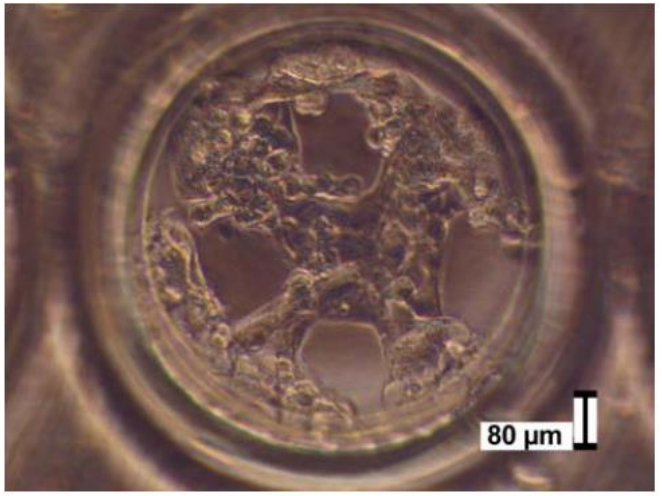

Fig. 6 Hep G2 cells in photochemically patterned microcavity made from PS (day 2 of cultivation). Same pattern as above. Phase contrast microscopy

Coating effect…

Table 1: List of polymers and coatings used in cell culture test and their observable effect on cell adhesion

CellChip	Polymer	Coated with collagen	Cell adhesion
Laser-perforated	Polycarbonate	–	–
	Polycarbonate	+	+
Membrane-bonded	Polycarbonate	–	–
	Polycarbonate	+	+
Thermoformed	Polystyrene	–	–
	Polystyrene	+	+
	Polycarbonate	–	–
	Polycarbonate	+	+
	Cyclo-olefin Polymer	–	–
	Cyclo-olefin Polymer	+	+

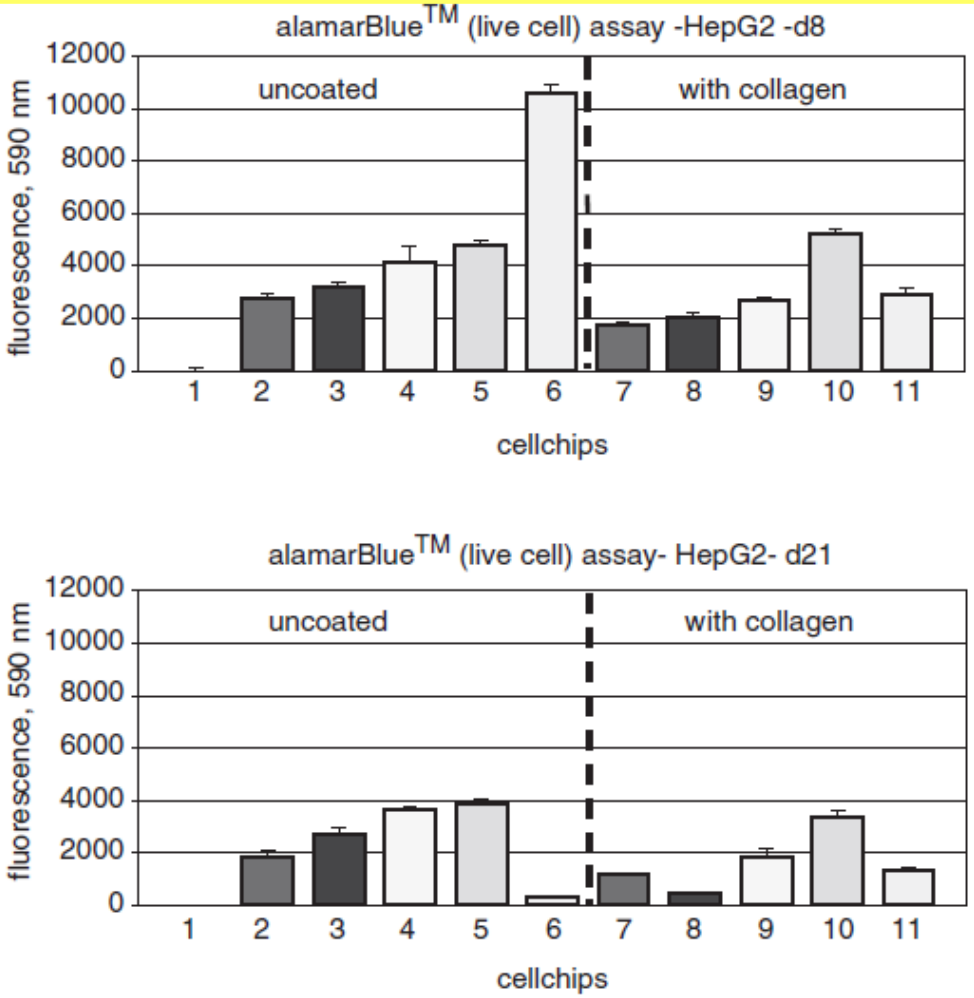

Fig. 11 *Results of alamarBlue™ assays on days 8 and 21*
1: blank; 2/7: laser-perforated PC CellChip; 3/8: membrane-bonded PC CellChip; 4/9: thermoformed PC CellChip; 5/10: thermoformed PS CellChip; 6/11: thermoformed COP CellChip

S. Giselbrecht, *et al. IEE Proc.-Nanobiotechnol.* **2004**, *151*, 151-157

Organs-on-a-chip: Example

Freshly isolated adult rat hepatocytes show an initial loss of total CYP450 content and of associated activities (mixed function oxidases). However, in the aggregate system, this level did not decrease further but remained stable or even increased throughout the culture period of 10–13 days.

What type of cell culture?

Erik Eschbach, et al. *J. Cell. Biochem.* **2005**, *95*, 243–255

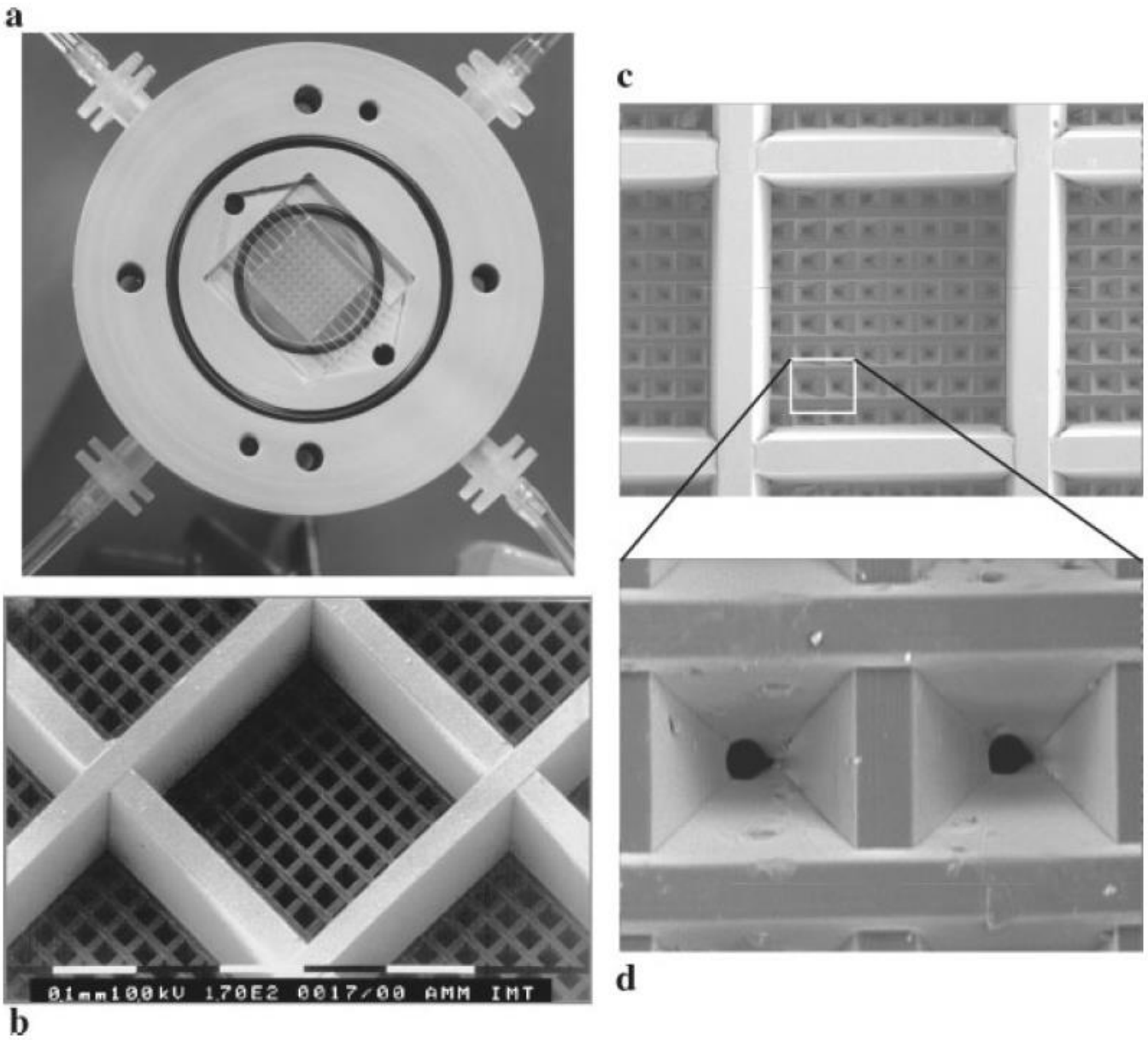

Fig. 1. The microstructured scaffold is formed by injection moulding of PMMA. **a**: Open bioreactor housing with inserted microstructure for application in superfusion or perfusion mode. The 900 microcontainers are arranged in the middle 1-cm^2 surface. **b**: SEM picture of the CellChip's surface, each container has $300 \times 300 \times 300$ µm^3. **c**: The bottom of the microcontainer is treated with an excimer laser resulting in an array of 8×8 pores. **d**: Higher magnification of the bottom. Each pore has a diameter of 2.7 µm.

Erik Eschbach, et al. *J. Cell. Biochem*. **2005**, *95*, 243–255

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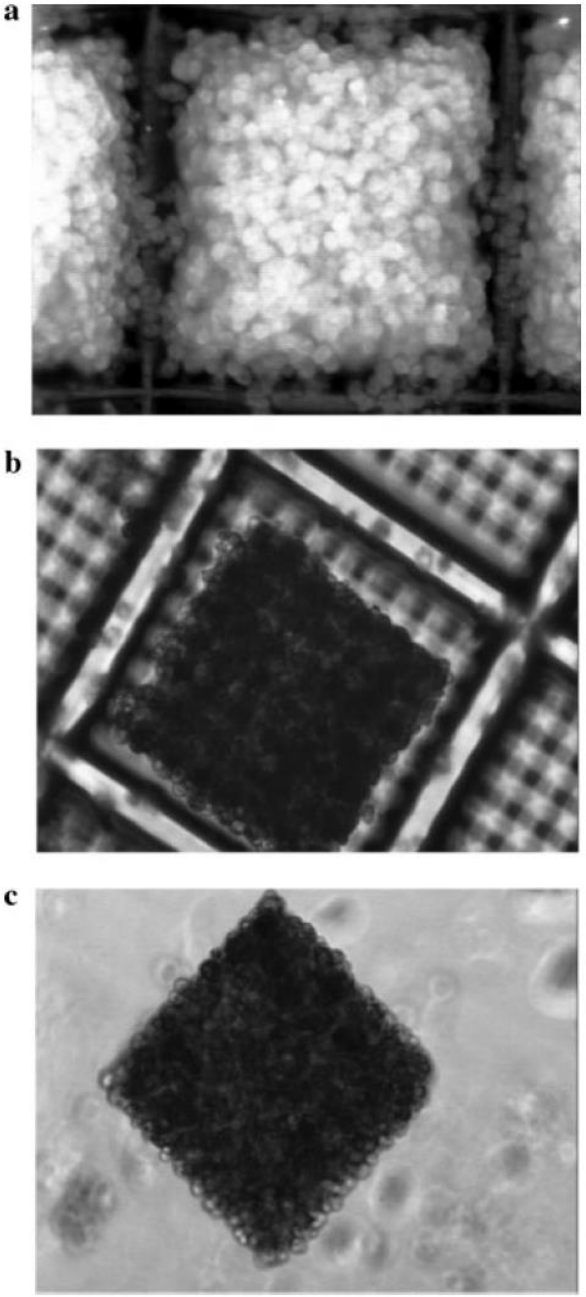

Fig. 2. Rat liver cells aggregated in the microcontainers after (a) coating the surface with collagen I, (b) without any coating. c: Aggregates can be flushed out from uncoated containers as freefloating cuboids.

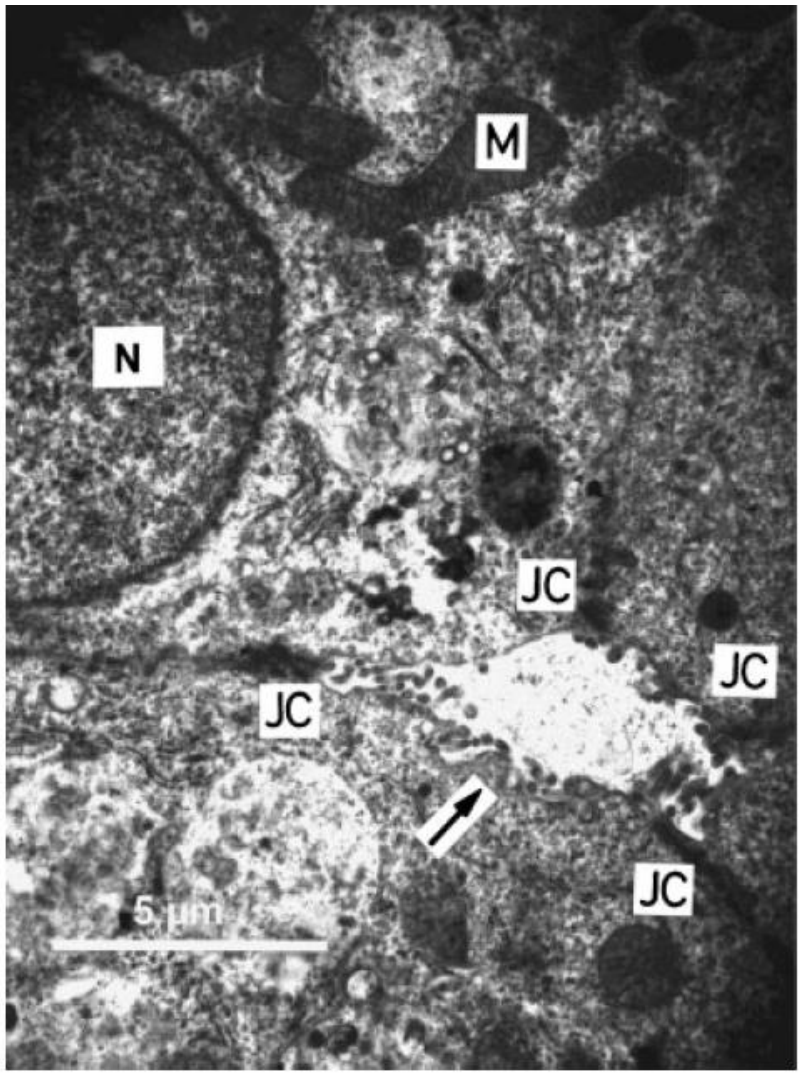

Fig. 3. Electron micrograph of the ultrathin section of an aggregate of rat liver cells (day 6). The arrow indicates a bile canaliculi-like cavity lined by protruding microvilli and sealed by junctional complexes (JC). M, mitochondrium; N, nucleus. Bar length = 5 µm.

Erik Eschbach, et al. *J. Cell. Biochem.* **2005**, *95*, 243–255

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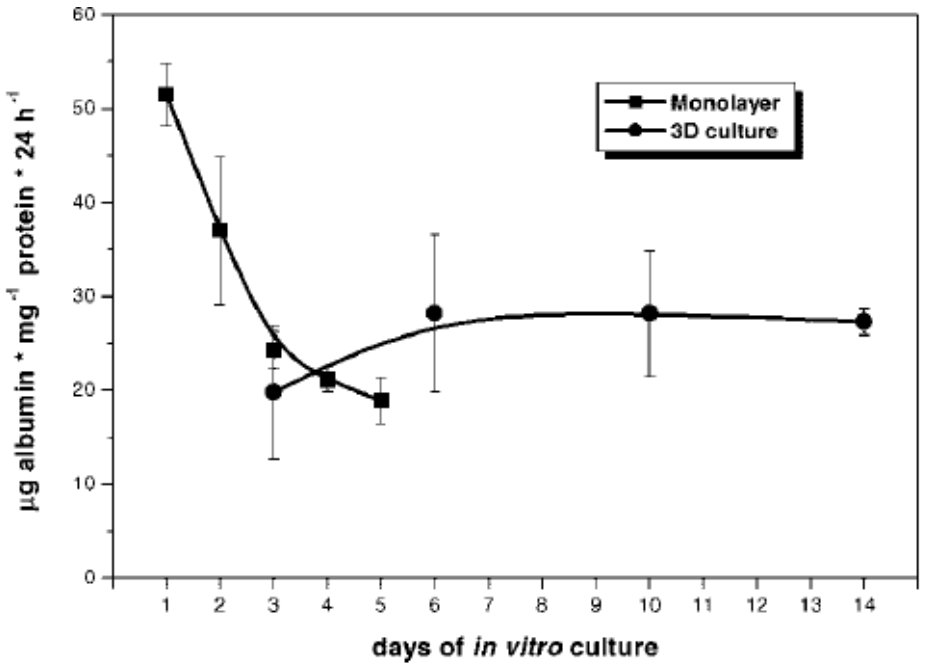

Fig. 4. Albumin secretion of hepatocytes cultured as multicellular aggregates determined by ELISA. During these experi-

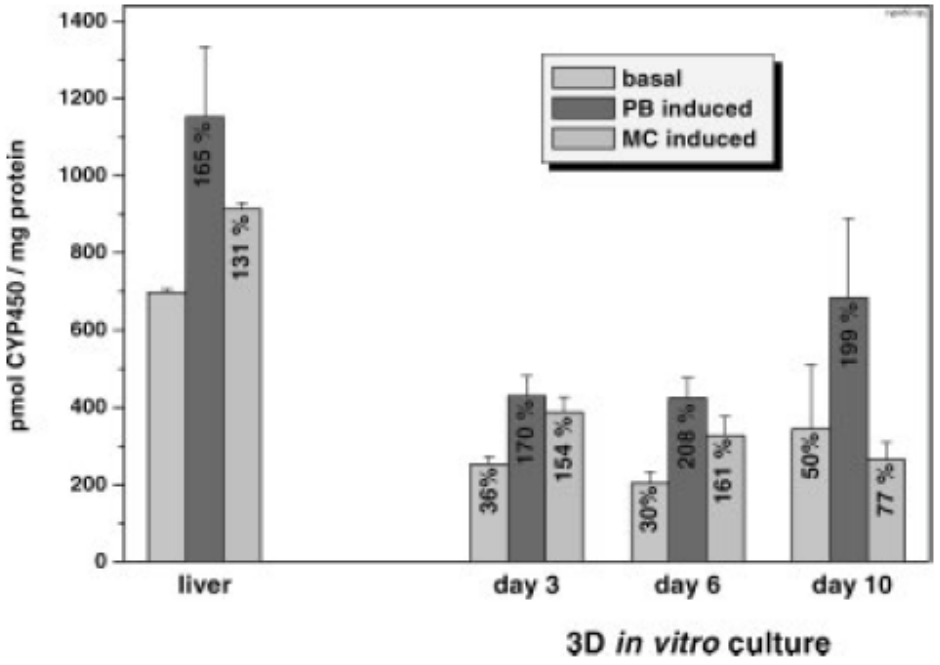

Fig. 5. Basal and induced CYP450 amounts (pmol/mg protein) in microsomal preparations from liver and aggregates at different days of in vitro culture. Values represent the mean of at least three independent experiments. Error bars indicate the standard error of the mean. Numbers within the columns represent the induction by phenobarbital (PB) and 3-methylcholanthrene (MC) expressed as percent of the respective basal values.

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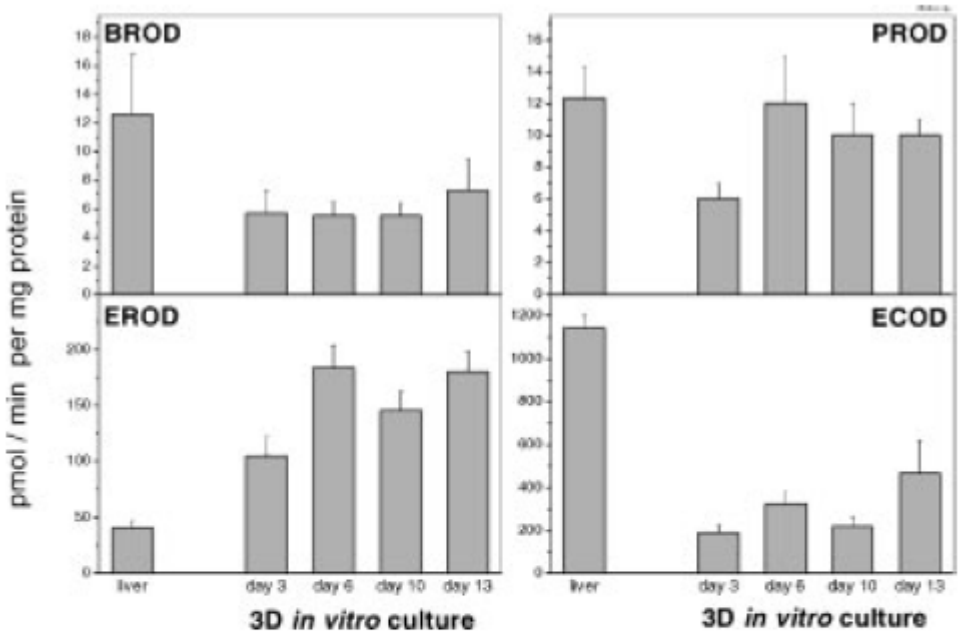


Fig. 6. Basal activities (pmol/min*mg protein) of cytochrome P450 dependent mixed function oxidases in microsomal preparations from liver and aggregates at different days of in vitro culture. Values represent the mean of at least three independent experiments. Error bars indicate the standard error of the mean. BROD, 7-benzyloxyresorufin O-debenzylase; PROD, 7-pentoxyresorufin O-depenthylase; EROD, 7-ethoxyresorufin O-deethylase; ECOD, 7-ethoxycoumarin O-deethylase.

Erik Eschbach, et al. *J. Cell. Biochem.* **2005**, *95*, 243–255

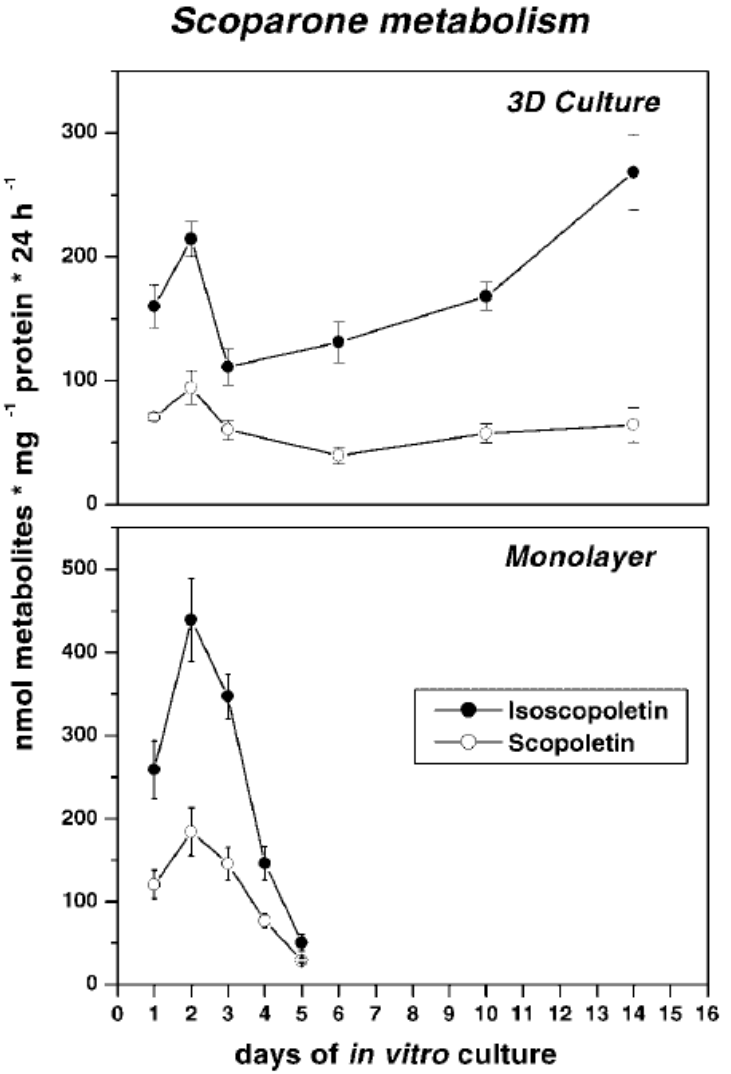
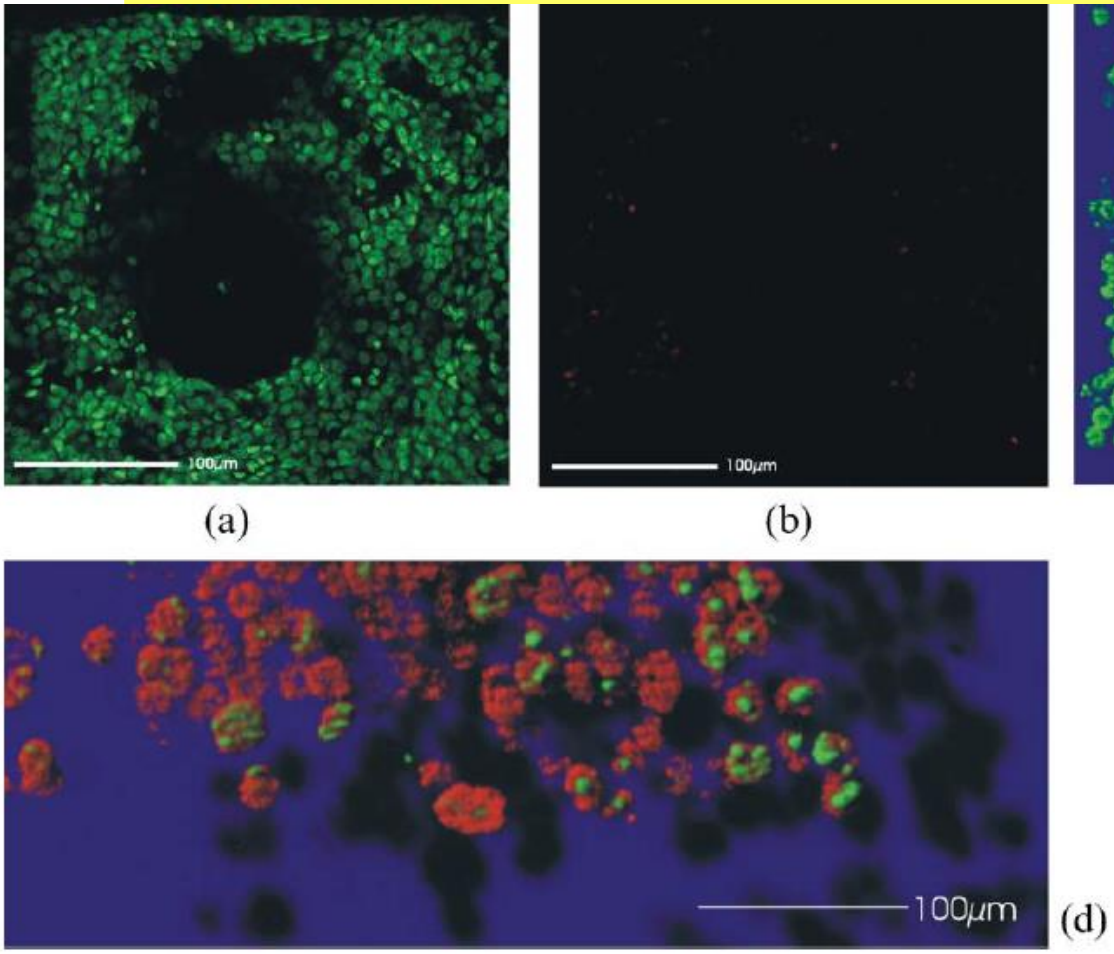


Fig. 9. Metabolism of the model substrate scoparone into scopoletin and isoscopoletin in aggregate culture in comparison to monolayer determined by HPLC. Each sample was prepared twice and injected twice for HPLC analysis. Calculation of scoparone metabolism was done in nanomole metabolites per milligram protein per 24 h. At least three independent experiments were performed to calculate mean values for each single day of in vitro culture. Error bars indicate the standard error of the mean.

Better characterization of the cells



E. Gottard, *et al. Lab Chip* **2007**, *7*, 777–785

Fig. 3 Confocal image of C3A cells cultivated for 24 h in a cf-chip without active perfusion. Nuclei were simultaneously stained with Syto 16 (green) and propidium iodide (red). (a) Syto 16 stained nuclei of cells in one micro-container. (b) The same micro-container as shown in 3(a) with nuclei of dead cells stained with prodidium iodide. Note that after 24 h only a few cells are stained. (c) Optical section of a fluorescence image obtained by structured illumination of one micro-container of a cf-chip (top view) cultured with Hep G2 cells for 3 days. Nuclei were stained with Syto 16 (green). (d) Optical cross section of the same micro-container. In addition to the (green) nuclei, as a cytoplasmic marker, inducible heat shock protein 72 was stained (red).

Better characterization of the cell cultures

Table 1 List of Hep G2 genes upregulated at least 2-fold in 3D-chip culture (cf-chip)

Gene	Fold change in 3D	SD
Cytochrome oxidase III	6.24	0.509
Alpha-fetoprotein	5.99	0.339
Cytochrome oxidase II	5.92	0.152
NADH-3	5.53	1.409
NADH-1	4.41	0.590
GAPD	4.04	0.268
Complement component 3	3.74	0.048
ATPase-6/8-2	3.65	1.593
Alpha-2 macroglobulin	3.52	1.201
NADH-dehydrogenase-4	3.25	0.734
Alpha-1 antitrypsin	3.16	0.429
Beta-2-glycoprotein I (=Apolipoprotein H)	2.92	0.479
NADH-dehydrogenase-3	2.91	0.321
Apolipoprotein A-II	2.89	0.492
Ferritin-L-chain	2.68	0.702
EF-1 alpha	2.62	0.411
NADH-dehydrogenase-2	2.37	0.307
Fatty acid binding protein	2.30	0.340
Transthyretin	2.17	0.248
11-beta hydroxysteroid dehydrogenase	2.05	0.102
Vitamin D-binding protein	2.00	0.193

[a] Hep G2 monolayer cells served as controls and were set to equal 1. All data displayed are statistically significantly different on a $p < 0.05$ basis.

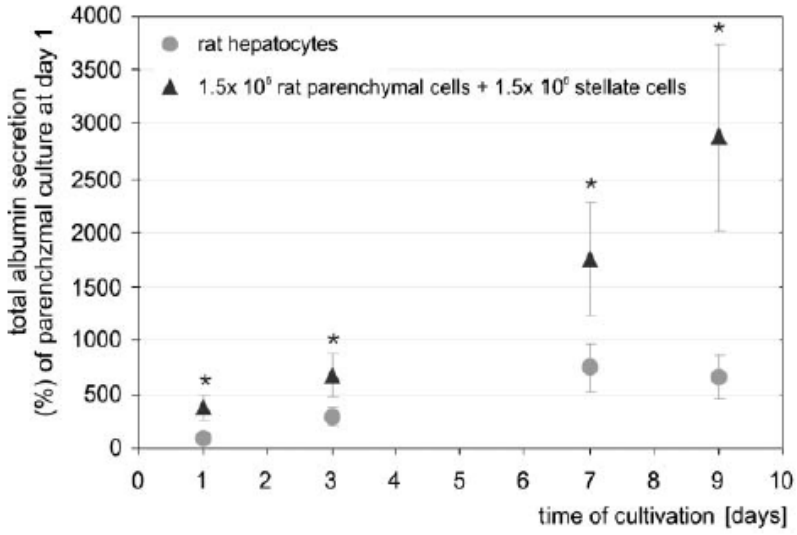


Fig. 4 Comparison of primary rat hepatocytes cultured three-dimensionally as parenchymal cells or in co-culture with stellate cells in a cf-chip over a period of 9 days. * = $p < 0.05$ in Mann–Whitney U-Test.

E. Gottard, *et al. Lab Chip* **2007**, *7*, 777–785

Improvement of the method

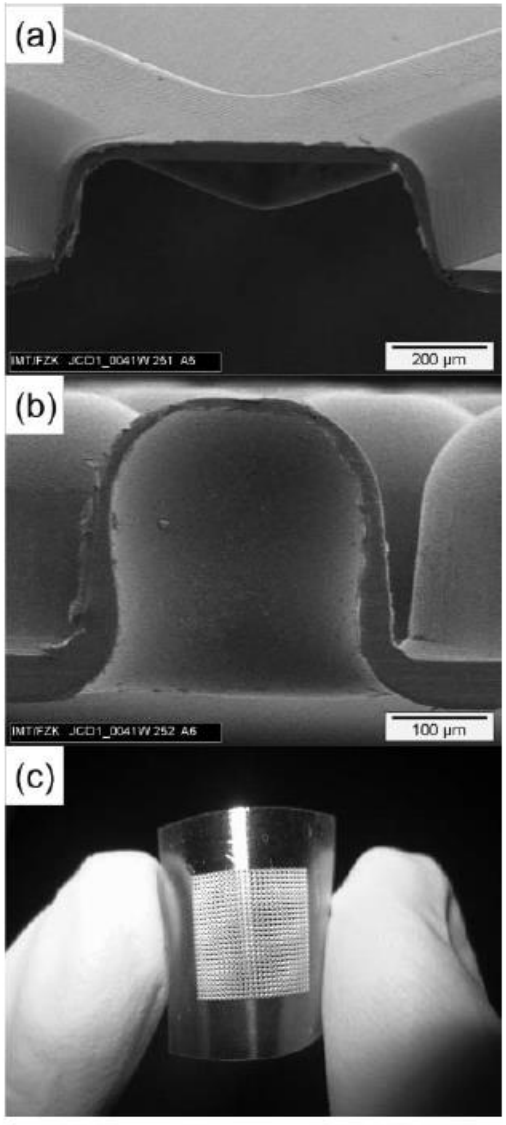


Fig. 3 (a) Thermoformed microchannel intersection from PC (SEM micrograph; cross sectional view; channel width: approximately 500 µm). (b) Microcontainer of the cell culture chip from PC (SEM micrograph; cross sectional view; inner container diameter and depth: approximately 300 µm; wall thickness at container bottom: approximately 8 µm; undercut: approximately 13 µm). (c) Flexible PC cell chip comprising 625 microcontainers on an area of 1 cm^2 (bent between the thumb and the forefinger of a hand).

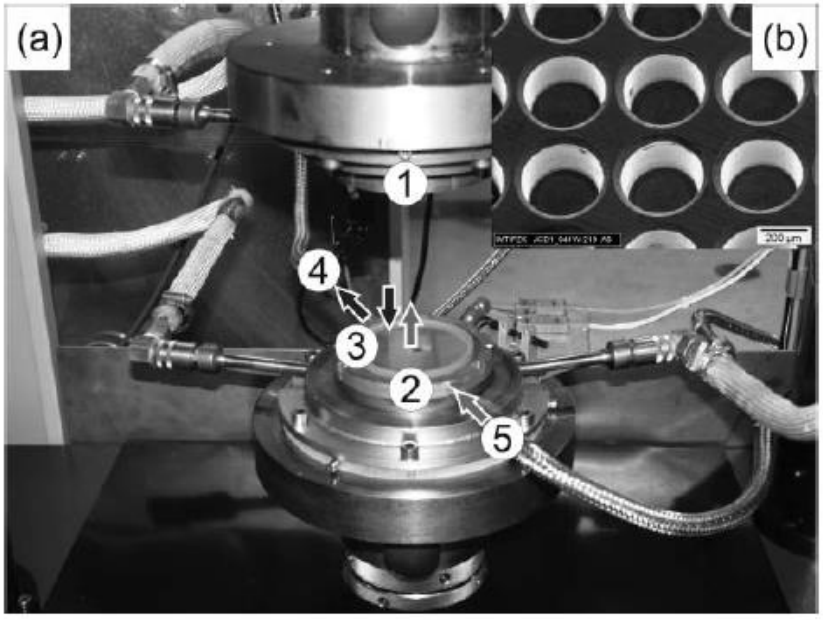
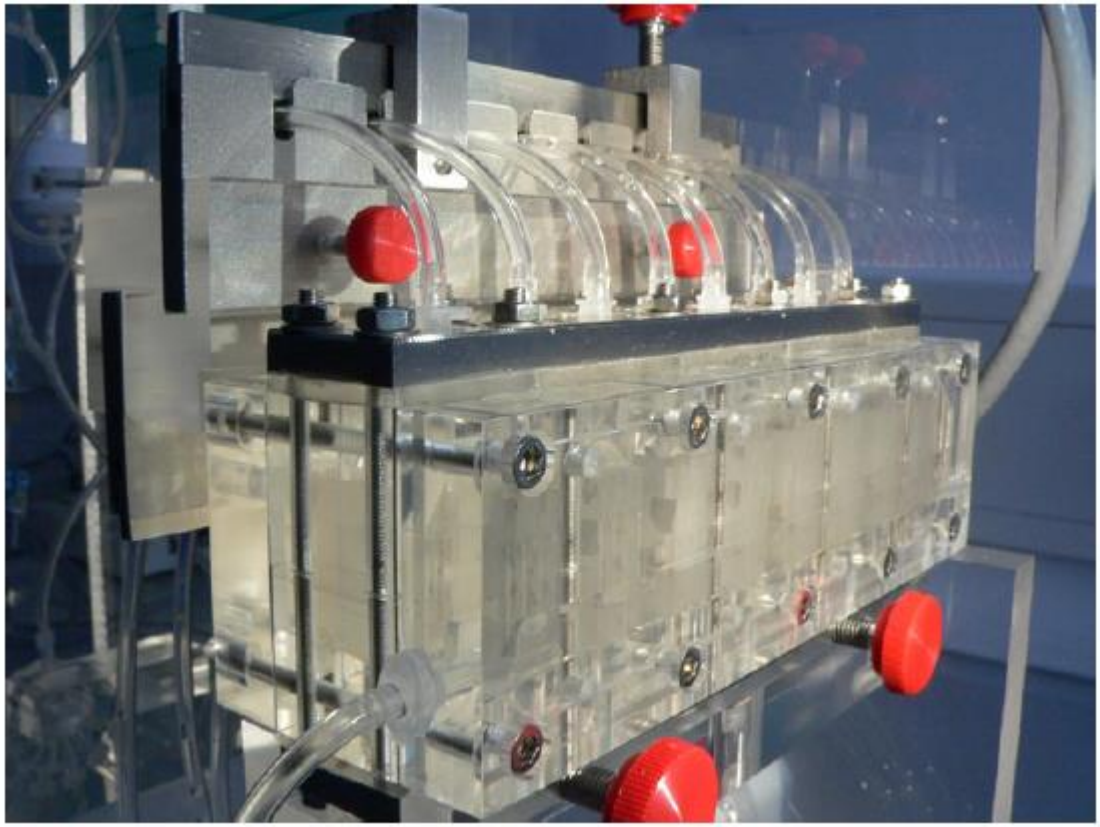


Fig. 2 (a) Hot embossing machine (Jenoptik, Jena, Germany; type WUM) used for microthermoforming still on a laboratory scale, equipped with a microthermoforming tool ((1) micromould, (2) counter plate with openings for evacuation and gas pressurisation, (3) seal, (4) vacuum, (5) compressed nitrogen). (b) Mould cavities of the micromould from brass for thermoforming the cell culture chip (scanning electron microscope (SEM) micrograph; microcavity diameter: 350 µm; depth: 300 µm).

R. Truckenmüller,, *et al. Lab Chip* **2008**, *8*, 1570–1579

Throughput improvement

Multi-unit bioreactor consisting of eight compartments, each containing four chips. Every compartment has an individual medium supply.



B. Altmann, *et al. Biomed. Mater.* **2008**, *3*, 034120

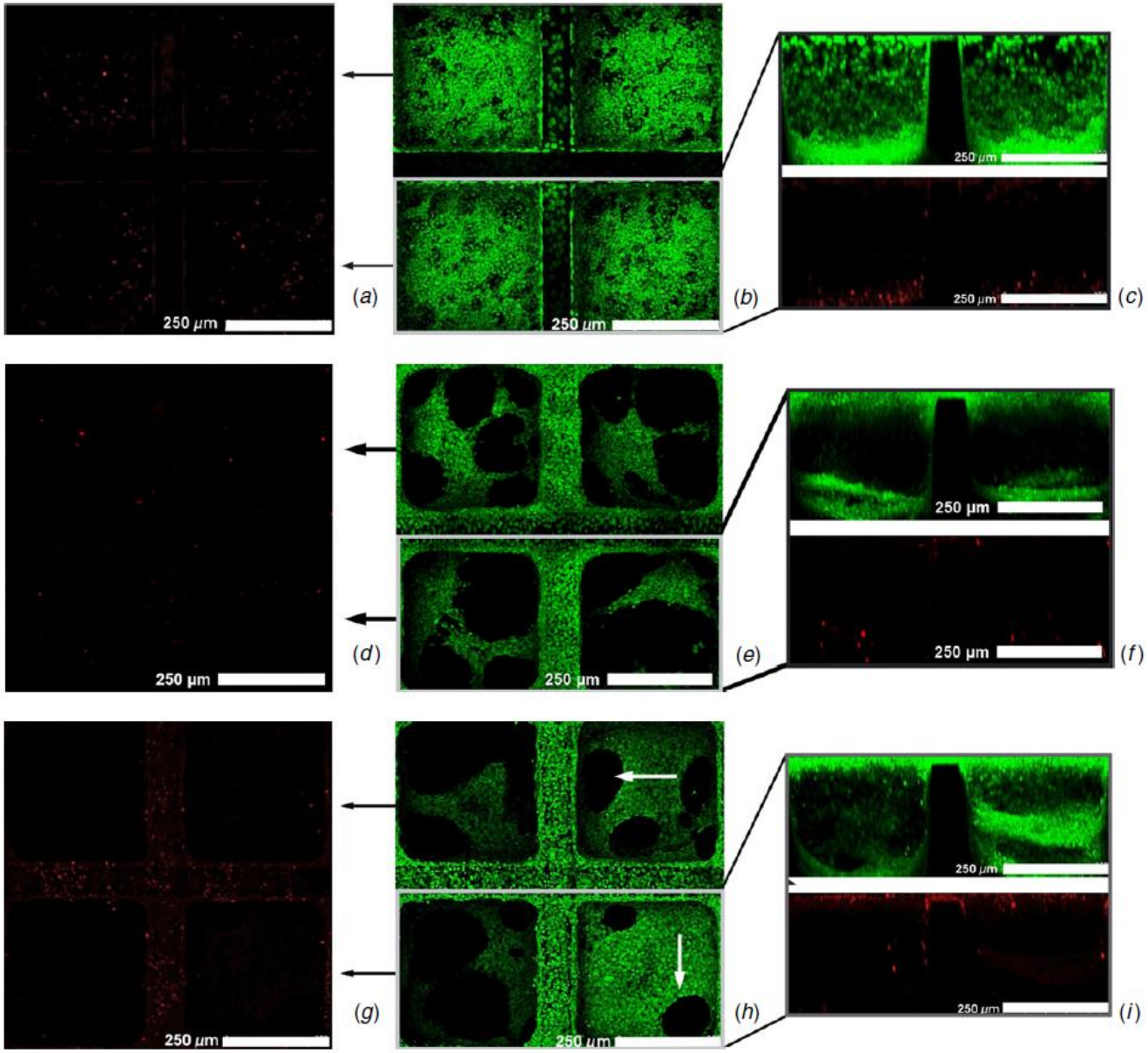


Figure 4. Confocal images of the cell line HepG2 in cf-chips cultivated for 24 h ((*a*)–(*c*)) and 6 days ((*d*)–(*f*)) without active perfusion, and cells after 6 days in perfused culture configuration (*g*)–(*i*). (*a*), (*b*), (*d*), (*e*), (*g*) and (*h*) Each image shows a maximum projection (top view) of about 100 pictures of a *z*-stack with an overall height of 300–310 μm and a distance of 3 μm between each scanning position. The cells were simultaneously stained with the nuclear dyes Syto16 for living cells (green laser line: (*b*), (*e*) and (*h*)) and the dead cell marker propidium iodide (PI) (red laser line: (*a*), (*d*) and (*g*)). (*c*), (*f*) and (*i*) Projection of the corresponding images on the left side and in the middle (side view). The upper images show cells labeled with Syto16 and the images underneath dead cells stained with PI.

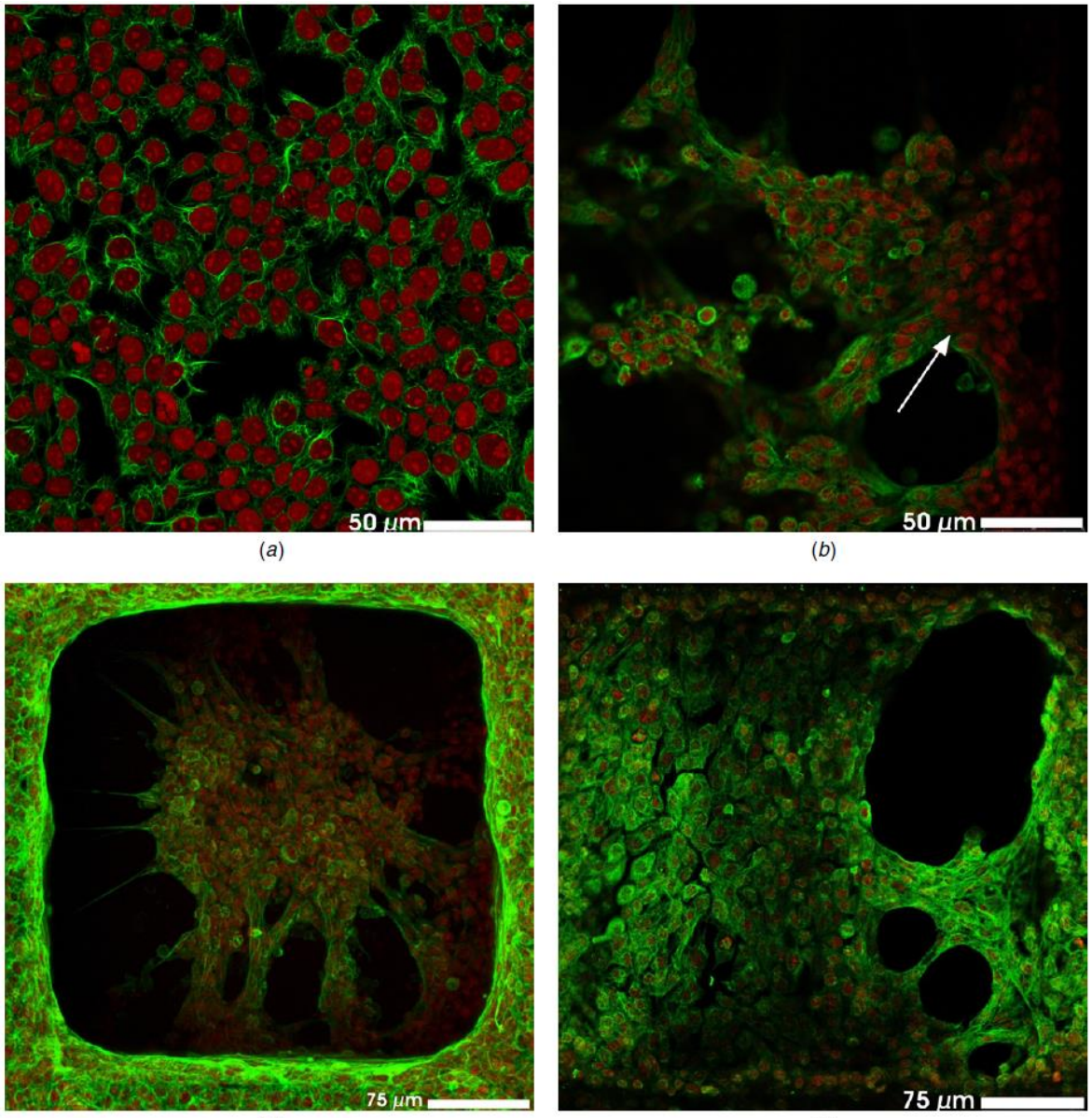


Figure 5. Immunohistochemical analysis of an optical section of keratin 18 filaments (green) in HepG2 cells cultured as a monolayer (*a*) or in a cf-chip (chip with a flat bottom) ((*b*), (*c*) and (*d*)). Nuclei were counter-stained with propidium iodide (PI, red). (*b*) HepG2 cells were cultured for 24 h in a non-perfused cf-chip (*z*-position: 200 μm). (*c*) Maximum projection of a *z*-stack with an overall height of 300 μm. HepG2 cells were cultured for 6 days in a non-perfused cf-chip. (*d*) Labeled cells in a chip after 6 days of perfused culture with a flow rate of 59.9 μl min^{-1} (*z*-position: 100 μm). No unspecific binding was detectable (data not shown).

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The current gold standard for the prediction of human hepatotoxicity is still animal studies that are largely hampered by high cost, poor predictivity and a common ethical problem. While 85% of cardiovascular, 88% of gastrointestinal and 90% of hematological toxicity can be predicted by animal toxicity tests, hepatotoxicity prediction still suffers from less than a 50% accuracy and is therefore recognized as inappropriate for drug screening campaigns. For an improved risk assessment, there is a need for predictive and inexpensive in vitro models that reflect the in vivo (human) situation better than classical cell cultures (e.g. monolayer). One option is an organ-like, three-dimensional in vitro cell culture. The patented approach of the Institute for Biological Interfaces-1 of the Karlsruhe Institute of Technology (KIT) is a system based on a microstructured chip, which allows an actively perfused 3D cell culture. Due to the slice-format of the chip, it is compatible with standard microscopes of any laboratory.

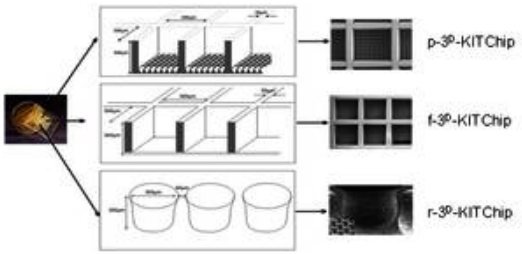

Figure: 3^{D}-KITChip variants: p-, f- and r-3^{D}-KITChip (with kind permission of Dr. E. Gottwald, KIT)

...but it still is a tedious process.

Troubleshooting Table

Problem	Explanation	Potential Solutions
Empty microcontainers on the chip.	Entrapped air bubbles.	De-aerate properly by increasing the duration of the alcohol series steps.
Cells float off the chip.	Inappropriate cell counts due to initially too high cell numbers or too long cultivation periods with proliferating cells.	Predetermine optimal cell numbers for each cell type separately prior to the experiment.
	Inappropriate surface modification or ECM deposition.	For UV-based surface modifications, determine the optimal irradiation dose.
Entrapped air bubbles in the bioreactor circulation.	After transferring the bioreactor system to the 37°C chamber, dissolved gases in the medium outgas.	Switch the three-way connectors of the bioreactor circulation to superfusion. This leads to the export of the air bubbles.
During long term experiments the medium in the reservoir will decrease.	The use of dry gas mixtures will lead to an evaporation of medium through the sterile filters.	Incorporate a wash flask into the gas inlet tract containing sterile water.
Generation of small bubbles on top of the chip.	Accumulation of cellular respiration products.	Switch the three-way connectors of the bioreactor circulation to superfusion. This leads to the export of the air bubbles.

Chip-Based Tissue Engineering in Microbioreactors

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CHAPTER 5