Micro bioreactor for muliwell plates with an active micro fluidic system for 3D cultivation

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ABSTRACT

Recently it has been shown that 3D cultivation of cells and tissues show great potential for bio/medical research [1]. Within this scope are developments of micro bioreactors with micro fluidic support structures [2]. We have designed a micro system - suitable for parallelization - to cultivate cells three dimensionally. This system is based on the CellChip bioreactor from FZK (Forschungszentrum Karlsruhe). Our system is miniaturized in such a way that it is implementable into a 24 well plate. A single channel system with an integrated micro pump is manufactured as a prototype. The aim of our system is reduction of costs, animal experiments and the acceleration of drug screening. This system uses CellChips to grow cells organ-like, i.e. three dimensionally. In this chip the cells are located in containers. Up to 2 million cells like HepG2 can be cultivated in this system. The system is characterized with respect to oxygen consumption of the cells and cell viability. 24-channel-systems are under construction.

Keywords: three dimensional cell culture, micro pump, micro-fluidics, bioreactor, multi-well-plate screening

1 DESIGN AND FUNCTIONAL PRINCIPLE

Based on the CellChip bioreactor form FZK (Fig.1), we have designed and build a novel miniaturized system to cultivate cells three dimensionally.

Fig. 1 Bioreactor with CellChip from FZK

A closed loop of culture medium is integrated in our micro bioreactor. Also a micro pump is used to generate a fluid flow in the system. The pump is actuated by compressed air. In Fig. 2 you can see a schematic of our micro reactor.

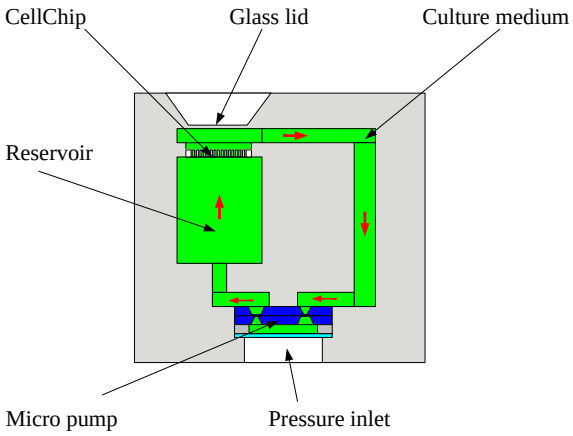

Fig. 2 Schematic of the micro bioreactor

A new smaller CellChip is used to cultivate cells in our system. The dimensions of this CellChip (Fig. 3) are 8 x 8 x 1 mm³. The chip contains 169 micro containers to carry cells. The size of these containers is 300 x 300 x 300 µm³. A glass lid above the CellChip allows for microscopical analysis of the cells. If necessary, we can exchange the glass lid with a transparent plastic lid. By this way we can increase the oxygen content in the culture medium, but optical resolution decreases.

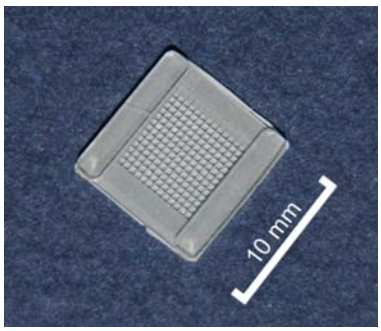

Fig. 3 The new smaller CellChip

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1.1 The modular body

In order to be able to gain diverse information on fluidic parameters, biological variables etc. we needed a flexible experimental setup. Therefore we choose a modular design for the bioreactor. As material we used polycarbonate, because it is transparent and can easily be machined with a milling machine.

The inner part of the system carries the CellChip. This carrier contains a reservoir of culture medium and fluidic channels. Its size is given by the grid dimensions of a 24 well plate. The carrier is housed by a body, which – in case of the single channel bioreactor – is directly connected to a module either with an internal micro pump or with connectors for an external pump. Fig. 4 shows the micro bioreactor with the integrated micro pump.

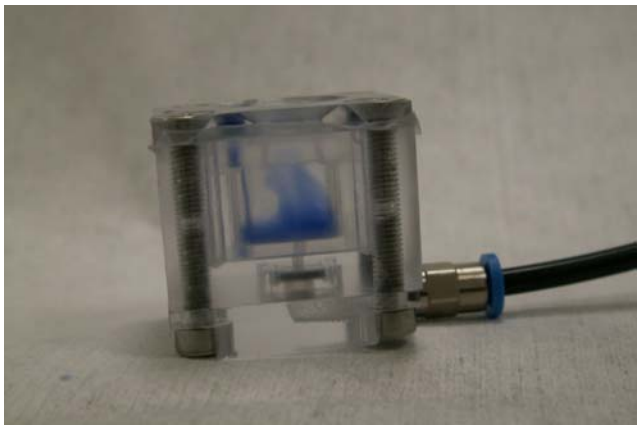

Fig. 4 Single channel micro bioreactor with a blue tracer

1.2 The micro pump

Like other bioreactors, also the micro bioreactor can be operated with an external pump for supplying the cells with culture medium. This works for a single channel system but is not practicable for a multi well system. Therefore we have developed a robust internal micro pump which can be integrated into the system. By this means, each channel of a multi well system is controlled by its own micro pump.

The pumping principle is based on a membrane pump. Our pump has two micro machined check valves. They are made of silicon. The membrane of the pump consists of PDMS (Sylgard 184). Without the housing the pump has a size of 5 x 5 x 1 mm³.

Our micro pump can reach a maximum flow of 1ml per minute. Also, this pump can drive gases and fluids or a mixture of both. This is of advantage, especially if gas bubbles cannot completely be avoided. In addition, the actuation of the pump with compressed air facilitates gas exchange between the culture medium and the compressed air and hence, to some extent, allows oxygenation of the culture medium.

1.3 Multi well plates

The single channel system is the first step towards a multi channel system (multi well plate). A possible design of such a multi channel system is shown in Fig. 5

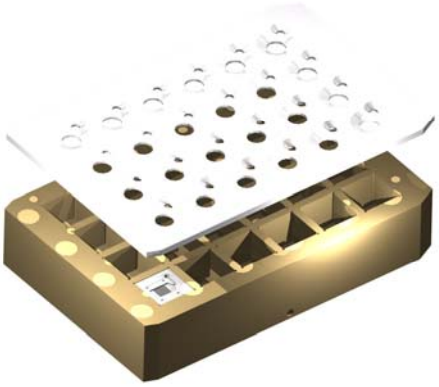

Fig. 5 CAD model of a 24 channel bioreactor system

One challenge of a multi well system lies in the fact that all channels need to have identical characteristics, i.e. every micro pump must have the same flow rate while being addressed by only one shared driving pressure connection. With micro technologies we should be able to address this challenge since it is possible to produce micro valves with small tolerances.

2 METHODS

2.1 Cell Culture

HepG2 cells (ATCC) were cultivated in Minimum Essential Medium (Sigma, Taufkirchen, Germany) with 10% fetal calf serum, 100 U/ ml penicillin, 100 µg/ ml streptomycin, 2 mM L-glutamine and 1 mM sodium pyruvate. CellChips with a micro-structured area of 0.5 x 0.5 cm² were used for three-dimensional cultivation of the cells. 1.5 x 10^6 cells were inoculated per single-channel-bioreactor and per CellChip as described elsewhere [4]. Total volume was 1 ml per bioreactor. Bioreactors with and without cells were incubated at 37°C, 5% CO2.

2.2 Determination of Viability

Viability of cells was compared for three different culture systems: CellChips within actively perfused bioreactors, CellChips within non-perfused bioreactors and non-perfused CellChips placed in a multi well plate (no bioreactor housing). After 48 h (day 2) of cultivation, cells were enzymatically (trypsin) removed from the CellChips and viability of cells was determined by trypan-blue exclusion using a hemocytometer.

In order to get more detailed information on the cellular viabilities on day 2, cells were then plated as monolayers.

After 24 h (day 3) adherence was checked microscopically and viability was estimated as percentage of adherence.

2.3 Determination of oxygen content

For determining the oxygen content of the medium during cell cultivation fiberoptical oxygen minisensors (PreSens, Regensburg, Germany) were used. The sensor was implemented into the main chamber of the bioreactor. Media were incubated at 37°C, 5% CO_2 for 2 h before filling into the bioreactors. Oxygen contents of an actively perfused bioreactor (perfusion via a pneumatically driven micro pump) and a non-perfused (static) bioreactor were compared. As control a non-perfused bioreactor without cells was used. Oxygen content was measured every minute and data were logged using appropriate software (OXY, PreSens).

3 RESULTS AND DISCUSSION

Viability of HepG2 cells cultivated in a perfused bioreactor was higher than that of the other systems (tab.1) on day 2 and also day 3. Adherence of the cells on day 3 was very good for cells removed from the perfused bioreactor and good for cells removed from non-perfused CellChips placed in a 24-well plate. Cells removed from the non-perfused bioreactor adhered poorly.

culture system	day 2	day 3
perfused bioreactor	75	75
non-perfused bioreactor	10	< 10
non-perfused CellChip without bioreactor housing	65	< 50

Table 1: Viability (%) of HepG2 cells after three dimensional cultivation within and after removal from different culture systems (day 2) as well as after replating as monolayers (day 3).

For long-term cultivation of cells within a closed bioreactor system appropriate oxygenation is vital. Therefore the oxygen content within the bioreactors was monitored while cultivating HepG2 cells. A non-perfused bioreactor without cells served as control and demonstrated maximum oxygen content as well as stability of the sensor signals (fig 6). Oxygen content did not change significantly within perfused bioreactors, which demonstrates sufficient oxygenation of the system. The pneumatically driven pump increases oxygen diffusion through the silicone membrane into the medium.

In contrast, oxygen content within a non-perfused system dropped to about 50 % of the initial value (4.8 mg/l) before rising again to 3.4 mg/l. The latter may be explained by a decreased viability of the cells and therefore reduced oxygen consumption which allows an overall increase of oxygen content over time.

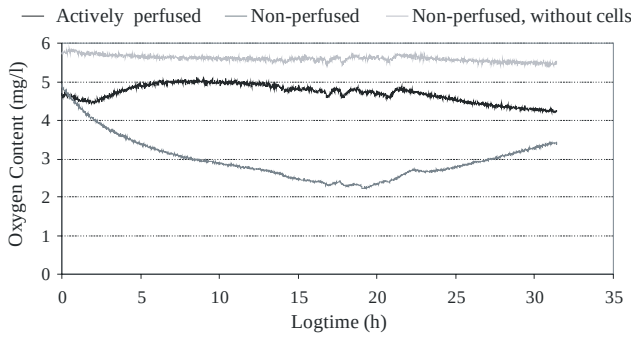

Fig. 6: Oxygen content of medium within an actively perfused bioreactor ($1.5x10^6$ HepG2 cells), a non-perfused bioreactor ($1.5x10^6$ HepG2 cells) and a non-perfused bioreactor without cells.

In conclusion, oxygenation in a non-perfused bioreactor is not sufficient and leads to strongly decreased cell viability. Both, oxygen supply and cell viability, can be improved by active perfusion of the system by a pneumatically driven micro pump as presented in this work.

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