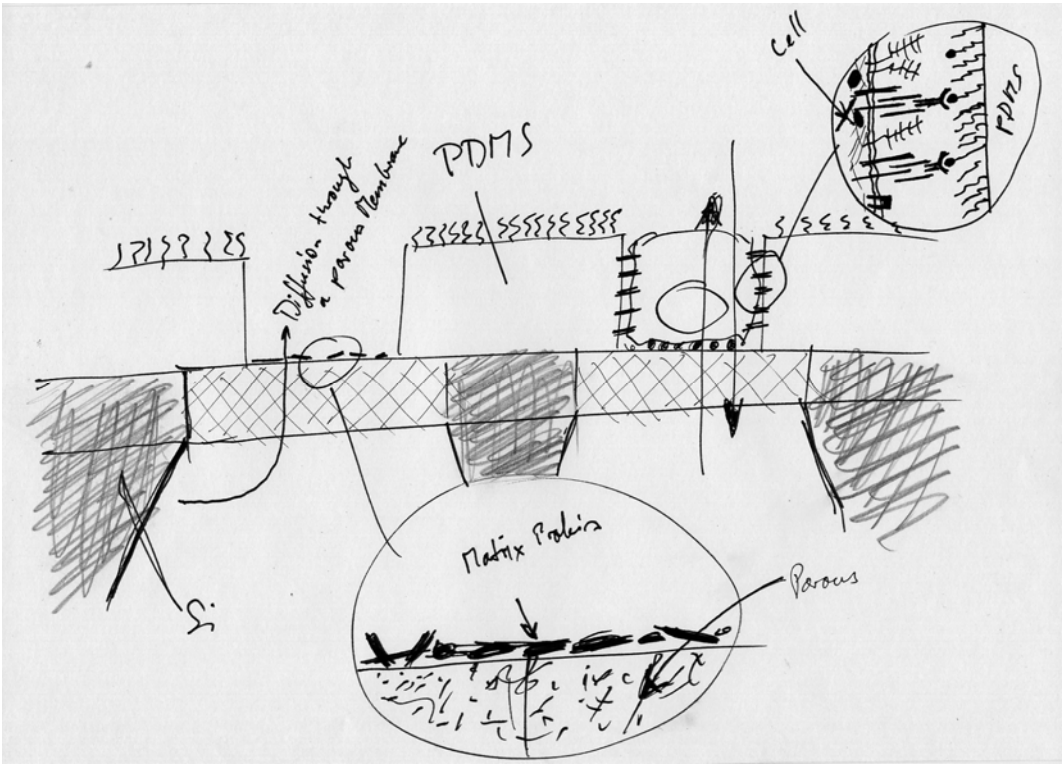

Figure 8.3 Vision of an integrated device consisting of a microfluidic part below which exhibits patterns of a porous membrane and a PDMS stencil aligned on top. The latter produces microwells to accommodate single cells. The membrane could be coated with matrix proteins prior to assembling of the device. Subsequent use of the inverted printing method introduced in this thesis can lead to a selective coating of the side walls and a non-interactive plateau surface. The sidewalls could exhibit adhesion sites mimicking a cell-cell contact. The device allows the investigation of transport phenomena through single epithelial cells (or small clusters).

The main subject of this thesis was the culturing of single cells in a defined 3-D microenvironment. Functional biological units like organs, though, always consist of colonies of different cell types. The use of microfabrication to grow clusters of cells to construct miniaturized functional units that can serve as physiological models for drug testing is of high interest(Bhatia 1999; Hecker, Baar et al. 2005; Fukuda, Sakai et al. 2006). The combination of replication and selective surface modification techniques can further be applied to create arrays of microwells that contain for example natural ECM, as shown Figure 8.4. The adhesion of these gels to the surrounding might be tailored using designed surface chemistry. In addition the tailoring of the mechanical properties of such microwells as well as their shape should lead to highly elaborated culture models in an array format. The growth of 3-D aggregates inside ECM gels is already a standard technique and has been shown to be more relevant, for example, in

breast cancer research(Weaver, Petersen et al. 1997); miniaturized systems, though, are still rarely used.

A more complete understanding of how cells behave in microengineered environments and the integration of different miniaturized cell cultures and microfluidic components to mimic different interacting parts of an organism is crucial for the development of reliable culture models, such has been introduced by Shuler et al.(Park and Shuler 2003; Khamsi 2005). These so-called cell culture analogs, or loosely termed rat on a chip(Khamsi 2005), could allow screening of biological cues in a model (still relying on tissue of sacrificed animals but making a more intelligent and efficient use of them) that is intermediate between standard cell culture tests and animal models, thus reducing the number of variants of drug to be tested in animals.

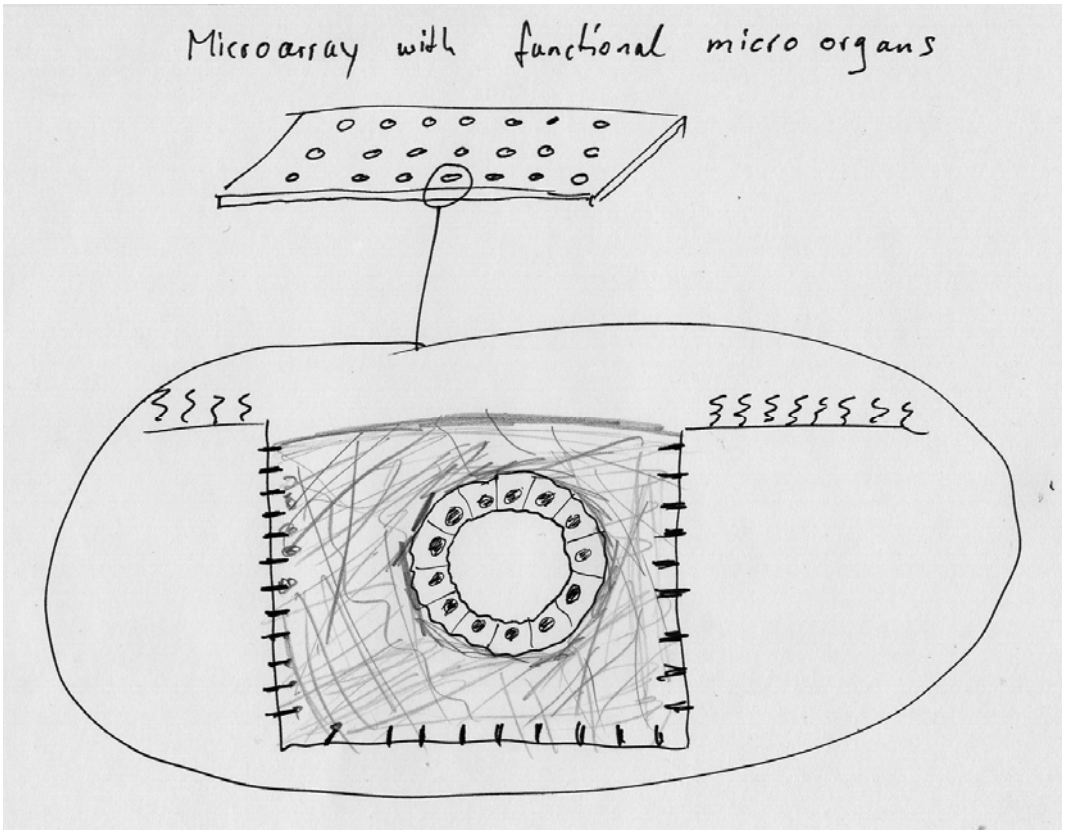

Figure 8.4 Gel in well concept for functional microorgan arrays. Microfabricated substrates, as has been developed in this thesis, could be used to grow functional cell aggregates in a 3-D environment, like natural ECMs, contained in microwells with a functional interface to control adhesion of the ECM proteins, mechanical properties and restrict outgrowth of the cells on top.

There are still numerous technologies used in the microelectronics and MEMS industry that could be combined with biology to create hybrid devices containing living matter interfaced with various sensors and actuators. Microscale cell cultures

can be integrated into implantable devices for the monitoring of body functions or release of substances such as insulin(Service 2002). Furthermore, engineered cell cultures could be integrated with fuel cells, which create energy from hydrogen produced by enzymes of the biological units in the system(Tye, Hall et al. 2005). There is still a long and very challenging way to go before such dreams become reality, although they have been dreamt by scientists, futurists or science fiction writers decades ago already.

8.3 Ethical aspects of miniaturized cell cultures

We would like to make some final comments about ethical considerations which are involved with the work described in this thesis. We have mainly worked on developing new technologies for the miniaturized culturing of cells. Miniaturization and automatization of biological experiments are technologies which can find a broad application in various fields. It is a general issue of enabling technologies that the final use of them is not known or decided by the investigators, who are mainly driven by the fascination of "getting things to work". We have so far applied our technologies mainly on fundamental biological studies, but other applications can easily be envisaged. The combination of cell cultures and microrobotics will have an implication also in fields like reproductive medicine. Microfluidic devices have already been applied for gender selection used for in-vitro fertilization(Cho, Schuster et al. 2003) and have been commercially applied. The technologies mentioned in this thesis will lead to improved systems for the sorting and selection unfertilized eggs or embryos, enabling cloning experiments and pre implantation diagnostics (PID) at high-throughput and higher success rates. In addition, the understanding and control of the microenviromental influences on human stem cells might enable platforms for the miniaturized engineering of tissue, as foreseen in regenerative medicine by the use of therapeutic cloning(Hwang, Roh et al. 2005). Or finally the implantation of engineered biohybrid microdevices consisting of microelectronics and biological material, even from other human beings or different organism can be thought of.

All these issues are currently discussed in politics, media, academia and ethical committees. The discussion is needed and most important is has to be done between all members of society. I have the impression that many people are not aware of the fact that these technologies are already available in labs all over the worlds and are

not mere science fiction. It is thus a decision society has to make now and not a matter of feasibility. Therefore I want to encourage everybody in research to speak openly about their work, their fears, their dreams and their opinions about life and humanity. Only by an open debate and sufficient information society is able to frame their destiny by creating ethical concepts, laws and regulations using democratic means.

Corrigendum:

Due to the controversy about the human embryonic stem cell lines reported by Hwang et. al.(Hwang, Roh et al. 2005), which have been finally proven to be fraudulent and the papers been retracted(Kennedy 2006), we would like to mention that at the time of print of this thesis no successful attempt to create patient specific embryonic stem cells have been reported. The potential and ethical considerations of using embryonic stem cells in controlled microenvironments mentioned above are still valid.