‘Lab-on-a-chip’ for cell engineering: towards cellular models mimicking *in vivo*

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One of the main scopes of modern cell engineering is development of cellular models that can replace animals in drug screening and toxicological tests, so called alternative methods. Construction of the alternative model is a very challenging task due to a richness of factors creating the *in vivo* environment. The monolayer cell culture — cultivation of adhesive cells on artificial surfaces such as glass or polymer — lack most of the *in vivo*-like interactions, but still is the only tool for the majority of applications.

One of the most prospective approaches on mimicking *in vivo* environment is “Lab-on-a-chip” technology. Microfluidic devices offer lots of advantages over traditional *in vitro* culture, e.g. much higher cell volume-to-extracellular fluid volume ratio or possibility of regulation of hydrodynamic stress.

This presentation aims to introduce latest advances of our team in microfluidic cell culture devices. Our novel approach is to cultivate three dimensional multicellular aggregates (spheroids) in microenvironments arranged in a microfluidic system. The geometry and materials of the system allow for cultivation, observation and analysis of multicellular spheroids. The results presented concern multicellular tumor spheroids (MCTS) rising from human cancer cells, which are considered to represent most of the conditionings of cancer tumor *in vivo*. The fully developed MCTS microdevice will be a reliable tool for anticancer drug screening, as the results most likely will be in a close accordance with the results obtained *in vivo*.

Keywords and phrases: Lab-on-a-chip, microfluidic tools, cancer, cell culture, Multicellular Tumor Spheroid (MCTS).

Introduction

Using animals in toxicological tests, drug development process and biomedical research is still a very problematic and controversial issue. Unfortunately, according to our current knowledge, there is no possibility of complete elimination of animal tests. Since the 60s of XX-th century the 3R concept has been introduced [1]. The 3R concept states for Reduction of number of animals used for tests, Refinement of methods reducing animals’ suffering and Replacing animals with alternative methods. Especially the last statement is among scientists’ interests nowadays.

Alternative methods, defined as models unable to feel or suffer, are mainly based on *in vitro* cell and tissue culture. Every new alternative method should be validated as being reproducible and in good accordance with the same tests performed *in vivo*. To coordinate validation process in European Union, the European Centre for Validation of Alternative Methods was established in 1991 [2]. For almost twenty years only 27 methods have been approved, most of them concerning skin irritation tests of cosmetic ingredients. This number is disproportionate to the number of research projects concerning this subject and show how challenging the task is.

Cell culture

Cell culture means the maintenance of cell explant viability *in vitro* for more than 24 hours [3]. The history of cell and tissue culture reaches the decline of XIX-th century, when scientists focused on developmental biology and embryology observed cell differentiation of animal embryos *ex vivo* [4]. Next decades was the time of rapid development of methods concerning cell culture.

The milestone in this field was the development of HeLa cell line in 1951 [4]. A scientist George Gey took

cells from cervical cancer of 30 year old patient and observed unexpected viability of the cells. HeLa line (name taken from the first letters of patient's name — Henrietta Lacks) was the first stable human cell line. It was a turning point not only for cell culture itself, but also for cancer research. HeLa cells are the most viable and widely used cell line up to now.

Nowadays there is a number of commercially available stable cell lines, including human cell lines. The research is taken using both stable and primary (freshly explanted) cell lines. The advancement of cell culture enabled the development of genetics, molecular biology, medicine and — already mentioned — toxicology and pharmacology.

The third dimension

A cellular model that is most widely used is a monolayer cell culture (Fig. 1). The model is simple and easy to handle, so the automation and standardization of the research methods were possible. It is still the best model for basic cell metabolism and function investigation. However, there are many disadvantages of the model for more complex research. Cell cultured in flat culture dishes lacks most of the factors present *in vivo* [5]:

- flat broad surface, which hardly corresponds with micrometer scale *in vivo* niches
- lack of the Extracellular Matrix (ECM) — a three--dimensional protein scaffold
- the structure of cytoskeleton is changed *in vitro*
- the cell to cell interactions are limited or disabled, both direct — junctions and desmosomes — and chemical — autocrine, paracrine and endocrine signalling
- high volume of cell culture medium leads to dilution of secretion products
- periodically changed medium do not mimic continuous nutrients and oxygen supply and waste products' disposal provided by vascular system
- all cells are exposed to the same conditions, no variety of concentrations in deeper cell layers
- no tissue to tissue interactions.

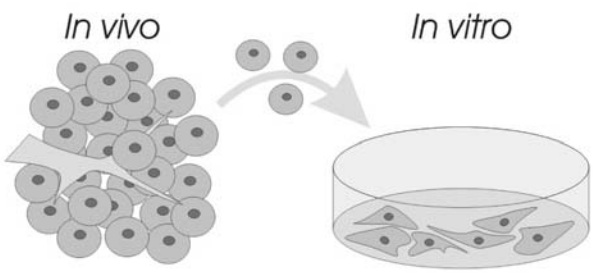

Fig. 1. Mammalian cells explanted from living tissue lack vascular system and spatial interactions.

All these factors are essential for cell function. There is number of evidence that cells explanted from specific organs (e.g. hepatocytes from liver) shortly dedifferentiate and loose specific functions [6]. It is the main reason of differences between drug response *in vivo* and *in vitro*. Therefore, research on cellular model more closely mimicking *in vivo* environment is fully understandable.

Among approaches leading to the *in vivo*-like cellular model, three dimensional cell cultures seem to be most interesting and prospective [5]. Different solutions have been developed for three dimensional cell culture: artificial vessels, polymeric scaffolds, Extracellular Matrix analogues. Multicellular aggregates, so called spheroids (also known as tumoroids or organoids), present many interactions characteristic for *in vivo* environment, such as locally present ECM, number of cell to cell junctions, chemical gradients and different rates of cell proliferation. Multicellular Tumor Spheroids (MCTS), spherical aggregates of carcinoma cells, are especially valuable for cancer research and anticancer drug screening [7]. MCTS present metabolism, growth rate and function very similar to cancer tumors. However, most of the known methods of MCTS formation cause variation in size or is cost, labor and energy consuming. Moreover, the fully reproducible method of single MCTS observation was not developed yet [7].

"Lab-on-a-chip" for cell engineering

Microfluidic devices (also called "Lab-on-a-chip") found their application in many fields of biomedical research [8, 9]. Microfluidics has already been used for single cell handling, cell culture, analysis and observation of cell to cell interactions and migration [9]. Apart from cost reduction and portability, the main advantage of "Lab--on-a-chip" devices is the ability of utilization of phenomena inaccessible in a larger scale. Most of them lead to obtainment of systems that can closely mimic cellular microniches [10]. The dimensions of microdevices strongly correspond with the *in vivo* microenvironments. Small cross section of microchannels causes laminar flow obtainment, which helps controlling fluid flow. It is widely used for perfusion culture of endothelial cells, mimicking conditions present in blood vessel lumen [11]. Microfluidic is also suitable for co-culture establishment, with the precise tissue to tissue interaction control [12] and three dimensional cultures [13]. Many of the cell-based assays' performed using microfluidic modules shown their good accordance with the results obtained *in vivo* [10].

Results and discussion

The aim of our research is the development of microfluidic systems suitable for human carcinoma cell

culture and anticancer drug screening. The microfluidic device for adherent cells' culture and cytotoxicity tests was published before [14]. The microdevice with cell culture microchambers and Concentration Gradient Generator (CCG) for precise medium and drug dosing was used for model cytotoxic agent test (Fig. 3). The results were reproducible and standard deviations achieved for different concentrations were lower than for standard 96-well plate assay. The microdevice based on monolayer cell culture model.

Our current research is focused on cellular models more closely mimicking cancer tumor *in vivo*. The model chosen is Multicellular Tumor Spheroid model. Example cultivation of MCTSs is shown in Fig. 2.

Spheroid culture using nonadhesive plates leads to the suspension of many spheroids of different sizes. Facing the need of single MCTS observation, the microfluidic device for single MCTSs formation and analysis was designed and prototype systems were fabricated (Fig. 3c). The material chosen for the fabrication was poly(dimethylsiloxane) — PDMS, due to its hydrophobicity preventing cell adhesion, gas permeability and transparency. Elements of the system were fabricated by soft lithography and replica molding techniques [15], to achieve microchannels 300 μm wide and 50 μm deep. The three dimensional structure was achieved by bonding of three layers of polymer microstructures using the oxygen plasma treatment. The microsystem consists of an array of 0.2 μL chambers connected with the network of microchannels providing medium supply. First attempts of human carcinoma cells' (HT-29) culture were performed using the prototyped system and Multicellular Tumor Spheroid formation was observed (Fig. 3d).

Our further plans range evaluation of Multicellular Tumor Spheroids' culture protocols and performance of cytotoxicity tests of model anticancer drugs. A number of factors concerning cell seeding and culture should be elaborated and optimised, e.g. microsystem inner environment preparation, cell suspension density, cells' introduction, frequency and way of medium exchange. In the further study, coupling of the system with the

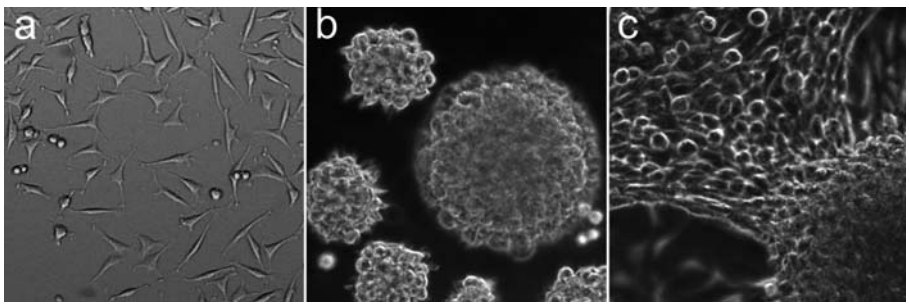

Fig. 2. A 549 cells (human lung carcinoma cells): (a) cultured as a monolayer in polystyrene culture dish; (b) Multicellular Tumor Spheroids in PDMS-covered plate; (c) spheroids cultured on adhesive surface, show high proliferation rate and ability of maintaining three dimensional structure.

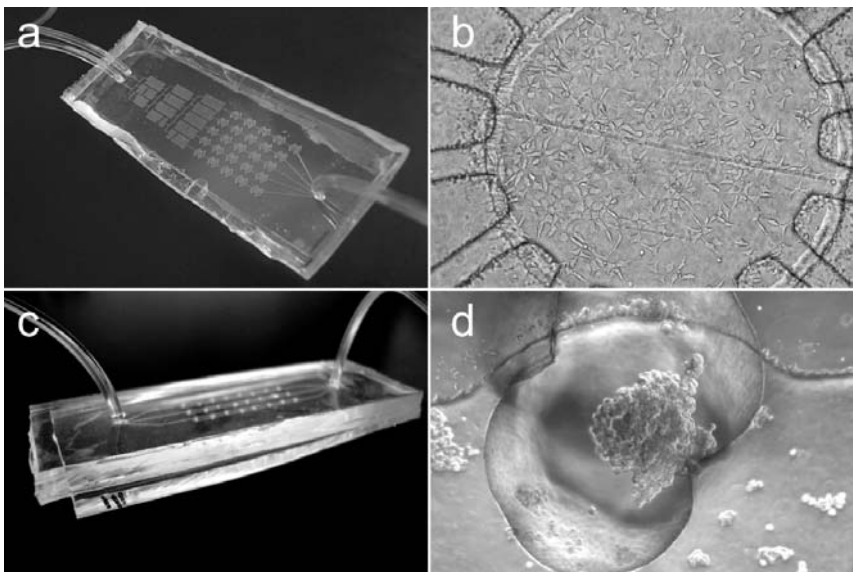

Fig. 3. Cell culture microsystems: (a) for monolayer cell culture and cytotoxicity tests; (b) adherent growing cells in microchamber; (c) three dimensional MCTS microsystem; (d) spheroid formed in microchamber.

CCG is planned, to perform cytostatic drugs' activity assays. We hope that our microsystem will find its application in anticancer drug screening.

Acknowledgments

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References

[1] Russell, W.M.S., and Burch, R.L. *The Principles of Humane Experimental Technique*. London: Methuen, 1959.
[2] European Centre for the Validation of Alternative Methods. [Online] http://ecvam.jrc.it/. 2010.
[3] Freshney, R.I. *Culture of Animal Cells*. New York, 2000.
[4] Masters, J.R. "HeLa cells 50 years on: the good, the bad and the ugly". *Nature Reviews* 2 (2002): 315.
[5] Yamada, K.M., and E. Cukierman. "Modeling Tissue Morphogenesis and Cancer in 3D". *Cell* 130 (2007): 601.
[6] Guillouzo, A., and C. Guguen-Guillouzo. "Evolving concepts in liver tissue modeling and implications fot in vitro toxicology". *Expert Opinion on Drug Metabolism & Toxicology* 4 (2008): 1279.
[7] Lin, R.Z., and H.Y. Chang. "Recent advances in three-dimensional multicellular spheroid culture for biomedical research". *Biotechnology Journal* 3 (2008): 1172.
[8] Chudy, M., et al. "Miniaturized tools and devices for bioanalytical applications: an overview". *Analytical and Bioanalytical Chemistry* 395 (2009): 647.
[9] El-Ali, J., P.K. Sorger, and K.F. Jensen. "Cells on chips". *Nature* 442 (2006): 403.
[10] Walker, G.M., M.S. Ozers, and D.J. Beebe. "Insect Cell Culture in Microfluidic Channels". *Biomedical Microdevices* 4:3 (2002): 161.
[11] Van der Meer, A.D., et al. "Microfluidic Technology in Vascular Research". *Journal of Biomedicine and Biotechnology* 2009.
[12] Folch, A., and M. Toner. "Microengineering of Cellular Interactions". *Annual Review of Biomedical Engineering* 2 (2000): 227.
[13] Huang, P.C., et al. "Engineering microscale cellular niches for three-dimensional multicellular co-cultures". *Lab on a Chip* 9 (2009): 1740.
[14] Ziolkowska, K., et al. "PDMS/glass microfluidic cell culture system for cytotoxicity tests and cells passage". *Sensors and Actuators*, B: *Chemical* 145 (2010): 533.
[15] Duffy, D., et al. "Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane)". *Anal. Chem*. 70 (1998): 4974.