„Lab-on-a-chip" devices for Multicellular Tumor Spheroid cultivation and testing.

Karina Ziółkowska

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.

The goal of our research is the development of microfluidic systems suitable for human carcinoma cell culture and anticancer drug screening. The aim of this project is to develop a microfluidic chip for Multicellular Tumor Spheroid (MCTS) formation, culture and analysis. The MCTS is recognized as the best cellular model for anticancer therapy testing developed so far, as it presents morphology and physiology similar to tumor *in vivo* with the network of cell-cell interactions, three dimensional structure and nutrients, metabolites and oxygen gradients.

The MCTS microchip consist of three dimensional structure of microchannels and microwells fabricated in poly(dimethylsiloxane). Various techniques are applied for 3D microchip fabrication: soft lithography, replica molding, micromilling and PDMS double casting. The construction of the device enables single spheroid formation and observation, which is a significant advantage over other MCTS cultivation methods. The microchip is suitable for studies on hydrodynamic stress influence on cultured cells as well as for cytotoxicity studies of anticancer drugs.

The presented MCTS microsystem can be a reliable, inexpensive and easy to handle alternative for current spheroids' cultivation methods.

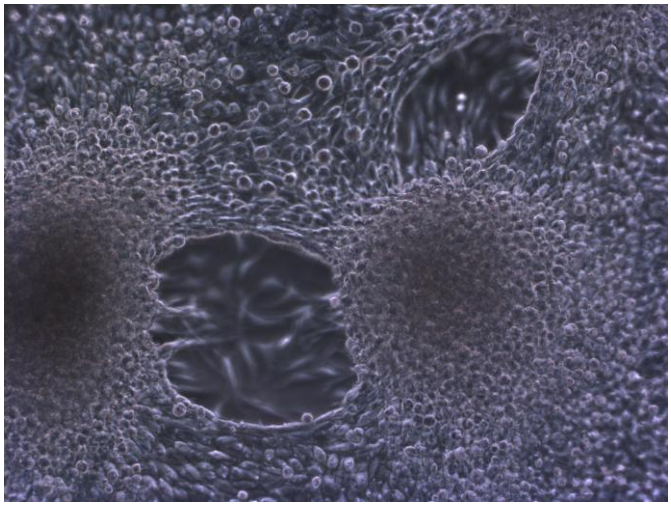

Figure 1. Three dimensional culture of A 549 human epithelial cells.

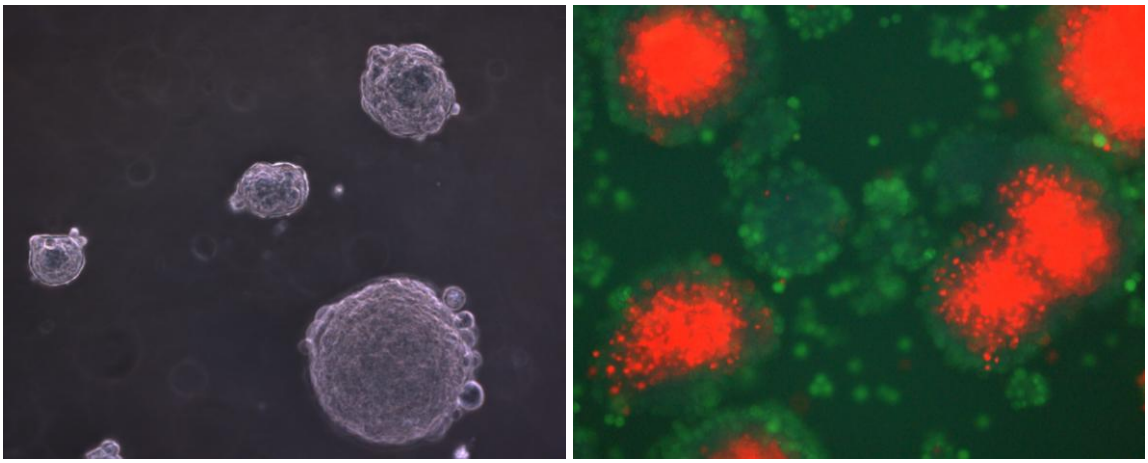

Figure 2. Multicellular Tumor Spheroids cultured on a PDMS covered plate. Left: HT-29 MCTSs; right: cell viability assay of A549 MCTS's – layered structure visible, with the necrotic core.

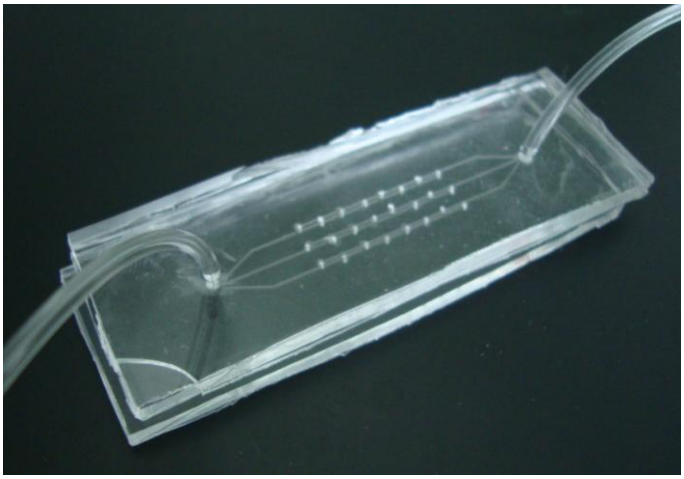

Figure 3. An overview of three-dimensional MCTS microchip

Figure 4. SEM micrograph of microfabricated three-dimensional structure in PDMS

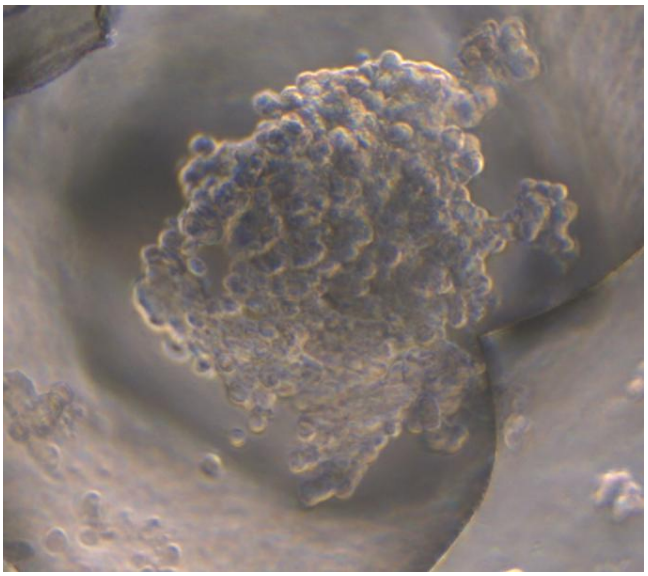

Figure 5. HT-29 MCTS formed inside polymeric microwell