MULTIWELL PLATE READER-COMPATIBLE MICROFLUIDIC SYSTEM FOR LONG-TERM MULTICELLULAR SPHEROID CULTURE AND MONITORING

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ABSTRACT

Development of novel cellular models that can replace animals in preclinical trials of drug candidates is one of the major goals of cell engineering. Current *in vitro* screening methods hardly correspond with the *in vivo* situation, whereas there is lack of assays for more accurate cell culture models. Therefore, development of automated assays for 3D cell culture models is urgently required.

In this work, we present a novel microfluidic system for long-term 3D cell culture and analysis. The system is compatible with commercially available spectrofluorimetric multiwell plate readers. Microfluidic chip consists of cell culture microchambers and microwells connected with concentration gradient generator. Culture of tumor spheroids of human cancer cells was monitored for over two weeks using the developed system and alamarBlue® as an indicator of metabolic activity. Four phases of growth were identified, and results were in a good correspondence with macro-scale potentiometric cell culture monitoring. The developed microfluidic system is a promising tool for drug-screening applications.

KEYWORDS

Tumor spheroid, long-term cell culture, metabolic activity, high throughput screening, automation

INTRODUCTION

Nowadays, drug development sector faces a quandary between throughput and accuracy of accessible *in vitro* screening assays. A majority of the applied tests base on monolayer cell culture, which is an easy to handle model suitable for automation and is willingly used in High Throughput Screening (HTS) systems [1]. However, monolayer culture lacks essential interactions present *in vivo*, which strongly limits a prediction of an effect of a drug on humans [2]. 3D tissue-like cell culture models are attractive alternative in the field of mimicking *in vivo*, e.g. Multicellular Tumor Spheroid (MCTS) which exhibits numerous similarities to cancer tumor [3]. However, its widespread use has been limited so far, due to imperfections of available culture methods and assays [3].

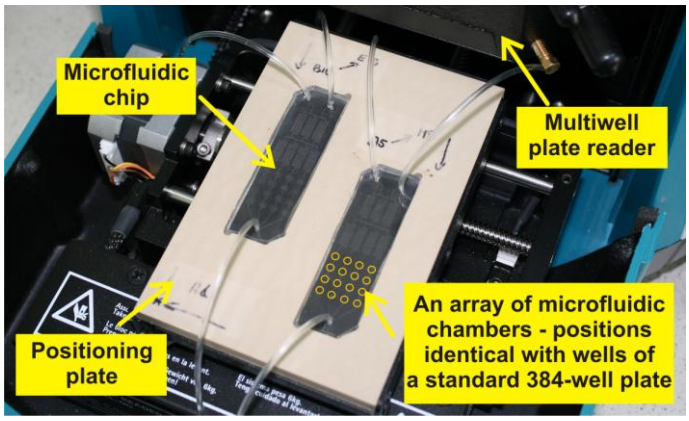

Figure 1: Overview of the 3D cell culture monitoring system.

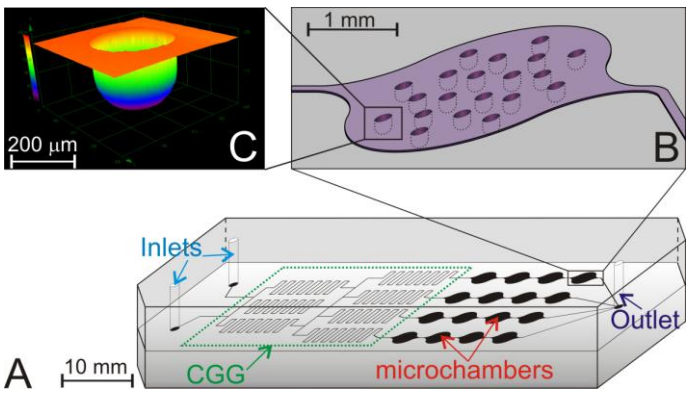

Figure 2: (A) Scheme of a microfluidic chip. (B) Microchamber (a depth of 50 µm). (C) Profile of a microwell (a depth of 200 µm, Ø=200 µm).

In this work, we present a microfluidic 3D cell culture system compatible with commercially available 384-well plate readers. The system (Fig.1) consists of a positioning plate suitable for two microfluidic chips placement (one for a blank probe). The chip (Fig.2) consists of a 4x4 array of microfluidic chambers coupled with a Concentration Gradient Generator (CGG) structure. Each chamber contains 20 U-shaped microwells for MCTS culture [4]. When the chip is placed in the positioning plate, microchambers are detection cells of the multiwell plate reader.

The chip was applied for MCTS culture of HT-29 cells (human colon carcinoma). The challenge of our work laid in automation of monitoring of 3D cell culture by coupling of an *in vivo*-like model with an instrumental analysis method. We decided to use alamarBlue® as the fluorescent indicator of cellular metabolic activity [5]. The reagent's fluorescence increases proportionally to decrease of medium red-ox potential, caused by metabolizing cells. The system was successfully used for two-week monitoring of spheroid growth in the microfluidic chip. Additionally, off-chip potentiometric measurements were proceeded for comparison.

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EXPERIMENT

The 3D microstructure was fabricated using double casting in PDMS with a thermal aging step [6]. A 3D master for the replication step and the positioning plate were fabricated by micromilling.

Application of a ball-end mill resulted in semi-spherical microwells for spheroid formation and culture. Cells introduced to the microfluidic system gravitated towards centers the microwells, aggregated and formed spheroids within 24 hours (Fig.3). Therefore, each microwell contained single spheroid and good uniformity of sizes was observed in the whole microfluidic system. Initial size of spheroids could have been controlled by setting of inoculum density. HT-29 spheroids were cultured in the microfluidic system for over two weeks (Fig.3). Microscopic observations of spheroid morphology and sizes allowed identification of following growth phases: (1) lag phase (aggregation, spheroid compaction), (2) intense proliferation and growth, and (3) plateau.

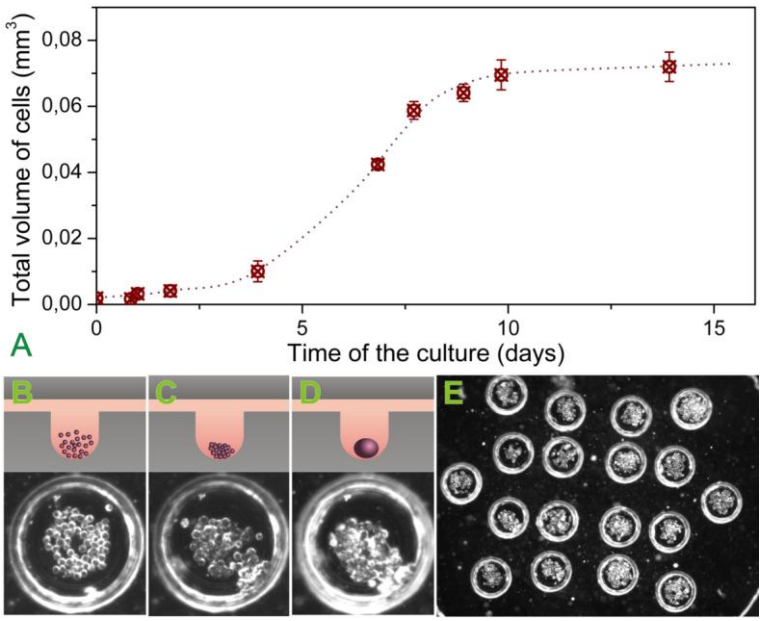

Figure 3: Long-term MCTS culture: (A) growth curve, (B) cell seeding (scheme and micrograph), t=0h, (C) aggregation, t=24h, (D) MCTS formation, t=48h. (E) MCTSs in a microchamber, uniform seeding was achieved.

To verify applied detection system, cell culture media with (1) off-chip reduced and (2) native alamarBlue® were introduced via CGG to the array of microchambers (Fig.4). It was expected to achieve four different concentrations of reduced reagent, exhibiting proportional fluorescence intensities (Fig.4:B). The signal was detected in a spectrofluorimetric multiwell plate reader. Linear dependence consistent with *in silico* modeling of CGG was obtained (Fig.4:A). It was an evidence, that both the geometry of the chip and the experimental set up were designed correctly.

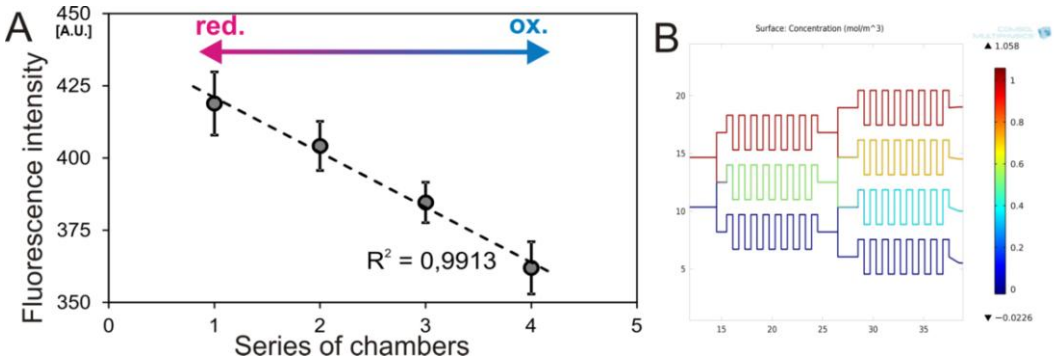

Figure 4: (A) Verification of the detection system: 1st series of microchambers contained off-chip reduced probe (highest fluorescence) and 4th series contained blank (lowest fluorescence). Fluorescence intensity in 2nd and 3rd series was in the linear range between the extremes. (B) Results of in silico *modeling of CGG.*

A long-term cultures of HT-29 spheroids of different cell seeding densities were monitored using the developed system (Fig.5). Each measurement point corresponded to 24h incubation with alamarBlue®. Characteristic, repetitive curve was observed for the culture. No toxic effect of alamarBlue® was observed during long-term monitoring. Results obtained during first three days were in a good correlation with off-chip potentiometric spheroid culture monitoring using platinum electrode and Ag/AgCl2 reference. It is an outcome of reaching equilibrium of anabolic and catabolic processes during aggregation and compact spheroid formation. Apart from the growth phases identified by microscopic observations, two stages of the lag phase were observed. First, freshly seeded cells exhibited high catabolic activity (red-ox potential decrease and high fluorescence intensity related to the first 24 hours of culture). Next, cell-to-cell aggregation and binding of adhesive proteins promoted cell cycle and biosynthesis, which effected in growing participation of anabolism and slight increase of red-ox potential (decrease of fluorescence intensity during second day of culture). After achieving equilibrium between anabolic and catabolic processes, further growth of fluorescence intensity was strictly related to the growing number of cells in the system.

CONCLUSIONS

We present a novel 3D cell culture monitoring system compatible with HTS systems. To our best knowledge, it is the first microfluidic-based well plate compatible spheroid analysis system. It is suitable for long-term MCTS culture and analysis. It provides an unique opportunity of continuous observation and quantitative analysis of sequencing days of culture, which will be beneficial for evaluation of repeated doses of xenobiotics.

ACKNOWLEDGEMENTS

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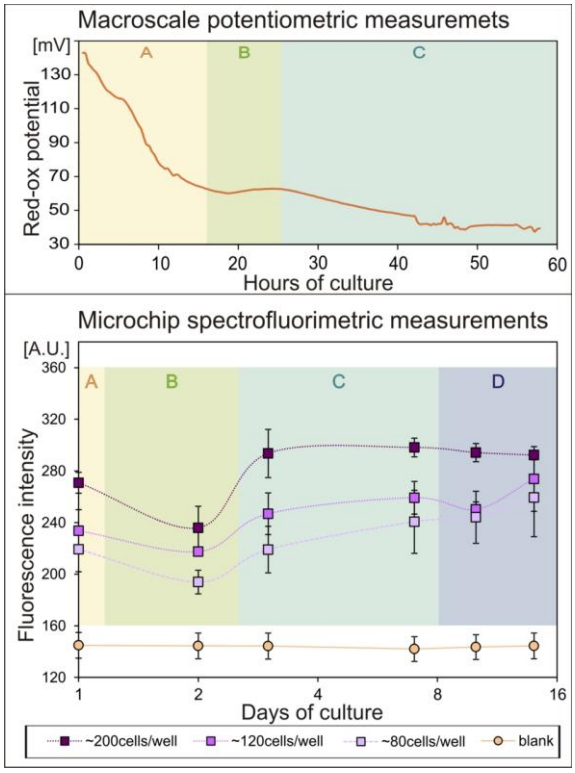

Figure 5: Results of on-chip spectrofluorimetric monitoring of long-term MCTS culture for different cell seeding densities. Four phases of growth occurred: (A) aggregation and predominance of catabolism, (B) spheroid formation, ECM secretion, greater anabolism, (C) spheroid growth and (D) coincidence of curves due to space limitations. Results of potentiometric monitoring of the macro-scale culture were given for comparison: the same growth phases were observed (A, B, C).