Case study

Monitoring Spheroid Growth and Response to Treatment via Impedance

Tools:
MicroPick S/O, AtlaZ

IMTEK-Laboratory for MEMS Applications, University of Freiburg, featured by Nanion Technologies

Viktoria Zieger (left), Scientific Associate and Dr. Sabrina Kartmann, Head of Division Laboratory Automation Hahn-Schickard & IMTEK-Laboratory for MEMS Applications, University of Freiburg. Their focus is the automation of cell aggregate handling for an increased throughput and standardization of physiologically relevant preclinical drug screenings.

In modern biomedical research, three-dimensional (3D) cell aggregates such as spheroids or organoids are increasingly important as they replicate the physiological environment of solid tumors or tissue. Their 3D structure promotes natural cell interactions, making them ideal for studying, for example, tumor biology or for performing drug testing. 3D cell aggregates more accurately mimic nutrient, oxygen, and waste gradients in tissues, providing a superior model for evaluating drug penetration, response, and resistance, thereby offering more reliable preclinical predictions than 2D models.

Conventional drug screenings with 3D cell aggregates, for example spheroid cultivation in ultra-low attachment plates paired with endpoint viability assays [1], limit the precision and flexibility of drug response assessments, particularly in terms of the kinetics of effect and depth of analysis. Executing assay protocols and performing multiple microscopy steps to record drug responses entail a substantial manual workload. As a result, it becomes challenging to perform time-resolved monitoring of the drug's mechanism of action.

The Department of Microsystems Engineering (IMTEK) at the University of Freiburg and Hahn-Schickard, in collaboration with Nanion, introduce a novel approach for time-resolved, label-free spheroid analysis. To enhance automation in spheroid handling and improve the standardization of drug screenings with 3D *in vitro* models, the MicroPick S/O platform was utilized. It consists of a liquid handling platform specifically designed for the automated deposition of 3D cellular aggregates. The system enables controlled quantities and precise sizes and types of cellular aggregates to be plated into specific target areas with high throughput. The platform utilizes the novel Pick-Flow-Drop principle, which allows for a gentle and selective uptake of individual cellular aggregates from a reservoir (pick), their transport via a microfluidic capillary (flow), and precise deposition at the desired location (drop) [2]. With a plating efficiency of 98.4% and a throughput of up to 21 spheroids per minute, the system excels in efficient sample handling. Notably, it achieves a plating position accuracy of less than 25 µm [3],

Nanion's AtlaZ platform
AtlaZ allows for cellular research on cell adhesion and proliferation, cytotoxicity, morphology and barrier function. The assays are real-time and label-free and are performed in up to six 96-well plates simultaneously or independently.

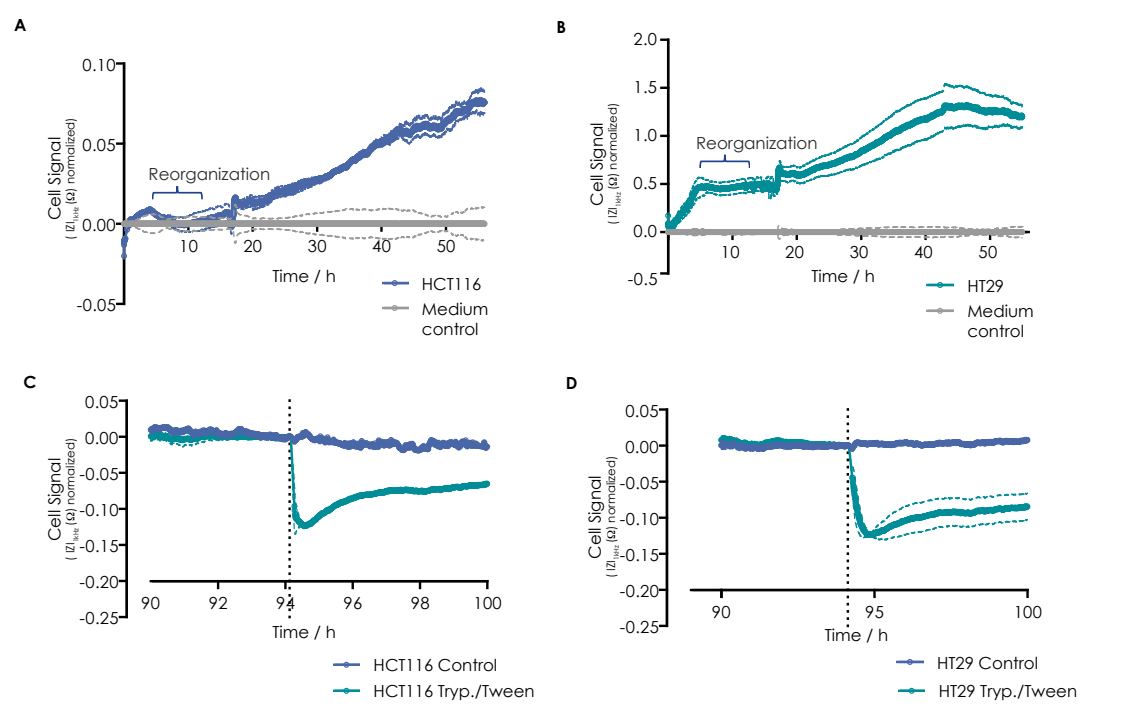
"The combination of the AtlaZ system and the MicroPick S/O has yielded a highly effective synergy between automated spheroid handling and a functional analysis of 3D cell aggregates. Our collaboration with Nanion has proven to be exceptionally valuable."

Tobias Hundertmark, PhD candidate, IMTEK-Laboratory for MEMS Applications, University of Freiburg

making the platform particularly well-suited for placing individual spheroids onto electrodes in dedicated recording plates. Here, specifically, we used 96-well plates with embedded electrodes (with a 600 µm diameter, NSP-Z plates) for electrical impedance spectroscopy read-outs using Nanion's AtlaZ system.

Cultivating spheroids in suspension, using methods such as ultra-low-attachment plates or hanging drops, ensures consistent spheroid size and cell count, enhancing reproducibility in 3D *in vitro* testing. Once mature, transferring these spheroids into hydrogel further improves physiological relevance, as the hydrogel better mimics the *in vivo* cellular environment compared to suspension cultures. Consequently, 3D spheroids in hydrogels are valuable tools for cell-based assays and drug screening applications. After plating in hydrogel, spheroids typically undergo cellular reorganization [4]. This potentially affects growth patterns and the formation of cell-cell and cell-matrix contacts linked to aggregate densification [5]. These cellular changes can vary depending on the cell type, spheroid size, and hydrogel composition used. Such altered cellular behavior due to the adapted cultivation conditions should be taken into account when evaluating drug responses in 3D *in vitro* screenings. Currently, detecting cellular reorganization often relies on labor-intensive techniques, such as high-resolution fluorescent microscopy, which limits throughput and hinders the broader application of drug response assessments.

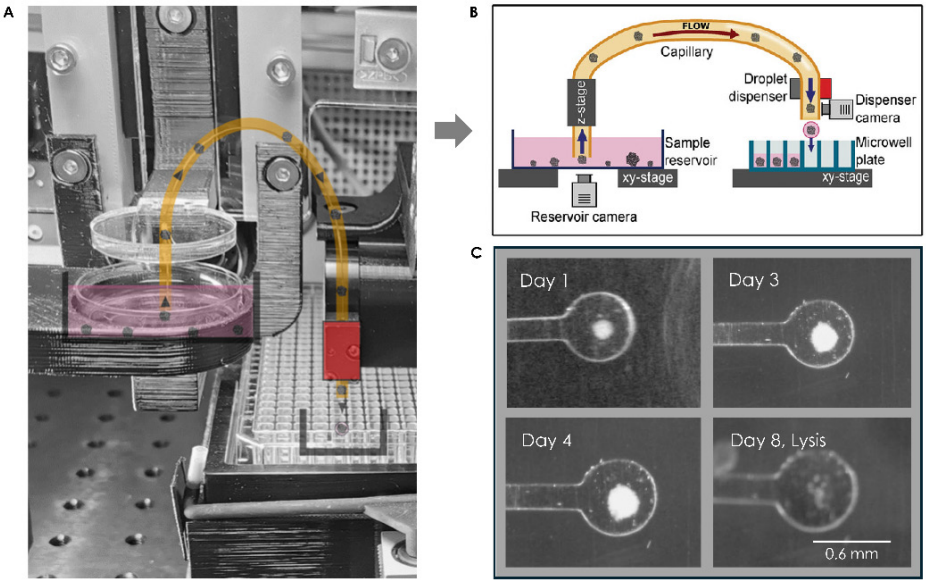
The following aspects should be considered when investigating 3D cell aggregates such as spheroids or organoids using an impedance measurement system:



AtlaZ recordings of HCT116 and HT29 spheroid growth and killing. A, B *Growth curves of HCT116 and HT29 spheroids over 55 hours, respectively. As spheroids increase in size, although the surface area covering the electrode only slightly increases, the impact on the resistive properties of the multiplied cells in the sphere shape is much more.* ***C,D*** *Lysis of HCT116 and HT29 spheroids upon application of 30% Trypsin & 0.6% Tween, respectively. The error bars depict SD, n = 2 (HT29) or 3 (HCT116) wells each.*

- Electrode contact: Impedance measurements typically require cells to be in direct contact with electrodes. Spheroids and organoids have a smaller contact area compared to 2D cultures, which spread out over a larger surface. Furthermore, when 3D cell aggregates grow larger, its volume increases more rapidly than its surface area, resulting in an increasing volume-to-surface area ratio.
- Signal penetration: The densely packed nature of spheroids impacts the electrical signals which penetrate deep into the structure. This aspect can be employed in the AtlaZ system by utilizing the full strength of the methodology, electrical impedance spectroscopy (EIS). Next to the impedance, parameters Resistance (R), Capacitance (C) and Phase (φ) are recorded. In the experiments here, analyzing the Impedance IZI at 1 kHz, i.e. focusing on the resistive impact on the Cell Signal, proved to be the most meaningful.
- Data interpretation: The complex geometry of 3D cultures can make interpreting impedance data more challenging compared to 2D monolayers. Here, the Cell Signal reveals cellular proliferation and also cellular reorganization within spheroids in a holistic way. The methodology EIS allows the detection of cell-surface area changes and the strength of cell-cell contacts when analyzing the resistance. This is invaluable when investigating 3D cell aggregates, as the spheroids undergo the cellular changes and reorganizations as described above and EIS is a method which captures such morphological changes. Thus, a reorganization and densification of the spheroids could be monitored.

In this study, spheroids from two human colorectal carcinoma cell lines, HT29 and HCT116, were cultured in ultra-low attachment plates, harvested, and then automatically deposited with the MicroPick S/O into individual wells of an NSP-Z plate preloaded with a basal membrane matrix. The plates were incubated at 37 °C for 30 minutes to allow the matrix to polymerize before adding culture medium to the wells. Over the course of several days, spheroid behavior and proliferation was continuously monitored using the AtlaZ measurement unit. Within the first five hours after plating, an increase of the Cell Signal (Impedance signal as recorded at 1 kHz) was observed, corresponding to the spheroids settling through the matrix



Pick-Flow-Drop principle for transferring 3D cell aggregates into a measurement plate. *3D cell aggregates, such as spheroids or organoids, can be automatically and selectively deposited at designated target locations for screening.* ***A*** *Prototype of the MicroPick S/O platform,* ***B****, schematic outline of the setup and* ***C****, HCT116 spheroids centered on the recording electrode of AtlaZ NSP-Z plates over the experimental timeframe.*

"The AtlaZ system enables time-resolved and label-free proliferation analysis of 3D cell aggregates. The effortless monitoring of the treatment response allows an in-depth assessment which is particularly useful when testing drug efficacy and toxicity for a large number of samples and compounds."

Victoria Zieger, Scientific Associate, IMTEK-Laboratory for MEMS Applications, University of Freiburg

and adhering to the electrode. After this initial rise, the signal remained steady or slightly decreased, likely reflecting the cellular reorganization within the hydrogel [6]. The resistance did not increase because of the reorganization and at t = 16 h after plating the now increasing Cell Signal hints to the formation of stronger cell-cell contacts and spheroid densification. This process was also observed via bright-field microscopy [7] and is discussed in more detail in related literature [8]. Following this period of cellular reorganization, the Cell Signal began to increase further, indicating cell proliferation and spheroid growth. As a proof-of-concept, cell lysis was induced by replacing the cell culture medium in the wells with a solution containing 30% Trypsin and 0.6% Tween. Following this treatment, the Cell Signal showed a significant decrease compared to control wells, indicating cell death. After reaching a minimum impedance level, the signal began to increase slightly, possibly indicating that surviving cells had begun to proliferate again.

In this case study, the MicroPick S/O platform enabled precise and controlled spheroid plating, achieving a level of positional accuracy unattainable with standard pipetting. The automated high-throughput handling of cell aggregates is particularly crucial in preclinical drug development, where large sample numbers must be processed efficiently. The integration of automated cell aggregate handling with real-time analysis through EIS significantly reduces the time and effort required for 3D *in vitro* drug screenings. This approach boosts throughput and data richness without the need for invasive assays, such as those using fluorescent markers. As a result, it opens new opportunities for drug discovery and development, delivering fast, reliable data in preclinical testing.

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Acknowledgments

We thank Viktoria Zieger, Tobias Hundertmark and Sabrina Kartmann from IMTEK-Laboratory for MEMS Applications University of Freiburg, for sharing their knowledge and equipment for 3D cell aggregate handling. We are grateful for valuable insights and a wonderful collaboration. The MicroPick S/O is jointly developed at IMTEK-Laboratory for MEMS Applications, University of Freiburg and Hahn-Schickard, Freiburg.

The development of Micropick S/O was supported by the German research ministry "Bundesministerium fuer Bildung, Wissenschaft, Forschung und Technologie" (BMBF) during the project ADAPT (Project Nr. 161L0235A-C (2020-2023)) and ADAPT-2 (Project Nr. 16LW0335K (2023-2025)), coordinated by Dr. Sabrina Kartmann.

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