SenseCard—a Disposable Parallel-sensing Card for Advanced In-vitro Models

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Due to increasing regulatory constraints and lack of physiological relevance of animal tests, there is an increasing demand for in vitro microsystems mimicking organ-level functions. Cell-on-a-chip approaches have proven their versatility and strength to reproduce the cell/tissue/organ microenvironment. While academia and SMEs have so far mainly been working on proving the biological relevance of their systems, they are now looking for solutions to monitor the cellular response, to get functional read-outs and to facilitate the use of such systems. With our SenseCard we provide a simple solution to sense molecules like glucose in such cell cultures.

The emerging market of organ-on-a-chip and 3D cell culture is mainly looking for a comprehensive solution, which allows to verify the validity of their systems for drug screening or toxicology. Therefore, many methods can be used for determining the concentration of molecules in the cell culture. However, methods based on optical readout (fluorescence, luminescence, or colorimetry) typically require relatively large amounts of sample. Others, based in molecular recognition and marking / or separation using magnetic beads, are not adequate for quantitative concentration measurements and are typically implemented for single-use positive / negative detection of target molecules. Electro-chemical sensors are on the other hand particularly well suited for measuring the concentration of molecules in an accurate, quantitative way, from a small sample-volume and can be re-used several times if the sample is efficiently evacuated and renewed without cross-contamination between successive measurements.

CSEM's SenseCard combines the strength of the electro-chemical sensors and parallel fluidics. It allows to perform 8 parallel measurements simultaneously with up to 40 cycles within 6h. This allows to closely monitor the cell culture without the need of many manual steps like dilution. Furthermore, with the recalibration possibility, SenseCard does not suffer from potential offset due to the culture media matrix effects.

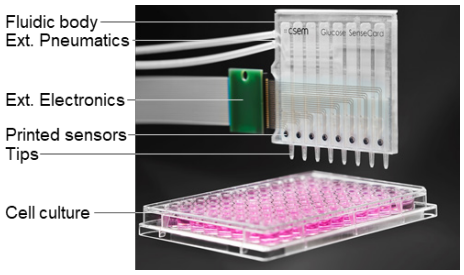

Figure 1: SenseCard on top a well plate containing a cell culture.

The current SenseCard (see Figure 1) holds 8 electro-chemical sensors in a row fitting on top of a standard 96-well plate. With pneumatics, 5 ul of culture media is aspirated into each of the 8 sensor chambers in parallel (simultaneously). To enable the parallel aspiration, a fluidic body was designed with appropriate restrictions to compensate for manufacturing tolerances. Meaning, after each channel a restriction is placed which dominates the flow resistance in this channel. With this feature it is possible to aspirate 8 samples of the culture media with one single pull-stroke of a pipette. During the aspiration, the sensor chamber is filled and the excessive liquid (not in contact with the sensor) can be returned to the cell culture with one single push stroke on the pipette. After the measurement, the analyzed samples are moved to the on-card waste with another single pull-stroke. Before the first measurement, a 3-point-calibration should be performed to tune the electronics for the applied culture media. Additionally, after each measurement, a wash cycle and ideally a one-point-calibration step follows to verify the cards function.

The complete fabrication process is optimized for low-cost mass production. The sensor's electrodes together with the electrical connections can be roll to roll (R2R) printed onto a heat stabilized PET foil. The subsequent electro-chemical functionalization can be handled with automated dispensing equipment. Finally, the fluidic body can be injection molded and the assembly completed with a lamination step. The complete assembly is finally sterilized via gamma radiation.

In Figure 2 we present results taken with the electro-chemical sensors functionalized for glucose. In a first step, three different glucose concentrations (0.5 mM, 1 mM and 5 mM) were used for calibration. In a second step, daily measurements over a period of 4 days have been performed in a 3T3 cell culture. Due to the sensor operational stability of 6h, every day a new sensor was calibrated and used for the culture media measurement. The calibration curve shows a good linearity while the culture results show, that the glucose consumption is rapidly increasing after the first day due to the fact that the cells proliferate constantly.

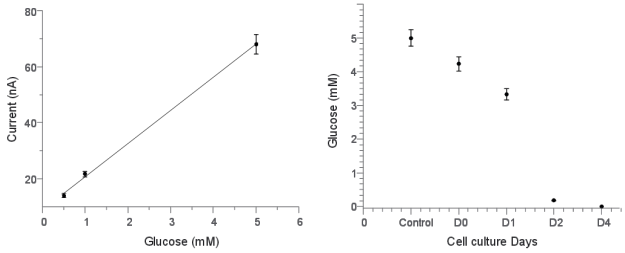

Figure 2: Electro-chemical sensor calibrated (left) and tested in cell culture over several days (right).

The patent pending SenseCard provides a solution which allows the operators to focus on their cell culture instead of the tedious work of diluting and pipetting. The gamma sterilized SenseCard with 8 parallel sensors generates results within a half a minute and can be calibrated to the applied cell culture media with or without serum. Due to the design, the measured samples are directly disposed in the card's waste. Furthermore, the rest of cell culture is never in direct contact with the sensor to prevent any contamination also by the sensor. Finally, the reuse of the SenseCard for up to 40 measurement cycles (40x8 = 320 measurements) and its operational stability of 6 hours allows to closely monitor the cells behavior during one day.

In a next step, the card will be used to monitor 3D cell cultures and might be equipped with on-card washing buffer to further reduce manual steps. Depending on user needs, we continue the development of additional electro-chemical sensor functionalization for, *e.g.*, lactate or others.

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