

out of a reservoir with an applied negative pressure. Since the lifting pressure is strongly dependent on the spheroid size and the used medium, and since strong local forces act on the spheroids for a short time, the generalization of the method is difficult. In contrast, drop-on-demand (DoD) methods where the droplets are dispensed *via* a microvalve (spheroOne® Cellenion SASU, France) or by mechanical deformation of an elastic tube¹ have proven to be compatible with a large range of different samples and offer a simplified implementation.

In this work, we have combined for the first time an advanced 3D bioprinting process¹ for deposition of spheroids or arbitrary 3D cell culture models, with a microsensor platform with embedded amperometric oxygen sensor electrodes. Here, breast cancer spheroids were automatically deposited *via* DoD directly into microsensor wells of nanoliter-range volumes, bringing sensor and microtissue in close contact. In this paper, the spheroid formation and deposition using bioprinting techniques are discussed. Electrochemical oxygen sensors allowed the precise and reproducible determination of single spheroid metabolic rates, including changes upon cancer drug exposure. Our approach explores advanced and automated microtissue manipulation together with the integration of sensors in 3D cell culture, towards more efficient and parallelized procedures, *e.g.* in drug screening.

2 Concept: bioprinting of spheroids onto microsensors

For successful access to the oxygen consumption and metabolism of bioprinted single spheroids *via* microsensors,

numerous boundary conditions must be fulfilled: (i) sensor and spheroid must be brought into close proximity to each other in a controlled manner; (ii) remain in that position for hours to ensure a reliable sensor result and (iii) have approximately the same size in the micrometre-range; (iv) the culture volume must be below microlitre-range for single spheroids³⁰ to guarantee a measurable concentration change; (v) the volume in which measurements are taken must be sealable from the external environment to prevent an undesired influx of additional oxygen and (vi) long-term stability of the sensor within the complex measurement electrolyte must be ensured over the whole experiment. At the same time, the platform has to be compatible with the DoD bioprinting process.

The electrochemical sensor platform consists of a glass chip with an array of nine circular (\varnothing 200 μm) platinum microelectrodes on its surface, fabricated on a wafer-level⁹ (Fig. 1b). Dedicated microwells (\varnothing 500 μm) patterned with permanent epoxy-based photoresist confine single spheroids at the electrodes and allow the real-time acquisition of the oxygen concentration directly within their microenvironment for monitoring both, culture conditions and cellular respiration. Breast cancer spheroids are cultured outside the platform and individually printed by DoD directly into the microelectrode wells, using a bioprinting technique¹ developed at the Laboratory for MEMS Applications (Fig. 1a and b). The bioprinting accomplishes the otherwise demanding and time-consuming tasks of trapping and precisely positioning spheroids in small volumes and close to the sensor electrodes. Its high degree of automation allows parallelization and offers flexibility as well as powerful liquid handling options, *e.g.* for drug delivery. Spheroid integrity and viability are maintained without the use of artificial



Fig. 1 Single spheroid deposition set-up and oxygen-sensing microwells. (a) Scheme of the bioprinting process: spheroids, homogeneously distributed in a reservoir, are transferred fully automatically into microwells with embedded oxygen sensors by means of a droplet dispenser. All components are software-controlled. (b) PipeJet® nanodispenser with the protruding capillary nozzle above the sensor glass chip with microwell array and electrode layout. (c) Cross-sectional view of one oxygen sensor well showing all relevant components and materials of the system.



concentration. The chronoamperometric part consisted of the consecutive application of (1) $0.7 V_{Ag/AgCl}$ for 1 s, (2) $-0.4 V_{Ag/AgCl}$ for 1 s, (3) $-0.3 V_{Ag/AgCl}$ for 3 s. It was followed by an open-circuit phase for 25 s or 55 s for active potentiometric measurement,⁴⁵ as well as to minimize electrode on-time in which oxygen is consumed (4 s at negative potential), which amounts 13.3% and 6.7% of the total time, respectively. Unless otherwise stated, sensor currents were recorded at an acquisition rate of 4 Hz, and the mean current value of the penultimate 500 ms during the final cathodic pulse (3) was used for amperometric data evaluation. Potentiometric values were taken 20 s after the end of the chronoamperometric measurement.

Sensor calibration was performed in 0.1 M PBS and cell culture medium by changing the ratio of air and nitrogen within the electrolyte by means of the gas mixing station GMix41 (Hitec-Zang, Germany). Microelectrode wells were covered with a glass slide in order to isolate their inner volume and to prevent gas exchange with the surrounding electrolyte, as required for respiration measurements. An oxygen concentration of $186.5 \mu\text{M}$ was assumed for both, PBS and cell culture medium, at atmospheric oxygen conditions.

For cell-related experiments, the spheroids were first printed into the microelectrode wells using the method mentioned above, followed by pipetting 0.5 ml cell culture medium onto the glass sensor chip so that a bubble-free wetting of all electrodes and microelectrode wells was ensured. Excessive convection during filling was avoided to maintain integrity of the spheroids within the microelectrode wells. To determine respiration rates during the oxygen measurement, the microelectrode wells were periodically opened and closed for 10 minutes, respectively. Oxygen sensors were calibrated after the measurements by performing a one-point calibration and taking the signal at air saturation as reference. Antimycin A (Sigma Aldrich, USA) was used for drug exposure experiments.

3.5 Data presentation and statistical analysis

The box plots shown extend from first to third quartile of the data, the median is shown as centre line. The whiskers extend from the edges of each box to the last data point in the $1.5\times$ interquartile range and outliers are shown as circles. The heights of bars in bar plots correspond to the mean values of the data with error bars as \pm standard deviation (s.d.). Mean values are stated with \pm s.d.

Statistical assessment was based on the two-sided *t*-test if not otherwise stated, whereas normal distribution was tested with the D'Agostino–Pearson test for normality. *p*-Values ≥ 0.05 were considered not being significant while significant *p*-values were categorized as $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$.

4 Results and discussion

4.1 Accuracy and precision of the printing position

Verification of sufficient accuracy and precision of the bioprinting process to provide pinpoint spheroid deposition

into the microelectrode well was investigated with a NBS 1952 Resolution Test Target Slide (Thorlabs, USA) used for calibrating the camera. For this purpose, the impact position of an ejected drop was first determined and subsequently used as a reference position. The target cross of the NBS slide was then positioned at these reference coordinates with the motorized *xy*-stage. Drop impact position reproducibility (precision) and deviation from target (accuracy) were determined by dispensing individual drops of either pure PBS or PBS containing a single spheroid. Images of the resulting drop position were captured with the calibrated print position camera. The center of the impact position was determined with OpenCV Library and Python programming, and the deviation of the drop center to the target cross was identified (Fig. 2a). The mean distance to the target of 30 individual pure PBS drops was $7.2 \mu\text{m} \pm 3.3 \mu\text{m}$, the mean distance of 23 individual PBS drops containing a single spheroid was $25 \mu\text{m} \pm 13 \mu\text{m}$. With regard to the target position, a printing accuracy and precision of pure PBS droplets of $2.0 \mu\text{m} \pm 3.6 \mu\text{m}$ and $3.2 \mu\text{m} \pm 4.2 \mu\text{m}$ in *x* and *y* direction was achieved, respectively. For PBS drops containing a single spheroid, the printing accuracy and precision in *x* direction was $-3 \mu\text{m} \pm 22 \mu\text{m}$ and in *y* direction $-10 \mu\text{m} \pm 13 \mu\text{m}$. A rough analysis of the spheroid size in printed droplets using ImageJ showed no correlation between the spheroid diameter and the droplet deflection. In addition, the volume and the largest horizontal expansion of the droplet just above the sensor well of 19 free-flying PBS drops and of 19 free-flying PBS drops with a spheroid were determined with the nozzle camera⁴³ (Fig. 2b). At its widest point, the PBS drop diameter was $334.1 \mu\text{m} \pm 6.4 \mu\text{m}$ with a total volume of $22.22 \text{ nl} \pm 0.22 \text{ nl}$. Interestingly, the mean diameter of drops containing a spheroid of $342.9 \mu\text{m} \pm 9.8 \mu\text{m}$ and the mean volume of $22.15 \text{ nl} \pm 0.28 \text{ nl}$ did not differ significantly from pure PBS drops. We assume that the slightly poorer accuracy and precision of the printing process for drops with spheroids are caused by varying spheroid position in the capillary just before the drop is ejected, the drop inhomogeneity as well as the slightly different weight, varying density and viscosity due to the biological cell material the drop contained. The largest horizontal expansion of the free-flying drop shortly before it enters the microelectrode well was a relevant indicator to assess if the accuracy and precision of the bioprinting process was sufficient for spheroid deposition into the well. If the droplet impact position was too far from the center of the microelectrode well, the droplet would hit the well edges and potentially stick to it, possibly resulting in the spheroid not being positioned within the well. With the ascertained maximum droplet expansion, the distance of a perfectly centered free-flying drop to the border of the microelectrode well would be $82.95 \mu\text{m} \pm 3.2 \mu\text{m}$ for a pure PBS drop and $78.6 \mu\text{m} \pm 4.9 \mu\text{m}$ for a PBS drop containing one spheroid, respectively. Regarding this matter, the previously determined deviation of the impact position of the drops to the target was small compared to this margin. Therefore, the





Fig. 2 Accuracy and precision of the impact position regarding single spheroid deposition. (a) Impact position of individually printed drops ($n_{w/o}$ spheroid = 30, n_{with} spheroid = 23) in relation to an initially determined reference printing position (red cross). The mean impact position is displayed by the black cross. The insets show exemplary drops of pure PBS or of PBS containing a spheroid positioned on the NBS 1952 resolution test target slide (crosshair of the slide has a length and width of 610 μm). For clarity, the largest mean diameter of a free-flying droplet just above the sensor well is shown in blue and the size of the sensor well is shown in grey. (b) Side view of a PBS drop ejected from the nozzle and falling into the sensor well. (c) Exemplary image of a spheroid successfully deposited into a sensor well.

successful, highly precise deposition of single spheroids into sensor wells could be achieved and is exemplarily shown in Fig. 2c.

4.2 Automated single spheroid deposition

To automatically deposit single spheroids into the microelectrode wells, we used an in-house developed spheroid-printing algorithm based on Gutzweiler, *et al.*¹ The nozzle region close to the orifice of the capillary where the droplets were ejected was optically monitored with the nozzle camera system. Passing spheroids were detected and tracked. The algorithm was able to predict how many spheroids would be contained in the next ejected droplet to make a decision on where to deposit it. Dispensed droplets can be categorized into single-spheroid droplets (SSD) containing exactly one spheroid, multi-spheroid droplets (MSD) containing more than one spheroid and zero-spheroid droplets (ZSD) containing no spheroids.

In order to quantify the single-spheroid printing rate (SSPR), the system was set to eject droplets into 30 different wells of a 96 micro well plate until a droplet with at least one spheroid was dispensed. The experiment was repeated three times. Afterwards, the number of spheroids in each well was counted with a 4 \times microscope. The SSPR was calculated according to:

$$\text{SSPR} = \frac{f}{\sum_{i=1}^n n_{iD}} \cdot n_{\text{SSD}}, \quad (1)$$

where $f = 5.78 \text{ Hz} \pm 0.26 \text{ Hz}$ was the droplet dispensing frequency of the system, n_{iD} was the total number of droplets which were dispensed into each well i , and n_{SSD} was the number of wells with exactly one spheroid. Furthermore, by dividing the number of wells with exactly one spheroid by the number of all filled wells, it was possible to determine the portion of single-spheroid droplets in relation to all droplets containing at least one spheroid. The results for three different spheroid concentrations in the reservoir are summarized in Table 1. For all wells, the minimum number of spheroids per well was one, and the maximum was three.

Next, the printing algorithm accuracy (PAA) was analysed. The PAA describes the accuracy of the software algorithm in determining the number of spheroids ejected with the last drop. Accurately detecting which spheroids were ejected depends on both the spheroid detection in the capillary and the tracking algorithm. In order to assess the PAA, the spheroid counts per well extracted from the software were compared with the counted spheroids under the microscope. The PAA for different spheroid concentrations in the reservoir can be found in Table 1 and was determined as:

Table 1 Analysis for different reservoir concentrations of the single spheroid printing rate (SSPR), the probability of single-spheroid droplets (SSD) in relation to multi-spheroid droplets (MSD) and the printing algorithm accuracy (PAA). n_{SSD} is the observed number of droplets containing exactly one spheroid, and n_{MSD} is the observed number of droplets containing more than one spheroid within one run

Reservoir concentration [spheroids per ml]	SSPR [min^{-1}]	$\frac{n_{\text{SSD}}}{n_{\text{SSD}} + n_{\text{MSD}}} [\%]$	PAA [%]
300	1.9 ± 0.6	88.9 ± 1.9	93.3 ± 3.3
1000	6.1 ± 1.5	88.9 ± 3.8	93.3 ± 3.3
2000	15.2 ± 6.9	81.1 ± 3.8	86.7 ± 8.8



$$\text{PAA} = \frac{\text{number of wells with correctly counted spheroid number}}{\text{total number of filled wells}} \quad (2)$$

The data in Table 1 shows that with an increased spheroid concentration in the reservoir, the single spheroid printing rate also increased, as expected. Likewise, however, the probability of MSDs which were not suitable for single spheroid printing increased. In addition, the PAA deteriorated with a higher spheroid concentration due to the increased number of spheroids passing the nozzle at the same time. Thus, it was more complex to distinguish individual spheroids and to precisely determine the number of spheroids that were ejected with the last droplet.

A concentration of 1000 spheroids per ml has proven to be an advantageous spheroid concentration in the reservoir. The high accuracy with which the spheroids were detected and automatically deposited, as well as the high probability that the drops contained no more than one spheroid, guaranteed a low-loss and reliable single spheroid deposition process with sufficient efficiency. For the oxygen measurement, only SSDs were deposited into the microelectrode wells. Empty droplets or droplets containing more than one spheroid were printed into a collection container.

4.3 Spheroid vitality and integrity after deposition

During the bioprinting process, the spheroids were subject to different possible stress factors, namely gentle mixing, aspiration, and transport through a thin capillary, ejection, and impact on a target. To rule out that the spheroid processing had negative effects on the sample and therefore producing misleading sensor signals, the cell viability and the integrity of the cell aggregates were examined.

Cell viability of 58 processed spheroids was analyzed and compared to 50 harvested, but unprocessed spheroids as control (Fig. 3a and b) in three independent samples. In total, the viability analysis was carried out with 1478 cells of the control sample and 1773 cells of the processed sample.

Fig. 3b shows that the mean viability of processed spheroids of $93.1\% \pm 5.8\%$ did not differ significantly from the viability of the control sample of $93.9\% \pm 5.0\%$. This behavior was in accordance with the estimated shear stress due to sample mixing and bioprinting, which did not exceed 25 mPa and 60 mPa, respectively, and was therefore below or in the order of physiological relevant shear stress values.⁴⁶

In addition, the integrity was examined by determining the spheroid area before and after the bioprinting process. The contours of 50 spheroids each of the control and the processed sample were identified and the resulting area was normalized by the median of the control (Fig. 3c). The mean diameter of the unprocessed spheroids of $199 \mu\text{m} \pm 46 \mu\text{m}$ did not differ significantly from the mean diameter of the bioprinted spheroids of $200 \mu\text{m} \pm 26 \mu\text{m}$ indicating that spheroids remained intact after deposition.

4.4 Oxygen sensor characteristics

The dissolved oxygen concentration was measured using platinum-based amperometric microelectrodes at the microwells' bottom. Highly stable and reproducible sensor performance was achieved by applying an advanced chronoamperometric protocol, in which the platinum surface is first electrochemically cleaned by an anodic and cathodic potential pulse prior the actual oxygen measurement by its reduction at $-0.3 \text{ V}_{\text{Ag}/\text{AgCl}}$. Transient current responses are shown for atmospheric and anoxic conditions in Fig. 4a. This method allows the oxygen reduction reaction to take place on a renewed platinum surface each time the measurement is performed, eliminating electrode fouling even in serum-containing electrolytes. Additionally, an electrode covering PHEMA-based hydrogel membrane was used to improve sensor long-term stability by preventing the settlement of blocking substances and a direct contact between spheroid and electrode surface.

Sensor calibration (Fig. 4b and c) was performed in the used cell culture medium and PBS with a high precision (relative error $<2.5\%$ across the whole measurement range) and a limit of detection of below $1 \mu\text{M}$, based on three times



Fig. 3 Influence of the bioprinting process on spheroid viability. (a) Representative live/dead staining of cells in a spheroid. Cyan-colored cells were living, and red-colored cells were dead. (b) Cell viability of unprocessed control spheroids and processed and bioprinted spheroids ($N_{\text{Control}} = 3$, $n_{\text{Control}} = 50$; $N_{\text{Printed}} = 3$, $n_{\text{Printed}} = 58$). (c) Spheroid area of unprocessed and processed spheroids normalized to the median of the unprocessed control sample ($n_{\text{Control}} = n_{\text{Printed}} = 50$). P values ≥ 0.05 were considered not being significant (n.s.).





Fig. 4 Oxygen sensor characteristics. (a) Transient current responses at an acquisition rate of 10 Hz during the execution of the chronoamperometric protocol used for oxygen measurement within anoxic and atmospheric oxygen concentrations in PBS. (b) Oxygen sensor signal during six calibration cycles in PBS, in which the dissolved oxygen concentration was varied by means of a gas-mixing station. (c) Oxygen calibration in cell culture medium and PBS, showing no significant difference in sensitivity. Error bars are s.d. (d) Comparison of the oxygen concentration within an open and closed microwell. While atmospheric conditions in an open microwell resulted in a constant sensor current, the current in a closed microwell decreased rapidly due to the exclusion of additional oxygen influx.

the standard deviation of the blank signal. At $-0.34 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$, the sensor sensitivity was only slightly lower in cell culture medium than in PBS ($-0.37 \mu\text{A cm}^{-2} \mu\text{M}^{-1}$). Moreover, we demonstrated in a prior work,²⁴ that such oxygen sensors can be operated in cell culture medium over a period of one week without a measurable change in sensitivity. The sensor method allows a one-point calibration, as the sensor current is nearly zero under conditions without oxygen and increases linearly until an oxygen concentration at atmospheric conditions, eliminating time-consuming calibration measurements at various concentrations.

Since oxygen is consumed by the sensor electrodes and is only available to a limited extent within the closed low-volume microelectrode wells, minimizing oxygen uptake by the electrodes was intended. Therefore, compared to the previously reported chronoamperometric oxygen measurement protocols,^{9,13,18} pulse durations were reduced by 50% to a total sensor on-time of 4 s. In addition, the diffusion limiting pHEMA membrane lowered the oxygen flux to the electrode during sensor on-times, further limiting consumption.

Oxygen dissolves at the electrolyte surface and spreads within the liquid by diffusion and convection. In an

uncovered microelectrode well, oxygen from the medium could reach the microelectrode well unhindered, resulting in a high steady-state current corresponding to an atmospheric oxygen concentration, as shown in Fig. 4d. Under these conditions, the sensor currents in PBS and cell culture medium were nearly the same, indicating that the same amount of oxygen dissolves in both liquids and consequently implying the same strength of the salting out effect. Once the volume in the well was sealed by a glass cover, additional influx of oxygen from the microelectrode wells' electrolyte-environment was drastically reduced. Thus, due to consumption by the electrode, the oxygen concentration began to decrease nearly linearly during the first 15 min. The concentration did not drop continuously to zero, as a small amount of oxygen still reached the inside due to a remaining gap between microelectrode well wall and cover. Instead, a concentration equilibrium established at approximately 50 μM . Since an ion flux in the electrolyte between working and counter electrode is required during the electrochemical measurement, a complete isolation of the inner volume from the electrolyte environment is impossible. Additionally, this would increase the resistance between the working and reference electrode, resulting in an undesired high potential





Fig. 5 Oxygen monitoring and respiration of single MCF-7 spheroids. (a) Comparison of sensor currents during amperometric oxygen measurements with an untreated spheroid and after exposure to 100 μM antimycin A. In addition, the oxygen concentration course of a blank measurement without incorporated spheroid is shown. (b) Transient amperometric oxygen measurement in a typical long-term drug response experiment, in which the microwell is periodically covered with a glass slide. (c) Potentiometric sensor signals during OCP phases, showing a high stability of electrode potentials and a decreased equilibrium potential at low dissolved oxygen concentrations. (d) Oxygen consumption rates of three representative MCF-7 spheroids before and after the exposure to 100 μM antimycin A. The blank signal was subtracted for data evaluation.

drop. Therefore, a trade-off between gas tightness and ionic conductivity has to be found. However, since the measurements in the following did not exceed 10 minutes under closed conditions, and a nearly linear decrease in oxygen concentration was observed during this duration (Fig. 4d), it can be assumed that the amount of additional inflowing oxygen molecules is negligible for monitoring the oxygen uptake of single spheroids.

4.5 Monitoring the oxygen microenvironment of single spheroids

Fig. 5a compares the course of the oxygen concentration in a microelectrode well with an incorporated spheroid to the same microelectrode well without spheroid, before and after the addition of the aerobic metabolism-suppressing drug antimycin A. Oxygen concentration was measured continuously, once per minute. While the microelectrode well was open, the sensor currents showed a constant value that corresponded to the oxygen concentration at air saturation. In the case of the untreated spheroid, the oxygen concentration during open conditions was slightly below that atmospheric steady-state value of the blank, due to additional oxygen consumption of the spheroid. By covering the microelectrode well with a glass slide, the inner volume was

isolated from its surrounding cell culture medium. This limited the amount of oxygen molecules in the well so that almost no additional oxygen could enter from the environment and solely the sensor electrode and the spheroid were responsible for a change in oxygen concentration. Due to the high cellular oxygen consumption of the untreated spheroid, the concentration decreased linearly and reached anoxic conditions after 8 minutes. After addition of 100 μM antimycin A, which stopped the aerobic metabolism of the spheroid by interrupting the mitochondrial respiratory chain,⁴⁷ the decrease in oxygen was significantly reduced (by approx. 95%) and close to the course without spheroid. After 10 minutes, the glass slide was removed, resulting in an increase in sensor current as fresh cell culture medium could enter its inner volume and re-establish atmospheric conditions.

By periodically covering and opening the microelectrode wells every 10 minutes, a long-term measurement was recorded (Fig. 5b). The oxygen concentration temporarily exceeded the atmospheric baseline upon addition of the drug-containing medium, due to its lower temperature (room temperature vs. 37 °C) and consequently higher solubility of oxygen. Oxygen consumption rates could be estimated from the slopes of the decreasing sensor signals during closed conditions and are shown in Fig. 5d for three representative



Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Tobias Lange for his assistance with cell culture. Partial funding by the German Federal Ministry of Education and Research, grant number 161L0235B, is gratefully acknowledged.

References

- L. Gutzweiler, S. Kartmann, K. Troendle, L. Benning, G. Finkenzeller, R. Zengerle, P. Koltay, G. B. Stark and S. Zimmermann, *Biofabrication*, 2017, **9**, 025027.
- V. Brancato, J. M. Oliveira, V. M. Correlo, R. L. Reis and S. C. Kundu, *Biomaterials*, 2020, **232**, 119744.
- B. Pinto, A. C. Henriques, P. M. A. Silva and H. Bousbaa, *Pharmaceutics*, 2020, **12**, 1–38.
- L. A. Low, C. Mummery, B. R. Berridge, C. P. Austin and D. A. Tagle, *Nat. Rev. Drug Discovery*, 2021, **20**, 345–361.
- K. Moshksayan, N. Kashaninejad, M. E. Warkiani, J. G. Lock, H. Moghadas, B. Firoozabadi, M. S. Saidi and N. T. Nguyen, *Sens. Actuators, B*, 2018, **263**, 151–176.
- J. L. Leight, A. P. Drain and V. M. Weaver, *Annu. Rev. Cancer Biol.*, 2017, **1**, 313–334.
- D. Liu, S. Chen and M. Win Naing, *Biotechnol. Bioeng.*, 2021, **118**, 542–554.
- J. Kieninger, A. Weltin, H. Flamm and G. A. Urban, *Lab Chip*, 2018, **18**, 1274–1291.
- J. Marzioch, J. Kieninger, A. Weltin, H. Flamm, K. Aravindalochanan, J. A. Sandvik, E. O. Pettersen, Q. Peng and G. A. Urban, *Lab Chip*, 2018, **18**, 3353–3360.
- A. Moya, M. Ortega-Ribera, X. Guimerà, E. Sowade, M. Zea, X. Illa, E. Ramon, R. Villa, J. Gracia-Sancho and G. Gabriel, *Lab Chip*, 2018, **18**, 2023–2035.
- E. Tanumihardja, R. H. Slaats, A. D. Van Der Meer, R. Passier, W. Olthuis and A. Van Den Berg, *ACS Sens.*, 2021, **6**, 267–274.
- W. Shang, C. Y. Chen, K. Lo, G. F. Payne and W. E. Bentley, *Sens. Actuators, B*, 2019, **295**, 30–39.
- A. Weltin, K. Slotwinski, J. Kieninger, I. Moser, G. Jobst, M. Wego, R. Ehret and G. A. Urban, *Lab Chip*, 2014, **14**, 138–146.
- P. Wolf, M. Brischwein, R. Kleinhans, F. Demmel, T. Schwarzenberger, C. Pfister and B. Wolf, *Biosens. Bioelectron.*, 2013, **50**, 111–117.
- P. Pellegrini, J. T. Serviss, T. Lundbäck, N. Bancaro, M. Mazurkiewicz, I. Kolosenko, D. Yu, M. Haraldsson, P. D'Arcy, S. Linder and A. De Milito, *Cancer Cell Int.*, 2018, **18**, 1–15.
- H. Azizgolshani, J. R. Coppeta, E. M. Vedula, E. E. Marr, B. P. Cain, R. J. Luu, M. P. Lech, S. H. Kann, T. J. Mulhern, V. Tandon, K. Tan, N. J. Haroutunian, P. Keegan, M. Rogers, A. L. Gard, K. B. Baldwin, J. C. de Souza, B. C. Hoefler, S. S. Bale, L. B. Kratchman, A. Zorn, A. Patterson, E. S. Kim, T. A. Petrie, E. L. Wiellette, C. Williams, B. C. Isenberg and J. L. Charest, *Lab Chip*, 2021, **21**, 1454–1474.
- H. Zirath, M. Rothbauer, S. Spitz, B. Bachmann, C. Jordan, B. Müller, J. Ehgartner, E. Priglinger, S. Mühleder, H. Redl, W. Holnthoner, M. Harasek, T. Mayr and P. Ertl, *Front. Physiol.*, 2018, **9**, 815.
- J. Kieninger, K. Aravindalochanan, J. A. Sandvik, E. O. Pettersen and G. A. Urban, *Cell Proliferation*, 2014, **47**, 180–188.
- J. Kieninger, Y. Tamari, B. Enderle, G. Jobst, J. A. Sandvik, E. O. Pettersen and G. A. Urban, *Biosensors*, 2018, **8**, 44.
- K. Domansky, W. Inman, J. Serdy, A. Dash, M. H. M. Lim and L. G. Griffith, *Lab Chip*, 2010, **10**, 51–58.
- M. A. Ortega, X. Fernández-Garibay, A. G. Castaño, F. De Chiara, A. Hernández-Albors, J. Balaguer-Trias and J. Ramón-Azcón, *Lab Chip*, 2019, **19**, 2568–2580.
- Y. S. Zhang, J. Aleman, S. R. Shin, T. Kilic, D. Kim, S. A. M. Shaegh, S. Massa, R. Riahi, S. Chae, N. Hu, H. Avci, W. Zhang, A. Silvestri, A. S. Nezhad, A. Manbohi, F. De Ferrari, A. Polini, G. Calzone, N. Shaikh, P. Alerasool, E. Budina, J. Kang, N. Bhise, J. Ribas, A. Pourmand, A. Skardal, T. Shupe, C. E. Bishop, M. R. Dokmeci, A. Atala and A. Khademhosseini, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, E2293–E2302.
- S. Eggert, M. S. Gutbrod, G. Liebsch, R. Meier, C. Meinert and D. W. Huttmacher, *ACS Sens.*, 2021, **6**, 1248–1260.
- J. Dornhof, J. Kieninger, H. Muralidharan, J. Maurer, G. A. Urban and A. Weltin, *Lab Chip*, 2022, **22**, 225–239.
- P. M. Misun, J. Rothe, Y. R. F. Schmid, A. Hierlemann and O. Frey, *Microsyst. Nanoeng.*, 2016, **2**, 16022.
- N. Rousset, R. L. Sandoval, M. M. Modena, A. Hierlemann and P. M. Misun, *Microsyst. Nanoeng.*, 2022, **8**, 14.
- D. Bavli, S. Prill, E. Ezra, G. Levy, M. Cohen, M. Vinken, J. Vanfleteren, M. Jaeger and Y. Nahmias, *Proc. Natl. Acad. Sci. U. S. A.*, 2016, **113**, E2231–E2240.
- S. Russell, J. Wojtkowiak, A. Neilson and R. J. Gillies, *Sci. Rep.*, 2017, **7**, 1–11.
- K. Ino, M. Şen, H. Shiku and T. Matsue, *Analyst*, 2017, **142**, 4343–4354.
- A. Weltin, S. Hammer, F. Noor, Y. Kaminski, J. Kieninger and G. A. Urban, *Biosens. Bioelectron.*, 2017, **87**, 941–948.
- R. Mukomoto, Y. Nashimoto, T. Terai, T. Imaizumi, K. Hiramoto, K. Ino, R. Yokokawa, T. Miura and H. Shiku, *Analyst*, 2020, **145**, 6342–6348.
- A. Sridhar, H. L. De Boer, A. Van Den Berg and S. Le Gac, *PLoS One*, 2014, **9**, e93618.
- K. Ino, Y. Yamada, Y. Kanno, S. Imai, H. Shiku and T. Matsue, *Sens. Actuators, B*, 2016, **234**, 201–208.
- H. Kurosawa, H. Utsunomiya, N. Shiga, A. Takahashi, M. Ihara, M. Ishibashi, M. Nishimoto, Z. Watanabe, H. Abe, J. Kumagai, Y. Terada, H. Igarashi, T. Takahashi, A. Fukui, R. Suganuma, M. Tachibana and N. Yaegashi, *Hum. Reprod.*, 2016, **31**, 2321–2330.
- Y. Date, S. Takano, H. Shiku, K. Ino, T. Ito-Sasaki, M. Yokoo, H. Abe and T. Matsue, *Biosens. Bioelectron.*, 2011, **30**, 100–106.
- Y. Xiang, K. Miller, J. Guan, W. Kiratitanaporn, M. Tang and S. Chen, *Arch. Toxicol.*, 2022, **96**, 691–710.



