

Online oxygen monitoring using integrated inkjet-printed sensors in a Liver-On-a-Chip system

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Electronic Supplementary Information (ESI)

Sheet

resistance measurement

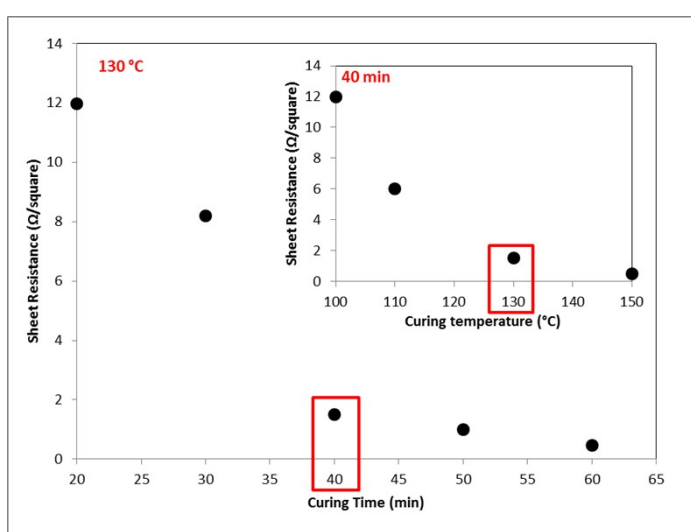
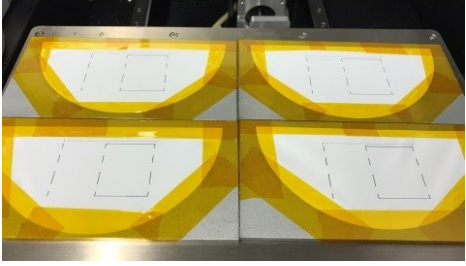


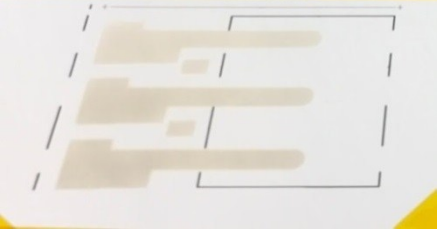
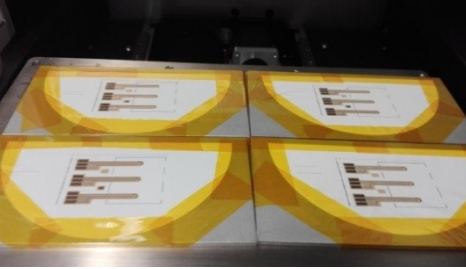
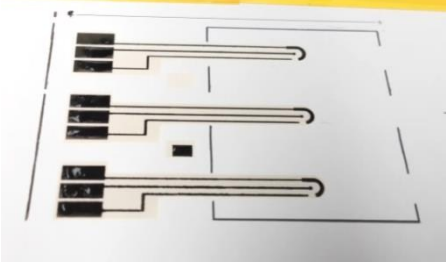
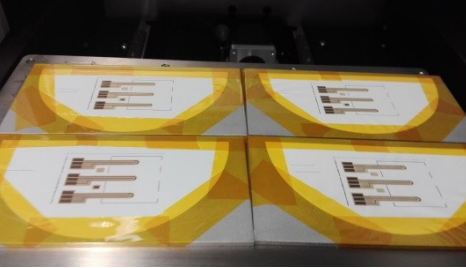
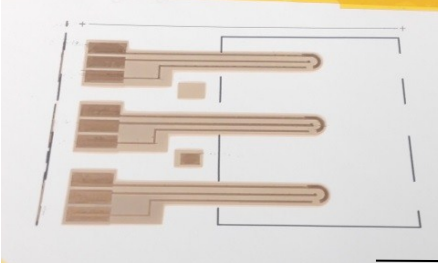

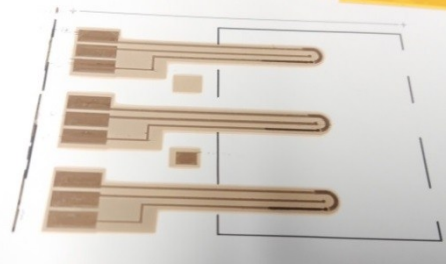


Fig. S1 Sheet resistance of the gold ink at different sintering time and temperature.

IJP Fabrication procedure

The printer operates with piezoelectric inkjet printheads, which have 16 individually addressable nozzles with nominal droplet volumes of 10 pL. Printing patterns were generated using Electronic Design Automation (EDA) layout software. The printing was carried out in a standard laboratory environment in ambient conditions, without non-particulate filtered enclosure systems and without precise control of temperature or humidity. Different treatment processes were performed to change the properties of the printed layers. The dielectric SU-8 was cured with a Dymax BlueWave 75 UV spot lamp. A standard laboratory hotplate was employed for the drying and sintering of the conductive inks. An oxygen plasma treatment was carried out to adjust the surface energy of the printed layers (SmartPlasma, Plasma technology GmbH). The MobileDrop (GH11, Krüss GmbH) contact angle analysis was employed to determine the surface energy of the layers.

DS: Drop Space

<p>Printing of the alignment patterns of the membrane</p> <p>Ink: Silver DS: 30 μm $T_{\text{substrate}}$: 40 $^{\circ}\text{C}$ Layers: 1</p>		
<p>Printing of the primer layer. Cured with UV radiation 15 seconds.</p> <p>Ink: SU-8 Ds: 15 μm $T_{\text{substrate}}$: 40 $^{\circ}\text{C}$ Layers: 2</p>		
<p>Printing of the gold electrodes</p> <p>Ink: Au Ds: 15 μm $T_{\text{substrate}}$: 40 $^{\circ}\text{C}$ Layers: 1</p>		
<p>Drying of the gold pattern at 100 $^{\circ}\text{C}$ for 10 minutes.</p>		
<p>Printing of the silver electrodes</p> <p>Ink: Ag Ds: 30 μm $T_{\text{substrate}}$: 40 $^{\circ}\text{C}$ Layers: 1</p>		


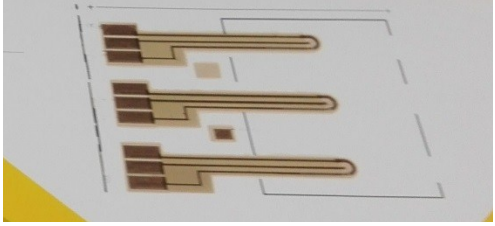

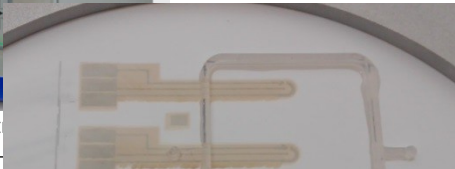

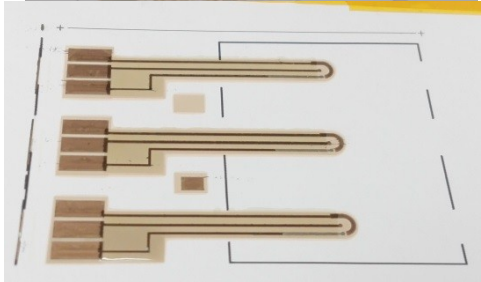
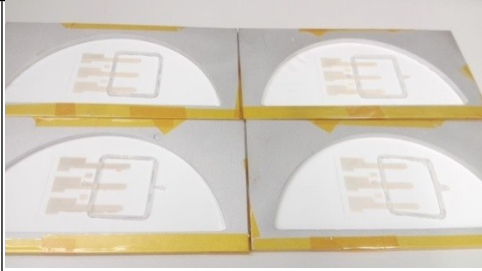

<p>Sintering of both conductive inks at 130 °C during 40 minutes</p>		
<p>Plasma treatment to allow the printing of the SU8 passivation layer. Conditions: 24 W, 30 s. and 0.5 bar O₂.</p>	 <p>http://www.plasma-t</p>	
<p>Passivation layer to isolate the tracks.</p> <p>Ink: SU-8 Ds: 15 μm T_{substrate}: 40 °C Layers: 1</p>		
<p>Attachment of the silicon gasket to define the cell culture area</p>		

Fig. S2 Step-by-step fabrication procedure of the IJP oxygen sensors.

Experimental procedure

Sensors characterization and calibration

Two point calibration measurements were taken with a cell culture medium buffer at atmospheric and at a Zero oxygen reading. Zero oxygen buffer was achieved by the addition of sodium sulphite to the medium. The calibration solution used was at the same temperature and salinity of the sample. The DO value of both calibration buffers was controlled with a commercial oxygen probe. A commercial Clark-type oxygen sensor was also incorporated inside the ExoLiver as a validation of our sensors and it was calibrated following the same procedure. All the elements were sterilized with ethanol and UV light without any loss of sensor function and response.

Experimental set-up

The inkjet-printed membrane was assembled in the ExoLiver as described in⁴³ and the perfusion of the dynamic culture was begun. Dynamic stimulation of the culture started with $0.1 \text{ dyn}\cdot\text{cm}^{-2}$ and the flow rate was gradually increased to $1.15 \text{ dyn}\cdot\text{cm}^{-2}$ ($1.5 \text{ mL}\cdot\text{min}^{-1}$), with a total amount of 43 mL unidirectional recirculating culture media. ExoLiver, reservoir, filters and most of the tubing were placed inside an incubator in order to maintain physiological conditions ($37 \text{ }^\circ\text{C}$, $5\% \text{ CO}_2$). The peristaltic pump and all the instrumentation to measure the sensor properties were placed outside the incubator.

Fig. S3 Experimental set-up to monitor the DO sensor inside an OOC system.

Biocompatibility test

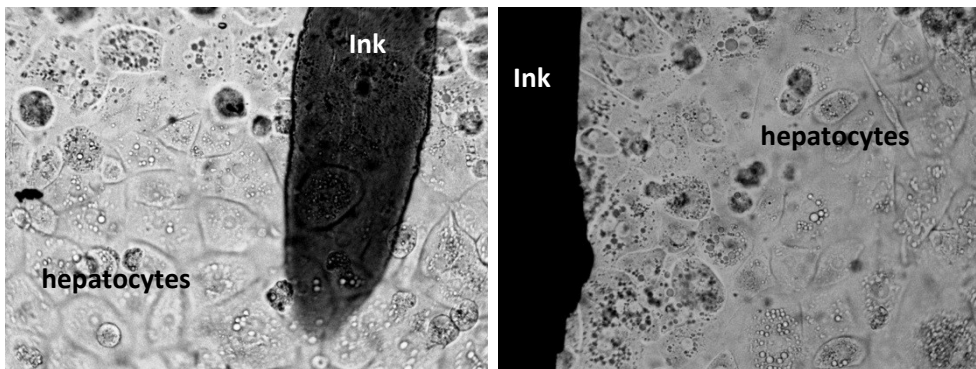


Fig. S4 Primary human hepatocytes cultured along over a printed Au, Ag and SU-8 printed patterns. Representative images of the cells above the printed materials were taken using a bright field microscope (Olympus BX51) at 20X magnification.

Study of the primer layer

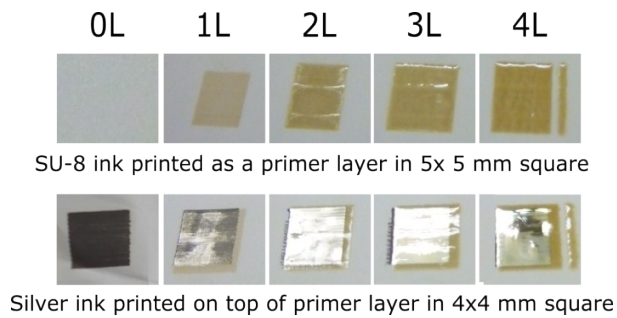


Fig. S5 Silver ink printed onto different number of primer layers.

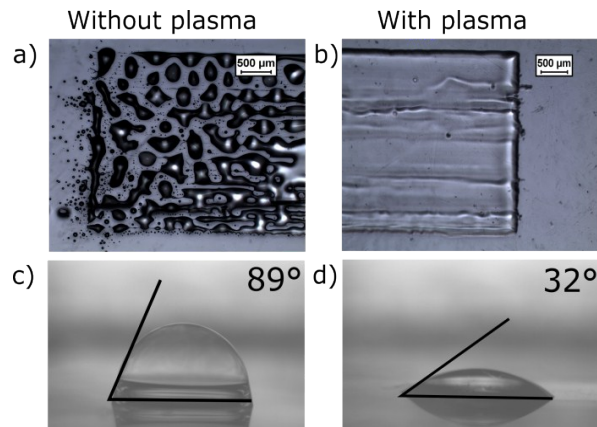


Fig. S6 A pattern of SU-8 printed over cured SU-8 A) before and B) after the treatment of the surface with plasma. Contact angle of deionized water on a SU-8 layer measured C) before and D) after the treatment of the substrate with oxygen plasma.

Electrochemical characterization

Fig. S7a shows the CV in ferro(II)/ferri(III)-cyanide obtained for the printed gold WE (300 μm). The anodic/cathodic peak current values (I_p) are directly proportional to the WE area as shown in the theoretically Randles-Sěvčik equation. To determine if the electrode is working properly we compared the obtained I_p with the theory, using Randles-Sěvčik's equation.

$$I_p = 0.4463 \cdot \left(\frac{F^3}{RT} \right)^{\frac{1}{2}} \cdot n^{\frac{3}{2}} \cdot A \cdot D^{\frac{1}{2}} \cdot R_0 \cdot v^{\frac{1}{2}} \quad (1)$$

where n is the number of electrons participating in the redox process, F is the Faraday Constant [$\text{C}\cdot\text{mol}^{-1}$], A is the electrochemically active electrode area [m^2], R_0 is the initial concentration of analyte [$\text{mol}\cdot\text{m}^{-3}$], D is the diffusion coefficient [$\text{m}^2\cdot\text{s}^{-1}$], v is the scan rate [$\text{V}\cdot\text{s}^{-1}$], R is the gas constant [$\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$] and T is the temperature [K].

Our printed gold electrodes have achieved a cathodic I_p of about $1.2 \pm 0.2 \mu\text{A}$ and an anodic I_p of $-0.96 \pm 0.3 \mu\text{A}$. The theoretical Randles-Sěvčik current peak is $0.90 \mu\text{A}$. The deviation is attributed to the increased of the effective electrodes area due to their roughness and the differences in final printed area between electrodes.

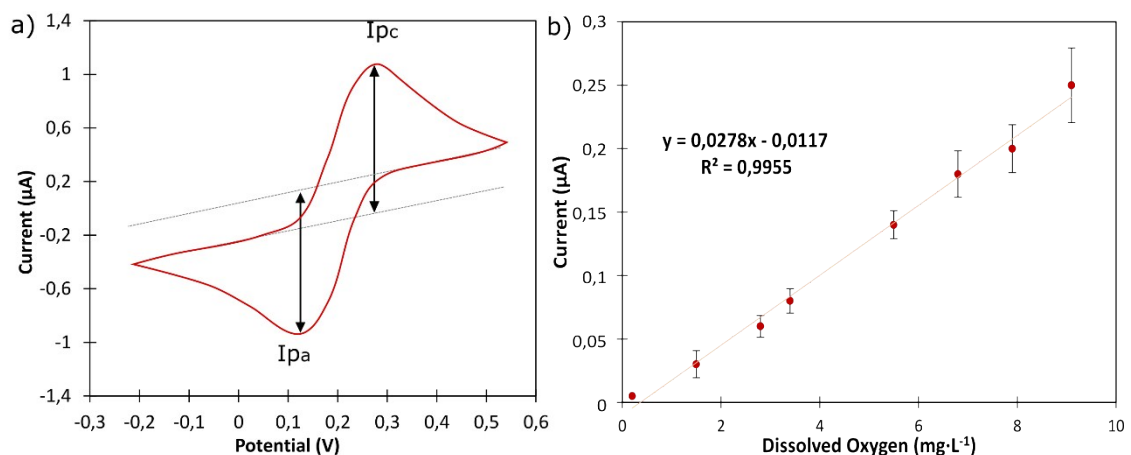


Fig. S7 a) Cyclic voltammetry of gold WE= 300 μm in ferro/ferricyanide (10 mM) and b) its calibration curve at different concentrations of oxygen in the range from 0-to 9 $\text{mg}\cdot\text{L}^{-1}$.

Fitting of the bioreactor system modelling

Qualitatively simulated profiles reproduce the experimental trend of DO along the lower channel throughout the monitoring (Fig. S8). Thus, highlighted that ExoLiver model is able to predict changes in OCR caused by drug additions, as well as well DO concentration gradients along the lower channel. For more quantitative comparison with experimental results, the normalized mean square errors (NRMSE) between the experimental DO profiles and the DO simulated profiles were determined (Table 1). The deviation obtained are below the 8%, reproducing with great accuracy the biological behavior.

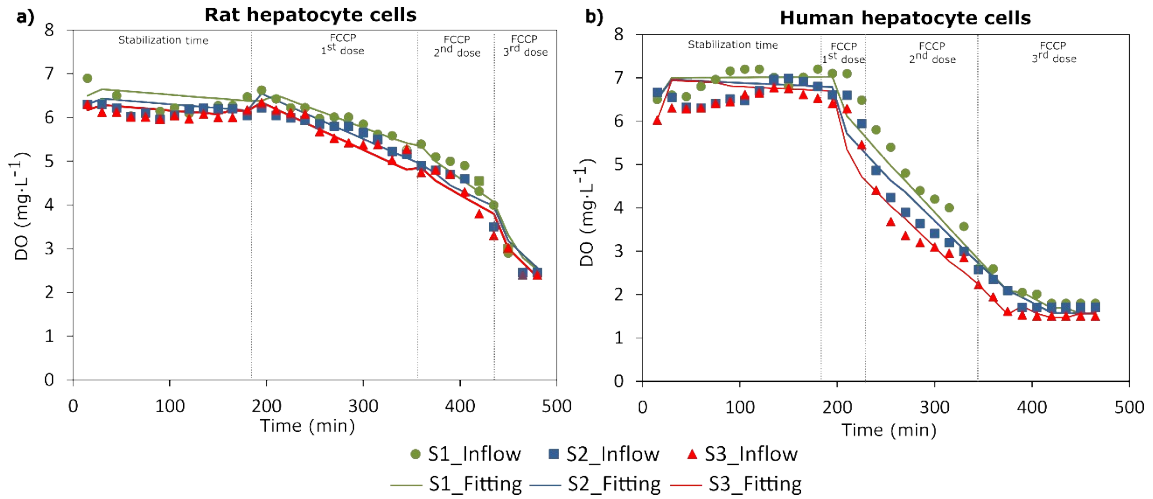


Fig S8. Experimental and fitting results of the DO monitored during 8 hours (green is the sensor placed in the inflow, blue r the middle, red the outflow) for a) rat hepatocyte cells and b) human hepatocyte cells, for several addition of FCCP drug.

Table S1. Normalized root mean square errors (NRMSE) between the experimental DO profiles and the DO simulated profiles.

	Inflow	Middle	Outflow
Rat cells	5.7%	5.9%	4.9%
Human cells	6.0%	6.4%	6.5%