BIOGRAPHY - HIGH RESOLUTION ROLL-TO-ROLL PRINTING OF BIOCOMPATIBLE GRAPHENE/PROTEIN MULTILAYERS FOR BIOMEDICAL APPLICATIONS

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1 *Two-color printing machine for rotary gravure printing (SAUERESSIG GmbH + Co. KG) with corona unit and NIR drying systems.*
2 *Multi-well plates: Printed foil bonded to a 96-well (left) and a 24-well (right) plate.*

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BIOGRAPHY

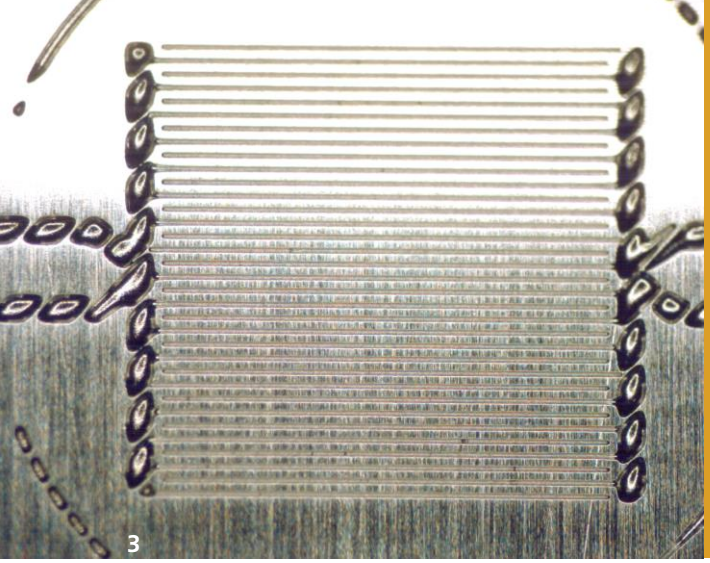
PRINTED GRAPHENE SENSORS FOR ELECTRIC CELL IMPEDANCE SENSING

Starting situation

There is a growing demand for cell-based assays for determining the interaction between cells and chemical and biological materials or compounds. Sensing systems and methodologies are required that provide continuous results in real time, at high throughput and at low cost.
The measurement of impedance using electrically conductive interdigital structures is a non-invasive methodology which has the potential to fulfill these requirements. Sensors for cell-based applications with those interdigital structures already exist today. Various commercial variants such as single biochips (cellasys GmbH, Kronburg / Germany), 24-well plates (HP Medizintechnik GmbH, Oberschleißheim / Germany), 96-well plates (Applied Biophysics, Troy / USA) or 384-well systems (ACEA Biosciences, San Diego /USA) are available. They use electrically conductive structures made of biocompatible metals, like e.g. gold or platinum, and are usually produced using photolithographic methods. Since cell-based sensors are used to examine viable cells, a biocompatible interface to a fluidic system is provided. This is usually done by bonding so-called wells to the printed circuit board comprising the electrically conductive interdigital structures.
The sales prices of these disposable systems are in the three-digit euro range per unit (e.g. per microtiter plate) and are mainly due to the complex production method of the biocompatible interdigital structures.

Our approach

A cost-effective production of the sensors is made possible by the use of a roll-to-roll printing technology. Printed foils are bonded to bottomless well plates in order to set up sensors in well plate format (Figure 2).



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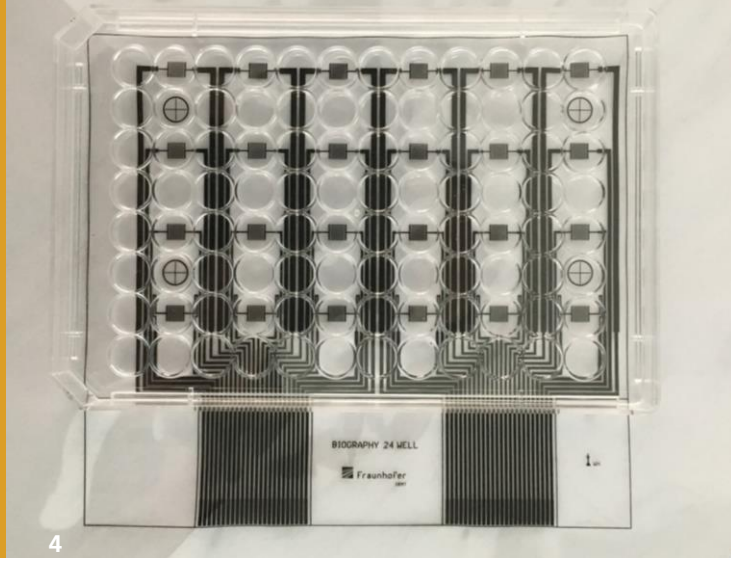

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The sensors are produced by sequential printing of two substances. Interdigital structures are produced from conductive ink comprising graphene platelets. In a second step, an additional protein layer is printed onto the electrode structures to improve adhesion of cells, for example as a confluent monolayer. For this purpose, the printing system has a second printing unit.
Register control, i.e. synchronization of the printing units to each other is used for precise alignment of the layers to each other. This is crucial for the quality and function of the sensors.

Printing machine

In order to match both prints, the units are adjustable in position transversely to the printing direction. Doctor blade pressure and angle are independently adjustable on both units. As a result, the print quality can be optimized in a wide range depending on the characteristics of the ink and the printing cylinder.
The drives for the printing unit, winder, unwinder and web guide are all digitally controlled. This allows an exact control of the web guide with respect to web tension and speed. The surface energy of the film materials can be increased with the built-in corona unit, in order to improve the wettability of the films. The unit is located immediately before the first printing unit.
NIR (near infrared) drying systems are installed as drying units, since the drying of the graphene ink could not be ensured with a conventional hot air drying or only with long drying paths, which were not feasible in the compact printing system. The radiated power is infinitely variable and thus also enables a gentle drying of the biological substances.
For the realization of register control, the system uses a photo sensor and a digital path length correction. The web length can be digitally controlled in printing operation.

Printing cylinders

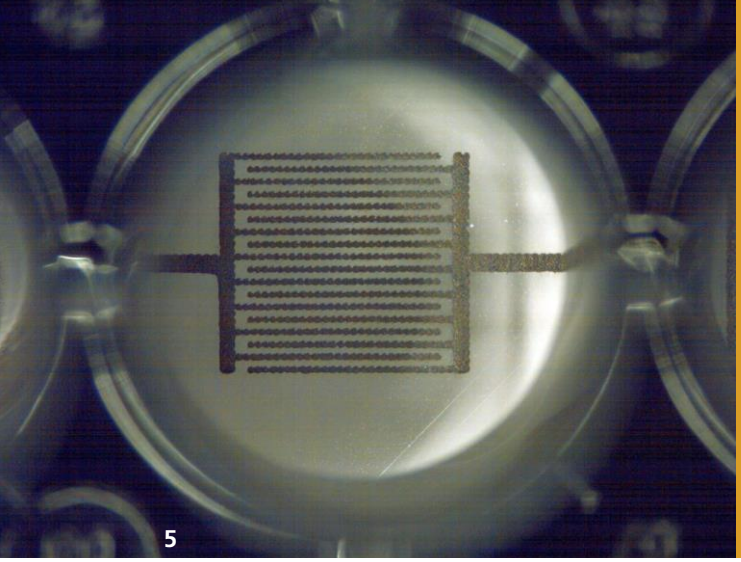
The two printed layers of the sensors to be produced differ in the printed pattern and in the properties of the printing substances. The interdigital structures comprise fine lines, which must have no interruptions or short circuits and at the same time have to achieve sufficient conductivity. In contrast, printing errors in the protein layer are less critical. While the viscosity of the graphene ink can be easily adjusted to a standard value for gravure printing, the printing of the biological substances is mainly influenced by the low viscosity of the protein inks.
An ultrashort pulse laser engraving system available at SAUERESSIG is used for the production of very shallow and small wells in the micrometer range. They allow the defined transfer of the protein ink to the substrate. The printing cylinder transferring the graphene ink is produced by means of a laser etching process.

Graphene ink

A new graphene-containing ink was developed. It meets the requirements concerning printability and application in a cell-based sensor. The reformulated ink is pasty, electrically conductive and biocompatible as a printed layer. Biocompatibility was demonstrated in cytotoxicity tests according to ISO 10993-5 with different cell lines. The initially pasty ink was modified such that it could also be applied through rotary gravure printing. For accurate printing at high speed and with good definition the ink needed to be formulated to have suitable viscosity and fast drying. An ink is composed of three main component types: Resin (or binder), solvents and additives such as pigments or other functional additives. The combination of targeted properties was made possible by careful and considered selection of the constituents, specifically the addition of Haydale's Graphene Nanoplatelet (GNP) material as a key active ingredient. GNPs are high-aspect ratio carbon nanomaterials which are processed and surface-functionalised using Haydale's patented HDPlas® plasma-based technology.
The low energy HDPlas® process is able to add functional chemistry in a uniquely benign manner, which causes very minimal damage to the nanomaterial structure, thereby maximising the ability to impart the nanomaterial properties to the final ink.

3 *Interdigitated electrode structures on the gravure printing cylinder (line width 50 µm, depth 80 µm).*

4 *24 printed interdigital electrodes. Pitch: Two times the pitch of a 96-well plate.*



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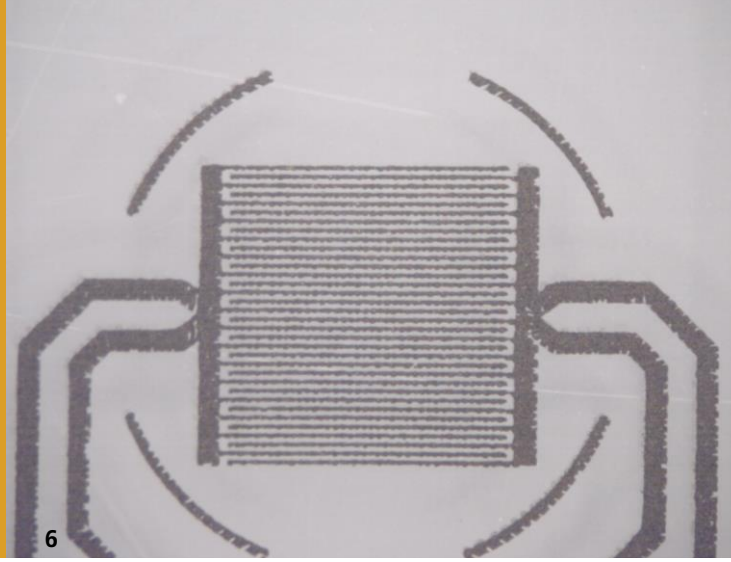

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The chemical functionalisation promotes efficient dispersion into the carrier resin used in the ink; it is this effective dispersion that allowed the ink to more readily exhibit the desired characteristics described above. The ink was made using a variety of mixing and dispersion methods; GNPs were mixed into the resin vehicle, which then followed by a series of milling procedures to ensure adequate uniformity through the ink.
The resultant biocompatible ink is that of a paste-like viscosity, which can then be diluted down using an appropriate solvent. Solvent selection is influenced by a number of factors, such as chemical compatibility, printing speed and drying factors such as temperature and time. At a solvent concentration between 20 and 30 weight percent, the viscosity of the ink was about 0.1 Pa*s. The surface tension was between 31 and 36 mN/m.

Print substrate and printing

For the targeted biological applications of the printed sensors, all materials used must be biocompatible, i.e. they must not harm the cells to be examined. From the point of view of printing technology, the films must have a significantly higher surface energy than the printing ink. The surface energies before and after corona activation were determined for the following biocompatible film materials: Polystyrene (PS), cyclic olefin copolymer (COC), polyethylene terephthalate (PET) and polypropylene (PP). Out of this list, PET exhibited the highest surface energy which was 42 mN/m before and between 55 and 60 mN/m after corona treatment.

Based on test structures, the square resistance was determined for prints made with the two-color printing machine described above and the new graphene ink. The test cylinder used had a well depth of 60 µm. The measured square resistance of the 5 to 6 µm thick printed layer was around 100 Ω/sq. Normalized to a layer thickness of 25 µm, this results in a square resistance of around 20 Ω/sq.
Electrically conductive interdigital structures of various geometries were successfully printed (Figures 5 and 6). Even the smallest electrode structures with a width of 50 µm and a distance of 50 µm between adjacent electrodes could be realized by gravure printing.

Technical Data

Two printing units; each with an integrated NIR drying unit

Web width:	300 mm
Width of printable area:	250 mm
Printing speed:	Up to 40 m/min
Power of corona unit:	Up to 600 W
Smallest electrodes printed:	50 µm
Thickness of printed graphene layer:	4-6 µm
Square resistance of printed structures:	10-20 Ω/sq at 25 µm)
Graphene ink viscosity:	0.1 Pa*s.
Graphene ink surface tension:	31-36 mN/m

Application Examples

The sensors developed within BIOGRAPHY can be used both for toxicity studies and for validating the effectiveness of anti-infective agents, like for example antiviral substances. In both cases, so-called indicator cell lines are used which form a confluent cell monolayer on the interdigital electrode-based sensor and electrically insulate it. When added substances destroy the electrically insulating, confluent cell layer, this indicates, for example, the cytotoxicity of these substances. This is recognizable by the reduction of the electrical resistance of the interdigital structure. In the second application, viruses are added instead of toxic substances. This leads to a morphological change of the cells or to a detachment of the cells from the cell monolayer, the so-called cytopathic effect (CPE). The addition of antiviral substances inhibits the infection of the cells or the viral replication in the cells and thus the CPE. This circumstance can be used to deduce from the changes in the measured impedance values the achieved inhibition of the CPE and thus the efficacy of the antiviral substances.

5 *Magnified view of printed interdigital electrodes integrated with a well of a 96-well plate. Electrode width and gap: 100 µm.*

6 *Printed interdigital electrodes. Electrode width and gap: 50 µm.*

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