



ADVANCED FUNCTIONAL &
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High-resolution gravure printing of graphene for biomedical applications

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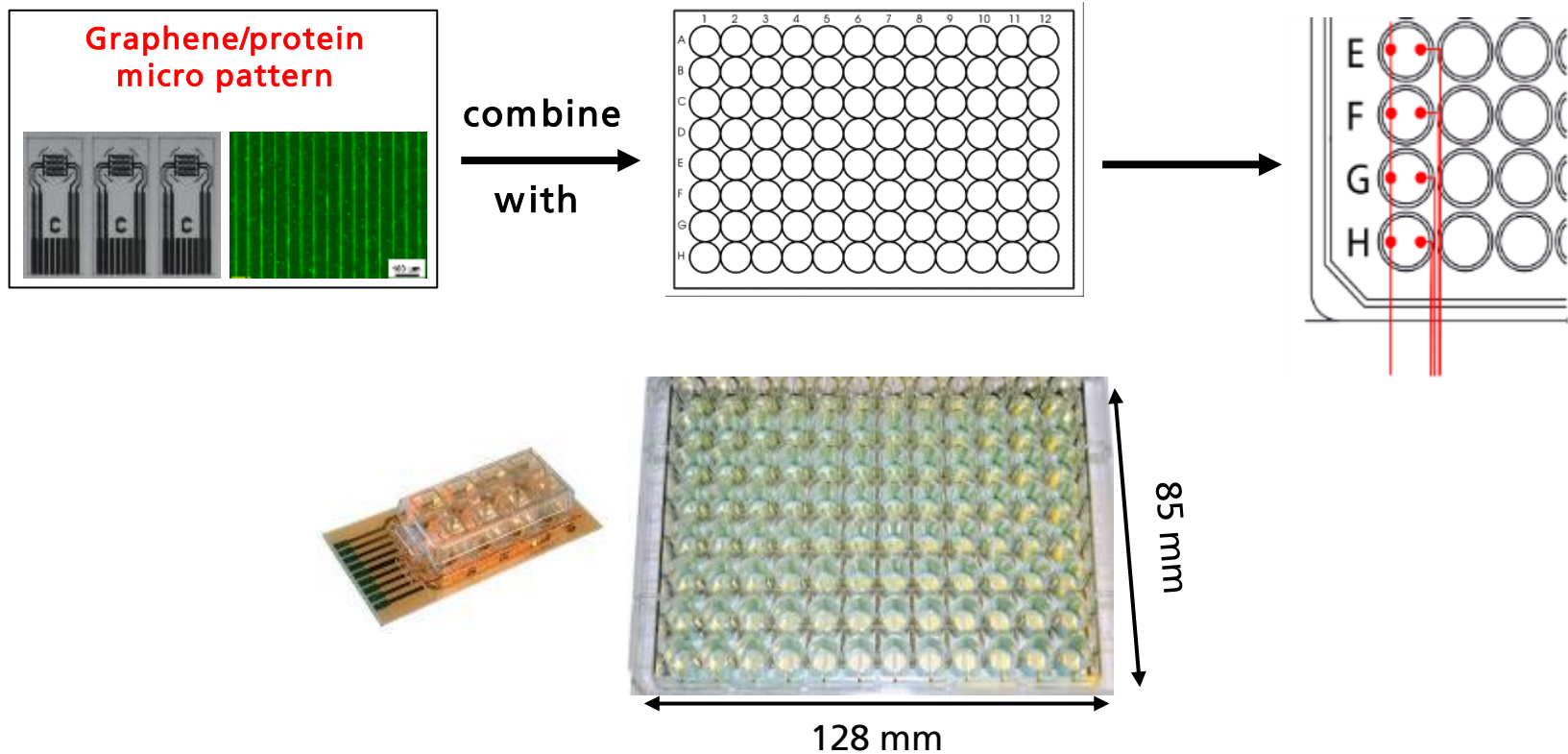
Düsseldorf, 3rd March 2016

Basic idea

- Roll-to-roll gravure printing of biosensors
 - Electrically conductive structures
 - Adherence proteins
-
1. Printing of graphene electrodes on polymer foils for impedimetric or electrochemical measurements
 2. Drying of printed graphene
 3. Printing of protein patterns on conductive electrodes for improved cell adherence and proliferation (expensive → resource efficiency)
 4. Drying of printed protein

Biosensor

Combination of surface functionalized foils with cell culture substrates, e.g. with bottom-less well plates



Objectives

- Biocompatible and electrically conductive **graphene ink** suited **for gravure printing**
- Fabrication of **gravure printing cylinders** with structure size $< 10 \mu\text{m}$
- **Roll-to-roll process** for graphene/protein printing
- **Two-colour roll-to-roll production line** for surface functionalisation of large area polymer foils
- **Evidence of suitability** of printed biosensors for
 - Vaccine and antiviral drug validation
 - Cell based toxicity tests

Project consortium

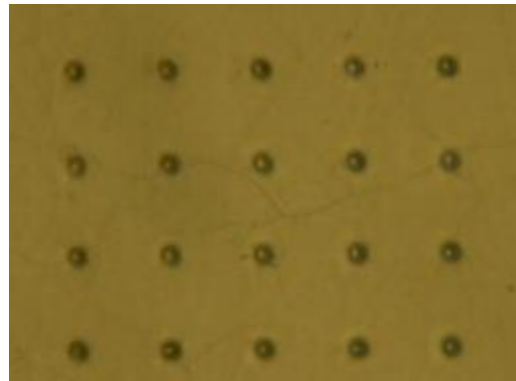
Name	Tasks
Fraunhofer IBMT	R2R gravure printing process (graphene and proteins), biocompatibility tests
SAUERESSIG GmbH + Co. KG	R2R two-colour printing machine, laser-based method for micro patterning of gravure printing cylinders
Haydale Ltd.	Biocompatible graphene ink for gravure printing
AiCuris GmbH & Co. KG	Application: antiviral drug validation
cellasys GmbH	Application: repeated dose toxicity



- Basis: screen printing graphene ink (HDPlas® IGSC02002)
 - Viscosity: ~ 5.5 Pa*s
 - Thickness before / after drying: ~ 13 μm / 7 μm
 - Sheet resistivity: ~ 12 $\Omega/\text{sq.}$ (normalised to 25 μm)
- Target values for gravure printing on polymer foil
 - Viscosity: ~ 100 mPa*s (gravure printing ink ~ 40 mPa*s)
 - Surface tension: < 58 mN/m (gravure printing ink ~ 32 mN/m)
- Modifications
 - Solvent type
 - Solvent content
 - Resin content
 - Milling procedure

Fabrication of gravure printing cylinders

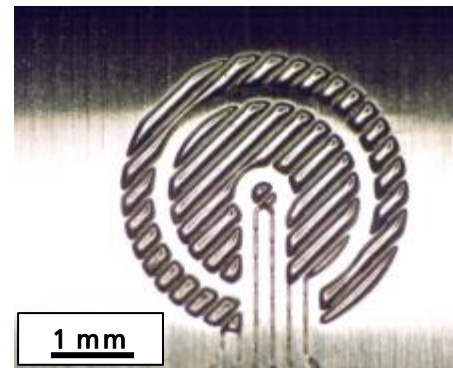
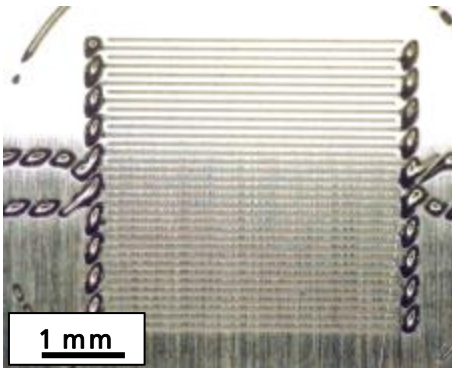
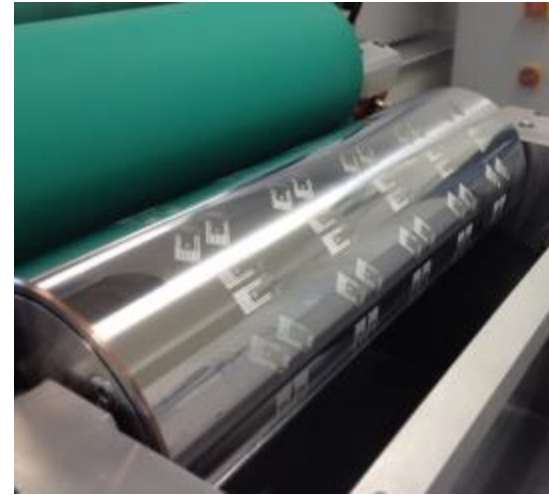
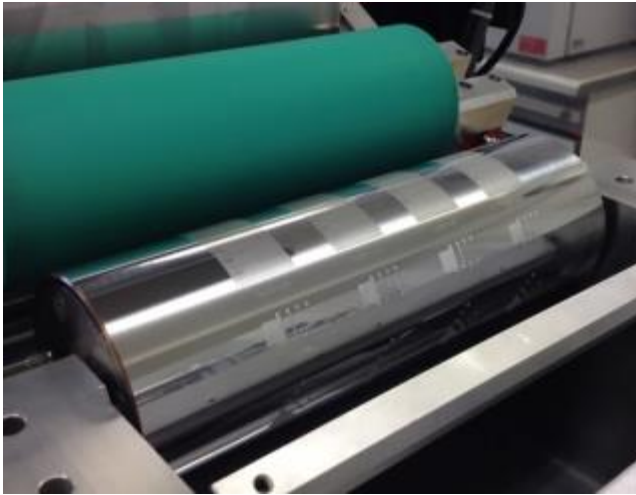
- Different common fabrication methods
- Electromechanical engraving and direct engraving in zinc
→ not suitable for printing of graphene or proteins
- Masking and chemical etching
→ strongly defined structures, high ink transfer (low electrical resistance)
- Direct engraving in copper with ultrashort pulse laser
→ microstructures < 10 μm



Directly structured dots
with diameter 8 μm

Fabrication of gravure printing cylinders

- First prototypes of graphene cylinders



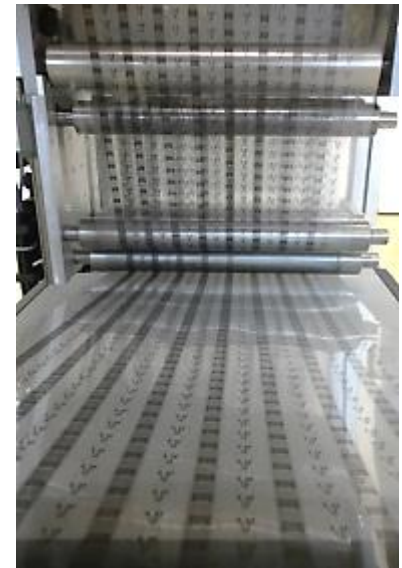
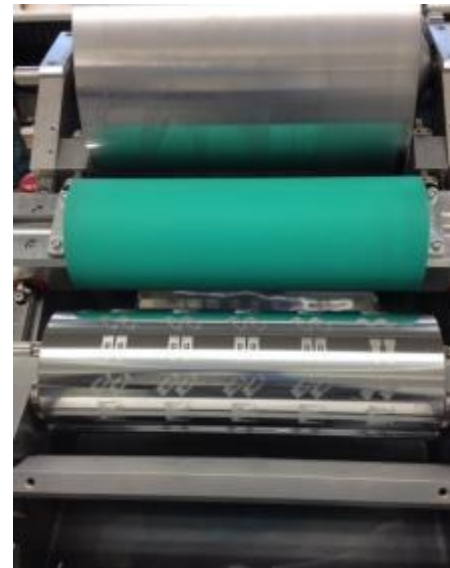
Etched IDE structure, line width 40 μ m

R2R gravure printing with graphene ink

- PET foil (50 μm , 300 mm)
- Corona activation (600 W)
- Ink volume: ~ 10 ml
- Speed: 25 m/min

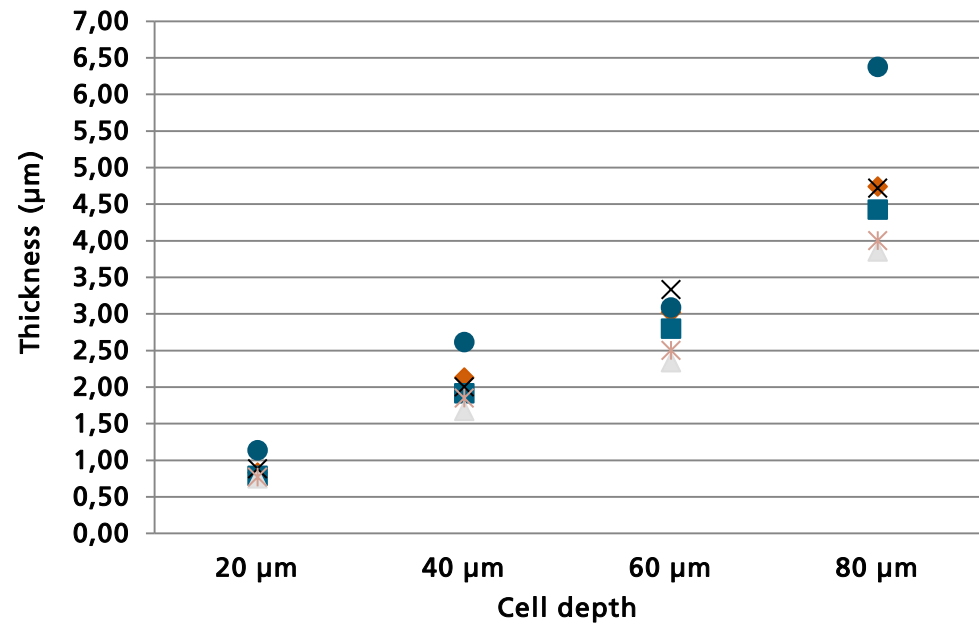
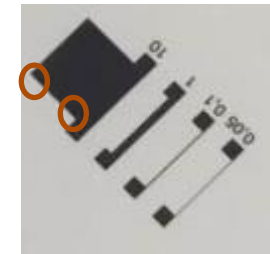
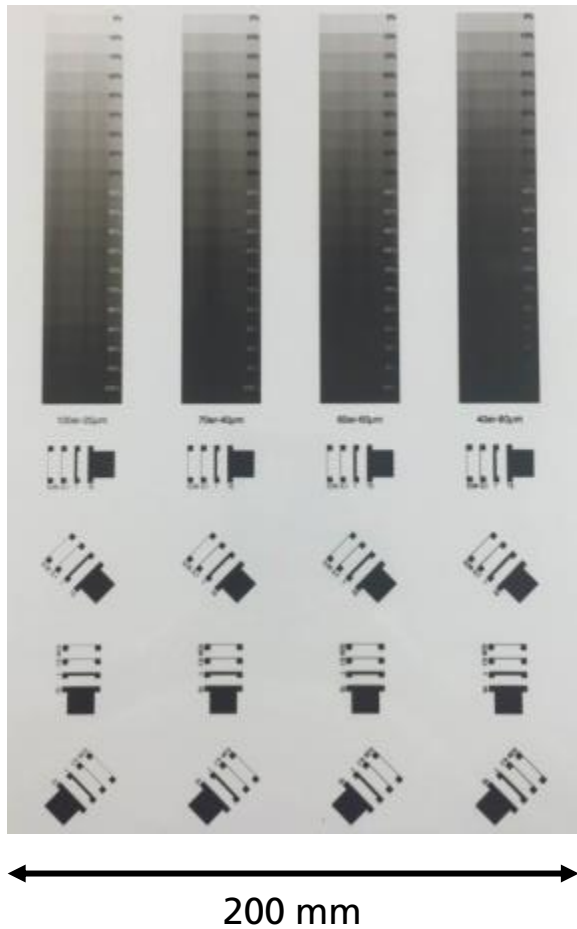


One-colour printing machine for flexo and gravure printing (FREE-1)



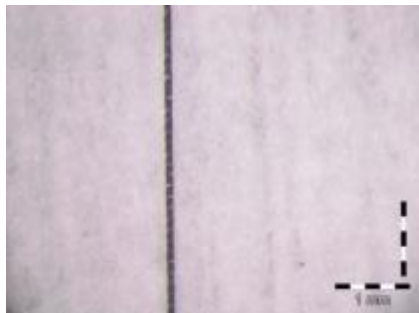
R2R gravure printing with graphene ink

- Printed graphene patterns with first test cylinder

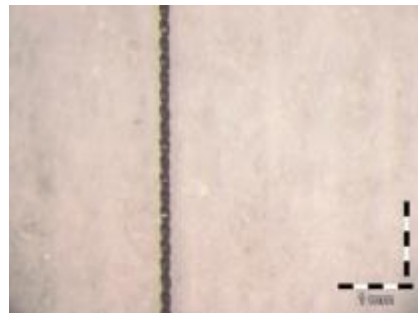


R2R gravure printing with graphene ink

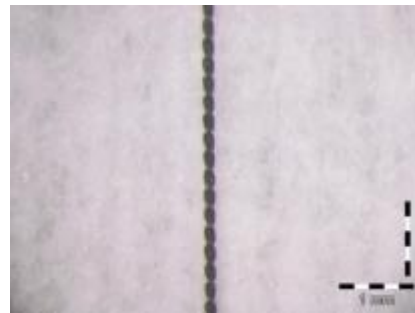
- Printed lines (width 0.05 mm and 1.0 mm), various cell depths



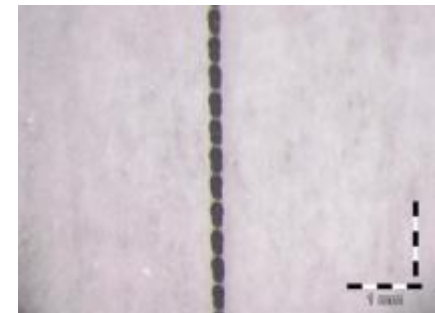
20 μm



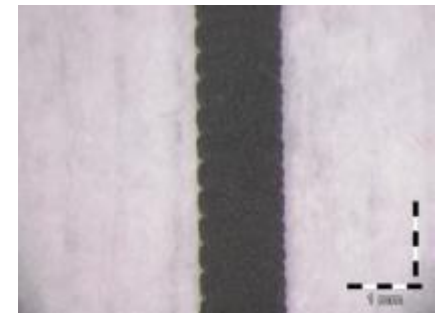
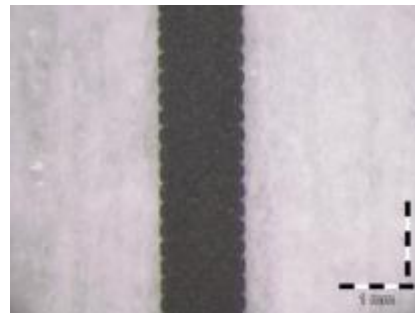
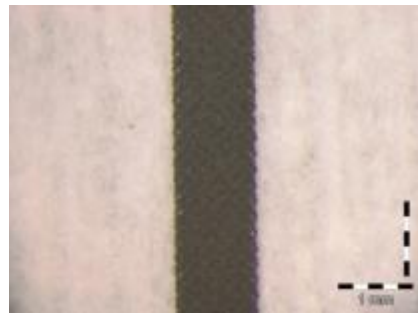
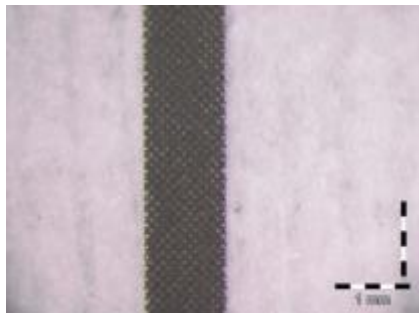
40 μm



60 μm

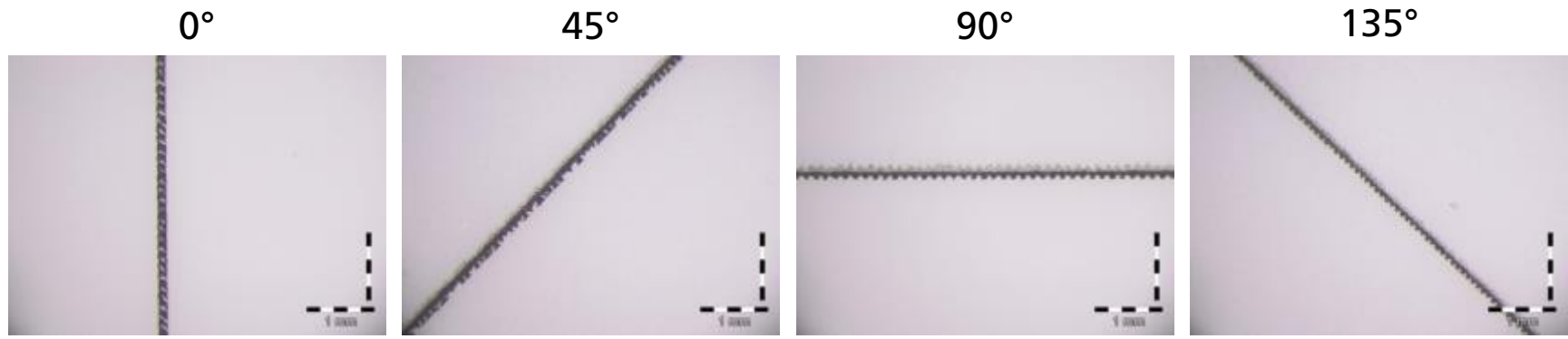


80 μm

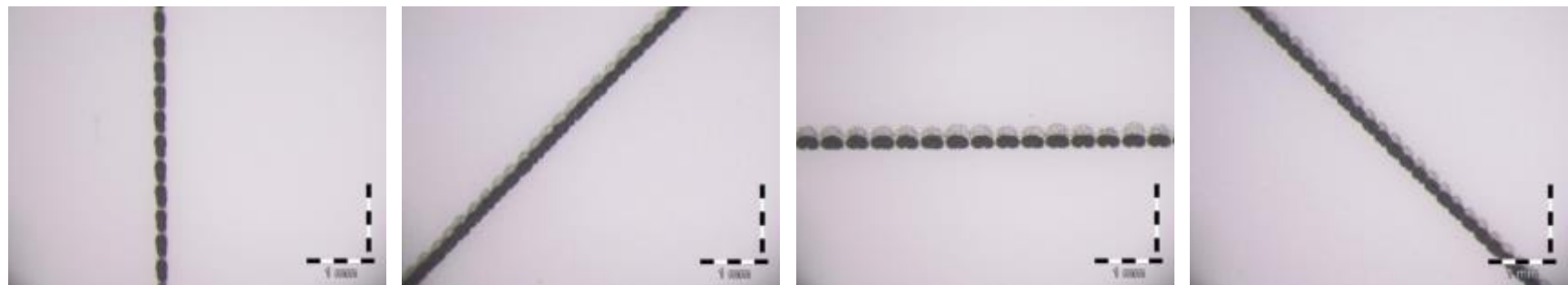


R2R gravure printing with graphene ink

- Orientation dependency (0.05 mm lines, different cell depths)



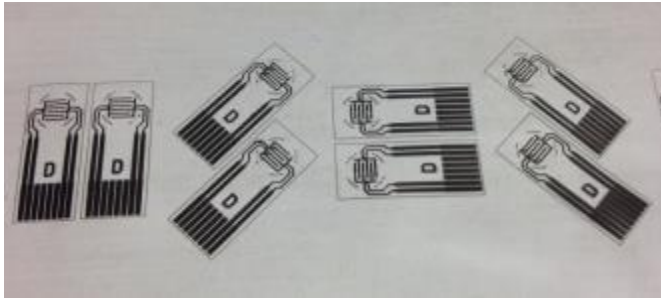
Cell depth 20 μm



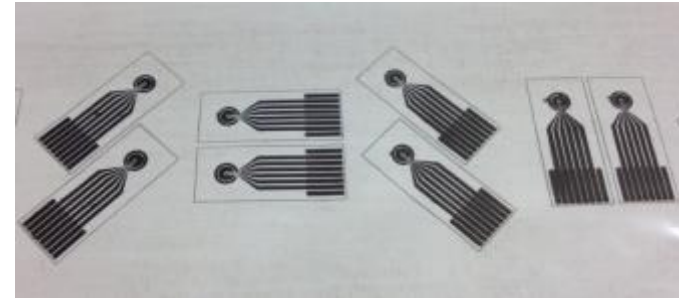
Cell depth 80 μm

R2R gravure printing with graphene ink

- Printing with first biosensor printing cylinder



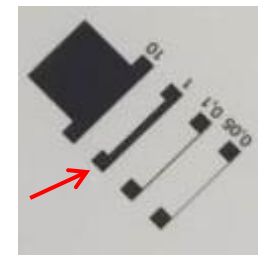
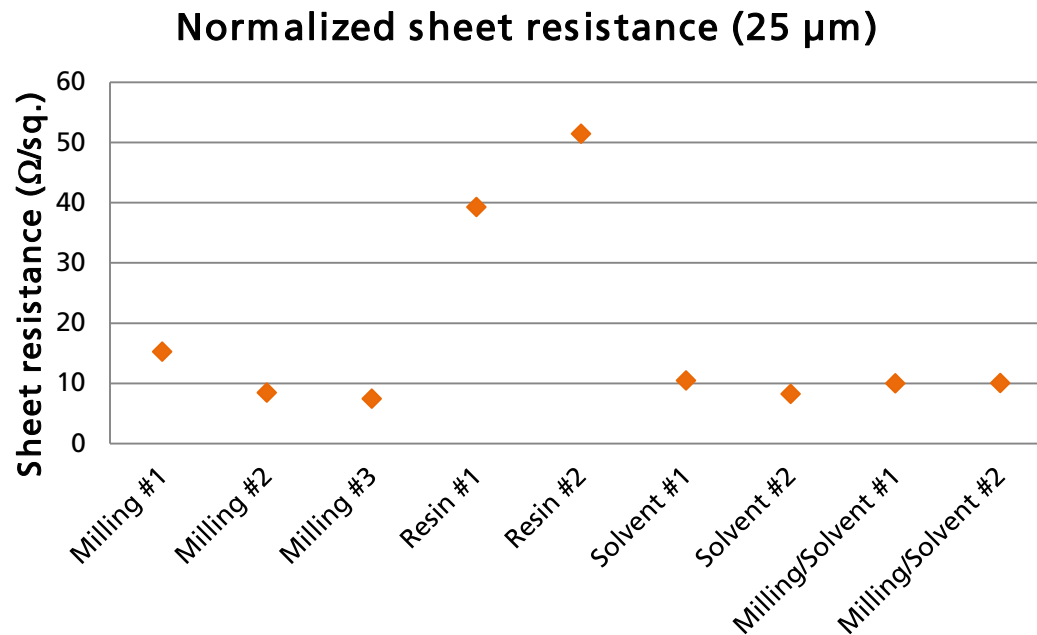
IDE structures
(nominal width 50 μm)



IDE structures
(nominal width 100 μm)

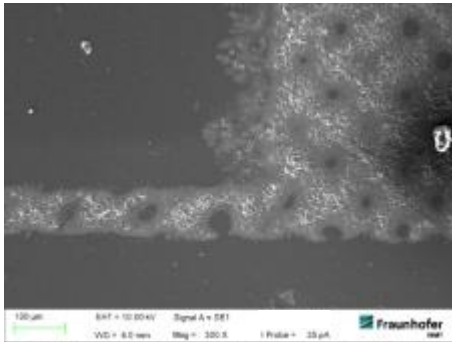
Electrical characterization

- Resistivity measurements
- Line width: ~1.05 mm, length: 10 mm
- Angles: 45° and 135°, cell depth: 60 μm
- Graphene ink thickness: ~ 4 μm

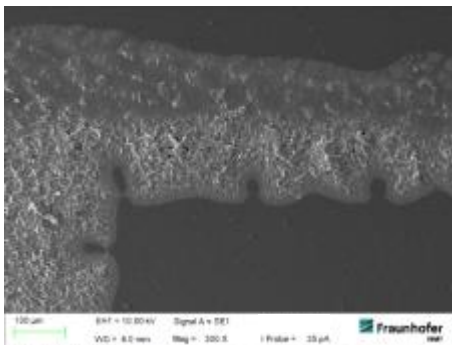
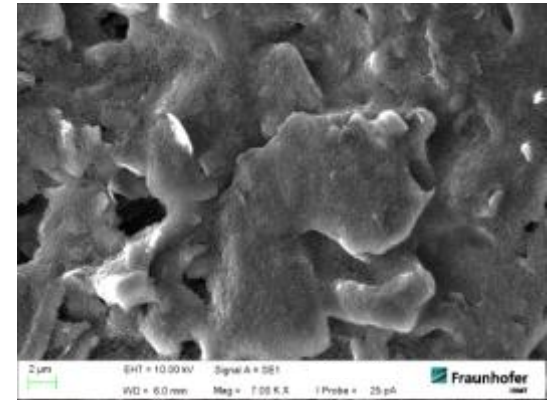
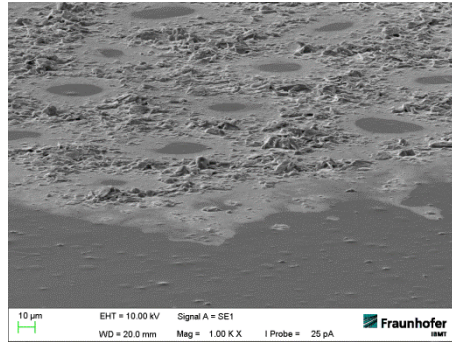


$$R_{sq.} = 10 - 20 \Omega/sq.$$

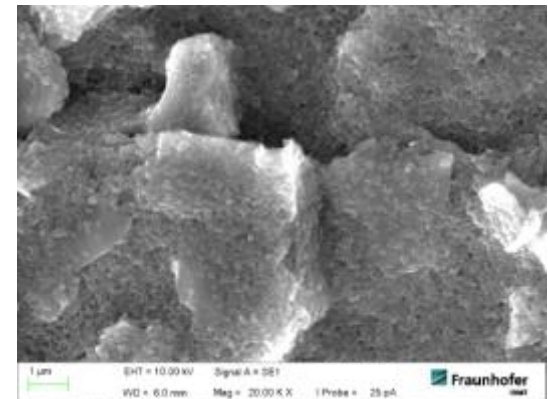
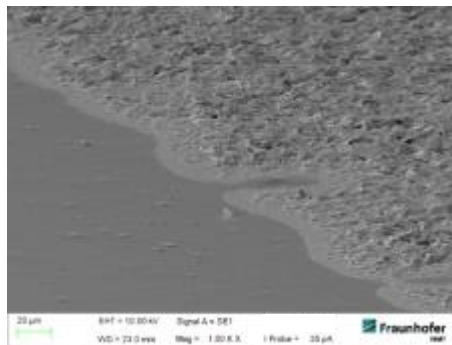
SEM images of graphene patterns



Cell depth 20 µm



Cell depth 80 µm



Cytotoxicity testing of graphene ink

- Samples printed on PET foil and sterilized by ethylene oxide (EO)

1. PET foil → reference (PET)

2. Graphene (pure)

3. Graphene + solvent 1

4. Graphene + solvent 2

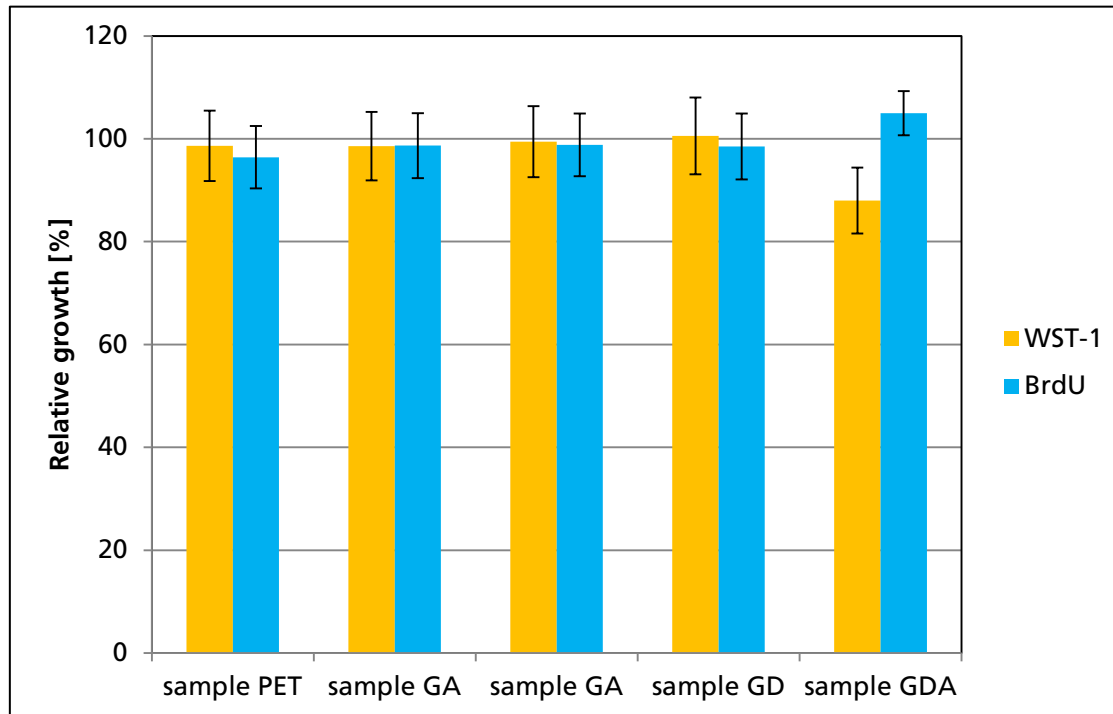
5. Graphene + solvent 3



10 mm

- Determination of cellular behaviour after exposure to testing substance
- Cells: human fibroblasts (MRC5)
- Following ISO 10993:
 1. Quantitative determination of the metabolic activity (WST-1)
 2. Quantitative determination of the rate of cell division (BrdU)
 3. Qualitative direct contact test (live/dead staining)

Indirect contact test WST-1 and BrdU

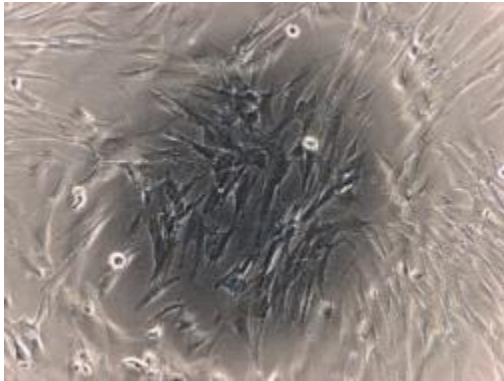


WST-1 = metabolic activity

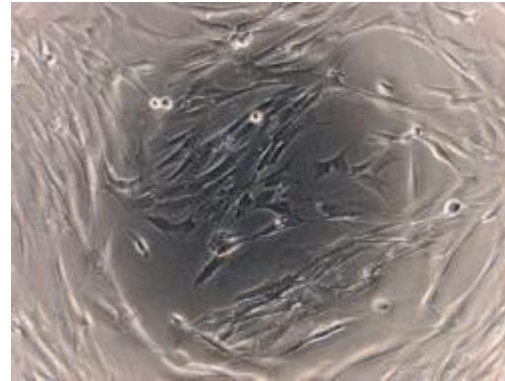
BrdU = cell proliferation

Cell morphology and direct contact test

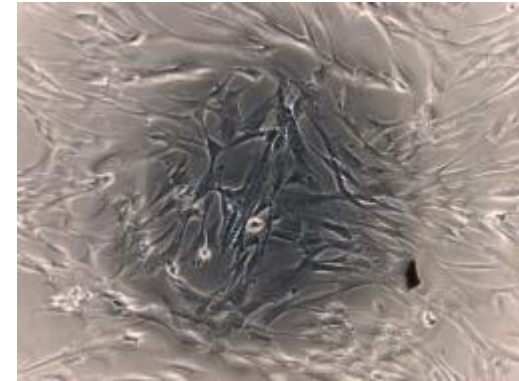
- Cell morphology after 24 h incubation



Control

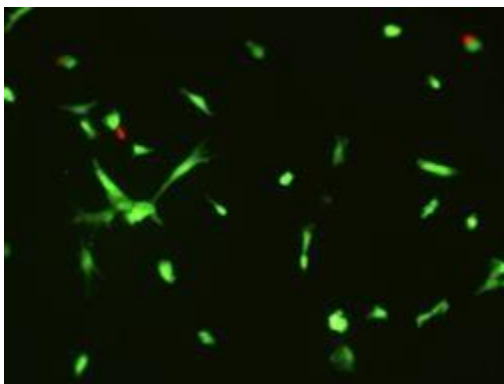


PET

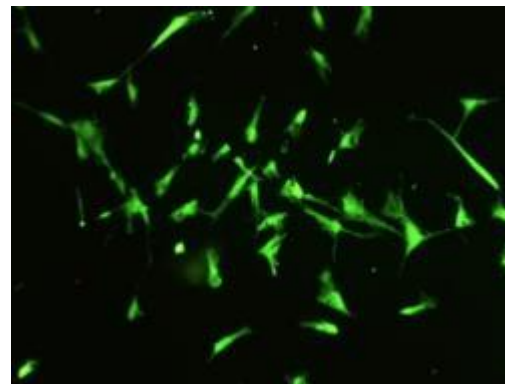


Graphene

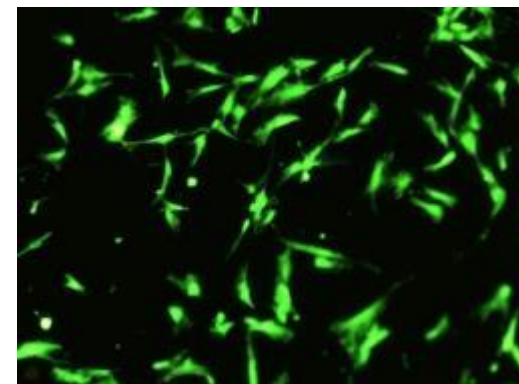
- Direct contact test after 48 h incubation



Graphene (pure)



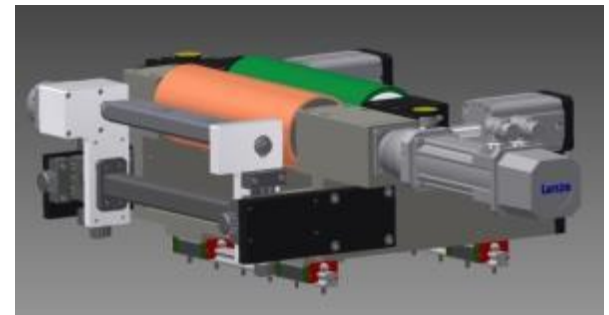
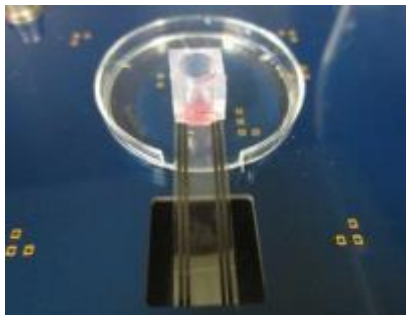
Graphene + collagen type IV



Graphen + matrigel

Summary

- Successful initial gravure printing with graphene
- Developed graphene ink is not cytotoxic
- Enhancement of cell adhesion by additional protein coating on graphene structures
- Next steps
 - Set-up of first biosensors for validation
 - Two-colour printing machine



Acknowledgements

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Thank you for your attention.
