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Dry native protein assays on nitrocellulose coated glass substrates by non-contact Laser-Induced-Forward Transfer process

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Background

To produce nano-scale native protein trehalose patterns on substrates in constant concentration by Laser-Induced-Forward Transfer (LIFT), ultrathin coatings on LIFT targets are needed [1]. The LIFT process is used as a non-contact method to transfer protein patterns on substrates at a distance of 100 µm to 300 µm. Biochips generated as biosensors can be spotted with variable protein combinations in different concentrations. In this way protein based biosensor assays can be achieved by selected cell adsorption. Thin trehalose layers with embedded proteins like green fluorescent protein (GFP), streptavidine and the extra cellular matrix (ECM) protein laminin type1 were generated by a spin-coating technique on titanium coated targets. In the case of 2D biochips, combined protein types with different concentrations can be arranged. Robustness against long term storage is a key feature for applications, which needs multi-protein spotting (sensors; 3D cell cultures). To examine the protein stability the protein coatings were exposed to long term experiments [2,3]. Coating thickness was examined by means of ellipsometric spectroscopy on a statistically relevant number of samples [4]. During the LIFT process, the proteins are exposed to both mechanical and heat stress. Thus, subsequent tests are mandatory to grant the activity of the proteins after the transfer process. Our main focus is to examine the quality of the micro structured coatings transferred by the LIFT process using analysis by AFM and protein activity tests [3,4,5]. Activity tests are either done by application of a fluorescence scan (GFP and streptavidine with labeled biotin) or cell adhesion tests on transferred laminin type1 with fibroblasts .

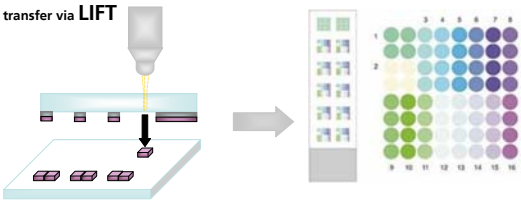
Aim

Our objective is to develop homogenous protein coatings containing native proteins of each configuration, which can be used to be patterned on 3D or 2D substrates via non-contact Laser-Induced-Forward Transfer devices.
Furthermore, we evaluate the patterned substrates applying a biosensor protein microarray, consisting of ECM protein laminin type1 for a selective fibroblast cell adsorption.

Protein printing via LIFT

Motivation

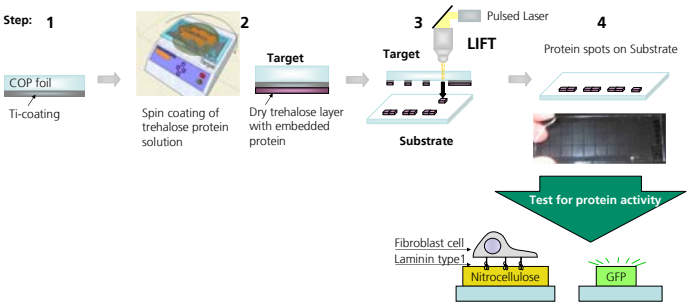
Non-contact transfer via **LIFT**



No cartridge – very Low volume of protein solution needed

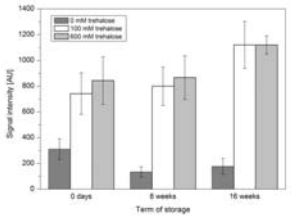
- New concentration compositions of different proteins
- New biosensors
- 3D cell culture

Principle workflow



Results – Robustness of proteins against drought stress

Long-term storage of streptavidine during drought stress with addition of trehalose

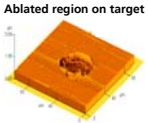


- Trehalose builds hydrogen bonds with proteins in drought stress and protects from denaturation.
- Trehalose = disaccharide can be found in various plants, mushrooms, hemolymph

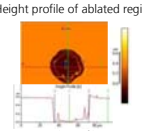
Analysis after LIFT process

AFM analysis

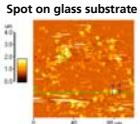
Ablated region on target



Height profile of ablated region



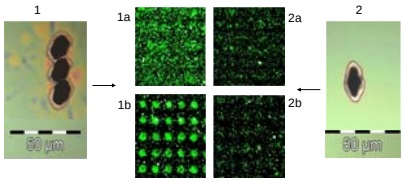
Spot on glass substrate



Results

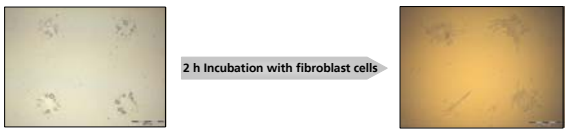
- Laser power: 6 mW
- Target
  - Homogenous protein coating
  - Ablated region:
    - Diameter 20 µm
    - Observable scope
- Height profile:
  - Sharp edges
  - Powerful ablation
  - Crater depth Ø 0.56 µm
- Opposite substrate
  - Spot well defined
  - Diameter 80 µm

LIFT transfer of active GFP



1 and **2:** Microscope images of a target. The biological layer consisted of native self-luminescent protein GFP (1 mg mL-1) and trehalose (600 mM).
1a-2b: Fluorescence scan images of an acceptor glass substrate
1: Three laser shots per protein spot (3S/S)
2: One laser shot per protein spot (1S/S)
1a: 3S/S, laser power of 10 mW, **1b:** 3S/S, laser power of 5 mW, **2a:** 1S/S, laser power of 10 mW, **2b:** 1S/S, laser power of 5 mW.

Selective adhesion of fibroblast cells on transferred laminin type1

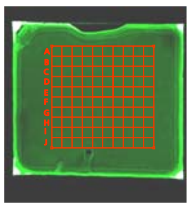


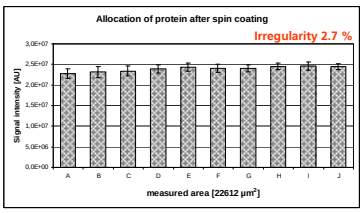
LIFT transferred laminin type1 spots on glass substrate

Selective cell adhesion on the protein spots

Homogenous protein films via spin-coating

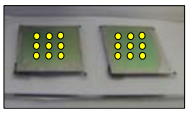
Homogenous allocation of GFP molecules within the trehalose coating



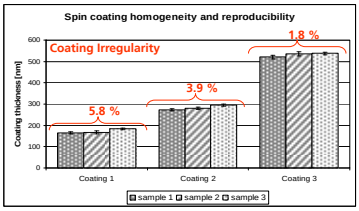


- Protein type: green fluorescent protein (GFP)
- Rotational speed: 3000 rpm
- Detection: fluorescence scan - quantitative analysis

Analysis of coating homogeneity and reproducibility via spectroscopic Ellipsometry



Measuring point



- ✓ Homogeneity
- ✓ Reproducibility

Conclusions

- ✓ Production of dry protein trehalose coating with long-term stabilizing character on a planar target
- ✓ Creation of reproducible homogenous ultrathin protein coatings by spin coating
- ✓ Transfer of native protein patterns on the opposite substrate via LIFT

Next steps

- ➢ Decrease of satellite fragments between the spots
- ➢ Design of a Compact Disc with various proteins to create multi type protein microarrays on biochips

References

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Acknowledgement

The authors would like to thank Eva Hoch and Julia Maierle for lab work, Joachim Mayer for AFM images and BMWI focus on INNONET (grant ID 16IN0491) and Fraunhofer Gesellschaft state Baden-Württemberg for financial support.