

## ELECTRONICALLY ADDRESSABLE POLYELECTROLYTE COATINGS AND THEIR POTENTIAL FOR CELL SHEET ENGINEERING

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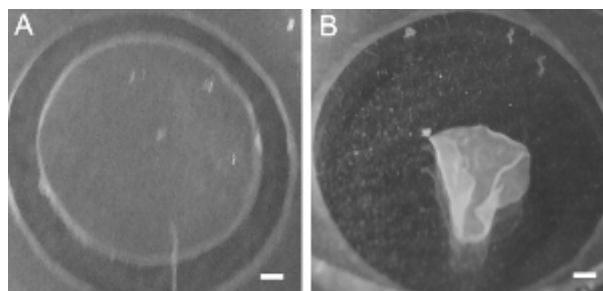
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**INTRODUCTION:** In the field of regenerative medicine, cell sheet based tissue engineering holds great promises for the construction of three dimensional functional tissues. In contrast to the conventional tissue engineering approaches, this technique allows for the fabrication of cell-dense structures with preservation of critical cell surface proteins. The technique has been initially developed using thermo-responsive polymer-grafted dishes that enable cell detachment upon temperature reduction. As an alternative, we developed a novel method for cell sheet recovery based on highly tunable, electrochemically addressable surfaces.

**METHODS:** Biodegradable mono- or multilayer films based on poly(L-lysine), poly(L-glutamic acid) and hyaluronic acid were built on an indium tin oxide semi-conductor substrate by a simple dip and rinse coating process. The layer-by-layer buildup [1] and the dissolution of the polyelectrolyte films upon electrochemical polarization of the substrate [2] were followed by electrochemical optical waveguide light mode spectroscopy [3]. For cell sheet detachment by electrochemical polarization, the electronically addressable surfaces with confluent cell monolayers were subjected to an external potential, using the indium tin oxide substrate as a working electrode in a three-electrode configuration system.

**RESULTS:** Various biocompatible polyelectrolyte mono- and multilayers were built up, and optionally bio-functionalized by deposition of fibronectin onto films ending with a positively charged polymer layer or by the adsorption of RGD-modified poly(L-lysine)-*graft*-(polyethylene glycol) onto films ending with a negatively charged polymer layer. When seeded at high densities onto coatings consisting in 1 to 6 layers of poly(L-lysine) and poly(L-glutamic acid) or hyaluronic acid, confluent osteoblasts and fibroblasts detached as cell sheets without the need for any external stimulus. In contrast, the cells grown onto PLL-*g*-PEG/PEG-RGD monolayers under similar conditions as well as the cells seeded at lower densities did not spontaneously detach. In those cases, the cell sheets could be recovered by

subjecting the substrate to electrochemical polarization (Fig.1).



*Fig. 1: Cell sheet recovery by electrochemical polarization: monolayer of confluent fibroblasts grown onto ITO-PLL-PGA-PLL-*g*-PEG/PEG-RGD before (A) and after (B) the application of an external potential. Bars are 1mm.*

**DISCUSSION & CONCLUSIONS:** These biocompatible polyelectrolyte coatings allowed for non-invasive cell sheet harvesting by fine-tuning of the bio-interface or by their use as electronically addressable sacrificial layer. Both strong cell-cell cohesion and weak cell-substrate interactions are probably required for the spontaneous cell sheet detachment. While the cell type and the cell density influence the cell-cell cohesion, the highly tunable properties of polyelectrolyte multilayers allow for a fine control of the cell-substrate interactions. In cell sheet recovery by electrochemical polarization, a local pH change leads to the dissolution of the polyelectrolyte coating, and the applied current and the buffer capacity determine the confinement of this pH change. These easy-to-build substrates are applicable to any surface geometry and offer a promising new tool for cell sheet-based tissue engineering.

**REFERENCES:** <sup>1</sup> G. Decher, J.D. Hong, et al (1992) *Thin Solid Films* **210/211**:831-835. <sup>2</sup> F. Boulmedais et al (2006) *Advanced Functional Materials* **16**: 63-70. <sup>3</sup> J.P. Bearinger et al (2003) *Biotechnology and Bioengineering* **82**:465-473.

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