

## Engineering and Differentiation of Stem Cell Sheets

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**INTRODUCTION:** Cell sheet engineering has emerged as a novel technique for creation of artificial, three-dimensional model tissues without the need for biodegradable scaffolds. Others could show that cell culture on temperature-responsive polymer films allows non-enzymatic harvesting of intact cell sheets, which can be further surgically transplanted for tissue reconstruction [1,2]. We have begun to explore new methodology that builds on the use of electric charge to accomplish non-enzymatic harvest of cell sheets. For this purpose, we developed polymer electrolyte films, and tested whether they would perform as substrates for growth of human stem cells effectively.

**METHODS:** We explored charge-sensitive arrangements of polyelectrolyte multilayer films (PEMs) that were coated on the conductive surface Indium Tin Oxide (ITO). The PEM layers were obtained by piling up to 8 alternating layers of poly-L-lysine (PLL) and hyaluronic acid (HA). The top layers, either PLL or HA, were coated with fibronectin, or alternatively we used of PLL-PEG-RGD as cell-adhesive surface. The studies were performed with human placenta-derived mesenchymal stem cells (HPMSC) that we isolated from chorionic villi. The cells were seeded at 15000 cells/cm<sup>2</sup>.

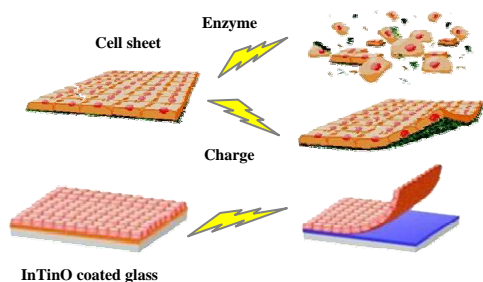


Fig. 1. Working hypothesis

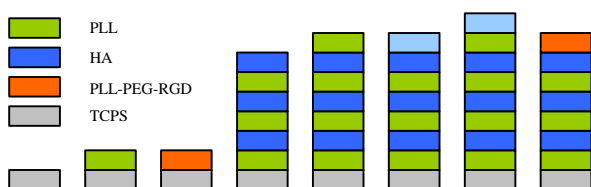


Fig. 2 PEM surfaces under this study

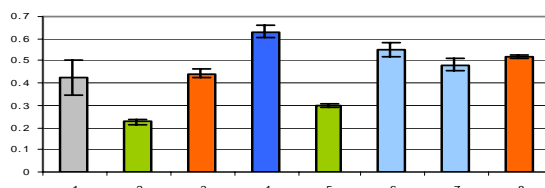
**RESULTS:** Cells were seeded on PEM films and cultured for 48hrs, then examined for morphology (phase microscopy), viability (live/death stain) and vitality (WST-assay). Stratification of nine different surfaces revealed following principal requirements [I] HPMSC adhered and grew out on [HA] but not on [PLL] surfaces [II] Display of fibronectin of upper layer of [PEM] is necessary to mediate efficient adhesion and outgrowth of HPMSC.



A: Crystal Violet Staining of Cell Sheets



B: Viability - Live/Death Staining



C: Vitality - WST-1 Test

Fig. 3. Representative results of positive and negative outgrowth of HPMSCs on PEM surfaces

**DISCUSSION & CONCLUSIONS:** We showed that the charge-sensitive arrangements of PEM can serve as a substrate for confluent layers of adult human mesenchymal stem cells. Future directions of this research are to evaluate whether stem cells grown on PEMs can be stimulated to differentiate into chondrocytes, osteocytes or cardiomyocytes *in vitro*, and whether differentiated sheets can be collected in intact form.

**REFERENCES:** <sup>1</sup> T. Okano, et al. (2005) Biomaterials 26: 6415-6422. <sup>2</sup> Y. Miyahara, et al. (2006) Nature Medicine 12, (4): 459-465.

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