Proceedings of German Society for Stem Cell Research (PGSSCR)

Engineering and differentiation of stem cell sheets

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Published online on 16 May 2007

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 mesenchymal stem cell sheets, which can be further surgically transplanted for tissue reconstruction (Miyahara et al. Nat Med 2006; 12:459-65). We have begun to explore new methodology that builds on the use of electric charge to accomplish non-enzymatic harvest of cell sheets.

Materials and Methods:

We used charge-sensitive arrangements of polyelectrolyte multilayer films (PEMs) that were obtained by piling up to 8 alternating layers of poly-L-lysine (PLL) and hyaluronic acid (HA). Some PEM layers were additional formed with a top layer of PLL-PEG-RGD or adsorbed fibronectin. Tests were performed with placenta-derived mesenchymal stem cells (HPMSC). The cells were cultured for 48hrs, then examined for morphology (phase microscopy), viability (life/death stain) and vitality (WST-assay).

Results:

Stratification of nine different surfaces revealed following principal requirements (1) HPMSC adhered and grew out on HA but not on PLL surfaces (2) Fibronectin precoating, or alternatively, PLL-PEG-RGD layers, are necessary to mediate efficient adhesion and outgrowth.

Discussion and Conclusions:

These are first steps into development of new technology for cell sheet engineering. We showed that the charge-sensitive arrangements of PEM cell do indeed function as substrates for human mesenchymal stem cells. Next steps are whether stem cell sheets grown on PEMs can be differentiated prior to transplantation which is under current investigation.