Evaluating the Influence of Cell-Substratum Adhesivity on Human Mesenchymal Stem Cell Neural Differentiation

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Statement of Purpose: The influence of the polymeric substrate on stem cell differentiation has not been studied extensively. We examined a library of structurally related polymeric substrata varying in their adhesivity to cells.[5] Human mesenchymal stem cell (MSC) differentiation along the neural lineage was conducted on polycarbonates, poly desaminotyrosyl-tyrosine ethyl ester (DTE) carbonate, desaminotyrosyl-tyrosine octyl ester (DTO) carbonate, and DTE-co-5% polyethylene glycol (PEG) carbonate (E-P). These polymers are increasingly hydrophobic with E-P being the most hydrophobic or anti-adhesive.

Methods: Polymers were prepared by solvent casting and spin coating for 96-well and 24-well culture plates, respectively, adapted from earlier techniques.[1] The relative wettability was determined by air-water contact angle measurements of the polymer surface. The wells were pre-conditioned with control media (CM), which is 20% fetal bovine serum (FBS), 1% antibiotic antimycotic, and low-glucose Dulbecco's Modified Eagle Media (DMEM). MSCs were isolated from human, whole bone marrow, purchased from Cambrex Inc. and obtained by previously described protocol.[2] Cryopreserved MSCs were thawed and seeded at 4000 cells/cm^2 in all polymer surfaces and cultured in CM for 24 hours. The media was replenished with pre-induction media followed by the induction media every 24 hours adopted from previous protocol.[3] Neuron specific enolase (NSE) and cell proliferation was determined by immunostaining and Picogreen ® dsDNA reagent at 24 hours after induction, respectively. One-way analysis of variance (ANOVA) and Tukey-Kramer test were used to determined the statistic significance between the groups ($p<0.05$).

Results / Discussion: The air-water contact angle values were 69.7, 80.7, and 85.1 for poly DTE, DTO, and E-P carbonate respectively. The E-P copolymer was the most hydrophobic surface and DTE carbonate was the most hydrophilic surface. The percentage of neuron-like cells on the polycarbonates was negatively correlated to the polymer surface air-water contact angle measurement (Figure 1). Cell number in the control media was generally higher and was also negatively correlated to contact angle. Cells grown in the induction media had similar cell numbers although the least number of cells was present on E-P carbonate ($p<0.05$). 24 hours after induction, the MSC in the induction group on all polymer substrates showed neuron-like morphology and also NSE expression (Figure 2). The spreading of MSCs on polymer surfaces was least on the E-P carbonate and most on the DTE carbonate.

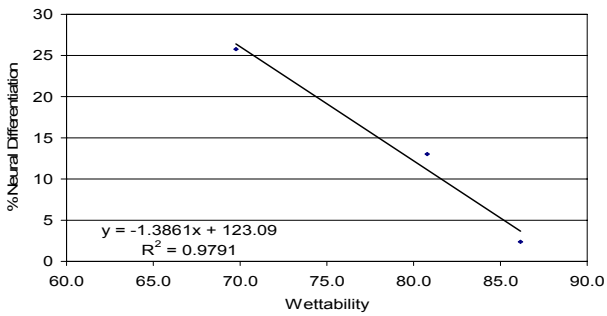

Figure 1: Percentage of neural differentiation vs. wettability for polycarbonates. DTE carbonate yielded highest percentage of differentiation where the copolymer yielded the lowest ($p<0.05$).

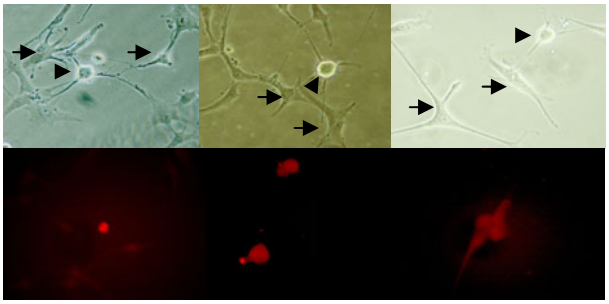

Figure 2: 24-hour post-induction morphology and fluorescent images(x40). From left to right: DTE, DTO, E-P carbonate. Arrow head and arrow indicates neuron-like and immature neuron-like morphology respectively.

Conclusions: Cell proliferation on polymers such as polycarbonates is significantly dependent on the wettability of the surface[1,4] and exhibited negative correlation between cell number and air-water contact angle measurements (Figure 1), suggesting most hydrophobic polycarbonates are a less stimulating substrate for MSC proliferation and neural differentiation. On 5% PEG surfaces, MSCs, like other cell types, tended to aggregate rather than spread out[5], signifying cell-cell cohesion due to decreased cell substrate adhesivity decreases neuronal differentiation of MSCs.

References:

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