Osteogenic Differentiation of Human Mesenchymal Stem Cells on Tyrosine Derived Polycarbonates

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Statement of Purpose: The potential for regenerative medicine using adult human mesenchymal stem cells (hMSCs) is promising, especially in the area of orthopedics. With the advent of a combinatorial library of well-characterized tyrosine derived polymers, one could take advantage of the known surface properties to tailor a specific response from stem cells. Tyrosine derived polycarbonates are a new generation of biomaterials that have been proven to be biodegradable and non-toxic (1). Tyrosine derived polycarbonates possess the same backbone, however differ in the alkyl ester pendent chain length. Tyrosine derived polycarbonate polyethylene glycol (PEG) copolymers has an additional PEG group in its backbone that has been shown to have a negative effect on cell attachment yet providing increased cell motility (2). In this study we used poly(DTE carbonate), poly(DTO carbonate) and poly(DTE co 5% PEG 1K carbonate) which all differ in their surface chemistries through small changes in the pendent chain length and backbone, resulting in increasing levels of hydrophobicity and lower cell adhesion, respectively. The purpose of this study was to determine whether small changes in the surface chemistries of these polymers have an effect on hMSC osteogenic differentiation, cell adhesion and motility.

Methods:

Polymer Fabrication: The polymers were synthesized based on previously published protocols (3). Polymer films were prepared by solvent cast method.

Cell Culture: hMSCs were isolated from human, whole bone marrow obtained from the iliac crest of anonymous donors. The cells were cultured in Dulbecco's Modified Eagle Medium containing 10% Fetal Bovine Serum and 1% Antibiotic-Antimycin. For osteogenic induction (OS), cells were cultured in DMEM containing 10 mM of beta-glycerophosphate, 50 µM of ascorbic acid, and 100 nM of dexamethasone.

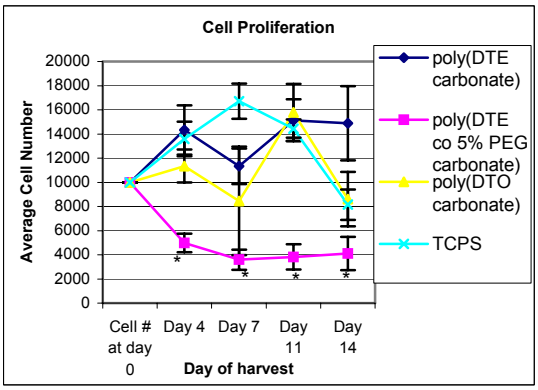
At day 0 of the experiment, 10,000 cells were seeded into each well (n=4). After 24 hours, cells were induced in OS medium. Cells were harvested at days 4, 7, 11 and 14 for cell proliferation, alkaline phosphatase activity and calcium production.

Cytoskeleton Observations: At day 0, 10,000 cells were seeded into solvent cast 96 well plates containing the polymers. Cells were induced in OS medium after 24 hours. Cells were fixed and stained with phalloidin stain at timepoints: 1 hour, 5 hours, 24 hours, 4 days, 7 days, 11 days and 14 days.

Cell Motility: Cells were seeded onto spin coated disks at a low seeding density. Cell speed was measured by time-lapse photography of the cultures using a confocal microscope based on methods previously published (2).

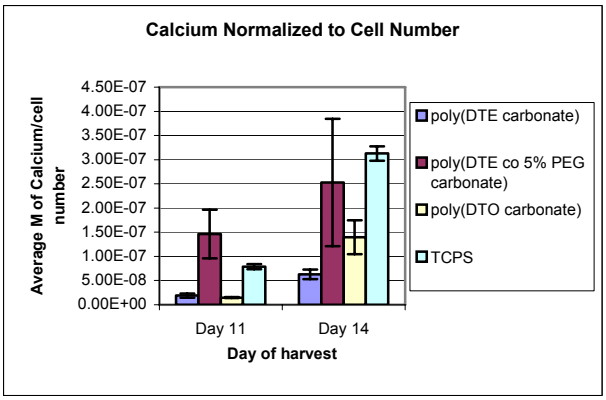
Results / Discussion:

Cell proliferation was measured with PicoGreen ds DNA assay. Tissue culture polystyrene (TCPS) was used as a control. It is evident that poly(DTE carbonate) and poly(DTO carbonate) support cell growth. However, cell proliferation is not supported on poly(DTE co 5% PEG 1K carbonate) and is significantly different from all other polymers including TCPS ($p<0.05$) as indicated by the asterisks.



Cells on poly(DTE co 5%PEG 1K carbonate) had significantly lower alkaline phosphatase activity compared to the other materials (results not shown).

Calcium production was normalized to cell number. Unlike cell proliferation and alkaline phosphatase activity, cells on poly(DTE co 5% PEG 1K carbonate) produced more calcium compared to all other polymers.



Conclusions:

Cell attachment is a function of the surface chemistry of polymers and therefore has an effect on cell differentiation, cytoskeleton arrangement and cell motility. A pronounced effect was observed with polycarbonate PEG-ylation. Cells proliferated less yet continued to differentiate and produce a significant mineralized ECM. Cells on poly(DTE co 5% PEG 1K carbonate) clustered and formed discrete aggregates whereas cells on the other polymer surfaces formed a spread out, confluent layer. Based on studies performed, this data suggests that the interplay between cell-cell adhesion and cell-substratum adhesion directed by polymeric surfaces can modulate stem cell differentiation.

References:

1. (Bourke S., ADR, 2003, 55:447-466)
2. (Tziampazis E., Biomaterials, 2000, 21:511-520)
3. (Ertel S.I., JBMR, 1994, 28: 919-930)