HUMAN ARTICULAR CHONDROCYTE GROWTH AND GENE EXPRESSION PATTERNS ARE MODULATED BY SUBSTRATA FOR CELL TRANSPLANTATION

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Introduction
Engineered tissues have the potential to provide treatments for patients suffering from the loss of tissues or their function. In tissue engineering strategies for cartilage repair, chondrocytes isolated from biopsy tissue can be either seeded directly onto biodegradable polymer carrier scaffolds, or expanded in culture in vitro prior to seeding onto scaffolds. The resulting cell-material constructs are then implanted back into the defect site with the desired goal to mimic the important properties of cartilage, thereby enabling better functionality. In order to understand the effects of selecting certain materials for such cell transplantation therapies, it is crucial to understand the cell-material interactions that occur during this process, from cell attachment to downstream signal transduction events.

Segmented copolymers of poly(ethylene glycol) terephthalate (PEGT) and polybutylene terephthalate (PBT) have been shown to be biocompatible in vivo and in vitro. The overall copolymer properties are determined by its two components – the PEG segment is hydrophilic and provides hydrogel-like properties, whereas PBT provides hydrophobic, protein-binding domains and stiffness to the system. During synthesis, the molecular weight of PEG and weight ratio of the PEGT/PBT components can be defined to allow the copolymer to be tailored for specific surface and mechanical properties, as required for individual applications. PEGT/PBT co-polymers have been evaluated for dermal and bone repair applications. We have shown in a previous study that a series of PEGT/PBT substrata were able to differentially support chondrocyte attachment, growth and maintenance of morphology. Therefore, not only are these interesting materials for cell transplantation applications, but they constitute a useful system to study the cellular effects of controlled variations in substrate chemical properties.

The objective of this study was to (1) compare the effects of varying PEG molecular weight and PEGT/PBT ratio on the attachment, proliferation and phenotypic characteristics of non-osteoarthritic primary human chondrocytes.

Materials and Methods
Human articular cartilage was harvested from the articulating surface of the femoral head of a 70 year old female patient undergoing hip-replacement surgery. Chondrocytes were harvested by 22 hour digestion in collagenase type II at 37 °C, and were seeded at a density of 10,000 cells/cm² on non-porous films of each of the following compositions:

(i) PEG mol wt=1000 g/mole, 70% PEG (1000PEGT70/PBT30) → hydrophilic
(ii) PEG mol wt=300 g/mole, 55% PEG (300PEGT55/PBT45) → hydrophobic

Tissue culture polystyrene (TCPS) was used as a control.

Cells cultivated on the polymer substrata were analyzed for attachment (Day 1), and proliferation (Day 19) by Cyquant assay for total DNA content. Other cultures were analyzed by quantitative real time RT-PCR for mRNA expression of type II and I collagen (CII and CI). 18s ribosomal RNA expression was utilized as a housekeeping gene. To compare differentiated versus dedifferentiated chondrogenic phenotypes, we calculated the ‘Differentiation Index’, which is the ratio of CII/CI mRNA expression.

Chondrocyte morphology was also observed by scanning electron microscopy (SEM) after cellular fixation in Karnovsky’s fixative.

Results
Cell attachment increased with increasing surface hydrophobicity (Fig. 1). The total number of cells at Day 19 also increased with surface hydrophobicity but this was not observed when cell proliferation was calculated as per the number of cells that actually attached (Fig. 2). Chondrogenic phenotype was lost as substrate hydrophobicity increased. Cell spreading (not shown) coincided with decreased chondrogenic phenotype (Fig. 3).

Discussion
Decreasing PEG molecular weight and PEGT content causes increased cell attachment, although once cells had attached, proliferation was independent of substrate composition. Cell attachment, spreading and proliferation leads to differential downstream gene expression of markers characteristic of the differentiated, and dedifferentiated, chondrogenic phenotype. This is a useful model system by which the effects of controlled variations in substrate composition on cell function can be examined. These differences are also important when selecting materials for cell transplantation applications.

References