

INTERACTION OF HUMAN OSTEOBLASTS WITH PHOSPHORYLCHOLINE POLYMERS

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INTRODUCTION: It has been a long-term objective of biomaterials research to develop materials, which can interact with cells and living tissue to replace, repair and enhance biological function. Clearly biomaterials must be thoroughly investigated prior to any clinical use. Although in-vivo studies are essential for a complete picture of healing processes around implants, in-vitro studies are also valuable and can provide answers to questions on cell attachment, proliferation and differentiation on biomaterials. In this study the interaction of primary human osteoblasts (Hobs) with a phosphorylcholine (PC) polymer, containing 20% cationic charge, was investigated. PC polymers are made of hydrophilic PC-based groups and hydrophobic groups such as alkyl methacrylates. They can closely mimic the structure of the naturally occurring cell-surface biomembrane lipids and possess excellent biocompatibility. It has been shown that incorporation of cationic groups into PC polymers increases protein adsorption and cell attachment.

MATERIALS AND METHODS: 25 ml tissue culture flasks were coated with a 1wt% ethanolic solution of PC (designated PC20) polymer and cured at 72°C for 72 hours. Uncoated tissue culture flasks were used as controls.

Hobs were seeded into flasks at a density of $1.4 \times 10^4/\text{cm}^2$ and cultured in McCoy's medium containing 10% foetal calf serum, 1% glutamine and 30µg/ml vitamin C. Cultures were treated with hydrocortisone, in a water soluble cyclodextrin encapsulated form, to stimulate cell differentiation or β-cyclodextrin as a control (HC and DC respectively). Images of cells on PC and control surfaces were captured using an optical microscope and 3-chip colour camera connected to a computer with frame grabber and image analysis software.

Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to measure the cellular expression levels of alkaline phosphatase (ALP) messenger RNA (mRNA). Total RNA was isolated from adherent cells at 12, 24, 36, 48 and 168 hours, following seeding, using phenol/chloroform. PCR was performed using a GeneAMP 5700 Sequence Detection System. mRNA levels for ALP was measured relative to that of the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RESULTS: Cell adhesion and spreading on the surfaces began about 1 hour after seeding. By 24 hours in culture most cells were fully spread on both PC20 and control surfaces. At the 48 hour time point some mineral deposits were visible on PC20 (Figure.1). The mRNA level for ALP decreased with time in all cultures, however it remained higher in those treated with hydrocortisone compared to those exposed to β-cyclodextrin. There were no significant differences in ALP gene expression between cells on PC20 and control substrates.

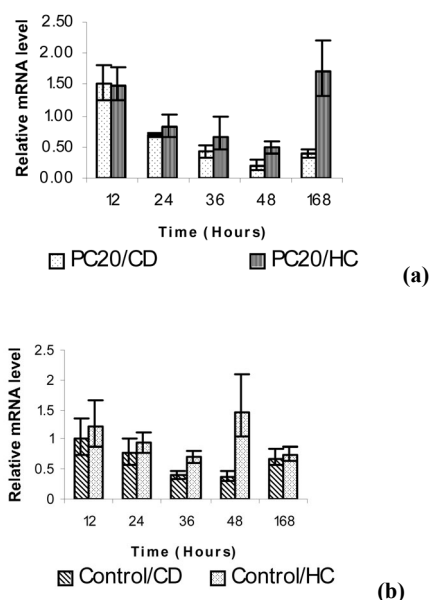


Fig. 1: Graphs showing mRNA levels for ALP on PC20 (a) and control (b) surfaces. mRNA levels expressed have been expressed relative to tissue culture plastic exposed to β-cyclodextrin (Control/CD) at 48 hours.

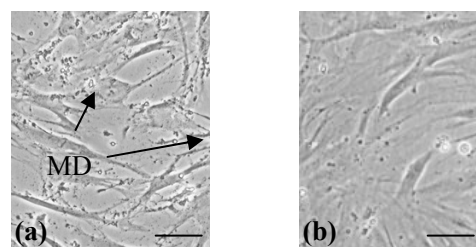


Fig. 2: Optical images of Hobs on PC20 (a) and control (b), Scale bar = 100µm. MD= mineral deposit.

DISCUSSION: The results show that PC polymer is capable of promoting mineral deposition on Hobs. Cationic charge promotes cell adhesion and spreading. The PCR results however indicate that, whilst cell behaviour was altered on PC surfaces, an early change in mRNA levels for markers of osteoblast differentiation was not seen. The higher mRNA level from cells treated with HC indicated that the cells were responding to hydrocortisone. We will measure the message levels for Collagen type I and osteocalcin in future experiments and investigate the mechanism of mineral deposition process.

ACKNOWLEDGEMENTS: BBSRC for funding.