HUMAN OSTEOBLAST INTEGRIN EXPRESSION ON DEGRADABLE POLYMERIC MATERIALS FOR TISSUE ENGINEERED BONE

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INTRODUCTION: The replacement of bone loss due to trauma or disease has been of great interest in the field of orthopaedics. Conventional treatment continue to rely on the use of allografts or autografts, despite problems inherent in their use. We have focused attention on the development of polymer and polymer composite based matrices for tissue engineered bone. It is the purpose of our work to gain an understanding of the mechanisms by which osteoblasts successfully attach and mature in the matrix environment.

Cell-matrix adhesion is largely mediated by integrins, transmembrane receptors, composed of \(\alpha\) and \(\beta\) subunits that dimerize in specific combinations that both are dependent on and determine the extracellular matrix or surface structure on which the receptor binds (e.g., \(\alpha_5\beta_1\), for collagen and \(\alpha_6\beta_1\) for fibronectin). Integrin expression has recently been shown to be involved in cell signaling pathways that influence cellular proliferation and gene expression. The objective of the current study was to examine the expression of primary human osteoblast integrin receptors (\(\alpha_5\), \(\alpha_6\), \(\alpha_{10}\), \(\alpha_{11}\) and \(\beta_1\)) involved in cell adhesion to biomeodified polystyrenes commonly utilized in our tissue engineered polymeric scaffolds.

METHODS & MATERIALS: Poly(lactic-co-glycolide) (PLAGA) in a ratio of 50:50 and Poly(lactic acid) (PLA) polymers were obtained from Purac and initially dissolved in a solution of methylene chloride using a vortex set at a constant speed. The dissolved polymer was then poured into a Teflon coated petri dish and allowed to settle slowly overnight at -20°C. After evaporation of the solvent, thin film matrices were bored into circular discs of 14mm diameter and lyophilized to remove residual solvent.

Primary human osteoblastic cells were obtained using a modification of a previously developed procedure. For adhesion studies, 10,000 cells/cm² were plated on PLAGA, PLA and tissue culture polystyrene (TCPS). At 3, 6, and 12 hours, adhered cells were quantified for each surface after incorporation of a fluorescent dye (BCECF-AM) that was measured using a Spectrafluor Plus instrument (Tecan). Parallel cultures were also plated to examine osteoblastic phenotypic expression of alkaline phosphatase (Sigma kit) and osteocalcin activity (ELISA activity, sensitive to ng concentrations, Biomedical Technologies). For Western blot analysis, cells were grown on the matrix at a concentration of 5 x 10⁴ cells/cm². At 12 hrs, cells were lysed with 1% Triton X-100 and the protein concentration was determined using the BCA assay. Equal protein amounts were fractionated by SDS-PAGE and integrin expression was analyzed by western immunoblotting. Density of the bands was determined by an image analyzer (Kodak).

RESULTS: We first determined how osteoblastic cells adhered to the substrates. Rates of human osteoblast cell adherence on PLAGA were comparable to control TCPS and significantly higher than PLA polymer at 3, 6 and 12 hrs (Fig 1a). Cells consistently exhibited a high rate of adherence to the substrate that supported greater cell adhesion. Furthermore, the integrin subunits expressed at the highest levels in osteoblastic cells adhered to degradable polymeric surfaces were found to be the same integrins normally found to adhere to the ECM molecules collagen and fibronectin (\(\alpha_5\), \(\alpha_6\), \(\beta_1\)).

We believe that the ability of the degradable polymeric materials to promote cell adhesion of human osteoblasts may be directly dependent on the repertoire of integrins expressed on that material. This interaction has been shown to play a significant role in cell-cell adhesion, signaling and spreading in a variety of other settings. Further studies will continue to investigate the role of integrins in regulatory events surrounding human osteoblast adherence and growth on biomeodified materials suitable for bone tissue engineering.

![Image](image_url)

Figure 1. (a) Human osteoblastic cell adhesion on PLAGA, PLA and TCPS at 3, 6 and 12 hours. N=5 for all points. (b) Human osteoblastic cell osteocalcin expression at 24 hrs on PLAGA, PLA, and TCPS. * denotes p<0.05 for comparison between PLAGA and PLA using student t-Test.

Figure 2. Alkaline phosphatase staining in human osteoblasts grown on polystyrene PLA and PLAGA. Note the morphology of the cells with characteristic processes extending over the matrices. Staining is seen to be associated with the cell membrane as well as intracellular sites.

Figure 3. Western analysis for integrins \(\alpha_5\), \(\alpha_6\), \(\alpha_{10}\), \(\alpha_{11}\), \(\alpha_6\) and \(\beta_1\) on bioerodible polymers. Lane 1, 3, 5, 7, 9, and 11 represent integrins isolated from osteoblastic cells grown on PLA matrices; lanes 2, 4, 6, 8, 10 and 12, integrins expressed by osteoblastic cells adhered to PLAGA.

REFERENCES

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