INTRODUCTION

The anterior cruciate ligament (ACL) connects the femur to the tibia, and functions as a joint stabilizer. ACL tears and ruptures are the most common knee ligament injuries, and affect over 250,000 people per year in the United States alone[1]. Current ACL reconstruction procedures cannot fully restore function without associated side effects such as donor site morbidity, muscle atrophy, tendinitis, and arthritis. Although semitendinous autografts are superior to other clinical alternatives, they often fail at the insertion site between the graft and the bone tunnel [6]. Thus, the degree of graft integration with osseous tissue is a critical factor governing its clinical success.

The long term objective of our research program is to design functional interfaces which can facilitate the integration of bone with soft tissues. The natural insertion site between ACL and bone is divided into three principal zones. After the removal of extraneous tissue and facia lata, the intact ACL and trabeicular bone chips were excised. The cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM, Life Technologies), supplemented with 10% fetal bovine serum, L-glutamine, and 1% antibiotics (Life Technologies).

Osteoblasts and ACL Fibroblasts Co-culture: Co-culture was established by first dividing the surfaces of each well in a multi-well plate into three parallel sections using sterile agarose inserts. ACL cells and osteoblasts were seeded on the left and right surfaces respectively, with the middle section left empty. Cells were seeded at 50,000 cells per well and left to attach for 30 min prior to rinsing with PBS. Cells were grown to confluency at 37°C and 5% CO2 under humidified conditions. The media was supplemented with 10 µg/ml L-ascorbic acid and 1mM β-glycerophosphate at day 7 [8]. The agarose inserts were removed at day 7, and cell migration into the interface was monitored over time, as seen in Table I. Osteoblast control also expressed type II, III and X collagen at day 14, while the control fibroblast did not. Control groups were fibroblasts alone and osteoblasts alone. Media was changed every 2-3 days. Analyses were performed at 1, 3, 7, 14, 21 days.

RESULTS

Cell and Cell Culture: Bovine osteoblasts and ligament fibroblasts (ACL) were isolated from explant cultures of tissue from 4-6 month old calves. After the removal of extraneous tissue and facia lata, the intact ACL and trabeicular bone chips were excised. The cells were cultured in Dulbecco’s Modified Eagles Medium (DMEM, Life Technologies), supplemented with 10% fetal bovine serum, L-glutamine, and 1% antibiotics (Life Technologies).

Osteoblasts and ACL Fibroblasts Co-culture: Co-culture was established by first dividing the surfaces of each well in a multi-well plate into three parallel sections using sterile agarose inserts. ACL cells and osteoblasts were seeded on the left and right surfaces respectively, with the middle section left empty. Cells were seeded at 50,000 cells per well and left to attach for 30 min prior to rinsing with PBS. Cells were grown to confluency at 37°C and 5% CO2 under humidified conditions. The media was supplemented with 10 µg/ml L-ascorbic acid and 1mM β-glycerophosphate at day 7 [8]. The agarose inserts were removed at day 7, and cell migration into the interface was monitored over time, as seen in Table I. Osteoblast control also expressed type II, III and X collagen at day 14, while the control fibroblast did not. Control groups were fibroblasts alone and osteoblasts alone. Media was changed every 2-3 days. Analyses were performed at 1, 3, 7, 14, 21 days.

Response of Co-cultured Osteoblasts and Fibroblasts: For all groups, cell growth (n=6) was measured using a quantitative fluorometric assay (PicoGreen, Molecular Probes). Alkaline phosphatase (ALP) expression cell growth (n=6) was measured using a quantitative fluorometric assay (PicoGreen, Molecular Probes). Alkaline phosphatase (ALP) expression was ascertained by Fast-blue staining, and mineralization was visualized with osseous tissue is a critical factor governing its clinical success.

DISCUSSION

With the goal of reconstructing the interface between bone and ligament, we examined the interaction between osteoblasts and ligament fibroblasts using a co-culturing model. A continuous and distinct interfacial zone was developed between these cells. Osteoblasts maintained their phenotype during co-culture, and fibroblasts continued the expression of type II collagen at day 14, while the control fibroblast did not. However, type X collagen was not expressed in these cultures, likely due to the low concentration of b-GP used here.

REFERENCES