INTRODUCTION: The human anterior cruciate ligament (ACL) fails to heal after rupture, even with primary repair. Current methods of treatment involve replacement of the ruptured ligament with a tendon graft – a procedure, which restores immediate stability to the knee, but in a significant number of patients, fails to provide long-term stability. Our work focuses on development of a collagen-based scaffold to facilitate ligament regeneration in the gap between the ruptured ends of the human ACL. We have previously demonstrated the ability of human ACL cells to migrate from the ligament tissue into a 3-D collagen-glycosaminoglycan (CG) scaffold in vitro (1). In the present study, our hypothesis was that selected growth factors would affect the rates of cell migration, proliferation and collagen production in the CG matrix. We chose four growth factors previously found to affect ligament cell behavior (TGF-β1, PDGF-AB, EGF, and bFGF), and used them in concentrations found to have maximal effect in previous studies using 2-D culture.

MATERIALS AND METHODS: Human anterior cruciate ligaments were retrieved from the knees of six patients undergoing total knee arthroplasty. Each ligament was divided into sixty-two explants. For each ligament, two of the explants were reserved for histology, and the remaining sixty explants were placed onto a 9 mm diameter CG scaffold. The CG sponge-like matrix was prepared by freeze-drying a mixture of bovine tendon type I collagen and chondroitin 6-sulfate followed by dehydrothermal cross-linking as previously described (2). The CG scaffold was more than 90% porous with an average pore diameter of 90µm. For each of the six ligaments, the sixty explant/scaffold constructs were divided into five groups based on the culture conditions: control (2% FBS only); TGF-β1 (2% FBS and 10 ng/ml TGF-β1); EGF (2% FBS and 50 ng/ml EGF); PDGF-AB (2%FBS and 10 ng/ml bFGF); and PDGF-AB (2% FBS and 50 ng/ml PDGF-AB). Four constructs in each group were harvested for analysis at 2, 3 and 4 weeks, one for histology and three for biochemistry. The three specimens from each patient at each time point used for biochemistry analysis were averaged to yield n=6 for each group. The 324 specimens from each patient at each time point used for biochemistry analysis were averaged to yield n=6 for each group. The 324 specimens from each patient at each time point used for biochemistry analysis were averaged to yield n=6 for each group. The 324 specimens from each patient at each time point used for biochemistry analysis were averaged to yield n=6 for each group. The 324 specimens from each patient at each time point used for biochemistry analysis were averaged to yield n=6 for each group.

RESULTS: EGF and bFGF treatment resulted in an increased number of cells in the CG scaffolds at the two-week time point (two-factor ANOVA, p < 0.0001 for growth factor effect, p < 0.0001 for time effect; Fig 1). This effect was not, however, seen at the later periods. The other growth factors had no effect on the number of cells in the CG matrices.

DISCUSSION: There was a differential effect of the selected growth factors on the migration, proliferation, and loss of cells from the CG matrices. The short-term effects of bFGF and EGF seen at 2 weeks may have been due to the promotion of migration as well as cell proliferation. The subsequent 50% reduction in cell number to the control level indicated a loss of cells from the matrix or cell death. That the elevation in DNA synthesis stimulated by PDGF-AB after 3 weeks is interesting in the light of the more pronounced degradation displayed in that group. This might be explained by an increase in collagen turnover, promoted by this growth factor. These findings suggest the need to employ additional cross-linking of the CG matrix to resist degradation. Consistent with the literature, TGF-β1 was also found to stimulate collagen synthesis. This work suggests that certain growth factors (viz., PDGF-AB) may enhance ligament cell proliferation and collagen synthesis within a CG matrix implanted as a bridging scaffold at the site of an ACL rupture. The addition of EGF and bFGF with shorter release time may also enhance cell migration into the scaffold in the first two weeks after implantation.


Growth factor treatment also had an effect on collagen synthesis in the scaffold (two-factor ANOVA, p < 0.07; Fig. 3). Post-hoc testing demonstrated a significant difference between the PDGF and control groups (p < 0.004).