Introduction: Ruptures of the anterior cruciate ligament (ACL) are common knee injuries that do not heal, even with surgical repair. Our research is directed towards developing novel, biological approaches that enable suture repair of this ligament. We have previously shown that cells of the ACL are able to proliferate after ligament rupture, to migrate into an adjacent provisional scaffold [1] and to take up a foreign transgene when delivered within a vector-laden hydrogel in vitro [2]. Based upon these data, in this study we further characterized the expression of foreign transgenes by ACL cells when cultured as monolayer, within collagen hydrogels and when transduced in situ while migrating out of ACL tissue pieces in explant culture. Moreover we report the healing response induced by the delivery of the IGF-1 transgene under these culture conditions.

Materials and Methods: Generation of adenoviral vectors: Serotype 5, E1/E3 deleted adenoviral vectors of the first generation were used encoding human IGF-1 (Ad.IGF-1), the green fluorescent protein (Ad.GFP), and the firefly luciferase (Ad.Luc) gene. ACL cell isolation: Anterior cruciate ligaments were harvested aseptically from 4-6 week-old calves, minced into pieces of about 1 mm3, and sequentially digested in trypsin, and collagenase solution. The recovered cells from the digests were plated in monolayer culture using DMEM with 10% FBS. Monolayer and hydrogel culture and adenoviral transduction: For the monolayer experiment, ACL cells were seeded at 5*10^4 cell/mL in 12 well plates, and transduced with Ad.GFP and Ad.IGF-1 at 10, 100 and 300 multiplicities of infection (m.o.i.), or were left uninfected. For the hydrogel cultures, aliquots 3*10^5 ACL cells were transduced with various m.o.i. of Ad.IGF-1, Ad.GFP and Ad.Luc, respectively, or were left uninfected. 24 hours after transduction, cells were resuspended in 200 μL of a bovine collagen hydrogel and maintained completed DMEM. In vitro ACL repair model: Fascicles of approximately 3 mm diameter were dissected longitudinally from six bovine ACLs, and divided transversely into halves. The proximal and the distal halves were divided into pieces of approximately 3 mm which were placed at opposite ends of 5 mm diameter semicircular silicone tubes leaving a 5 mm gap which was then subsequently filled with collagen hydrogel, containing 108 infectious particles of Ad.IGF-1, Ad.GFP or no viral particles, respectively, and the constructs were placed into 12-well plates and cultured as stated above. Quantitation of transgene expressions: GFP was visualized by fluorescence microscopy, firefly luciferase expression was quantified in a luminometer, and the IGF-1 transgene expression was determined in the supernatants using an IGF-1 immunoassay kit (R&D Systems). Cell proliferation and collagen production assays: DNA content was determined fluorometrically on proteinase K digested samples using Hoechst dye. Monolayer and gel cultures were also radiolabeled with (H3)thymidine and (C14)proline. Apoptosis: Constructs were evaluated the Annexin 5 detection kit (Sigma). Histology, immunohistochemistry, and RT_PCR: Histologic analyses of the gels was performed on H&E, Azan, Masson Goldner, and Van Gieson stained 5 μm paraffin sections. For collagen synthesis, immunohistochemical staining for collagen type III, elastin, tenascin and vimentin were performed using monoclonal antibodies and the DAB Method for visualiza-

Results: We showed that monolayers of ACL fibroblasts were efficiently transduced by adenovirus vectors and continued to express transgenes when subsequently incorporated into the hydrogel. Transfer of IGF-1 cDNA increased the cellularity of the gels and led to the synthesis and deposition of increased amounts of types I and III collagen, elastin, tenascin and vimentin (Fig. 1). The cells remained viable, even when subjected to high viral loads as evidenced by negative staining for Annexin 5. Similar results were obtained when collagen hydrogels were preloaded with adenovirus prior to insertion into an experimental ACL lesion in vitro (Fig. 2). Finally we demonstrated that indeed adenoviral vectors stayed infective within the hydrogels for at least three weeks, after infections with collagenased vector-laden hydrogels on sensitive 293 cells and ACL fibroblasts.

Discussion: These data confirm the promise of using vector-laden hydrogels for the in situ delivery of genes to cells within damaged ligaments and suggest novel possibilities for the biological repair of the ACL.


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