EARLY EFFECT OF AUTOLOGOUS PLATELETS AT THE TENDON-BONE INTERFACE

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Introduction Soft tissue anterior cruciate ligament (ACL) reconstruction is dependent on a stable tendon-bone interface. Initially, graft fixation is considered the limiting factor, but ultimate stability and clinical outcome is dependent on graft incorporation within the bone tunnels and intra-articular graft. There is some evidence that bone-to-bone healing, with patellar tendon grafts, is superior to tendon to bone healing seen with soft tissue (hamstring) grafts. This is presumably because of the direct bone-to-bone healing in the patellar tendon grafts. Tendon to bone healing is dependent on bone ingrowth and mineralization of the fibrovascular interface between tendon and bone. Augmentation of bone ingrowth at this interface may improve tendon-graft incorporation and influence clinical outcomes. Platelets contain a number of factors implicated in fracture healing and bone repair, including platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β). Concentrated platelets have been shown to accelerate bone ingrowth into bone graft substitutes and aid in spinal fusion. We hypothesised that the introduction of concentrated platelets at the tendon-bone interface may help optimise graft incorporation. This pilot study examined the effects of platelet sequestration and concentration (Autologous Growth Factors™, AGF™, Interpore Cross International) on tendon-bone healing in an ovine ACL model.

Method Six adult sheep were allocated to control or platelet treatment following ethical approval and underwent an ACL reconstruction using an extensor tendon graft passed through 4.5mm tunnels in the tibia and femur. Endobutton fixation (Smith & Nephew Endoscopy, MA) was used on the femoral side with tibial fixation over a bony post. 400-450ml of blood was withdrawn and 60ml of buffy coat isolated using a 2-stage sequestration protocol. Buffy coat was further processed into 20ml of AGF which was injected into the bony tunnels simultaneously with thrombin (100 units/ml). Animals were killed at 2 weeks. The knee joint was inspected grossly and examined using computed tomography (CT). The tibial and femoral bony tunnels were harvested, fixed in buffered formalin and decalcified in 10% formic acid – formalin solution. Decalcified tissues were embedded in paraffin and 5 micron serial sections cut for H&E staining and immunostaining for PDGF, VEGF, and BMP –2, -7. Non-immunized mouse and goat IgG were applied as negative controls. A Biotin-streptavidin system was applied for amplifying the signals. The color was developed using a peroxidase-DAB system (DAKO, Australia). The intensity and location of the immunostaining was analyzed using light microscopy by 3 independent blinded observers.

Results No adverse clinical events were noted following surgery. Macroscopic inspection of the joint revealed no adverse effects to the articular cartilage surface. The tendon appeared to be stable within the tunnels. CTS at 2 weeks revealed a more active tendon-bone interface in the animals treated with concentrated platelets compared to controls (Figure 1). Histology revealed a disorganized tendon-bone interface in both groups at 2 weeks. The interface in AGF treated samples presented more active cellular response compared to controls (figure 2a,c: control; 2b,d: AGF). Immunostaining revealed an increased expression of VEGF at the tendon-bone interface with AGF treatment compared to controls. PDGF, BMP 2 and 7 were expressed in both groups but no major differences were noted at the healing tendon bone interface at 2 weeks. Expression for these factors was found in osteoblasts lining the adjacent cancellous bone as well as fibroblastic tissue at the interface. No differences were detected between control and treatment.

Discussion No adverse effects within the joint space were seen. CTS revealed increased signal intensity at the tendon-bone interface compared to controls. VEGF expression was increased in the treated group. These pilot results need to be addressed in light of the type of fixation and graft used. The Endobutton-suture-graft composite provides a suspensory fixation with respect to the femoral fixation. This type of fixation may be associated with increased micromotion compared to the use of interference screws for patellar tendon or soft tissue grafts and could play a factor in the results. Altering the biology of the tendon-bone interface through biological therapy may allow for faster graft incorporation and a stronger interface.

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