

The Use of Green Fluorescent Protein to Determine Chondrocyte Fate in a Sheep Model of ACI

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INTRODUCTION: The use of autologous chondrocyte implantation (ACI) is becoming increasingly widespread clinically, however a number of questions still exist as to its clinical efficacy and how implanted chondrocytes might contribute to the repair. Furthermore, little is understood about the fate of the implanted chondrocytes once the joint is closed and weight bearing motion is resumed. A recent study which looked at the fate of chondrocytes implanted within an alginate matrix into osteochondral defects in rabbits found that the number of implanted chondrocytes decreased in number with time¹.

METHODS: In our study a 5mm defect was created in the medial femoral condyle of a sheep knee. Chondrocytes isolated from cartilage harvested during this procedure were cultured *in vitro* and labelled by using a gene encoding for a green fluorescent protein (GFP) which is expressed by the transduced cells. As is the case clinically, these cultured autologous chondrocytes were implanted into the defect using a sutured periosteal graft, which was sealed with fibrin glue. At the time of re-implantation 1% of the 3.7×10^6 cells expressed GFP. One week after ACI the animal was killed and the defect was retrieved, decalcified and freeze sectioned in entirety.

RESULTS: Histology of the retrieved tissue revealed the presence of numerous GFP positive chondrocytes at the site of the defect (Figure 1). Counting of cells showed that the total number of GFP+ cells in the MFC defect to be 695 which would equate to approximately 7×10^4 cells in total. This indicates that approximately 2% of the cells initially injected below the periosteum were retained with 98% being lost into the synovial capsule. No GFP positive cells could be found in any of the samples of synovial fluid, synovium or meniscus.

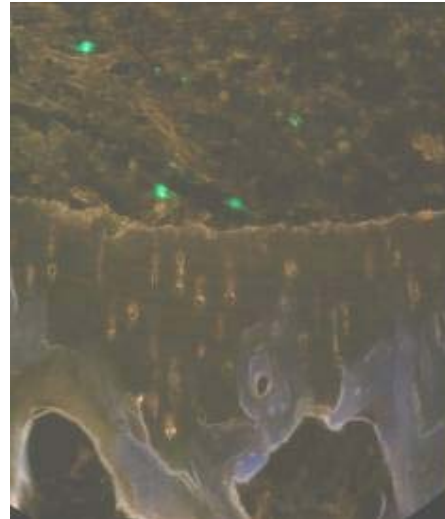


Fig. 1: Detection of GFP+ cells in defect.

DISCUSSION & CONCLUSIONS: This model was designed to determine what proportion of implanted chondrocytes remain at the defect site following ACI. Following wound closure and resumption of the stresses that occur between the femoral condyles and the tibial plateau it seems inevitable that when chondrocytes are implanted below a periosteal flap as in ACI some are lost into the surrounding synovial capsule. The fact that only 2% of the implanted chondrocytes injected could be detected does indeed indicate that the vast majority are lost into the synovial fluid. This experiment does show categorically however that implanted chondrocytes persist at the implant site for at least one week. Further work currently underway will examine the longer term fate and contribution of GFP labelled chondrocytes to cartilage repair.

REFERENCES: 1. Mierisch *et al.* JBJS [Am]2003;85-A:1757-67