

Chondrocyte damage following osteochondral grafting using metal and plastic punches: comparative study in an animal model

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ABSTRACT

Purpose. Osteochondral impaction grafting to manage isolated chondral defects in femoral condyles usually uses a metal punch to impact the grafts into predrilled cavities. Damage to the chondrocytes during impaction is a concern, however, and new methods are being sought to minimise the damage. We studied if impaction with a plastic punch instead of a metal punch reduces the extent of chondrocyte damage in an animal model.

Methods. 32 osteochondral plugs were prepared from knees of 10 freshly slaughtered sheep knees; the contralateral condyles were then prepared to receive the osteochondral grafts. 20 plugs were impacted into predrilled holes: 10 using a metal punch and 10 using a plastic punch. The 12 remaining plugs were used as controls. The plugs were recovered, incubated for 24 hours in calf serum, and stained with 3-[4,5-

dimethylthiazol]-2,5-diphenyltetrazolium to measure the content of viable cells. Digital photographs of the stained cartilage were then analysed on a 0-to-255 grey-scale.

Results. We found no significant difference in the extent of chondrocyte damage caused by impaction using metal and plastic punches. The content of viable cells in plugs impacted by metal and plastic punches, however, was significantly lower than that in the control plugs, as reflected by higher means of light intensity of 52.9 ($p < 0.001$) and 32.4 ($p = 0.005$), respectively.

Conclusion. Impaction grafting clearly damages chondrocytes of the osteochondral plug. The use of a plastic punch does not reduce the extent of chondrocyte damage during the impaction grafting procedure.

Key words: chondrocyte damage; chondroplasty; metal punch; plastic punch; osteochondral impaction grafting

INTRODUCTION

Osteochondral impaction grafting is gaining popularity as a method of managing isolated chondral defects in femoral condyles. The short-term results are encouraging and the surgical techniques are becoming progressively more refined and less invasive.¹⁻⁵ Most of the available systems use a metal punch to impact the osteochondral grafts into predrilled cavities. The procedure is arthroscopically assisted and a formal arthrotomy is avoided. Damage to the chondrocytes during impaction grafting is a concern,⁶ however, and new methods are being sought to minimise this harmful effect.

This study was designed to investigate if using a plastic punch instead of a metal punch reduces the extent of chondrocyte damage because of osteochondral impaction grafting.

METHODS

32 osteochondral plugs were prepared from the medial femoral condyles of knees from 10 freshly slaughtered sheep knees using the Cor (Mitek; Johnson and Johnson, Boston, Massachusetts, US) osteochondral impaction grafting system. Two osteochondral plugs were implanted into predrilled cavities in each of the 10 condylar condyles, by using a metal punch for one graft and a plastic punch for the other. Implantation involved expulsion of the plugs from the harvesting tube and impaction by gentle tapping. To simulate the clinical situation, the impaction force was not measured; however, to make the experiment reproducible, an attempt was made to limit to the number of times that the plugs were tapped to 5. 12 plugs were used as controls and were not implanted.

Implanted plugs were retrieved immediately without interfering with the cartilaginous cap. They were bathed in antibiotic solution for one minute and then incubated in calf serum for 24 hours. The antibiotic solution used was 1% solution of 10000 units of penicillin G, 10000 µg of streptomycin sulphate and 25 µg of amphotericin B in 0.85% saline. The plugs were washed in saline and then incubated in 1 mg/ml aqueous solution of 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium (MTT) in a dark container for 90 minutes. This yellow solution changes to an insoluble purple formazan product by cleavage of the tetrazolium ring by dehydrogenase enzymes⁷ in mitochondria of living cells. The amount of formazan generated is directly proportional to the number of viable cells⁸; dead cells do not cause this chemical reaction. The MTT assay has been used to assess cell

viability in both cell culture⁹ and tissue explant^{10,11} studies.

All plugs were washed again with saline. The cartilaginous caps were removed and photographed by a digital camera attached to a Leica-MZ8 Macro-scope (Leica, Heerbrugg, Switzerland). Images were converted to a grey-scale of 0 to 255 using Image-Pro Plus 3.0 (Media Cybernetics, Maryland, US). Live cartilage appeared black and dead cartilage appeared white. An area that was consistent for all samples was selected and analysed using the same programme to determine the light intensity, which was inversely related to the amount of staining.

Statistical analysis was performed using Statistics Package for Social Science (Windows version 10.0; SPSS Inc., Chicago, Illinois, US). Determination of statistical significance was based on an alpha error of 0.05. One-way analysis of variance was used to determine if there were differences between the staining intensity in the samples of the 3 study groups.

RESULTS

The mean light intensity for the control group of plugs was 67.4, whereas the means for the plugs that had been impacted by metal and plastic punches were 120.3 and 99.8, respectively (Table 1). The control plugs had significantly lower mean intensities than metal- and plastic-punched plugs, by 52.9 and 32.4, respectively (Table 2). Because the light intensity was a reciprocal indicator of MTT staining, the control plugs contained more live cells than the plugs in the other 2 groups, indicating that the use of either punch caused more cell damage. Although the metal-punched plugs had, on average, a lower content of living cells than did plastic-punched plugs, the difference (an increase in light intensity of 20.5) was not statistically significant.

DISCUSSION

Torzilli et al.⁶ demonstrated an impact stress—dependent inhibition in chondrocyte metabolic activity and also a decrease in cell population when cartilage is impacted with a load beyond a certain critical threshold stress, which was done on bovine cartilage in vitro. We did not measure the load, because an attempt was made to simulate as close as possible to a true clinical situation—namely, where a surgeon would impact the osteochondral graft using a punch and mallet without knowing the exact stress value. Impaction grafting clearly damages chondrocytes of the osteochondral plug. Our study shows that using a

Table 1
Light intensities in stained impacted plugs according to method of impaction

Treatment	n	Mean light intensity* (SD)	95% Confidence interval
Control	12	67.4 (22.5)	53.1–81.7
Metal punch used	10	120.3 (22.9)	103.9–136.7
Plastic punch used	10	99.8 (19.6)	85.8–113.8

* Light intensity is inversely proportional to the staining of viable cells; the grey-scale is from 0 (black) to 250 (white).

plastic punch instead of a metal punch does not reduce the extent of chondrocyte damage during osteochondral impaction grafting.

Assessing the clinical success of osteochondral impaction grafting is difficult. Chondral defects are often asymptomatic, and are often incidental findings during arthroscopic examination. Alternatively, these defects may be due to osteochondritis dissecans, which usually improve symptomatically after simple debridement and drilling.¹² The natural course of solitary chondral defects is uncertain. It is presumed that large weightbearing defects progress to more generalised osteoarthritis; there is, however, little evidence for this.¹³

Lack of symptoms and uncertainty regarding the nature of progression of chondral defects make clinical and radiographic postoperative assessment difficult, if not impossible. Follow-up arthroscopy is useful in visualising the graft, but the quality and biomechanical characteristics of the graft cannot be determined without biopsy. If biopsy is performed, it will invariably cause further damage to the transplanted

Table 2
Light intensities in stained impacted plugs

Comparison groups	Mean difference in light intensity* (SE)	p value
Control—Metal	–52.9 (9.32)	<0.001
Control—Plastic	–32.4 (9.32)	0.005
Metal—Plastic	20.5 (9.73)	0.132

* Light intensity is inversely proportional to the staining of viable cells.

cartilage. It is reasonable to assume that using hyaline cartilage as to plug any defect would be beneficial.

Every effort should be taken to maintain the optimal condition for the survival of the transplanted chondrocytes. Impacting grafts with a metal or a plastic punch clearly contradicts this philosophy, as demonstrated by this study. Future research should be aimed at less traumatic methods of fixing the osteochondral plugs in place.

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