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Chondrocyte function after osteochondral transfer: comparison of concave and plane punches

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Abstract
Background An incongruity between instrument and articular surfaces in osteochondral transfer (OCT) results in unevenly distributed impact forces exerted on the cartilage which may cause a loss of functional chondrocytes. We tested whether a plane instead of a concave design of the punch of an osteotome can reduce these cartilage damages.
Methods Osteochondral cylinders were transferred from a donor to a recipient site within porcine humeral heads. Histological sections of the cartilage were assessed for metabolic active chondrocytes by in situ hybridization detecting coll α_1(II) mRNA subsequent to OCT and 24 h thereafter.
Results The percentage of cartilage harbouring functional chondrocytes in the transferred grafts was 85 ± 10 and $91 \pm 4\%$ subsequently to OCT using punches with concave or plane surfaces, respectively, and $83 \pm 10\%$ (concave) and $82 \pm 10\%$ (plane) after 24 h. In the superficial layer of the cartilage the percentages were $72 \pm 13\%$ (concave) and $84 \pm 8\%$ (plane) subsequently to OCT, and $68 \pm 15\%$ (concave) and $70 \pm 3\%$ (plane) after 24 h. The analysis did not reveal any statistically significant differences.
Conclusions The OCT leads to considerable loss of functional chondrocytes which could not be prevented by the use of a plane instead of a concave punch. Since functional chondrocytes might be of crucial importance for the survival and integration of the graft into the recipient site further work is needed to optimize the OCT procedure.

Keywords Osteochondral transplantation · Cartilage · Harvest damage · Instrumental design

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Introduction

Osteochondral transfer (OCT) is a treatment option for larger (1–4 cm^2) isolated, full-thickness cartilage lesions [1] predominantly located at the medial femoral condyle of the knee [2]. For treatment of cartilage defects within the weightbearing portions of the femoral condyles, grafts are taken from the less weightbearing intercondylar notch or the periphery of the patellar groove; the latter providing a significantly better topographic match to the medial femoral condyle [3–6].

Functional impairment of chondrocytes was observed at the edges of the grafts after OCT [7–9] with incomplete bridging between the host's and the recipient's cartilage [10–15]. As inhibition of cell death mechanisms occurring in transferred grafts considerably improved binding to the recipient cartilage [16, 17], functional chondrocytes at the edges of transferred cartilage may be of importance for its integration at the recipient site. In order to evenly distribute the impact forces exerted on the cartilage, the punches within the osteotomes used for OCT are designed with a concave surface to match the convexity of the articular cartilage at the medial or lateral aspects of the patellar groove (main source of donor tissue) [18, 19].

However, differences in the shape of each single donor site [18, 19] cannot be accounted for by the instrumental design and it appears that the cartilage surface of the graft is often less convex than the concavity of the punch surface. We proposed that this incongruity between the instrument and cartilage surfaces might lead to cell damage at the periphery of the grafts reducing the amount of functional chondrocytes and limiting graft integration. In an attempt to prevent the loss of functional chondrocytes at the periphery of the grafts we tested in vitro a punch with a plane surface to reduce the pressure applied to the periphery of the grafts and compared it to a punch with a conventional concave surface.

Materials and methods

Osteochondral transfer

Fresh forelegs from 3 to 6-month-old pigs were provided from a local slaughterhouse. The osteochondral grafts were harvested from the humeral head using a commercially available device (Osteochondral Autologous Transfer System; Arthrex Inc., Naples, FL, USA). This device is currently used in clinics and the surgical technique for the graft harvest is described elsewhere [20]. During the surgical procedure the joint was irrigated with physiological saline.

Osteochondral grafts (6 mm in diameter, 10 mm in length) were harvested from a porcine humeral head (ex vivo model) using an osteotome with a punch inside with either a concave or a plane surface ("first harvest", Fig. 1). Subsequently, the grafts were implanted "press-fit" and "unbottomed" into an adjacent recipient hole (5 mm in diameter, >10 mm in length) by many light blows rather than by a few heavy ones with a light hammer head as recommended previously in order to minimize the cartilage damage during re-insertion [21]. The transferred graft was harvested with the surrounding host tissue by a standard osteotome (11 mm in diameter, 10 mm in length: "second harvest", Fig. 2) to assess the cartilage damage. The surgeon (JDB) was blinded to the used punch. All tissues for one experiment were harvested from the same animal. Tissues assessed after 0 h were harvested from one humeral head whereas the tissues assessed after 24 h were derived from the contralateral humeral head of the same animal. The tissues for the different experimental groups ("concave" vs. "plane") were obtained from the same animal. The experiment was performed three times independently with tissues obtained from three different animals ($n = 3$).

Histological analysis

In one series, the tissues were fixed immediately after the "second harvest" in 4% paraformaldehyde/3% dextran in phosphate-buffered saline (PBS; Cambrex, Verviers, Belgium) to identify the function of chondrocytes and thus the initial quality of the osteochondral graft after OCT.

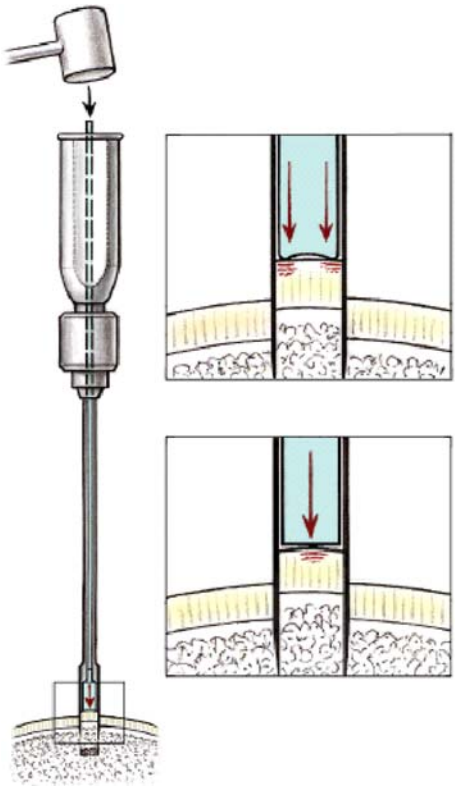

Fig. 1 Commercially available device as used in clinics for osteochondral transplantation using an osteotome with a punch inside. The picture details on the right are showing the concave punch (*above*) and the modified plane punch (*below*) within the osteotome with the hypothesized differences in the pressure distribution (*red arrows*) on the cartilage during the impaction procedure

In a second series, the tissues were cultured for 24 h after the "second harvest" in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Basel, Switzerland)/10% heat-inactivated fetale bovine serum (FBS H.I.; Oxoid AG, Basel, Switzerland)/1% penicillin/streptomycin (P/S; Sigma Aldrich, Buchs, Switzerland) at 37°C and 5% CO_2 before fixation to allow time dependent cell death mechanisms to occur.

After fixation, tissues were decalcified in 15% ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich)/0.5% paraformaldehyde in PBS (pH 8.0), dehydrated in graded ethanol, cleared in xylol, and embedded in low-melting-point paraffin (Histo-Comp®, Vogel, Giessen, Germany). Four sections (thickness 5 µm) were prepared (Microm Cool Cut, Carl Zeiss, Feldlach, Zürich, Switzerland) in different longitudinal section planes (at 0.5, 1.0, 2.0, 3.0 mm measured from the periphery of the graft). The sections were mounted on poly-L-lysine coated superfrost slides (Menzel Glasbearbeitungswerk GmbH & Co. KG,

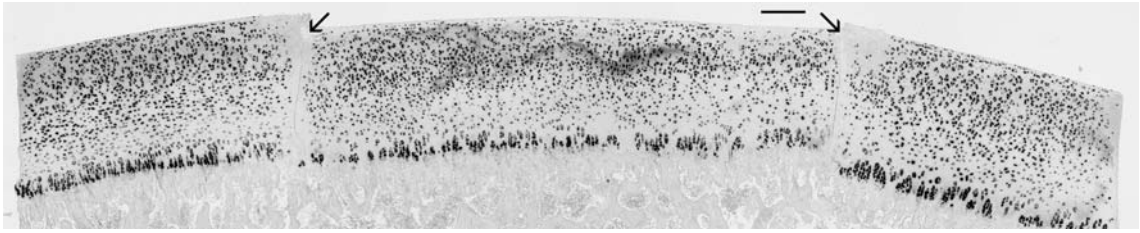

Fig. 2 Example of a histological section after osteochondral transfer using the plane punch with anatomic reconstruction of the articular surfaces (in situ hybridization for mRNA encoding col α_1(II)). The *black arrows* indicate the "host-graft interface" at the cartilage site. (*section plane* 3.0 mm, *scale bar* 500 μm)

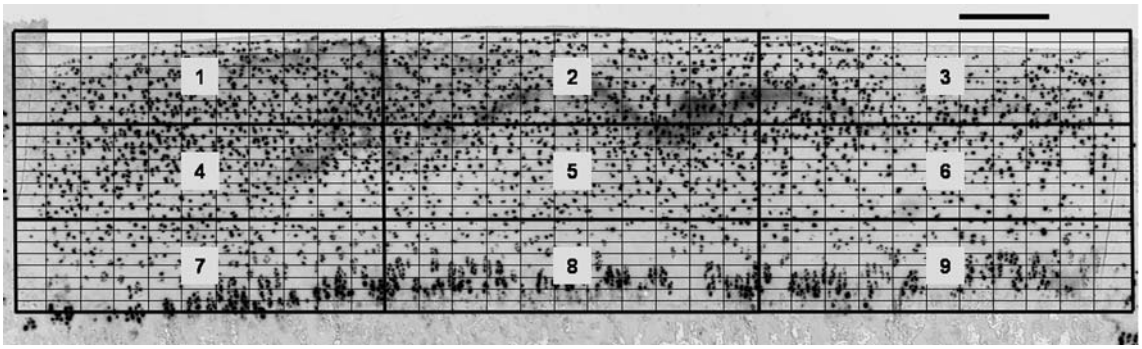

Fig. 3 Morphometric evaluation of osteochondral tissue. To quantify the area of the cartilage harbouring cells positive for col α_1(II) mRNA, a grid with nine *rectangles* was used (each rectangle contains 88 crossings of *grid lines*). The amount of crossings lying in areas containing cells positive for col α_1(II) mRNA were counted and expressed relative to all crossings lying within cartilage tissue

Braunschweig, Germany) and dried overnight at 42°C on a heating plate (MEDAX Nagel, Kiel, Germany).

After deparaffinization and rehydration, the sections were assessed for the function of chondrocytes by in situ hybridization for mRNAs encoding the α_1-chain of collagen type II [col α_1(II)] [22]. The DIG-labelled riboprobes [23, 24] were detected by immunohistochemical analysis using an anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche Diagnostics, Rotkreuz, Switzerland). Binding of the antibody was visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma-Aldrich). Thereafter, the sections were embedded with Aquamount (BDH Laboratory Supplies, Poole, England).

The four sections of each graft were histomorphometrically analysed (Fig. 3): the transferred cartilage was assessed by defining the percentage of the cartilage area which harbours functional chondrocytes [col α_1(II) mRNA positive]. Therefore, the cartilage was overlaid with a grid and the crossings covering cartilage areas containing functional chondrocytes were expressed as percentage of all crossings within the grid covering the cartilage tissue. For local mapping reasons the grid, and thus the cartilage, was divided into nine zones, each containing 88 crossings: superficial layers were the zones 1, 2, 3, whereas zones 4, 5, 6 and 7, 8, 9 were the middle and the basal layer, respectively. The zone 1, 4, 7 and 3, 6, 9 were the cutting edges.

Statistical analysis

For each position and position sums a linear mixed effects model was calculated to test the time and the technique effect in one model. Time effects were measured on independent animals but technique effects on the same animals. Replicated measurements for each animal within time and technique were averaged for the model. Due to the low number of animals a nonparametric procedure is more appropriate than a parametric. The main interest (technique effect) could be analysed alone by a Wilcoxon Signed-rank test; statistical significance was established at $P < 0.05$. Calculations were done with SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

The percentage of cartilage tissue harbouring functional chondrocytes subsequent to the OCT in the transferred grafts was 85 ± 10 and 91 ± 4% when the concave and the plane punches, respectively, were used. After 24 h in culture, the percentages were 83 ± 10% for the concave and 82 ± 10% for the plane punch.

The superficial layer (zones 1, 2, 3) was probably exposed the most to the impaction procedure. In this layer the proportion of cartilage harbouring functional chondrocytes subsequent to OCT was 72 ± 13% (concave) and 84 ± 8% (plane),

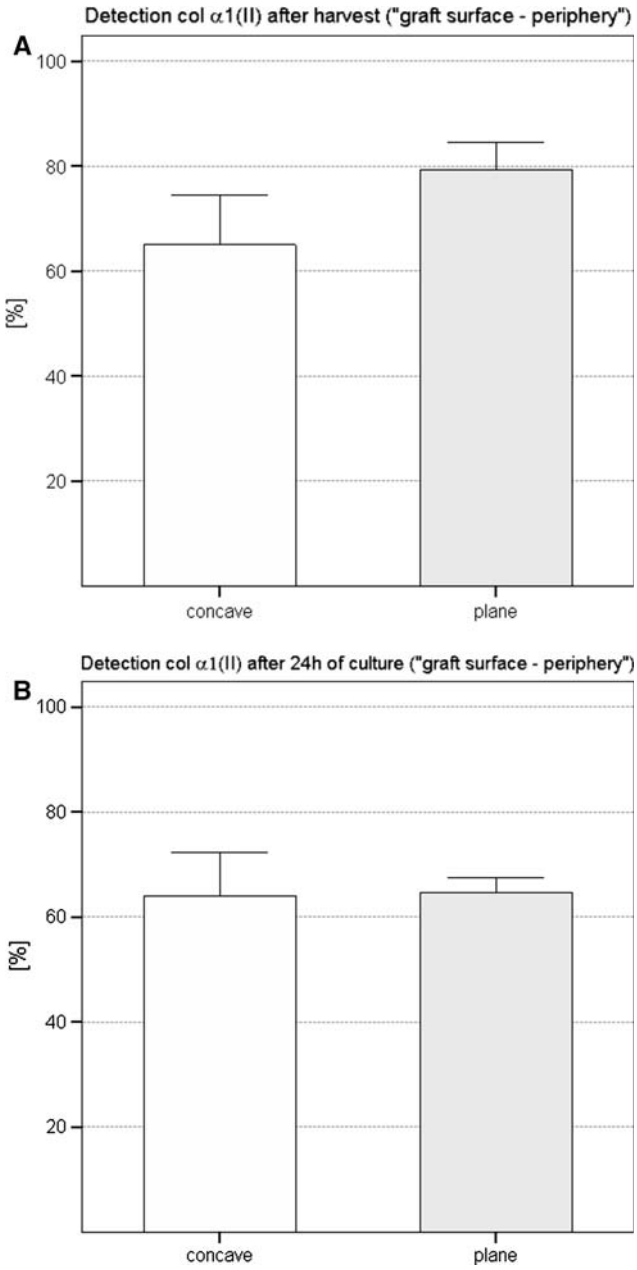

Fig. 4 Cartilage area containing cells positive for col α_1(II) mRNA relative to the total cartilage area was assessed by in situ hybridization **a** 0 h and **b** 24 h after osteochondral transplantation using a concave or a plane punch in the periphery of the graft surface (zones 1 and 3)

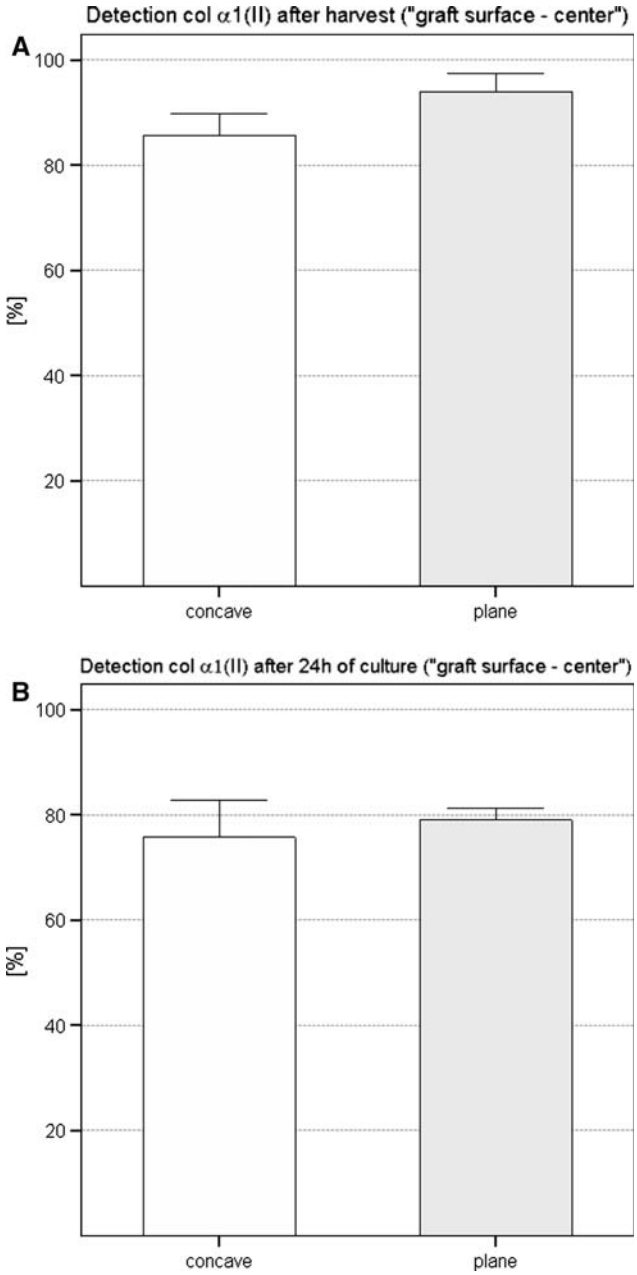

Fig. 5 Cartilage area containing cells positive for col α_1(II) mRNA relative to the total cartilage area was assessed by in situ hybridization **a** 0 h and **b** 24 h after osteochondral transplantation using a concave or a plane punch in the centre of the graft surface (zone 2)

and 24 h later 68 ± 15% (concave) and 70 ± 3% (plane). In the periphery of the graft surface (zone 1 and 3) the cartilage area containing cells positive for col α_1(II) mRNA relative to the total cartilage area was 65 ± 17% (concave) and 80 ± 9% (plane) (Fig. 4a), and 24 h later 64 ± 17% (concave) and 65 ± 5% (plane) (Fig. 4b). In the centre of the superficial layer (Zone 2) the percentage of cartilage harbouring functional chondrocytes was 86 ± 7% (concave) and 94 ± 6% (plane) subsequent to OCT (Fig. 5a) and 76 ± 14% (concave) and 79 ± 5% (plane) after 24 h in culture (Fig. 5b). The detailed results for the single zones 1–9 immediately and 24 h after OCT using either the concave or the plane punch are presented in Table 1. The statistical analysis did not reveal any significant differences in all groups ($P > 0.05$).

Discussion

The aim of this study was to investigate whether a different design of the ostechondral graft transfer instruments,

Table 1 Percentage of cartilage areas harbouring cells positive for col α_1(II) mRNA

(A)			(B)		
Zone	Concave 0 h	Plane 0 h	Zone	Concave 24 h	Plane 24 h
1	68 ± 27	78 ± 6	1	69 ± 20	62 ± 9
2	86 ± 7	94 ± 6	2	76 ± 14	79 ± 5
3	62 ± 26	81 ± 13	3	59 ± 19	68 ± 9
4	95 ± 3	94 ± 3	4	92 ± 5	91 ± 6
5	100 ± 0	100 ± 0	5	100 ± 1	100 ± 0
6	79 ± 35	96 ± 3	6	96 ± 5	95 ± 5
7	93 ± 5	86 ± 10	7	76 ± 14	76 ± 24
8	100 ± 0	97 ± 4	8	85 ± 20	83 ± 33
9	76 ± 35	90 ± 9	9	85 ± 17	76 ± 33

Influence of instrumental design of a concave versus plane punch within the harvest osteotome (A) immediately and (B) 24 h after osteochondral transplantation. Data are presented as the mean percentage of cartilage area harbouring cells positive for col α_1(II) mRNA ± SD. Values within the group "plane" were not significantly different to those obtained in the group "concave" ($P > 0.05$)

namely the concave punch surface in the osteotome, might improve the biological function of an osteochondral graft after transfer. Regardless whether a concave or a plane punch was used, we observed a loss of active chondrocytes in the peripheral cartilage zones (1, 3, 4, 6, 7 and 9) which either occurred during harvest or re-insertion of the graft into the recipient site. This is corroborated by previous reports presenting that the graft margin is compressed and subjected to increased abrasion or shear stresses as it is passed through the osteotome [9] and that the impaction of the graft into the recipient site leads to cell death and collagen rupture in the cartilage [25]. We intended to reduce the stress applied to the periphery of the graft surface by adapting the punch, which is in direct contact with the graft. The results showed no statistically significant differences in comparison with the standard technique. However, there is a tendency towards a higher proportion of active chondrocytes in the superficial layer immediately after OCT using the plane punch.

The low numbers of tissues assessed ($n = 3$) is a limitation of the present study. However, since the results did not reveal a tendency towards a notable beneficial effect of the plane punch we feel comfortable to reject our hypothesis.

Conclusion

In our hands, the use of a plane instead of a concave punch did not reduce the extent of cartilage damage during the extraction and impaction procedure in this experimental setup. However, the considerable loss of functional chondrocytes at the edges of the transferred grafts, which are of importance for the integration into the recipient site, requires the optimization of the transplantation technique to achieve ideal conditions for the survival of the cartilage after osteochondral transplantation.

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