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ORIGINAL

Autologous osteochondral grafts for the treatment of focal chondral lesions of the femoral head: an experimental study in rabbits

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ABSTRACT

The use of autologous osteochondral graft is a useful method for the treatment of cartilaginous lesions. In our study we compared the results of autologous osteochondral graft and simple drilling in cartilaginous lesions in rabbits. The graft was taken from the lateral femoral condyle and put in the hip joint. We evaluated the histological quality of the femoral head 24 weeks after the usage of the graft or the simple drilling for the treatment of the osteochondral defect. Autologous osteochondral graft had excellent results, with high quality new articular surface in comparison with the results of drilling. However, the usage of autologous osteochondral is far more difficult than drilling the osteochondral defect.

Keywords: Articular cartilage; Chondral lesions; Osteochondral grafts; Autologous; Rabbits.

Introduction

The usual response of physiological cartilage to defect formation of either traumatic or degenerative origin is restoration with lower quality tissue. The physiological and mechanical properties of the new tissue differ to those of articular cartilage resulting to a function alteration of the articular surfaces of the joint. Chondrocytes of the environment surrounding the defect, possess the ability of migration but are not capable of multiplication and production of macromolecules necessary for the synthesis of an organized basic network of normal hyaline cartilage.

Reconstruction of the articular surface and defect coverage is necessary in order to avoid the development of osteoarthritis of the joint. Autologous osteochondral transplantation is widely used for the treatment of chondral and osteochondral defects of the knee and ankle joint. Clinical and radiological results show that autologous osteochondral transplantation

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Figure 1: *Osteochondral graft 24 weeks after transplantation.*

is a method that offers reconstruction of the articular surface with mature autologous hyaline cartilage.[1-4] The application of this method on focal chondral pathology of the femoral head of the hip joint, has initially been described by Hangody et al.[2] but no report has been provided for the clinical or radiological outcome of the articular surface of the treated hip joints. On experimental level, to our knowledge, there is lack of literature reporting the usage of this specific method in the hip joints of animal models. Questions concerning the quality of the transplanted tissue, the coverage of the chondral defect, the integration of the transplant and the function of the joint are left open. This study is an effort from our side to give an answer to these questions and to provide the autologous osteochondral transplantation as a safe and effective method for extended cartilaginous lesions.

Materials and methods

After performing the experimental procedure in 3 pilot animals, 12 rabbits were used for the experimental study. They were divided into 2 different groups (A and B). All animals were driven to euthanasia 24 weeks after the transplantation. Group A

Figure 2: *Photograph of the cartilage 24 weeks after subchondral drilling.*

(C, G, H, J, K, and L) animals underwent autologous osteochondral transplantation of an iatrogenic produced osteochondral defect of 2,7mm diameter and 2mm depth. Group B (A, B, D, E, F, and I) underwent subchondral drilling of an iatrogenically produced defect of 2.7 mm diameter and 2 mm depth. More specifically, the surgical technique included sedation through 1-1.5 mg sodium phenobarbital injection. After thorough aseptic cleaning of the hip joint area, skin and soft tissues were prepared until the hip joint capsule was reached. The anterolateral part of the joint capsule was penetrated and opened in a direction parallel to the femoral neck offering vision to the craniolateral portion of the femoral head with the hip positioned in extreme internal rotation. An iatrogenic lesion of the craniolateral portion was created through manual drilling of the femoral head into a depth of 5 mm with a drill, aiming to avoid thermal destruction of the surrounding cartilage. The diameter of the drill was 2.5 mm.

In the autologous osteochondral transplantation group the ipsilateral knee joint underwent aseptic cleaning and preparation as well. A lateral parapatellar arthrotomy was performed and a 2.7 mm in diameter and 5mm in length osteochondral graft was taken from the lateral femoral condyle. The incision was sutured and the knee joint was closed in layers. The osteochondral graft was transplanted into the prepared site of the femoral head with care to bring it to the same level as the surrounding cartilage. The joint was irrigated and closed in layers. In the subchondral drilling group the iatrogenic

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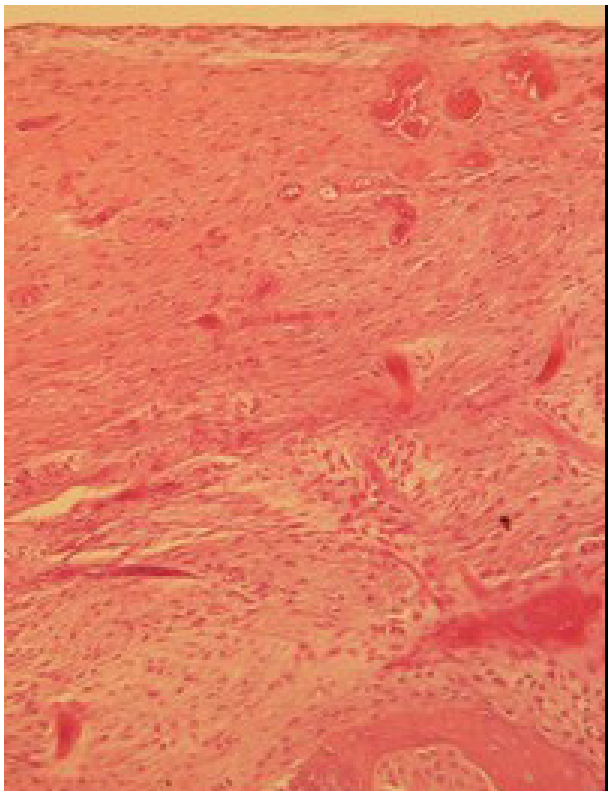

Figure 3: *Histological section 24 weeks after subchondral drilling shows fibrous and fibrocartilaginous tissue with multiple vessels.*

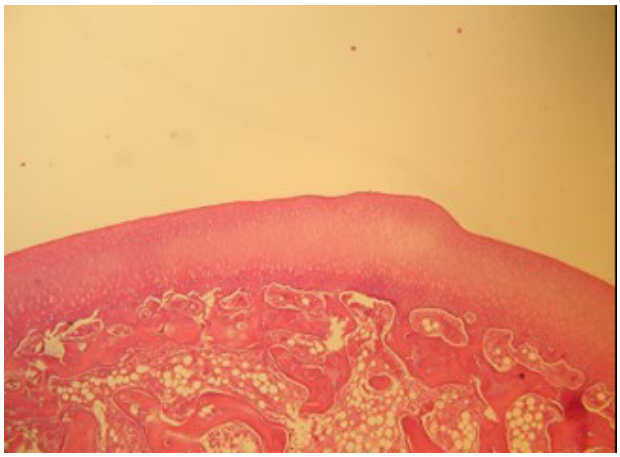

Figure 4: *Histological section 24 weeks after osteochondral grafting shows hyaline cartilage on the top and subchondral bone without any significant changes.*

lesion was left without further treatment. Immediate full weight bearing, without any restrictions of range of motion was allowed. After euthanasia of the animals, the proximal part of the femur was dissected and cleaned from the surrounding soft tissues and was contained in formaldehyde for 24 hours. The dissected parts underwent demineralization. Pieces of 0.3 – 0.4 cm thickness were taken and were positioned into special cassettes and were subjected to dehydration with dilutions of ethylic alcohol 50%, 70%, 96% and 100% as well as xylol where the cassettes were inserted for 45 minutes periodically. Paraffin embedment followed for 120 minutes at a temperature of 62° C. After that the pieces were shut into paraffin cubes and were cut into histological slices with thickness of 5 μ. These slices were painted with hematoxylin /eosin. The results were classified according to the classification system of the International Cartilage Research Society (ICRS).[2] This classification system controls and evaluates 6 important histological parameters. 1) normal appearance of the reconstructed articular surface, 2) quality of matrix, 3) chondrocyte distribution, 4) chondrocyte viability, 5) quality of subchondral bone and finally 6) the nature and site of chondral calcification zone. These parameters are rated with 3 for an excellent result and 0 for failure.

All variables were represented by the number of patients (N), mean value (mean), standard deviation (SD) and median value. Comparisons of variables between the 2 groups were performed with the Mann-Whitney test. All tests were two-sided with 95% significance level. Statistical analysis was done using the statistical package SPSS v. 12.00 (Statistical Package for the Social Sciences).

Results

The articular cartilage of animals sacrificed 24 weeks after the osteochondral transplantation showed a smooth and subwhite surface with 0.05 cm of thickness (Figure 1). Animals sacrificed 24 weeks after subchondral drilling showed an irregular surface with a diameter of 0.3 cm at the treated site. The surrounding cartilage was white and grey (Figure 2). Histological examination of specimens from the six rabbits sacrificed 24 weeks after subchondral drilling showed an irregular articular surface. The defect was covered by fibrous tissue in 2 animals, by fibrocartilaginous tissue in one ani-

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TABLE 1.

ICRS visual histological assessment score of animals treated with subchondral drilling 24 weeks later (Group B).

Subchondral drilling	A	B	D	E	F	I
Articular surface	3	3	0	3	0	0
Martix	2	2	0	2	1	0
Cell orientation	0	0	0	0	1	0
Cell viability	3	3	3	3	3	3
Subchondral bone	2	2	2	2	2	2
Cartilage mineralization	3	2	0	3	0	0

TABLE 2.

ICRS visual histological assessment score of animals treated with osteochondral grafts 6 weeks later (Group A).

Osteochondral grafts	C	G	H	J	K	L
Articular surface	3	3	3	3	3	3
Matrix	3	3	2	3	3	3
Cell distribution	2	2	2	2	2	2
Cell population viability	3	3	3	3	3	3
Subchondral bone	2	3	2	3	3	3
Cartilage mineralization	3	3	2	3	3	3

mal and in the remaining 3 animals by combined hyaline cartilage and fibrocartilage. The tissue of all specimens consisted of viable cells arranged clusters in 3 animals. In 2 animals they are arranged in clusters only. Subchondral bone showed increased remodeling in 4 animals. In 1 animal the subchondral bone underwent necrosis and fibrous tissue formation. In 3 animals we observed normal cartilage calcification. None of the joints treated with subchondral drilling showed full restoration of the articular surface with hyaline cartilage or normal subchondral bone (Figure 3, Table 1). Histological examination of specimens from 6 rabbits sacrificed 24 weeks after osteochondral graft transplantation showed smooth articular surface in all animals consisting of mature viable chondrocytes with column and cluster distribution. In 5 of the 6 animals the cartilage covering the operated site has the appearance of hyaline cartilage, and in one animal the appearance of hyaline cartilage and fibrocartilaginous tissue was observed. The subchondral bone in 4 animals showed no significant changes. In 2 animals the subchondral bone showed increased remodeling. Normal calcification of the cartilage was detected in 5 animals (Figure 4, Table 2).

Statistical analysis showed a statistically significant difference for all variables: articular surface ($p= 0.049$), matrix ($p= 0.003$), cell distribution ($p< 0.0005$), subchondral bone ($p= 0.010$), cartilage mineralization ($p= 0.000$), except for cell population viability between the 2 groups. Additionally, no histological evidence of osteonecrosis of the femoral head in any of the animals was observed (Table 3).

Discussion

Autologous osteochondral transplantation is being

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TABLE 3.

Comparison of the mean values of each parameter of the ICRS visual histological score

	Group	N	Mean	SD	p-value
Articular surface	GROUP B	6	**1.50**	1.64	**0.049**
	GROUP A	6	**3.00**	0.00	
Matrix	GROUP B	6	**1.17**	0.98	**0.003**
	GROUP A	6	**2.83**	0.41	
Cell distribution	GROUP B	6	**0.17**	0.41	**< 0.0005**
	GROUP A	6	**2.00**	0.00	
Cell population viability	GROUP B	6	**3.00**	0.00	**1.000**
	GROUP A	6	**3.00**	0.00	
Subchondral bone	GROUP B	6	**2.00**	0.00	**0.010**
	GROUP A	6	**2.67**	0.52	
Cartilage mineralization	GROUP B	6	**1.33**	1.51	**0.040**
	GROUP A	6	**2.83**	0.41	

applied in the treatment of chondral as well as osteochondral defects of the knee and the ankle joint. Good clinical as well as radiological reports showed that it is a method offering successful reconstruction of the articular surface with grafts consisting of bone and hyaline cartilage.[1-4,5] The application of this method of treatment in the hip joint has been initially reported by Hangody et al.[2] nevertheless without any published reference on the clinical or radiological follow up of their results. This leaves questions concerning the histological quality of the tissue covering the chondral defect, the congruity of the newly formed articular surface or the integration of the graft into the transplanted site of the specific joint, open for answers. This experimental study aimed to investigate histologically the results of the reconstruction of the articular surface of the hip joint through the application of subchondral drilling and autologous osteochondral transplantation, in case of an osteochondral lesion of the femoral head. The most vulnerable and important part of the articular surface of the hip joint is the craniolateral weight bearing area of the femoral head. This area receives the main part of weight bearing forces when the animal is standing as well as walking.

In order to achieve a realistic simulation of the reconstructed area under weight bearing forces, the autologous osteochondral grafting as well as the drilling were performed at the proximal, lateral quarter of the femoral head, based on the hypothesis that it is an area of increased loading pressure. The animals were free to full range of motion and full weight bearing of the hip joint. One major concern of the study was the influence of the dissection and the extreme position of internal rotation of the hip joint on the vascular supply of the femoral head. Since there was no evidence of osteonecrosis of the femoral head an assumption can be made that the vascular supply of the joint capsule compensates well the single lateral incision as well as the internal rotation without any negative influence on the osseous structure of the femoral head. Macroscopically as well as microscopically the osteochondral defect treated with autologous osteochondral transplantation was fully covered from tissue showing definite difference to the tissue produced after subchondral drilling. In order to compare the histological results of the two treatment methods we used the International Cartilage Research Society Scoring system. This system allows histological es-

timation of the quality of the newly formed tissue. Clear superiority of the score of the group treated with autologous osteochondral transplantation was shown in comparison to the group treated with subchondral drilling after a period of 12 weeks. The comparative analysis of the parameters observed in the histologic outcomes of each method justifies the results. More specifically, the articular surface of all the rabbits belonging to the group of autologous osteochondral transplantation was smooth becoming a high histological average ICRS score 3 (excellent). On the other hand the subchondral drilling group showed in 3 out of 6 animals an irregular articular surface, result of the uncontrolled action of the multipotential mesenchymatic cell gatherings of the blood clot produced at the defect site. The histological score was very low in these cases and influenced in a negative way the overall histological outcome although the rest 3 animals showed a smooth articular surface. The average of the histological scores of this group parameter was 1 (poor). The histological assessment of the matrix in the autologous transplantation group showed the existence of tissue of equal quality with the articular surface of a normal joint since hyaline cartilage was observed in 5 out of 6 animals and fibrocartilage in 1 animal a very good result since the primary goal of such procedures was accomplished. The average of the histological scores of this group parameter is the number 2.8 (excellent). On the other hand the subchondral drilling group showed existence of mixed hyaline and fibrocartilage tissue as well as fibrocartilage in 4 out of 6 rabbits. Histological investigation of the rest 2 animals showed fibrous tissue. The average of the histological scores of this group is the number 1.2 (poor). This observation agrees with the reports of De Palma and Mitchel according to which the procedure of cartilage defect reconstruction does not end up into a rebuilding of normal cartilage. Instead of normal cartilage the defects fill up with a mesenchymal type of tissue converting into fibrocartilage. It is well known that this type of tissue is less resilient to stress forces leading finally to degenerative changes of the joints.

Another important difference was observed in the distribution of the cells, an important structural component of normal cartilage. The presence of chondrocytes is a fact in the tissue produced by subchondral drilling as well in the cartilage of the osteochondral grafts.[6-8] The main parameter that characterizes normal hyaline cartilage concerns the distribution of those cells inside the matrix which produce the 4 zones of the chondral tissue. In all osteochondral grafts the cells were well distributed in columns or columnar-clusters and the average score of this parameter was 2. On the other hand the subchondral drilling group showed individual or disorganized cells in 5 out of 6 specimens and clusters in 1 specimen. The average score of this parameter was 0.2.

No difference was observed in the viability of the cells of both groups since all cells were viable and contributed in the reconstruction and remodeling of the treated area. The average score of this parameter was 3 for both groups. The subchondral bone in the group treated with autologous osteochondral grafts was histologically predominantly normal with an average score of 2.8 showing good integration of the grafts without any resorption of the osseous part. The group treated with subchondral drilling showed evidence of increased remodeling with an average score of 2.

Cartilage mineralization was normal in all the specimens of the graft group with a scoring average of 3. In the subchondral drilling group 3/6 specimens showed abnormal /inappropriate location. The results concerning all the histological parameters of subchondral bone and cartilage mineralization show the advantage of the osteochondral graft which provides mature, organized tissue to the defect site in comparison to the subchondral drilling which relies on the random rebuilding of the tissues.[9,10]

Conclusion

Autologous osteochondral transplantation in the treatment of an osteochondral defect of the femoral head provides superior results concerning the quality of the new articular surface tissue as well as structure. The results reward the demanding technique and the difficulty in the effort of implantation of the graft in comparison to the easiness of subchondral drilling which lacks the advantage of the ready to use structural and histological properties of the graft providing inferior histological results.

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