A Comparative Study Between Autogenous Tibial Periosteal Transplants and Mosaicplasty for Full Thickness Articular Cartilage Defects in the Knee Joints of Pigs

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ABSTRACT

An experimental study was conducted to demonstrate and compare macroscopic and microscopic healing of full thickness osteochondral defects after autogenous tibial periosteal grafts and mosaicplasty in the knee joints of pigs.

Similar full thickness articular cartilage defects were created in the medial femoral condyles of both knees in 20 healthy young adult pigs. In 10 pigs, periosteal transplants were performed on the left knees and the right knees used as controls. In the remaining 10 pigs, mosaicplasty was performed on the left knees and the right knees again used as controls. The pigs were sacrified in two groups at 6 weeks and 3 months ensuring equal numbers of mosaicplasty and periosteal transplant knees for direct comparison. The knees were inspected for healing, colour and stability of the graft. Microscopic sections were taken and evaluated using a histological score developed by O'Driscoll.

Macroscopically, both mosaicplasty and periosteal transplanted defects healed with a translucent bluish-white colour indicating articular cartilage formation. All the controls but two healed with a pinkish colour indicating fibrocartilage and granulation tissue. There was better restoration of the bony contour in the mosaicplasty knees when compared to the periosteal transplanted knees. The control knees remained uniformly depressed. Histologically, the mosaicplasty knees scored significantly better than the periosteal transplant knees. The control knees had the consistently and significantly worst results.

We conclude that both mosaicplasty and periosteal transplants in the knee of a pig model healed with mature stable hyaline cartilage.

INTRODUCTION

Traumatic articular cartilage injuries to the knee are common and difficult to treat. There have been many clinical and experimental studies on the healing and biological resurfacing of such full thickness osteochondral defects. Despite these, the ability to regenerate mature hyaline cartilage able to withstand daily functional stresses in the knee remains a utopian dream today. We have only had limited success with small osteochondral defects.

Traditionally, there are many approaches to biologic resurfacing. Osteochondral allografts[1-4] and autografts[2,5-9], perichondral[10,11] and periosteal grafts[12-24], autologus chondrocyte[25,26] and mesenchymal cell transplants[27], subchondral abrasion and microfracture[28] have all been studied extensively. Other factors such as meniscal tears, associated ligamentous instability and limb alignment are also important considerations before attempting biological resurfacing.

In this experiment, we set out with the aim of directly comparing the macroscopic and microscopic healing of periosteal transplants and mosaicplasty for articular defects in the knee joints of pigs.

MATERIALS AND METHODS

An experimental study with 20 healthy young adult pigs was conducted. Under general anaesthesia and using aseptic techniques, a medial parapatellar approach was performed on both knees. Similar full thickness cartilage defects were created in the weight bearing aspects of the medial femoral condyles. A dowel was used to create the defects 10mm in diameter and 8mm deep. In 10 pigs, autogenous periosteal grafts were harvested from the upper tibia with care taken to include the cambium layer. These were stitched to the defect with 4-O' Dexon. The deep cambium layer of the graft was superficial and facing the joint. In the right knee, the defects were irrigated and closed. These served as our controls.

In the remaining, 10 pigs, mosaicplasty was performed in the left knees. The donor site for the multiple osteochondral cylindrical pegs was the medial supracondylar ridge of the femur. The right knees were again used as control.

Post-operatively, the pigs were kept in large individual cages which allowed free movement and let out for half hour walks three to four times a day. They were given dry pellet food and water and covered with post-operative antibiotics. There were no wound infections. The pigs were able to stand on all limbs by 48 to 72 hours. They were walking with a slight limp by 5 to 7 days. At two weeks, they were walking normally. Unfortunately, one pig died on the 18th day from an unrelated cause (intraabdominal sepsis).

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Eight pigs were sacrificed at 6 weeks and ten pigs at 3 months ensuring equal numbers of mosaicplasty and periosteal transplant knees for direct comparsion. The knees were inspected for macroscopic healing. We looked at the size and filling of the defect, the restoration of the contour, the colour and stability of the graft. The margins were then inspected for incorporation to the surrounding articular cartilage.

Microscopic sections were taken and stained with Haematoxylin and eosin (H&E), Periodic acid Schiff (PAS), Alcian blue, Masson trichrome and Van Gieson. H&E was the stain for routine light microscopy. PAS stained glycogen and basement membrane. Alcian blue was used specifically for acid mucopolysaccharides. Van Gieson stained elastin tissue black and Masson trichrome was used to colour collagen fibres green. An independent scoring was performed by a histopathologist* using the following parameters: Filling of the defect, nature of predominant tissue, reconstitution of the osteochondral junction, bonding to adjacent cartilage, matrix staining, cellularity and structural integrity. An aggregate score was then determined for each specimen. Each parameter and the aggregate score were then statistically analysed against the corresponding control knees using the Wilcoxan Rank Sum Test. We also analysed the periosteal transplant against the mosaicplasty knees.

RESULTS

At six weeks, all mosaicplasty and most of the periosteal transplant knees healed with a translucent bluish white colour indicating articular cartilage formation. All but one of the control knees healed with pinkish colour indicating granulation tissue and fibrocartilage. All mosaicplasty knees and half the periosteal transplant knees healed with good restoration of the contour and level of the articular cartilage. The control knees had defects which were uniformly depressed (Table 1).

We evaluated the histological specimens using a grading scale developed by O' Driscoll (Table 2). The results were tabulated (Table 3). Immature hyaline cartilage was seen in two periosteal transplant and two mosaicplasty knees. Mature hyaline cartilage was seen in two mosaicplasty knees. The control knees, however, healed with undifferentiated mesenchymal cells and fibrous tissue. Statistical analysis revealed that :

1. Mosaicplasty knees were significantly better than the periosteal transplant knees (P=0.03).
2. The control knees had the significantly poorest histological scores (P =0.03 in periosteal transplant knees and P=0.006 in the mosaicplasty knees).

At three months, all mosaicplasty and periosteal transplant knees healed with a bluish white colour. All controls but two healed with a pinkish colour. All the mosaicplasty and periosteal transplant knees healed with good restoration of the bony contour. The controls remained uniformly depressed (Table 4).

We tabulated the histological scores (Tables 5). Mature hyaline cartilage was demonstrated in all mosaicplasty (Figure 1) and two periosteal transplant knees. Immature hyaline cartilage was seen in another two periosteal transplant knees (Figures 2a & 2b). Again, most of the control knees healed with undifferentiated cells (Figure 3). Immature hyaline cartilage were seen in four control knees. Statistical analysis again revealed that :

Table 1 Macroscopic findings at 6 weeks

Periosteal Transplant

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
P1	Blue White	Smooth	Level	Almost Complete
P2	Faint Red	Smooth	Depressed	Partial
P3	Blue White	Slightly Rough	Level	Almost Complete
P4	Blue White	Smooth	Depressed	Partial

Mosaicplasty

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
M1	Blue White	Smooth	Depressed	Partial
M2	Blue White	Smooth	Level	Complete
M3	Blue White	Smooth	Level	Almost Complete
M4	Blue White	Smooth	Level	Almost Complete

Periosteal Control

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
C1	Pink White	Smooth	Depressed	None
C2	Pink White	Smooth	Depressed	None
C3	Faint Red	Smooth	Depressed	None
C4	Faint Red	Smooth	Depressed	None

Mosaicplasty Control

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
Cm1	Faint Red	Slightly Rough	Depressed at Centre	Partial
Cm2	Pink White	Smooth	Depressed at Centre	None
Cm3	Blue White	Smooth	Depressed at Centre	None
Cm4	Pink White	Smooth	Depressed at Centre	Partial

1. Mosaicplasty knees were significantly better than periosteal transplant knees (P=0.04).
2. The control knees had the significantly poorest histological scores (P=0.04 in periosteal transplant and P=0.008 in the mosaicplasty knees).

Fig. 1 - Mosaicplasty knee at 3 months. Mature hyaline cartilage has filled the defect except for a small area which is not well bonded at the edge (Haematoxylin & Eosin, x 20)

Table 2 Histological grading scale

Particulars of Study		Score
Filling of Defect :		
125%	(Above Articular Surface)	1
100%	(At Articular Surface)	0
75%	(At Osteochondral Junction)	1
50%	(At Subchondral Level)	2
25%	(At Bone)	3
0%	(None)	4
Nature of Predominant Tissue :		
Mature Hyaline Cartilage		0
Immature Hyaline Cartilage		1
Undifferentiated Mesenchymal Cells		2
Fibrous Tissue		3
Reconstitution of Osteochondral Junction :		
Yes		0
Almost		1
Not Close		2
Bonding To Adjacent Cartilage :		
Bonded at Both Ends		0
Bonded at One End or Partially at Both		1
Not Bonded		2
Matrix Staining :		
Normal		0
Reduced Staining		1
Significantly Reduced		2
Faint Staining		3
No Stain		4
Cells :		
Normal		0
Diffuse Hypercellularity		1
Cloning		2
Hypocellularity		3
Structural Integrity :		
Intact		0
Partially Disrupted		1
Completely Disrupted		2

DISCUSSION

We have known about the osteogenic capacity of periosteum ever since the classical studies of Duhamel[29]. Other investigators[30,31] have shown us that the deep cambium layer was responsible for bony regeneration infracture healing. Ham[31] demonstrated that this cambiumlayer was able to differentiate into both bone and cartilageforming cells. This fascinating dual potential ofperiosteum has since been confirmed by many investigators[3,15-23,32,33]. Salter[34], Rubak[20-22] and O'Driscoll[15-18] have demonstrated that free periosteal grafts with the cambium layer superficial in the knee joints of rabbits healed with tissue that resembled articular cartilage. They concluded that continuous passive motion and not immobilisation were vital to the chondrogenic ability of the transplanted periosteum.

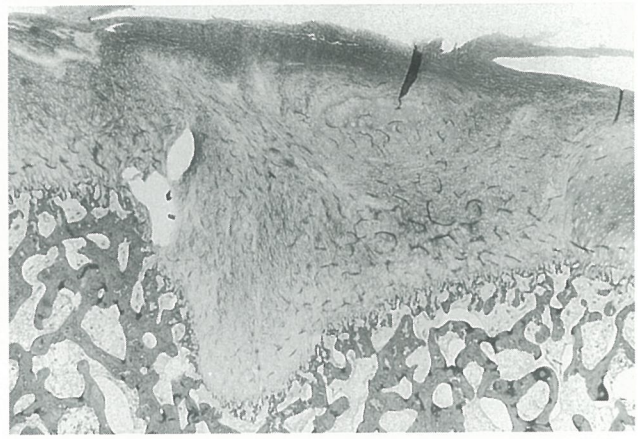

Fig. 2a - Periosteal transplant knee at 3 months. Immature hyaline cartilage is seen filling the defect with focal poor bonding at the edge (Haematoxylin & Eosin, x 20)

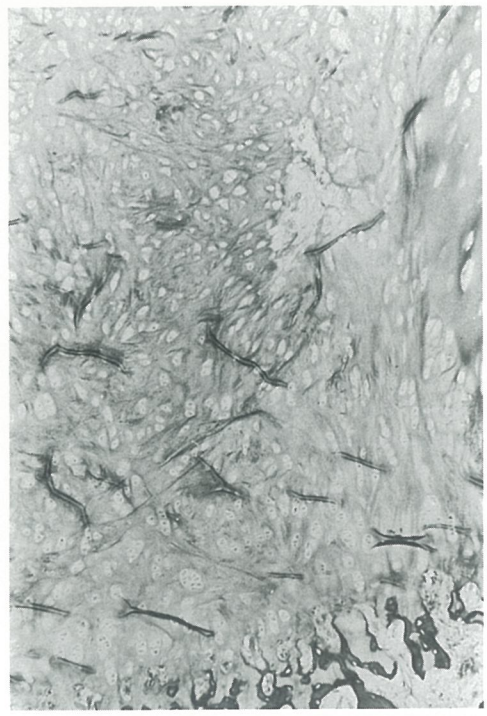

Fig. 2b - High power view of Fig 2a. Showing immature hyaline catilage above and at the preosseous interface (Haematoxylin & Eosin, x 100)

Clinical studies with follow up arthroscopy have demonstrated good healing of full thickness defects in the femoral condyles after autogenous tibial periosteal transplants. Niederman[14] reported the use of glued periosteal grafts for osteochondritis dissecans of the medial femoral condyle in 3 patients and osteonecrosis of the lateral femoral condyle in one patient. Follow-up arthroscopy was performed at 3, 6 and 12 months. At one year, the defects had healed well with stable cartilage-like tissue and indistinct borders. Other studies utilising MRI have also shown complete filling of articular defects. Lorentzon[35] reported the use of autogenous periosteal grafts in full-thickness patellar cartilage defects. Thirteen patients were followed up with repeat MRI at one year which showed complete filling of the defects. These patients had good to excellent clinical results.

In this study, we demonstrated that transplanted periosteum was able to differentiate into immature hyaline cartilage which matured at 3 months. The synovial fluid environment in which the graft lies provide a source of nutrition as well as possible chondrogenic factors to stimulate this fascinating transformation. We believe that articular loading and mobilisation also helped in the eventual formation of mature hyaline cartilage. The newly formed cartilage was stable and well incorporated. It had almost complete bonding to the adjacent articular margin, good reconstitution of the osteochondral junction and a well-maintained structural integrity. In the control groups, the defects remained uniformly depressed. The deeper aspects of the crater were partially filled with a connective tissue which resembled primitive mesenchymal cells, fibrous tissue and fibrocartilage. There were two control knees with immature hyaline cartilage cells at three months.

Fig. 3 - Control knee at 3 months. The depressed defect is filled with predominantly fibrous tissue and undifferentiated cells. (Haematoxylin & Eosin, x 20)

Table 3. Histological scores at 6 weeks

Mosaicplasty								
Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
M1	0	1	1	0	0	1	0	0.43
M2	1	0	1	0	1	1	0	0.57
M3	1	1	1	1	1	1	0	0.86
M4	0	0	1	0	1	1	0	0.43
Mean Score	0.50	0.50	1.00	0.25	0.75	1.00	0.00	0.57
Periosteum Transplant								
Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
P1	3	2	2	1	1	1	1	1.71
P2	0	2	1	0	1	1	0	0.71
P3	3	1	1	0	1	1	1	1.14
P4	2	1	1	0	1	1	0	0.86
Mean Score	2.00	1.50	1.25	0.25	1.00	1.00	0.50	1.07
Mosaicplasty Control								
Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
Cm1	3	2	2	1	2	2	2	2.00
Cm2	2	2	1	1	1	2	1	1.43
Cm3	3	3	2	2	3	3	2	2.57
Cm4	2	3	1	1	1	3	1	1.71
Mean Score	2.50	2.50	1.50	1.25	1.75	2.50	1.50	1.86
Periosteum Control								
Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
C1	4	3	2	2	3	3	2	2.71
C2	3	3	2	2	3	3	2	2.57
C3	3	2	1	1	1	2	1	1.57
C4	4	2	1	2	2	2	0	1.85
Mean Score	3.50	2.50	1.50	1.75	2.25	2.50	1.25	2.18

Osteochondral grafts have been used to resurface articular cartilage defects for many years. Depalma[4] and Campbell[2] have shown us in early animal experiments on dogs that transplantation of autogenous osteochondral grafts could survive with creeping substitution of the cancellous bone. Functional, mature and viable hyaline cartilage was also well demonstrated up to a year after transplantation. Other authors have also shown us that good clinical results were possible with autogenous osteochondral grafts. Yamashita[36] treated large articular cartilage defects in two adult patients by transplanting autogenous osteochondral grafts harvested from the antero-medial portion of the medial femoral condyle. These were held by small cancellous screws. The patients improved clinically and biopsies taken at 2-3 years at the time of screw removal revealed that there was evidence of bony union and the formation of fibrocartilage at the edges of the graft. The hyaline cartilage of the graft survived and looked macroscopically and histologically normal. Matsusue[37] performed simultaneous arthroscopic anterior cruciate ligament reconstruction and multiple autogenous cylindrical osteochondral grafts harvested from the lateral supracondylar ridge for osteochondral defects. He also demonstrated good clinical results and survival of mature hyaline cartilage at 2 years.

In recent years, Hangody[38-40] has treated full thickness articular cartilage defects of the knee and ankle joints with mosaicplasty. With the use of multiple small cylindrical osteochondral pegs, he was able to fill the defect with up to 80% of mature hyaline cartilage. The intervening spaces filled in with fibrocartilage grouting from the underlying cancellous bed. Good clinical results were obtained. Ultrasound, MRI and CT arthrography revealed good congruity and satisfactory cartilage thickness in the grafted defects.

In our study, we demonstrated that transplanted mature hyaline cartilage in mosaicplasty survived even at three months. Proper press fitting of the grafts into the defects resulted in good restoration of the level of the articular surface and the overall contour of the femoral condyle. We believe that active mobilisation was possible after placing well-fitted grafts into the defect. This helped in the nutrition of the transplanted cells and eventually to the survival of these mature cartilage cells. The grafts were stable and well incorporated. They had almost complete bonding to the adjacent articular margin, good reconstitution of the osteochondral junction and a well-maintained structural integrity.

Lastly, when we compare the two forms of biologic resurfacing, mosaicplasty proved to be better at restoring the congruity and level of the articular cartilage defects. It was more consistent in providing mature hyaline cartilage to fill the defects. In the periosteal transplant knees, we were only able to demonstrate that the defects healed with mature stable hyaline cartilage in 40% of our specimens.

In the control groups, the defects always remained uniformly depressed with the deeper aspects of the crater filled with connective tissue that resembled primitive mesenchymal cells, fibrous tissue, fibrocartilage and immature hyaline cartilage.

Table 4. Macroscopic findings at 3 months

Periosteal Transplant

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
P6	Blue White	Rough	Partial Depressed	Partial
P7	Blue White	Rough	Depressed at Centre	Partial
P8	Blue White	Smooth	Depressed	None
P9	Blue White	Smooth	Level	Partial
P10	Blue White	Smooth	Level	Partial

Mosaicplasty

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
M6	Blue White	Smooth	Level	Complete
M7	Blue White	Smooth	Elevated	Partial
M8	Blue White	Rough		Partial
M9	Blue White	Smooth	Level	Complete
M10	Blue White	Smooth	Level	Complete

Periosteal Control

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
C6	Pink White	Smooth	Depressed	None
C7	Pink White	Smooth	Depressed	None
C8	Pink White	Smooth	Depressed	None
C9	Pink White	Smooth	Depressed	None
C10	Blue White	Smooth	Depressed	None

Mosaicplasty Control

Pig Group	Colour	Regenerated Tissue	Level of New Tissue	Restoration of Contour
Cm6	Pink	Rough	Depressed	None
Cm7	Pink	Smooth	Depressed	None
Cm8	Pink	Smooth	Depressed	None
Cm9	Pink	Rough	Depressed	None
Cm10	Blue	Rough	Depressed	None

CONCLUSION

Both mosaicplasty and periosteal transplants in the knee of a pig model healed with mature stable hyaline cartilage at 3 months. In both groups, the newly formed tissue had almost complete bonding to the adjacent articular margin, good reconstitution of the osteochondral junction and a well-maintained structural integrity which was stable and well incorporated. Macroscopically, there was better restoration of the bony contour and superior filling of the defect in the mosaicplasty group. Histologically, mosaicplasty provided significantly better results.

These results are encouraging although the long-term durability of the grafts and their clinical correlation are unknown.

In future studies, we plan to do larger series with longer follow-ups. Experimental work comparing the different forms of biological resurfacing and finally more clinical studies must be performed to help us fully understand this fascinating field of cartilage regeneration.

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Table 5. Histological scores at 3 months

Mosaicplasty

Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
M6	1	0	0	0	1	1	0	0.43
M7	1	0	0	0	0	0	1	0.29
M8	1	0	0	0	0	0	0	0.14
M9	1	0	0	2	0	0	2	0.71
M10	1	0	0	1	0	0	0	0.29
Mean Score	1.00	0.00	0.00	0.60	0.20	0.20	0.60	0.37

Periosteal Transplant

Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
P6	3	3	2	0	3	3	2	2.29
P7	1	0	0	0	1	1	0	0.43
P8	0	1	0	0	1	1	0	0.43
P9	1	1	0	0	1	1	0	0.57
P10	1	0	0	0	1	0	0	0.29
Mean Score	1.20	1.00	0.40	0.00	1.40	1.20	0.40	0.94

Mosaicplasty Control

Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
Cm6	2	3	1	0	3	3	1	1.86
Cm7	2	1	1	1	1	1	1	1.14
Cm8	2	3	0	0	3	3	0	1.57
Cm9	1	3	1	1	3	3	1	1.86
Cm10	2	1	1	1	1	1	0	1.00
Mean Score	1.80	2.20	0.80	0.60	2.20	2.20	0.60	1.49

Periosteum Control

Group	Defects	Tissue	O-C	Bonding	Matrix	Cells	Integrity	Score
C6	3	2	1	1	1	1	1	1.43
C7	1	3	0	0	3	3	0	1.43
C8	2	1	1	1	1	1	1	1.14
C9	1	3	0	0	3	3	1	1.57
C10	2	1	0	1	1	1	1	1.00
Mean Score	1.80	2.00	0.40	0.60	1.80	1.80	0.80	1.31

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