

The Results of Autogenous Tibial Periosteal Transplants for Full Thickness Cartilage Defects in the Knee Joints of Pigs

PCC Chang,*^{FAMS, MBBS FRCS (Edin)}, R M Pradhan,**^{MBBS, MS (Orth)}, A K Mitra,***^{MBBS, FRCS (Glas)},
CSSim,†^{MBBS, FRCPA}, BK Tay,‡^{FAMS, MBBS, FRCS Ed (Orth)}

Abstract

An experimental study was conducted to demonstrate macroscopic and microscopic healing of full thickness cartilage defects with mature stable hyaline cartilage after autogenous tibial periosteal transplants in the knee joints of pigs.

Similar full thickness osteochondral defects were created in the medial femoral condyles of both knees in 10 healthy young adult pigs. Periosteal transplants were performed on the left knees and the right knees used as controls. The pigs were sacrificed in two groups at 6 weeks and 3 months. The knees were inspected for healing and stability of the graft. Microscopic sections were taken and evaluated using a histological score developed by O'Driscoll.

Macroscopically, almost all defects with periosteal grafts healed with a translucent bluish-white colour indicating articular cartilage formation. There was good restoration of the bony contour and filling of the defects were superior to controls. Histologically, we were able to demonstrate immature hyaline cartilage which matured at 3 months. The newly formed tissue 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.

We concluded that periosteal transplants in the knee of a pig model healed with mature stable hyaline cartilage.

Ann Acad Med Singapore 1999; 28:8-14

Key words: Hyaline cartilage, Osteochondral defects, Osteochondritis dissecans, Traumatic osteochondral fractures

Introduction

Articular cartilage injuries to the knee are commonly seen by team doctors, sports physicians and orthopaedic surgeons. Despite many clinical as well as experimental studies on the healing of such defects, the ability to regenerate mature hyaline cartilage capable of withstanding daily functional stresses in the knee remains a utopian dream today. We have only had limited success with small osteochondral defects.

There are many approaches to biologic resurfacing. Osteochondral allografts¹⁻⁴ and autografts,^{2,5-9} perichondral^{10,11} and periosteal grafts,¹²⁻²⁴ chondrocyte transplants,^{25,26} mesenchymal cell transplants,²⁷ subchondral abrasion and microfracture²⁸ have all been extensively studied.

In this experiment, we set out with the aim of

demonstrating mature stable hyaline cartilage after periosteal transplants for articular defects in the knee joints of pigs.

Materials and Methods

An experimental study with 10 healthy young adult pigs was conducted. Each pig weighed between 30 to 35 kg. 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 10 mm in diameter and 8 mm deep. In the left knee, periosteal grafts were harvested from the upper tibia with care taken to include the cambium layer. This was stitched to the defect with 4-0 Dexon. The deep cambium layer of the graft was superficial and facing the joint. In

* Consultant

** Fellow

*** Senior Consultant

‡ Senior Consultant and Head

Department of Orthopaedic Surgery

† Senior Consultant

Department of Pathology

Singapore General Hospital

Address for Reprints: Dr P C C Chang, Department of Orthopaedic Surgery, Singapore General Hospital, 1 Hospital Drive, Singapore 169608.

the right knee, the defects were irrigated and closed. These served as controls.

Postoperatively, 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 postoperative ampicillin and cloxacillin.

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 the 5th to 7th days. At 2 weeks, they were walking normally. Unfortunately, 1 pig died on the 18th day from an unrelated cause (intra-abdominal sepsis).

Four pigs were sacrificed at 6 weeks and 5 pigs at 3 months. 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 into 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 (Table I). 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 Wilcoxon Rank Sum test.

Results

At six weeks, half the periosteal transplant knees healed with good restoration of the bony contour and level of the articular cartilage. Most healed with a translucent bluish white colour indicating articular cartilage formation. On the other hand, the control knees remained uniformly depressed and healed with a pink colour indicating granulation tissue and fibrocartilage (Table II).

We evaluated the histological specimens using a grading scale developed by O' Driscoll (Table I). Immature hyaline cartilage was demonstrated in two periosteal transplant knees. The control knees healed with undifferentiated mesenchymal cells and fibrous tissue. There was also better matrix staining, good bonding to adjacent cartilage and diffuse hypercellularity in the periosteal transplant knees. Statistical analysis with the

TABLE I: 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 closed	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

Wilcoxon Rank Sum test was performed and the test knees scored significantly better (Table III).

At three months, we again found that the periosteal transplant knees healed with better restoration of the bony contour (Fig. 1) and with a bluish white translucent stable graft which resembled articular cartilage macroscopically. The controls however remained uniformly depressed (Fig. 2) and predominantly with a pinkish hue indicating fibrocartilage formation (Table IV).

Histologically, there was mature hyaline cartilage in two periosteal transplant knees (Figs. 3a & 3b) and immature hyaline cartilage in another two knees. Unfortunately, one knee only had fibrous tissue as the predominant tissue. In the control knees, there were two specimens with immature hyaline cartilage but undif-

TABLE II: MACROSCOPIC FINDINGS AT 6 WEEKS

Periosteal test knees							
Pig group	Incision	Size of graft	Shape of graft	Colour	Regenerated tissue		Stability
					Smooth	Rough	
P1	Healed	7/7 mm	Circular	Blue white	Smooth		Stable
P2	Healed	6/6 mm	Circular	Faint red	Smooth		Stable
P3	Healed	6/7 mm	Oval	Blue white		Slightly rough	Stable
P4	Healed	8/8 mm	Circular	Blue white	Smooth		Stable

Pig group	Relation to margin	Level of new tissue			Restoration of contour		
		Elevated	Level	Depressed	Complete	Partial	None
P1	Incorporated		Level		Almost complete		
P2	Partial incorporated			Depressed medially		Partial	
P3	Incorporated		Level		Almost complete		
P4	Incorporated			Partial depressed		Partial	

Control knees							
Pig group	Incision	Size of graft	Shape of graft	Colour	Regenerated tissue		Stability
					Smooth	Rough	
C1	Healed	7/7 mm	Circular	Pink white	Smooth		Stable
C2	Healed	6/5 mm	Oval	Pink white	Smooth		Stable
C3	Healed	6/6 mm	Circular	Faint red	Smooth		Stable
C4	Healed	7/7 mm	Circular	Faint red	Smooth		Stable

Pig group	Relation to margin	Level of new tissue			Restoration of contour		
		Elevated	Level	Depressed	Complete	Partial	None
C1	Incorporated			Depressed			None
C2	Incorporated			Depressed			None
C3	Incorporated			Depressed			None
C4	Incorporated			Depressed			None

TABLE III: HISTOLOGICAL SCORES AT 6 WEEKS

Periosteal transplant knees								
Pig group	Filling of defects	Nature of predominant tissue	O-C junction	Bonding	Matrix staining	Cells	Structural integrity	Aggregate 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

Control knees								
Pig group	Filling of defects	Nature of predominant tissue	O-C junction	Bonding	Matrix staining	Cells	Structural integrity	Aggregate 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

TABLE III: contd.

Statistical analysis		
Filling of defect	0.100	ns
Predominant tissue	0.041	s
Reconstitution of O-C junction	0.1128	ns
Bonding	0.01	s
Matrix staining	0.028	s
Cells	0.026	s
Structural integrity	0.126	ns
Aggregate score	0.026	s

ns: not significant; s: significant



Fig. 1. Macroscopic appearance of a periosteal transplanted knee at 3 months. There is good filling of the defect with a white stable translucent graft.

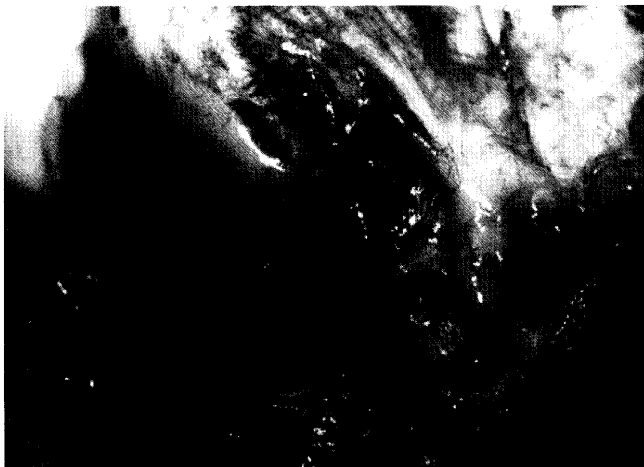


Fig. 2. Macroscopic appearance of a control knee at 3 months. There is a deep crater with poor restoration of the bony contour.

ferentiated mesenchymal cells or fibrous tissue (Figs. 4a & 4b) in the rest. There was better filling of the defect, reconstitution of the osteochondral junction, matrix staining and cellularity in the periosteal transplanted knees. Statistical analysis again showed that the periosteal transplanted knees did significantly better (Table V).

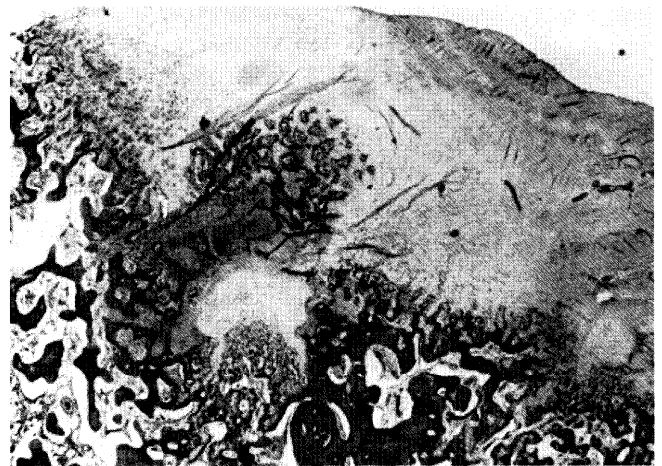


Fig. 3a. Periosteal transplant at 3 months. There is near complete replacement of the defect with irregular mature hyaline cartilage (Haematoxylin and eosin x20).

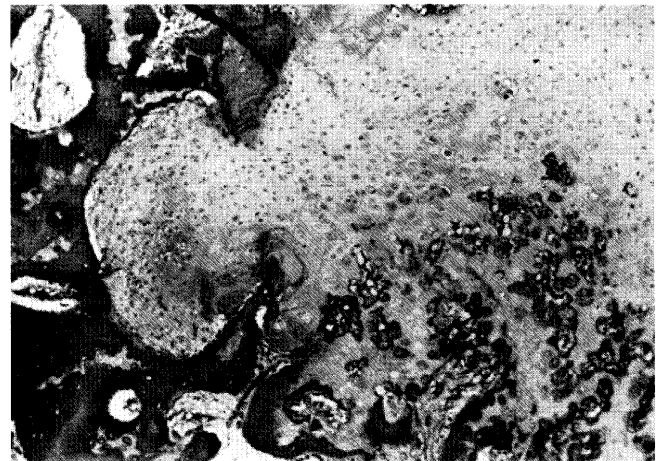


Fig. 3b. Periosteal transplant at 3 months. High power view of the well formed hyaline cartilage as shown in figure 3a (Haematoxylin and eosin x100).

Discussion

The osteogenic capacity of periosteum has been documented ever since the classical studies of Duhamel.²⁹ Other investigators^{30,31} have shown that the deep cambium layer is responsible for bony regeneration in fracture healing. Ham³² demonstrated that this cambium layer was able to differentiate into both bone and cartilage forming cells. This fascinating dual potential of periosteum has since been confirmed by many investigators.^{15-23,33,34}

Rubak,²⁰ O'Driscoll¹⁵ and Salter³⁵ 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.

Clinical studies with follow-up arthroscopy have demonstrated good healing of full thickness defects in the femoral condyles after autogenous tibia1 periosteal trans-

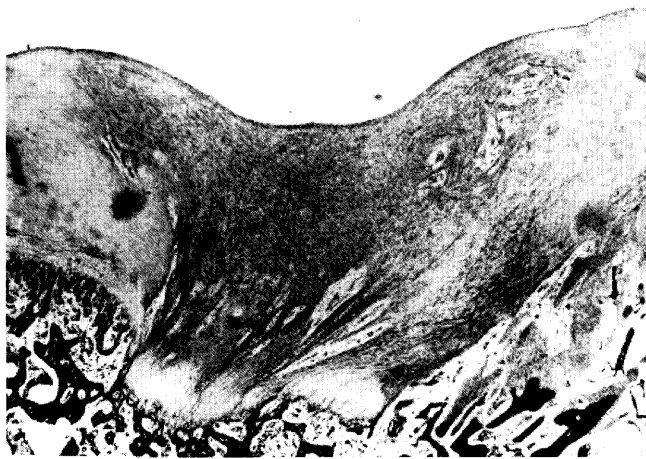


Fig. 4a. Control case at 3 months. The superficial portion of the defect is filled with predominantly fibrous tissue. Some new bone and preosseous cartilage is seen in the depth of the defect (Haematoxylin and eosin x20).

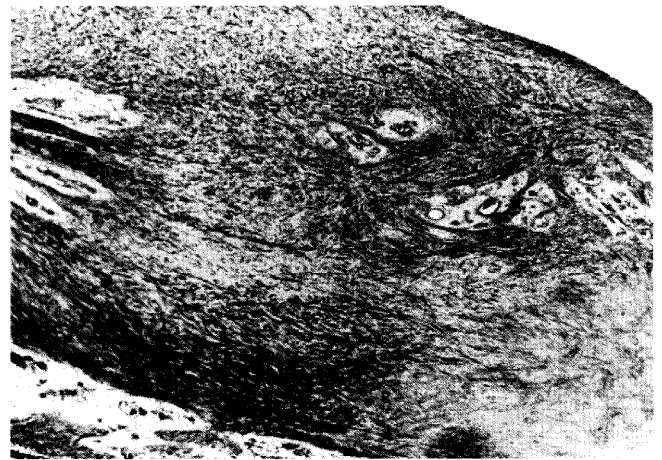


Fig 4b. Control case at 3 months. High power view of the fibrous tissue seen in figure 4a (Haematoxylin and eosin x40)

TABLE IV: MACROSCOPIC FINDINGS AT 3 MONTHS

Periosteal test knees							
Pig group	Incision	Size of graft	Shape of graft	Colour	Regenerated tissue		Stability
					Smooth	Rough	
P6	Healed	9/10 mm	Oval	Pink white		Rough	Stable
P7	Healed	14 x 14 mm	Circular	Blue white		Rough	Stable
P8	Healed	8 x 7 mm	Oval	Blue white	Smooth		Stable
P9	Healed	11 x 1 mm	Circular	Blue white	Smooth		Stable
P10	Healed	8 x 10 mm	Oval	Blue white	Smooth		Stable

Pig group	Relation to margin	Level of new tissue			Restoration of contour		
		Elevated	Level	Depressed	Complete	Partial	None
P6	Incorporated			Partially depressed		Partial	
P7	Incorporated		Level	Depressed at centre		Partial	
P8	Incorporated			Depressed			None
P9	Incorporated		Level			Partial	
P10	Incorporated		Level			Partial	

Control knees							
Pig group	Incision	Size of graft	Shape of graft	Colour	Regenerated tissue		Stability
					Smooth	Rough	
C6	Healed	8/8 mm	Circular	Pink white	Smooth		Stable
C7	Healed	8 x 9 mm	Oval	Pink white	Smooth		Stable
C8	Healed	9 x 9 mm	Circular	Pink white	Smooth		Stable
C9	Healed	10 x 10 mm	Circular	Pink white	Smooth		Stable
C10	Healed	8 x 7 mm	Oval	Blue white	Smooth		Stable

plants. Niederman et al¹⁴ 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 et al³⁶ reported the use of autogenous periosteal grafts in full-thickness patellar cartilage de-

TABLE IV: contd.

Pig group	Relation to margin	Level of new tissue			Restoration of contour		
		Elevated	Level	Depressed	Complete	Partial	None
C6	Incorporated			Depressed			None
C7	Incorporated			Depressed			None
C8	Incorporated			Depressed			None
C9	Incorporated			Depressed			None
C10	Incorporated			Depressed			None

TABLE V: HISTOLOGICAL SCORES AT 3 MONTHS

Pig group	Filling of defects	Nature of predominant tissue	Periosteal transplant knees					Structural integrity	Aggregate score
			O-C junction	Bonding	Matrix staining	Cells			
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	

Pig group	Filling of defects	Nature of predominant tissue	Control knees					Structural integrity	Aggregate score
			O-C junction	Bonding	Matrix staining	Cells			
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	

Statistical analysis

	<i>P</i>	
Filling of defect	0.0143	s
Predominant tissue	0.0046	s
Reconstitution of O-C junction	0.0143	s
Bonding	0.8174	ns
Matrix staining	0.0109	s
Cells	0.0109	s
Structural integrity	0.8174	ns
Aggregate score	0.0086	s

ns: not significant; s: significant

fects. 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. Although mature hyaline cartilage was observed in only 40% of the periosteal transplant knees, the ability of periosteum to remarkably change into mature hyaline cartilage is indeed an amazing phenomenon. Chondrocyte transplantation under periosteal flaps may improve this success rate and is the subject of ongoing clinical and experimental trials.

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.

Conclusion

Periosteal transplants to full thickness articular cartilage defects in the knee of a pig model healed with mature stable hyaline cartilage. These results are encouraging although the long term durability of the graft and its clinical correlation are unknown.

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

Acknowledgements

The authors are grateful to the following for contributing to the successful completion of this study:

- Medical Research Council for their financial support.
- 2) Department of Experimental Surgery, especially Mr Robert Ng, Ms Ong In Chin and Ms Irene Kee for their invaluable help.
- 3) Mr Barry Pereira for his expert statistical advice.
- 4) Ms Cecilia Siong for her patient secretarial support.

REFERENCES

- Beaver R J, Mahomed M, Backstein D. Fresh osteochondral allografts for post-traumatic defects in knee. A survivorship analysis. *J Bone Joint Surg* 1992; 74(B):105-10.
- Campbell C J, Ishida H, Takashi H, Kelly F. The transplantation of articular cartilage. An experimental study in dogs. *J Bone Joint Surg* 1963; 45A:1579-92.
- Coventry F R, Akeson W H, Keown G H. Long term survival of chondrocytes in an osteochondral cartilage allograft. *J Bone Joint Surg* 1996; 78(A):1082-8.
- Depalma A F, Swyer B, Hoffman J D. Fate of osteochondral grafts. *Clin Orthop* 1962; 22:217-34.
- Aichorth I'. Osteochondral fractures and their relationship to osteochondritis dissecans of the knee. *J Bone Joint Surg* 1971; 53(B):448-554.
- Bovlals H J. Autograft replacement of small joint defects in the hand. *Clin Orthop* 1996; 327:63-71.
- Gillespie A F, Day B. Bone peg fixation in the treatment of osteochondritis dissecans of the knee joint. *Clin Orthop* 1979; 143:125-9.
- Lane J M, Brighton C T, Ottens H R, Lipton M. Joint resurfacing in the rabbit using an autologous osteochondral graft. *J Bone Joint Surg* 1977; 59(A):218-22.
- Lindholm T S, Osterman K, Kinnunen P. Reconstruction of the joint surface using osteochondral fragments. *Scand J Rheumatol (Suppl)* 1982; 44:5-46.
- Engvist O, Ohlson L. Reconstruction of articular cartilage with free autologous perichondral grafts. *Scand J Plast Reconstr Surg Hand Surg* 1979; 13:269-74.
- Skoog T, Johansson S H. The formation of articular cartilage from free perichondral grafts. *Plast Reconstr Surg* 1976; 51:1-6.
- Jaroma H J, Ritsila V A. Reconstruction of patellar cartilage defects with free periosteal grafts. *Scand J Plast Reconstr Surg Hand Surg* 1987; 21:175-81.
- Kreder H J, Moran M, Xeeley F W, Salter R B. Biological resurfacing of a major defect with cryopreserved allogenic periosteum under the influence of continuous passive motion in a rabbit model. *Clin Orthop* 1994; 300:288-96.
14. Niederman B, Boe S, Lavritzen. Glued periosteal grafts in the knee. *Acta Orthop Scand* 1985; 56:457-60.
15. O'Driscoll S W, Salter R B. The induction of neochondrogenesis in free intraarticular periosteal autografts under the influence of continuous passive motion. *J Bone Joint Surg* 1984; 66(A):1248-57.
16. O'Driscoll S W, Salter R B. The repair of major osteochondral defects in the joint surface by neochondrogenesis with autogenous osteoperiosteal grafts by continuous passive motion. *Clin Orthop* 1986; 208:131-40.
17. O'Driscoll S W, Keeley F W, Salter R B. The chondrogenic potential of free autogenous periosteal grafts for biological resurfacing of major full thickness defects in joint surface under the influence of continuous passive motion. *J Bone Joint Surg* 1986; 68(A):1017-34.
18. O'Driscoll S W, Keeley F W, Salter R B. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full thickness defects in the joint surface under the influence of continuous passive motion. *J Bone Joint Surg* 1988; 70(A):595-606.
19. Ritsila V, Alhopuro S, Rintala A. Bone formation with periosteum. *Scand J Plast Reconstr Surg Hand Surg* 1972; 6:51-6.
20. Rubak J M. Reconstruction of articular cartilage defects with free periosteal grafts. *Acta Orthop Scand* 1982; 53:175-80.
21. Rubak J M, Poussa M, Ritsila V. Effects of joint motion on the repair of articular cartilage with free periosteal grafts. *Acta Orthop Scand* 1982; 53:187-91.
22. Rubak J M, Poussa M, Ritsila V. Chondrogenesis in repair of articular cartilage defects by free periosteal grafts in rabbits. *Acta Orthop Scand* 1982; 53:181-6.
23. Tonna E A, Cronkite E I'. The periosteum: autoradiographic studies on cellular proliferation and transformation utilising tritiated thymidine. *Clin Orthop* 1963; 30:218-31.
24. Zarnett R, Delaney J I', O'Driscoll S W, Salter R B. Cellular origin and evolution of neochondrogenesis in major full thickness defects of a joint surface treated by free autogenous periosteal grafts and subjected to continuous passive motion in rabbits. *Clin Orthop* 1987; 222:267-74.
25. Britt B M, Lindahal A, Nilson A. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; 331:889-95.
26. Green W T. Articular cartilage repair; Behavior of rabbit chondrocytes during tissue culture and subsequent allografting. *Clin Orthop* 1979; 124:237-48.
27. Wakitani S, Gato Pineda S J. Mesenchymal cell based repair of large full thickness defects of articular cartilage. *J Bone Joint Surg* 1994; 76(A):579-92.
28. Steadman J R, Sterett W I. The surgical treatment of knee injuries in skiers. *Med Sci Sports Exercise* 1995; 23:328-33.
29. Duhamel H L (1739). Cited by Bassett. Current concepts of bone formation. *J Bone Joint Surg* 1962; 44(A):1217-44.
30. Feil H B. Chondrogenesis in cultures of endosteum. *Proc R Soc Lond (Biol)* 1932; 112:417-27.
31. Zuckman J, Maurer I', Berbeson C. The effects of autografts of bone and periosteum in recent diaphyseal fractures. An experimental study in rabbits. *J Bone Joint Surg* 1968; 50(B):409-22.
32. Ham A W. Repair of fracture. In: *Histology*. 5th ed. London: Lippincott, 1965:436-44.
33. Bassett A L. Current concepts of bone formation. *J Bone Joint Surg* 1962; 44(A):1217-39.
34. Kernek C B, Wray J B. Cellular proliferation in the formation of fracture callus in the rat tibia. *Clin Orthop* 1973; 91:197-209.
35. Salter R B, Simmonds D F, Malcolm B W. Biological effects of continuous passive motion on the healing of full thickness defects in articular cartilage. *J Bone Joint Surg* 1980; 62(A):1232-51.
36. Lorentzon R, Hildingsson C, Alfredsson H. Treatment of deep cartilage defects in the knee with periosteum transplantation. *Knee Surg Sport Traumatol Arthrosc* 1998; 6:202-8.