

Transplantation of the Autogenous Chondrocyte Graft to Physeal Defects: an Experimental Study in Pigs

P. GÁL¹, A. NEČAS², J. ADLER³, O. TEYSCHL¹, P. FABIÁN⁴, Š. BIBROVÁ¹

¹Department of Pediatric Surgery, Orthopaedics and Traumatology, Medical Faculty,
Masaryk's University, Brno, Czech Republic

²Department of Surgery and Orthopaedics, Small Animal Clinic, Faculty of Veterinary Medicine,
University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

³Tissue Bank, Medical Faculty, Masaryk's University, Brno, Czech Republic

⁴Department of Pathology, Medical Faculty, Masaryk's University, Brno, Czech Republic

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Abstract

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The aim of the experiment was to evaluate the possibility of preventing bone bridge formation in damaged distal femoral physis using implantation of autogenous chondrocyte graft. Ten pigs were used in the study. In each pig, a sample of cartilage of 3 × 3 mm in diameter was taken from non-weight-bearing surface of the lateral femoral condyle and used to culture chondrocytes for the preparation of the autogenous chondrocyte graft.

Fourteen days later, a canal of 4 mm diameter was drilled through the distal femoral physis in the area of the lateral condyle. This canal was then filled with the autogenous chondrocyte graft. The same defect, left unfilled, was created in the physis of the medial condyle. One animal succumbed during the transplantation of the chondrocyte graft due to complications of anaesthesia.

Remaining 9 animals were euthanised and examined histologically with respect to the healing of the transplant in the area of the growth plate. In all 9 cases the graft was healed in the distal femoral physis. Bone bridges were formed in canals unfilled with the autogenous chondrocyte graft.

We conclude that transplantation of the autogenous chondrocyte graft into iatrogenically damaged physis in pigs can prevent bone bridge formation and growth arrest. This finding may have clinical implications in potential transplantation of chondrocyte autografts in children.

Growth plate, injury, bone bridge, pig

Growth plate fractures in children, in particular those ones of types III to V according to the classification by Salter–Harris (1963), belong to injuries of poor prognosis. Damage to the regular chondrogenesis in the physis can result in altered growth of the bone affected due to the formation of a bone bridge. In dependence on its location we recognise either a peripheral bone bridge resulting in angular deformities of the bone affected, a central bone bridge shortening the bone without its angular deformity or a combined kind of a bridge causing both angular deformities and shortening of the bone (Rockwood et al. 1984). Combined bone bridges are least favourable for the patient (Rockwood et al. 1984). Iatrogenic damage to the physis during surgical repositioning of a physeal fracture can lead to the formation of osteonecrotic bone bridges and then can form a bone bridge in the fissure after incomplete repositioning of fragments (von Laer 1984). Clinical consequences of physeal bone bridging are often very serious (Bak and Boeckstyns 1997) and their therapy includes complex corrective surgeries (Hove and Engesaeter 1997) or complicated resections of bone bridges (Langenskiöld 1981).

Trauma to the distal femoral physis is a typical example of injury to the growth plate in children. This injury, common in preadolescents and adolescents, is not so frequent but can

Address for correspondence:

Doc. MUDr. Petr Gál, Ph.D.
Department of Pediatric Surgery, Orthopedics and Traumatology
Medical Faculty, Masaryk's University
Cernopolní 9, 662 63 Brno, Czech Republic

Phone: +420 5 4512 2360
E-mail: petrgal@med.muni.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

be accompanied by serious clinical consequences (Rockwood et al. 1984; Pešl and Havránek 1995). It is due to the fact that the distal femoral physis is responsible for up to 70% of growth of this bone to the length and about 40% of growth of the whole pelvic limb (Rockwood et al. 1984). Apart from changes in the growth of the bone and limb affected, nerve and vascular bundles in the popliteal area can be damaged due to extensive traumatisation of the limb during car accidents in particular (Kleinman and Marks 1998). Even though it is a tradition that the implant may not cross the growth plate (Pešl and Havránek 1995), the most precise anatomical reposition and fixation of fragments using Steinmann pins is consistent with adequate therapy. Regarding the age of the injured and the considerable traction of muscles surrounding the fracture, it is to be supposed that, in polytraumatic patients in particular, it is necessary to search for more rigid forms of fixation. Trauma during insertion of substitutes of anterior cruciate ligaments in the stifle joint, rupture of which occurred prior to the closure of growth plates (Lo et al. 1997), may be another cause of damage to the distal femoral physis. Damage to the distal growth plate of tibia tends to be similarly complicated, because adequate stability of the fragments reduced is often achieved only through compress osteosynthesis by pins inserted transphyseally (Toupin and Lechevallier 1997). There is a risk of bone bridge formation with all the complications mentioned in these physeal injuries. Problems of damage to the growth plate and its therapy are topical both in human and veterinary traumatology (Donigian et al. 1993; Gomes and Volpon 1993; Kershaw and Kenwright 1993; Johnson et al. 1994; Viljanen et al. 1997; Partio et al. 1997; Pereira et al. 1997; Sukhiani and Holmberg 1997; Janary et al. 1998; Lorinson et al. 1998; Oni 1999).

It was the aim of this experimental study to verify the possibility of preventing bone bridge formation following insertion of the implant through the distal femoral physis using the autogenous chondrocyte graft.

Materials and Methods

Domestic swine was chosen as an experimental animal because the structure of growth plates in piglets is similar to humans. The experiment was performed on the growth plate of distal femur. Ten three-month-old piglets were included in the experiment. All procedures were performed on anaesthetized animals. They were pre-medicated with a mixture of xylazine (2 mg/kg body weight), ketamine (2 mg/kg body weight), zolazepam (2 mg/kg body weight) and tiletamine (2 mg/kg body weight) intramuscularly and then kept under inhalation anaesthesia using $N_2O + O_2$ (2:3). Analgesia was enhanced using butorphanol (0.2 mg/kg body weight). Amoxicillin clavulanate (1 ml/20 kg body weight i.m. once daily for 5 days) was used as a prophylaxis.

The samples of cartilage, 3 × 3 mm in diameter, were collected from a non-weight-bearing surface of the lateral femoral condyle and used to culture chondrocytes for the preparation of autogenous chondrocyte grafts. The collected material was placed into a sterile Gey's Balanced Salt Solution (GBSS) containing antibiotics (penicillin + streptomycin) and further processed under sterile conditions in a laminar box. With a scalpel blade we divided the samples of cartilage into 1-2 mm³ bits and placed them into a 50 ml glass with a magnetic mixing device. We performed a series of washings in the GBSS. The last washing was performed with added 4 ml of 0.2% trypsin for 5 min. Trypsin solution was then withdrawn, another dose of trypsin was added to the sample and it was incubated at 37 °C for 30 min and slowly mixed with aid of the electromagnetic mixing device. Trypsin solution was withdrawn again and the sample was washed twice with the GBSS. The next phase included incorporation of 2 ml of 0.2% solution of collagenase for 5 min, followed by its removal and addition of 4 ml of fresh 0.2% collagenase. The sample was then incubated at 37 °C for 30 min and slowly mixed with the electromagnetic mixing device. Collagenase was removed again and the procedure of addition of 4 ml of fresh collagenase and 30 min-incubation was repeated. Supernatant was withdrawn and centrifuged at 600 g for 10 min. The cellular sediment was suspended in the culture medium (HAM's F-12 + fetal bovine serum + antibiotics) and plated to culture bottles. The procedure with 90-min culture using fresh collagenase was repeated until complete processing of the sample.

The culture was kept at 37 °C and 5% CO₂. The culture medium was changed every 48 h. After the required cell count (approx. 5 million/ml) was obtained, we performed the passage of cells using 0.1% trypsin + 0.02% EDTA.

The sediment of cells was then re-suspended in the solution of Tissukol (Immuno) and used to form a cast in the syringe of 4 mm diameter corresponding to defects created.

Fourteen days later, a simulation of drilling the peripheral bone bridge was made under general anaesthesia. A defect of 4 mm in diameter was created in the growth plate of the lateral condyle and filled with the autogenous chondrocyte graft (Plate X, Fig. 1). The same defect, made in the medial condyle, was left without filling.

Ten weeks later the animals were euthanised (Thiopental 3 g *pro toto* i.v.). Distal parts of femurs were extracted and placed in a fixative 10% solution of buffered formalin for 48 h. They were then decalcified in a solution of hydrochloric acid and ferric chloride (changed every 12 h) for 5 to 8 d. Following complete decalcification, the resected samples were divided using a kitchen cutting device into sections, wide 4 to 5 mm, in a way to reveal the longitudinal axis of the canal drilled. Parts of the growth plates with the lesions were excised together with at least 1 cm of the surrounding tissue. They were histologically processed and stained with hematoxylin and eosin. Sections 6 µm in depth were examined and photographed using an optic microscope Nikon Eclipse 1000. We evaluated incorporation of the transplant into the canal drilled through the growth plate. We compared this canal with the one left unfilled.

Results

One animal died during the transplantation of the chondrocyte graft due to complications of anaesthesia. In all 9 remaining animals the graft was incorporated into the canal and iatrogenically damaged growth plate was reconstructed (Plate X, Fig. 2). Bone bridges (Plate X, Fig. 3) were formed in canals unfilled with the autogenous chondrocyte graft in all cases. These results are summarised in Table 1.

Table 1
A chondrocyte graft healed in the transplantation area of the distal femoral physis. Comparison of histological findings of canals drilled through the same physis and left without filling by the graft.

Animal No.	Evaluation of the canal filled with the chondrocyte graft	Evaluation of the canal left without the chondrocyte graft filling
1	healed, physis reconstructed	bone bridge
2	healed, physis reconstructed	bone bridge
3	healed, physis reconstructed	bone bridge
4	healed, physis reconstructed	bone bridge
5	healed, physis reconstructed	bone bridge
6	healed, physis reconstructed	bone bridge
7	healed, physis reconstructed	bone bridge
8	death of the animal	
9	healed, physis reconstructed	bone bridge
10	healed, physis reconstructed	bone bridge

Histological examination of samples with the autogenous chondrocyte graft revealed cancellous bone containing yellow bone marrow in the epiphyseal part. In the diaphyseal part the cancellous bone had about 50% of blood-forming marrow. The epiphyseal growth plate between them was physiologically moderately undulated, had the same width throughout its course and columnar arrangement of chondrocytes. From the epiphyseal side it was bordered by fine trabeculae of cancellous bone. From the diaphyseal side under the line of erosion there was a layer of fine trabeculae of a newly built bone. This bone is under the activity of osteoblasts and osteoclasts being rebuilt into a definitive cancellous bone of diaphysis. In the area of the implant, the growth plate in a funnel-shape manner projects into the diaphysis by about two- to three-fold its normal width. Bone trabeculae of the epiphyseal surface at this point were slightly thickened. The growth plate itself at the point of invagination was weakened to about one half; it, however, did not lose its columnar arrangement. The axis of chondrocyte columns was always longitudinal to the long axis of the bone. The structure of the bone matter beyond the line of erosion was similar to undisturbed areas. A number of histological specimens evidence that the chondrocytes transplanted remain vital deep in the diaphysis and result in the elongation of the epiphyseal growth plate in the area of the defect in a form of a narrow beak-like projection, often longer than 10 mm. Even in this projection the chondrocytes arrange to columns. These

are, however, not straight but run first perpendicular to the axis of the projection (i.e., perpendicular to the axis of the bone) and turn to the correct direction in the second third of the bone. In this area, the primitive fine trabeculae are also being rebuilt, under the influence of the activity of osteoblasts and osteoclasts, to form the cancellous bone.

Discussion

When inserting implants transphyseally to immobilise growth plate fractures, we can produce iatrogenic osteonecrotic bone bridge. Therefore many authors in their studies seek for an optimal therapeutic procedure. Many papers (Donigian et al. 1993; Partio et al. 1997) have a common feature of seeking an optimal therapeutic procedure for the treatment of injuries to the growth plate to prevent the formation of a bone bridge. Other papers are aimed at removing the existing bone bridge, thus preventing growth disturbances of the bone. After resection of the bone bridge the defect is most often filled with allogenic fat, graft of fascia or tendon, or silicone (Langenskiöld 1981). Rarely, the growth in the affected physis is artificially arrested. This is accompanied by the same procedure on the contralateral side. Transplantation of the growth plate grafts is another possibility, currently in the stage of extensive experimental research (Park et al. 1994; Lee et al. 1998).

In this study we selected implantation of the autogenous chondrocyte graft, carried on fibrin glue, into the damaged distal growth plate of femur as a method of preventing the bone bridge formation. Domestic pig was selected as experimental animal. Until recently, similar studies have been performed in small animals only (Jouve et al. 1998). We filled the defect created by transphyseally inserted Steinmann pin in the distal growth plate of femur with the autogenous chondrocyte graft and achieved a 100% success in preventing the bone bridge formation in the area of iatrogenically damaged physis. Tissukol was found to be an excellent culture and carrying medium of chondrocytes. Its selection was based on the need to find a medium, commonly used in human medicine, that has optimal strength and adhesive power, as well as solubility, and that ensures the survival of chondrocytes. Rahfoth et al. (1998) used a medium of similar structure (i.e., agar gel).

Results of our study suggest that transplantation of autogenous chondrocyte graft into the area of iatrogenically damaged physis prevents bone bridge formation. This method of preventing the bone bridge formation has the advantage of employing a relatively easy accessible autologous graft and commonly used fibrin glue Tissukol. This method seems to be cheaper than the use of absorbable implants which are being tested for these indications. If this method will be improved in the future, it could be employed even outside the field of traumatology, e.g., in oncologic orthopaedics. To our knowledge, no similar study has been published on transplantation of the autogenous chondrocyte graft as prevention of the bone bridge formation following injury to the physis in the pig. It is, however, necessary to consider this study to be only preliminary in the development of this application for the clinical practise. Even though the results of chondrocyte transplantation in the physis seem promising with respect to the possible prevention of the bone bridge formation, it is necessary to study the survival of chondrocytes, immunological aspects (Hyc et al. 1997) as well as the possibility of influencing the proliferation of chondrocytes pharmacologically (Wakita et al. 1998).

Transplantace autogenního chondrocytárního štěpu do defektu ve fýze: experimentální studie u prasat

Cílem experimentální studie u 10 prasat bylo zjistit možnost zábrany vzniku kostního můstku v iatrogeně poškozené distální fýze femuru implantací autogenního chondrocytárního štěpu.

Z nezátěžové plochy laterálního kondylu femuru byl odebrán vzorek chrupavky o průměru 3 × 3 mm a z něj vykultivovány chondrocyty k přípravě autogenního chondrocytárního štěpu.

Za 14 dnů byl v oblasti laterálního kondylu vyvrtán přes distální fýzu femuru kanál o průměru 4 mm, který byl následně vyplněn autogenním chondrocytárním štěpem. Stejný defekt ve fýze byl vytvořen v mediálním kondylu a tento byl ponechán bez vyplnění. Jedno zvíře při transplantaci chondrocytárního štěpu uhynulo z důvodu komplikací při anestezii.

Po 10 týdnech bylo zbylých 9 zvířat utraceno a histologicky bylo sledováno vhojení transplantátu v oblasti růstové ploténky. Ve všech 9 případech došlo ke vhojení štěpu do distální fýzy femuru. V případě kanálů nevyplněných autogenním chondrocytárním štěpem se vytvořil kostní můstek.

Z výsledků studie lze vyvodit závěr, že transplantace autogenního chondrocytárního štěpu do iatrogeně poškozené fýzy u prasat může zabránit vzniku kostního můstku a úzavěru růstové ploténky. Toto zjištění může být prvním krokem ke klinickému použití transplantace chondrocytárních autoštěpů u dětí.

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Fig. 1. Implantation of the autogenous chondrocyte graft into the canal predrilled into the distal femoral physis.

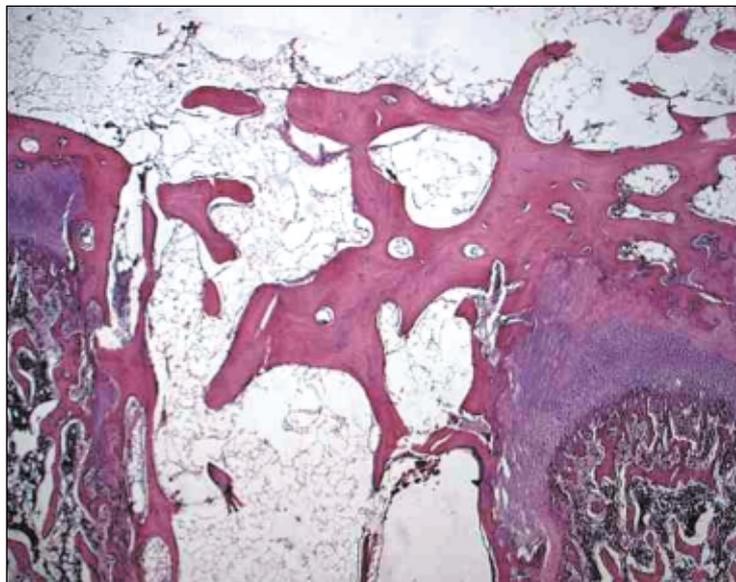


Fig. 2. A chondrocyte graft healed in the drilled canal reconstructing the distal femoral physis in the area of iatrogenic damage – a histological specimen.

Plate XI

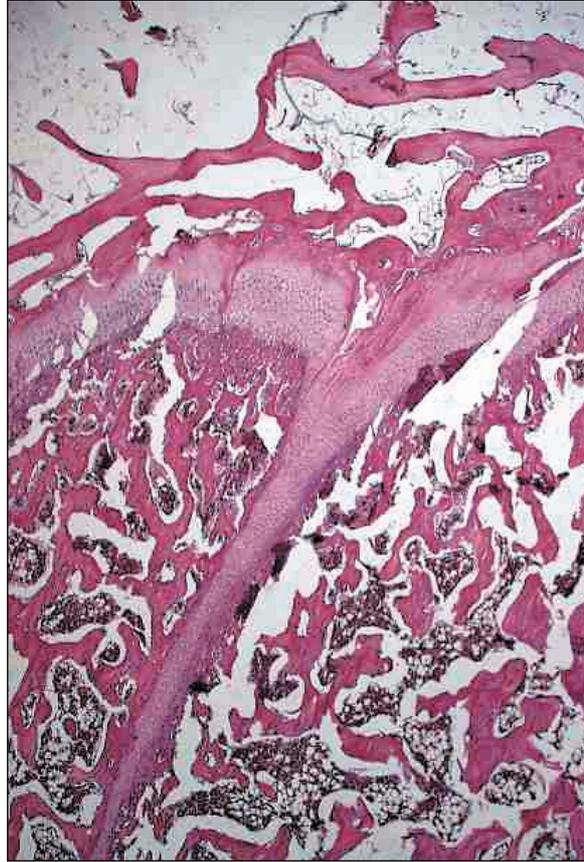


Fig. 3. A bone bridge in the distal femoral physis formed after drilling a canal and leaving it without the autogenous chondrocyte graft filling – a histological specimen.