Basic Science

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Autologous cartilage and fibrin sealant may be superior to conventional fat grafting in preventing physeal bone bridge formation – a pilot study in porcines

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Abstract

Purpose: The article compares physeal recovery after insertion of autologous cartilage and a conventional fat graft in a standardized porcine physeal gap model. Presence of a bone bridge was the primary outcome.

Methods: Ten porcines in two groups of five were included in a paired design. A standardized physeal gap in the distal femur was made in all animals. One group (n = 5) was randomized for deposition of autologous cartilage and a Tisseel® or Tisseel® alone. The autologous cartilage was harvested from the femoral articular surface. The other group was randomized for fat grafting or no grafts at all. All animals were housed for 14 weeks. Magnetic resonance imaging (MRI) was performed at 14 weeks prior to euthanasia. The physis was harvested for histology.

Results: MRI – Three bone bridges were seen in the fat grafted gaps. All empty gaps formed a bone bridge. No gaps filled with autologous cartilage and Tisseel® resulted in bone bridges. One gap filled with Tisseel® only caused a bone bridge. Histology – The cartilage grafted gaps recovered with physeal-like cartilaginous tissue in histological analysis.

Conclusions: Fat grafts seems ineffective in preventing bone bridges. The use of autologous cartilage may be superior to the current treatment. However, donor site complications were not investigated. The study serves as a proof of concept study and requires further investigation.

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Keywords: bone bridges; cartilage graft; fat graft

Introduction

Of all paediatric extremity fractures, 20% involve the physis.[1] One-third of physeal fractures will result in bony healing across the cartilaginous physis, forming a physeal bone bridge.[2–4] The bone bridge formation might result in growth arrest of the affected part of the physis. This can lead to gait abnormality based on angular bone deformities and leg length discrepancies, depending on the location of the bridge.[5–7] Hence, it is of great importance to treat this condition in order to allow normal bone growth. The current surgical treatment of bone bridge formation is excision of the bridge and interposition of materials such as silicone, bone wax or fat.[8] The size and location of the bone bridge determine the treatment as bridges larger than 50% of the physeal area often should be offered an alternative to excision of the bridge. However, interposition of materials after excision have been associated with variable outcomes, and has mainly proven to be ineffective.[9] There is no apparent explanation for the ineffective and inconsistent outcome in the treatment applied today. Further, no changes in treatment options have been effectuated until now. Thus, there is a need for evaluation of the current treatment for bone bridge formation in experimental models to improve the current clinical outcome. Restoration of the cartilaginous physis and continuous growth would be the ultimate goal.

The aim of this study was to evaluate the bone bridge formation after interposition of autologous cartilage in a physeal gap in a porcine model and compare it with interposition of a fat graft, which is commonly used in the clinic.

Materials and methods

Animal model

A juvenile porcine model was established. Ten skeletally immature female pigs (Yorkshire–Landrace–Duroc) were included in this study (n = 10). At baseline the mean body weight was 26.8 kg (range 23.2 kg to 31.3 kg). Hind legs only were used for intervention. All open procedures were performed in operating theatres with a sterile environment. All animals were housed for 14 weeks post-operatively. Presence or absence of bone bridge formation was chosen as primary outcome.

Physeal gap

A standardized physeal gap was created as a standardized gap model in both hind legs. The distal femoral physis was approached from the medial side. A k-wire was inserted into the physis. Fluoroscopy in two planes verified the position of the k-wire. A 6 mm diameter cannulated drill was manually driven into the physis until it reached the 15 mm ruler mark on the probe. A standardized cylindrical empty gap (6 mm x 15 mm) was created. The gaps were then rinsed with a sterile 0.9% NaCl solution. The created gaps represented physeal penetration and mimicked the clinical setting after a complete excision of a bone bridge. An identical procedure was performed in the contralateral hind leg. The longitudinal cross-sectional area through the centre of gap was determined to calculate the damaged physeal area in percentage. The diameter of the cylinder (6 mm) was multiplied by the height (15 mm) = 0.9 cm^2.

Design

A matched paired design was used in this study. We assumed no difference between left and right femurs, nonetheless treatments were alternated between right and left leg. The animals were divided into Group A (n = 5) and B (n = 5). Group A was randomized to filling of the physeal gap with a fat graft in one leg and left empty in the contralateral. Group B was randomized to filling with autologous cartilage and Tisseel® (fibrin sealant) or Tisseel® alone. The fat grafted gaps were compared to the empty gaps while the Tisseel® and cartilage-filled gaps were compared to the gaps filled with Tisseel® only. Each animal acted as its own control.

Filling of gaps

In Group A, the fat was harvested locally from the subcutis. The fat graft was deposited into the gap until full with a press fit technique. The empty gaps were left untouched after rinsing with 0.9% saline water.

In Group B, the autologous cartilage was harvested perioperatively from the same leg it was deposited into. The distal femoral joint cartilage was exposed through an infrapatellar approach through the patellar ligament without harming the physis. A 6 mm diameter punch was used to harvest cartilage at two sites from a non-weight bearing area of the joint. The non-weight bearing part of the physis was defined as the femoral articular cartilage anterior to the tibial plateau with the leg in full extension. The cartilage was kept in a sterile NaCl 0.9% solution until the gaps were created. The harvested circular cartilage was then cut perioperatively into chips. It was cut into small pieces of approximately 2 mm with a surgical knife and subsequently deposited into the gap until full according to randomization. The gap was then sealed with Tisseel®, a commercially packed binary fibrin sealant system by Baxter.[10] The system consists of two syringes with sealer protein and Thrombin respectively joining in a shared tip. When applied, sealer protein and Thrombin is mixed in the tip of the syringe to form an adherent mass. The Tisseel® was applied using the commercially packed syringes in the system. The contralateral leg was filled with Tisseel® using the same technique. All gaps were filled according to pre-operative randomization.

Magnetic Resonance Imaging (MRI)

MRI (MAGNETOMSkyra, 3.0 Tesla, Siemens Healthcare GmbH, Germany) was performed 14 weeks after surgery before euthanasia. T1 and T2-weighted 3D sequences were carried out in addition to T1-weighted quantitative water content sequences.[11] Presence and absence of a bone bridge was determined on MRI.

Physeal cross-sectional area of the distal femoral physis was determined in the transversal plane after aligning the axis. The cross-sectional area of the physis was determined as the area inside the perimeter of the physis.

Water content was calculated using the software Siswin v.0.9.[11] A gap ratio (GR) was determined to assess the water content at the injury site in comparison to the totalphysis.

$$GR = \frac{water\ content\ of\ gap}{water\ content\ of\ physis}$$

GR > 1 suggests increased water content in the injury site when compared to physeal water content. If GR = 1, the water content is similar and less if GR < 1.

Anaesthesia and euthanasia

All operations were performed under general anaesthesia. Intravenous access was obtained through the ear. Intubation was carried out after administration of Hypnomidate (0.5 mg/kg). Continuous anaesthesia and analgesia were achieved with infusion of Propofol (10 mg/kg/hour) and Fentanyl (60 µg/kg/hour). Lidocaine (50 mg) was placed subcutaneously after wound closure.

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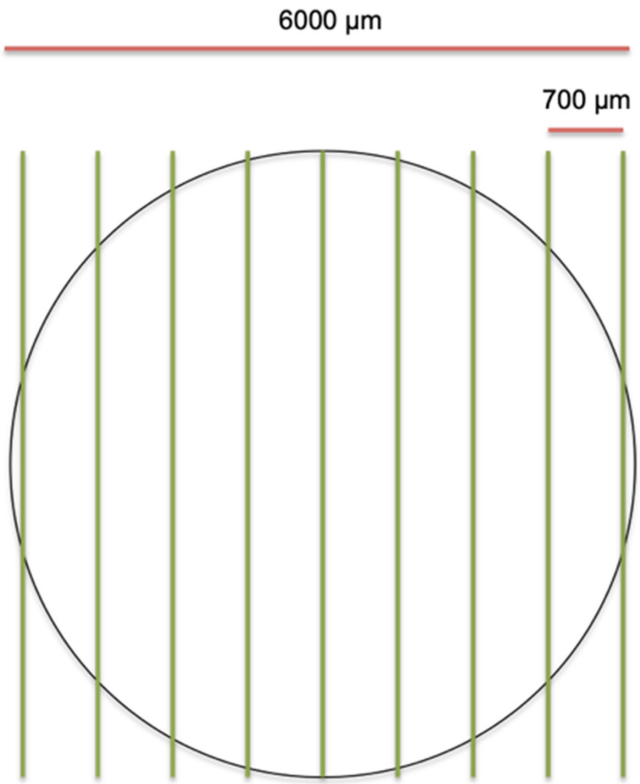

Fig. 1 Medial part of the distal femoral physis in the sagittal plane. Total size (6000 µm) and the distancebetween each level (700 µm) examined of the block is shown.

Procainpenicillin IM (15000 IE/kg) was administered prior to operation. The antibiotics were repeated for three-days post-operatively.

All animals were euthanized using a lethal dose of IV pentobarbital (200 mg/ml) at study termination (14 weeks). The distal femoral physis were harvested for further analyses.

Histology

A tissue sample measuring approximately 1 cm x 1 cm x 1,5 cm, containing the healed physeal gap, was harvested from each hind limb for histology after euthanasia. The sample was dehydrated in ethanol, starting with a 70% concentration increasing until 96%. Clearing was done using isopropanol and xylene. Finally, the samples were embedded at –20°C in methyl methacrylate. Each sample was cut into nine sections of 7 µm per section throughout the defect. The distance between each section was 700 µm (Fig. 1). Hematoxylin/Eosin (HE) stain was used. The sample was cut parallel to the coronal plane. Histomorphometry was performed[12] to determine the quantitative repair tissue fractions in the physeal gap. Visio software (Visiopharm®, Denmark) was used for quantitative analysis. The region of interest was marked in each section. The software imposed a 3 x 3-point grid and 33% of the region of interest was counted at 20x magnification. The software randomly selects 33% of the defined region of interest to be counted. Tissue fractions were determined for each section in every sample. All viable cartilage was counted as one with no regard to orientation or organization of the cells. Tissue fractions were recounted two months after primary investigation in eight randomly chosen samples. A mean coefficient of variation was determined with correlated 95% confidence interval. The coefficient of variation was 0.11 (0.03; 0.19).

Statistics

All analyses were carried out using STATA 13 (SataCorp., 2013 Stata Statistical Software: Release 13, College Station, Texas, USA). Student's t-test was carried out after checking for normality. P-values below 0.05 were considered significant.

Results

No infections or other complications were observed during the study period. All animals gained a mean of 12.4 kg (10.1 kg to 15.0 kg). No animals suffered from lack of weight bearing beyond the first post-operative day.

The mean cross-sectional area of the physis in all groups was 12.42 cm^2. The mean proportionate size of the gaps was 7.2 % of the physeal cross-sectional area (Table 1).

Bone bridge formation Group A

All empty grafted gaps formed a bone bridge after standardized physeal injury. The interposition of fat only prevented bone bridge formation in two animals (Table 2, Fig. 2). Bone bridge formation was confirmed by histology (Fig. 3).

Table 1 Physeal cross-sectional area of the distal femoral physis (determined on 3T MRI) and proportionate gap size

	Gap filling	Physeal area (cm^2)	Gap size (%)
Group A			
	Fat (n = 5)	12.16 (10.53; 13.79)	7.4
	Empty (n = 5)	11.90 (10.29; 13.51)	7.6
Group B			
	Autologous cartilage chips and Tisseel® (n = 5)	13.0 (11.57; 14.43)	6.9
	Tisseel® (n = 5)	12.62 (11.61; 13.63)	7.1
Combined			
		12.42 (11.88; 12.96)	7.2

Table 2 Bone bridge formation upon physeal gap and grafting

	Gap filling	Bone bridge formation
Group A		
	Fat (n = 5)	3/5
	Empty (n = 5)	5/5
Group B		
	Autologous cartilage chips and Tisseel® (n = 5)	0/5
	Tisseel® (n = 5)	1/5

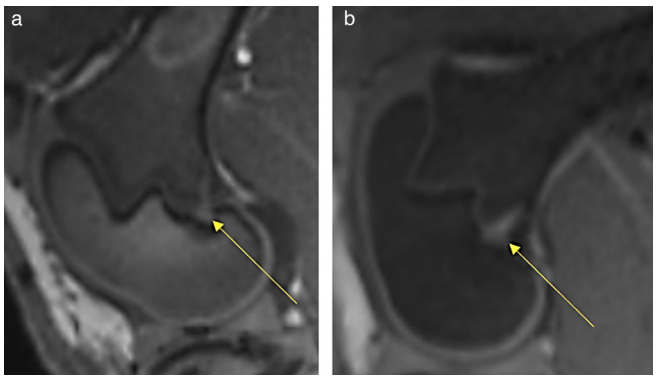

Fig. 2 Sagittal slices of T1 MRI image from the operated distal femora. **(a)** Bone bridge formation, marked with yellow arrow, on MRI T1 in an empty physeal gap. **(b)** No bone bridge seen on MRI T1 in a fat grafted gap, the fat graft is marked with a yellow arrow.

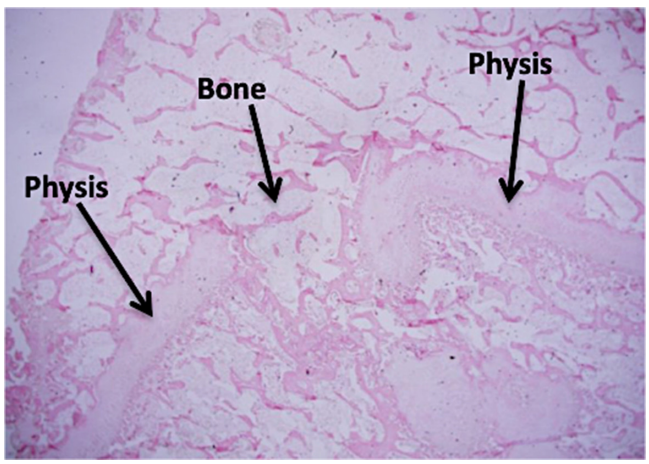

Fig. 3 Bone bridge formation in empty physeal gap seen on histology. 1.25 x magnification, HE stain.

Bone bridge formation Group B

No bone bridges were seen in the gaps filled with cartilage chips and Tisseel®. One bone bridge was detected in the gaps filled with Tisseel® alone (Table 2, Fig. 4).

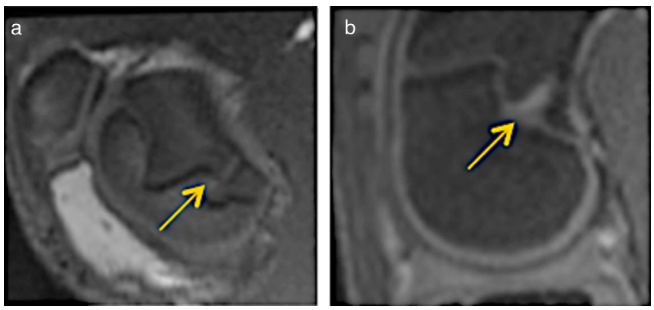

Fig. 4 Sagittal slices of T1 MRI image from the operated distal femora. **(a)** Bone bridge formation seen on MRI T1 in Tisseel®-filled gap, marked with yellow arrow. **(b)** No bone bridge is formed. Physeal healing without bony tissue on MRI T1 in cartilage-filled gap, marked with yellow arrow

Histomorphometry Group A and B

Tissue fractions of the gaps determined by histomorphometric analysis show more fibrous tissue in the fat grafted gaps compared to empty gaps in Group A. In Group B, more cartilage and less fibrous tissue were observed in the cartilage-filled gaps (Table 3).

Descriptive histology

Gaps filled with cartilage healed with cartilaginous tissue in continuation of the physis, suggesting physeal regeneration (Fig. 5). The cells were more disorganized, although with all zones of the physis. The cartilaginous healing resulted in slightly hypertrophic physis compared to uninjured part of the physis (Fig. 5).

MRI with water content quantification

Water content analyses showed similar water content in the fat grafted gaps when compared to the whole physis and less water content in the empty gaps in Group A. In Group B, similar water content was observed in the Tisseel®-filled gaps and higher water content in the cartilage-filled gaps when compared to the whole physis (Table 4, Fig.6).

Table 3 Histomorphometric tissue fractions of physealhealing in the gaps according to material deposition.

Group	Cartilage tissue %	Bone %	Fibrous tissue %	Fat %	Empty* (%)	Total
Group A						
Fat grafted gap	14.98 (8.79; 21.17)	40.73 (29.48; 51.96)	16.72 (5.49; 27.94)	0 (0; 0)	27.57 (12.97; 42.18)	100
Empty gap	16.04 (9.83; 22.24)	45.83 (22.60; 69.07)	38.13 (18.08; 58.18)	0 (0; 0)	0 (0; 0)	100
p-value**	0.81	0.64	0.04	N/A	0.01	
Group B						
Autologous cartilage and Tisseel®	63.54 (51.46; 75.42)	12.02 (4.08; 19.95)	19.93 (12.71; 27.03)	4.49 (–1.07; 10.03)	0.02 (–0.2; 0.06)	100
Tisseel®	45.60 (34.40; 56.81)	7.43 (3.39; 11.49)	43.57 (32.84; 54.28)	3.40 (–1.72; 8.52)	0 (0; 0)	100
p-value**	0.03	0.3	0.01	0.77	0.33	

Note. Values are reported as means with corresponding 95% confidence intervals.
*These fractions represent the areas with empty space without cells
**t-test

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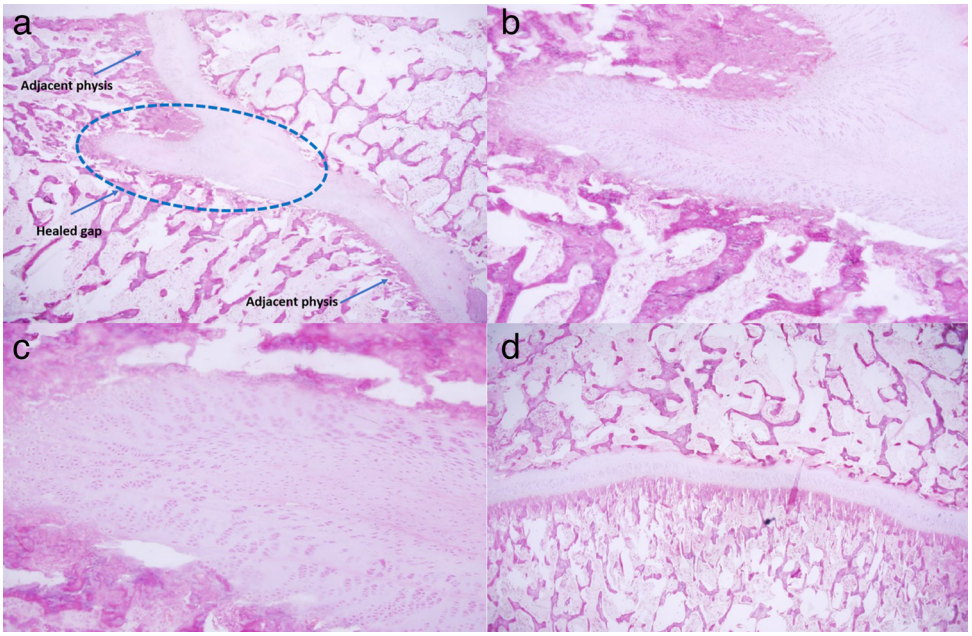

Fig. 5 **(a)** Physeal healing with cartilage at 1.25x magnification in gap filled with autologous cartilage and Tisseel®. Gap interposition is highlighted in the blue area. Adjacent physis is marked. **(b)** Blue area on (a) is showed with 4x magnification. Displays cartilaginous tissue. **(c)** Blue area on (a) is showed with 10x magnification. Displays healing with cartilage and columnar arrangement of cells. **(d)** Normal distal femoral porcine physis at 1.25x magnification.

Table 4 Quantitative water content MRI. Gap Ratio (GR) for Group A and B

	Gap filling	Mean GR (CI95)
Group A		
	Fat (n = 5)	0.89 (0.61; 1.17)
	Empty (n = 5)	0.69 (0.44; 0.93)
Group B		
	Autologous cartilage and Tisseel® (n = 5)	1.50 (1.43; 1.58)
	Tisseel® (n = 5)	1.41 (0.96; 1.86)

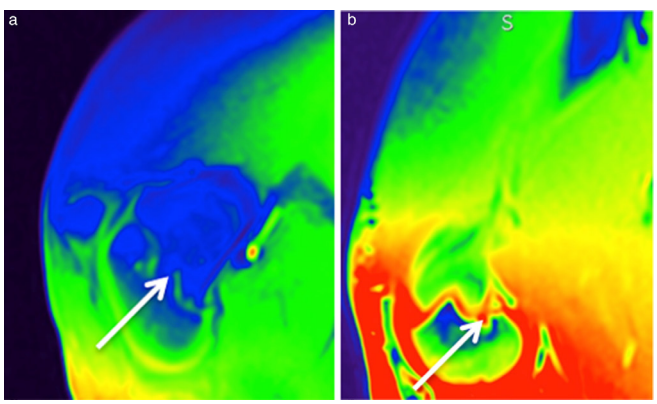

Fig. 6 Sagittal slices of T1 map MRI images from the operated distal femora used for water content quantification and description. **(a)** Physeal gap (empty group) is marked with a white arrow and is of same colour as the bone (blue) while the physis is green. Shows decreased water content in gap on MRI (Gap ratio < 1). **(b)** Physeal gap (cartilage-filled) is marked with a white arrow and is of same colour as the physis (red). Shows similar water content in gap on MRI, (Gap ratio = 1).

Discussion

In this study we present an experimental standardized physeal gap in a porcine model forming a histologically confirmed bone bridge. This model intends to mimic the gap after complete resection of a bone bridge where prevention of osseous healing by grafting is needed. However, in a clinical setting it can be difficult to ensure that the bridge is resected completely thus making our gap model closer to a physeal penetrating injury. The remaining osseous tissue after incomplete resection is likely to influence the healing in the gap. Applying fat grafts in our study, two-thirds of the animals still developed a bone bridge, agreeing with a previous study.[9] Only one bridge was confirmed when the gap was filled with Tisseel® and none when the cartilage chips were added.

Several factors could influence the inconsistent effect of fat grafts in our study. The mechanical load could cause the fat graft to float out of the gap, due to insufficient sealing inside the gap, eventually leaving the gap empty.[13] This may be solved with better sealing of the fat graft. Another reason for the variable results of filling the physeal gap with fat could be the degradation of fat tissue after deposition. Histomorphometry supports this process as fat cells were lacking in the examined sections. The histomorphometry also elucidates the tissue fractions composing the physeal healing after injury. No difference was observed in bony and cartilaginous fractions between empty and fat grafted gaps, which may support the MRI findings in our study, where bone bridges were seen in several fat grafted gaps.

However, the empty gap healed with a larger fraction of fibrous tissue. A proportion of the fat grafted gaps were composed of empty areas with no cells. This may be due to artefacts caused by the preparation process, but could also indicate absorption or leaking of the graft from the physeal gap. In contrast to our experimental results, Escott et al[14] reported a fairly good clinical outcome for interposition of fat tissue after excision of small bone bridges (fat grafted gap). The review consisted of treatment of bone bridges at multiple sites and not only in the distal femoral physis, which may be more susceptible to bony union.[15,16] It is important to emphasize that we evaluated physeal healing in regard to presence or absence of a bone bridge and not functional growth outcomes as in clinical studies where some bridges may not be clinically significant due to size or fracture of the bridge.[17,18] The gaps in our study are relatively small, comprising approximately 7% of the physeal cross-sectional area and may for that reason not have an impact on growth.

The interposition material must be sufficiently sealed and stay inside the gap. Additionally, the materials used to fill the physeal gap should not possess osteoconductive properties in order to prevent reformation of the bone bridge. A potential gap sealant could be Tisseel®, which was chosen due to easy clinical applicability and the ability to create haemostasis in addition to the sealant effect.[19] Angiogenesis and presence of vascular endothelial growth factor (VEGF) has proven essential to the formation of bone after injury.[20–23] Decreasing the blood supply and haematoma is therefore essential in preventing bone bridges. This may support the inconsistency results of fat grafting, as fat has poor haemostatic properties. The adherent and haemostatic effect of the Tisseel® may explain why fewer bridges were seen when Tisseel® was deposited alone into the gaps compared to the fat grafted gaps. The interposition of solid materials could also decrease the haematoma and thus the presence of VEGF as a result of compression inside the physeal gap. This would lead to decreased bone formation and ultimately lower risk of bone bridge formation, which may be the case in the gaps filled with Tisseel®. As seen in Table 3, the Tisseel®-grafted gaps mainly consist of fibrous and cartilaginous tissue. This confirms the lack of bone bridge formation. However, it is still unknown if the fraction of fibrous tissue will result in osseous tissue in the long term. The use of autologous cartilage chips can unite the properties of fat tissue with a chondroconductive environment to prevent the formation of bone bridges. This was documented in this study. The cartilage chips deposited in the physeal gap filled the gap and gave no significant empty space for bone to form. Concurrently histology showed cartilaginous healing at the physis in continuation to it. The Tisseel® combined with cartilage chips probably anchored the placement of the chips simultaneously decreasing the bleeding, and thereby lowering the presence of VEGF. Viable cartilage has the potential to give a permanent protection against bony healing compared to degradable grafts, however the cartilage was disorganized compared to the normal physis and it is unknown how this cartilaginous bar influences the physeal growth. Despite the interesting outcome, a disadvantage of applying autologous cartilage is the unknown long-term effects from the donor site of the joint.

MRI water content quantification showed less water content in the gap when a bone bridge was formed. Water content of the gaps with no bone bridge formed showed similar water content in the gap in comparison to whole physis. The gaps filled with cartilage showed higher water content on MRI than the rest of the physis. Still, the cartilage was expected to present high water content if the tissue survives as it mostly consists of water. Less water in the gap indicates formation of bone or at least decreased cartilage and physeal health as cartilage is mainly composed of water.[11] The combined findings in the histological analysis and on MRI, including water content quantification, suggest that autologous cartilage chips in combination with Tisseel® may be suitable in preventing bone bridges after physeal injuries in the distal femur.

There are limitations to this study. The number of animals included in each group (n = 5) can result in uncertainties in the percentage of bone bridge formation due to a type II error. However, it shows variability in the prevention of bone bridges if a physeal gap is filled with fat tissue and consistency in the gaps filled with cartilage. In addition to this, in our study we created a straight cylindrical gap through a wavy physis. The tissue in the resected gap may have contained both bony and physeal tissue instead of continued physeal tissue. This could influence a possible bone bridge formation as the proportion of residual physis in the gap might vary. Still, the bone bridge was seen in all animals with an empty physeal gap. This suggests that the physeal gap is sufficient and capable of forming a bone bridge. Moreover, evaluation of the growth was not carried out, thus it is unknown what impact the healed gaps have on growth. Hence, the actual clinical outcome is difficult to predict. The clinical setting after resecting a bridge is different to our gap model as bone remnants from the bridge may influence on the reoccurrence of the bridge. In our model there are no bone remnants due to the lack of bone bridge formation prior to grafting. Finally, this is a translational animal model and differences in physiology, bone healing, growth and even weight bearing may influence the outcome.

In conclusion, the results show that filling of a physeal gap with fat tissue is ineffective and autologous cartilage chips may be superior to current conventional fat grafting after excision.

Our study serves mainly as a proof of concept study for further investigations that include an extended follow-up period, larger sample size and evaluation of growth.

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COMPLIANCE WITH ETHICAL STANDARDS

FUNDING STATEMENT

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

ETHICAL STATEMENT

Ethical approval: The study was carried out in porcines and accordingly submitted to and approved by the Danish National Committee for the Protection of Animals used for Scientific Purpose prior to initiation of the study. All procedures performed in this study involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.
Informed consent: Not required.

ICMJE CONFLICT OF INTEREST STATEMENT

None declared.

AUTHOR CONTRIBUTIONS

AAA: Primary investigator, Data analysis and interpretation.
BMM: Study design, Data interpretation.
JMSM: Study design, MRI set-up.
HSJ: Study design, MRI set-up.
CF: Study design, Histology set-up.
OR: Study design, Data interpretation.

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