

# Does fibrin clot really enhance graft healing after double-bundle ACL reconstruction in a caprine model?

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## Abstract

**Purpose** Graft healing following anterior cruciate ligament (ACL) reconstruction is a complex process characterized by phases of healing that lead to ACL remodelling. Our hypothesis is that fibrin clot addition to ACL reconstruction will result in advanced graft remodelling and healing when compared to a control group at 12 weeks as observed by histology, immunohistochemistry and magnetic resonance imaging (MRI).

**Methods** Eleven Spanish Boar goats underwent double-bundle ACL reconstruction: 8 were analysed and 3 were excluded. Group 1 was treated with DB ACL reconstruction utilizing autologous fibrin clots ( $n = 4$ ), and group 2 was treated with standard DB ACL-R ( $n = 4$ ). Histological and radiographic analysis was performed at 12 weeks. Each animal underwent 3-T MRI immediately after euthanization for evaluation of graft signal intensity utilizing the signal noise quotient (SNQ). Specimens were then sectioned and stored for standard histological and immunohistochemistry testing.

**Results** The mean ligament tissue maturity index score was significantly higher for group 1 ( $15 \pm 2.3$ ) compared with group 2 ( $7.7 \pm 5.2$ ) ( $p < 0.05$ ). The mean vascularity

(cell/mm<sup>2</sup>) for group 1 was  $7.1 \pm 1.3$  and  $9.3 \pm 3.1$  for group 2 (n.s.). The mean collagen type 1 (% 50× field) for group 1 was  $35.8 \pm 22.1$  and  $19.9 \pm 20.5$  for group 2 (n.s.). The mean SNQ for the AM bundle was  $1.1 \pm 0.7$  for group 1 and  $3.1 \pm 1.8$  for group 2 (n.s.). The mean SNQ for the total PL bundle was significantly lower for group 1 ( $1.1 \pm 0.7$ ) compared with group 2 ( $3.7 \pm 1.3$ ) ( $p < 0.05$ ). There was a significant correlation between the vascularity and the ligament tissue maturity index score as well as between collagen type 1 and SNQ, both AM and PL bundles ( $p < 0.05$ ).

**Conclusion** The use of fibrin clot in ACL reconstruction in a caprine model demonstrated improved healing with respect to histological analysis of the intra-articular ACL reconstruction segment and decreased signal intensity on MRI. It may lead to improved graft healing and maturation. By accelerating the intra-articular healing and ligamentization, the outcome of patients after ACL-R can be improved with faster return to sports and daily activity.

**Keywords** Anterior cruciate ligament · Fibrin clot · ACL graft healing · Double-bundle reconstruction · Graft remodelling

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## Introduction

The primary goal of anterior cruciate ligament (ACL) reconstruction is to replicate native anatomy and restore knee stability [1, 6, 35]. Early graft failure, including re-rupture or persistent instability, is reported in 2–3 % of the cases [15, 18, 29]. Biological factors play a major role in graft failure as evidenced by a high re-rupture rate during the first 12 months following ACL reconstruction [38]. This phenomenon is also detected in double-bundle (DB) ACL

reconstruction where single-bundle re-ruptures have been observed [13].

ACL graft healing involves stages of inflammation, vascularization as well as cellular and fibrous remodelling [11]. The process of graft remodelling begins with an inflammatory response driven by recruitment of macrophages and cytokines in the first day following surgery [17]. This leads to progressive cellular repopulation of the tendon graft and formation of fibrous scar tissue at the bone-to-tendon junction. The intra-articular segment of the graft heals faster and undergoes a stage of early revascularization when compared to healing at the bone-to-tendon junction. At 20 weeks postoperatively, the intrinsic vasculature of the graft is matured [4]. Vascularization is followed by progressive remodelling and finally complete healing of the graft [11].

Efforts to improve ACL graft healing through biological enhancements have focused on the use of bone morphogenic protein, gene therapy and exogenous growth factors. Increased bone formation, decreased tunnel widening, increased collagen fibrils and increased load to failure have been described [7, 19, 33, 36]. Recent research has focused on platelet-derived therapies [16, 22, 27, 30, 32]. However, the use of platelet-rich plasma (PRP) alone was shown to be insufficient in improving primary ACL repair [21].

Murray et al. showed that the addition of a scaffold to PRP enhances primary ACL repair and ACL graft healing [12, 16]. Fibrin clots have been described for the enhancement of healing of musculoskeletal tissues, such as meniscus and cartilage lesions [5, 26, 28]. Fibrin clots are readily available and display properties necessary for structural support as well as a delivery mechanism of platelet-derived growth factors. The collagen matrix stabilizes the platelets and protects the clot from premature dissolution by the synovial fluid. Therefore, the objective of this study was to examine the effect of a fibrin clot on ACL healing following double-bundle (DB) ACL reconstruction in a caprine model. A DB reconstruction technique was chosen due to the fact that the native ACL consists of two functional bundles that are attached to each other histologically. Therefore, it would be necessary not only to improve the healing/ligamentization within a bundle but also to achieve improved healing between the two bundles. By accelerating the intra-articular healing and ligamentization, the outcome of patients after ACL reconstruction can be improved with faster return to sports and daily activity. It was hypothesized that the addition of a fibrin clot to ACL reconstruction would result in advanced healing when compared to a control ACL-R group. Analysis was performed by histology, immunohistochemistry and magnetic resonance imaging (MRI).

## Materials and methods

ACL reconstruction in the goat model has been successfully established with a low rate of osteoarthritis and graft failure postoperatively [10, 39]. Eleven mature female Spanish Boar goats, between 3 and 4 years of age, were used in this study. Animals were obtained from a licensed US Department of Agriculture dealer and were housed in the animal facilities at our institution. Each animal underwent caprine arthritis encephalitis virus testing prior to boarding to ensure the absence of accelerated progression of knee osteoarthritis.

In each animal, DB ACL reconstruction was performed on the right hindlimb utilizing an Achilles tendon autograft technique. The left hindlimb was observed as a normal control. Group 1 ( $n = 5$ ) underwent DB ACL reconstruction utilizing an autologous fibrin clot, and group 2 ( $n = 6$ ) underwent standard DB ACL reconstruction. Animals were randomly assigned to each group. Both histological and MRI analysis were performed by blinded observers in the Department of Orthopaedic Surgery (DH, KI).

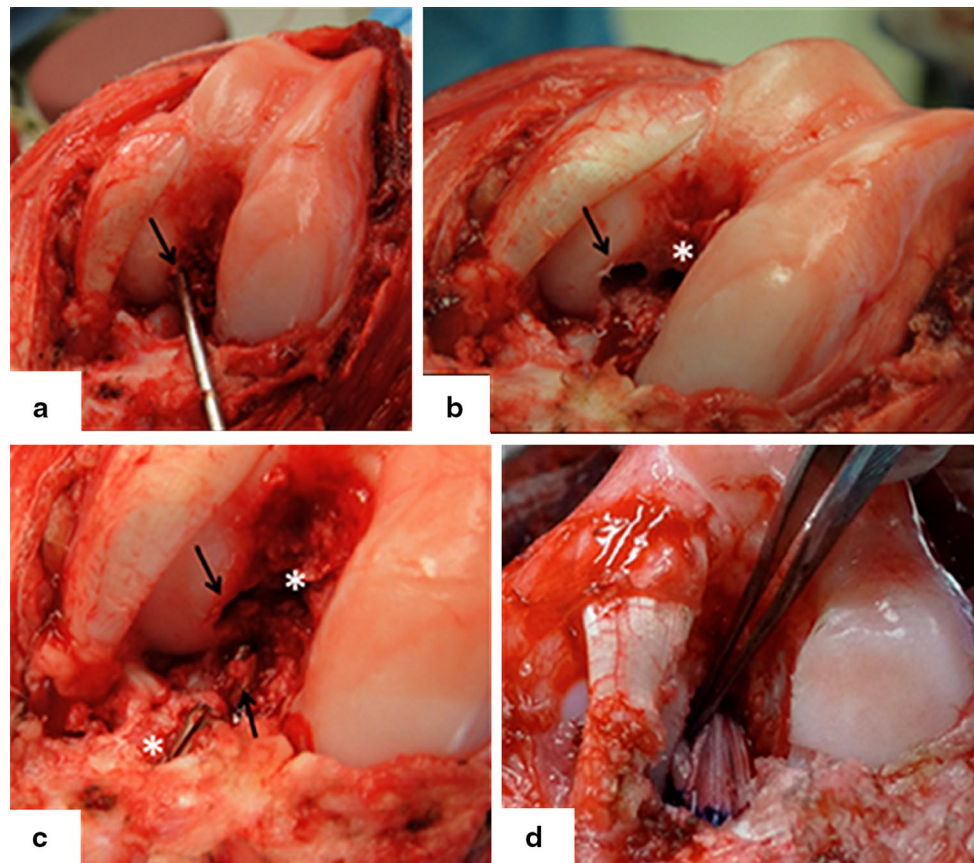
### Surgical technique

Prior to induction of anaesthesia, the animals were clinically examined for signs of trauma or pathology to the knee that might interfere with the surgical procedure. A posterolateral incision was made over the Achilles tendon, and the middle third of the Achilles tendon was harvested yielding a graft of approximately 10 cm in length by 5–6 mm in width. After harvesting, the tendon was re-approximated and the tendinous sheath was repaired.

The graft was split longitudinally in line with the orientation of the fibres to form two separate grafts. Each individual graft was looped incorporating a whipstitch at the distal end and a suture through the looped portion, both utilizing Ultrabraid sutures (Smith and Nephew Endoscopy, Andover, MA). Each graft was sized to 5 mm diameter.

### Double-bundle ACL reconstruction

A standard medial para-patellar incision was used, and after preparation, the patella was dislocated laterally. The native insertion site of the anteromedial (AM) and posterolateral (PL) bundles was identified, marked to allow for anatomic ACL reconstruction and then excised using a No. 15 scalpel. A guide pin was inserted into the centre of the PL insertion on the femur. Using a 5-mm reamer (Smith and Nephew Endoscopy, Andover, MA), the femoral PL tunnel was reamed through the lateral cortex (Fig. 1a). The AM position was identified and marked with a guide pin in identical fashion (Fig. 1b). The tibial PL and AM position



**Fig. 1** Intra-operative photographs of the DB ACL reconstruction. **a** Placement of the PL guide wire (*downwards arrow*) in the centre of PL footprint. **b** The AM (*asterisk*) and PL (*downwards arrow*) tunnels

in their anatomic footprints. **c** Both the femoral and tibial AM and PL tunnels are shown. **d** Both AM and PL grafts are passed

were identified, and an ACL tip guide (Acufex, Smith & Nephew, Andover, MA) was used to place a guide wire in the PL and AM tibial insertion sites (Fig. 1c). Five-mm tibial tunnels were reamed for the PL and AM tunnels leaving a bone bridge between the tunnels. The two grafts were passed from their respective tibial tunnel to the femoral tunnel, starting with the PL graft (Fig. 1d). Femoral fixation was achieved with sutures tied over a titanium button (Smith and Nephew Endoscopy, Andover, MA). Tibial fixation was achieved with sutures tied over a 3.5-mm cortical screw and washer. Standard layered closure was performed, assuring adequate medial stabilization in order to prevent patellar dislocation. Postoperative lateral radiographs were obtained to document correct tunnels and hardware positioning.

#### Fibrin clot preparation

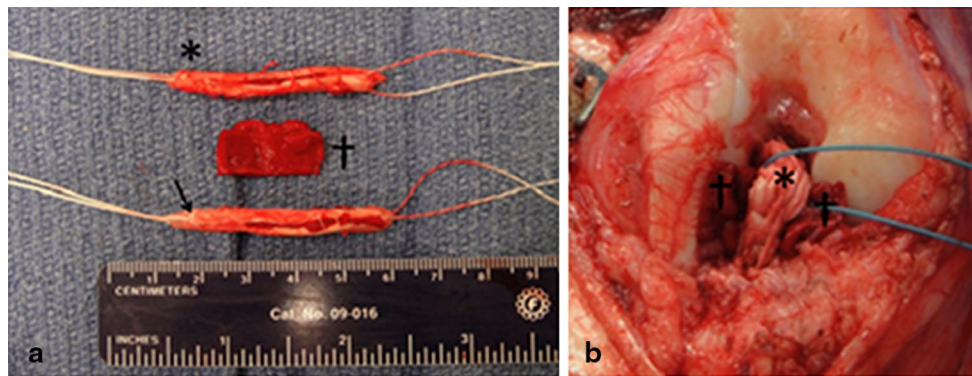
Ten minutes prior to drilling the tibial tunnels, 50 cc of venous whole blood was drawn from a peripherally located, commonly used permanent venous catheter and placed in a 250-ml sterile, borosilicate glass beaker. Using a sterile stir

rod, the whole blood was gently stirred in a twisting clockwise fashion for 10–15 min until coagulation and formation of a fibrin clot was achieved. Once clotted, the fibrin clot was removed from the beaker and placed on sterile gauze. The clot was separated into two small portions and one larger portion. The two smaller portions were incorporated into the proximal (femoral) ends of the graft (Fig. 2a), with the larger portion of the fibrin clot placed between the two intra-articular grafts (Fig. 2b).

#### Double-bundle ACL reconstruction with fibrin clot

The DB ACL reconstruction was performed in the same manner as described above with the following additional steps: the AM and PL grafts had a fibrin clot incorporated into the proximal portion as described above. After passage of the grafts, a looped suture was placed between the AM and PL bundle in order to place gentle traction on the AM bundle in an anterior direction (Fig. 2b). This created adequate space for the placement of the fibrin clot, which was introduced between the AM and PL bundles, taking care to maximize the surface area of contact between the clot and





**Fig. 2** **a** Achilles tendon autograft on back table with AM (*asterisk*) and PL (*downwards arrow*) graft. Fibrin clot (*dagger*) to be placed between the two bundles. **b** Intra-operative photograph with fibrin clot placed between the AM and PL bundles

grafts. Using a 2-0 vicryl, three simple stitches were placed through the AM bundle into the PL bundle to secure the clot in place.

Postoperatively, free cage activity was allowed and gait was documented as described previously utilizing a lameness score [39]. Vital signs and general animal health were monitored. Goats were killed after 12 weeks with an overdose injection of sodium pentobarbital.

### Histology

The knee joints were harvested and assessed macroscopically for signs of graft failure, osteoarthritis, fibrous formation and any signs of abnormal pathology. The intra-articular ACL reconstruction segments were harvested from the femoral and tibial origins and immediately frozen in liquid nitrogen for cryosectioning. Serial sections of 8  $\mu\text{m}$  thickness were followed by routine histological staining (haematoxylin and eosin [H&E]) for visualization with light microscopy. Specimens were sectioned in the axial and coronal planes. For histological evaluation, images were obtained at low- and high-power magnification with a Leica DFC 300 FX digital camera (Leica Microsystems, Wetzlar, Germany) coupled to a Leica DMLB light microscope. For histological evaluation, three central sections of every animal were analysed by two observers who were blinded to each other and to the assigned treatment for the animal. The ligament tissue maturity index, as described by Murray et al. [23], was used to quantify histological specimens. A native ACL, harvested from the contralateral knee, was used as a reference for these criteria.

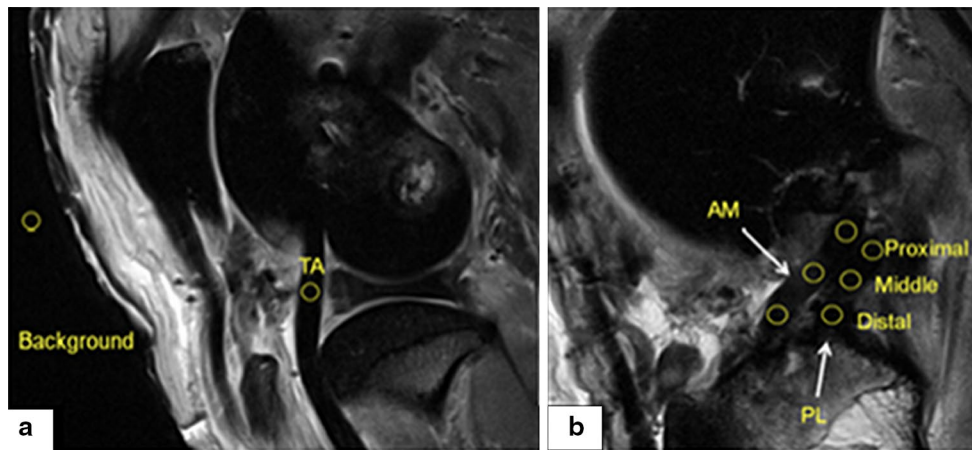
### Immunohistochemistry

For detailed immunohistochemical analysis of the neoligament vascularity, cryosections were stained with monoclonal antibodies against alpha-smooth muscle actin ( $\alpha\text{-SMA}$ )

and collagen type 1. After fixation with methanol, all samples were treated with 0.3 % hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity. Non-specific binding of primary antibodies was reduced by blocking with normal donkey serum.  $\alpha\text{-SMA}$  antibody binding was detected with Alexaflour 488 Donkey, and counterstaining was performed using DAPI. As in histological analysis, three sections of every animal were analysed (counting of the blood vessels) and the median from these sections was used for statistical analysis. Photomicrographs were taken on three different slides treated with  $\alpha\text{-SMA}$ . On these slides, vascularity was determined by counting all the vessels in a 6-mm<sup>2</sup> area and the vessel count per mm<sup>2</sup> was calculated. Collagen type 1 antibody binding was detected with Alexaflour 594 Donkey, and counterstaining was performed using DAPI. Photomicrographs were taken and the two best, most representative sections of every animal were analysed for the percentage of collagen type 1 staining per 100 $\times$  magnification field, and the mean was used for statistical analysis.

### Magnetic resonance imaging

Immediately after euthanization, the right hindlimb was harvested and imaged on a 3-T MRI (Siemens, Erlangen, Germany). Sagittal T2, TE-18, slice thickness 2-mm sequences were used for analysis. MRI signal intensity was determined utilizing the signal to noise quotient (SNQ) [3]. Using the OSIRIX free imaging software (version 3.7.1, Pixmeo Sari, Bernex, Switzerland), three positions on the AM and PL bundle were determined: proximal, midsubstance and distal. A 4-mm<sup>2</sup> circle region of interest (ROI) was placed in the centre of the three locations. The ROI size was determined as the maximum circular area that could be used in all animals and stay within the limits of the graft in all three regions. The mean signal intensity and standard deviation were determined for each region. A single



**Fig. 3** Method for determining the signal noise quotient (SNQ). **a** Sagittal T2 image showing the placement of 4-mm<sup>2</sup> region of interest (ROI) on tibialis anterior (TA) and background signal. **b** Three ROIs are placed on the AM and PL bundles: proximal, middle and distal

4-mm<sup>2</sup> circle ROI was determined in the tibialis anterior (TA) and on the background 5 mm anterior to the patella as a normal control (Fig. 3). SNQ was calculated as (1) AM SNQ = (AM signal – TA signal)/Background signal and (2) PL SNQ = (PL signal – TA signal)/Background signal.

The study protocol was approved by the Institutional Animal Care and Use Committee of our institution (Division of Laboratory Animal Resources, Protocol no. 0912030).

#### Statistical analysis

Data analysis began with the calculation of descriptive statistics including frequency counts and percents for categorical variables and measures of central tendency (means, medians) and dispersion [standard deviation, inter-quartile ranges (IQR)] for continuous variables. Because of the small sample sizes, the data are reported as medians and inter-quartile ranges, and we used nonparametric statistical methods to analyse the data. Independent sample Mann–Whitney *U* tests were used to compare the ligament tissue maturity index score, vascularity, collagen type 1 and the SNQ between the two groups. Spearman’s correlation coefficients were used to determine the relationships between the ligament tissue maturity index score, vascularity, collagen type 1 and the SNQ. All statistical analyses were performed using SPSS for Windows, version 16 (SPSS, Chicago, IL). Statistical significance was determined to be  $p < 0.05$ .

#### Results

Three animals were excluded from this study. One animal in the “control” group sustained systemic sepsis in

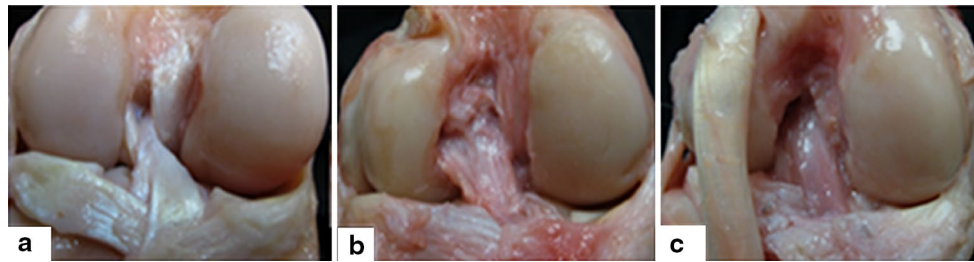
the immediate postoperative period and was euthanized at 1 week. One animal in the “fibrin clot” group sustained a neurological insult due to a fall on postoperative day #2 and was euthanized at 1 week. One animal in the fibrin clot group developed septic arthritis. The animal was therefore excluded from further analysis. The remaining animals tolerated surgery well and displayed uneventful skin healing. Normal gait was achieved by 4 weeks postoperatively. Eight animals were included in the analysis for this study.

#### Macroscopic analysis

In both groups, newly formed scar tissue could be found adjacent to the pre-patellar fat pad and the neoligament. After removal of the tissue, it was observed that the fibrin clot group had more continuity between the AM and PL bundle; however, excessive macroscopic examination was not undertaken in order to preserve new tissue formation between the bundles for histological analysis. All grafts appeared intact with stable connections to femur and tibia (Fig. 4).

#### Histology

On H&E analysis, there was a consistently more organized and ligamentous-appearing tissue in the “fibrin clot” group when compared to the “control” group, although the morphology and microstructure of both groups contrasted with the native caprine ACL. More fusiform cells could be found in the “fibrin clot” group throughout the grafts, whereas ovoid cells were found in the “control” group. There was a more pronounced septum between the AM and PL bundles in the “fibrin clot” group (Fig. 5). However, the median and inter-quartile range (IQR) for the ligament tissue maturity index score were not significantly different (Table 1).



**Fig. 4** Macroscopic evaluation. **a** Native caprine ACL. **b** DB ACL-R with fibrin clot at 12 weeks. **c** DB ACL-R without fibrin clot at 12 weeks

#### Immunohistochemistry ( $\alpha$ -SMA)

In contrast to the native ACL, samples from both groups show increased vascularity in the central regions of the graft (Fig. 6). Vessels in the “fibrin clot” group were smaller and distributed more regularly than in the “control” group with their orientation along the bundles (Fig. 6c, d). Arterioles were noticed in 7 out of 8 goats in areas between the two bundles of the graft as well as in the adjacent newly formed tissue (Fig. 6b). There was no significance between the two groups in regard to vascularity content (Table 1). There was a significant negative correlation between the vascularity and the ligament tissue maturity index score ( $r = -0.9$ ,  $p = 0.005$ ).

#### Immunohistochemistry (collagen type 1)

The native goat ACL had a collagen type 1 of 86.58 % and was orientated and organized in the axial section (Fig. 7a). Orientation and organization of collagen fibres and percentage per field were not significant between the groups (Figs. 7b, c, 8a–d, n.s.; Table 1).

#### Magnetic resonance imaging (Fig. 9)

There was a significant difference in the proximal AM, proximal PL and distal PL SNQ between the two groups ( $p < 0.05$ ; Fig. 5; Table 2). There was a significant negative correlation between total AM SNQ and collagen type 1 ( $r = -0.8$ ,  $p = 0.02$ ), as well as total PL SNQ and collagen type 1 ( $r = -0.7$ ,  $p = 0.05$ ).

## Discussion

The most important finding of the present study was that the addition of a fibrin clot resulted in evidence of advanced healing on histology and decreased signal intensity on MRI when compared to a control group at 12 weeks following DB ACL reconstruction in a caprine model. These results support the hypothesis that the addition of a fibrin clot to

ACL-R would result in advanced healing when compared to standard DB ACL reconstruction without a fibrin clot.

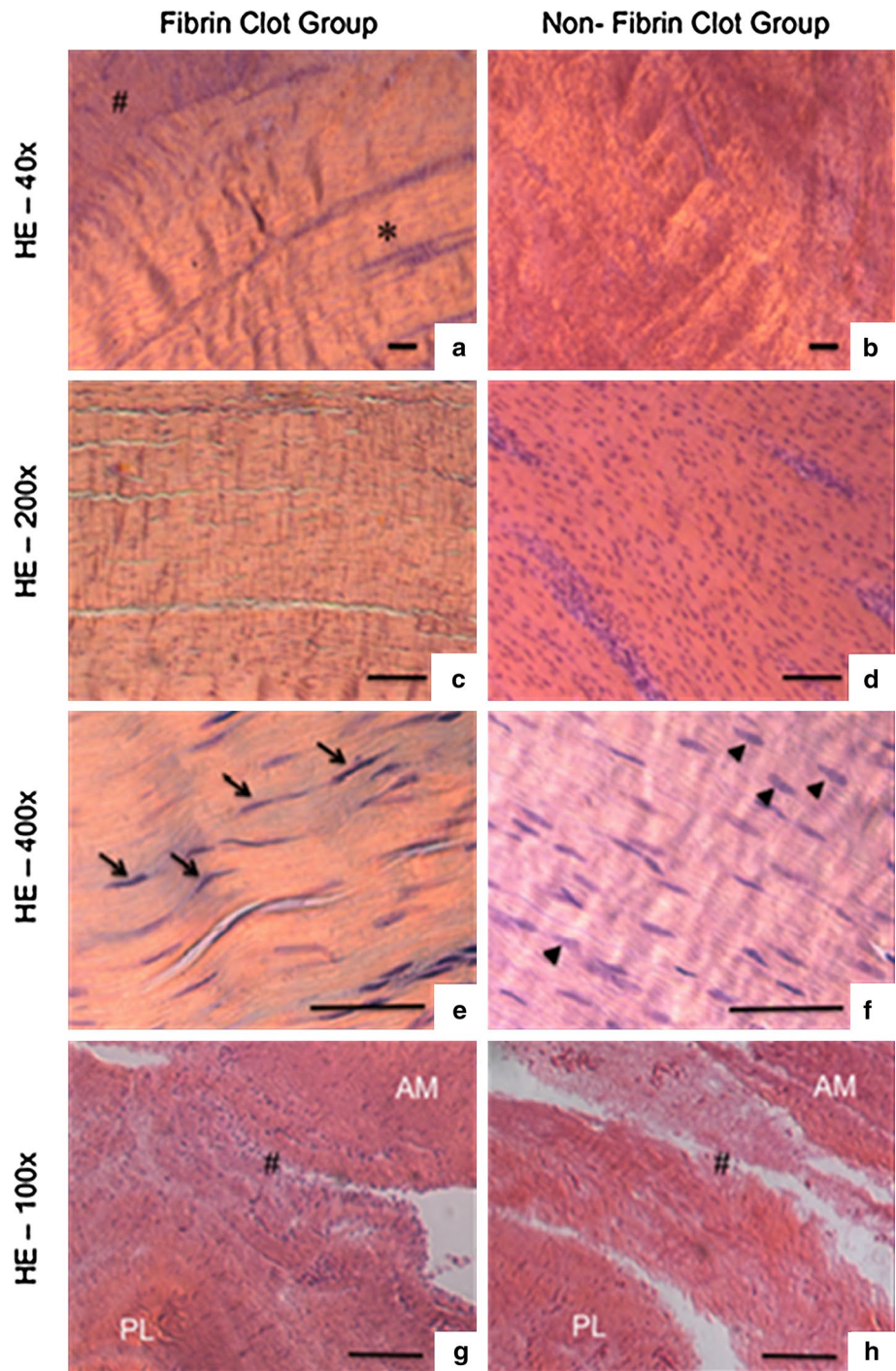
The ligament maturity index in the fibrin clot group indicates, however, statistically not significant in the present study, that the remodelling tissue appears more ligamentous compared with the samples without a fibrin clot. Addition of a fibrin clot resulted in more fusiform cells indicating the capability of producing and organizing collagen [2, 8]. Although not statistically significant, the trend towards a higher collagen content in the fibrin clot group is supported by this. It is therefore likely that improved biomechanical properties after DB ACL reconstruction with a fibrin clot would be achieved [9, 25].

The histological and immunohistochemical results in both groups showed higher vascularity of the graft after 12 weeks compared with the native ACL. The addition of a fibrin clot resulted in decreased vascularity and more mature appearing vessels; however, the number of vessels between both groups was not significant. A recent study showed in a porcine model that the vascularity of remodelling tissue increases until 6 weeks postoperatively and then decreases until 12 weeks after surgery, indicating a more matured graft [16, 20]. Furthermore, higher vascularization correlates to decreased mechanical properties [34, 37]. The current study demonstrated that there was a significant negative correlation between vascularity and ligament tissue maturity index score, with a higher vascularity associated with a lower ligament tissue maturity index score.

The mean percentage of collagen type 1 per group was higher in the fibrin clot group than in the non-fibrin clot, although this was not statistically significant. As evident by the native ACL collagen type 1 content (86.58 %), a higher collagen type 1 concentration is associated with a more ligamentous-like structure and increased tensile properties when compared to lower collagen type 1 percentages. An increase in signal intensity on MRI (both AM and PL bundles, Fig. 9) was shown to correlate with a decrease in collagen type 1 percentage. This correlation may prove to be clinically useful when evaluating patients with increased signal intensity in the ACL on MRI after reconstruction as this may be related to a decrease in



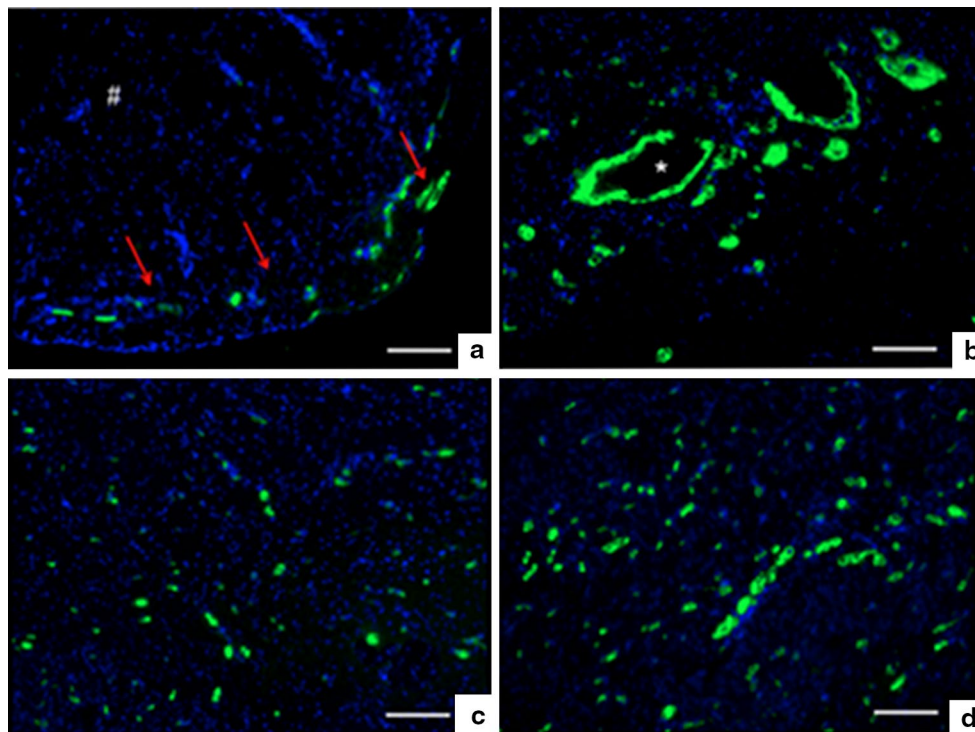
**Fig. 5** Hyphen indicates representative samples of histological staining of the graft (haematoxylin and eosin [H&E]). Notice the different appearance of the remodelling tissue between the two groups under polarized microscope. **a** Stained tissue appears more tendon-like (*asterisk*) with some areas of increased remodelling and higher cell density (*hash*). **c** On higher magnification, bundle-like structures and crimp can be noticed. **d** Remodelling tissue in the non-fibrin clot group appears inhomogeneous with the appearance of a large number of cells. **e** The fibrin clot group shows consistently more spindle cells (*arrows*). **f** In the non-fibrin clot group, more ovoid cells can be found indicating increased remodelling. **g** Area between the bundles (*hash*) showing dens scar tissue formation. **h** Shows loose tissue with less active ovoid cells. Bar size: **a, b, g, h** 100  $\mu\text{m}$ ; **c–f** 40  $\mu\text{m}$ ; longitudinal section: **a–f**, cross section: **g + h**



**Table 1** Ligament tissue maturity index score, vascularity (vessels/ $\text{mm}^2$ ) and collagen type 1 (% per 100 $\times$  field) per group

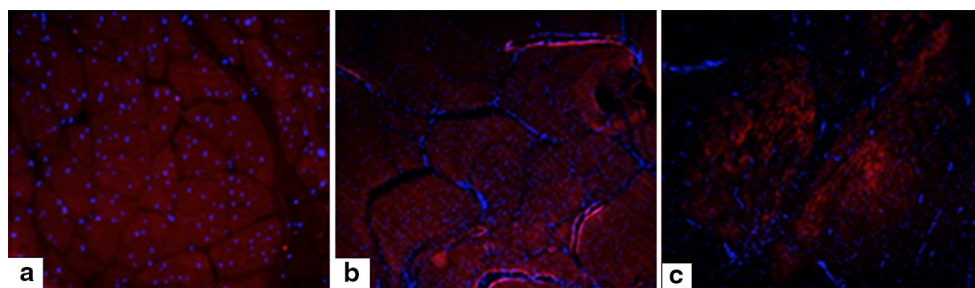
	Group 1 (median (IQR))	Group 2 (median (IQR))	<i>p</i> value
Ligament tissue maturity index score <sup>a</sup>	15 (13–17)	6 (4–12)	n.s.
Vascularity (vessels/ $\text{mm}^2$ ) <sup>a</sup>	7.5 (6.3–7.9)	8.1 (7.3–11.3)	n.s.
Collagen type 1 (% per 100 $\times$ field)	36.3 (19.0–60.0)	13.9 (12.4–36.8)	n.s.

<sup>a</sup> Expressed as median and inter-quartile range



**Fig. 6** Hyphen indicates representative immunohistochemistry,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) where green is a positive stain, cross-sectional samples. **a** Native ACL: minimal vascularity inside the tendon (*hash*) and increased vascularity at the border of the ligament (*red arrows*). **b** Arterioles (*asterisk*) between bundles of a sam-

ple from the fibrin clot group. **c, d** Intraligamentous examples of fibrin clot group showing less vascularity compared to an representative sample from the non-fibrin clot group. Bar size in all samples: 40  $\mu$ m



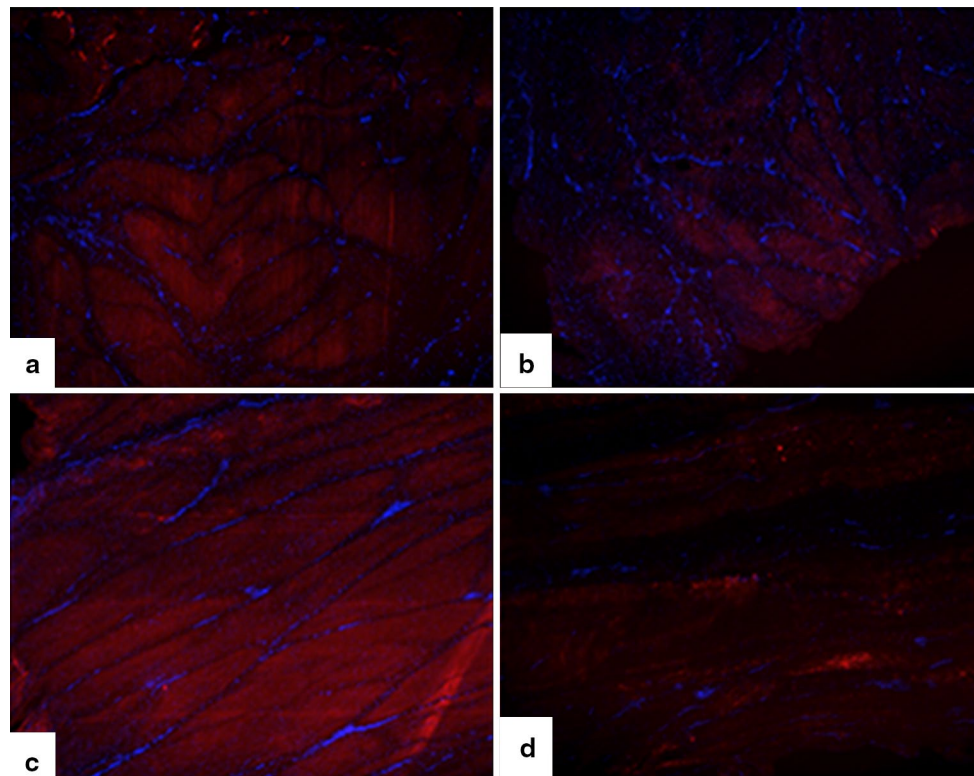
**Fig. 7** Immunohistochemistry for collagen type 1 at  $\times 100$  magnification. **a** Axial section of native goat ACL. **b** Axial sections of fibrin clot group demonstrating increased collagen content as well as more organized and orientated tissue when compared to the non-fibrin clot group (**c**)

collagen type 1 content and potentially decreased biomechanical properties. Previous literature has shown that the use of PRP in ACL-R resulted in lower signal intensity when compared to a control group [24, 27, 31]. This study showed that signal intensity on MRI of the “fibrin clot” group was significantly lower in two regions (one in AM and one in PL) and the PL bundle as a whole compared to the “control” group (Table 2). Studies have shown that high intensity on MRI is correlated with an increase in vascularity and a decrease in mechanical properties of the graft [37].

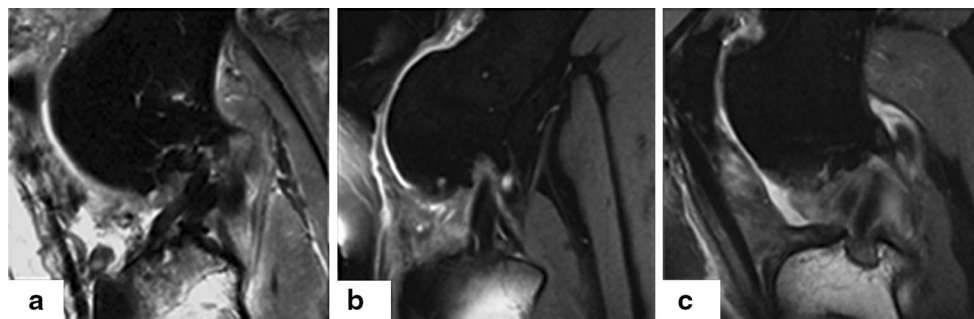
Complete healing and incorporation of the graft require the two bundles created with double-bundle ACL reconstruction to heal to one another and allow load sharing as in the native ACL. The current study found evidence that addition of a fibrin clot in between the AM and PL bundles resulted in increased tissue formation between the bundles as well as a more mature graft. This could aid in healing of the bundles and provide a basis for bundle synergy.

Limitations of this study include lack of biomechanical testing on the effect of the fibrin clot on ACL reconstruction. Furthermore, there is a small sample size in the





**Fig. 8** Immunohistochemistry for collagen type 1 at  $\times 50$  magnification for axial and longitudinal section for the fibrin clot group (a, c) and the non-fibrin clot group (b, d). The fibrin clot group demonstrated increased collagen content and organization



**Fig. 9** Sagittal T2 MRI. **a** One week following DB ACL-R without fibrin clot showing low signal intensity. **b** Twelve weeks following DB ACL-R with fibrin clot showing low signal intensity in AM and

PL bundles. **c** Twelve weeks following DB ACL-R without fibrin clot showing high signal intensity in the AM and PL bundles

present study. However, while the results indicate favourable effects of a fibrin clot, further studies with a larger number of animals in each group will be necessary to achieve significance. This study did not have a PRP control group, and therefore, a direct comparison of PRP over a fibrin clot could not be made. However, previous studies attempted to use PRP in the caprine model and found it to be unsuccessful due to the similarities in the caprine red blood cell and platelet [32]. The properties of the fibrin clot, such as type of growth factors and their concentration inside the clot as

well as their release over time, were not investigated. These issues will also have to be investigated in further studies. The growth factor concentrations in the fibrin clot are also unknown and would be beneficial as studies have shown that delivery of platelet-derived growth factors alter the mechanical properties during graft remodelling [36].

This study showed that there is potential to improve healing and ligamentization of the graft and usage of a fibrin clot is one option; however, significant improvement could not be demonstrated.

**Table 2** Median values and inter-quartile range of the signal noise quotient (SNQ) per group

SNQ	Group 1 (median (IQR))	Group 2 (median (IQR))	<i>p</i> value
Proximal AM <sup>a</sup>	1.1 (.4–1.2)	3.3 (2.7–4.2)	<i>p</i> = 0.03*
Mid AM <sup>a</sup>	1.1 (.8–1.3)	2.5 (1.4–5.2)	n.s.
Distal AM <sup>a</sup>	0.9 (.6–2.6)	2.4 (1.3–3.6)	n.s.
Total AM <sup>a</sup>	1.0 (.6–1.6)	2.5 (1.8–4.3)	n.s.
Proximal PL <sup>a</sup>	1.4 (.7–2.6)	5.2 (3.6–6.8)	n.s.
Mid PL <sup>a</sup>	0.8 (.4–1.2)	2.7 (1.5–4.1)	n.s.
Distal PL <sup>a</sup>	1.0 (.30–1.6)	2.7 (2.3–3.8)	<i>p</i> = 0.03*
Total PL <sup>a</sup>	1.2 (.6–1.6)	3.6 (2.6–4.8)	<i>p</i> = 0.03*

AM anteromedial bundle, PL posterolateral bundle, *mid* midsubstance

<sup>a</sup> Expressed as median and inter-quartile range

\* Statistical significance

## Conclusion

Fibrin clots are readily available, require no additional cost and can be prepared in as little as 10 min [14]. Data from this study indicated that the addition of a fibrin clot to DB ACL reconstruction resulted in a slightly higher histological score of ligament and decreased MRI signal intensity indicating improved healing. There were also trends indicating that a fibrin clot increased collagen content and decreased vascularity although these were not statistically significant. Bundle-to-bundle healing in this study was improved providing the necessary basis for load sharing.

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