The influence of avascularity on the mechanical properties of human bone-patellar-tendon-bone grafts

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Our aim was to analyse the effect of avascularity on the morphology and mechanical properties (tensile strength, viscoelasticity) of human bone-patellar-tendon-bone (BPTB) grafts in vitro. These were harvested at postmortem and stored submerged in denaturated human plasma at a constant pH, pO₂, pCO₂, temperature and humidity under sterile conditions. Mechanical testing was performed two and four weeks after removal of the graft. The mean ultimate strength was 1085.7 ± 255.8 N (control), 1009.0 ± 314.9 N (two weeks cultured) and 1076.8 ± 414.8 N (four weeks cultured). There was no significant difference in linear stiffness or deformation to failure between the groups. There was a difference in viscoelasticity between the control group and the avascular grafts and the latter had significant lower peak load-to-load ratios after 15 minutes compared with the control group. After two and four weeks the graft contained viable fibroblasts. There was regular cellularity in the superficial layers and decreased cellularity in the midportion. The structure of the collagen including the crimp pattern appeared to be normal in polarised light.

We conclude that avascularity does not significantly affect ultimate failure loads or stiffness of BPTB grafts. Slight changes in viscoelasticity were induced, but the significance of the increased stress relaxation is not fully understood.

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A bone-patellar-tendon-bone (BPTB) autograft is presently the most widely used graft for reconstruction of the anterior cruciate ligament (ACL).¹ The advantages are the high tensile strength at the time of surgery and secure bone-to-bone fixation.

The natural history of BPTB autografts has been well documented in animal experiments²⁻¹² and in biopsy studies in man¹³⁻¹⁵ using histological and microvascular techniques. The transplanted patellar tendon undergoes changes in microstructure and structural properties. Four stages of autograft transformation have evolved: avascular necrosis, revascularisation, cellular proliferation, and remodelling.¹⁶ Some investigators have focused on the later stages analysing revascularisation, cellular proliferation, and remodelling.^{4,13,14} In accelerated rehabilitation after reconstruction of the ACL¹⁷ the behaviour of the graft in the immediate postoperative period is of more significance.

The graft is transplanted without a blood supply and therefore avascularity may be one of the major determinants of its environment within the first weeks after surgery. In animal models the graft tissue remains avascular for different periods of time. Revitalisation begins at two weeks in the rabbit⁸ and four weeks in the dog⁵ with round to ovoid cells visible at the margins of the graft⁸ and a synovial membrane rich in vessels surrounding it.⁵ In the rabbit the number of cells within the graft dramatically increases at four weeks.⁸ In the dog intrinsic vessels are seen in the graft at eight weeks.⁵

Data from animal experiments suggest a dramatic decrease in tensile strength after surgery^{6,7,9-11} reflecting the development of avascular necrosis. In these studies, however, the tibia and femur were both fixed to the testing machine thus evaluating both the graft and its graft fixation. When tested in the first weeks after surgery these constructs failed at the site of fixation, representing the weakness of fixation rather than the structural properties of the graft tissue. These studies are based on animal experiments and focus on ultimate failure loads. The viscoelasticity of the grafts, which may be as important for graft function as strength, was not examined.

We have therefore analysed the effect of avascularity on the morphology and mechanical properties, such as the tensile strength and viscoelasticity, of human BPTB grafts in vitro.

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Materials and Methods

We used a tissue-culture model to study avascularity in vitro and the experiments were performed in two series.

Series 1

The objective was to study ultimate failure loads and mode of failure of avascular grafts under high rates of strain and to assess the histological appearance after two and four weeks of tissue culture.

Specimens. Human BPTB specimens were obtained at postmortem within 24 hours of death from 11 pairs of knees. There were nine male and two female donors with a mean age of 57 years (42 to 69). We harvested two grafts from each patellar tendon, under sterile conditions, one each from the central and medial thirds, 10 mm in width with bone plugs from the patella and tibia. They were immediately placed in Petri dishes filled with physiological saline.

The grafts of one knee were assigned randomly to one of two tissue culture groups. Those of the opposite knee of each donor were used as a paired control and were tested immediately.

Tissue culture. The specimens were cultured in Petri dishes submerged in human plasma for two or four weeks. The plasma had been denaturated previously by increasing the temperature to 56°C for 20 minutes. An antibiotic for cell culture (penicillin/streptomycin, 3%) was added to avoid bacterial infection.

The specimens were incubated at 37°C in a humidified atmosphere of 5% CO₂/95% air (cell culture incubator; Nunc Inc, Wiesbaden, Germany). They were checked twice a week for bacterial growth and for the pH of the plasma which was changed each week.

Mechanical testing. We used a universal testing machine (type 1474; Zwick Inc, Ulm, Germany) to examine the tensile properties of the BPTB grafts at room temperature. Specimens were kept moist with saline solution. Both ends were attached to the machine by custom-made clamps. Each specimen was preconditioned cyclically (10 cycles between 40 N and 100 N) and then allowed 15 minutes of recovery. It was then loaded to failure at a rate of displacement of 500 mm/min. Load-deformation curves were registered for each test. From each curve we calculated the maximum load, linear stiffness and deformation to maximum load. The deformation was measured from clamp

to clamp. The cross-sectional area was measured using calliper gauges. Maximum stress and elastic modulus were calculated.

Statistical analysis was performed using the Wilcoxon test for paired data.

Failure mode. There were four modes of failure: ligamentous disruption, avulsion of the tendon from the bone plug, fracture of the bone plug and complex failure with a longitudinal separation of tendon tissue and avulsion from the opposite bone plugs.

Statistical analysis was performed using the chi-squared test.

Histological analysis. Specimens were prepared after testing and stained with haematoxylin and eosin and Masson-Goldner. They were analysed for cell viability and collagen structure (polarised light).

Series 2

The objective was to examine ultimate failure loads and failure modes of avascular grafts under low rates of strain and to assess viscoelasticity after two weeks of tissue culture.

Specimens. BPTB specimens were obtained at postmortem within 24 hours of death from eight pairs of knees. There were six male and two female donors with a mean age of 59 years (40 to 68). We harvested two tendon grafts from each patellar tendon under sterile conditions, one each from the central and medial thirds, 10 mm in width with bone plugs from the patella and tibia. They were immediately placed in Petri dishes filled with physiological saline.

The grafts of one knee were assigned to the tissue-culture group and those of the opposite side were used as a paired control and were tested immediately.

Tissue culture. The specimens were cultured in Petri dishes submerged in human plasma for two weeks as described for series 1.

Mechanical testing. We used the universal testing machine and procedure as described for series 1. Specimens were subjected to a static relaxation test. A strain inducing a graft load of 100 N was applied and maintained for 15 minutes. The percentage of peak load maintained was assessed every minute. The final load-to-peak-load ratios were calculated. The grafts were then returned to their resting lengths for 15 minutes of recovery.

The specimens were also subjected to a cyclic creep test. They were loaded for ten cycles between 40 N and 120 N.

Table I. Series 1. Structural and material properties (mean $\pm$ SD) of human BPTB grafts. The cross-head speed was 500 mm/min

	Control group	Avascular group	
		2 weeks	4 weeks
Ultimate load (N)	1085.7 $\pm$ 255.8	1009.0 $\pm$ 314.9	1076.8 $\pm$ 414.8
Linear stiffness (N/mm)	118.5 $\pm$ 30.8	110.9 $\pm$ 55.1	113.5 $\pm$ 40.7
Relative strain (%)	25.7 $\pm$ 5.6	24.4 $\pm$ 4.8	24.5 $\pm$ 4.7
Ultimate stress (MPa)	33.9 $\pm$ 7.3	31.7 $\pm$ 9.6	32.8 $\pm$ 12.0
Elastic modulus (MPa)	186.2 $\pm$ 44.7	185.6 $\pm$ 64.1	173.3 $\pm$ 1.7

Fig. 1

Photomicrograph of a human patellar tendon after two weeks in tissue culture showing a longitudinal section of the superficial layers. Viable spindle-shaped nuclei of fibroblasts (arrow) indicate a normal cellular pattern (haematoxylin and eosin $\times 60$).

Fig. 2

Photomicrograph of a human patellar tendon after two weeks in tissue culture showing a longitudinal section of the central area. The central core of the graft has areas of acellularity. Only some nuclei of fibroblasts can be seen which are less easily stained (arrows) (haematoxylin and eosin $\times 60$).

Fig. 3

Photomicrograph of a longitudinal section of a human patellar tendon after two weeks in tissue culture under polarised light. The crimp pattern of the collagen appears to be normal (haematoxylin and eosin $\times 23$).

Fig. 4

Photomicrograph of the peritendineum area showing endothelial cells of blood vessels which are necrotic (arrow) and have lost their integrity forming clusters within the vessels. The surrounding tendon tissue appears to be normal with a regular distribution of viable fibroblasts (haematoxylin and eosin $\times 60$).

The strain rate was 100 mm/min. The cyclic creep was given as the increase in length at 120 N between the first and tenth cycles relative to the resting length. After another 15 minutes of recovery the specimens were loaded until failure at a rate of strain of 10 mm/min.

Statistical analysis was performed using the Wilcoxon test for paired data.

Results

Series 1

Mechanical testing. There was no significant difference in the ultimate strength, linear stiffness and strain to maximum load between the control group and the cultured specimens (Table I). The failure modes are given in Table II. There was no significant difference between the groups.

Histological findings. After two and four weeks the graft contained viable fibroblasts in the superficial layers (Fig. 1). The midportion showed a relative decrease in tissue cellularity with acellularity in scattered areas (Fig. 2). The structure of collagen including the crimp pattern appeared to be normal under polarised light (Fig. 3). The arrangement of the bundles was disturbed only in spotted areas in

Table II. Series 1. Mean failure modes of human BPTB grafts

	Control group	Avascular	
		2 weeks	4 weeks
Tendon rupture	7	2	1
Tendon avulsion	5	3	2
Bone plug fracture	8	4	5
Complex failure	2	2	3

the mid-portion of some grafts. The endothelial cells of the blood vessels were necrotic and had lost their integrity forming clusters within the vessels (Fig. 4).

Series 2

Mechanical testing. There was no significant difference in the structural and material properties between the groups (Table III). Failure modes are given in Table IV. There was no significant difference between the groups.

Cyclic creep was $1.19 \pm 0.33\%$ in the control group and $1.29 \pm 0.42\%$ in cultured specimens. The difference was not statistically significant.

The final load-to-peak-load ratio (Fig. 5) after 15 minutes was $70.9 \pm 5.2\%$ in the control group and $65.1 \pm 5.6\%$ in the avascular group. The difference was statistically significant ($p < 0.01$).

Discussion

Animal studies have shown that BPTB grafts undergo changes in structure and a dramatic loss of tensile strength. In a canine model Butler et al⁶ found that the lowest maximum force (14% of the opposite unaffected ACL) and the lowest stiffness (9% of the opposite unaffected ACL) occurred immediately after surgery. The tensile strength increased gradually to 28% of the control at 26 weeks. In monkeys¹¹ the ultimate load was 16% and the stiffness 24% of the control ACL at seven weeks. Both increased at approximately the same rate up to 29 weeks after operation. In a goat model McPherson et al⁷ observed a maximum force to failure of 1% of the intact ACL immediately after surgery. Yoshiya et al,⁹ in a canine model, showed that the femur-patellar-tendon graft-tibia complex tested immediately after reconstruction failed at a load which was only 10% of that of the control patellar tendon. They stated that this was due to weakness at fixation sites. After three months, the value increased up to 20% of the control, and specimens failed in the graft substance. Ballock et al¹⁰ using a rabbit model showed that the stiffness was 15% and

Table III. Series 2. Structural and material properties (mean $\pm$ sd) of human BPTB grafts. The cross-head speed was 10 mm/min

	Control group	Avascular group (2 weeks)
Ultimate load (N)	932.6 $\pm$ 355.3	951.2 $\pm$ 399.5
Linear stiffness (N/mm)	97.2 $\pm$ 32.2	106.1 $\pm$ 33.4
Relative strain (%)	28.9 $\pm$ 9.6	28.3 $\pm$ 8.0
Ultimate stress (MPa)	29.1 $\pm$ 10.3	29.1 $\pm$ 11.1
Elastic modulus (MPa)	148.6 $\pm$ 63.6	150.1 $\pm$ 51.9

Table IV. Series 2. Mean failure modes of human BPTB grafts

	Control group	Avascular group (2 weeks)
Tendon rupture	0	0
Tendon avulsion	2	3
Bone plug fracture	13	11
Complex failure	1	2

the ultimate load 7% of the control immediately after surgery. Kasperczyk et al¹² suggested that the decrease in tensile strength within the first weeks is caused by a deterioration of collagen tissue due to avascular necrosis. Examination of the experimental detail of these studies^{6,7,9-11} shows that the authors had attached the femur and tibia to the testing machine. There were various methods of graft fixation and it is possible that the strengths reported related to fixation failure and not graft tissue failure. Therefore these data do not support the conclusion that the strength of a BPTB graft decreases dramatically immediately after surgery because of avascular necrosis of graft tissue.

In our study avascularity did not significantly alter the structural properties (maximum load, linear stiffness, deformation to maximum load) or material properties (maximum stress, elastic modulus) during maximal loading.

Fig. 5

Mean ($\pm$ SD) loss of tension because of viscoelastic relaxation which was significantly higher in avascular tendons ($p < 0.01$).

The failure loads of the specimens were relatively low compared with data given for young donors (mean age 26 and 28 years) in some studies.^{18,19} In these the mean maximum loads at failure were 2900 N¹⁸ and 3057 N.¹⁹ The cross-head speed was very high in both studies (100% of the graft length/s). In addition, Noyes et al¹⁸ harvested grafts of 14 mm width which is not the standard surgical technique. In man the maximum width of a BPTB graft is usually 10 mm.

The tensile strength and the linear stiffness of tendons and ligaments decrease with age.^{20,21} In addition, the failure loads of cadaver specimens depend on the activity level of the donors before death.²²⁻²⁴ In donors aged from 17 to 54 years Blevins et al²⁵ observed a maximum load of 1693 N and a maximum stress of 35.9 MPa which is not much higher than the results in our study although their donors were younger. Petermann et al²⁶ reported an ultimate tensile strength of 1151 N (369 to 3008) in donors aged from 32 to 80 years. In donors with a mean age of 60 years Bechthold et al²⁷ found an ultimate stress of 23.76 MPa which is lower than that in our study.

Woo et al²¹ investigated the structural properties of the femur-anterior-cruciate-ligament-tibia complex in younger (22 to 49 years) and older (60 to 97 years) donors. They found mean ultimate loads of 1954 N (younger donors) and 642 N (older donors) with a linear stiffness of 292 N/mm and 179 N/mm, respectively. In our study the ultimate strength of the avascular BPTB grafts exceeded that of the femur-anterior-cruciate-ligament-tibia complex of the older donors of Woo et al.²¹

The design of our study using paired specimens reduces any bias related to the history of the donors and quality of the tendon. The model which we used isolated 'avascularity' in vitro and evaluated its impact on the structure and mechanical properties of the graft tissue. There may, however, be other environmental conditions which impair the mechanical performance of the graft in the knee after surgery.

The graft is not cyclically stressed as it is in the joint after operation, although ligaments become weaker when immobilised.²²⁻²⁴ Therefore the lack of stress should not produce higher values of ultimate strength compared with the situation in vivo.

It is possible that there are aggressive factors similar to cytokine within synovial fluid after surgery. If these environmental factors have an impact on the morphology of the graft they should destroy graft tissue starting at the surface, as this is the area which is exposed to synovial fluid. Animal experiments^{2-5,8} have shown, however, that the superficial layers of the graft remained intact with viable cells and a normal structural arrangement of the collagen fibres. Changes in histomorphology affected the central areas of the tissue with focal areas of cell death, hypocellularity and fragmentation of collagen. This is consistent with the histomorphology of avascular grafts in vitro. These experimental data may not support the theory of aggressive factors in

synovial fluid, but are consistent with the view that avascularity is a significant environmental factor with respect to the histomorphology and biomechanics of the graft in the first weeks after surgery. The tendon tissue lacks a blood supply. Cells rely exclusively on diffusion for the supply of nutrients and oxygen. Nutrition of the cells in the central core of the graft may be insufficient due to the long diffusion distance. This would account for the pattern of histomorphological changes observed in animal studies.

There was a difference in the viscoelastic behaviour of the control and avascular groups. Avascular grafts had significant lower peak-load-to-load ratios after 15 minutes compared with the control group. Viscoelasticity may have an important role in the function of the graft, but its impact is not yet fully understood. The higher stress relaxation of avascular grafts may reduce the preload of the graft after surgery inducing more laxity of the joint. The concept of preconditioning of the graft^{28,29} is based on this theory. With an increased stress relaxation of avascular grafts preconditioning may be even more important to maintain the preload of the graft. On the other hand stress relaxation may protect collagen tissue during avascularity by reducing the load induced by rehabilitation.

We conclude that avascularity does not significantly affect ultimate failure loads or stiffness of BPTB grafts. Slight changes in viscoelasticity are induced, but the significance of the increased stress relaxation is not yet fully understood.

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