Bone & Joint Research

Supplementary Material

10.1302/2046-3758.125.BJR-2022-0340.R2

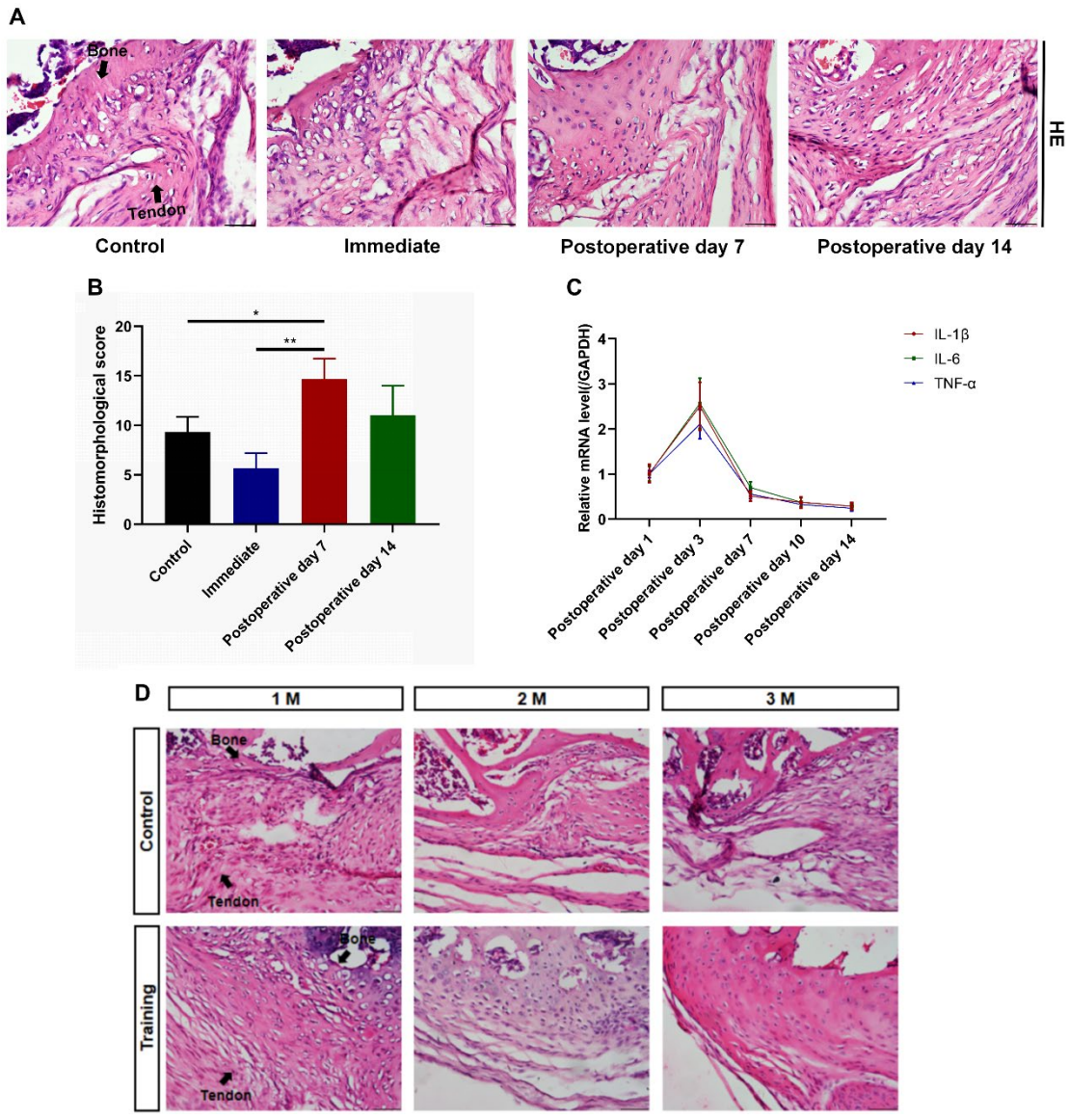

Fig a. Typical haematoxylin and eosin (HE)-stained image of each group and proinflammatory factor messenger RNA (mRNA) expression levels at different

times after surgery. a) Typical HE-stained image of treadmill training initiated at different times after surgery. b) Histomorphological score of treadmill training initiated at different times after surgery. Control = full free activity in the cage; Immediate = treadmill training initiated immediately; Postoperative day 7 = treadmill training initiated on postoperative day 7; Postoperative day 14 = treadmill training initiated on postoperative day 14. Our pre-experiment used the same treadmill training protocol in this study; mice were euthanized at one month after surgery. c) Changes in proinflammatory factor mRNA expression levels in tendon-bone insertion at different times after surgery. d) Typical HE-stained image of 1M-C, 1M-T, 2M-C, 2M-T, 3M-C, and 3M-T. Data are shown as the mean and standard deviation. N = 5 for all groups, *$p < 0.05$ and **$p < 0.01$ compared with postoperative day 7. Scale bar: 50 μm. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-1β, interleukin-1 beta; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha.

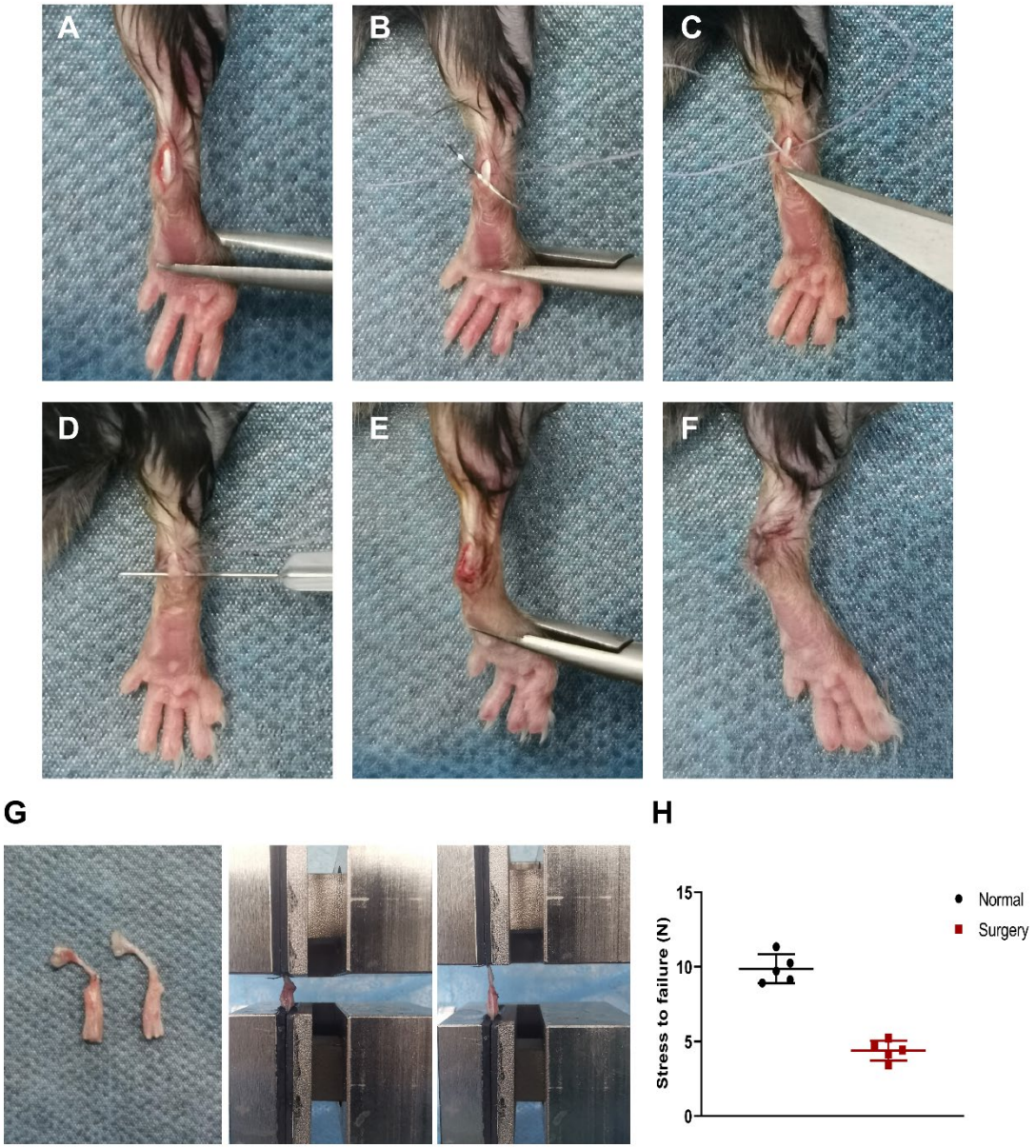

Fig b. Surgical procedures and biomechanical test of Achilles tendon detachment and repair. a) A cutaneous incision was made over the anterolateral side of the calcaneus to fully expose the Achilles tendon-bone insertion. b) The Achilles tendon was placed with 6-0 polydioxanone (PDS) suture in a figure-eight fashion. c) The Achilles tendon was sharply transected at its insertion on the surface of calcaneus. d) A transverse bone tunnel was established on the calcaneus. e) A 6-0 PDS suture was passed through the bone tunnel and tightened to approximate the Achilles tendon to its original footprint. f) Interrupted suturing was used to

close the cutaneous incision. g) Representative image of the biomechanical test process. h) Stress to failure data of surgical mice and normal mice. Data are shown as the mean and standard deviation. N = 5 for all groups.

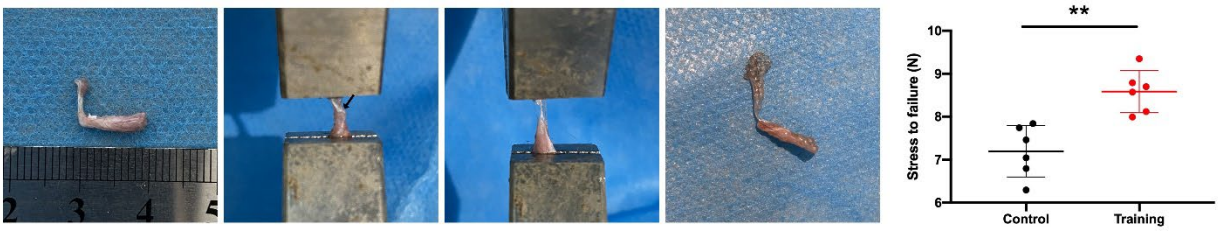

Fig c. Setting for the biomechanical test and results of maximal stress to failure. Black arrows indicate the tendon-bone insertion. Statistical significance was assessed using an independent-samples *t*-test or one-way analysis of variance. Data are shown as the mean and standard deviation (SD). *p < 0.05, **p < 0.01, and ***p < 0.001 compared with the control groups.

Table i. Histomorphometric scoring system for tendon-bone insertion healing.

Item	Score criteria			
	0	1	2	3
Tendon-to-bone interface				
Fibrocartilage cell number*	0% to 25%	25% to 50%	50% to 75%	≥ 75.0%
Fibrocartilage cell alignment	None	Unorganized	Moderate alignment	Highly aligned
Collagen fibre Continuity*	0% to 25%	25% to 50%	50% to 75%	75% to 100%
Collagen fibre Orientation*	0% to 25%	25% to 50%	50% to 75%	75% to 100%
Tidemark*	0% to 25%	25% to 50%	50% to 75%	75% to 100%
Cellularity*	> 400%	300% to 400%	200% to 300%	< 200%
Vascularity†	> 15	10 to 15	6 to 10	≤ 5
Inflammation	Abundant inflammatory cells	Moderate inflammatory cells	Minimal inflammatory cells	No inflammatory cells
Total scores	0 to 24			

*The percentage is the relative value compared with the uninjured tendon-bone interface tissue.
†Number of blood vessels per low-power field (×10) from each section low-power field.

Table ii. Target gene sequence list.

Gene	Primer	Sequence (5→3)
COL2A1	Forward	CAGGATGCCCGAAAATTAGGG
	Reverse	ACCACGATCACCTCTGGGT
COL10A1	Forward	TTCTGCTGCTAATGTTCTTGACC
	Reverse	GGGATGAAGTATTGTGTCTTGGG
SOX9	Forward	GAGCCGGATCTGAAGAGGGA
	Reverse	GCTTGACGTGTGGCTTGTTC
IL-1β	Forward	GCAACTGTTCCTGAACTCAACT
	Reverse	ATCTTTTGGGGTCCGTCAACT
IL-6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
TNF-α	Forward	CACCACCATCAAGGACTCAAAT
	Reverse	TCAGGGAAGAATCTGGAAAGGT
GAPDH	Forward	TGTGTCCGTCGTGGATCTGA
	Reverse	CCTGCTTCACCACCTTCTTGAT

COL2A1, type II collagen; COL10A1, collagen type X alpha 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-1β, interleukin-1 beta; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha.

Table iii. Scores for each item in the histomorphometric scoring system for each sample.

Group	Fibrocartilage cell number	Fibrocartilage cell alignment	Collagen fibre continuity	Collagen fibre orientation	Tidemark	Cellularity	Vascularity	Inflammation	Total scores
1 M-C1	1	2	2	1	1	1	2	2	12
1 M-C2	1	1	1	2	1	2	2	2	12
1 M-C3	1	1	1	0	1	2	2	1	9
1 M-C4	2	1	2	1	0	1	2	2	11
1 M-C5	0	1	1	1	0	1	1	2	7
1 M-T1	2	2	1	2	2	2	2	2	15
1 M-T2	1	2	2	2	1	2	3	2	15
1 M-T3	2	1	1	1	1	1	2	2	11
1 M-T4	2	1	2	1	2	2	2	2	14
1 M-T5	2	2	1	1	1	2	2	1	12

Group	Fibrocartilage cell number	Fibrocartilage cell alignment	Collagen fibre continuity	Collagen fibre orientation	Tidemark	Cellularity	Vascularity	Inflammation	Total scores
2 M-C1	1	1	1	1	0	1	2	1	8
2 M-C2	1	2	1	1	1	1	3	2	12
2 M-C3	1	1	1	1	1	1	3	2	11
2 M-C4	2	2	1	1	1	1	3	1	12

2 M-C5	1	2	1	1	1	2	2	1	11
2 M-T1	3	1	2	2	2	1	3	2	16
2 M-T2	2	1	1	1	2	1	3	2	13
2 M-T3	1	2	2	2	2	2	3	2	16
2 M-T4	1	2	2	2	2	2	3	2	16
2 M-T5	2	2	1	1	2	2	2	3	15

Group	Fibrocartilage cell number	Fibrocartilage cell alignment	Collagen fibre continuity	Collagen fibre orientation	Tidemark	Cellularity	Vascularity	Inflammation	Total scores
3 M-C1	2	1	2	1	1	2	3	1	13
3 M-C2	1	0	1	1	0	1	3	1	8
3 M-C3	1	1	2	1	1	2	2	2	12
3 M-C4	2	1	2	1	1	2	3	1	13
3 M-C5	1	1	1	2	0	2	3	2	12
3 M-T1	2	3	2	2	2	3	3	2	19
3 M-T2	2	2	2	1	1	2	3	2	15
3 M-T3	2	2	1	2	2	2	3	2	15
3 M-T4	2	2	2	2	1	2	3	2	16
3 M-T5	2	3	2	1	2	2	3	2	17

1 M-C, one month control group; 1 M-T, one month treadmill group; 2 M-C, two months control group; 2 M-T, two months treadmill group; 3 M-C, three months control group; 3 M-T, three months treadmill group.

Table iv. Micro-CT raw data of tendon-bone insertion healing. All values are presented as means and standard deviations.

Variable	1 M-C (n = 5)	1 M-T (n = 5)	2 M-C (n = 5)	2 M-T (n = 5)	3 M-C (n = 5)	3 M-T (n = 5)
BV/TV, %	6.302 (0.907)	8.002 (1.703)	9.336 (1.692)	12.575 (2.106)*	12.976 (1.654)	16.377 (2.092)†
Tb.Th, mm	0.060 (0.003)	0.074 (0.012)‡	0.078 (0.009)	0.072 (0.017)	0.091 (0.009)	0.099 (0.016)
Tb.N, 1/mm	1.056 (0.140)	1.290 (0.235)	1.379 (0.296)	1.389 (0.485)	1.143 (0.292)	1.377 (0.221)
Tb.Sp, mm	0.295 (0.055)	0.303 (0.059)	0.312 (0.042)	0.314 (0.029)	0.332 (0.045)	0.306 (0.033)
BMD, g/cm^3	0.062 (0.003)	0.094 (0.019)‡	0.082 (0.004)	0.116 (0.028)*	0.097 (0.018)	0.174 (0.037)†

*Statistical difference compared with 2 M-C.

†Statistical difference compared with 3 M-C.

‡Statistical difference compared with 1 M-C.

1 M-C, one month control group; 1 M-T, one month treadmill group; 2 M-C, two months control group; 2 M-T, two months treadmill group; 3 M-C, three months control group; 3 M-T, three months treadmill group; BMD, bone mineral density; BV/TV, bone volume/total volume; Tb.N, trabecular bone number; Tb.Sp, separation; Tb.Th, trabecular bone thickness.

NOTE: Please save this file locally before filling in the table, DO NOT work on the file within your internet browser as changes will not be saved. Adobe Acrobat Reader (available free here) is recommended for completion.

The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item		Recommendation	Section/line number, or reason for not reporting
Study design	1	For each experiment, provide brief details of study design including:	
		a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.	Methods
		b. The experimental unit (e.g. a single animal, litter, or cage of animals).	Methods
Sample size	2	a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.	Methods
		b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done.	Methods
Inclusion and exclusion criteria	3	a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori.* If no criteria were set, state this explicitly.	Methods
		b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.	There were no exclusions.
		c. For each analysis, report the exact value of *n* in each experimental group.	Results
Randomisation	4	a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.	Methods
		b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.	Methods
Blinding	5	Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	Methods
Outcome measures	6	a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).	Methods
		b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.	It is not a hypothesis-testing study.
Statistical methods	7	a. Provide details of the statistical methods used for each analysis, including software used.	Methods
		b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.	Methods
Experimental animals	8	a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.	Methods, Results
		b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.	Methods
Experimental procedures	9	For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:	Methods
		a. What was done, how it was done and what was used.	Methods
		b. When and how often.	Methods
		c. Where (including detail of any acclimatisation periods).	Introduction
		d. Why (provide rationale for procedures).	
Results	10	For each experiment conducted, including independent replications, report:	Methods
		a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).	It is animal research, there is no effect size.
		b. If applicable, the effect size with a confidence interval.	

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Item		Recommendation	Section/line number, or reason for not reporting
Abstract	11	Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.	Methods, Discussion
Background	12	a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach. b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.	Introduction Methods, Discussion
Objectives	13	Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.	Introduction
Ethical statement	14	Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.	Methods
Housing and husbandry	15	Provide details of housing and husbandry conditions, including any environmental enrichment.	Methods
Animal care and monitoring	16	a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress. b. Report any expected or unexpected adverse events. c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.	Methods There were no expected or unexpected events in this study. Methods
Interpretation/ scientific implications	17	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.	Introduction Discussion
Generalisability/ translation	18	Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).	Discussion
Protocol registration	19	Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.	Methods
Data access	20	Provide a statement describing if and where study data are available.	Data sharing
Declaration of interests	21	a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated. b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study.	ICMJE COI statement Funding statement