

ARRIVE Essential 10 Checklist

Local Probiotic Treatment Attenuates Suprascapular Nerve Injury–Associated Inflammatory and Histological Changes in a Rat Rotator Cuff Repair Model

ARRIVE Essential 10 item / Item no.	Recommendation	Reported in manuscript
Study design 1	For each experiment, provide brief details of study design including: a. The groups being compared, including control groups. b. The experimental unit.	Reported in Materials and Methods, sections Study Design and Ethical Approval and Animals and Experimental Groups. Four groups were compared according to suprascapular nerve injury and local probiotic treatment status. Each animal was accepted as a single experimental unit.
Sample size 2	a. Specify the exact number of experimental units allocated to each group and total number of animals. b. Explain how sample size was decided.	Reported in Animals and Experimental Groups and Power Analysis. A total of 36 rats were used, with 9 animals per group. Within each group, 6 specimens were allocated to the primary histological and immunohistochemical analyses and 3 specimens were allocated exclusively to biomechanical testing. The a priori sample-size calculation was based on histological tendon-healing scores. Using the prespecified large effect size (Cohen's $d \approx 1.93$), $\alpha = 0.05$, and 6 animals per group for the primary histological analysis, the estimated power was approximately 85%.
Inclusion and exclusion criteria 3	a. Describe inclusion/exclusion criteria. b. Report exclusions. c. Report exact n for each analysis.	Reported in Animals and Experimental Groups and Results. Male Sprague-Dawley rats aged 10–12 weeks and weighing 250–300 g were included. All 36 animals completed the 21-day observation period without mortality, and no animals were excluded. Exact n values were $n = 6/\text{group}$ for histological and immunohistochemical analyses and $n = 3/\text{group}$ for biomechanical testing.
Randomisation 4	a. State whether randomisation was used and method. b. Describe strategy to minimise confounders.	Reported in Animals and Experimental Groups. Animals were randomly allocated to four experimental groups using a computer-generated randomization sequence. Within each group, specimens were randomly allocated to histological/immunohistochemical or biomechanical analysis.
Blinding 5	Describe who was aware of group allocation at different stages.	Reported in Histological Evaluation, Immunohistochemical Evaluation, and Biomechanical Evaluation. Histological assessments were conducted by an independent histologist blinded to group allocation. Immunohistochemical analyses and biomechanical testing were also performed by investigators blinded to group allocation.
Outcome measures 6	a. Clearly define all outcome measures. b. Specify primary outcome measure used for sample size.	Reported in Abstract, Power Analysis, Histological Evaluation, Immunohistochemical Evaluation, Biomechanical Evaluation, and Statistical Analysis. The primary outcome was histological tendon healing assessed using Bonar and Movin scores. Secondary outcomes were TNF- α H-score and biomechanical parameters including maximum failure load, energy to failure, and axial stiffness.
Statistical methods 7	a. Provide statistical methods, including software. b. Describe assumption testing and approach if assumptions not met.	Reported in Statistical Analysis and Results. Bonar and Movin scores were primarily analyzed using Kruskal–Wallis tests followed by pairwise Mann–Whitney U tests with Holm correction; Cliff's delta with bootstrap 95% CIs was reported for between-group effects. TNF- α H-scores were analyzed using one-way ANOVA with Holm-adjusted pairwise t-tests, with mean differences, 95% CIs, and Hedges' g. Because the study used a 2×2 factorial design, two-way ANOVA models were used to estimate nerve-injury and probiotic main effects and their interaction. For Bonar and Movin factorial models, residual normality was assessed with Shapiro–Wilk testing and homogeneity of variance with Levene's test; no significant violations were detected. Effect estimates, 95% CIs, and partial eta squared (η^2) were reported for factorial main and interaction effects. Biomechanical analyses were supplemented with Kruskal–Wallis sensitivity analyses and factorial findings were interpreted as exploratory because $n = 3/\text{group}$. Analyses were performed using IBM SPSS Statistics Version 29.0 and Python 3.13.5 with SciPy 1.17.0 and statsmodels 0.14.6.
Experimental animals 8	a. Provide species, strain, sex, age, weight. b. Provide further relevant information.	Reported in Animals and Experimental Groups. Thirty-six male Sprague-Dawley rats aged 10–12 weeks and weighing 250–300 g were used. Experiments were performed at the Acibadem Experimental Animals Center under institutional and national regulations.
Experimental procedures 9	Describe procedures in enough detail to allow replication: what, how, when, where, and why.	Reported in Surgical Procedure, Probiotic Application, Tissue Harvesting, Histological Evaluation, Immunohistochemical Evaluation, and Biomechanical Evaluation. The manuscript describes anesthesia, antibiotic prophylaxis, suprascapular nerve transection, supraspinatus tendon transection and repair, local probiotic application, postoperative analgesia and wound care, euthanasia and tissue harvesting, histological/IHC processing, and biomechanical testing.

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Results 10	For each experiment, report summary/descriptive statistics and variability; effect size if applicable.	Reported in Results, Tables 1–3, and Figures 5–7. Descriptive statistics and variability are reported for histological, immunohistochemical, and biomechanical outcomes. Pairwise effect sizes and 95% CIs are reported in Table 2, including Cliff's delta with bootstrap 95% CIs for Bonar and Movin scores and mean differences with 95% CIs together with Hedges' g for TNF- α H-scores. For the 2 $\times$ 2 factorial analyses, main effects and interaction effects are reported as marginal mean differences or difference-in-differences with 95% CIs and partial eta squared (η^2). The manuscript also states that pairwise 95% CIs were not adjusted for multiplicity, whereas p values were Holm-adjusted.