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

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Abstract

Background

Suprascapular nerve dysfunction may impair rotator cuff healing by exacerbating local inflammation, particularly tumor necrosis factor-alpha (TNF-α) expression, and inducing denervation-related changes. This study aimed to determine whether local probiotic administration could counteract these detrimental effects in a rat rotator cuff repair model.

Methods

Thirty-six male Sprague-Dawley rats were randomly allocated into four groups (n = 9 per group): nerve injury (NI) with local probiotic treatment, no NI with local probiotic treatment, NI without probiotic treatment, and no NI without probiotic treatment. All animals underwent supraspinatus tendon transection and modified Mason-Allen repair. In the NI groups, the suprascapular nerve was sharply transected at the suprascapular notch. Local probiotic treatment was applied directly to the repair site in the designated groups. At 21 days postoperatively, tendon healing was evaluated histologically using Bonar and Movin scores and immunohistochemically using TNF-α H-scores. Biomechanical properties, including failure load, energy to failure, and axial stiffness, were also assessed.

Results

The untreated NI group exhibited the poorest histological healing, with the highest Bonar score (8.33 ± 0.82) and Movin score (11.00 ± 1.41). Local probiotic treatment in the presence of NI was associated with lower Bonar (5.50 ± 1.87) and Movin (7.50 ± 1.22) scores. Bonar and Movin scores differed significantly among groups ($p = 0.001$ and $p < 0.001$, respectively), and the untreated NI group had significantly higher scores than all other groups. TNF-α H-scores also differed significantly among groups ($p < 0.001$), with the highest expression in the untreated NI group (52.73 ± 4.36) and lower expression in the NI with probiotic treatment group (43.92 ± 3.82). The lowest TNF-α expression was observed in the nerve-intact probiotic-treated group (26.47 ± 3.91). Biomechanical findings were consistent with the histological and immunohistochemical results; the untreated NI group showed the lowest failure load (15.48 ± 2.21 N) and energy to failure (29.66 ± 21.63 N·mm), whereas the NI with probiotic treatment group showed higher values (27.31 ± 4.15 N and 118.11 ± 26.72 N·mm, respectively). Axial stiffness did not differ significantly among groups.

Conclusion

Local probiotic treatment attenuated nerve injury-associated inflammatory and histological deterioration in a rat rotator cuff repair model, with biomechanical findings showing a consistent favorable pattern.

These findings suggest that probiotic-based local modulation may improve the repair-site biological environment under adverse denervation conditions.

Introduction

Rotator cuff (RC) repair remains biologically challenging, particularly in chronic and large-to-massive tears in which tendon retraction, muscle atrophy, fatty infiltration, and impaired tendon-bone healing may compromise structural and functional outcomes [1, 2]. Although repair failure is commonly attributed to tendon quality and fixation strength, the RC should be viewed as an integrated muscle-tendon-nerve unit, in which the suprascapular nerve contributes to muscle function, trophic maintenance, and the biological integrity of the muscle-tendon unit. In this context, suprascapular nerve dysfunction constitutes a well-documented biological burden, particularly in large or retracted tears, by contributing to denervation-related muscle degeneration and sustaining a pro-inflammatory microenvironment that undermines the repair site [3].

Experimental evidence supports the detrimental role of suprascapular nerve injury. In rat models, concurrent nerve injury aggravates muscle degeneration, impairs regenerated enthesis maturation, reduces collagen organization, and compromises overall biomechanical properties after repair [4, 5]. These findings indicate that suprascapular nerve injury affects rotator cuff repair beyond the loss of muscle activation and may promote a less favorable inflammatory repair-site environment. Increased pro-inflammatory cytokine activity may contribute to impaired collagen organization, delayed enthesis maturation, and inferior biomechanical properties. Therefore, local modulation of excessive inflammatory signaling may represent a promising strategy to improve tendon-bone healing under nerve injury conditions.

Among the key mediators of this detrimental cascade, tumor necrosis factor-alpha (TNF-α) stands out as a central cytokine that simultaneously drives neuroinflammatory signaling, skeletal muscle catabolism, and adverse tendon-bone healing responses. In rat RC repair models, TNF-α blockade significantly improves early healing strength, confirming that excessive inflammatory signaling is detrimental during the repair phase [6]. Consequently, local TNF-α expression serves not only as an optimal marker to evaluate the biological modulation of the repair environment, but also as a rational therapeutic target for interventions aiming to restore tissue homeostasis following nerve injury.

Beyond their established gastrointestinal effects, probiotics are now recognized as potent systemic modulators of immune and inflammatory pathways, with growing evidence linking the gut microbiota to peripheral nerve biology, neuroimmune signaling, and skeletal muscle metabolism via the gut-nerve and gut-muscle axes [7, 8]. In experimental models, probiotic interventions improve functional recovery, reduce neuropathic pain, and decrease oxidative stress following peripheral nerve damage [9, 10]. Importantly, these immunomodulatory properties are often retained even in inactivated or lysed bacterial preparations, highlighting a viability-independent signaling mechanism that is particularly attractive for local therapeutic applications.

However, whether direct local application of probiotics can mitigate the inflammatory and histological deterioration triggered by suprascapular nerve injury in the setting of RC repair has not been investigated. We hypothesized that local probiotic treatment at the repair site would suppress the adverse inflammatory response—as reflected by reduced TNF-α expression—and shift the local microenvironment toward a less inflammatory state, thereby attenuating adverse tendon histological changes.

The aim of this study was to evaluate the efficacy of local probiotic administration in attenuating suprascapular nerve injury-associated inflammatory and histological deterioration in a rat RC repair model.

Materials and Methods

Study Design and Ethical Approval

This was a controlled experimental animal study designed to evaluate tendon healing using histological, immunohistochemical, and biomechanical outcomes. The study protocol was approved by the Animal Experiments Local Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (ACU-HADYEK). All experimental procedures were carried out at the Acıbadem Experimental Animals Center in accordance with institutional and national regulations for the care and use of laboratory animals.

Animals and Experimental Groups

A total of 36 male Sprague-Dawley rats, aged 10 to 12 weeks and weighing 250 to 300 g, were included in the study. Animals were randomly allocated to four experimental groups using a computer-generated randomization sequence, with 9 animals in each group. Each animal was accepted as a single experimental unit; accordingly, the total sample size was 36. Within each group, 6 randomly selected specimens were allocated for histological and immunohistochemical evaluation, while the remaining 3 specimens were reserved exclusively for biomechanical testing.

The study groups were defined as follows:

Group 1: suprascapular nerve injury (+) / probiotic (+)

Group 2: suprascapular nerve injury (–) / probiotic (+)

Group 3: suprascapular nerve injury (+) / probiotic (–)

Group 4: suprascapular nerve injury (–) / probiotic (–).

The experimental design and specimen allocation are shown in Fig. 1.

Power Analysis

An a priori power analysis was performed before study initiation using PASS software (Version 11; NCSS, Kaysville, UT, USA). The primary outcome measure was defined as histological tendon healing assessed using the Bonar and Movin scoring systems [11], which assess parameters including cellular morphology, collagen organization, vascularity, and extracellular matrix composition.

Based on previously published animal models of rotator cuff healing [12–15], a minimum sample size of six specimens per group was estimated to provide adequate power to detect a significant difference in the primary histological endpoint using a two-sided alpha level of 0.05. Accordingly, six specimens from each group were allocated for histological and immunohistochemical evaluation. To allow additional biomechanical testing without compromising the histological sample allocation, three further specimens per group were included, resulting in a total of 9 animals per group and 36 animals overall.

Surgical Procedure

All animals underwent the same anesthetic and perioperative preparation. General anesthesia was induced with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (10 mg/kg) administered intramuscularly. Preoperative antibiotic prophylaxis was provided with cefazolin (10 mg/kg). After shaving, the surgical field was prepared with iodine scrub and 70% alcohol. A 3-cm skin incision was then made over the shoulder through the deltoid region, and the soft tissues were carefully dissected. The operative field was gently widened with a mosquito clamp to allow adequate exposure. The suprascapular nerve was meticulously isolated at the suprascapular notch. In the nerve injury (NI) groups, the nerve was sharply transected just anterior to the notch using microsurgical scissors, whereas it remained anatomically intact in control cohorts [4, 5]. The supraspinatus tendon was then identified and sharply transected in a single pass with a scalpel. Primary tendon repair was performed using a modified Mason-Allen suture technique with 5 – 0 Prolene (Ethicon; Johnson & Johnson, New Brunswick, NJ, USA). In the probiotic-treated groups, the probiotic preparation was then applied locally to the repair site as described below. Skin closure was subsequently completed with primary suturing, followed by sterile dressing. Animals were allowed unrestricted cage activity. Postoperative analgesia was ensured via subcutaneous administration of carprofen (5 mg/kg) once daily for the first 48 hours, and standardized wound care was performed every other day throughout the follow-up period [4, 16]. The surgical model of suprascapular nerve injury and rotator cuff repair is shown in Fig. 2.

Probiotic Application

Immediately following tendon repair, topical probiotic treatment was administered exclusively to the repair site in designated groups. To prevent mechanical interference with tendon-bone healing, approximately 1 g of lyophilized probiotic powder was evenly distributed as a thin layer onto the hemostatic wound bed using a sterile curette. The preparation (SFA AR-GE, Istanbul, Türkiye) comprised

a purified lyophilized culture containing Lactobacillus acidophilus (ATA-LTC-La060301), Lactiplantibacillus plantarum (ATA-LTC-Lp230198), Lacticaseibacillus rhamnosus (ATA-LTC-Lr010902), and Bifidobacterium animalis (ATA-LTC-Ba030100), yielding a total viable concentration of approximately 1.2×10^{10} CFU/g. To maintain the purity of the biological intervention, no additional carrier or vehicle was utilized. Representative local application of the probiotic preparation is shown in Fig. 3.

Representative intraoperative image showing local application of the probiotic preparation to the repaired supraspinatus tendon footprint. Image is representative and was not used for quantitative measurements.

Tissue Harvesting

Following the 21-day observation period, all animals were euthanized via intravenous administration of saturated KCl under deep anesthesia. The repaired shoulder complexes were meticulously harvested en bloc to preserve the integrity of the tendon-bone interface for downstream analyses. Representative en bloc harvesting of the repaired shoulder complex at the end of the follow-up period is shown in Fig. 4.

A total of 36 specimens were obtained. Within each experimental cohort (n = 9), six randomly selected specimens were allocated for histological and immunohistochemical profiling, while the remaining three were preserved exclusively for biomechanical testing.

Representative image showing en bloc harvesting of the repaired shoulder region at the end of the follow-up period for subsequent analyses. Image is representative and was not used for quantitative measurements.

Histological Evaluation

Specimens designated for histological evaluation were immediately fixed in 10% neutral buffered formalin (pH 7.4) at + 4°C for 7 days. Subsequent decalcification was achieved using Morse's solution (22.5% formic acid and 10% sodium citrate) for 5 weeks, with twice weekly solution exchanges to ensure optimal tissue preservation. Tissues were then dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sagittal sections (5 μm) were mounted on poly-L-lysine-coated slides and stained with hematoxylin-eosin (H&E) and Masson's trichrome. Histological examination was performed using a Leica DM 6000 microscope equipped with Leica Application Suite software (Leica Microsystems, Wetzlar, Germany). Tendon healing was defined as the primary outcome measure and was evaluated using the semi-quantitative Bonar and Movin scoring systems [11], which assess parameters including cellular morphology, collagen organization, vascularity, and extracellular matrix composition. To eliminate observer bias, all histological assessments were conducted by an independent histologist completely blinded to the group allocations.

Immunohistochemical Evaluation

Immunohistochemical profiling was performed on 5-μm paraffin-embedded sections using a primary antibody against TNF-α. Stained sections were analyzed using the established H-score method [17]. For quantitative assessment, three randomly selected fields per specimen were evaluated at ×20 magnification under the aforementioned Leica DM 6000 microscope. The staining intensity was graded on a scale of 0 to 3, and the proportion of positively stained cells at each intensity level was calculated to determine the total H-score. Consistent with the histological evaluation, all immunohistochemical analyses were performed in a strictly blinded manner.

Biomechanical Evaluation

Biomechanical testing was performed in the Biomechanics Laboratory of the Department of Mechanical Engineering at Yeditepe University using a tensile loading protocol adapted from previous experimental RC repair models [12, 13]. To prevent macroscopic slippage at the tissue-clamp interface, the repaired tendon-bone constructs were rigidly secured using custom surgical needle holders, ensuring precise alignment with the primary loading axis. Testing was executed using an Instron 3382 Universal Testing Machine equipped with Bluehill® software (Instron Corp., Norwood, MA, USA) for synchronized device control and continuous load-displacement data acquisition. A monotonic tensile load was applied at a constant displacement rate of 0.1 mm/s until macroscopic construct failure. Failure was strictly defined as either a sudden load drop exceeding 30% or the development of plastic deformation greater than 20 mm. The acquired load-displacement curves were analyzed to extract the maximum failure load (Fmax, N), displacement at failure (mm), and axial stiffness (N/mm) for each evaluated specimen.

Statistical Analysis

Data normality was evaluated using the Shapiro-Wilk test. Continuous variables are reported as mean ± standard deviation or median (interquartile range) based on their distribution. As semi-quantitative variables, Bonar and Movin scores were analyzed using the Kruskal-Wallis test, followed by Dunn's post-hoc test with Holm correction for multiple comparisons. Normally distributed continuous data (TNF-α H-scores and biomechanical parameters) were compared across groups using one-way analysis of variance (ANOVA) with appropriate post-hoc testing. Given the limited sample size for biomechanical testing (n = 3/group), Kruskal-Wallis tests were concurrently performed as non-parametric sensitivity analyses. Statistical significance was set at $p < 0.05$. In alignment with the a priori power analysis, histological scores were treated as the primary endpoints, while immunohistochemical and biomechanical data were interpreted as secondary outcomes.

Results

All 36 animals completed the 21-day observation period without mortality. No wound infection, dehiscence, or probiotic-related local adverse reactions were observed.

Histological findings

Histological evaluation revealed significant intergroup differences for both Bonar (Kruskal-Wallis p = 0.001) and Movin (Kruskal-Wallis p < 0.001) total scores. The untreated nerve injury group (Group 3) exhibited the most severe histological degeneration, with the highest mean Bonar (8.33 ± 0.82) and Movin (11.00 ± 1.41) scores. Post-hoc analysis with Holm correction showed that Group 3 had significantly higher Bonar and Movin scores than Groups 1, 2, and 4. In contrast, the intact-nerve probiotic-treated group (Group 2) displayed the lowest mean Bonar (2.83 ± 1.83) and Movin (4.00 ± 2.10) scores. The nerve-injured probiotic-treated group (Group 1) demonstrated lower histological scores than Group 3 and was not significantly different from the intact-nerve control group (Group 4), suggesting that local probiotic application attenuated the deleterious histological effects associated with suprascapular nerve injury. Descriptive statistics for all groups are summarized in Table 1, and representative histological sections are presented in Fig. 5.

Immunohistochemical findings

Immunohistochemical analysis revealed significant differences in local TNF-α expression among the four groups (ANOVA p < 0.001; Kruskal-Wallis p < 0.001). Mirroring the histological outcomes, the untreated nerve injury group (Group 3) showed the highest mean TNF-α H-score (52.73 ± 4.36), whereas the intact-nerve probiotic-treated group (Group 2) showed the lowest H-score (26.47 ± 3.91). Holm-adjusted pairwise comparisons confirmed that TNF-α H-scores differed significantly between all group pairs, with the mean values ordered as Group 3 > Group 1 > Group 4 > Group 2. This gradient suggests that local probiotic treatment attenuated injury-associated TNF-α elevation and was associated with lower TNF-α expression in the absence of nerve injury. Representative immunohistochemical sections are presented in Fig. 6.

Histological and immunohistochemical outcomes are summarized in Table 1. Significant post-hoc comparisons for histological and immunohistochemical outcomes are presented in Table 2.

Table 1
Histological and immunohistochemical outcomes

Outcome	Group 1 NI+/Pro+	Group 2 NI−/Pro+	Group 3 NI+/Pro−	Group 4 NI−/Pro−	Overall p value
Bonar total score	5.50 ± 1.87	2.83 ± 1.83	8.33 ± 0.82	4.33 ± 1.37	0.001
Movin total score	7.50 ± 1.22	4.00 ± 2.10	11.00 ± 1.41	6.83 ± 1.33	< 0.001
TNF-α H-score	43.92 ± 3.82	26.47 ± 3.91	52.73 ± 4.36	34.87 ± 5.21	< 0.001

NI = suprascapular nerve injury; Pro = probiotic treatment.

Values are expressed as mean ± standard deviation. Intergroup comparisons were evaluated using the Kruskal-Wallis test for histological scores and one-way ANOVA for TNF- α H-scores.

Table 2
Post-hoc comparisons for histological and immunohistochemical outcomes

Outcome	Significant post-hoc comparisons after Holm correction
Bonar total score	G3 > G1, G3 > G2, G3 > G4
Movin total score	G3 > G1, G3 > G2, G3 > G4
TNF-α H-score	All group pairs significant; mean order: G3 > G1 > G4 > G2
Note: Higher Bonar and Movin scores denote inferior histological healing, whereas elevated TNF-α H-scores reflect greater pro-inflammatory expression.	

The quantitative distributions of these histological and inflammatory metrics across all experimental cohorts are visualized in Fig. 7.

Biomechanical findings

Consistent with the histological and immunohistochemical profiles, the untreated nerve injury group (Group 3) demonstrated the least favorable biomechanical profile, with the lowest maximum failure load (15.48 ± 2.21 N) and energy to failure (29.66 ± 21.63 N·mm). Local probiotic treatment was associated with more favorable biomechanical values: the highest maximum failure load was observed in the intact-nerve probiotic group (Group 2, 27.46 ± 7.52 N), whereas the highest energy to failure was recorded in the nerve-injured probiotic group (Group 1, 118.11 ± 26.72 N·mm).

One-way ANOVA indicated significant intergroup differences for maximum failure load ($p = 0.038$) and energy to failure ($p = 0.025$). However, because biomechanical testing was performed in only three specimens per group, Kruskal-Wallis sensitivity analyses were also performed and did not reach statistical significance for failure load ($p = 0.094$) or energy to failure ($p = 0.075$). Axial stiffness did not differ significantly among groups. Therefore, biomechanical findings were interpreted as secondary supportive outcomes showing a consistent directional pattern with the histological and immunohistochemical results. Full biomechanical results are detailed in Table 3.

Table 3
Biomechanical outcomes

Outcome	Group 1 NI+/Pro+	Group 2 NI−/Pro+	Group 3 NI+/Pro−	Group 4 NI−/Pro−	ANOVA p	Kruskal-Wallis p
Failure load, N	27.31 ± 4.15	27.46 ± 7.52	15.48 ± 2.21	25.40 ± 2.53	0.038	0.094
Energy to failure, N·mm	118.11 ± 26.72	94.96 ± 43.73	29.66 ± 21.63	71.22 ± 6.75	0.025	0.075
Axial stiffness, N/mm	3.61 ± 1.30	5.43 ± 0.47	4.93 ± 1.23	6.30 ± 1.38	0.106	0.204

NI = suprascapular nerve injury; Pro = probiotic treatment.

Discussion

The principal finding of this study is that suprascapular nerve injury significantly impairs histological tendon healing, exacerbates local TNF-α expression, and compromises the biomechanical integrity of the repaired rotator cuff. Crucially, local probiotic administration attenuated these adverse degenerative changes. While untreated nerve injury created the most deleterious local environment—evidenced by the highest histological degeneration scores and the lowest construct strength—probiotic intervention markedly reduced this inflammatory and structural deterioration. Notably, the inflammatory severity gradient observed across groups (Group 3 > Group 1 > Group 4 > Group 2) suggests that local probiotic therapy not only partially reversed the injury-induced deterioration but also exerted an additional anti-inflammatory benefit even in the absence of nerve injury. These findings support the hypothesis that targeted local probiotic therapy can effectively mitigate the detrimental effects of denervation on the tendon-bone interface in a RC repair model.

Traditionally, RC repair failure has been attributed to mechanical and structural factors such as tendon quality, tear size, and fatty infiltration. However, particularly in massive and retracted tears, the RC should be viewed as an interconnected muscle-tendon-nerve unit, in which neural integrity may contribute to optimal biological and functional recovery.

Clinical evidence suggests that concomitant suprascapular neuropathy exacerbates pain, accelerates denervation-related muscle atrophy, and impedes functional recovery [18–22]. Rather than representing separate clinical entities, tendon rupture and nerve dysfunction engage in a dynamic interplay: structural repair can facilitate neurological recovery, while untreated neuropathy can undermine the biological success of the repair [18–20]. Consequently, the baseline neural status emerges as a critical—yet often overlooked—determinant of the biological healing capacity of the rotator cuff unit. This recognition underscores the need for therapeutic strategies, such as local probiotic application, that directly target the inflammatory consequences of nerve injury at the repair site.

Recent experimental models provide direct experimental support for this clinical concept. Preclinical in vivo studies confirm that suprascapular nerve injury independently deteriorates bone-tendon junction healing, resulting in inferior biomechanical properties, compromised muscle quality, and deficient enthesis regeneration compared to nerve-intact controls [4, 15]. Eshima et al. also showed that suprascapular nerve injury may worsen muscle quality and regenerated enthesis formation in a rat RC tear model [5]. Our histological and biomechanical findings are in full agreement with these observations: the untreated nerve injury cohort (Group 3) consistently exhibited the poorest outcomes across all measured parameters. However, a key novelty of the present study is the demonstration that these deleterious effects may be biologically modulated by local probiotic intervention, as evidenced by the marked improvement observed in Group 1.

Clinical Relevance and the Role of Inflammation

These findings may be relevant to the biological challenges observed in massive, chronically retracted rotator cuff tears. Chronic degeneration alters the biological landscape of the muscle-tendon-bone complex, resulting in inferior structural healing compared to acute repair settings [23]. These findings help explain why structural healing may remain difficult even after technically adequate repair in chronic tears. In addition, in massive and retracted RC tears, excessive tendon retraction and high-tension repair may theoretically increase traction on the suprascapular nerve, potentially contributing to neuropathic changes or further compromising the muscle-tendon-nerve unit [18, 20, 22]. This secondary neuropathy introduces an additional biological burden, compounding denervation-related muscle degeneration and indirectly undermining the tendon-bone interface. Although our study employed an acute transection model to isolate the specific effects of neural compromise, the data indicate that nerve injury alone can create a less favorable inflammatory and structural environment at the repair site—an observation that likely underestimates the severity of the clinical scenario, where chronic traction neuropathy coexists with long-standing tissue degeneration.

Inflammation appears to serve as the critical biological conduit linking suprascapular nerve injury to impaired tendon healing. While physiological inflammation is essential for tissue repair, excessive pro-inflammatory signaling—particularly TNF-α-mediated pathways—is known to impede early enthesis regeneration [6]. Our study strongly corroborates this paradigm: the untreated nerve injury group exhibited profound upregulation of local TNF-α expression, and this molecular profile was closely mirrored by the histological findings. The exaggerated inflammatory response correlated with severe architectural disorganization, marked extracellular matrix alteration, and elevated Bonar and Movin scores, supporting an association between the local cytokine milieu and the structural quality of the healing enthesis.

Existing literature confirms the therapeutic value of mitigating excessive inflammation in rotator cuff repair. Targeted pharmacological strategies—such as TNF-α blockade or IKKβ suppression—have been shown to blunt the detrimental hyper-inflammatory phase and enhance early tendon-to-bone healing strength [6, 24]. These studies establish a clear precedent for anti-inflammatory intervention at the repair site.

In addition, inflammation has been increasingly recognized as an active component of RC tendon degeneration and repair rather than a merely secondary phenomenon [25]. Building upon this premise, the rationale for local probiotic application in our model is rooted in their potent immunomodulatory capabilities. Unlike systemic anti-inflammatory agents, locally applied probiotic preparations may modulate the local immune milieu without systemic exposure risks. Emerging evidence indicates that such microbiota-targeted interventions can suppress pro-inflammatory cytokine production, attenuate oxidative stress, and promote a regulatory, pro-resolving immune phenotype at the tissue level [7, 8, 26]. Our findings extend this paradigm by demonstrating, for the first time, that local probiotic delivery can effectively interrupt the inflammatory cascade triggered by suprascapular nerve injury, thereby preserving the structural integrity of the healing enthesis.

In peripheral nerve injury models, Rodenhouse et al. demonstrated that antibiotic-induced gut dysbiosis impaired functional recovery after traumatic peripheral nerve crush injury in mice, whereas probiotic treatment with butyrate-producing bacteria improved recovery [9]. Shabani et al. also reported that probiotic supplementation reduced neuropathic pain behavior and improved oxidative stress parameters in a chronic constriction injury model of the sciatic nerve [10]. Although these studies used systemic or gut-directed approaches and the present study used local probiotic application, they support the broader biological concept that probiotic-based interventions may modulate inflammatory and oxidative pathways relevant to nerve injury and tissue repair.

The present study advances this paradigm by shifting the focus to the direct local application of probiotics at the rotator cuff repair site. The marked reduction in TNF-α expression observed with localized delivery supports the possibility that locally applied probiotic preparations exert immunomodulatory effects within the repair microenvironment.

Importantly, rather than implying direct suprascapular nerve regeneration, our findings indicate that local probiotic therapy effectively counteracts the hostile, denervation-induced inflammatory cascade, thereby protecting the structural integrity of the healing tendon.

The emerging concept of the "gut-muscle axis" further supports the biological plausibility of our findings, as microbiota-derived metabolites are known to influence muscle mass and function via immune and metabolic pathways [8, 27]. Our results extend this concept by suggesting that localized probiotic delivery, bypassing the gut entirely, can protect the muscle-tendon unit from denervation-induced deterioration.

Another plausible mechanism underlying the protective effect observed in our study involves macrophage-mediated immune regulation. Effective tissue repair requires a meticulously coordinated transition from an early pro-inflammatory phase to a reparative state. Specific probiotic strains, including Lactobacillus species, have been shown to promote this transition by modulating macrophage polarization, shifting the balance from a pro-inflammatory M1 phenotype toward a pro-regenerative M2 phenotype [28]. Although specific macrophage surface markers, such as CD68, CD86, CD163, or CD206, were not quantified in the present study, the reduction in TNF-α expression observed in probiotic-treated cohorts is compatible with a less pro-inflammatory local environment. This finding may indirectly support the possibility of altered immune-cell activity at the repair site. This modulated cytokine environment may be biologically relevant, as excessive and persistent pro-inflammatory signaling can impair extracellular matrix organization and tendon-bone interface maturation, as reflected by the elevated Bonar and Movin scores observed in the untreated nerve injury group.

The histological and biomechanical data together provide convergent evidence supporting this intervention. Local probiotic application partially, yet significantly, reversed the adverse structural consequences of suprascapular nerve injury, preserving collagen organization and extracellular matrix integrity in close alignment with the dampened TNF-α response. Biomechanical testing, although a secondary exploratory endpoint, was consistent with these findings: probiotic-treated constructs

demonstrated superior mechanical resilience. Notably, the divergence between peak failure load (Group 2) and peak energy to failure (Group 1) suggests that probiotic treatment may also influence the material quality of the healing tissue—a finding that warrants further investigation. Collectively, this structural-mechanical correlation indicates that mitigating the early inflammatory cascade at the repair site helps preserve the functional integrity of the repaired tendon.

Translational Relevance and Limitations

The translational relevance of this study lies in its challenge to the conventional paradigm of biological augmentation in RC repair. Rather than targeting the tendon-bone interface in isolation, our findings suggest that future strategies should address the broader neural and inflammatory status of the musculotendinous unit. This is particularly relevant in massive, retracted tears, where suprascapular neuropathy and muscle degeneration coexist and jointly compromise the repair environment. While direct clinical extrapolation requires prudence—as our model does not demonstrate primary neural regeneration or direct macroscopic muscle recovery—these findings establish a compelling preclinical rationale. Targeted, probiotic-mediated local immunomodulation emerges as a promising adjunctive strategy to salvage biologically compromised repairs by attenuating denervation-associated inflammatory deterioration.

We acknowledge several limitations. First, the acute rat model used herein does not fully recapitulate the chronic tendon retraction, profound fatty infiltration, and insidious suprascapular nerve dysfunction characteristic of human massive RC tears. Second, our assessments focused on the tendon-bone interface and local TNF-α expression; direct evaluation of supraspinatus muscle quality, detailed nerve histomorphometry, or electrophysiological recovery was not performed. These data therefore reflect local anti-inflammatory protection rather than direct neural healing. Third, the limited sample size allocated for biomechanical testing (n = 3 per group) restricts these outcomes to exploratory trends rather than permitting definitive biomechanical conclusions. Fourth, TNF-α served as the sole molecular marker of inflammation; broader cytokine profiling was beyond the scope of this proof-of-concept study. Finally, as only a single early time point (21 days) was evaluated, long-term in vivo studies are essential to determine whether this early inflammatory modulation translates into sustained, mature enthesis regeneration.

CONCLUSION

This study demonstrates that targeted local probiotic application effectively attenuates the deleterious inflammatory and histological consequences of suprascapular nerve injury in a rat RC repair model. By suppressing the excessive pro-inflammatory response and preserving structural integrity, local probiotic intervention offers a novel therapeutic strategy to protect the repair site from denervation-induced deterioration. These findings establish a compelling preclinical framework and pave the way for future mechanistic investigations into macrophage polarization, dose-dependency, and long-term functional outcomes in chronic, massive RC tear models.

Abbreviations

RC
rotator cuff
ANOVA
analysis of variance
CFU
colony-forming unit
H&E
hematoxylin and eosin
IHC
immunohistochemistry
NI
suprascapular nerve injury
Pro
probiotic treatment
SSPN
suprascapular nerve
TNF-α
tumor necrosis factor-alpha.

Declarations

Competing interests:

The authors declare that they have no conflict of interest.

Ethics approval and consent to participate:

The study protocol was approved by the Animal Experiments Local Ethics Committee of Acıbadem Mehmet Ali Aydınlar University (ACU-HADYEK). All experimental procedures were performed in accordance with institutional and national regulations for the care and use of laboratory animals.

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Author Contribution

OG, BEK, and OY conceived and designed the study. OG, AC, and OY performed the experimental work and collected the data. AK, OG, EB, and OY wrote the main manuscript text. AK, BEK, EB, AV, and AC critically revised the manuscript for important intellectual content. All authors reviewed and approved the final manuscript.

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

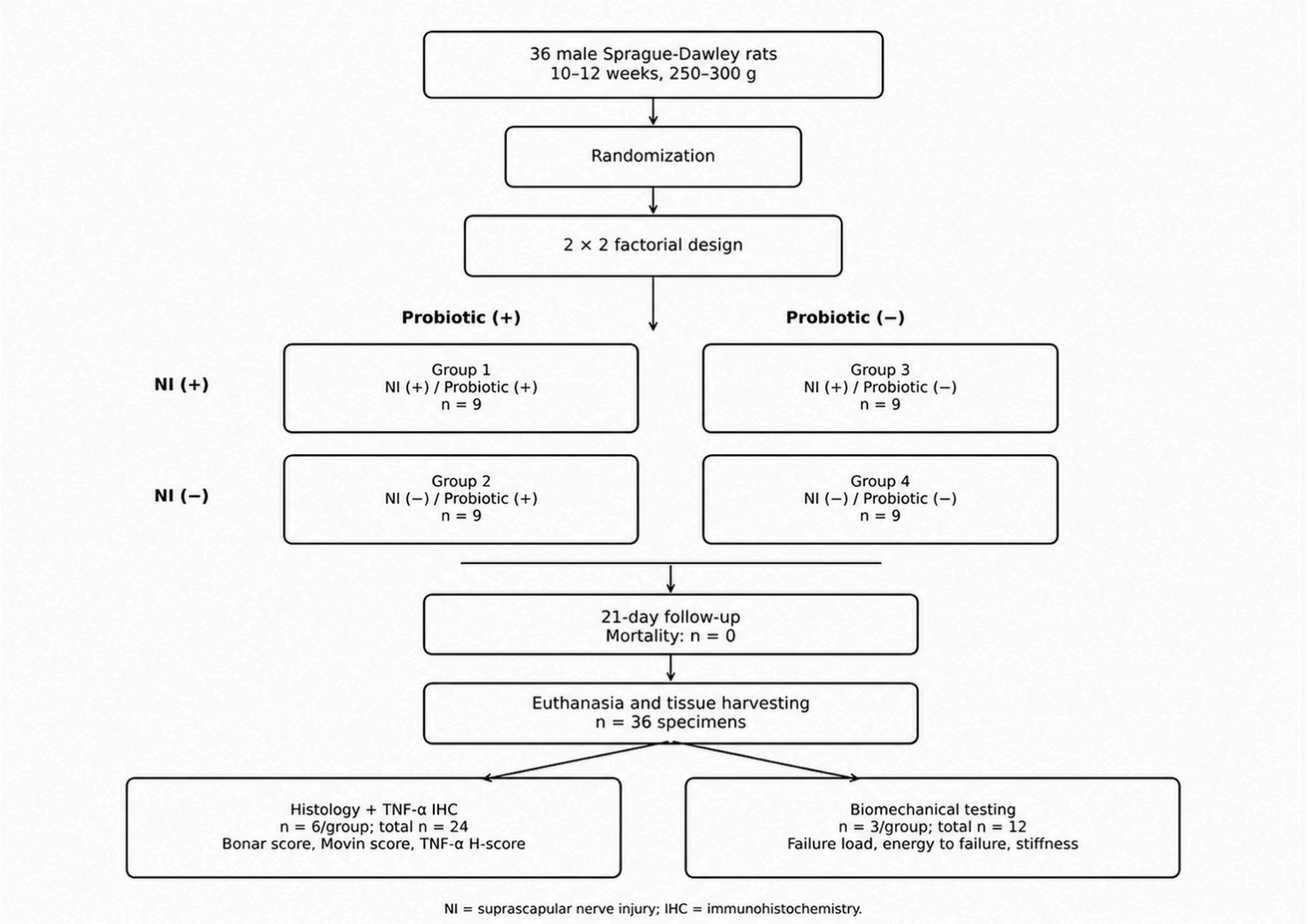


Figure 1

Experimental design and specimen allocation. Thirty-six male Sprague-Dawley rats were randomized into four groups according to suprascapular nerve injury and local probiotic treatment. In each group, six specimens were allocated to histological and TNF-α immunohistochemical analyses, and three specimens were allocated to biomechanical testing. NI = suprascapular nerve injury; IHC = immunohistochemistry.

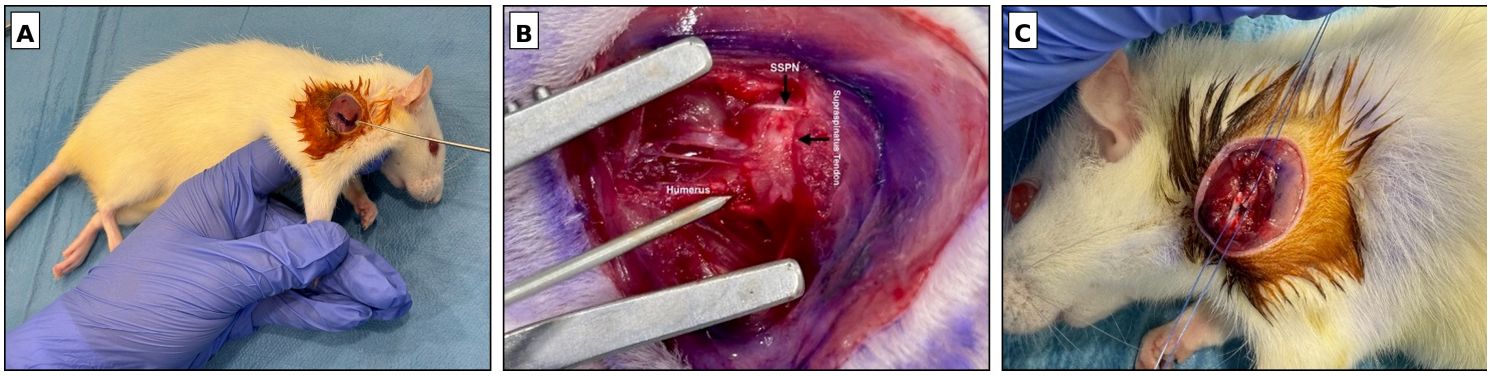


Figure 2

Surgical model of suprascapular nerve injury and RC repair. Representative intraoperative images showing (A) approach to the supraspinatus tendon, (B) suprascapular nerve exposure and transection, and (C) supraspinatus tendon exposure and transection.



Figure 3

Local probiotic application to the repair site.



Figure 4

En bloc tissue harvesting of the repaired shoulder specimen.

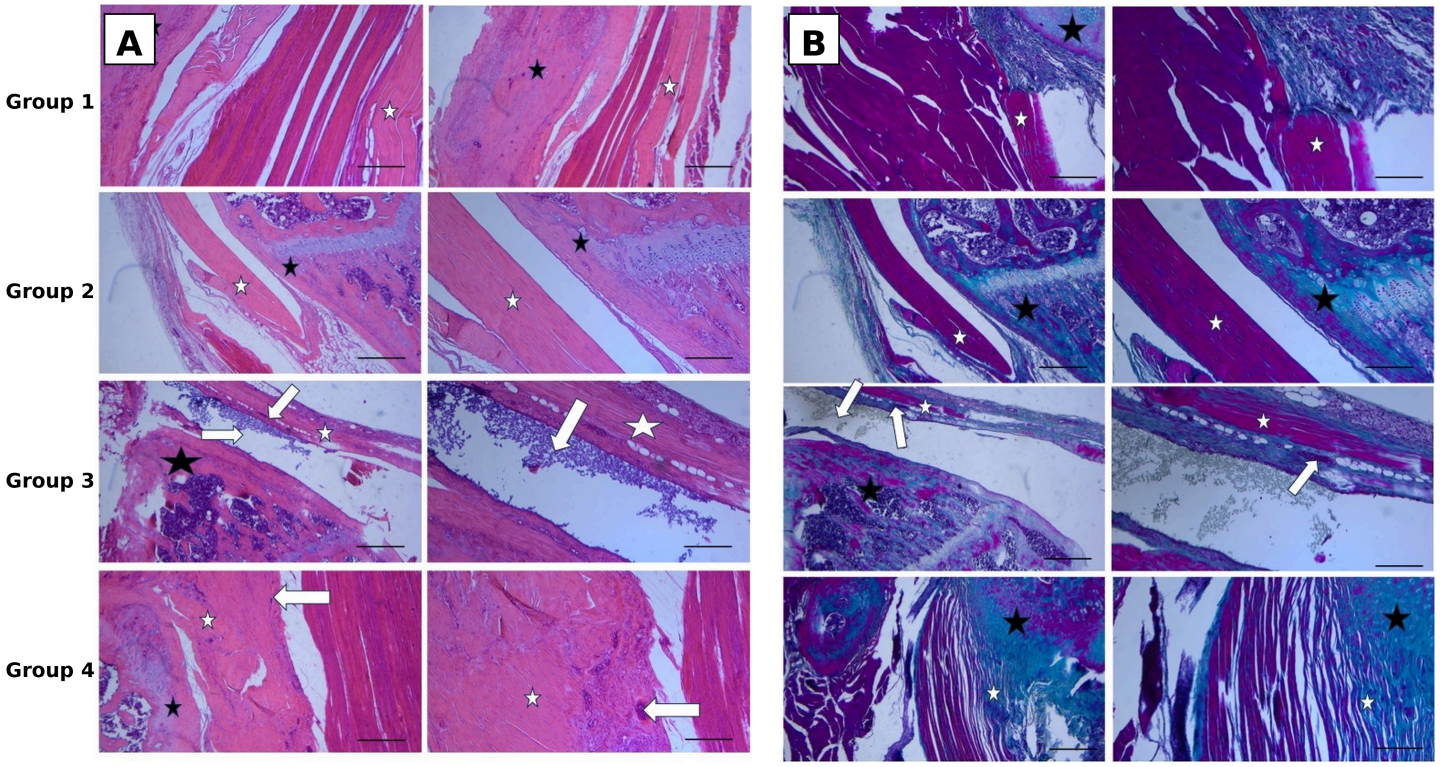


Figure 5

Histological evaluation of the tendon-bone interface. Representative sagittal sections from the four experimental cohorts stained with (A) hematoxylin and eosin (H&E) and (B) Masson's trichrome. Group designations are indicated on the left. Black asterisks denote bone, white asterisks indicate the tendon substance, and white arrows highlight vascular structures. H&E staining reveals distinct variations in cellular infiltration, vascularity, and overall tissue organization across groups. Concurrently, Masson's trichrome delineates differences in collagen bundle alignment and extracellular matrix maturation, with the most profound degenerative changes and structural disorganization observed in the untreated nerve injury group. Scale bar = 1000 μm.

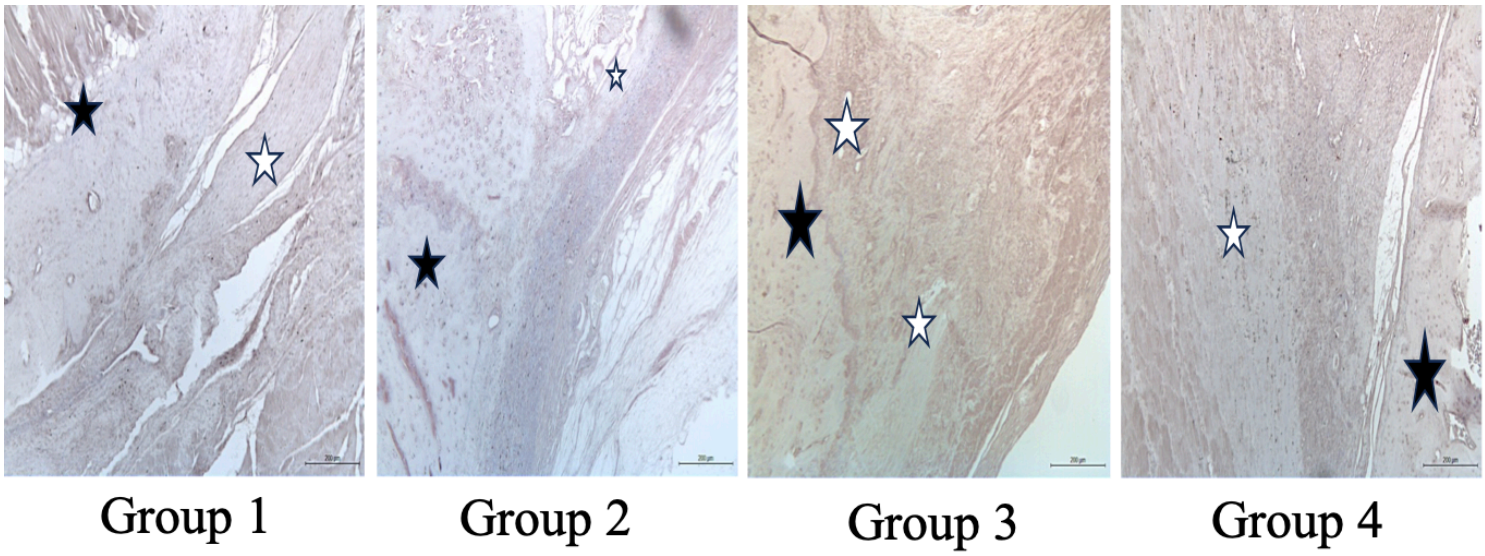


Figure 6

Immunohistochemical evaluation of TNF-α expression. Representative sections demonstrating TNF-α immunoreactivity at the tendon-bone interface across the four experimental cohorts. Black asterisks denote bone, and white asterisks indicate the tendon substance. Differences in TNF-α staining intensity can be observed among the groups, with the most pronounced immunoreactivity in the untreated nerve injury cohort and lower staining intensity in probiotic-treated groups. Tissues were probed using a polyclonal TNF-α antibody. Scale bar = 200 μm.

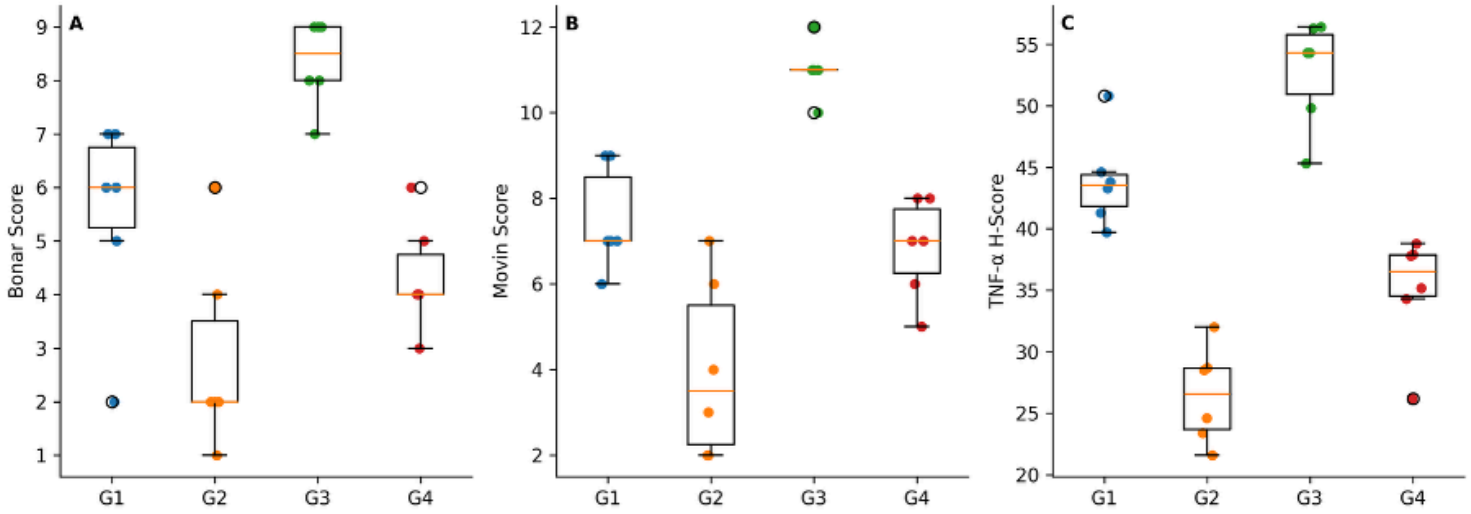


Figure 7

Quantitative histological and immunohistochemical outcomes. Box-and-whisker plots illustrating the distribution of (A) Bonar scores, (B) Movin scores, and (C) TNF-α H-scores across the four experimental cohorts. Individual data points overlaying the plots represent distinct specimens. Elevated Bonar and Movin scores correspond to inferior histological tendon healing, whereas increased TNF-α H-scores reflect greater pro-inflammatory expression. Group designations: G1 = suprascapular nerve injury (+) / probiotic (+); G2 = suprascapular nerve injury (−) / probiotic (+); G3 = suprascapular nerve injury (+) / probiotic (−); G4 = suprascapular nerve injury (−) / probiotic (−).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1.ARRIVEEssential10Checklist.pdf