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ORIGINAL ARTICLE

Comparison of tendon healing using local platelet-rich plasma, erythropoietin, and erythropoietin-bevacizumab in a rat Achilles tenotomy model

Ahmet Emrah Açan, MD[1], Aslı Karakılıç, MD[2], Mert Emre Aydın, MD[1], Özgür Bulmuş, MD[2], Emrah Özcan, MD[3], Gülay Turan, MD[4], Özge Özmen, MD[4], Reşit Buğra Hüsemoğlu, MD[5]

[1]Department of Orthopedics and Traumatology, Balıkesir University Faculty of Medicine, Balıkesir, Türkiye
[2]Department of Physiology, Balıkesir University, Faculty of Medicine, Balıkesir, Türkiye
[3]Department of Anatomy, Balıkesir University, Faculty of Medicine, Balıkesir, Türkiye
[4]Department of Pathology, Balıkesir University, Faculty of Medicine, Balıkesir, Türkiye
[5]Department of Biomechanics, Dokuz Eylül University, Institute of Health Sciences, İzmir, Türkiye

Achilles tendon injuries have limited regenerative capacity, often healing with scar tissue that lacks biomechanical strength, prolonging recovery and increasing re-injury risk.[1] Return-to-sport rates vary widely (18.6 to 100%), depending on injury severity and treatment.[2] with many individuals experiencing long-term deficits.[3,4]

Regenerative medicine utilizing biological agents has gained attention for tendon healing. Platelet-rich plasma (PRP), a platelet concentrate rich in growth factors, promotes cellular proliferation, matrix remodeling, and angiogenesis.[5] The PRP has

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Correspondence: Ahmet Emrah Açan, MD. Balıkesir Üniversitesi Tıp Fakültesi, Ortopedi ve Travmatoloji Anabilim Dalı, 10145 Balıkesir, Türkiye.

E-mail: dremrahacan@hotmail.com

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ABSTRACT

Objectives: This study aims to evaluate the effects of platelet-rich plasma (PRP), erythropoietin (EPO), and EPO-bevacizumab (EPO-BEVA) combination on tendon healing in a rat Achilles tenotomy model.

Materials and methods: Fifty-six male Wistar albino rats (14 to 16 weeks old) were randomly assigned to control, PRP, EPO, and EPO-BEVA groups including 14 rats in each group. Bilateral Achilles tenotomy was performed under anesthesia, followed by respective treatments. Platelet-rich plasma (0.1 mL/tendon) was prepared using a Ficoll-based extraction kit. The EPO (500 U/kg) and EPO-BEVA (175 U EPO + 1.25 mg BEVA) were administered locally. Biomechanical analysis assessed maximum force, stiffness, tensile stress, and Young's modulus. Histological evaluation included Bonar scoring, collagen organization, tenocyte morphology, and vascularity. Cross-sectional area (CSA) was measured.

Results: At Week 2, the EPO-BEVA group exhibited superior stiffness (14.79±6.9 N/mm) than PRP (8.64±1.5 N/mm, p=0.015) and greater tensile stress (8.2±1 MPa) than control (6.16±1.3 MPa, p=0.031). The CSA was reduced (4.79±0.8 mm^2) compared to EPO (6.56±1.1 mm^2, p=0.038), indicating qualitative tendon improvements. Histological analysis showed enhanced matrix organization and reduced vascularity in the EPO-BEVA group, with lower Bonar scores (5.29±1.4 vs. 9.29±1.1 in control, p=0.002). By Week 4, maximum force remained higher in EPO-BEVA (46.67±5.8 N) than control (34.84±3 N, p=0.004), with sustained Young's modulus superiority compared to EPO (3.2±1.2 MPa *vs*. 1.78±0.5 MPa, p=0.014), although the stiffness differences were no longer significant.

Conclusion: Our study results showed that EPO-BEVA enhanced tendon healing via vascular and matrix modulation, although the lack of a BEVA-only group limits conclusions on synergy. Future studies with larger sample sizes, including BEVA monotherapy, optimized dosing strategies, and long-term evaluations are needed to better clarify these effects and refine treatment strategies in regenerative medicine.

Keywords: Bevacizumab, erythropoietin, platelet-rich plasma, tendon healing.

demonstrated improved histological organization and biomechanical strength in tendons.[6,7]

Another promising agent is erythropoietin (EPO), a glycoprotein known for erythropoiesis but also possessing anti-inflammatory, angiogenic, neuroprotective, and regenerative properties.[8-13] Studies on the role of EPO role in tendon healing report both promising[14,15] and conflicting results.[16]

Bevacizumab (BEVA) is a monoclonal antibody which inhibits vascular endothelial growth factor (VEGF)-driven neovascularization, widely used in oncology and ophthalmology.[17,18] Recently, a limited number of experimental studies have reported on the use of intratendinous BEVA injections in orthopedics, demonstrating their potential to enhance tendon healing in experimental tendinosis and tenotomy models by regulating angiogenesis and improving matrix organization.[19-21]

While previous studies have explored the independent effects of EPO in tendon healing, its potential interaction with BEVA remains unexplored. In the present study, we hypothesized that combining EPO's pro-angiogenic and matrix-stimulating properties with BEVA's ability to regulate VEGF-driven angiogenesis could create a more controlled healing environment.[14,15,19-21] We, therefore, aimed to evaluate the effects of PRP, EPO, and EPO-BEVA combination on tendon healing in a rat Achilles tenotomy model.

MATERIALS AND METHODS

In this study, a total of 57 male Wistar albino rats (14 to 16 weeks old) were used. The study protocol was approved by the Balıkesir University Animal Ethics Committee (date: 16.05.2024, no: 2024/516). All procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and adhered to international guidelines on animal research ethics. The rats were divided into four groups: control, PRP, EPO, and EPO-BEVA including 14 rats in each group. One rat was used for PRP preparation. Each group was further split into two subgroups (Weeks 2 and 4 sacrifice timepoints, n=7 per subgroup).

Surgical procedure and experimental design

Under anesthesia (80 mg/kg ketamine, 8 mg/kg xylazine, intraperitoneal), the rats' lower limbs were shaved, sterilized (10% povidone-iodine), and draped. A single dose of cefazolin sodium (0.1 mg/kg, intramuscular) was administered for prophylaxis. Achilles tenotomy was performed percutaneously through a 2-mm lateral stab incision using a No.11 scalpel blade, 5 mm proximal to insertion. The plantaris tendon was also transected to prevent a splinting effect.[16,22] The skin was sutured (4/0 monofilament, Prolene). Thereafter, local injections of 0.1 mL PRP, EPO, and EPO-BEVA combinations were administered using an insulin syringe with a 30-gauge needle (Ayset Tıbbi ürünleri A.S., Adana, Türkiye) under the guidance of a mini ultrasound device (Vscan™, General Electric Healthcare, WI, USA), which was utilized both to ensure the accurate execution and verification of the full-thickness tenotomy and to facilitate precise injection placement (Figure 1). All surgical procedures, injections, and tendon harvesting were performed by the same researcher.

The dosages of EPO was selected based on the literature as 0.1 mL injection containing 175 U of EPO (Binocrit, 4000 IU/0.4 mL, Sandoz Pharmaceuticals, Istanbul, Türkiye) was administered to provide a local dose of 500 U/kg.[15] In the EPO-BEVA combination group, a total volume of 0.1 mL was administered, consisting of 0.05 mL containing 175 U of EPO, matching the dose used in the EPO group, and 0.05 mL of BEVA (100 mg/4 mL, Avastin, Roche Pharma AG, Grenzach, Germany.[20] For PRP preparation, 8.5 mL

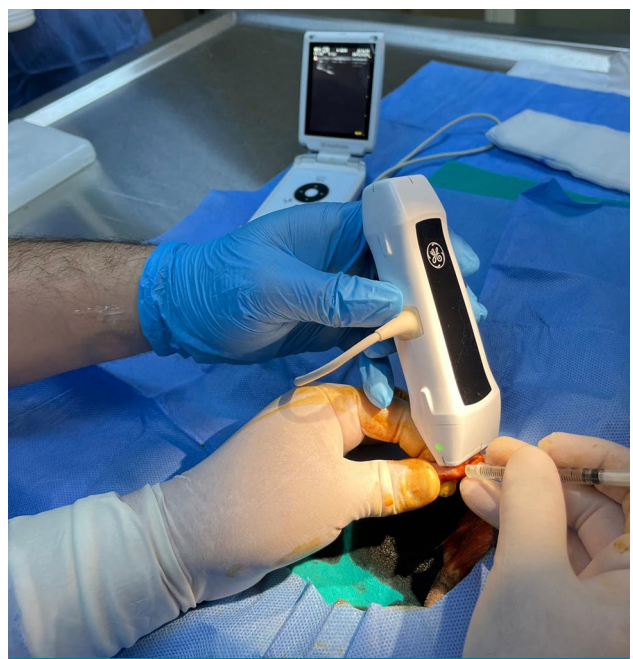


FIGURE 1. Real-time ultrasound-guided local injection procedure. Intraoperative imaging confirmed accurate needle placement within the tendon proper, ensuring precise delivery of the therapeutic agent.

of blood was collected via intracardiac puncture from a rat that was anesthetized and specifically reserved for this procedure. The blood was, then, transferred into a tube containing 1.5 mL of sodium citrate as an anticoagulant and processed using a Ficoll-based cell extraction kit (Easy PRP KIT, Neotec Biotechnology, İstanbul, Türkiye). The sample underwent centrifugation at 1,200 rpm for 5 min in a standard laboratory centrifuge to separate red blood cells. A second centrifugation at 1,200 rpm for 10 min was performed to concentrate platelets. This process yielded 2 mL of PRP, which was sufficient for 0.1 mL injections per rat and was used within 1 h of preparation.[23]

Postoperative care and tissue collection

Post-surgery, the rats had unrestricted movement, except for a one-day immobilization cast. They were maintained under controlled conditions (standard feed, water, natural day/night cycle, regulated temperature & humidity). Euthanasia was performed via cervical dislocation under high-dose anesthesia at Weeks 2 or 4. Achilles tendons were harvested, preserving the calcaneal insertion and extending to the musculotendinous junction. Right tendons were used for biomechanical testing, and left tendons for histology. All procedures were performed by the same researcher.

Cross-sectional area (CSA) measurement

Before −80°C storage for biomechanical testing, the anteroposterior (AP) and mediolateral dimensions at the tendon callus midpoint were measured using a digital caliper (0.01 mm precision, Mitutoyo CD-15D, Japan). The CSA was calculated assuming elliptical geometry.[21,22]

Biomechanical testing

Stored tendons were thawed at 4°C overnight, brought to room temperature, and hydrated with saline-soaked gauze before testing. The calcaneal bone was clamped proximally, and the tendon's distal end was secured with sandpaper for enhanced grip.[15,24] A 0.2 N preload was applied before the electromechanical actuator (5 kN AG-X, Shimadzu Corporation, Tokyo, Japan) pulled the specimen at 0.1 mm/s until failure. The maximum tensile force, stiffness, and displacement values were recorded using software-integrated measurement tools. Maximum tensile stress and Young's modulus were calculated using pre-measured CSA values.

Histological evaluation

Left-side Achilles tendons were fixed in 10% neutral formaldehyde for 48 h before sectioning into 5 to 6-µm longitudinal slices and staining with hematoxylin and eosin (H&E). A blinded pathologist examined the specimens under a light microscope, scoring them using the Bonar system.[15,20,22] This system assesses tenocyte morphology, ground substance, collagen fiber organization, and vascularity, assigning grades from 0 (normal) to 3 (severe changes) (Figures 2-4).

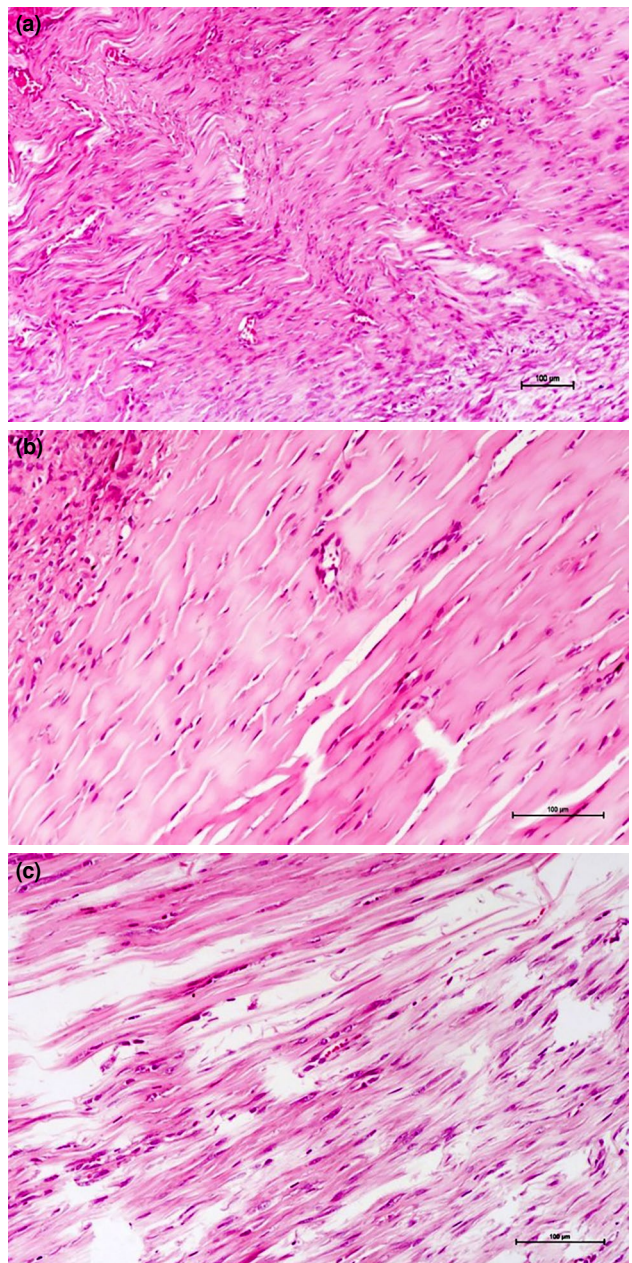


FIGURE 2. Histopathological examination of collagen fiber organization using the Bonar scoring system. **(a)** Score 1: Mild irregularity in collagen fibers, (H&E, ×100). **(b)** Score 2: Moderate irregularity in collagen fibers, (H&E, ×200). **(c)** Score 3: Pronounced separation of collagen fibers, (H&E, ×200).

Statistical analyses

Study power analysis and sample size calculation were performed using the G*Power version 3.1.2 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). Using an effect size of 0.40, confidence level of 0.85, power of 0.8169), minimum 52 rats were required.

Statistical analysis was performed using the IBM SPSS for Windows version 30.0 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean±standard deviation (SD), median (min-max) or number and frequency, where applicable. The differences among four groups were compared using the Kruskal-Wallis test and within pairwise comparison were applied between groups

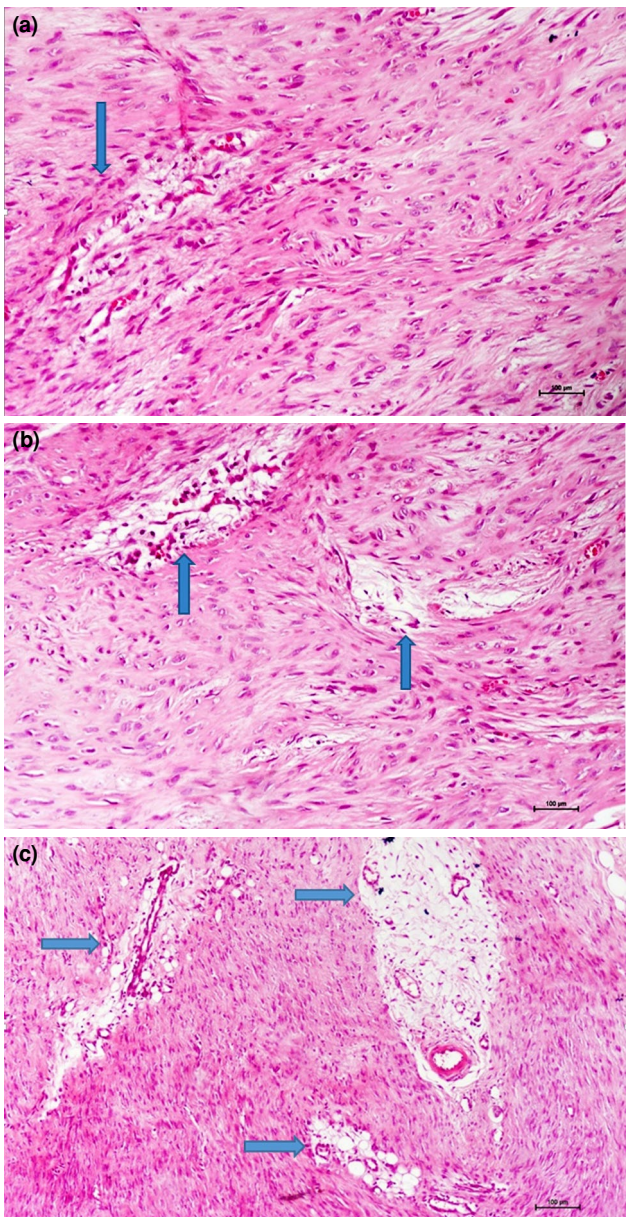


FIGURE 3. Histopathological examination of tenocytes using the Bonar scoring system. **(a)** Score 1: Mild presence of increased mucin, (H&E, ×100) (blue arrow). **(b)** Score 2: Moderate presence of increased mucin, (H&E, ×100) (blue arrows). **(c)** Score 3: Markedly increased presence of mucin, (H&E, ×100) (blue arrows).

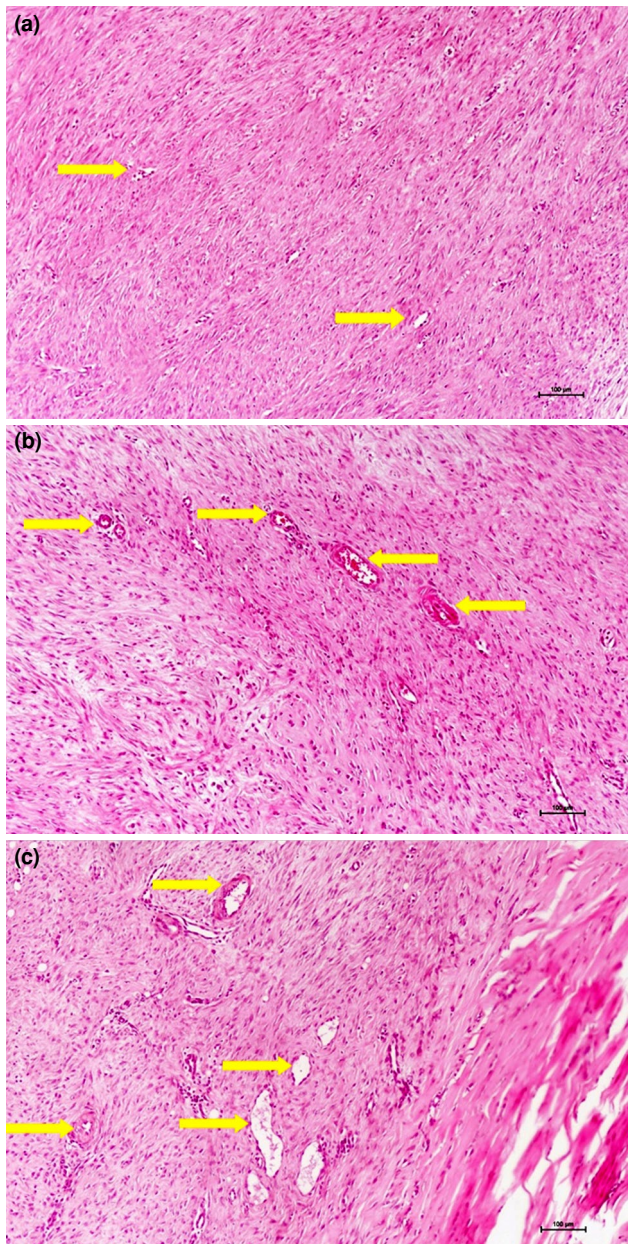


FIGURE 4. Histopathological examination of ground substance using the Bonar scoring system. **(a)** Score 1: Mildly increased vascularity, (H&E, ×100) (yellow arrows). **(b)** Score 2: Moderately increased vascularity, (H&E, ×100) (yellow arrows). **(c)** Score 3: Markedly increased vascularity, (H&E, ×100) (yellow arrows).

for post-hoc tests. A p value of <0.05 was considered statistically significant.

RESULTS

On the day of surgery, one rat in the control group and one in the EPO group died under anesthesia, while one rat in the EPO group and one in the EPO-BEVA group died the day after surgery. To maintain group sizes, surgery was repeated for these four rats the next day.

The CSA of the tendon tissues were calculated and compared among the groups. At Week 2, the mean CSA values were 5.11±1 mm² for the control group, 5.66±0.8 mm² for the PRP group, 6.56±1.1 mm² for the EPO group, and 4.79±0.8 mm² for the EPO+BEVA group. Statistical analysis revealed a significant difference between the EPO and EPO+BEVA groups (p=0.038). At Week 4, the mean CSA values increased to 5.62±0.9 mm² for the control group, 5.88±1 mm² for the PRP group, 6.9±1.2 mm² for the EPO group, and 5.32±1.1 mm² for the EPO+BEVA group; however, no statistically significant differences were observed among the groups at Week 4.

Biomechanical evaluation at Week 2 revealed that the mean maximum force values were significantly higher in the EPO group (38.95±3.2 N) and the EPO+BEVA group (38.91±5.8 N) compared to the control group (30.51±2.5 N), indicating a statistically significant (p=0.003 and p=0.01, respectively). The stiffness of the tendons was also significantly greater in the EPO+BEVA group (14.79±6.9 N/mm) compared to the PRP group (8.64±1.5 N/mm; p=0.015). Furthermore, the EPO+BEVA group exhibited a significantly higher maximum tensile stress (8.2±1 MPa) compared to both the control (6.16±1.3 MPa) and EPO (6.06±0.9 MPa) groups (p=0.031 and p=0.025, respectively). Similarly, the Young's modulus in the EPO+BEVA group (3.24±1.9 MPa) was significantly elevated compared to the PRP (1.55±0.3 MPa) and EPO (1.48±0.4 MPa) groups (p=0.006 and p=0.002, respectively) (Table I).

At Week 4, the mean maximum force remained significantly higher in the EPO+BEVA group (46.67±5.8 N) compared to the control group (34.84±3 N; p=0.004). Young's modulus also showed a statistically significant increase in the EPO+BEVA group (3.2±1.2 MPa) compared to both the control (1.85±0.3 MPa) and EPO (1.78±0.5 MPa) groups (p=0.031 and p=0.014, respectively). However, the stiffness and the maximum tensile stress parameters

TABLE I
Biomechanical results of the experimental groups

	Maximum force (N)		Maximum elongation (mm)		Stiffness (N/mm)		Maximum tensile stress (MPa)		Youngs modulus (MPa)	
	2nd week	4th week	2nd week	4th week	2nd week	4th week	2nd week	4th week	2nd week	4th week
	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)
Control	30.51±2.5 (30)	34.84±3 (34.8)	3.49±0.7 (3.5)	3.44±0.5 (3.3)	9.1±2.3 (7.8)	10.36±2 (10.7)	6.16±1.3 (5.8)	6.31±1 (6.3)	1.81±0.4 (1.9)	1.85±0.3 (1.8)
PRP	35.16±2.2 (35.5)	38.57±5.7 (36.1)	4.21±1 (3.8)	3.62±1.1 (3.7)	8.64±1.5 (9.1)	11.34±2.7 (11.2)	6.3±0.8 (6.3)	6.73±1.5 (6.8)	1.55±0.3 (1.6)	1.95±0.4 (1.9)
EPO	38.95±3.2 (38.3)	42.83±6.3 (41.3)	4.36±1.2 (4.1)	3.71±0.8 (3.9)	9.46±2.5 (9.5)	12.24±3.8 (10.9)	6.06±0.9 (6.3)	6.29±0.9 (6.2)	1.48±0.4 (1.6)	1.78±0.5 (1.6)
EPO+BEVA	38.91±5.8 (39.4)	46.67±5.8 (46.3)	2.92±0.8 (3.2)	3.02±0.7 (2.8)	14.79±6.9 (12.6)	16.53±5.9 (15.4)	8.2±1 (8.3)	9.16±2.5 (8.7)	3.24±1.9 (2.6)	3.2±1.2 (2.6)
p value	0.002*	0.005*	0.039*	0.317	0.013*	0.069	0.011*	0.033*	0.001*	0.009*

SD: Standard deviation; PRP: Platelet-rich plasma; EPO: Erythropoietin; BEVA: Bevacizumab; * p<0.05: Kruskal Wallis test.

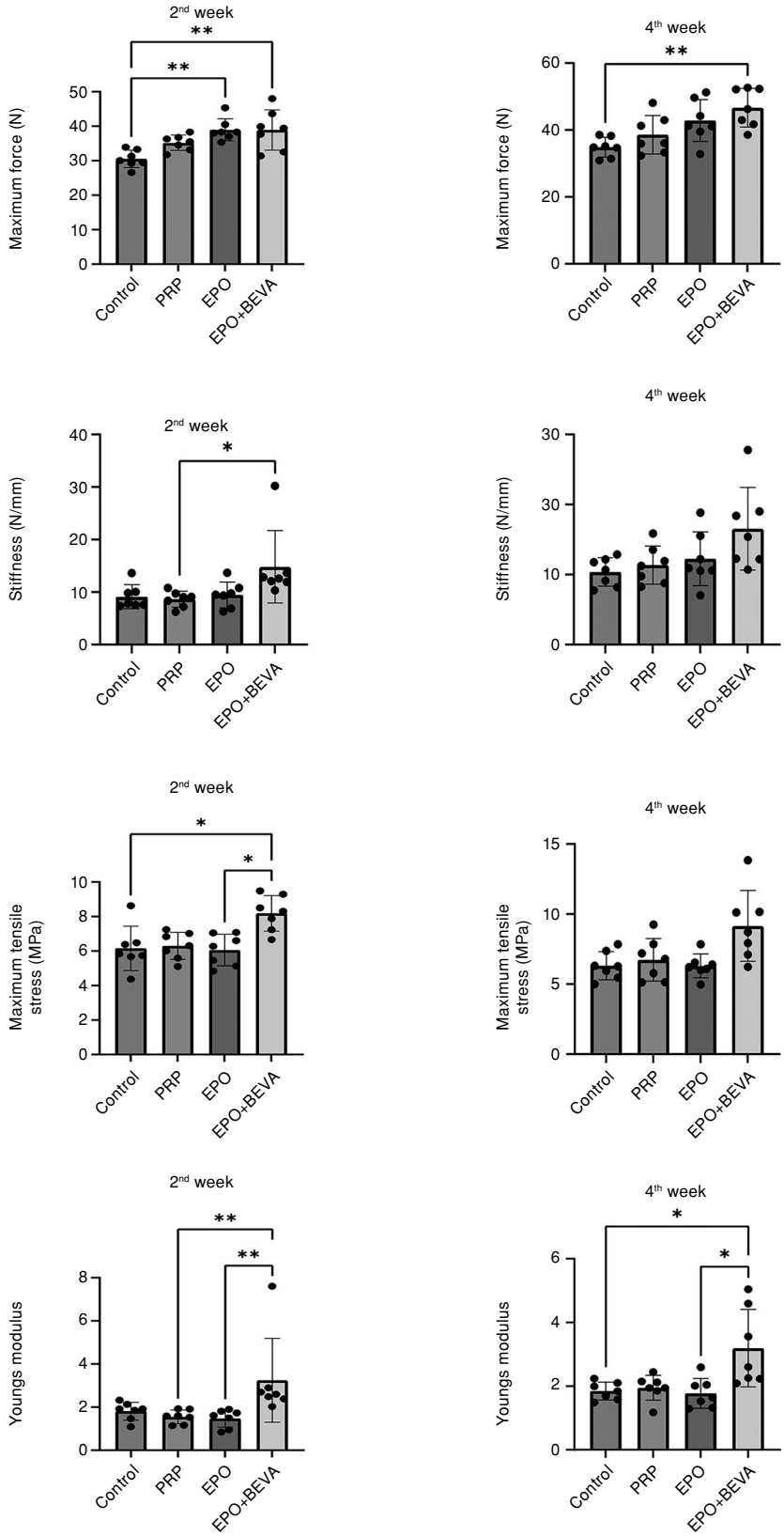


FIGURE 5. The graphs illustrates the mechanical variables for each group at 2nd and 4th week.
PRP: Platelet-rich plasma; EPO: Erythropoietin; BEVA: Bevacizumab; * p>0.05; ** p<0.01.

did not demonstrate statistically significant differences between the groups at Week 4 (Figure 5).

Histological evaluation using Bonar scores showed that at Week 2, total histological scores were significantly lower in EPO-BEVA (5.29±1.4) than control (9.29±1.1, p=0.002). At Week 4, this trend persisted, with scores of 4.29±1.4 (EPO-BEVA) than 9.14±1.8 (control, p=0.0001). While individual parameters (tenocyte count, extracellular matrix, collagen content) showed no statistical significance, the vascularity score was significantly lower in EPO-BEVA than control at Week 4 (p=0.006) (Table II).

DISCUSSION

In the present study, we investigated the combined effects of EPO and BEVA on Achilles tendon healing in a rat model. While BEVA has been shown to regulate angiogenesis and matrix organization [19-21] and EPO promotes extracellular matrix remodeling,[14,15] the extent to which their combined administration influences tendon repair remains unclear. The superior biomechanical and histological outcomes, including significantly lower total Bonar scores in the EPO-BEVA combination group, were promising and aligned with our hypothesis that combining EPO's pro-angiogenic and matrix-stimulating properties with BEVA's ability to regulate VEGF-driven angiogenesis may create a more controlled healing environment.

Following Rotter et al.'s[25] report on systemic EPO's regenerative effects in a muscle-nerve injury model, Uslu et al.[14] conducted the first study on systemic EPO in tendon healing. They reported that low-dose systemic EPO (500 U/kg for 10 days) improved patellar tendon healing, increasing ultimate breaking strength, collagen organization, fibroblast proliferation, capillary formation, and messenger ribonucleic acid (mRNA) expression of collagen (Col) I, Col III, tumor growth factor-beta1 (TGF-β1), and VEGF. However, Bilal et al.[16] and Köker et al.[26] found no histological benefits of systemic EPO in a rat Achilles tenotomy model. Oztermeli et al.[15] compared local and systemic EPO (500 U/kg/day for 10 days) in a rat rotator cuff model. By day 10, systemic EPO improved maximum load to failure and Bonar scores, while by Day 28, histological improvements were more pronounced in the local EPO group despite the lack of biomechanical differences. They concluded that systemic EPO enhanced early-phase healing biomechanically, while local EPO improved late-phase histological organization. Oztermeli et al.[15] also found that EPO did not significantly

TABLE II
Histological results of the experimental groups

	Tenocyte		Extracellular matrix		Collagen		Vascularity		Total score	
	2nd week	4th week	2nd week	4th week	2nd week	4th week	2nd week	4th week	2nd week	4th week
	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)	Mean±SD (median)
Control	2.29±0.8 (2)	2.14±0.7 (2)	2.14±0.7 (2)	2.14±0.7 (2)	2.29±0.8 (2)	2.14±0.7 (2)	2.57±0.5 (3)	2.71±0.5 (3)	9.29±1.1 (9)	9.14±1.8 (9)
PRP	1.86±0.7 (2)	1.71±0.8 (2)	1.86±0.7 (2)	1.71±0.8 (2)	1.86±0.7 (2)	1.71±0.8 (2)	2±0.6 (2)	1.71±0.8 (2)	7.57±1.5 (8)	6.86±1.2 (7)
EPO	1.71±0.8 (2)	1.57±0.5 (2)	1.71±0.8 (2)	1.57±0.5 (2)	1.71±0.8 (2)	1.57±0.5 (2)	2.14±0.9 (2)	1.71±0.5 (2)	7.29±2.1 (7)	6.43±1.3 (6)
EPO+BEVA	1.29±0.8 (1)	1±0.8 (1)	1.29±0.8 (1)	1±0.8 (1)	1.29±0.8 (1)	1±0.8 (1)	1.43±0.7 (2)	1.29±0.8 (1)	5.29±1.4 (5)	4.29±1.4 (5)
p value	0.149	0.077	0.227	0.077	0.149	0.077	0.063	0.008*	0.005*	0.0001*

SD: Standard deviation; PRP: Platelet-rich plasma; EPO: Erythropoietin; BEVA: Bevacizumab; * p<0.05: Kruskal Wallis test.

impact early vascularity, regardless of local or systemic administration. However, in later stages, locally injected EPO significantly reduced vascularity scores (0 points on the Bonar scale) compared to systemic EPO and control groups (both 1.33 points). The authors concluded that local EPO improved late-phase vascularization, accelerating angiogenesis reorganization in tendinous tissue.

Although EPO has been reported to enhance vascularization, our study found no significant vascular improvements compared to controls, suggesting that EPO's effect may depend on dose, administration route, or Achilles tendon-specific characteristics. Unlike previous studies that primarily assessed systemic administration, which prolongs biological activity, our study used a single local injection. Future research should explore alternative dosing regimens and routes of administration to clarify EPO's role in tendon healing. Additionally, variability in histological results may stem from differences in scoring methods, tendon types, and tenotomy and repair techniques (patellar, rotator cuff, Achilles). Moreover, the percutaneous tenotomy model used in this study (2-mm stab incision, no tendon repair) may provide a more standardized approach compared to open tenotomy models with various repair techniques, while also minimizing solution leakage.

Angiogenesis is essential for tissue healing, but excessive angiogenesis in tendons can lead to disorganized matrix formation and increased re-injury risk.[21,27-30] Dallaudière et al.[19,20] first demonstrated BEVA's anti-angiogenic potential in an Achilles tendinosis rat model, while Tempfer et al.[21] later evaluated local BEVA in a tenotomy model. They administered BEVA (25 mg/mL) with a peptide (3:2 ratio) at 75 µL on Days 4 or 11 into a 2-mm Achilles tendon defect. Their results showed improved matrix organization and fiber orientation with decreased angiogenesis, consistent with Dallaudière et al.'s findings.[19,20] They suggested that reducing angiogenesis post-tendon injury might enhance repair, in contrast to studies advocating for growth factor-induced vascular ingrowth.[21] Riggin et al.[24] further investigated vascular modulation in tendon healing using local BEVA or VEGF administration in a full-thickness Achilles tendon rat model. The VEGF (5 µg) and BEVA (250 µg) were administered daily from Days 0-2 (early group) or Days 4-6 (late group). While VEGF had minimal impact on healing, BEVA administered late reduced mechanical properties and sustained vascularity reduction, whereas early BEVA did not impair mechanics and improved collagen organization. These findings suggest that administering BEVA at the time of injury in our study may have contributed to improved mechanical and histological outcomes. Moreover, hypoxia-inducible factor 1-alpha (HIF-1α), a key regulator of angiogenesis and cellular response to hypoxia, plays a crucial role in tendon healing by modulating VEGF expression. While EPO has been reported to influence HIF-1α pathways in ischemic tissues, its specific effects on tendon repair remain unclear. Future studies should investigate whether EPO-BEVA interactions modulate HIF-1α expression and whether this contributes to the vascular remodeling effects observed in our study.

On the other hand, Kusaba et al.[28] explored BEVA's anti-angiogenic effects on tenogenic differentiation in an *in vitro* rat tendon-derived cell model. They found that BEVA upregulated scleraxis (Scx), tenomodulin (Tnmd), and Col1a1 gene expression, promoting tenogenic differentiation in tendon proper- and paratenon-derived cells. Imai et al.[31] reported that PRP enhanced tenogenic differentiation via Scx upregulation, but downregulated Tnmd alongside increased VEGF production. Kusaba et al.[28] hypothesized that combining BEVA with PRP could provide a synergistic effect, as BEVA's VEGF inhibition may counteract PRP's Tnmd-downregulating effects, though this remains untested.

Our study builds upon this parallel concept, hypothesizing that combining BEVA with EPO could balance vascular modulation while enhancing matrix organization. Previous studies suggest that EPO promotes beneficial late-phase vascular effects, while BEVA may mitigate excessive angiogenesis during early inflammatory phases by preventing the upregulation of matrix-degrading enzymes (matrix metalloproteinases [MMP2, MMP9]).[5,21] Literature further supports this hypothesis, indicating that VEGF expression peaks on Day 7 post-injury, with neoangiogenesis lasting from Days 7-8 to the third week. Temfer et al.[21] reported that locally injected BEVA was detectable on Day 7 but absent by Day 14, suggesting that timely administration of BEVA may regulate early-stage angiogenesis without disrupting later vascular remodeling. Consistent with this, our findings demonstrated that the EPO-BEVA group exhibited significantly reduced vascularity at both Weeks 2 and 4 compared to the control group. This suggests that BEVA's VEGF inhibition may have prevented excessive early angiogenesis, allowing for a more controlled vascular remodeling process that

optimizes tendon regeneration. Furthermore, this modulation may have delayed EPO-induced early angiogenesis to later stages, preventing excessive neovascularization in the inflammatory phase while enhancing EPO's role in late-stage vascular reorganization, supporting the rationale of our hypothesis.

From a biomechanical perspective, the mean maximum force values at Week 2 were significantly higher in the EPO and EPO+BEVA groups compared to the control group. The mean CSA values were 5.11±1 mm² (control), 5.66±0.8 mm² (PRP), 6.56±1.1 mm² (EPO), and 4.79±0.8 mm² (EPO+BEVA), with a statistically significant difference between the EPO and EPO+BEVA groups. Despite the reduced CSA in the EPO+BEVA group, the increase in maximum force values, along with significantly lower Bonar scores, suggests that qualitative tendon improvements may compensate for decreased CSA. The higher maximum force in the EPO group may be partially explained by its increased CSA, although this difference was not statistically significant compared to the control and PRP groups. Similar findings were reported by Uslu et al.[14] for systemic EPO administration and by Oztermeli et al.[15] in the systemic EPO group, but not with local EPO. However, none of these studies evaluated tendon callus volume or CSA.[14-16,26] In contrast, Tempfer et al.[21] observed similar biomechanical outcomes, reporting increased stiffness and maximum tensile load in BEVA-treated tendons, independent of CSA, as indicated by increased maximum tensile stress and Young's modulus. By Week 4, the mean maximum force remained significantly higher in the EPO+BEVA group than the control group, but not in the EPO group. This aligns with Oztermeli et al.[15] who reported improved histological outcomes with EPO on Day 28, particularly in the local group, without differences in maximum load to failure. These biomechanical findings, along with histological results, further support our hypothesis.

Despite the promising findings, this study has several limitations. First, the absence of a BEVA-only group limits our ability to determine whether the observed benefits stem from combination therapy (EPO+BEVA) or BEVA alone. Including additional groups, such as BEVA monotherapy or PRP+BEVA, could have provided a more comprehensive understanding of its effects. Second, the study did not assess time- or dose-dependent effects, which may have better elucidated BEVA's therapeutic potential. Another limitation is the focus on short-term outcomes (Weeks 2 and 4 post-surgery), offering limited insight into long-term effects. Extended follow-up is needed to assess the durability of the observed benefits. Additionally, functional assessments such as the Achilles Functional Index were not performed due to the bilateral tenotomy model, which restricts locomotor evaluation. While the Bonar scoring system is widely used, its semi-quantitative nature and observer variability remain limitations. Advanced molecular and imaging techniques could provide a more detailed understanding of tendon healing mechanisms. In particular, the absence of biochemical markers such as MMPs and the collagen I/III ratio in our study limits the ability to assess tendon remodeling at the molecular level. Future studies incorporating these markers could offer a more comprehensive evaluation of tendon healing dynamics. Finally, the variability in PRP preparation methods, including differences in centrifugation protocols, platelet concentration, and activation techniques, may affect its biological activity and limit reproducibility across studies. Additionally, the absence of specific data on platelet concentration and growth factor content in our study restricts the ability to fully determine PRP's biological activity. Future research should focus on standardizing PRP formulations and characterizing their composition to improve reproducibility, comparability, and translational relevance across different studies.

In conclusion, the combination of EPO and BEVA demonstrated superior biomechanical and histological outcomes compared to PRP or EPO alone, representing a promising therapeutic approach for tendon healing. However, the absence of a BEVA-only control group limits our ability to determine whether these effects result from a synergistic interaction or BEVA's independent influence. While these findings highlight the potential of EPO-BEVA combination in tendon repair, its clinical translation as an adjunctive treatment via percutaneous injection under ultrasound guidance requires careful evaluation. However, its systemic absorption may pose risks such as endothelial dysfunction, increased blood viscosity, or thromboembolic complications. Future studies with larger sample sizes, including BEVA monotherapy, optimized dosing strategies, and long-term evaluations to clarify its sustained effects on tendon remodeling and functional recovery, are needed to determine the safest and most effective application protocols.

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