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Synergistic Effects of Platelet-Rich Plasma and Amniotic Membrane on Tendon Healing in a Rabbit Model

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Abstract

TENDON repair remains a significant clinical challenge due to the slow rate healing process. While both platelet rich plasma (PRP) and amniotic membrane (AMNIO) therapies have been individually utilized for promoting tendon regeneration, their combined effects are still largely unexplored. Achilles tendon injuries were induced in 48 male New Zealand white rabbits and treated with one of four interventions: control (CTRL), PRP injection, AMNIO wrap, or a combination of PRP and AMINO (PRP+AMNIO). Tendon healing was assessed at 4-, 8-, and 12-weeks post-surgery using macroscopic evaluation and histological analysis, including hematoxylin and eosin (H&E) and Masson's trichrome staining to evaluate collagen deposition, inflammation, and tissue morphology. All data were statistically analyzed. The PRP+AMNIO group demonstrated superior tendon healing compared to CTRL, PRP, and AMNIO groups. Macroscopic evaluation revealed a significantly improved tendon structure in the PRP+AMNIO group, showing 5.5-, 5-, and 4.7-fold improvements compared to the CTRL group at weeks 4, 8, and 12, respectively ($p<0.0001$). Compared to the CTRL group, the PRP+AMNIO group showed reduced inflammation and hemorrhage, with reduction of up to 7.5-fold ($p<0.0001$). Collagen fiber linearity significantly improved in the PRP+AMNIO group by week 12, with a 5.7-fold increase compared to the CTRL group ($p=0.0007$). Moreover, the PRP+AMNIO group exhibited significantly higher collagen deposition than the PRP and AMNIO groups alone at weeks 8 and 12 ($p<0.0001$). In conclusion, compared to the control group, the combination of a-PRP injection with AMNIO wrap improves rabbit Achilles tendon recovery by reducing inflammation, promoting collagen deposition, and enhancing tendon morphology.

Keywords: Achilles Tendon Injury; Collagen Deposition, Platelet Rich Plasma, Inflammation, Regenerative Medicine.

Introduction

Tendon injuries are common and challenging conditions that often lead to prolonged healing times and incomplete recovery [1]. Due to a lack of cells and low growth factor activity, the healing process of injured tendons is frequently longer and weaker in the early stages [2]. Despite advancements in medical treatment, effective therapeutic interventions to accelerate tendon repair remain limited.

Platelet-rich plasma (PRP) therapy has been widely used in regenerative medicine due to its ability to deliver concentrated growth factors directly to the injury site, promoting tissue regeneration and reducing inflammation [3,4]. Moreover, PRP can simulate the healing process by promoting the production of an extracellular matrix rich in type I collagen [5]. In an experimental study by Bosch et al., autologous PRP significantly enhanced tendon repair and improved both structural properties and blood flow, as observed through ultrasonography [6]. Additionally, the combination of PRP and tendon stem cells (TSCs) has been shown to synergistically enhance tendon healing, particularly under loaded conditions [7].

Preclinical studies have demonstrated PRP's ability to stimulate tendon healing processes, improve fiber organization, and increase tensile

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strength [8,9]. An *in-vitro* research has shown that PRP treatment can increase cell proliferation and growth factor expression in tendon models [10].

Clinically, PRP has been applied to various tendinopathies, including Achilles, patellar, and elbow, as well as rotator cuff lesions [11]. While some clinical trials have reported improvements in pain control and function, others have found no significant differences compared to controls [4].

Amniotic membrane (AMNIO) also has shown promising results in promoting tendon healing and preventing adhesion. Studies have demonstrated that AMNIO enhances the maturation of fibroblasts and collagen fibers, improving the material properties of lacerated tendons in early healing stages [12]. When incorporated into collagen scaffolds, AMNIO exhibits immunomodulatory effects, downregulating pro-inflammatory cytokine expression in mesenchymal stem cells13. Composite silk scaffolds with AMNIO wrapping accelerate cellular migration and angiogenesis in neotendons [14].

In a clinical trial, freeze-dried AMNIO transplantation significantly improved the range of motion and reduced complications in flexor tendon repairs compared to control and poly-DL-lactic acid treatments [15,16].

Moreover, AMNIO is not limited to serving as a protective barrier or bandage; its high concentration of regenerative factors makes it highly effective in promoting tissue regeneration. Studies have shown that solubilized AMNIO, when combined with materials like hyaluronic acid, enhances tissue healing, emphasizing its broader potential in regenerative medicine beyond simple covering applications [17-20].

While both PRP and AMNIO have been individually utilized in several studies for tendon repair, no study to date has explored the potential benefits of combining these two traditional techniques. This study aims to fill that gap by investigating the synergistic effects of PRP and AMNIO in promoting tendon healing, providing a novel approach to an age-old challenge.

Material and Methods

Ethical approval

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Alexandria University, Egypt (approval number AU0132201111114).

Animals

This study was conducted on 48 mature fully vaccinated male New Zealand white rabbits, about three months old and weighing 2.5-3 kg. Animals' health was checked and housed in a separate cage under the same environmental condition. The animal was acclimatized to the experiment place for 2 weeks before surgery, with Light/ dark cycle 12-12hrs and free access to water and commercial diet *ad libitum.*

Sample size calculation

The sample size used in this study was guided by previously published rabbit models investigating tendon healing with PRP or amniotic membrane applications. Notably, studies such as Yang et al. [12] and Zukawa et al. [21] utilized comparable experimental group sizes, providing a solid basis for our design. Zukawa et al. employed 12 experimental groups in a rabbit flexor tendon model, while Yang et al. assessed tendon healing outcomes following amniotic membrane transplantation.

To ensure adequate statistical power and account for biological variability, sample size was further confirmed using G*Power 3.1 software. This analysis supported the inclusion of 48 tendons, distributed evenly across 12 groups (4 tendons per group), with assessments conducted at three distinct time points. Each tendon was obtained from a separate rabbit, resulting in a total of 48 animals included in the study.

Study design

This study was conducted on 48 apparently healthy male New Zealand white rabbits, about three months old and weighing 2.5-3 kg. Unilateral Achilles tendons injuries were induced in all rabbits. Based on treatment, these rabbits were randomly assigned to one of four groups (12 animals each). These treatment groups included control group (CTRL), AMNIO transplantation group (AMNIO), platelet-rich plasma group (PRP), and combination of platelet-rich plasma injection and AMNIO transplantation group (PRP+AMNIO). Each group was further subdivided into 3 subgroups (4 animals each) according to the evaluation time. In subgroups 1, 2 and 3, the evaluation was conducted at 4 weeks, 8 weeks, and 12 weeks post-surgery, respectively.

PRP preparation

Autologous PRP was prepared from each animal using a double centrifugation method with slight modifications, as previously described [23]. Blood (5.4 mL) was collected from the ear vein of each rabbit into vacutainer tubes containing 3.2% sodium citrate as an anticoagulant. The first centrifugation was performed at 1600 RPM for 10 minutes, yielding three layers: red blood cells, a buffy coat containing platelets and leukocytes, and plasma. The plasma and buffy coat were transferred to a sterile plain tube and subjected to a second centrifugation at 2000 RPM for 10 minutes, resulting in the separation of platelet-poor plasma (PPP) and PRP. The PRP was then collected in a 1 mL syringe.

Surgical procedure

All rabbits were fasted for 6 h, with water withheld for 2 h prior to surgery. Anesthesia was induced via intramuscular injections of Xylazine HCl at a dose of 5 mg/kg (Xylaject 2%®, ADWIA, Egypt) as a sedative and Ketamine HCl at a dose of 35-65 mg/kg (Keiran®, EIMC Pharmacuticals Co., Egypt) as a general anesthetic. Aseptic techniques were strictly adhered to throughout the surgery, including the shaving and disinfection of the surgical area with Povidone-iodine solution (Betadine antiseptic solution®, Pharoania Pharmaceuticals, Cairo, Egypt).

A 3cm longitudinal skin incision was made along the posterior aspect of the hind limb. The Achilles tendon was exposed through skin and subcutaneous incision as described by Bürgisser et al. [22]. Sterile saline solution was used to irrigate the tissue and maintain tendon hydration. The Achilles tendon was bluntly dissected from the fascia and surrounding tissue, then a full thickness tenotomy was performed at the midpoint between the calcaneal insertion and gastrocnemius junction. The tendon ends were sutured using a 4-strand core suture technique (modified Kessler) with 2-0 non-absorbable Prolene suture material. For CTRL group, the sutured tendon was injected with 0.5 mL physiological saline solution in an infiltrative manner.

In PRP group, 0.5 mL PRP was injected directly into the tendon repair site in an infiltrative manner. In AMNIO group, the sutured tendons were wrapped with AMNIO (approximately 2×2 cm in size) (Commercial dry membrane, REGE PRO, Egypt). For combination PRP+AMNIO group, tendon repair site was infiltrated with 0.5 mL PRP and then wrapped with AMNIO.

Postoperatively, rabbits were monitored until they fully recovered from anesthesia and were then transferred to their cages. Limb immobilization was achieved using a bandage cast wrapped from the toes to the groin, maintaining the ankle at 150° plantarflexion. The bandage was renewed twice weekly and kept in place for 3 weeks, after which the rabbits were allowed free movement in their cages. Post-operative care included cefotaxime (30 mg/kg) and meloxicam (0.1 mg/kg) administered intramuscularly for 5 consecutive days.

Tendon healing assessment

Tendon healing was assessed by the clinical findings and macroscopic as well as histologic examinations.

Gross morphological assessment

Gross morphological assessment of the repaired tendon at the rupture site was conducted using the macroscopic grading system described by Stoll et al. (Table 1) [24]. A healthy tendon is characterized by a white, reflective appearance with a smooth surface, and should exhibit no swelling or adhesions to the surrounding tissue.

Histopathology assessment

Rabbits were euthanized by intravenous administration of ≥ 100 mg/kg of Pentobarbital sodium (Nembutal®, Akorn Pharmaceutical, 369 Bayview Ave, Amityville, NY 11701, USA). The animal was monitored until lack of heartbeat was noted for > 60 seconds prior to tissue harvest. Achilles tendons were excised, rinsed with phosphate-buffered saline (PBS, pH 7.4), and fixed in neutral buffered formalin for 48 hours. The samples were processed using conventional paraffin embedding techniques as described by Bancroft and Layton [25]. Four-micron thick sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Sections were photographed using a Leica DM500 microscope linked to a Leica EC3 digital camera (Leica, Germany). Lesions, including hemorrhage, inflammatory infiltrates, and collagen fiber alignment, were assessed in 10 randomly selected fields per animal. A blinded scoring system was used as shown in Table 2 [26]. The percentage area of collagen fibers stained by Masson's trichrome was calculated using Image J software (NIH, Bethesda, MD, USA), and inverse mean density was determined from 10 random fields per group.

Statistical analysis

Gross macroscopic data were analyzed using a one-way ANOVA for overall analysis without consideration of individual time points. Microscopic data were analyzed using a two-way ANOVA, with Tukey's test applied for multiple comparisons. Coefficient of variation (CV) was calculated from the averaged replicate data for each group. Statistical significance was set at $p \leq 0.05$. All numerical data were presented as means ± standard deviation (SD). All statistical analyses and graphical presentations were generated using GraphPad Prism (version 10).

Results

Clinical findings

Postoperative clinical observations revealed that rabbits remained stationary for the first three days, using their hind limbs minimally. Thereafter, they walked when stimulated, and by day seven, they moved freely. No signs of infection were observed in all rabbits. By the fourth week, all animals showed no complications, moving freely without bandages.

Macroscopic findings

As shown in Figure 1, there were significant differences ($p < 0.05$) in the quality of healing responses among the various treatment groups based on macroscopic parameters. The combination therapy group PRP+AMNIO demonstrated superior outcomes, with a low CV of 15.75%, indicating consistent results in parameters such as the connection of the tendon to the skin, tendon surface, and level of defect. In contrast, the control group

exhibited significantly higher variability, with CVs of 21.65%, 43.30%, and 100.0%, respectively (Table 3). The PRP+AMNIO group also exhibited low inflammation that's achieved by decreased Oedema, swelling and redness with high precision, as indicated by a low CV of 33.33%, compared to 86.60%, 86.60%, and 91.65% in the CTRL, AMNIO, and PRP groups, respectively. Furthermore, the shape of the tendon in the PRP+AMNIO group was significantly improved, with a score 4.6-fold higher than that of the control group (p=0.0103).

The overall macroscopic evaluation score demonstrated significantly superior results in the PRP+AMNIO group, showing 5.5-, 5-, and 4.7-fold improvements compared to the CTRL group at weeks 4, 8, and 12, respectively ($p < 0.0001$). In subgroup 1 (week 4), the PRP+AMNIO group also showed a 1.8-fold higher score than both the AMNIO and PRP groups ($p = 0.002$). Notably, at this time point, some tendons in the AMNIO group exhibited rupture. In subgroup 2 (week 8), the PRP+AMNIO group showed 2- and 5-fold higher scores compared to the PRP and AMNIO groups, respectively ($p < 0.0001$). However, in subgroup 3 (week 12), there was no significant difference among the PRP+AMNIO, AMNIO, and PRP groups ($p > 0.05$), as shown in Figure 2.

Histopathology findings

Findings of H&E staining (Hemorrhage, inflammation, and collagen alignment):

Samples from the CTRL group showed extensive hemorrhage, absence of linear collagen fibers, and severe inflammatory cell infiltration in subgroup 1. In subgroup 2, the CTRL group exhibited moderate hemorrhage and nonlinear collagen fibers with persistent inflammatory cell infiltration, which persisted in samples of subgroup 3 (Fig. 3).

The AMNIO group displayed severe to moderate hemorrhage, moderate linear collagen fibers, and moderate inflammatory cell infiltration in subgroups 1 and 2. In subgroup 3, the AMNIO group demonstrated mild hemorrhage, increased collagen fiber linearity, and reduced inflammatory infiltration (Fig. 3).

In the PRP group, samples in subgroup 1 showed moderate hemorrhage, moderate collagen linearity, and moderate inflammation. While subgroups 2 and 3 the PRP group exhibited mild hemorrhage, linear collagen alignment, and mild inflammation (Fig. 3).

The PRP+AMNIO group revealed mild hemorrhage, linear collagen fibers, and mild inflammation in subgroups 1 and 2. In subgroup 3, no hemorrhage, linear collagen fibers, and no significant inflammatory cell infiltration were seen (Fig. 3).

Quantitative analysis of histological sections revealed significantly less hemorrhage in the PRP+AMNIO treatment group compared to CTRL ($p < 0.05$), with reductions of 2.1-, 4.6-, and 6-fold at weeks 4, 8, and 12, respectively (Fig. 4A). There was also a notable decrease in inflammatory cell infiltration by 2.1-, 4.75-, and 7.5-fold at the same time points (Fig. 4B). However, the enhancement in collagen fiber linearity in the PRP+AMNIO group compared to CTRL was only significant by week 12, showing a 5.7-fold improvement ($p = 0.0007$, Fig. 4C). In contrast, AMNIO-alone treatment showed no significant difference compared to the CTRL group at any of the three time points. By week 12, both the PRP+AMNIO ($p<0.001$) and PRP-alone ($p<0.01$) groups demonstrated significantly higher collagen fiber linearity compared to the AMNIO-alone group.

Findings of Masson's trichrome staining (Collagen content and organization):

The CTRL group showed the lowest collagen distribution at 4 weeks, with gradual increases at 8 weeks and 12 weeks (Fig. 5). In the AMNIO group, collagen distribution was higher in subgroup 1 compared to the CTRL group, with increases observed in subgroups 2 and 3 (Fig. 5). The PRP group exhibited similar collagen distribution to the AMNIO group in subgroup 1, with gradual increases in subgroups 2 and 3. The PRP+AMNIO group showed the highest collagen distribution in subgroup 1, with continued increases in subgroups 2 and 3 (Fig. 5).

Quantitative analysis of Masson's trichrome staining findings revealed that AMNIO, PRP, and PRP+AMNIO groups exhibited significantly higher collagen content compared to the CTRL group ($p<0.0001$). However, no significant differences were observed between AMNIO, PRP, and PRP+AMNIO groups in subgroup 1 ($p>0.05$). Notably, the PRP+AMNIO group demonstrated significantly higher collagen deposition, with increases of 14.19-, 7.24-, and 1.67-fold compared to the CTRL group at weeks 4, 8, and 12, respectively ($p<0.0001$). Furthermore, the collagen content in the PRP+AMNIO group was significantly higher than in the AMNIO and PRP groups alone in subgroups 2 and 3 ($p<0.0001$) as shown in Fig. 6A. A schematic diagram summarizing the results of the combination therapy PRP + AMNIO is shown in Figure 6B.

Discussion

Tendon injuries are prevalent and often require extended healing periods, leading to various complications. Common issues include the development of adhesions and ruptures, as well as the formation of scar tissue, which is typically has inferior mechanical properties compared to healthy tendon tissue [27]. These adverse phenomena can significantly impair functionality and lead to chronic pain or re-injury, underscoring the need for effective

treatment strategies that not only promote healing but also enhance the quality of the repaired tissue. In this study, we explored the synergistic benefits of combining PRP and AMNIO for tendon repair.

In the current study, rabbit was chosen as an experimental animal. Rabbits are frequently used as animal models in research on tendon healing because they show less spontaneous tendon regeneration after tendon injury than smaller animal models like rats and mice [28]. Furthermore, rabbits are an inexpensive option for research because they are less expensive to purchase and keep. Additionally, because rabbits are larger, surgical models and techniques can be used more easily, improving the precision and repeatability of experimental operations [29]. Compared to larger animals, which frequently have restricted overhead reaching ability, rabbits have more upright position which closely reflects human anatomy [29]. Herein, we used the Achilles tendon, as it is the most commonly injured tendon in the animal body. It is a long, round, rope-shaped extraarticular tendon that lacks a synovial sheath and is instead covered by a paratenon [30]. These extra-synovial tendons are more prone to adhesions and scarring compared to intra-synovial tendons and may require additional surgical intervention to release the scarred tissue following injury or laceration [31].

In the present study, the injury and treatment were performed unilaterally in each rabbit to minimize the potential impact of prolonged immobilization. A similar technique was conducted in a previous study [22].

Herein this study, PRP was injected directly into the injury site without prior platelet activation, as type I collagen naturally present in tendons stimulates platelet activation and growth factor release [32].

After injury, tendons typically undergo an initial inflammatory phase followed by a proliferative phase in which collagen is laid down, and finally a remodeling phase [33]. The complete recovery observed by the 4th week in the current work suggests that the treatments administered whether PRP, AMNIO, or their combination facilitated this natural healing process, accelerating the resolution of inflammation and promoting tissue repair.

Our histopathological analysis revealed significant changes during tendon healing across the 4, 8, and 12-week time points. At 4 weeks, moderate hemorrhage and inflammatory cell infiltration were observed, along with vascularization, which is critical for delivering growth factors and nutrients to the injury site [34]. Increased vascularization during the early stages aids cell recruitment and supports the proliferative phase of healing. By the eighth week, vascularization regressed, aligning with the role of PRP in promoting cellular migration and proliferation, as supported by Lyras et al., indicating early tendon healing [35]. The reduction in cellular infiltration at 8 weeks signals the onset of the remodeling phase, with collagen fiber alignment improving by the twelve-week, reflecting the transition to tissue maturation [35].

The anti-inflammatory effects of PRP, mediated through the recruitment of inflammatory cells and suppression of the prostaglandin biosynthetic pathway [36], likely contributed to the observed healing. PRP, rich in growth factors such as transforming growth factor and platelet-derived growth factor, has been shown to enhance tenocyte proliferation and collagen matrix synthesis [37]. This aligns with findings by Virchenko and Aspenberg, who reported faster restoration of tendon strength with PRP treatment, suggesting that PRP accelerates the remodeling phase and promotes more rapid tendon maturation [38].

Beyond its regenerative capabilities, PRP not only promotes tissue healing through its rich content of growth factors but also acts as a defensive barrier, offering antimicrobial protection to safeguard the injury site from potential infections. Previous study has demonstrated that the integration of PRP with reduced graphene oxide (rGO) and carbon nanotube (CNT) nanoparticles exhibited a protective effect, further enhancing tissue regeneration compared to nanoparticles alone [39].

Our results indicate that PRP treatment facilitated quicker progression from the proliferative to the remodeling phase, improving tendon strength and collagen organization. In contrast, the control rabbits showed prolonged vascularization that reflected by presence of hemorrhage and inflammatory cell infiltration and fibroblast activity at 12 weeks, indicating delayed healing. This slower progression, with a more gradual decrease in type I collagen expression, is consistent with previous studies showing PRP's role in enhancing the mechanical properties of regenerated tendons [21].

Herein, we also investigated the effect of dry AMNIO on the healing of induced tendon ruptures. At 4 weeks, the repaired tendons appeared hypertrophic, with dense peritendinous adhesions and significant inflammation surrounding the membrane. In some cases, complete tendon rupture was observed, which we attributed to low collagen fiber content at this early stage. By the eighth week, hypertrophy and adhesions had lessened, and by the twelve-week, both were minimally present around the healed site. These findings partially align with the study by Zukawa et al., which reported that AMNIO prevents peritendinous adhesion without impairing tendon healing [21]. Similarly, Demirkan et al. found that the AMNIO acts as a temporary barrier, preventing early adhesions by extrinsic cells [40].

Despite the potential benefits of AMNIO, adhesion formation after transplantation remains a concern, as it can restrict tendon gliding. This issue may be influenced by factors such as postoperative immobilization and tendon range of motion. Histopathologically, we observed heavy infiltration of inflammatory cells at 4 and 8 weeks, which we interpreted as an inflammatory reaction to the AMNIO wrap. This response was accompanied by increased hemorrhage, which gradually diminished by 12 weeks. The inflammation could be due to the AMNIO's ability to accelerate cell migration and modulate inflammation, as noted in previous studies [21].

It has been demonstrated earlier that amniotic extract possess inflammatory and anti-inflammatory cytokines which are variable according to the process and preservation techniques [41]. Herein, AMNIO treated group did not show any significant difference in inflammatory cell infiltration from the control group however the collagen content was significantly higher.

Additionally, residual AMNIO was detected at 4 weeks but had been completely reabsorbed by the eighth and twelve-weeks, likely converting into fibrous tissue. This aligns with findings by Demirkan et al. [40]. While collagen fiber distribution in the AMNIO group was higher than in the control group at both 4 and 8 weeks, the fibers remained randomly arranged as fibroblasts migrated to the repair site, indicating ongoing tissue remodeling.

Tendon healing can be classified as intrinsic or extrinsic, with intrinsic healing providing superior mechanical strength due to the contribution of tenocytes, producing tissue that closely resembles native tendon. In contrast, extrinsic healing relies on cellular migration from surrounding tissues, often resulting in a higher proportion of immature fibers and type III collagen, which is associated with increased re-rupture rates and weaker repair [33]. In the context of our study, the combined PRP+AMNIO therapy appears to promote intrinsic healing by delivering growth factors that stimulate tenocyte activity and enhance collagen deposition. The anti-inflammatory properties of the PRP and AMNIO further reduce the need for extrinsic healing, preventing the excessive scarring and disorganized fiber alignment typical of that process. This likely explains the observed improvements in collagen organization and tissue strength in the PRP+AMNIO group, indicating a shift towards more effective, and intrinsic-driven tendon repair.

The combination PRP+AMNIO group showed a greater effect on tendon healing, with tendon adhesion gradually decreasing until reaching a minimal level by the twelve-week. Importantly, no tendon ruptures occurred in this group, and by the twelve-week, the tendon appeared only slightly thickened, suggesting a return to near-normal structure. This observation is relevant with histopathological analysis, where the tendon showed no hemorrhage or inflammatory infiltration, and collagen fibers were arranged in a highly linear manner, reflecting the excellent synergy between PRP and AMNIO in promoting effective tendon healing.

The adhesions in tendons treated with only PRP or AMNIO were significantly higher than those in the PRP+AMNIO group. We hypothesize that the reduced adhesions in the combination group may be due to the protective wrapping of the repaired site by the AMNIO, which could slow the dispersion of PRP, allowing for more localized healing. Histologically, at 4 and 8 weeks, the PRP+AMNIO group exhibited mild hemorrhage, mild linear collagen fiber formation, and mild inflammatory infiltration. By the twelve-week, there was no evidence of hemorrhage or inflammatory infiltration, and the collagen fibers were highly organized. Notably, the PRP+AMNIO group displayed the highest collagen distribution of all groups at 4 weeks, which continued to increase over 8 and 12 weeks, demonstrating the strong synergistic effects of PRP and AMNIO on tendon repair.

However, no previous studies have combined PRP and AMNIO in tendon repair. One study, which combined AMNIO with hyaluronic acid, demonstrated that this combination effectively prevents postoperative adhesions without compromising tendon repair in chicken flexor tendons [42].

This study demonstrates the enhanced effectiveness of two long-established therapeutic approaches, PRP and AMNIO transplantation, when used together. Both methods, long used individually for their regenerative properties, have demonstrated high potential in promoting tendon repair. However, this study uniquely highlights how these two traditional techniques can work in synergy, offering a valuable and efficient solution for improving tendon repair outcomes.

This study has few limitations as it did not include a detailed analysis of rabbit gait post-surgery, which could have provided valuable insights into the loading and functionality of the healing tendon. Also, it lacked tendon diameter measurements as the geometrical analysis of tendon diameter has been shown to be a useful parameter in evaluating tendon repair [43]. The study lacked quantitative evaluation methods, such as biochemical assays for collagen measurement, and objective mechanical assessments of tendon strength. These limitations prevent a comprehensive understanding of the functional recovery achieved with the treatments. Therefore,

future studies with larger sample sizes and more rigorous quantitative and mechanical assessments are needed to validate these results and explore the full therapeutic potential of these treatments.

Conclusion

Combining a-PRP injection and AMNIO wrap seems to be a promising treatment for tendon injuries, as evidenced by a highly significant improvement in collagen deposition, reduced inflammation, and enhancing overall healing quality, particularly at later stages of healing compared with a-PRP or AMNIO alone.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Alexandria University, Egypt (ethics approval number AU0132201111114).

TABLE 1. Macroscopic scoring system for tendon healing, modified from Stoll *et al.* [24].

Variable		Points
Inflammation	Non exist	**1**
	Exist (oedema, swelling, redness)	**0**
Adhesion of tendon to surrounding	Not adnated, slidable	**1**
	Adhesion, not slidable	**0**
Connection of tendon to skin	Not conjoined/slidable	**1**
	Adhesion, not fully slidable	**0**
Color of tendon	Reddish	**0**
	Less reddish	**1**
	Dull white (opaque)	**2**
	Bright white	**3**
Tendon surface at defect area	Intact, smooth	**1**
	Uneven, harsh	**0**
Shape of tendon	Normal	**3**
	Slightly thickened	**2**
	Moderately thickened	**1**
	Intensely thickened	**0**
Level of defect	At the niveau of tendon surface	**1**
	Prominent, above the level of tendon	**0**

TABLE 2. Histological scoring system for tendon healing, modified from Nixon *et al.* [26].

Variable	Score and criterion	
Inflammatory cell infiltrate	**1**	Normal
	2	Slightly increased
	3	Moderately increased
	4	Severely increased
Hemorrhage	**1**	Absent (normal)
	2	Sparse or uneven
	3	Multiple areas
	4	Predominantly hemorrhagic
Linearity of collagen fibers	**1**	Linear
	2	>50% linear
	3	20% to 50% linear
	4	Absence of linear areas

TABLE 3. Coefficient of Variation (CV) for Macroscopic Parameters of Tendon Evaluation

Parameters \ Groups	CTRL	AMNIO	PRP	PRP+AMNIO
Inflammation	86.60%	86.60%	91.65%	33.33%
Adhesion of tendon to surrounding	34.64%	34.64%	65.47%	34.64%
Connection of tendon to skin	21.65%	15.75%	34.64%	15.75%
Color	173.2%	0.000%	86.60%	43.30%
Tendon surface	43.30%	65.47%	57.28%	15.75%
Shape of Tendon	34.64%	56.77%	44.61%	19.92%
Level of defect	100.0%	173.2%	34.64%	15.75%

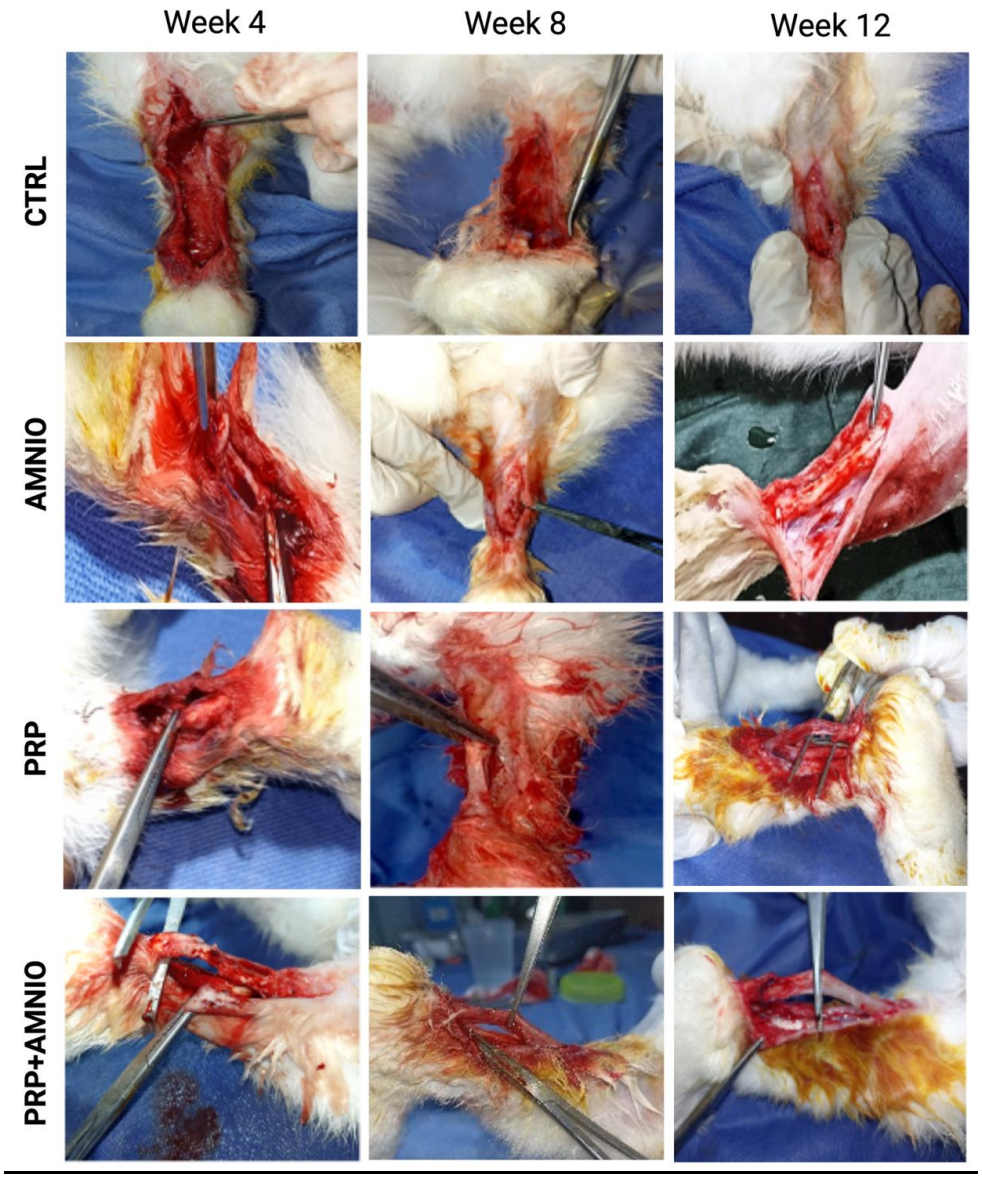

Fig. 1. Macroscopic evaluation of tendons from different treatment groups (CTRL, AMNIO, PRP, and PRP+AMNIO) at various time points (Weeks 4, 8, and 12). The images highlight differences in color and adhesion with surrounding tissues across the treatment groups.

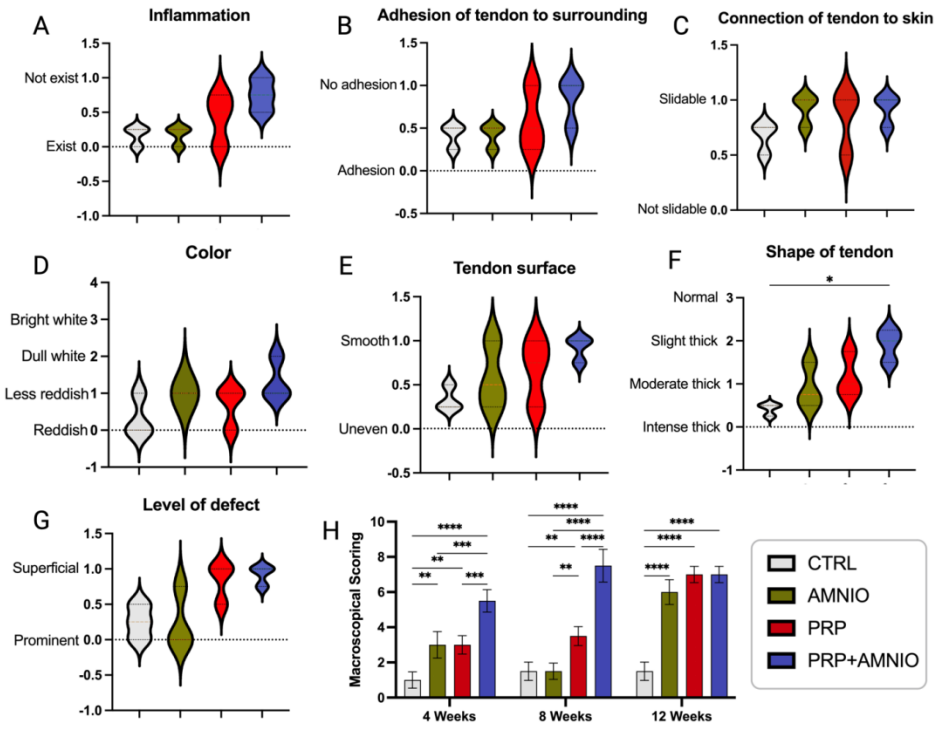

Fig. 2. Macroscopic scoring. Graphs show (A) Inflammation, (B) Adhesion to the surrounding tissue, (C) Connection of tendon to the skin, (D) Color, (E) Tendon surface, (F) Shape of the tendon, and (G) Level of defect. (H) Detailed overall scoring of gross morphology at three time points (Weeks 4, 8, and 12). * indicates P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

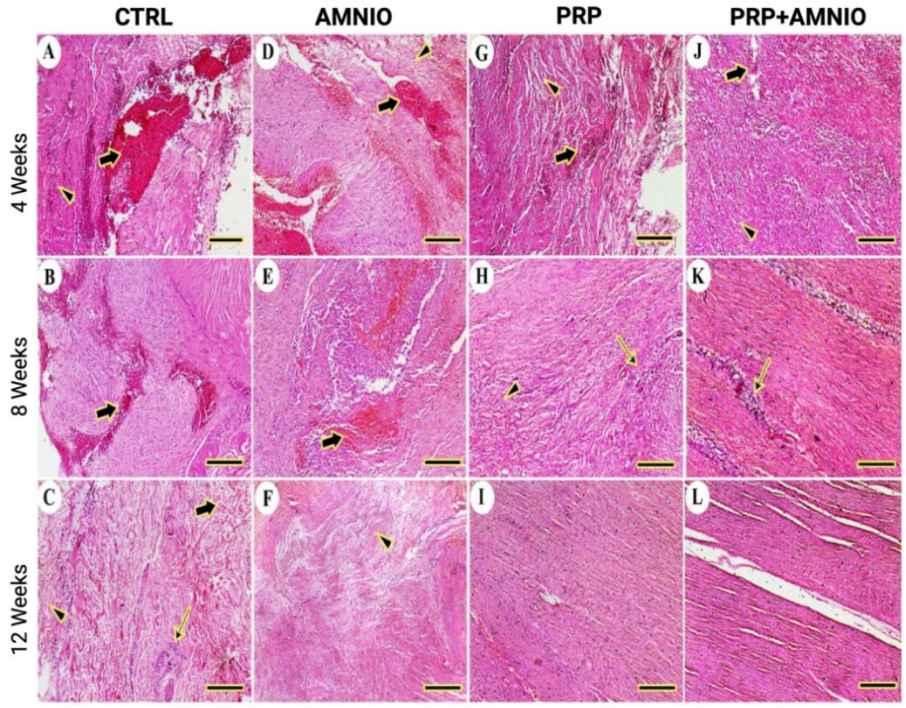

Fig. 3. Histopathological images of rabbit Achilles tendons stained with Hematoxylin and Eosin, illustrating differences between experimental groups. Control group: (A) 4 weeks, (B) 8 weeks, and (C) 12 weeks, showing excessive hemorrhage and infiltration of inflammatory cells. Treated groups: AMNIO group at (D) 4 weeks, (E) 8 weeks, and (F) 12 weeks; PRP group at (G) 4 weeks, (H) 8 weeks, and (I) 12 weeks; and PRP+AMNIO group at (J) 4 weeks, (K) 8 weeks, and (L) 12 weeks. Inflammatory cell infiltration (thin arrows), wavy nonlinear collagen fibers (arrowheads), and hemorrhage (thick arrows) are highlighted. Magnification: x100, Scale bar: 200 µm.

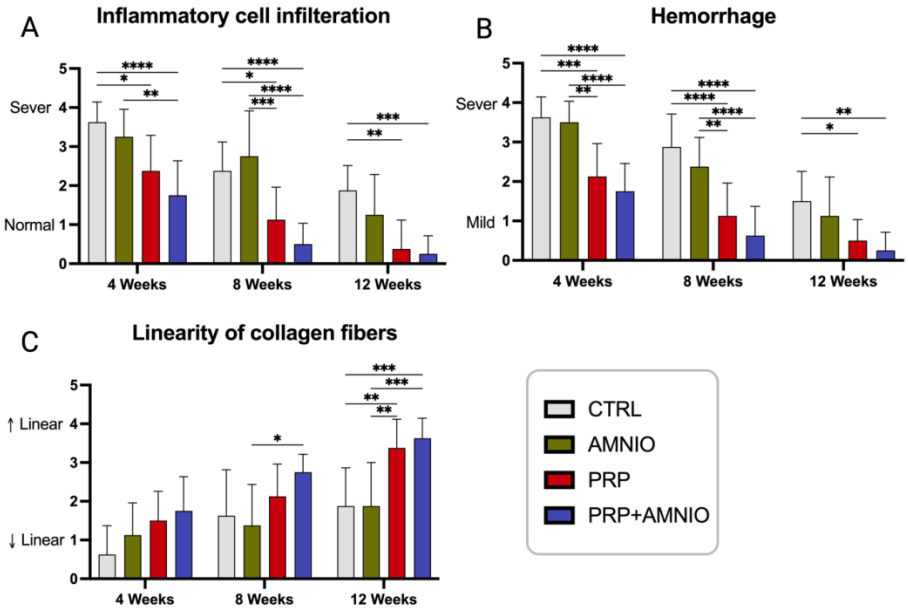

Fig. 4. Histological scoring (H&E staining). A schematic diagram summarizes the results of the combination therapy (PRP+Amnio). Quantitative analysis includes: (A) Hemorrhage, (B) Inflammation, and (C) Linearity of collagen fibers. * indicates P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

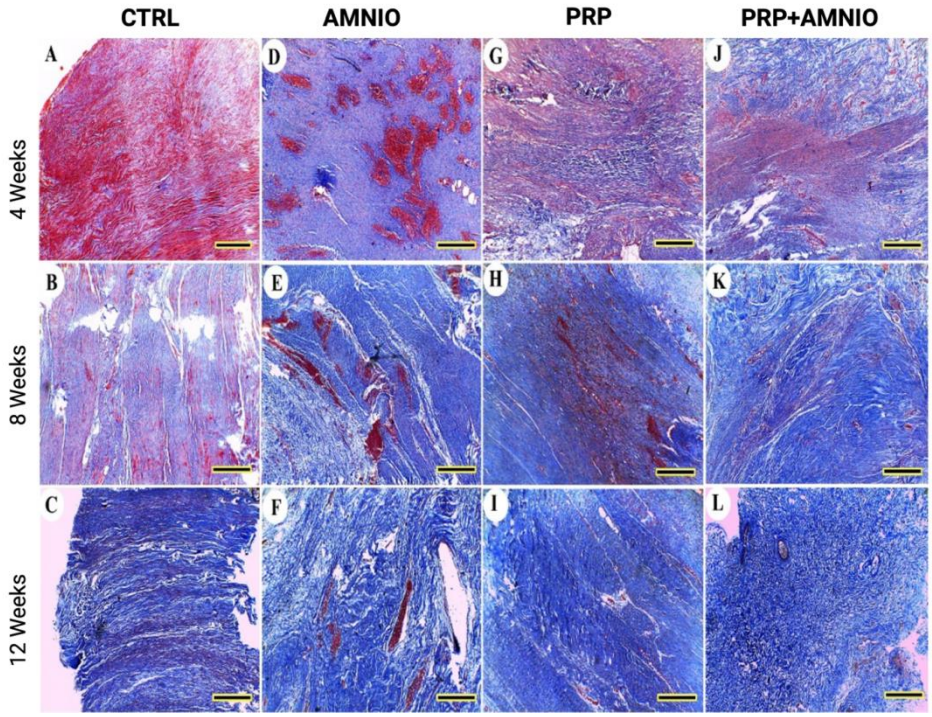

Fig. 5. Histological images of rabbit Achilles tendons stained with Masson's Trichrome, demonstrating collagen deposition across different experimental groups. Control group: (A) 4 weeks, (B) 8 weeks, (C) 12 weeks; AMNIO group: (D) 4 weeks, (E) 8 weeks, (F) 12 weeks; PRP group: (G) 4 weeks, (H) 8 weeks, (I) 12 weeks; PRP+ AMNIO group: (J) 4 weeks, (K) 8 weeks, (L) 12 weeks. Magnification: x100, Scale bar: 200 µm. (Indicate the difference in collagen deposition in the groups).

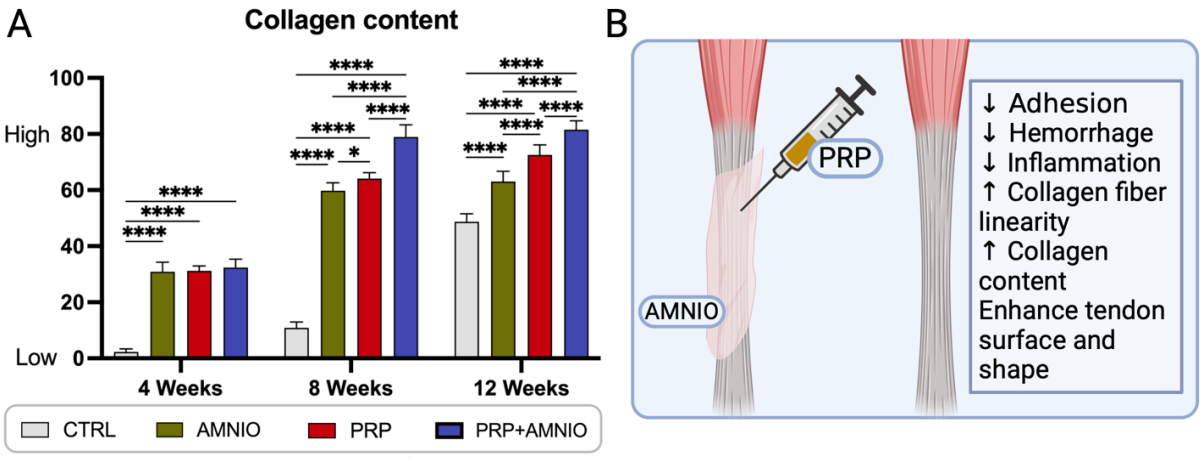

Fig. 6. (A) Quantitative analysis of collagen content in different treatment groups (CTRL, AMNIO, PRP, and PRP+AMNIO) as detected by Masson's Trichrome staining. * indicates P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. (B) A schematic diagram summarizing the results of the combination therapy (PRP + Amnio).

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التأثيرات التآزرية للبلازما الغنية بالصفائح الدموية والغشاء الأمنيوسي على إلتئام الأوتار في نموذج الأرانب

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الملخص

لا يزال ترميم الأوتار يمثل تحديًا سريريًا كبيرًا بسبب عملية الشفاء البطيئة. وفي حين تم استخدام علاجات البلازما الغنية بالصفائح الدموية (PRP) والغشاء الأمنيوسي (AMNIO) كل على حدة لتعزيز تجدد الأوتار، إلا أن تأثيراتهما المشتركة لاتزال غير مستكشفة إلى حد كبير. تم إحداث إصابات في وتر أخيل لدى 48 من ذكور الأرانب النيوزيلندية البيضاء وتم علاجها بأحد أربعة تدخلات: المجموعة الضابطة (CTRL)، حقن البلازما الغنية بالصفائح الدموية (PRP)، غلاف الغشاء الأمنيوسي (AMNIO)، أو توليفة من البلازما الغنية بالصفائح الدموية والغشاء الأمنيوسي (PRP+AMNIO). تم تقييم شفاء الأوتار في الأسابيع 4 و 8 و 12 بعد الجراحة باستخدام التقييم العياني والتحليل النسيجي، بما في ذلك صبغة الهيماتوكسيلين والإيوسين (H&E) وصبغة ماسون ثلاثية الألوان لتقييم ترسب الكولاجين والالتهاب ومورفولوجيا الأنسجة. تم تحليل جميع البيانات إحصائيًا. أظهرت مجموعة PRP+AMNIO تفوقاً في شفاء الأوتار مقارنة بمجموعات CTRL و PRP و AMNIO. كشف التقييم العياني عن تحسن كبير في بنية الوتر في مجموعة PRP+AMNIO، مُظهرًا تحسنًا بمقدار 5.5 و 5 و 4.7 أضعاف مقارنة بالمجموعة الضابطة (CTRL) في الأسابيع 4 و 8 و 12 على التوالي ($p<0.0001$). مقارنة بالمجموعة الضابطة، أظهرت مجموعة PRP+AMNIO انخفاضًا في الالتهاب والنزف، بانخفاض يصل مقداره إلى 7.5 أضعاف ($p<0.0001$). تحسنت استقامة ألياف الكولاجين بشكل معنوي في مجموعة PRP+AMNIO بحلول الأسبوع 12، مع زيادة بمقدار 5.7 أضعاف مقارنة بالمجموعة الضابطة ($p=0.0007$). علاوة على ذلك، أظهرت مجموعة PRP+AMNIO ترسبًا أعلى معنوياً للكولاجين مقارنة بمجموعتي PRP و AMNIO كل على حدة في الأسبوعين 8 و 12 ($p<0.0001$). في الختام، فإن الجمع بين حقن البلازما الغنية بالصفائح الدموية (a-PRP) وغلاف الغشاء الأمنيوسي (AMNIO) يعزز شفاء وتر أخيل في الأرانب من خلال تقليل الالتهاب، وتعزيز ترسب الكولاجين، وتحسين مورفولوجيا الوتر مقارنة بالمجموعة الضابط

الكلمات الدالة: إصابة وتر أخيل، الكولاجين، البلازما الغنية بالصفائح الدموية، الالتهاب، الطب التجديدي.