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Original Article

Nanofat Grafts Enhance Tendon Healing in a Chronic Achilles Tendinopathy Rat Model

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ABSTRACT

Objective: Chronic tendinopathy is a prevalent condition with long recovery times, often necessitating treatments beyond conventional methods like rest and stretching. This study aimed to evaluate the regenerative and anti-inflammatory effects of nanofat and microfat grafts in a collagenase-induced rat model of Achilles tendinopathy.

Materials and Methods: Using collagenase injections, a chronic Achilles tendinopathy model was induced in 27 Wistar albino rats. The rats were randomly divided into three groups: Group 1 received microfat grafts, Group 2 received nanofat grafts, and Group 3 (control) received phosphate-buffered saline (PBS). Nanofat and microfat grafts were prepared from the inguinal fat pads of the rats. Histological and immunohistochemical analyses were performed 12 weeks after treatment.

Results: The nanofat group exhibited significantly lower Modified Verhofstad scores compared to the control group, particularly for fibrosis, polymorphonuclear leukocyte (PMNL) infiltration, edema, collagen, and fibroblast density ($p<0.016$). Both the nanofat and microfat groups demonstrated significantly higher vascularity compared to the control group ($p<0.016$). The nanofat group showed significantly less PMNL infiltration, edema, and greater collagen density than the microfat group ($p<0.016$). Immunohistochemistry revealed the highest immunopositivity for type I and type III collagen in the nanofat group, while the control group showed the lowest levels. CD45 immunoreactivity was most diffuse in the control group and minimal in the nanofat group.

Conclusion: Nanofat, enriched with mesenchymal stem cells and growth factors, significantly improved histopathological outcomes and reduced inflammation compared to microfat and control treatments in a chronic Achilles tendinopathy rat model. These findings suggest that nanofat grafts may offer a minimally invasive and effective treatment for chronic tendinopathy.

Keywords: Achilles tendon, adipose-derived mesenchymal stem cell, microfat, nanofat, regenerative medicine, stem cell treatment, tendinitis, tendinopathy, tendinosis

INTRODUCTION

Tendinopathies, or tendonitis, are relatively common musculoskeletal conditions that typically result from repetitive overuse or forced activity. They mainly present as tenderness and pain in and around the affected tendon. This condition can lead to a loss of work hours/days for productive individuals, particularly those with active lifestyles. It may even result in premature retirement from sports or related professions [1]. Although common, the underlying pathological mechanisms remain poorly understood [2]. As a result, no treatment method has consistently demonstrated efficacy in the current literature [3]. While inflammatory processes play a more prominent role in the early stages of the disease, the chronic phase is marked by an imbalance between collagen production and degradation, particularly in the ratio of type III collagen to type I collagen [1].

The most common treatment methods are rest, strengthening, and stretching exercises [1, 3]. However, the prolonged duration of rest and the intensity of exercises can make it challenging for patients to adhere to treatment. Medical options include steroid injections and nonsteroidal anti-inflammatory drugs (NSAIDs), but these present two significant issues: first, inflammatory processes have not been conclusively proven to be central in the disease mechanism, and second, steroid injections are associated with adverse effects, such as reduced tendon rupture resistance and inhibited collagen synthesis [4, 5]. As a result, new treatment strategies that control pain while preserving tendon integrity are needed [6].

Recent advancements in regenerative medicine have led to experimental treatments such as platelet-enriched plasma (PRP) injections and bone marrow (BMSC) or mesenchymal stem cell (MSC) therapies [7]. PRP has been widely studied as a source of autologous growth factors and immunomodulators, including bFGF, TGF-ß, and BMP-12 [7-9]. Adipose tissue is now recognized as a promising source of MSCs, with adipose-derived stem cells (ASCs) offering advantages over BMSCs due to better accessibility and safety profiles [10]. The composition of fat tissue grafts, including mature adipocytes, MSCs, and growth factors, varies depending on the harvesting and processing methods. Additionally, adipose tissue is a known source of extracellular vesicles or exosomes, which serve as carriers of growth factors or RNA fragments that encode pro-regenerative proteins [11].

In terms of cellular composition, structural fat grafts (unprocessed macro-fat) contain the highest number of cells, predominantly adipocytes. Microfat grafts, which undergo moderate emulsification, contain fewer adipocytes and relatively more MSCs. Nanofat grafts, created through more vigorous emulsification, contain no intact adipocytes but include MSCs and higher concentrations of growth factors, exosomes, chemokines, and cytokines [12]. In summary, less processed fat grafts (such as structural and microfat) are typically used for volume reconstruction, whereas nanofat grafts are primarily employed for regenerative purposes [12].

In this experimental study, we investigated the effectiveness of nanofat grafts compared to control and microfat injection groups in a rat model of Achilles tendinopathy. Specifically, we aimed to determine whether nanofat and microfat injections promoted histological recovery in treating collagenase-induced tendinopathy in rats. We hypothesized that nanofat grafts, with their higher concentrations of mesenchymal stem cells (MSCs) and stromal vascular fraction-derived growth factors, would result in significantly better histological recovery and reduced inflammation compared to microfat grafts and control treatments.

MATERIALS AND METHODS

Animals and Study Design

This experimental study involved 27 adult male Wistar albino rats (8–10-weeks-old), each weighing between 250 and 330 grams. The rats were provided with a standard laboratory diet and had unrestricted access to water. They were housed in individual standard cages in a temperature-controlled environment maintained at 20-22°C. Before the commencement of the experiment, approval was obtained from the Ege University Animal Experiments Local Ethics Committee (approval date/issue: 28.05.2020/055), and the study was conducted at the institution's Center for Experimental Animals. This study was conducted in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines to ensure robust and transparent reporting of animal research [13].

Collagenase-induced Achilles Tendinopathy Model

The chronic tendinopathy model chosen for this study was the chemical method using collagenase, originally developed by Silver et al. for equine models; this model has since been further refined for use in rats with a predictable time course by Orfei et al. [14]. They found that a concentration of 3 mg/mL of collagenase (high dose) and a minimum waiting period of two weeks was required to produce a chronic tendinopathy similar to that seen in humans. We followed this protocol in our study, except they used Sprague-Dawley rats in their experiment, whereas we used Wistar-albino rats.

General anesthesia was administered using a mixture of Ketamine HCl (80-100 mg/kg) and Xylazine HCl (10-12.5 mg/kg). Analgesic and antibiotic prophylaxis, including paracetamol and cefazoline, were provided before the surgical procedure. A 1 cm longitudinal incision was made along the posterior border of the right ankle, and 30 µL of 3 mg/mL type I collagenase (derived from Clostridium histolyticum, Sigma

Aldrich™, St. Louis, MO, USA), diluted with phosphate-buffered saline to a total volume of 1 mL, was injected directly into the tendon using a 26 G needle.

Preparation and Administration of Nanofat and Microfat

On the 14th day post-collagenase injection, the animals were randomly divided into three groups: 1) Group I: microfat, 2) Group II: nanofat, and 3) Group III: control. All rats were anesthetized, scrubbed, and prepared for surgery similarly. The inguinal fat pads were excised for use in the microfat and nanofat groups, while PBS was used in the control group (Fig. 1a). Microfat group: In nine rats, the excised fat pads were minced with surgical scissors and emulsified with phosphate-buffered saline (PBS). The emulsified tissue was passed 20 times through a 2.4 mm Luer-lock cannula to achieve limited thinning. The prepared microfat graft was injected into the right Achilles tendon through the initial posteromedial incision. Nanofat group: The same procedure as the microfat group was followed for another nine rats, except the emulsified tissue was additionally passed 100 times through a smaller 1.2 mm Luer-lock cannula and filtered through a 0.1 mm strainer to prepare the nanofat graft (Fig. 1b). This graft was injected into the right Achilles tendon through the same posteromedial incision (Fig. 1c). Control group: In the control group of nine rats, following the excision of the inguinal fat pads, PBS was injected into the right Achilles tendon through the initial posteromedial incision.

Sacrification and Specimen Harvesting

Twelve weeks after treatment, the rats were euthanized using CO_2, and their right Achilles tendons were resected and preserved in 10% formaldehyde solution (Fig. 1d).

Histopathological and Immunohistochemical Assessments

The preserved tendons were longitudinally embedded in paraffin blocks, and 5 µm thick slices were prepared for histochemical analysis. These slices were stained with hematoxylin & eosin, Alcian blue, and Masson trichrome. Histological assessment of the tendon samples was conducted using the modified Verhofstad scale, which evaluates tissue healing and inflammation across six parameters: 1) Fibrosis, 2) Polymorphonuclear leukocytes (PMNLs), 3) Edema, 4) Fibroblast abundance, 5) Vascularity, and 6) Collagen (Table 1) [15, 16].

The slices were further treated with CD45 and vimentin markers to assess collagen composition (Types I and III), inflammation, and fibroblast proliferation. Each sample was examined by two independent histologists, who were randomly assigned and worked in a double-blinded manner using Olympus™ BX51 light microscopy (Orinpasu Kabushiki-kaisha, Shinjuku, Tokyo, Japan) and photographed with an Olympus™ C5050 camera. The summary of the study protocol is presented in Figure 2.

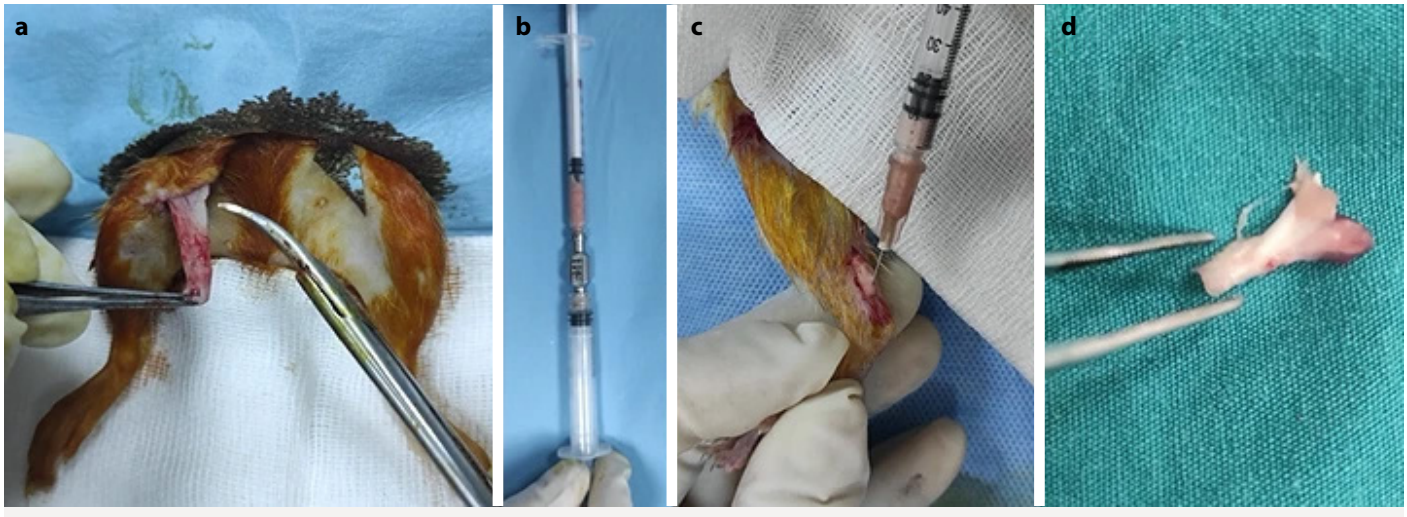

Figure 1. (a) Excision of Inguinal Fat Pads: Inguinal fat pads are excised from the rats as the source material for microfat and nanofat graft preparation. **(b)** Emulsification and Extraction Process: The excised fat tissue is passed through a Luer-lock cannula. Microfat grafts are prepared by passing the slashed tissue 20 times through a 2.4 mm cannula. For nanofat grafts, an additional 100 passages are made through a 1.2 mm cannula, followed by filtration through a 1 mm strainer to refine the grafts. **(c)** Nanofat Administration: The prepared nanofat graft is administered to the Achilles tendon via a posteromedial incision. **(d)** Resected Achilles Tendon: Post-treatment, the Achilles tendons are resected for histological and immunohistochemical analysis.

Table 1. Modified Verhofstad scale

Fibrosis	PMNs	Edema	Collagen density	Vascularity	Fibroblast proliferation
None	Normal	None	None	None	None
Superficial	Light	Light	Light	Light	Light
Pronounced	Pronounced	Pronounced	Pronounced	Pronounced	Pronounced
Massive	Massive	Dense	Dense	Dense	Dense

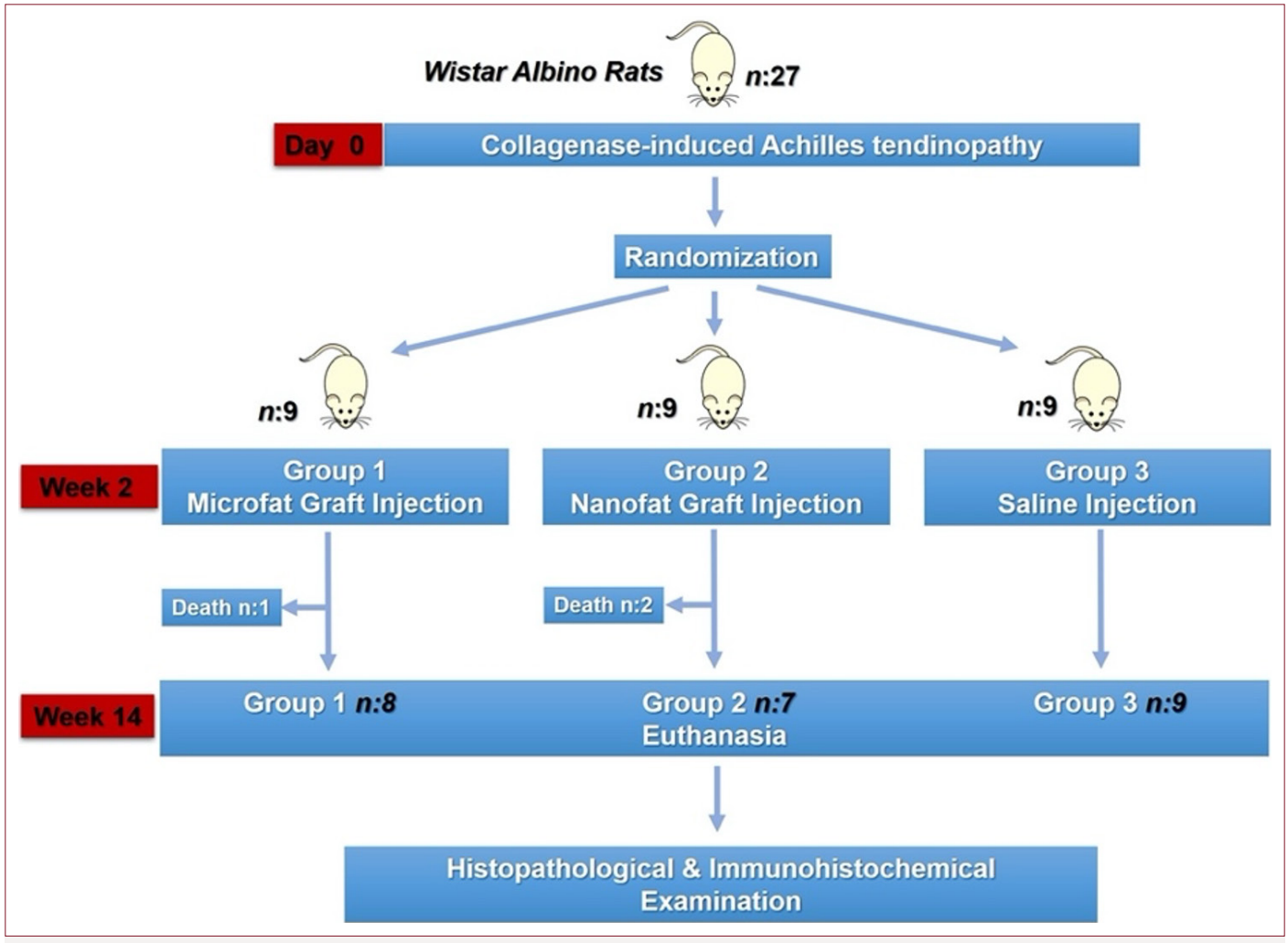

Figure 2. Flowchart of the study design.

Electron Microscopy

Scanning electron microscopy (SEM) was used to examine the ultrastructural composition of the fat grafts. Samples from both the nanofat and microfat preparations (one sample from each) were fixed with glutaraldehyde, dehydrated with alcohol and alcohol/hexamethyldisilazane, according to the Ege University Central Research Test and Analysis Laboratory Protocol. The samples were then coated with a 9 nm gold/palladium (Au/Pd) layer and examined using a Thermo Scientific/Apreo S™ scanning electron microscope.

Statistical Analysis

Statistical analyses were conducted using SPSS™ version 25 software (IBM Inc., Armonk, NY, USA). Due to the ordinal nature of the data and the inability to assume normal distribution,

non-parametric tests were used. Descriptive statistics, including median values and interquartile ranges (IQRs), were calculated for each group to summarize central tendencies and variability. For intergroup comparisons, the Kruskal-Wallis test was employed. This non-parametric method was selected as it is appropriate for comparing multiple independent groups when the data are not normally distributed. Post-hoc pairwise comparisons were conducted using the Mann-Whitney U test with Bonferroni correction to adjust for multiple comparisons. The corrected P-value threshold was determined by dividing the initial significance level (0.05) by the number of comparisons made.

RESULTS

Three rats, one from the microfat group and two from the nanofat group, died before the sacrifice. Additionally, two animals experienced bilateral wound dehiscence, and one had unilateral wound dehiscence at the inguinal incision site. A donor site hematoma was observed in one subject, which was promptly evacuated using a syringe. No other complications were encountered throughout the study.

Histopathological Findings

Qualitative Findings

The paratenon, a loose connective tissue surrounding the tendon proper, was identified in all specimens. Vascular elements, adipose cells, connective tissue cells, and the connective tissue matrix were observed within this paratenon. In the control group (Group 3), the blood vessels were notably dilated, and the paratenon area relative to the tendon was more prominent (Fig. 3).

A fibrotic region, likely corresponding to the injection site, was distinguishable in the control group, with signs of inflammation present within the collagen fibers. In the microfat group (Group 1), there was a slight reduction in edema within the collagen fibers, along with some polymorphonuclear leukocyte (PMNL) activity. In contrast, the nanofat group (Group 2) exhibited markedly reduced signs of inflammation, with minimal PMNL activity compared to the control and microfat groups.

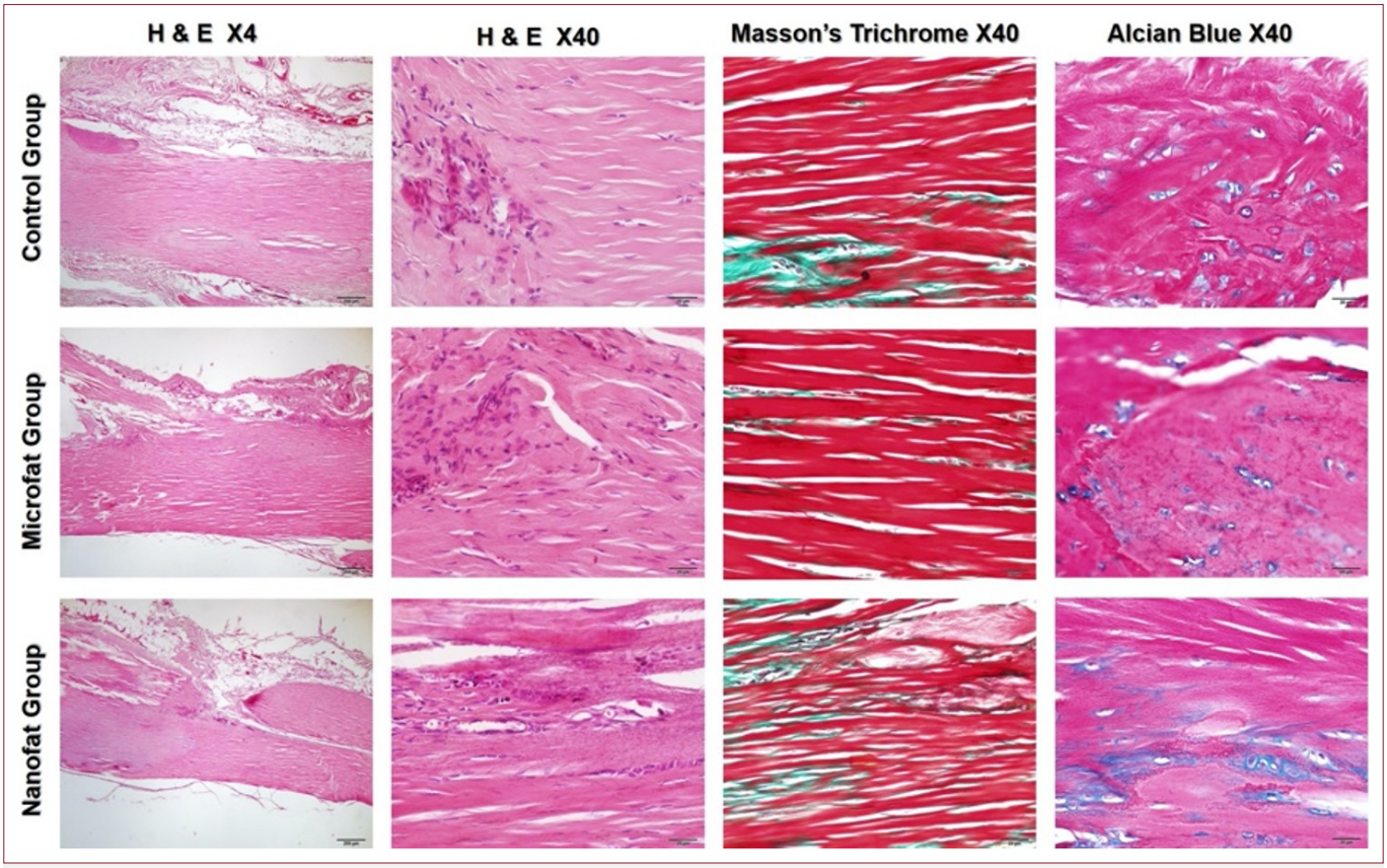

Figure 3. The appearance of the H&E, Masson trichrome and Alcian blue-stained preparations of the groups. The organization of the collagen fibers is visibly more uniform in the nanofat group, as well as the cellularity is markedly less predominant in the nanofat group compared to microfat and control groups.

Alcian blue staining of the specimens showed a tendency for fibroblast cells in all groups to be replaced by cells resembling chondrocytes, surrounded by a media similar to interterritorial matrix. In the control group, these cells were organized into large chondron-like structures, synthesizing a substantial amount of matrix. The organization was less pronounced in the microfat group, with the chondral cells appearing smaller. In the nanofat group, the synthesized matrix was scattered within the collagen fibers, and fewer chondral cells were observed (Fig. 3).

Quantitative Findings

The modified Verhofstad scores for the assessed specimens are provided in Table 2, with median values and interquartile ranges (IQR) shown in Table 3. Significant differences in the median Verhofstad scores were observed between the groups for all six parameters. Post-hoc analyses revealed significant differences between the microfat and nanofat groups in terms of PMNL infiltration (nanofat median: 1, IQR: 1-1; microfat median: 2, IQR: 2-3; p=0.015), tissue edema (nanofat median: 1, IQR: 1-1; microfat median: 2, IQR: 2-3; p=0.003), and collagen density (nanofat median: 1, IQR: 0-1; microfat median: 2, IQR: 2-3; p=0.003).

Immunohistochemical Findings

Type I collagen: In the control group, anti-type I collagen staining revealed low to medium immunoreactivity levels. In the microfat group, immunoreactivity was medium to high, while the nanofat group exhibited the highest levels (Fig. 4). Type III collagen: The highest level of immunoreactivity for

Table 2. The modified Verhofstad scores of the assessed specimens

	Fibrosis	PMNs infiltration	Edema	Collagen density	Vascularity	Fibroblast proliferation
Group 1 Control						
Sample 1	3	3	2	3	1	2
Sample 2	2	3	3	3	1	2
Sample 3	2	2	3	2	1	2
Sample 4	2	2	2	2	2	2
Sample 5	3	2	2	3	1	3
Sample 6	3	3	3	2	0	3
Sample 7	2	2	3	2	1	2
Sample 8	2	3	2	3	1	3
Sample 9	3	3	3	3	1	2
Group 2 Microfat						
Sample 1	2	2	1	2	2	2
Sample 2	3	3	2	2	2	2
Sample 3	2	3	3	1	3	1
Sample 4	2	2	2	2	2	2
Sample 5	1	1	3	3	3	2
Sample 6	1	1	2	3	2	2
Sample 7	2	2	3	1	2	1
Sample 8	1	2	2	2	1	1
Group 3 Nanofat						
Sample 1	1	1	1	1	2	1
Sample 2	1	0	1	0	3	1
Sample 3	2	0	2	1	3	1
Sample 4	2	2	1	1	2	1
Sample 5	1	1	1	0	2	2
Sample 6	0	1	0	1	3	1
Sample 7	1	1	1	0	3	2

Table 3. Median values and interquartile ranges for the groups

	Fibrosis	PMNs infiltration	Edema	Collagen density	Vascularity	Fibroblast proliferation
Control	2 (2-3)	3 (2-3)	3 (2-3)	3 (2-3)	1 (1-1)	2 (2-3)
Microfat	2 (1-2)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	2 (1-2)
Nanofat	1 (1-2)	1 (1-1)	1 (1-1)	1 (0-1)	3 (2-3)	1 (1-2)
P_1	>0.016	>0.016	>0.016	>0.016	**0.003**	>0.016
P_2	**0.002**	**<0.001**	**0.008**	**<0.001**	**<0.001**	**<0.001**
P_3	>0.016	**0.015**	**0.003**	**0.003**	>0.016	>0.016

A Kruskal-Wallis test was used for multiple group comparisons, and Mann-Whitney U test was employed for pairwise post-hoc analyses with Bonferroni correction. P_1 represents the comparisons between the P_1 control group and the microfat group, P_2 denotes the comparisons between the P_2 control group and the nanofat group, and P_3 illustrates the comparisons between the microfat and nanofat groups.

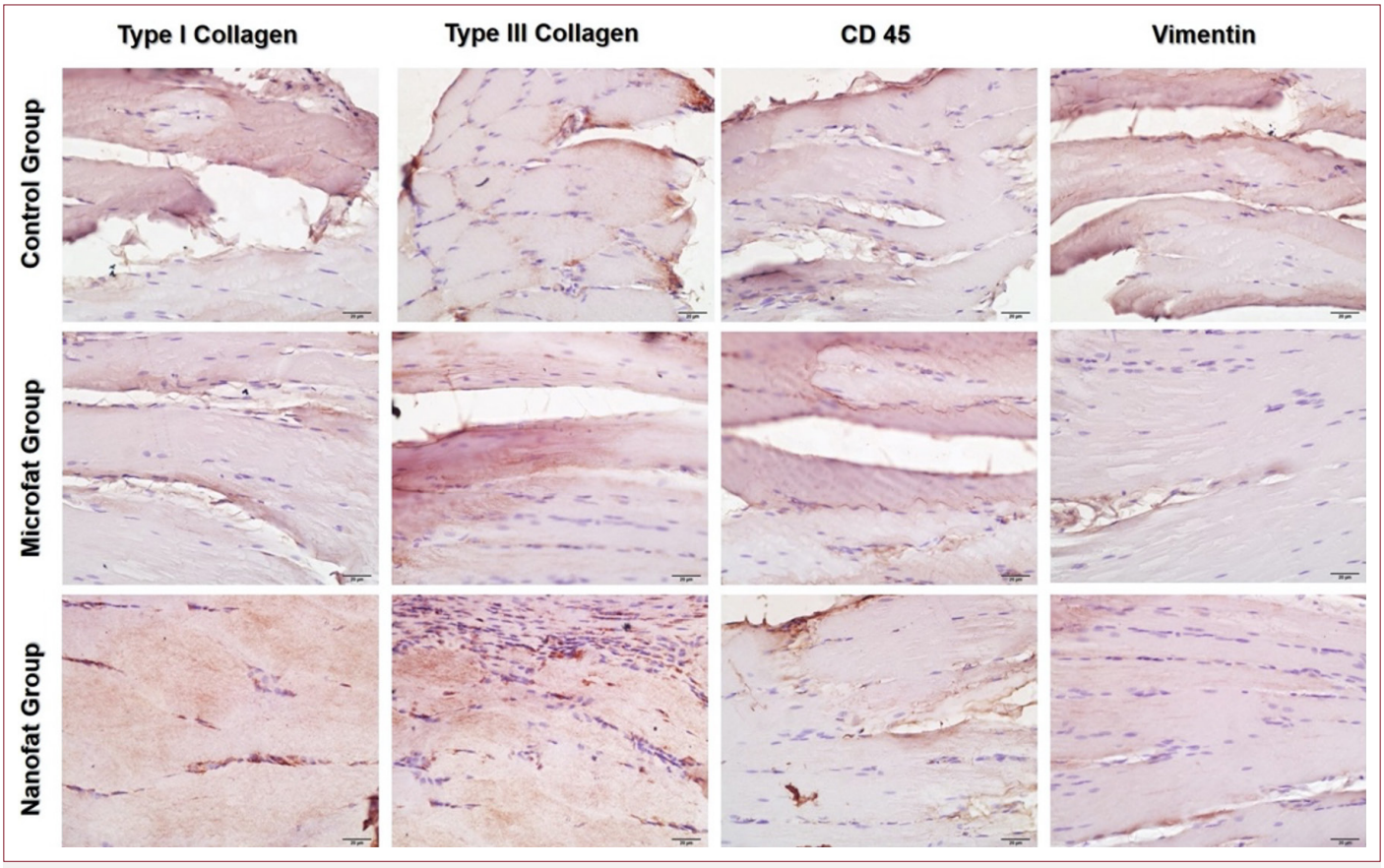

Figure 4. The appearance of the preparations stained with anti-type I collagen, anti-type III collagen, anti-CD45 and anti-vimentin primary antibodies of the experimental groups.

type III collagen was observed in the nanofat group, with the lowest levels found in the control group (Fig. 4). CD45 antibodies: CD45 immunoreactivity corresponded with the H&E staining. The control group showed the most diffuse staining, while the microfat group demonstrated focal dye retention. In contrast, immunoreactivity was scarce in the nanofat group (Fig. 4). Vimentin: The mesodermal cell marker vimentin was most prominent in the control group, while it was found at modest levels in both the microfat and nanofat groups (Fig. 4).

Electron Microscopic Findings

In the electron microscopy examination, intact adipocyte globules with diameters up to 50 micrometers, along with the matrix scaffold, were observed in the microfat sample. In contrast, the nanofat sample showed the presence of extracellular matrix, stromal vascular fraction (SVF), and extracellular vesicles with diameters around 2 micrometers (2000 nanometers) or smaller (Fig. 5).

DISCUSSION

In this study, we demonstrated that nanofat grafts significantly improved healing and reduced inflammation compared to microfat grafts and controls in a collagenase-induced rat model of chronic Achilles tendinopathy. Nanofat, with its higher concentration of mesenchymal stem cells (MSCs) and growth factors from the stromal vascular fraction, resulted in lower levels of polymorphonuclear leukocyte (PMNL) infiltration, reduced tissue edema, and increased collagen density, indicating a more robust regenerative response. These findings support the hypothesis that nanofat, with its superior regenerative and anti-inflammatory properties, may be a more effective treatment for chronic tendinopathy than microfat or standard interventions. Additionally, the higher immunoreactivity for type I and III collagen observed in the nanofat group further underscores its potential to enhance tendon healing at the molecular level.

The use of fat grafts as both structural and biological fillers, along with the discovery of their regenerative properties, marked a significant advancement in regenerative medicine. This breakthrough was largely driven by the identification of adipose-derived stem cells (ADSCs) in 2001, which opened new avenues for fat graft applications beyond traditional plastic surgery [10, 17-19]. Today, adipose-derived stem cells are used in various fields, including cardiology for cardiomyoblast formation, neurology for neural cell development, and orthopedics for treating conditions like arthritis [18, 19].

In particular, the regenerative potential of microfat grafts, which contain pluripotent stem cells and stromal vascular cells (SVC) alongside mature adipocytes, has been widely recognized. Unlike traditional fat grafts, which are primarily used for volumizing, microfat grafts emphasize their regenerative capacity, making them suitable for a range of treatments, particularly in the facial region [20]. Tonnard et al. [21] introduced further refinement of this technique by mechanically emulsifying fat grafts, resulting in nanofat, a preparation that lacks mature adipocytes but retains endothelial cells, macrophages, mast cells, stromal vascular fraction (SVF), and adipose-derived stem cells (ASCs). This preparation is highly regenerative and can be injected through fine needles, serving as a potent regenerative tool rather than a volumizing agent [22].

Our study's findings align with this regenerative potential, as the nanofat group demonstrated significantly improved histopathological tendon healing compared to both the control and microfat groups. Furthermore, the reduced presence of hyaline cartilage degeneration around the Achilles tendon—an avascular region—was most prominent in the nanofat group, likely due to the enhanced vascularity induced

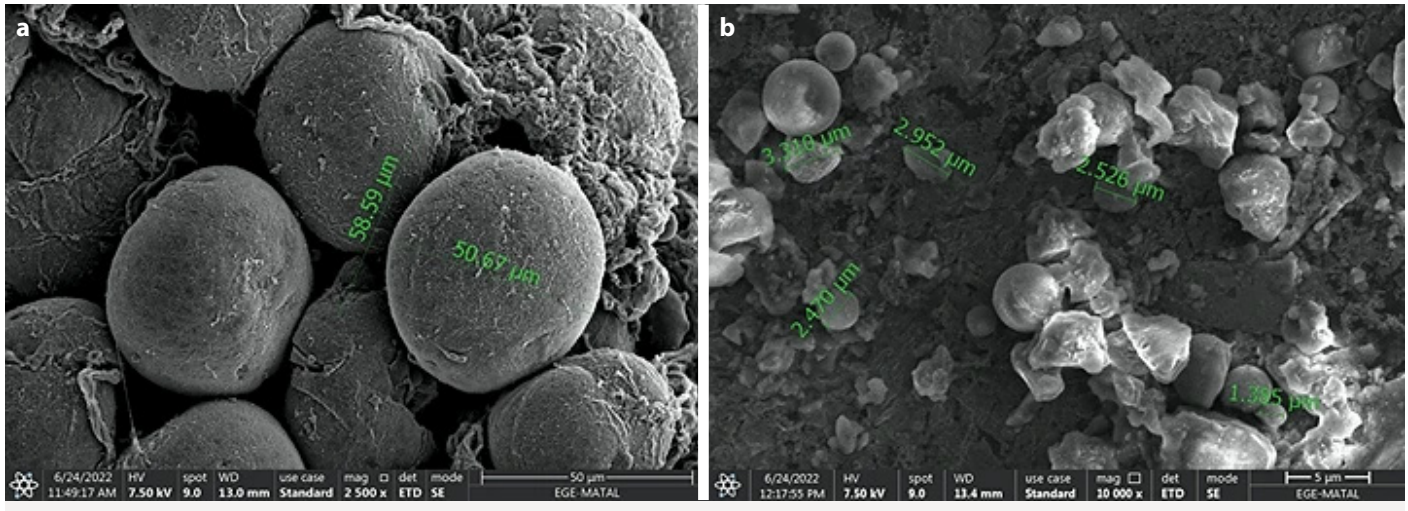

Figure 5. Scanning Electron Microscopic view of the; **(a)** microfat (to scale, magnification is 2500x), **(b)** nanofat samples (magnification is 10 000x). In microfat samples, the cellular and extracellular structures are preserved, while in the nanofat group, no cellular component can be identified (the diameter of the cell-like spheres are smaller than the smallest resident cell in the mesenchyme).

by mesenchymal stem cells (MSCs) and growth factors from the stromal vascular fraction. The increased dominance of type I collagen in the nanofat group, a key factor in tendon mechanical strength, further supports the regenerative advantages of nanofat in tendon healing.

Electron microscopy findings in our study were consistent with existing literature [23]. While microfat preparations contained scattered mature adipocytes and fat globules, nanofat preparations were predominantly composed of extracellular matrix, mesenchymal stem cells, and smaller extracellular vesicles such as exosomes [24]. Unlike previous applications of structural and cultured fat grafts in treating foot-related issues, nanofat grafts function primarily as biological regenerative agents rather than providing structural support [25].

Chronic tendinopathies, which affect a significant portion of the physically active population, including athletes and the elderly, can severely limit daily activities and performance. As societies increasingly embrace physical activity to combat health issues like cardiovascular disease and diabetes, finding effective treatments for tendinopathies is crucial. Traditional methods, such as steroid and non-steroidal anti-inflammatory drugs, provide short-term relief but are ineffective in addressing the chronic imbalance between collagen production and degradation, which is at the core of the condition. Newer therapies have yielded inconsistent or unproven results, including low-dose laser treatments, iontophoresis, and extracorporeal shock waves. Surgical options, while available, are often a last resort due to mixed outcomes. Given these challenges, our study's findings suggest that nanofat grafts may offer a promising, minimally invasive alternative for treating chronic tendinopathies by promoting tissue regeneration and reducing inflammation. This approach could potentially improve the quality of life for individuals struggling with chronic tendon conditions, enabling them to maintain an active lifestyle without the limitations imposed by traditional therapies.

Several limitations of this study should be acknowledged. First, using an animal model, specifically rats, poses inherent challenges in directly extrapolating the findings to human clinical scenarios despite the similarities in tendon healing mechanisms between mammals. Although animal models are widely used in preclinical studies to explore biological processes and treatment efficacy, physiological and anatomical differences may affect the translatability of the results to human tendinopathies. Second, the study did not include biomechanical testing to assess tendon endurance and functional recovery post-treatment. While the histopathological findings provide valuable insights into the healing process, the lack of biomechanical data limits the ability to evaluate the functional improvements in tendon strength and durability, which are critical for clinical applications. Third, the exact genetic and immunological composition of the nanofat and microfat grafts used in this study was not fully documented. Although both graft types were prepared using established techniques, the absence of detailed analysis on the specific cellular and molecular components limits the ability to understand the regenerative mechanisms involved fully. A more comprehensive characterization of the grafts, including the specific concentrations of growth factors, cytokines, and stromal vascular fraction cells, would enhance the understanding of their therapeutic potential. Finally, the study duration of 12 weeks, while sufficient to observe significant histological changes, may not capture the long-term effects and sustainability of the treatments. A longer follow-up period would be necessary to evaluate the potential for lasting tendon regeneration and functional recovery.

CONCLUSION

In conclusion, this study demonstrated that nanofat grafts, rich in mesenchymal stem cells, exosomes, and growth factors, significantly improved tendon healing and reduced inflammation compared to microfat grafts and control treatments in a collagenase-induced rat model of chronic Achilles tendinopathy. Nanofat applications resulted in superior histopathological outcomes, including reduced fibrosis, edema, and polymorphonuclear leukocyte infiltration, as well as increased collagen density and vascularity, which likely contributed to better tendon regeneration. The presence of a higher proportion of type I collagen in the nanofat group further supports its potential for enhancing tendon mechanical strength. While these findings highlight nanofat's regenerative capabilities, the study's limitations, including the use of an animal model and the absence of biomechanical testing, must be considered when extrapolating these results to clinical practice. Future research should focus on biomechanical assessments and long-term follow-up in human studies to better understand the clinical applicability of nanofat grafts in treating chronic tendinopathies.

DECLARATIONS

Ethics Committee Approval: The Ege University Animal Experiments Local Ethics Committee granted approval for this study (date: 28.05.2020, number: 2020-055).

Author Contributions: Idea/Concept – AB, YU, EKB; Design – AB, YU, EKB; Control/Supervision – AB, EKB; Data Collection and/or Processing – AB, KK, OFD, CT, YU, EKB; Analysis and/or Interpretation – AB, YU, EKB; Literature review – AB, KK, OFD, EKB; Writing – AB, KK, CT, YU, EKB; Critical Review – AB, YU, EKB; References and fundings – AB, YU, EKB; Materials – AB, KK, OFD, CT, YU, EKB.

Data Avaliability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest: The authors declared no conflict of interest.

Informed Consent: Not applicable.

Use of AI for Writing Assistance: The authors declared that they had not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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ABBREVIATIONS

MSC - Mesenchymal Stem Cell

PBS - Phosphate-Buffered Saline

PMNL - Polymorphonuclear Leukocyte

PRP - Platelet-Rich Plasma

BMSC - Bone Marrow Stem Cell

ASC - Adipose-Derived Stem Cell

SEM - Scanning Electron Microscopy

SVF - Stromal Vascular Fraction

NSAID - Nonsteroidal Anti-Inflammatory Drug

ARRIVE - Animal Research: Reporting of In Vivo Experiments

H&E - Hematoxylin & Eosin

TGF-ß - Transforming Growth Factor-Beta

bFGF - Basic Fibroblast Growth Factor

BMP-12 - Bone Morphogenetic Protein 12

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