

A new decellularized tendon scaffold for rotator cuff tear – Evaluation in Rabbits

Alex de Lima Santos (✉ alexdel@gmail.com)

Universidade Federal de Sao Paulo <https://orcid.org/0000-0002-4063-672X>

Camila Gonzaga da Silva

Universidade Federal de Sao Paulo

Leticia Siqueira de Sá Barreto

Universidade Federal de Sao Paulo

Katia Ramos Moreira Leite

Universidade de Sao Paulo

Marcel Jun Sugawara Tamaoki

Universidade Federal de Sao Paulo

Lydia Massako Ferreira

Universidade Federal de Sao Paulo

Fernando Gonçalves de Almeida

Universidade Federal de Sao Paulo

Flavio Faloppa

Universidade Federal de Sao Paulo

Research article

Keywords: Scaffold, Tissue Engineering, Extracellular Matrix, Decellularization, Tendon

Posted Date: December 1st, 2019

DOI: <https://doi.org/10.21203/rs.2.17861/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background Scaffolds have shown considerable progress in recent years. In orthopaedic surgery, scaffolds have been used as grafts in procedures involving tendon and ligament reconstruction. This paper aims to produce and evaluate decellularized tendon scaffolds from biomechanical, microscopic, macroscopic and in vivo perspectives.

Methods Bilateral gastrocnemius muscle tendons from 18 (eighteen) adult New Zealand rabbits were collected. Of these 36 (thirty-six) tendons, 11 (eleven) were used as controls (Group A), and 25 (twenty-five) were used in the decellularization protocol (Group B). The groups were subjected to histological, biomechanical and macroscopic analyses, and group B was subjected to an additional in vivo evaluation. Regarding the decellularization protocol, we used a combination of aprotinin, EDTA (ethylenediamine tetraacetic acid, 0,1% w/v), SDS (sodium dodecyl sulfate) and Triton X-100 (t-octylphenoxy polyethoxy ethanol) for six days. During the six days, the scaffold was kept at room temperature on a shaker with constant rotation.

Results The decellularized tendon scaffold showed an increased cross-sectional area and inter-fascicular distance, without changing parallelism or matrix organization. The nuclear material was not organized in the decellularized tendon scaffolds, as it was in the control. In the biomechanical analysis, a significant difference was not found between the groups after analysis of ultimate tensile load, stiffness, and elongation at the ultimate tensile load. During the in vivo evaluation, infiltration of mononuclear cells was noted.

Conclusions The evaluated decellularization protocol efficiently made a decellularized tendon scaffold, maintained the most important biomechanical characteristics and permitted cell infiltration.

1. Background

Musculoskeletal soft tissue injuries include those of tendons, ligaments, cartilage, and muscles. These injuries are very common, particularly in sports-related injuries [1]. The importance of these cases has increased progressively, and these injuries are commonly associated with a high socioeconomic cost and prolonged loss of work [2–4].

The treatment for these injuries ranges from “wait-and-see” approaches to surgery; normally, the treatment choice is made according to the type of injury [1]. For the surgical approach, principally tendon and ligament reconstruction, the use of a scaffold is necessary. An ideal scaffold for musculoskeletal injuries is (a) three-dimensional in structure with high porosity; (b) devoid of cellular material to minimize inflammatory potential, disease transmission, and host immune response; (c) cytocompatible; and (d) of sufficient biomechanical capacity to withstand rehabilitation until complete remodelling has occurred [5, 6].

Scaffolds are classified as synthetic or biological. Synthetic scaffolds are generally polymers that may permit satisfactory tissue integration and facilitate cell growth. In orthopaedic practice, poly-urethaneurea (SportMesh®), poly-L-lactide (X-repair®) and polytetrafluoroethylene (Gore-Tex®) have been approved by the FDA (Food and Drug Administration) and have been used in the USA [5, 7].

Biological scaffolds are tissues from humans or animals and function as supports that influence cell growth [8]. Biological scaffolds are classified according to the origin: autografts (donor and recipient are the same subject), allografts (donor and recipient are different subjects of the same species) and xenografts (donor and recipient are subjects of different species) [9]. Autografts are most commonly used; however, the limited availability of the dimension and sites of harvest is resulting in the use of different scaffolds in studies [9]. Allografts are an available option, but the potential chronic immune reaction and potential risk of disease transmission are limitation of this scaffold [10]. Finally, animal-derived xenografts have been associated with immune reactions, resulting in inflammation [10].

One approach to eliminate the risk of disease transmission, immune reactions and to improve the availability of scaffolds is the decellularization process [10]. Decellularized scaffolds are prepared from different protocols that include a combination of physical, chemical and enzymatic techniques [11–13]. Recent studies have not indicated a statistically significant difference among protocols that include a combination of SDS (sodium dodecyl sulfate), Triton X-100 (t-octyl-phenoxyethoxyethanol) and TnBP (1% tri-n-butyl phosphate) [14].

In the literature, grafting has been described, and the results have been mixed. In a recent systematic review, lower retear rates and higher scores were found for patients who received rotator cuff repair plus scaffolds. In this study, the author found the best results with autograft, allograft and synthetic scaffolds [15]. However, the xenografts showed the worst results in terms of rotator cuff repair [15]. Some years ago, a randomized clinical trial was terminated due to the presence of several local inflammatory reactions in the group with rotator cuff repair plus xenografts (Restore Orthobiologic Implant) [16].

In this study, we hypothesized that the decellularization protocol for tendons would be able to produce acellular tissue with appropriate biomechanical characteristics that could be used in allograft transplantation. This paper aims to produce and evaluate a decellularized tendon scaffold from biomechanical, microscopic, macroscopic and in vivo perspectives.

2. Methods

2.1. Animal grouping

The bilateral gastrocnemius muscle tendons were collected from 18 (eighteen) New Zealand rabbits weighing 3–3,5 kg, provided of a farm company (Granja R. G.). Of these 36 (thirty-six) tendons, 11 (eleven) were used as controls and 25 (twenty-five) were used in the decellularization protocol. Among the 11 (eleven) tendons used as controls, 4 (four) were subjected to histological and 7 (seven) to biomechanical evaluation. Of the 25 (twenty-five) tendons subjected to the decellularization protocol, 4

(four) underwent histological evaluation, 13 (thirteen) underwent biomechanical evaluation, and 8 (eight) were used for the in vivo analysis (Fig. 1). We used 8 (eight) additional rabbits for the in vivo analysis. The study was approved by the Institutional Review Board, and experiments were carried out according to the ARRIVE guidelines.

2.2. Gastrocnemius tendon harvest

Tendons were collected from the rabbits (3–3,5 kg) following the administration of anaesthesia and euthanasia with an anaesthetic overdose (ketamine 200 mg/kg + xylazine 40 mg/kg and tramadol 10 mg/kg). Before the anaesthesia and euthanasia the animals were kept in individual cages with a light-dark cycle of 12/12 hours and with food and water available in ad libitum. This study was conducted in accordance with the recommendations for the care and use of laboratory animals.

The leg was cleaned and disinfected using aseptic techniques. With the use of an aseptic technique, a longitudinal medial posterior skin incision was made directly over the flexor digitorum superficialis [17]. The gastrocnemius tendon was carefully dissected from the flexor digitorum superficialis and soleus tendons. A complete transverse laceration was made with a surgical blade through the distal bone insertion and proximal to the muscle-tendon transition. All the tendons were removed and stored at 4 °C in PBS (phosphate-buffered saline) with 1% penicillin/streptomycin, for the decellularization protocol and biomechanical evaluation of the control or with 10% formaldehyde for the histological evaluation of the control.

2.3. Preparation of decellularized gastrocnemius tendon scaffolds

Immediately after harvest, the gastrocnemius tendons were transferred under aseptic conditions to individual tubes. Our decellularization protocol had a duration of 6 (six) days, with daily changes in the decellularization agents, under aseptic conditions. During the decellularization protocol, the individual tube was placed onto a rotating shaker (MaxQ4000, Thermo Scientific, Waltham, MA, USA) at 200 rpm and at room temperature.

In the first step of the decellularization treatment, the tendons were washed with PBS for 60 min and then incubated in a solution containing 0.1% EDTA (ethylenediamine tetraacetic acid, 0,1% w/v) and aprotinin (10K IU/mg) for the next 24 hours (Figs. 2 and 3).

Following the decellularization protocol, the tendon was washed with PBS for 60 min and then incubated with 0.5% SDS (sodium dodecyl sulfate, Invitrogen, Carlsbad, CA, EUA) for 24 hours. On the third day, the washing procedure was repeated with PBS, and the tendon was incubated in a solution of 1% Triton X-100 (t-octyl-phenoxyethoxyethanol, Affymetrix, Maumme, Ohio) for 24 hours (Figs. 2 and 3).

On the fourth and fifth day of the protocol, the tendon was washed daily and maintained with constant agitation in PBS with 1% penicillin/streptomycin. On the sixth day, the scaffold was placed in PBS + 1%

penicillin/streptomycin and stored at 4 °C.

2.4. Biomechanics – Tensile testing

The decellularized tendon scaffold - group B (n:13) and the control - group A (n:7) were removed from storage, and the length and the cross-sectional area were measured. The cross-sectional area was measured in the middle of the scaffold or in the control. This measurement was performed with a manual decimal calliper.

The scaffolds or the controls were fixed with a customized jig system consisting of metal cages with adjustable pins. The specimen was mounted onto a machine for testing (AME equipment – 2KN/ Oswaldo Filizola) and preloaded at a predefined distance of 10 mm. The specimen was then loaded to failure at a constant speed of 10 mm/min until complete rupture. The data collected from DynaView Standard M Software were used to compute the material and structural properties of each specimen [18].

We documented the ultimate tensile load, the elongation at the ultimate tensile load and the cross-sectional area. We evaluated the results of the elastic modulus, ultimate tensile stress, and stiffness.

2.5. Histological Analysis

Mid-substance portions of the specimen were placed in 10% phosphate-buffered formalin at room temperature for 6 hours. The specimens were embedded in paraffin and microtomed to obtain transverse sections of 3 μ m thickness. The sections were mounted on slides and stained using haematoxylin and eosin (H & E), Masson's trichrome and DAPI (4,6-diamidino-2-phenylindole, Invitrogen, Carlsbad, CA, EUA).

The histological sections were analysed by using an Olympus IX 81 fluorescence optical microscope, and the images were taken with an Olympus DP72 camera. The histological evaluation was based on the presence and organization of nuclear components as well as the organization of the scaffold architecture [19]. Nuclear material was evaluated: complete loss, substantial loss or no loss. Architectural preservation was evaluated: normal architecture, minimal disruption or marked disruption.

2.6. Macroscopic Analysis

The macroscopic analysis was performed in all decellularized scaffolds, controls and protocols present in the study. We analysed the differences in colour, solidity, and size post-decellularization. All material collected was documented with photographs.

2.7.

In vivo host inflammatory response and tissue integration to decellularized tendon scaffold – Rotator Cuff Model

For the in vivo evaluation, we used eight male New Zealand rabbits weighing approximately 3–3,5 kg. We divided the animals into two groups: in “Group B – 2 weeks” the animals were euthanized after two weeks, and in “Group B – 8 weeks”, the animals were euthanized after eight weeks. All the procedures were performed with an aseptic technique, and the animals were anaesthetized with a combination of

ketamine 50 mg/kg + xylazine 10 mg/kg + tramadol 10 mg/kg and a combination of tramadol 10 mg/kg and meloxicam 2–3 mg/kg was used as an analgesic. The animals were kept in individual cages with a light-dark cycle of 12/12 hours and with food and water available in ad libitum. The animals were not maintained on postoperative cast immobilization.

2.7.1. Subscapularis Tendon Lesion and analysis

Following the anaesthetic protocol, the animals underwent injury to the subscapularis tendon [20]. A deltopectoral incision was made on the shoulder, and the subscapularis was exposed after dissection between the pectoralis major and deltoid. A complete parallel laceration, without detachment, was made in the subscapularis tendon. The laceration was not repaired and the decellularized scaffold was placed in the subscapularis tendon. We used Nylon 4/0 (Nylon 4 - 0: Shalon, Alto da Boa Vista, GO, Brazil) to mark the scaffold in the tendon. After eight or two weeks, the animals used for the in vivo evaluation were euthanized, and the subscapularis tendon was resected from the musculotendinous junction to the bone insertion.

The histological evaluation was performed on the scaffold topography, and we evaluated the cell infiltration, inflammatory response, and collagen arrangement. In a second approach, we evaluated the gross morphology of the integration between the scaffold and tendon.

2.8. Statistical Analysis

The number of animals used in the study was decided a priori after a review of the relevant literature, considering the biomechanical evaluation like the primary outcome [14]. We evaluated the data with the Kolmogorov-Smirnov test, related to the normal distribution of the results, with the use of SAS 3.71 software. The statistical analysis was performed using Student's t-test assuming different variance for the evaluation of the two groups. Differences were considered significant at $p \leq 0,05$.

3. Results

3.1. Biomechanics – Tensile testing

During tensile traction, all the specimens showed rupture in the space between the customized jig system. If the specimens slipped out of the jig system, the result was discarded. In this study, we do not have slippage in the tensile testing.

The ultimate tensile load, stiffness, and elongation at the ultimate tensile load did not significantly differ between the groups. The ultimate tensile load supported by the decellularized tendon scaffold was approximately 98% that of the control. The stiffness of the two groups was similar, and the elongation at the ultimate tensile load of the decellularized tendon scaffold was approximately 86% that of the other group (Table 1).

The cross-sectional area, ultimate tensile stress, and elastic modulus showed a statistically significant difference between the groups. The cross-sectional area of the decellularized tendon scaffold increased by 164% compared to the control. The elastic modulus and ultimate tensile stress of the decellularized tendon scaffold were reduced by 61% compared to the control (Table 1).

3.2. Histological Analysis

The control had abundant nuclear material, particularly in H&E and DAPI staining, as well as an intense organization of the extracellular matrix in H&E and Masson's trichrome staining (Fig. 4).

The decellularized tendon scaffold revealed no nuclear material in H&E staining, but DAPI staining showed the presence of nuclear material. However, in the decellularized tendon scaffold, the nuclear material was not organized as it was in the control. In conclusion, we noted a substantial loss of nuclear material (Fig. 4).

We noted an increase in the space between the collagen structures, but the parallelism of the fibres did not change in the decellularized tendon scaffolds; this was observed in H&E and Masson's trichrome staining. In conclusion, we noted minimal disruption in all the decellularized scaffolds (Fig. 4).

3.3. Macroscopic Analysis

The macroscopic analysis revealed an increase in the volume of the scaffold, without changes in the colour or consistency of the material after the proposed protocol (Fig. 3).

3.4. In vivo Analysis

3.4.1. Histological Analysis – In vivo

Using H&E-stained sections, we evaluated cell infiltration and the inflammatory reaction. Cell infiltration of fibroblast-like host cells was observed in the scaffold, and we found more infiltration after eight weeks than after two weeks. In the inflammatory evaluation, we did not find significant evidence of capsule or granuloma formation, but we found some inflammatory cells, such as lymphocytes and macrophages, in the regeneration area.

3.4.2. Macroscopic Analysis – In vivo

The subscapularis insertion was in the lesser tuberosities in all the specimens, and it was not possible to detach the scaffold of the subscapularis tendon in the two groups. We noted some differences between the two groups. In "Group B – 8 weeks" we have more connective tissue between the scaffold and the subscapularis tendon than in "Group B – 2 weeks".

4. Discussion

Our most important findings were that the tendon scaffold had a similar ultimate tensile load to a normal tendon, had high porosity, did not have organized nuclear material and permitted cellular infiltration.

Scaffolds made from decellularization protocols are currently used in clinical practice in various specialties. In developing countries, the high cost and regulatory services are limiting the use of these scaffolds compared to developed countries. Our purpose is to fill that gap and to reproduce the good results obtained from other studies [6, 21]. We thus evaluated a decellularization protocol with highly reproducible and low-cost technology. We see two options for the future: first, we could use rabbit-derived xenografts. In a second approach, we could use this protocol for human tissue and for use in allograft transplantation. Currently, we believe that decellularized allograft transplantation is a viable option, with a low chance of rejection.

Regarding the decellularization process, an efficient protocol should contain a combination of chemical (detergents), biologic (enzymatic or non-enzymatic agents) and physical or miscellaneous agents. This protocol needs to be applied for extended periods with a correct technique depending on the materials to be decellularized [12, 13]. Our protocol used a combination of two detergents (SDS + Triton X100), a non-enzymatic agent (EDTA), a protective compound for the extracellular matrix (aprotinin), and PBS for washing the scaffold. Furthermore, the scaffold was maintained under constant agitation, following the recommendations for tendinous structures [12, 13].

The histological analysis showed an acellular scaffold that reduced immunogenicity and antigenicity and may have maximized host-cell infiltration into the scaffold in vivo or in vitro. The biomechanical analysis showed statistically significant modifications in terms of the cross-sectional area, elastic modulus, and ultimate tensile stress of the scaffold. An increase in the cross-sectional area may be related to high porosity, which could help host-cell infiltration.

Despite the elastic modulus alteration, the ultimate tensile load, the elongation at the ultimate tensile load and the stiffness did not change with the protocol. During the first days of patient rehabilitation, the tendon scaffold should maintain its integrity. We do not know the most important characteristics for maintaining scaffold integrity, but having an ultimate tensile load and elongation similar to those of a normal tendon should be most important.

We noted some limitations. First, we did not measure immunogenicity or antigenicity, and we do not know if these biomechanical properties will be maintained after use of this decellularized tendon scaffold as a graft. Second, there is no consensus on the best sterilization protocol, and this is one of the difficulties of decellularized scaffolds. We attempted to use an ethylene oxide protocol as a pilot test, but the results were not satisfactory. One alternative to this problem is to use peracetic acid in the protocol, as it not require an additional sterilization protocol [6, 21, 22].

In the future, we hope to start testing scaffolds in several orthopaedic approaches. Superior capsular reconstruction and rotator cuff tendon interposition will be our next objectives. A different method for these studies may be to use mesenchymal stem cells (MSCs), as an adjuvant, in the same orthopaedic approaches [18].

For these next studies, we will maintain the final objective, which is to have off-the-shelf availability of reliable decellularized tendon scaffolds for use in clinical practice.

5. Conclusion

The evaluated decellularization protocol efficiently produced a decellularized tendon scaffold, maintained the most important biomechanical characteristics and permitted cell infiltration.

Declarations

Ethics approval and consent participate

This study was approved by the Ethics Committee of Animal Use of the Federal University of Sao Paulo. Protocol Number CEUA 5272080916.

We obtained written informed consent to use the animals in your study from the owner of the animal.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that have no competing interests.

Funding

The source of funding in this study was a donation of private institutes: Urology Research Center of the Federal University of São Paulo.

Authors' contributions

A.L.S., conceived the project idea, performed the experiments, analyzed the data and write the manuscript.

C.G.S., performed the experiments and analyzed the data.

L.S.S.B., performed the experiments and analyzed the data.

K.R.M.L., analyzed the data.

M.J.S.T., conceived the project idea and analyzed the data.

L.M.F., conceived the project idea and supervised the project.

F.G.A., conceived the project idea, analyzed the data and supervised the project

F.F., analyzed the data e supervised the project.

All authors discussed the results, read and approved the final manuscript.

Acknowledgements

Not applicable

References

1. Moraes VY, Lenza M, Tamaoki MJS, Faloppa F, Belloti JC. Platelet-rich therapies for musculoskeletal soft tissue injuries. *Cochrane Database Syst Rev.* 2013;:1–126.
2. Garrett WE. Muscle strain injuries. *Am J Sports Med.* 1996;24 6 Suppl:S2-8.
3. Longo UG, Berton A, Papapietro N, Maffulli N, Denaro V. Epidemiology, genetics and biological factors of rotator cuff tears. *Rotator Cuff Tear.* 2011;57:1–9.
4. Tashjian RZ. Epidemiology, Natural History, and Indications for Treatment of Rotator Cuff Tears. *Clin Sports Med.* 2012;31:589–604.
5. Laurencin CT, Ambrosio AMA, Borden MD, Cooper JA. Tissue Engineering: Orthopedic Applications. *Annu Rev Biomed Eng.* 1999;1:19–46.
6. Whitlock PW, Smith TL, Poehling GG, Shilt JS, Van Dyke M. A naturally derived, cytocompatible, and architecturally optimized scaffold for tendon and ligament regeneration. *Biomaterials.* 2007;28:4321–9.
7. Derwin KA, Badylak SF, Steinmann SP, Iannotti JP. Extracellular matrix scaffold devices for rotator cuff repair. *J Shoulder Elb Surg.* 2010;19:467–76.
8. Porrello ER. microRNAs in cardiac development and regeneration. *Clin Sci.* 2013;125:151–66.

9. Andreassi A, Bilenchi R, Biagioli M, D'Aniello C. Classification and pathophysiology of skin grafts. *Clin Dermatol*. 2005;23:332–7.
10. Keane TJ, Badylak SF. The host response to allogeneic and xenogeneic biological scaffold materials. *J Tissue Eng Regen Med*. 2015;9:504–11.
11. Schulze-Tanzil G, Al-Sadi O, Ertel W, Lohan A. Decellularized Tendon Extracellular Matrix—A Valuable Approach for Tendon Reconstruction? *Cells*. 2012;1:1010–28.
12. Lovati AB, Bottagisio M, Moretti M. Decellularized and Engineered Tendons as Biological Substitutes: A Critical Review. *Stem Cells Int*. 2016;2016:1–24.
13. Peter M. C, Gilbert TW, Badylak SF. An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2012;32:3233–43.
14. Xing S, Liu C, Xu B, Chen J, Yin D, Zhang C. Effects of various decellularization methods on histological and biomechanical properties of rabbit tendons. *Exp Ther Med*. 2014;8:628–34.
15. Bailey JR, Kim C, Alentorn-Geli E, Kirkendall DT, Ledbetter L, Taylor DC, et al. Rotator Cuff Matrix Augmentation and Interposition: A Systematic Review and Meta-analysis. *Am J Sports Med*. 2019;47:1496–506.
16. Walton JR, Bowman NK, Khatib Y, Linklater J, Murrell GAC. Restore orthobiologic implant: Not recommended for augmentation of rotator cuff repairs. *J Bone Jt Surg - Ser A*. 2007;89:786–91.
17. Doherty GP, Koike Y, Uhthoff HK, Lecompte M, Trudel G. Comparative anatomy of rabbit and human achilles tendons with magnetic resonance and ultrasound imaging. *Comp Med*. 2006;56:68–74. <http://www.ncbi.nlm.nih.gov/pubmed/16521862>.
18. de Lima Santos A, Silva CG da, de Sá Barretto LS, Franciozi CE da S, Tamaoki MJS, de Almeida FG, et al. Biomechanical evaluation of tendon regeneration with adipose-derived stem cell. *J Orthop Res*. 2019;;1281–6.
19. Caralt M, Uzarski JS, Iacob S, Obergfell KP, Berg N, Bijonowski BM, et al. Optimization and critical evaluation of decellularization strategies to develop renal extracellular matrix scaffolds as biological templates for organ engineering and transplantation. *Am J Transplant*. 2015;15:64–75.
20. Grumet RC, Hadley S, Diltz M V., Lee TQ, Gupta R. Development of a new model for rotator cuff pathology: The rabbit subscapularis muscle. *Acta Orthop*. 2009;80:97–103.
21. Seyler TM, Bracey DN, Plate JF, Lively MO, Mannava S, Smith TL, et al. The Development of a Xenograft-Derived Scaffold for Tendon and Ligament Reconstruction Using a Decellularization and Oxidation Protocol. *Arthrosc - J Arthrosc Relat Surg*. 2017;33:374–86.
22. Whitlock PW, Seyler TM, Parks GD, Ornelles DA, Smith TL, Van Dyke ME, et al. A novel process for optimizing musculoskeletal allograft tissue to improve safety, ultrastructural properties, and cell infiltration. *J Bone Jt Surg - Ser A*. 2012;94:1458–67.

Abbreviations

FDA: Food and Drug Administration

USA: United States of America

SDS: Sodium Dodecyl Sulfate

Triton X-100: t-octyl-phenoxyethoxyethanol

TnBP: 1% tri-n-butyl phosphate

EDTA: ethylenediamine tetraacetic acid, 0,1% w/v

PBS: phosphate-buffered saline

H & E: Haematoxylin and eosin

DAPI: 4,6-diamidino-2-phenylindole

Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

Figures

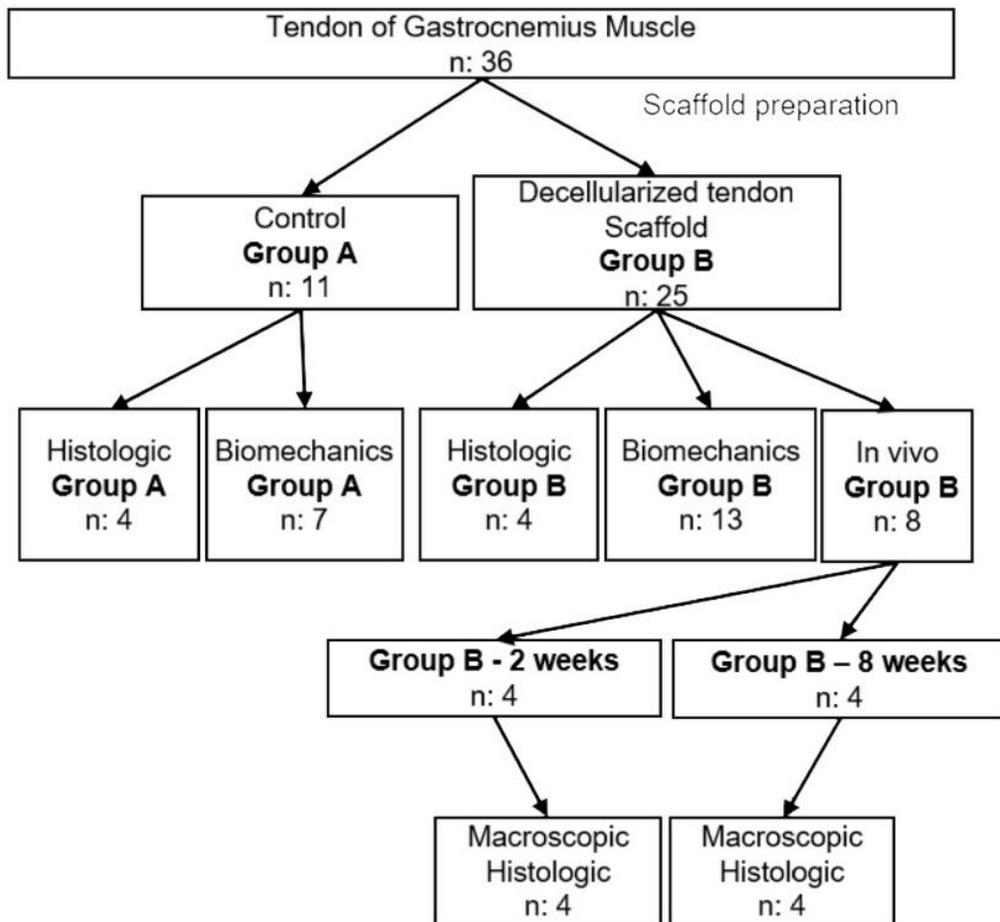


Figure 1

Animal Grouping Legend: -

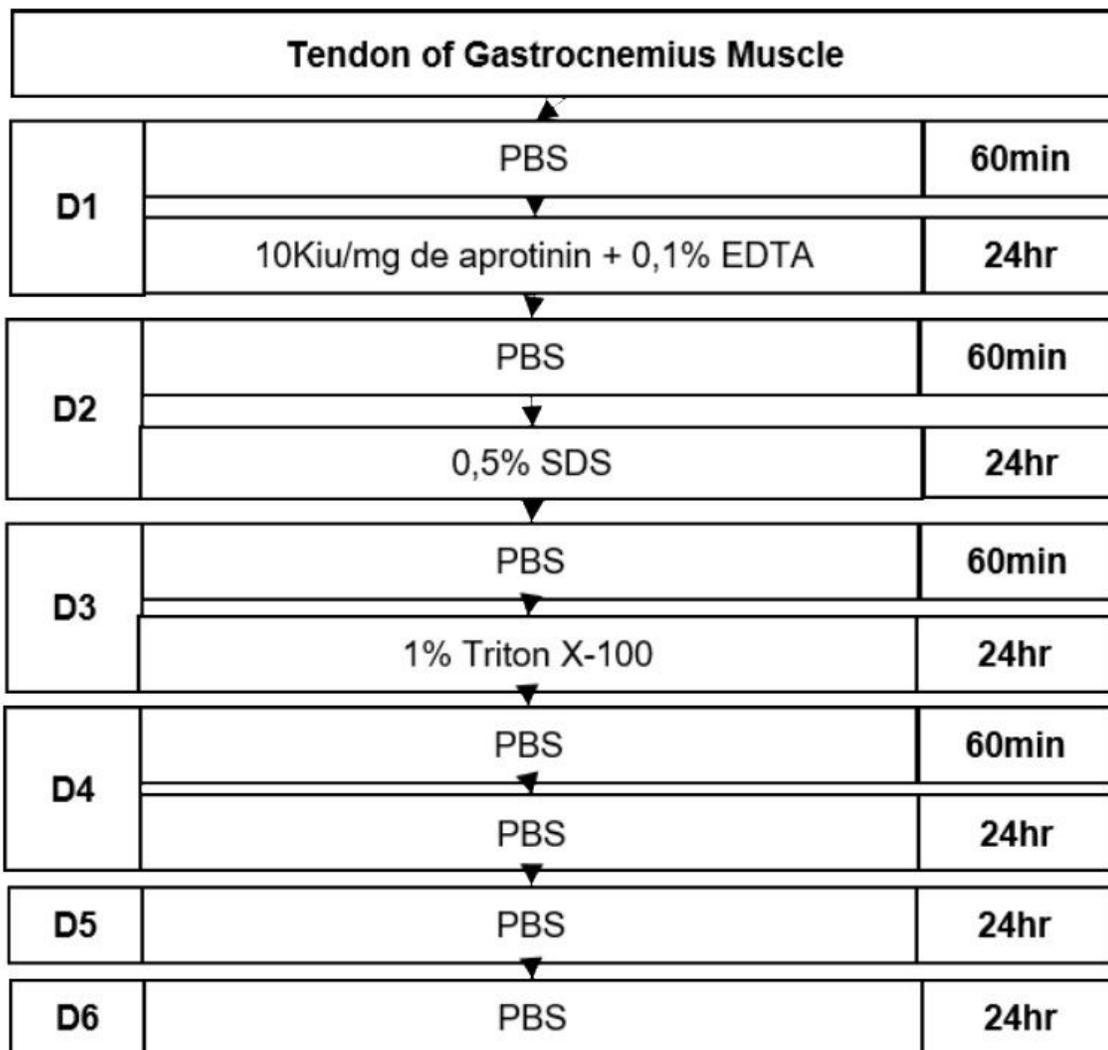


Figure 2

Preparation of decellularized tendon scaffolds Legend: -

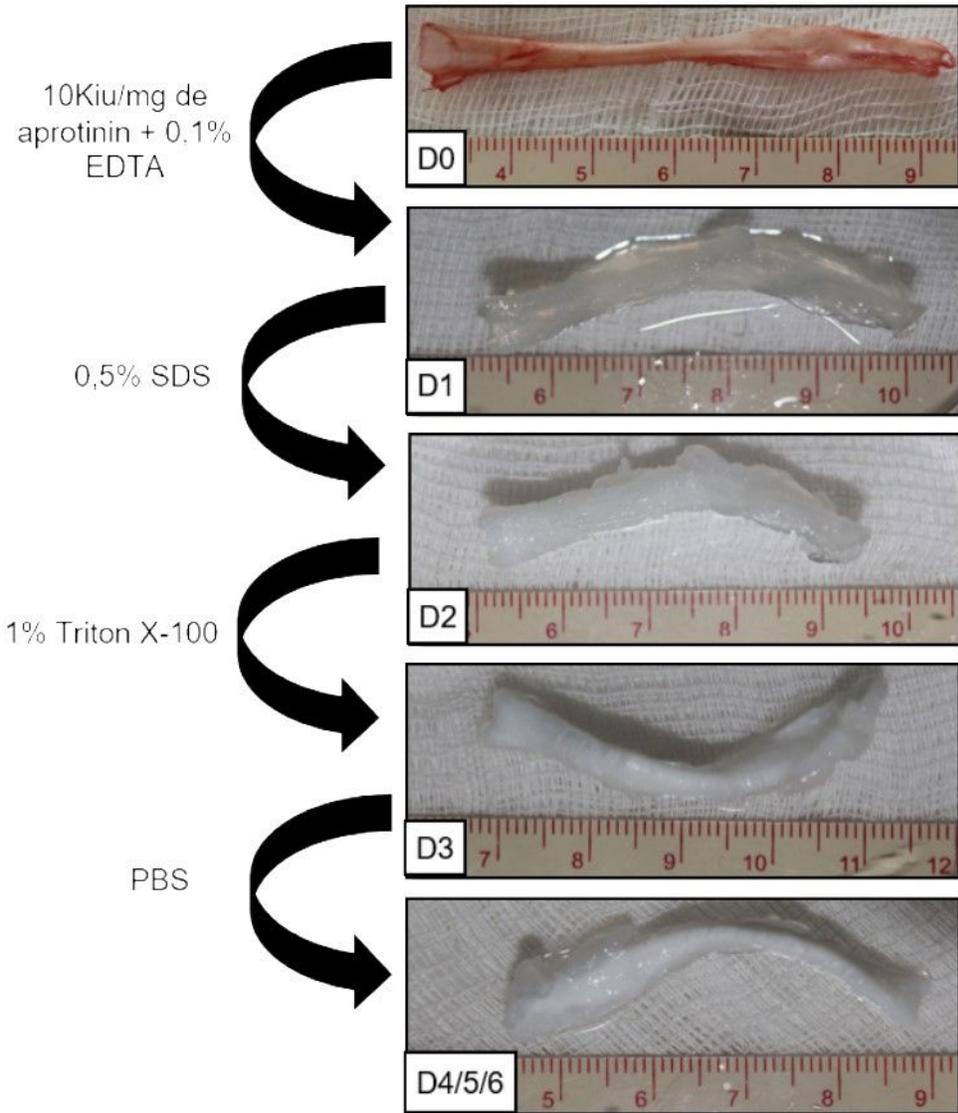


Figure 3

Preparation of decellularized tendon scaffolds Legend: -

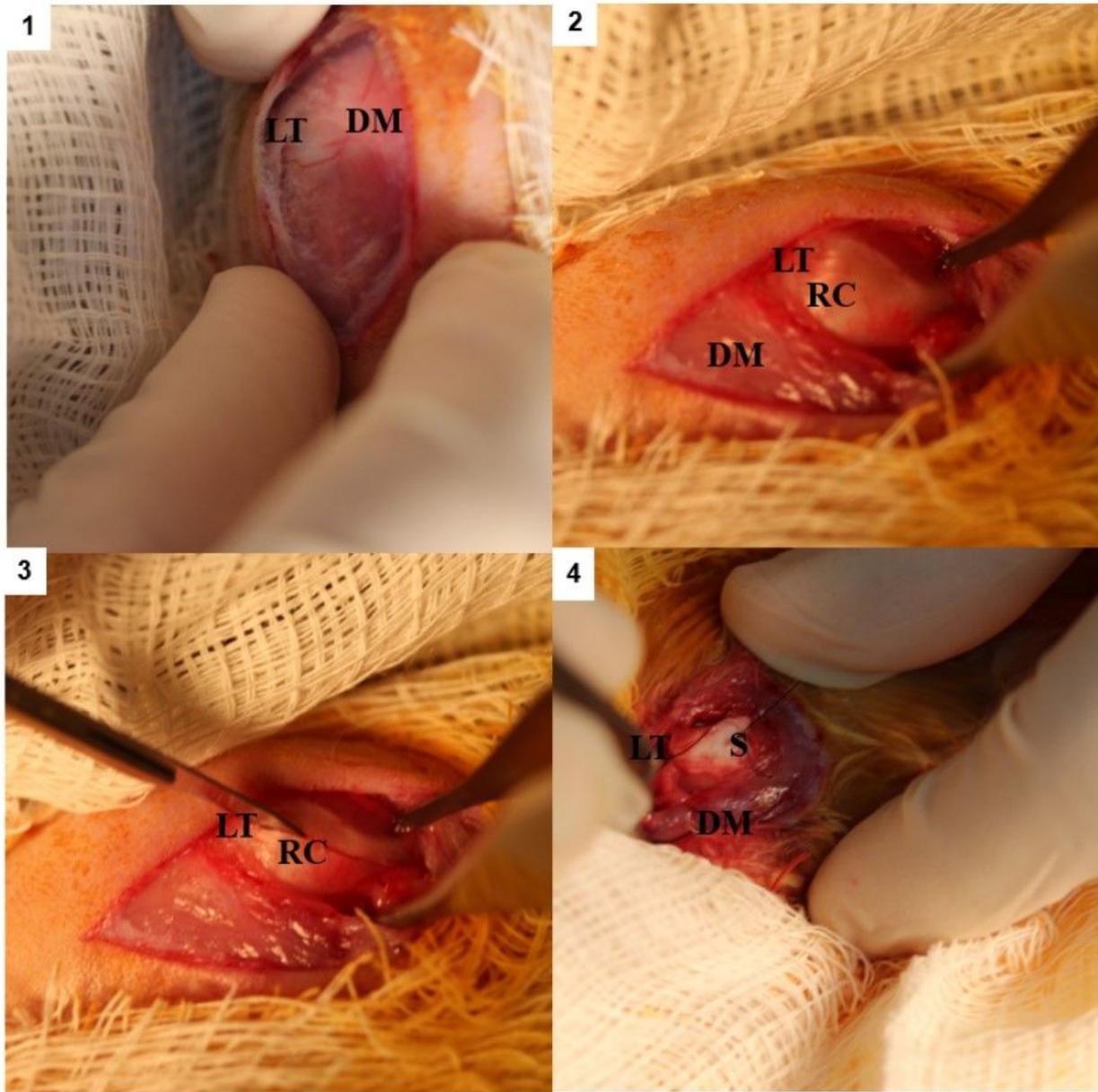


Figure 4

In Vivo Experimental Model – Subscapularis tendon lesion Legend: 1. Deltopectoral approach 2. Subscapularis Tendon exposition 3. Subscapularis tendon lesion 4. Scaffold positioning RC: Rotator Cuff S: Scaffold LT: Lesser Turbcle DM: Deltoid muscle

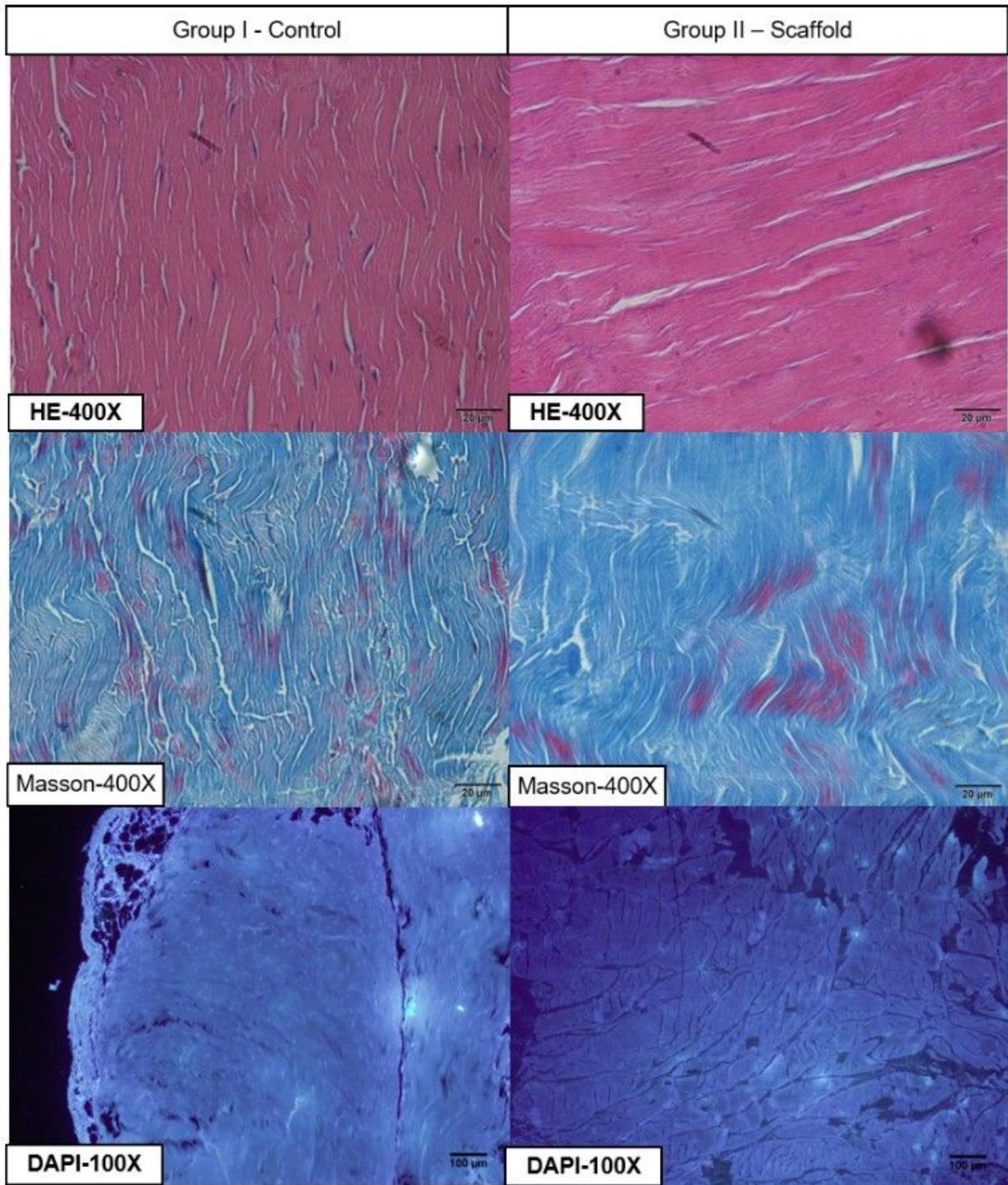


Figure 5

Histological Analysis – Tendon and Scaffold Legend: -

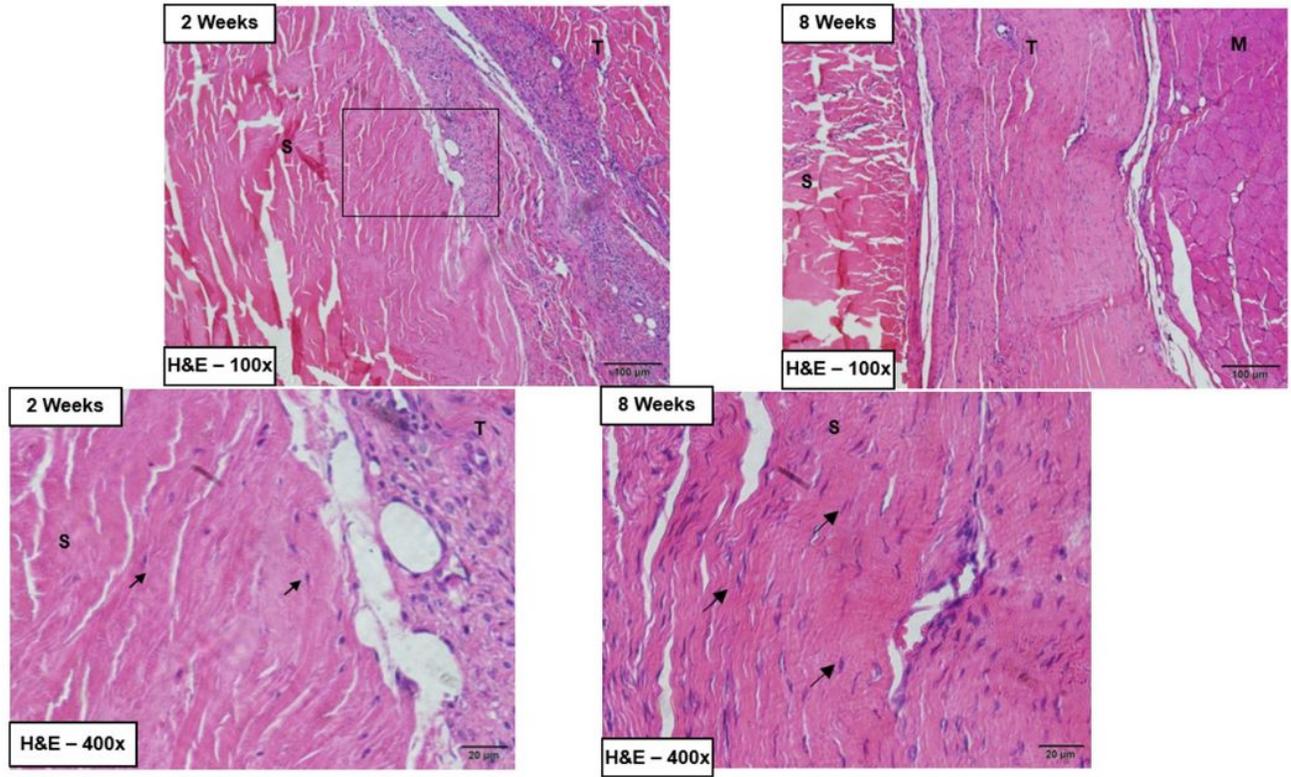


Figure 6

Microscopic Evaluation – In vivo Group B Legend: S: Scaffold T: Tendon M: Muscle arrow: Cell infiltration

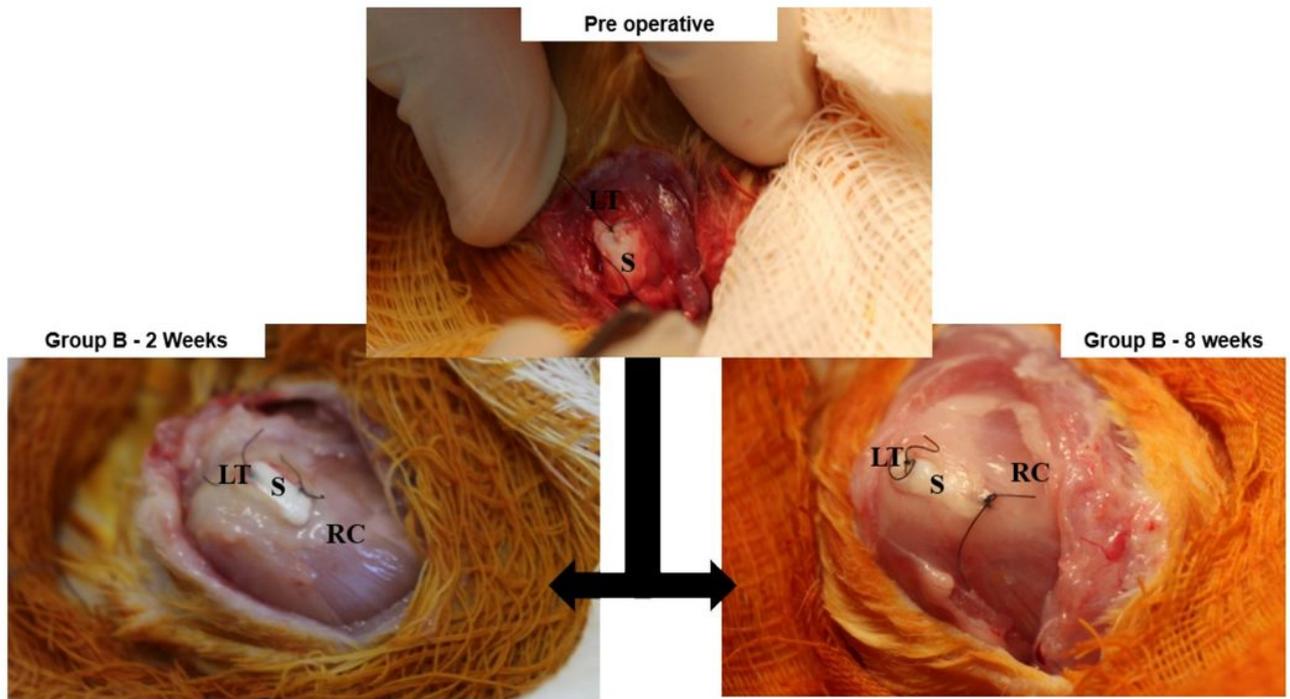


Figure 7

Macroscopic In Vivo Evaluation Legend: RC: Rotator Cuff S: Scaffold LT: Lesser Tubercle DM: Deltoid muscle