Clinics in Surgery

Research Article
Published: 10 Jul, 2020

Effects of Elastic High-Molecular Weight Poly (L-Co-D, L Lactic Acid) with 1,3-Trimethylene Carbonate Membrane in the Expression of Collagen and Glycosaminoglycans Tendon Healing, A Randomized Controlled Trial in Rabbits

Jose Carlos Garcia Jr.[1]*, Eliana Aparecida de Rezende Duek[2], Renan Pelluzzi Cavalheiro[3], Helena Bonciani Nader[4] and Edna Frasson de Souza Montero[5]

[1]NAEON Institute, Sao Paulo, Brazil

[2]Pontifical Catolic University of São Paulo, Brazil

[3]Federal University of São Paulo, Brazil

[4]University of São Paulo, Brazil

Abstract

Background: The use of membranes has been reported to present a positive impact on tendon healing. Thus refinements in absorbable materials and the best knowledge of the reparative process are important to drive clinicians into choose the more suitable therapeutic options.

Hypothesis/Purpose: Membranes presenting low crystallinity within an elastic structure may result in more biocompatible materials, improving healing at the same time that minimizes side effects related to the absorbable materials.

Study Design: It is a preclinical prospective randomized case-control study using animal model (New Zealand rabbits).

Methods: This study assessed the effectiveness of a new absorbable membrane that possesses mechanical and chemical features more suitable to tendons. The 46 rabbits underwent tenotomy and reconstruction of the right gastrocnemius tendons. They were randomly divided into three groups: case group (tendons wrapped with a membrane), control group, and intact tendon group. Animals of both the groups were allocated for extraction at seven, 14 and 28 days. Macroscopy, glycosaminoglycans, type I collagen (through polarized light) and histologic assessments were performed. Statistical tests respected the nature of the data in an adaptive model.

Results: At 14 and 28 days, the tendons of the case group presented less adherences (p=0.02 and p=0.03, respectively). Glycosaminoglycans (GAGs) presented statistical differences only for the central part of the tendon for Dermatan Sulfate and Hyaluronic Acid, both for the 7th day assessment p=0.02 and 0.04 respectively. Chondroitin Sulfate in the same part of the tendon presented a difference p=0.05. At 14 days, there was equivalence between the samples for CS and DS p>0.39.

The modified histologic Watkins classification for case and control groups averages: 14.67 ± 0.42 and 12.67 ± 0.56 (p=0.03) at 14 days, and 19.88 ± 0.83 and 17.25 ± 0.62 (p=0.02) at 28 days. The mean of gray value for codified red color on Image-J® for type I collagen were 2.41 ± 0.33 and 1.31 ± 0.18 (p=0.01) at 14 days, and 7.30 ± 0.63 and 2.92 ± 0.32 (p<0.0001) at 28 days.

Conclusion: These results suggest that the tendons wrapped with the new membrane presented changes that facilitated the healing process of the gastrocnemius tendons of New Zealand rabbits.

Keywords: Tendon healing; Absorbable implants; Tendon injuries; Glycosaminoglycan; Proteoglycan

OPEN ACCESS

Correspondence:
Jose Carlos Garcia Jr., NAEON Institute, Avenida Ibirapuera 2144, cj 82 - Sao Paulo-SP, ZIP: 04028-001, Brazil, Tel: +55 11 41078997; E-mail: josecarlos@naeon.org.br

Received Date: *17 May 2020*
Accepted Date: *02 Jul 2020*
Published Date: *10 Jul 2020*

Citation:
Jose Carlos Garcia Jr., de Rezende Duek EA, Cavalheiro RP, Nader HB, de Souza Montero EF. Effects of Elastic High-Molecular Weight Poly (L-Co-D, L Lactic Acid) with 1,3-Trimethylene Carbonate Membrane in the Expression of Collagen and Glycosaminoglycans Tendon Healing, A Randomized Controlled Trial in Rabbits. Clin Surg. 2020; 5: 2860.

What is known about this Subject?

Intact tendons possess low cellularity, and hence, have low capability to heal [1]. Tendons are

organized structures with high resistance, mainly to traction, due to the high density of type I collagen within its structure [2]. The perfect combination of resistance and elasticity enables tendons to dissipate energy to prevent lesions [3]. The risk of re-injury in tendons is high, even after initial successful repair [4-8]. Tendon healing, which changes the mechanical and biological properties, is a long process that may present definitive fibrotic modifications within the tendon structure.

Adhesions between the tendon and surrounding tissues may impede the normal excursion of the tendon [9,10], compromising the correct mechanical stimulus and thus creating structures less resistant to traction than before [11]. Unsuccessful repairs seem to present a direct correlation with these structural changes at the site of tendon lesion [12,13]. Biomechanical studies have suggested that tendon re-tears are more likely to occur just adjacent to the previous injury site [14]. The pathogen of tendons is not completely elucidated; however, extracellular matrix synthesis is abnormal and inflammation, even transient, is present [15-17].

In tendinopathy, all tendons are affected, exhibiting changes in their biological and mechanical features [9]. The extracellular matrix of tendons predominantly consists of, hyaluronic acid proteoglycans such as decorin, biglycan, fibromodulin, lumican, aggrecan, and versican, which can regulate the production of type I collagen [11]. An overall increase in proteoglycans and their sulfated Glycosaminoglycan (GAG) side chains is observed in the space surrounding the collagen fibers in lesioned tendons [18]. However, not all proteoglycans or GAGs are changed in tendinopathy. The levels of decorin and keratan sulfate appear to be unchanged in pathological tendons, in contrast to biglycan, versican and aggrecan [19-23]. GAGs, such as Chondroitin Sulfate (CS) and Dermatan Sulfate (DS), are highly expressed even in tendons presenting pathologic conditions [24,21,12]. Increased rates of GAGs are also associated with tendon pain increased tendon cross-sectional diameter and impaired collagen structure [25-8]. Biomembranes have been demonstrated to improve tendon healing, facilitating their reparative processes [29]. Less adherences and the possibility of earlier and better mechanical stimulus to the repaired tendon can result in the successful use of biomembranes [10].

What this Study Adds to Existing Knowledge?

A bioabsorbable membrane was created using Poly (L-co-D, L lactic acid) (PLDLA) with 1,3-Trimethylene Carbonate (TMC) of high molecular weight. The combination of these materials results in the most elastic, less crystalline, and quick absorption membrane. These characteristics make this membrane more biocompatible, potentially allowing a better reparative capability. In this study, the GAGs and the histological and macroscopic features of tendons were investigated at three different time points to determine the effectiveness of the bioabsorbable membrane on tendon healing.

Materials and Methods

Study design

It is a preclinical prospective randomized blinded case-control study using animal model (New Zealand rabbits).

Set for data collection

Surgical pathophysiology laboratory of the University which the author is filiated.

Polymer synthesis

The copolymers L and D,L-lactate were prepared using a mass polymerization reaction in a glass ampoule. The ratio of the monomers L-lactate (Sigma-Aldrich, Brazil) and D,L-lactate (Sigma-Aldrich, Brazil) for the syntheses was 70:30. The catalyst was tin (II) 2-ethylhexanoate, better known as Sn (Oct) 2.

The monomer: Catalyst ratio was approximately 5000, optimizing the production of high molar mass PLDLA [30]. The monomers (L-lactate and D,L-lactate) and the catalyst were inserted into the glass ampoule with TMC in a new 70:30 proportion. The glass ampoule was sealed under vacuum and immersed in an oil bath at 130°C for 72 h. Subsequently, the polymer was dissolved in chloroform ($CHCl_3$; Merck Millipore, Germany) and precipitated in methanol (CH_3OH; Merck Millipore, Germany). The PLDLA-TMC (70:30) membrane was made using the casting method and dried in a desiccator with a vacuum at 45°C for 8 h.

Animals and surgical procedure

The membranes were biologically tested in New Zealand white rabbits weighing 2000 ± 200 g with 3 months old. The rabbits were kept in a circadian cycle under controlled conditions. They were randomly divided as follows. Nine animals were used as positive control for GAGs; this group was named as the intact tendon group. All other animals underwent tenotomy of the right medial and lateral gastrocnemius tendon. Tenotomy was performed 2 cm proximal to its insertion on the bone. The rabbits underwent tendon reconstruction by Kessler suture using FiberWire 2 (Arthrex, FL, USA).

Two groups of six rabbits at each study time point were used. The rabbits were assessed at 7, 14, and 28 days to investigate the membrane in different phases of healing process. The adaptive statistical model required two more rabbits for each group for the assessment at 28 days, total of 40 rabbits for all study [31]. The soleus muscle was left intact. A plastic orthosis (Figure1) fixed with bands was used during the first two weeks to improve tendon protection at the initial healing phase. The orthosis did not impede, but restricted the movements of the rabbits. The tendons of the rabbits from the case group were wrapped using the new membrane. The membrane was fixed to the tendon with two 4.0 nylon sutures. All tendons were macroscopically assessed and extracted 0.5 cm to 3.5 cm from the calcaneus bone. A third group of six rabbits was used to extract intact tendons.

Macroscopy

The parameters used for the macroscopic assessment were as follows: Adherence (absent, minimal, mild, moderate, or high), continuity (organized or unorganized), and repair (absent or present). The tendons were horizontally split into two parts. One part was vertically subdivided into three subgroups for GAG analysis: Proximal, central (in the lesion site), and distal. The other part was used for histologic assessment. Collagen and fibers were not assessed in the 7-day groups because it was too early for this assessment. GAGs were also not assessed in the 28-day groups because changes in their expressions occur at earlier stages of healing.

GAG quantification

The three subdivisions of each tendon were separately assessed CS, DS, and Hyaluronic Acid (HA) were measured. An additional positive control group comprising six rabbits was also used to determine the normal tendon patterns of GAGs. The samples were pulverized using scissors and directly macerated. Peptides and nucleic acid fragments were removed by precipitation with 10% trichloroacetic acid at 4°C. After centrifugation (10 min, 3500 × g, 4°C), the supernatant containing GAGs was precipitated by adding

two volumes of methanol for 18 h at 4°C. The precipitate was separated and collected through centrifugation (10 min, 3500 × g, 4°C) and then dried. Subsequently, it was suspended in 40 ml of distilled water and analyzed for sulfated GAG. This method of recovering GAGs extracted from the tendons gives a yield of approximately 95% [32]. Sulfated GAGs were identified and quantified with agarose gel electrophoresis in 0.05 M 1,3-diaminopropane acetate buffer, pH 9.0. An aliquot of 5 ml of each sample was submitted to electrophoresis for 1 h at 100 V; GAGs were precipitated in the gel with 0.1% cetavlon (cetyltrimethylammonium bromide) for 2 h at room temperature. The gel was dried and stained with a 0.1% solution of toluidine blue in acetic acid ethanol and water (0.1:5:4.9, vol/vol). Quantification was carried out by densitometry at 530 nm of the toluidine blue-stained electrophoretic slide. The extinction coefficients of the GAGs were calculated using the standards of CS and DS. Identification of the sulfated GAGs was based on the migration of the compounds compared with those of the standards. The identities of GAGs present in the samples were further confirmed by treatment with specific enzymes. Electrophoresis was performed in triplicate. Results of the absolute amounts of GAGs were expressed per weight of tendon (μg mg^{-1}) [33-35].

HA was quantified by fluorometric noncompetitive Enzyme-Linked Immunosorbent Assay - Like Fluorescence Assays (ELISA) that can detect 2e 500 g/L of HA. The ELISA plates had a fixed probe, and 100 ml/well of standard HA solutions at various concentrations (0e 500 g/L) was added. The samples were diluted (1:100) in a Tris hydrochloride buffer 0.05 M with 1% bovine serum albumin and added to the ELISA plates in triplicate. The plates were incubated at 4°C for 12 h and then washed three times with Tris hydrochloride 0.05 M. Afterward, 100 ml of the probe (1 mg/ml) diluted (1:10.000) in the assay buffer was added. The plate was incubated for 2 h on a shaker and washed nine times with a wash buffer. Streptavidin labeled with europium diluted to 1:10,000 in assay buffer was added to each well (100 ml; Sigma, Germany). Streptavidin has an affinity for the biotin-conjugated probe. The plate was incubated 30 min on a shaker and washed nine times with Tris hydrochloride 0.05 M. To release the uropium bound to streptavidin (Sigma), an enhancement solution (280 ml/well) was added. The plates were agitated for 5 min, and the europium-free plate fluorescence was read on a fluorimeter. The results were expressed in ng/ml [36].

Histology

The colorations used for the assessments were eosin-hematoxylin, Masson's trichrome and red picrosirius. To evaluate tendon healing, the author modified the tendon maturing scoring system reported by Watkins et al. [37] (Table 1). Six histologic parameters including cellularity, fibrocytes, vascularity, fiber diameter, cells parallel, and fibers parallel were evaluated. One more category, i.e., collagen polarized light assessment colored with picrosirius red, was added to evaluate the type I/type III collagen ratio. Values between 7 and 28 points can be achieved using this score healing process. The reason for this scoring is that type III collagen first increases and then decreases, followed by its replacement with type I collagen, during the process of tendon healing. The proportion of type I/type III collagen has therefore a direct correlation with better mechanical properties of tendon healing [38-40]. The samples were scored in a blinded manner. The author also performed an objective assessment of type I collagen colored with picrosirius red by using polarized light. The assessment was conducted by photographing five different fields, within the regeneration site per sample, and Image-J® (National Institutes of Health, USA) was then used to divide each photo into three different colors and separate the red color only, process it to gray and thereafter quantify the Mean Gray Value (Mgv). It is the sum of the gray values of all pixels in the selection divided by the number of pixels.

Table 1: Tendon maturing scoring system.

	1	2	3	4
Cellularity	Marked	Moderate	Mild	Minimal
Fibrocytes	25%	25-50%	50-75%	>75%
Vascularity	15 bv/F	11-15 bv/F	6-10 bv/F	<6 bv/F
Fiber diameter	25%	25-50%	50-75%	>75%
Cells parallel	25%	25-50%	50-75%	>75%
Fibers parallel	25%	25-50%	50-75%	>75%
Polarized light	I(–) III(++)	I(+) III(++)	I(+) III(+)	I(++) III(+)

bv/F: Blood vessel/Field

The fields for the photos were chosen in a blinded manner at 400x amplification, 1443520 (1388×1040) pixels. Each field was considered a different sample for statistical purposes. This was the primary outcome for 14 and 28 days.

Statistics

Statistical tests were performed according to the nature of the data.

Randomization was performed by using the App Randomization Lite® (The Apps Factory-USA). The Rabbits received new random number after the tissue extraction in order to random the allocation using the same App mentioned above.

All assessments were blinded and conducted using Prism7 for Mac OS X (GraphPad, CA, USA) STATA (STATA Corp®, TX, USA) and Wizard 1.9.7 for Mac (Free Software Foundation-USA). A two-tailed $p<0.05$ was considered significant.

An adaptive statistical method was performed to optimize the sample size after an initial interim analysis based on six animals per group, considering an alpha of 0.05 within a statistical power of 90% [31].

The sample size was reached based on the primary outcomes for each period of assessment through the interim analysis.

For 14 and 28 days the primary outcome was the objective measure of Type I collagen, with an expected effect of at least 1.00 mgv for 14 days and 1.50 mgv for 28 days.

For 7 and 14 days the initial sample sizes were suitable, however for 28 days two more rabbits were required for each group.

Data will be described using mean ± standard error.

Results

Macroscopy

At 7 days none of the animals' tendons presented healing features that allowed them to be assessed by the standardized macroscopic parameters. They presented gelatinous to liquid consistency, markedly in its central part of the case group.

Adherences were different at 14 days (p=0.02) and 28 days (p=0.03) (Figure 2). Results are summarized in Charts 1 and 2. With regard to continuity, the assessment presented a difference favorable to the case group at 14 days (p=0.04) and 28 days (p=0.13). Repair was

Table 2: Results GAGs 7 days, 14 days and Intact Tendon.

7 days	Proximal	Central	Distal
Chondroitin case (µg/mg)	0.48 ± 0.05	0.44 ± 0.77	0.80 ± 0.04
Chondroitin control (µg.mg)	0.31 ± 0.08	0.69 ± 0.09	0.38 ± 0.04
P	0.0084	0.05	0.029
Dermatan case (µg/mg)	1.21 ± 0.11	0.42 ± 0.09	1.48 ± 0.20
Dermatan control (µg/mg)	0.90 ± 0.10	1.29 ± 0.67	1.17 ± 0.13
P	0.07	0.02	0.23
Hyaluronic Acid case	9.98 ± 2.79	17.72 ± 3.62	10.65 ± 3.13
Hyaluronic Acid control	8.52 ± 2.11	9.48 ± 1.61	10.91 ± 3.86
P	1	0.04	0.94
14 days	Proximal	Central	Distal
Chondroitin case (µg/mg)	0.67 ± 0.21	1.01 ± 0.13	0.65 ± 0.09
Chondroitin control (µg.mg)	0.67 ± 0.07	0.71 ± 0.25	0.69 ± 0.09
P	0.994	0.394	0.802
Dermatan case (µg/mg)	1.20 ± 0.13	1.39 ± 0.22	1.11 ± 0.05
Dermatan control (µg/mg)	1.08 ± 0.23	1.16 ± 0.38	1.27 ± 0.12
P	0.66	0.58	0.87
Intact Tendon			
Chondroitin (µg/mg)	0		
Dermatan (µg/mg)	1.50 ± 0.14		
Hyaluronic (ng/mg)	7.43 ± 0.53		

Figure 1: Plastic orthosis.

observed in all samples at 14 and 28 days.

Glycosaminogly can levels

Glycosaminoglycan measurements are shown in Table 2. When differences were not observed in the case and control groups at 14 days. Measurement was not performed at 28 days because in 14 days an equivalence between case and control have already been established for p>0.39. HA was just measured for seven days because of technical problems.

Microscopy

None of the animals presented healing features that allowed them to be evaluated at 7 days. According to the modified Watkins classification, the case and control groups presented averages of 14.67 ± 0.42 and 12.67 ± 0.56, respectively, (p=0.03) at 14 days. At 28 days, the case group presented an average of 19.88 ± 0.83 and the control group an average of 17.25 ± 0.62 (p=0.02).

The quantification of type I collagen by polarized light showed the following results. At 14 days, averages of 2.41 ± 0.33 mgv for the case group and 1.31 ± 0.18 mgv for the control group (p=0.01) were observed (Figure 3). At 28 days, averages of 7.30 ± 0.63 mgv for the case group and 2.92 ± 0.32 mgv for the control group (p<0.0001) was

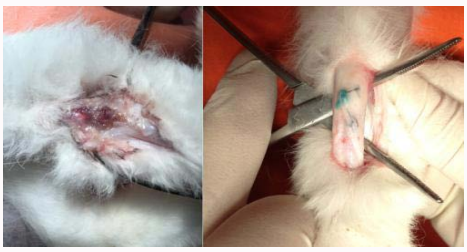

Figure 2: Macroscopy.

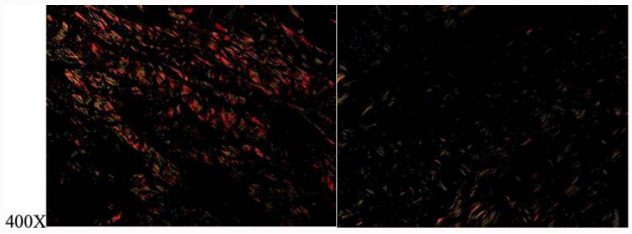

Figure 3: Type I Collagen 14 days: Control (right) and Case (left).

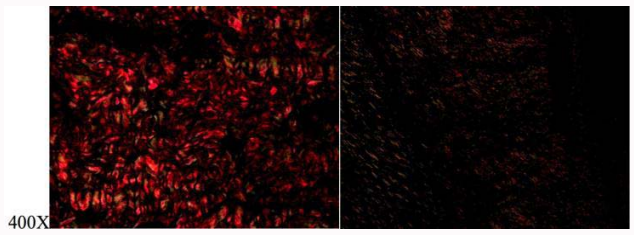

Figure 4: Type I Collagen 28 days: Control (right) and Case (left).

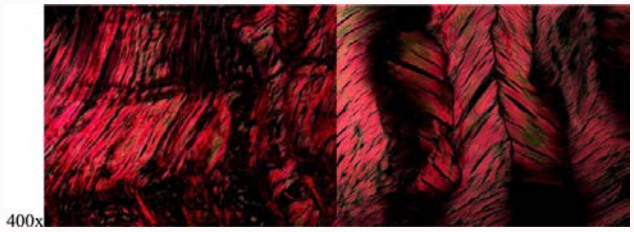

Figure 5: Type I Collagen Intact Tendon.

obtained (Figure 4). The intact tendon presented an average of 41.10 ± 0.32 mgv (Figure 5).

Discussion

The membranes used in this study were effective in preventing adherences, which is similar to the reports in literature [10,29]. However, other membranes are non-absorbable or when absorbable, their long absorption time can negatively affect the healing process [41]. The low crystallinity of this study's copolymer makes it more biocompatible [30,42].

The use of TMC provided additional elasticity, which is favorable in achieving a better fit to the tendon with less interference within the tendon's energy transmission. Some studies suggested that CS and DS concentrations are higher when the pathologic conditions of the tendon are considered [12,21,24]. This finding was confirmed for CS in the present study. However, the DS in normal tendons was higher than that ones for all groups. Assessments of CS at 7 days showed a statistically significant difference for distal samples favorable to the

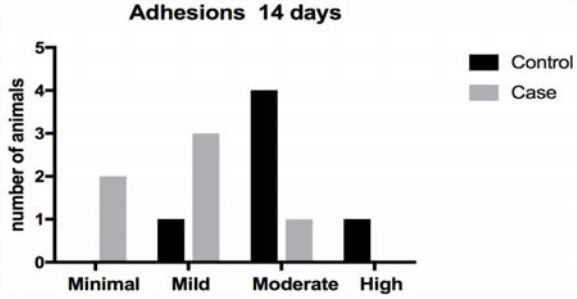

Chart 1: Adhesions/adherences 14 Days.

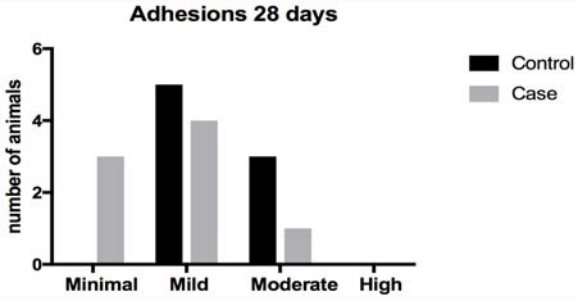

Chart 2: Adhesions/adherences 28 Days.

case group, a difference for proximal samples favorable to the case group, and a difference for central samples favorable to the control group. Assessments of CS at 14 days showed equivalence for all samples. These data demonstrate that CS was elevated in all areas, which contributed to the healing process. These data, associated posterior macroscopic and histologic findings, contribute to suggest that within the earlier phases of the healing process the GAGs levels can be affected by the membrane using, accelerating healing.

The membrane seemed to cause delay in the elevation of chondroitin concentration for the central part of the tendon, which improved in the following week, and an elevation on other parts during the first week?

The assessment at 7 days showed differences on DS concentrations between the groups favorable to the case group at the proximal tendon, statistically significant difference favorable to the control group in the central part of the tendon, and no difference at the distal tendon.

The liquid consistency, markedly within the tendon's central portion for the case group assessed at 7 days and a statistical difference on HA concentrations suggest that this hydrophilic substance has an important role during the very early healing process, as mentioned by other authors [43,44].

DS demonstrated equivalence between the case and control groups in the assessment at 14 days.

The membrane seemed to change the sulfated GAGs concentrations in all tendon areas for the first stages of the healing processes.

Some studies associated higher sulfated GAGs to inferior tendon biomechanics in the long term [18]. However, Özer et al. [44] suggested an improvement in tendon healing for rats treated with oral glucosamine and CS. Other authors found elevations on CS levels during the healing process for ligaments [46].

In the present study, healing process seems also to present a positive correlation with CS levels in short term assessments for tendons.

In this study, the DS level was lower in pathologic tendons than that in normal tendons at any time point, but the CS level was elevated; CS was not even present in intact tendons. Thus CS appears to have an important role in the early stages of healing process but not for mature tendons [45]. The modified Watkins [37] classification demonstrated that healing process was superior in the case group at 14 and 28 days. Tendon healing was also improved in other situations, such as tendon bone healing assisted by absorbable membranes [47].

With regard to the objective data from the polarized light measurement, an improvement of type I collagen at 14 and 28 days in the case groups was observed. The 28-day improvement established a definitive difference between the groups according to the Haybittle-Peto boundary [48]. Fewer adherences seem to be the key to achieve accelerated healing. It allows early mechanical stimulus. Once the mechanical stimulus is associated with better healing results and the energy direction is better canalized using the membrane the force lines will be better oriented, mitigating radial dissipation through the scars [49,50].

Limitations

This is a preclinical trial; therefore clinical trials are necessary to understand whether the good results presented in this study can be replicated for humans.

Macroscopic assessments cannot be blinded with possible bias for this outcome.

The absence of detectable harms for the animals enrolled does not warrant that harms will not take place for animals nor humans.

Conclusion

The absorbable membrane promoted early and better mechanical stimulus within more balanced responses, accelerating the healing process by mitigating radial energy dissipation through the scars. It also presented a short-term degradation that mitigated any long-term complications that absorbable devices can promote. The presented membrane could change the GAGs and histological structure, accelerating calcaneal tendon healing in New Zealand rabbits.

References

1. Enwenka CS. Inflammation, cellularity and fibrillogenisis in regenerating tendon: Implications for tendon rehabilitation. Physical Therapy. 1989;69(10):816-25.
2. O'Brien M. Structure and metabolism of tendons. Scandinavian J Med Sci Sports. 1997;7:5-61.
3. Kannus P. Structure of the tendon connective tissue. Scandinavean J Med Sci Sports. 2000;10(6):312-20.
4. Bey MJ, Derwin KA. Measurement of *in vivo* tendon function. J Shoulder Elbow Surg. 2012;21:149-57.
5. Dy CJ, Daluiski A, Do HT, Hernandez-Soria A, Marx R, Lyman S. The epidemiology of reoperation after flexor tendon repair. J Hand Surg (Am). 2012;37:919-24.
6. Hagglund M, Waldén M, Ekstrand J. Previous injury as a risk factor for injury in elite football: A prospective study over two consecutive seasons. Br J Sports Med. 2006;40:767-72.
7. Petersen J, Thorborg K, Nielsen MB, Hölmich P. Acute hamstring injuries

in Danish elite football: A 12-month prospective registration study among 374 players. Scandinavian J Med Sci Sports.2010;20(4):588-92.

8. Wilkins R, Bisson LJ. Operative *vs.* nonoperative management of acute Achilles tendon ruptures: A quantitative systematic review of randomized controlled trials. Am J Sports Med. 2012;40(9):2154-60.

9. Jacobsen E, Dart AJ, Mondori T, Horadogoda N, Jeffcott LB, Little CB, et al. Focal Experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. PLoS ONE. 2015;10(4):e0122220.

10. Zhao H, Guan HG, Luo ZP, Gu J, Luo ZP, Zhang W, et al. Collagen membrane alleviates peritendinous adhesion in the rat Achilles tendon injury model. Chinese Med J. 2013;126(4):729-33.

11. Yoon JH, Halper J. Tendon proteoglycans: Biochemistry and function. J Musculoskelet Neuronal Interact. 2005;5(1):22-34.

12. Smith MM, Sakurai G, Smith SM, Young AA, Melrose J, Stewart CM, et al. Modulation of aggrecan and ADAMTS expression in ovine tendinopathy induced by altered strain. Arthritis Rheumatol. 2008;58(4):1055-66.

13. Voleti PB, Buckley MR, Soslowsky LJ. Tendon healing: Repair and regeneration. Ann Review Biomed Eng. 2012;14:47-71.

14. Crevier-Denoix N, Collobert C, Pourcelot P, Denoix JM, Sanaa M, Gelger D, et al. Mechanical properties of pathological equine superficial digital flexor tendons. Equine Vet J Suppl. 1997;23:23-6.

15. Abate M, Gravare Silbernagel K, Siljeholm C, Di Iorio A, De Amicis D, Salini V, et al. Pathogenesis of tendinopathies: Inflammation or degeneration? Arthritis Res Ther. 2009;11(3):235.

16. Dakin SG, Dudhia J, Smith RKW. Resolving an inflammatory concept: The importance of inflammation and resolution in tendinopathy. Vet Immunol Immunopathol. 2014;158(3-4):121-7.

17. Sharma P, Maffulli N. Biology of tendon injury: Healing, modeling and remodeling. J Musculoskelet Neuronal Interact. 2006;6(2):181-90.

18. Jacobsen E, Dart AJ, Mondori T, Horadogoda N, Jeffcott LB, Little CB, et al. Focal Experimental injury leads to widespread gene expression and histologic changes in equine flexor tendons. PLoS ONE. 2015;10(4):e0122220.

19. Yoon JH, Halper J. Tendon proteoglycans: Biochemistry and function. J Musculoskelet Neuronal Interact. 2005;5(1):22-34.

20. Choi RK, Smith MM, Martin JH, Clarke JL, Dart AJ, Little CB, et al. Chondroitin sulphate glycosaminoglycans contribute to widespread inferior biomechanics in tendon after focal injury. J Biomech. 2016;49(13):2694-701.

21. Archambault JM, Jelinsky SA, Lake SP, Hill AA, Glaser DL, Soslowsky LJ. Rat supraspinatus tendon expresses cartilage markers with overuse. J Orthoped Res. 2007;25: 617-24.

22. Lo IKY, Marchuk LL, Leatherbarrow KE, Frank CB, Hart DA. Collagen Fibrillogenesis and mRNA levels in the maturing rabbit medial collateral ligament and patellar tendon. Connect Tissue Res. 2004;45(1):11-22.

23. Riley GP, Harrall RL, Constant CR, Chard MD, Cawston TE, Hazleman BL. Glycosaminoglycans of human rotator cuff tendons: Changes with age and in chronic rotator cuff tendinitis. Ann Rheumatic Dis. 1994;53(6):367-76.

24. Scott PG, MacEwan PA, Dodd CM, Bergmann EM, Bishop PN, Bella J. Crystal structure of the dimeric protein core of decorin, the archetypal small leucine-rich repeat proteoglycan. Proc Natl Acad Sci U S A. 2004;101(44):15633-8.

25. Attia M, Carpentier G, Etienne S, Thierart S, Scott A, Oystein L, et al. Histochemical analysis of GASs in altered tendon: A means to evaluate the degree of pathology. Br J Sports Med. 2013;47(9):e2.

26. Helland C, Bojsen-Møller J, Raastad T, Seynnes OR, Moltubakk MM, Jakobsen V, et al. Mechanical properties of the patellar tendon in elite volleyball players with and without patellar tendinopathy. Br J Sports Med. 2013;47(13):862-8.

27. Kongsgaard M, Qvortrup K, Larsen J, Aagaard P, Doessing S, Hansen P, et al. Fibril morphology and tendon mechanical properties in patellar tendinopathy: Effects of heavy slow resistance training. Am J Sports Med. 2010;38(4):749-56.

28. Åstrom M, Rausing A. Chronic Achilles tendinopathy: A survey of surgical and histopathologic findings. Clin Orthoped Related Res. 1995;316:151-64.

29. Zhao S, Xie X, Pan G, Shen P, Zhao J, Cui W. Healing improvement after rotator cuff repair using gelatin-grafted poly-(L-lactide) electrospun fibrous membranes. J Surg Res. 2015;193(1):33-42.

30. Motta AC, Duek EAR. Synthesis, characterization and "*in vitro*" degradation of Poly (L-lactic acid). Polímeros: Ciência e Tecnologia. 2006;16(1):26-32.

31. Chow SC, Chang M. Adaptive design methods in clinical trials - a review. Orphanet J Rare Dis. 2008.

32. Dietrich CP, Dietrich SM. Electrophoretic behavior of acidic mucopolysaccharides in diamine buffers. Anal Biochem. 1976;70(2):645-7.

33. Franciozi CE, Tarini VA, Reginato RD, Gonçalves PR, Medeiros VP, Ferretti M, et al. Gradual strenuous running regimen predisposes to osteoarthritis due to cartilage cell death and altered levels of glycosaminoglycans. Osteoarthritis Cartilage. 2013;21(7):965-72.

34. Martins JR, Passerotti CC, Maciel RM, Sampaio LO, Dietrich CP, Nader HB. Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. Anal Biochem. 2003;319(1):65-72.

35. Merli LA, de Medeiros VP, Toma L, Reginato RD, Katchburian E, Nader HB, et al. The low level laser therapy effect on the remodeling of bone extracellular matrix, Photochem. Photochem Photobiol. 2012;88(5):1293-301.

36. Martin JR, Passerotti CC, Maciel RM, Sampaio LO, Dietrich CP, Nader HB. Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. Anal Biochem. 2003;319(1):65-72.

37. Watkins JP, Auer JA, Gay S, Morgan SJ. Healing of surgically created defects in the equine superficial digital flexor tendon: collagen-type transformation and tissue morphologic reorganization. Am J Veter Res. 1985;46(10):2091-6.

38. Amiel D, Frank CB, Harwood FL, Akeson WH, Kleiner JB. Collagen alteration in medial collateral ligament healing in a rabbit model. Connect Tissue Res. 1986;16:357-66.

39. Liu SH, Panossian V, Al-Shaikh R, Tomin E, Shepherd E, Finerman GA, et al. Morphology and matrix composition during early tendon to bone healing. Clin Orthoped Related Res. 1997;339:253-60.

40. Liu SH, Yang RS, Al-Shaikh R, Lane JM. Collagen in tendon, ligament, and bone healing: A current review. Clin Orthoped Related Res.1995;318:265-78.

41. Ambrose C, Clanton T. Bioabsorbable implants: Review of clinical experience in orthopedic surgery. Ann Biomed Eng. 2004;32(1):171-7.

42. Anderson J, Rodriguez A, Chang D. Foreign body reaction to biomaterials. Semin Immunol. 2008;20(2):86-100.

43. Park JU, Tsuchiya T. Increase in gap junctional intercellular communication by high molecular weight hyaluronic acid associated with fibroblast growth factor 2 and keratinocyte growth factor production in normal human dermal fibroblasts. Tissue Eng. 2002;8(3):419-27.

44. Solis AM, Chen YH, Wong TY, Bittencourt VZ, Lin YC, Huang LL. Hyaluronan regulates cell behavior: A potential niche matrix for stem cells. Biochem Res Int. 2012.

45. Özer H, Taskesen A, Kul O, Selek HY, Turanli S, Köse K. Glukozamin kondroitinsülfatınonarılmış tenotomizesıçanAşiltendonlarıüzerineetkisi. Joint Dis Related Surg. 2011;22(2):100-6.

46. Bosh U, Gässler N, Decker B. Alterations of glycosaminoglycans during patellar tendon autograft healing after posterior cruciate ligament replacement. Am J Sports Med. 1998; 26(1):103-8.

47. Yokoya S, Mochizuki Y, Nagata Y, Deie M, Ochi M. Tendon-bone insertion repair and regeneration using polyglycolic acid sheet in the rabbit rotator cuff injury model. Am J Sports Med. 2008;36(7):1298-309.

48. Pocock SJ. When to stop a clinical trial for benefit. J Am Med Assoc. 2005;294(17):2228-30.

49. Juncosa-Melvin N, Matlin KS, Nirmalanandhan VS, Butler DL. Mechanical stimulation increases collagen type I and collagen type III gene expression of stem cell-collagen sponge constructs for patellar tendon repair. Tissue Eng. 2007;13(6):1219-26.

50. Qin TU, Sun YL, Thoreson AR, Steinmann SP, Amadio PC, An KN, et al. Effect of mechanical stimulation on bone marrow stromal cell seeded tendon slice constructs: A potential engineered tendon patch for rotator repair. Biomaterials. 2015;51:43-50.