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Flexor tendon repair using a reinforced tubular, medicated electrospun construct

Running title: Reinforced medicated electrospun flexor repair

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jor.25103.

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Flexor tendon repair using a reinforced tubular, medicated electrospun construct

Abstract

A reinforced tubular, medicated electrospun construct was developed for deep flexor tendon repair. This construct combines mechanical strength with the release of anti-inflammatory and anti-adhesion drugs. In this study, the reinforced construct was evaluated using a rabbit model. It was compared to its components (a tubular, medicated electrospun polymer without reinforcement and a tubular braid as such) on the one hand to a modified Kessler suture as a control group. Forty New Zealand rabbits were randomly divided into two groups. Surgery was performed in the second and fourth deep flexor tendons of one hind paw of the rabbits in the two groups using four repair techniques. Biomechanical tensile testing and macroscopic and histological evaluations were performed at 3- and 8 weeks postoperatively. A two-way analysis of variance with pairwise comparisons revealed that the three experimental surgical techniques (a reinforced tubular medicated electrospun construct, tubular medicated construct, and tubular braid as such) showed similar strength as that of a modified Kessler suture repair, which was characterized by a mean load at ultimate failure of 19.85 N (standard deviation [SD] 5.29 N) at 3 weeks and 18.15 N (SD 8.01 N) at 8 weeks. Macroscopically, a significantly different adhesion pattern was observed at the suture knots, either centrally or peripherally, depending on the technique. Histologically, a qualitative assessment showed good to excellent repair at the tendon repair site, irrespective of the applied technique. This study demonstrates that mechanical and biological repair strategies for flexor tendon repair can be successfully combined.

Graphical abstract

A reinforced medicated electrospun construct for flexor tendon repair was investigated in a rabbit tendon model. The construct was compared to its individual components and to a modified Kessler suture. Biomechanical tensile testing, macroscopic and histological evaluation performed at week 3 and week 8 post-operatively, showed similar strength as a modified Kessler suture repair, a distinct pattern of adhesion, and good to excellent histologic repair. This study demonstrates that biologic and mechanical repair can successfully be combined.

Keywords: flexor tendon repair; electrospinning; tubular braided construct; medication release; rabbit model

1. Introduction

The quest for a perfect repair strategy for flexor tendon lesions continues to be one of the most intriguing research topics in hand surgery. An online search for flexor tendon repair within hand surgery in the Web of Science shows 462 publications during the last decade. When reviewing these, it appears that the focus has shifted from mechanical improvements, such as better suture techniques or early mobilization protocols towards biological improvements to counter adhesion formation, increase gliding, and improve the biological environment for healing. The field of regenerative medicine has also focused on tendon repair, and scaffolds that can release drugs or even incorporate mesenchymal stem cells have also been developed¹⁻³. A known

limitation of these scaffolds is that the necessary strength for immediate rehabilitation cannot be provided⁴. Paradoxically, when using these scaffolds, complete immobilization would be necessary to protect the repair, which would neutralize all possible biological advantages. Currently, these scaffolds can only be used in addition to standard suture repairs⁴.

The idea of combining mechanical strength with a biological approach is a logical step. Therefore, in a prior study, a repair strategy that targets both of these aspects was developed⁵. A construct was designed that consists of an electrospun, acrylate-end-capped urethane-based precursor/poly(ϵ -caprolactone) (AUP/PCL) polymer that releases hyaluronic acid and naproxen. This construct is reinforced with an incorporated tubular braid that acts as a Chinese finger trap and should provide the necessary mechanical strength. When elongation of the repaired complex occurs, the tubular braid tightens and grasps the tendon because of the braided configuration. This type of design has been shown to mimic the stress-strain behavior of tendons⁴. Hyaluronic acid has been proven to promote gliding, while naproxen has the ability to counter adhesion formation around the repair complex^{2,6}. The concept, choice of materials, characterization, biocompatibility, and mechanical *ex vivo* tests have been investigated and described in a previous publication⁵. However, the biological effects of the medicated polymers and the influence of the construct on vascularization of a tendon cannot be tested in an *ex vivo* setting.

Therefore, in the present study, this newly designed construct was evaluated *in vivo* using a rabbit model. Not only the complete reinforced tubular, medicated construct, but also its two individual components, (i) a tubular electrospun AUP/PCL polymer that releases hyaluronic acid/naproxen without a tubular braid as reinforcement, and (ii) a tubular braid without medicated electrospun polymers, were compared with the

gold standard modified Kessler suture. The performance of each of these four repair techniques was evaluated by biomechanical tensile testing, macroscopic evaluation, and histological analysis.

2. Materials and methods

2.1 Design of the different repair constructs

Technique 1: ‘Reinforced, medicated construct’ (Fig. 1 A-C)

The design of the electrospun construct, consisting of (i) an AUP/PCL layer containing naproxen, (ii) an interspersed tubular braid, and (iii) an AUP/PCL layer containing hyaluronic acid, has been described previously⁵. In brief, an acrylate end-capped urethane-based precursor (AUP) material with a PCL backbone (MM 2000 g·mol⁻¹) was synthesized, and together with PCL processed into an electrospun tube using an in-house developed electrospinning (ES) set-up (Fig. 1A)⁵. After optimization of the electrospinning parameters, a polymer ratio of 1:3 for AUP/PCL was selected. The electrospinning solution comprised 4 wt% AUP and 12 wt% PCL in chloroform with 2 mol% photo-initiator TPO-L with respect to the amount of reactive double bonds. Electrospinning was performed on a 2.5-mm diameter mandrel. The mandrel was set at a rotating speed of 180 rpm, subjected to 15 kV DC voltage, with an 18-cm needle tip-to-mandrel distance and a 7-cm mandrel-to-ground distance using a 0.495 mm needle.

Figure 1. (A) Schematic overview of the electrospinning set-up (B) Illustration of the complete reinforced tubular, medicated electrospun construct showing the two electrospun layers with an interspersed tubular braid (C) Tubular reinforced, medicated electrospun construct (D) Tubular, medicated electrospun polymer without braid (E) Tubular braid without medicated electrospun polymers.

First, an AUP/PCL electrospun tube was created with an anti-inflammatory function, whereby naproxen (Fagron) was added as 1.47 wt% of the solution^{2,7}. Next, a tubular braid was mounted over this layer as reinforcement. This braid is made of polypropylene strands of 0.09 mm, has a length of 15 mm, and a diameter of 2.5 mm. Finally, a second AUP/PCL layer was created with an anti-adhesion function by adding hyaluronic acid (HA) (0.99 wt% of the total solution)^{3,8,9} (TRB Chemedica). Naproxen and HA were selected together because naproxen can increase the effectiveness of HA¹⁰. Finally, a post-electrospinning ultraviolet (UV) light treatment was performed by UV-A irradiation at $6 \text{ mW} \cdot \text{cm}^{-2}$ for 60 min.

Technique 2: 'Medicated construct' (Fig. 1D)

In this technique, the same protocol and processing parameters were used as described for technique 1 (*vide supra*), but there was no incorporation of a tubular braid. In other words, an electrospun AUP/PCL-medicated polymer tube was used, consisting of two electrospun layers with naproxen and hyaluronic acid. This construct did not have sufficient intrinsic strength⁵ and was therefore combined with a modified Kessler suture. Using this construct without reinforcement allows the evaluation of possible biological advantages of the AUP/PCL-medicated polymers without interference from the braid.

Technique 3: 'Tubular braid' (Fig. 1E)

In this technique, a tubular braid without any medicated electrospun polymers was used. This braid is made of polypropylene strands of 0.18 mm, has a length of 15 mm, and a diameter of 2.5 mm. Using this construct without medicated polymers allows the evaluation of the mechanical strength of the braid without medication interference.

Technique 4: 'modified Kessler'

The modified Kessler was used as a gold standard control technique.

2.2 Animals

Forty 18-24-weeks-old female New Zealand White rabbits with an average weight of 4.6 ± 0.3 kg were used (Cegav S.S.C., France). The ethics committee of the Faculty of Veterinary Medicine of Ghent University, Merelbeke, Belgium, approved the study (2019-60). All animals received humane care in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 85-23).

Before surgery, all rabbits were housed individually in standard rabbit cages for a minimum of 7 days. An overview of the grouping details is presented in Figure 2. The

rabbits were randomly divided into two groups of 20 rabbits for evaluation at 3 and 8 weeks after surgery to assess the early and late stages of tendon healing. Surgery was performed in the second and fourth deep flexor tendons of one hind paw using one of the four repair techniques described below. Techniques 1 and 2 were always used in the same paw because the constructs used for these techniques both release the same medication. Therefore, other combinations were not possible because undesired dispersion of medication could potentially influence the results. Whether a technique was used in the second or fourth tendons was randomly determined. This implies that 10 samples were available for analysis at 3 weeks and 10 at 8 weeks for each technique. Of these 10 samples, eight were used for mechanical testing, and two were used for histological analysis.

Figure 2. Overview of grouping details

2.3 Premedication and anesthesia

All rabbits were sedated with intramuscular (IM) injection of medetomidine (0.2-0.3 mg/kg) and buprenorphine (0.03-0.05 mg/kg). Subsequently, IM ketamine (10 mg/kg) was used to induce anesthesia and a laryngeal mask (V-gel®) was placed to

administer isoflurane. An intravenous (IV) catheter was inserted into the marginal ear vein (after applying EMLA® crème) to administer doxapram 5 mg/kg IV in case of apnea. No antibiotic prophylaxis was administered.

2.4 Injury induction

After positioning the rabbit in dorsal decubitus, the operative site on the right hind paw was clipped and surgically prepared (chlorhexidine scrub and alcoholic disinfection). A proximal unloading tenotomy of the common deep digital flexor was performed proximal to the heel, as described by Berglund et al.¹¹. Next, the flexor digitorum profundus (FDP) tendon rupture model described by Chou et al.¹² was used. Briefly, a longitudinal midline incision was made on the plantar side between the second and third metatarsi. After subcutaneous dissection, flexor digitorum superficialis (FDS) tendons were identified, and the second and fourth FDS were removed over a length of 2 cm to expose the underlying FDP tendons. A full transverse section was then performed midway through the second and fourth FDP tendons, which were subsequently repaired using one of the four techniques described below. The skin was closed using subcutaneous (vicryl 4-0 Ethicon) and intradermal sutures (monocryl 3-0 Ethicon). Finally, a custom-made splint using a thermoplastic strip was applied to immobilize the phalanges.

2.5 Surgical techniques

Reinforced, medicated construct: During surgery, the two ends of the transected tendon were pulled through the construct using a temporary simple suture (polydioxanone suture [PDS] 4-0, Ethicon). As a result, the tendon ends were pulled together to achieve an overlap of the tendon ends of 3 mm. The construct was then fixed to the tendon surface at the periphery of the construct using three simple surgical knots (PDS 4-0).

Medicated construct: During surgery, the first part of the modified Kessler suture (PDS 4-0) was placed on the proximal tendon end. Next, the suture threads were pulled through the polymer structure, which was then glided halfway over the proximal tendon end. Finally, the modified Kessler suture was completed at the distal end of the tendon. To complete the suture and tighten the knot, the tubular polymer was divided longitudinally until midway through the construct. The cut was closed after completion with a simple surgical knot using a PDS 4-0.

Tubular braid: During surgery, the same surgical steps as in technique 1 were used, including the insertion of the two ends of the tendon in the tubular construct with temporary sutures and fixation of the braided construct to the tendon surface with three simple surgical knots on both ends.

Modified Kessler: In this control group, the sectioned tendon was reconstructed using a two-stranded modified Kessler suture with PDS 4-0.

2.6 Postoperative protocol

During the first 3 weeks, the rabbits were housed individually. Subsequently, they were transferred to group housing to improve animal welfare. With no lack of access to food and water, a standard rabbit diet was provided in both housing conditions.

During the first 5 days, a daily dose of meloxicam 1 mg/kg was administered. During the first week, eating, drinking, production of fecal pellets and cecotropes, weight, and temperature were monitored daily. From the second week onwards, weight and temperature were measured twice per week. An inspection for swelling and wound or bandage problems was conducted daily.

2.7 Euthanasia

All rabbits were anesthetized using xylazine 5 mg/kg IM and ketamine 10 mg/kg IM. The animals were then euthanized by injecting sodium-pentobarbital (0.5 g/kg) IV in

the lateral ear vein. Immediately after euthanasia, the hind legs were transected. Within 1 h, dissection of the tendons was performed with either immediate macroscopic evaluation, tensile testing, or preservation for further histological analysis.

2.8 Biomechanical testing

Tensile testing was performed using a hydraulic tensile testing machine (LRX Plus with a 100N load cell, Lloyd Instruments, Bognor Regis, UK) using custom-made clamps with serrated teeth. Each tendon was secured between the clamps using sandpaper to prevent slippage. During testing, the tendons were kept moist by spraying them with a saline solution. The repaired tendons were first pre-tensioned at 0.5 N, and then a distraction was performed at a rate of 5 mm/min until complete failure¹³. Ultimate load, extension from preload at maximum load, and stiffness were recorded using Nexygen Plus software. As planned, 32/32 tendons available for tensile testing were used.

2.9 Macroscopic evaluation

A macroscopic evaluation of the repaired tendons (32/32) was performed immediately after euthanasia. The presence of adhesions around the repair site was evaluated using a frequently used visual scale¹⁴: grade 1, no adhesions; grade 2, adhesion area requiring blunt dissection alone for separation; grade 3, adhesion area less than or equal to 50% requiring sharp dissection for separation; grade 4, 51–97.5% adhesion area requiring sharp dissection for separation; grade 5, >97.5% adhesion area requiring sharp dissection for separation. One adaptation was made; instead of grading adhesion only centrally, a second grading was also applied more peripherally at 7.5 mm from the center on each side.

2.10 Histological analysis

After dissection, the tendons used for histological examination were retrieved and immersed in 10% formaldehyde. Because of an infected hind paw in one rabbit, two tendons, treated with two different techniques, were excluded from further analysis. A transverse cut immediately adjacent to the repair zone was performed, and the remainder of the sample was cut longitudinally. After technical and qualitative evaluation of the sections, one sample of each technique at each period was selected for further analysis (8/16 tendons available for histology). The samples were then serially dehydrated in ethanol and embedded in paraffin wax to produce tissue sections of 5- μ m thickness on positively charged glass slides. The slides were further heated in a 70°C oven followed by immersion in xylene solution for 5 min, and the process was repeated three times. The deparaffinized samples were then rehydrated and subjected to hematoxylin and eosin (H&E) and Masson's trichrome staining to analyze connective tissue, and immunohistochemical CD31 staining (Agilent, JC70A, 1/5 dilution) to analyze the number of blood vessels. The tissue sections were examined under an upright light microscope (Olympus BX3 microscope, Tokyo, Japan), and pictures were taken using an Olympus U-TVO.5XC-3 camera. Because of the limited number of samples available for histology, only a qualitative evaluation could be performed.

Tendon repair quality was examined on longitudinal histologic sections using a four-point grading scale based on Tang by a blinded observer¹⁵. The following four parameters were considered: the size of gap formation, newly formed collagen fibers, type of inflammation, and vascularization. The quality of tendon repair was classified as follows: (1) excellent – perfect continuity of the repaired zone, presence of collagen fibers, few inflammatory cells, and vascularization; (2) good - small gap formation, presence of newly formed collagen fibers, inflammatory cells, and

vascularization; (3) fair – the presence of gap formation, minor presence of newly formed collagen fibers, a moderate amount of inflammatory cells, and vascularization, (4) poor - large gap formation, no presence of newly formed collagen fibers, numerous inflammatory cells, and vascularization.

To estimate the influence of the different repair techniques on vascularization, the total number of blood vessels per mm² was counted manually by two independent observers (at 10x magnification) on transverse CD31- immunohistochemical staining sections at 3 and 8 weeks for each technique using LAS V4.1 software (Leica Microsystems).

2.11 Statistics

For the biomechanical tests, a total of 32 rabbits were chosen based on power analysis with a power of 0.80, an effect size of 0.65, and a significance level (alpha) of 0.05 for the biomechanical testing⁸. Furthermore, eight additional rabbits were added for histological analysis. Because normality (Shapiro-Wilk test) and equality of variances (Levene's test) could be assumed, parametric testing was performed. For each dependent factor (ultimate load, extension from preload, stiffness), a two-way analysis of variance (ANOVA, tests of between-subjects effects) was conducted to investigate the effects of both postsurgical periods, techniques, and possible interactions. Further pairwise comparisons were conducted to determine the significance. Pearson's chi-square (Fisher's exact) test was conducted to investigate the significance of the macroscopic evaluation of adhesion formation. For histological evaluation, only qualitative descriptions were provided. All statistical analyses were performed using SPSS version 26.0 (SPSS Inc., Chicago, IL, USA).

3. Results

In this section, first, the results of the biomechanical tests are presented. Second, macroscopic differences between the techniques are demonstrated. Finally, histological findings are illustrated.

3.1 Biomechanical testing

The results of biomechanical testing are shown in figure 3.

For the ultimate load at failure, the two-way ANOVA showed statistically significant differences between the postsurgical periods ($p = 0.05$) and the interaction between the postsurgical periods and techniques ($p = 0.018$). There was no statistically significant difference between the techniques ($p = 0.551$). Subsequently, a pairwise comparison was conducted for the interaction between the postsurgical periods and techniques, showing a statistically significant difference only for the medicated construct between 3 and 8 weeks ($p = 0.001$).

For the extension from preload at maximum load, the two-way ANOVA showed a significant difference between the techniques ($p = 0.008$). There was no statistically significant difference between the postsurgical periods ($p = 0.498$) and the interaction between the postsurgical periods and techniques ($p = 0.184$). Subsequently, a pairwise comparison was conducted to compare the techniques showing statistically significant difference only between techniques using the medicated construct and the tubular braid ($p = 0.006$).

For stiffness, the two-way ANOVA showed no statistically significant difference between the postsurgical periods ($p = 0.094$), techniques ($p = 0.221$), and their interaction ($p = 0.054$).

Figure 3. Results of biomechanical testing of the four techniques at 3 and 8 weeks.

(A) Ultimate load at failure, (B) extension from preload at maximum load, (C) stiffness. The data are shown as the mean $\pm$ standard deviation (SD). Asterisks indicate statistically significant differences after pairwise comparisons: * p=0.001, ** p=0.006. Blue: reinforced, medicated construct, orange: medicated construct, grey: tubular braid, yellow: modified Kessler.

3.2 Macroscopic evaluation

Macroscopic samples of each technique are shown in figure 4, and the results of the macroscopic evaluation are shown in figure 5. All techniques showed a slight decrease in adhesion formation between 3 and 8 weeks postoperatively. A significantly different pattern can be distinguished with the techniques using the tubular braid (reinforced, medicated construct and tubular braid as such), which predominantly showed peripheral adhesions, whereas the techniques using the Kessler suture (medicated construct and modified Kessler) mainly showed central adhesions.

Figure 4. Macroscopic samples. (A) Reinforced, medicated construct (B) Medicated construct (C) Tubular braid (D) Modified Kessler

Figure 5. (A) Mean total scores on adhesion formation at 3 weeks postoperatively. (B) Mean total scores on adhesion formation at 8 weeks postoperatively. Techniques with (*) are each individually significantly different ($p < 0.05$) from those with (#).

3.3. Histological analysis

Qualitative measurements of the tendon repair quality grading are shown in Table 1. Histological images of the longitudinal sections stained with H&E and Masson's staining are shown in figures 6 and 7, respectively. The reinforced, medicated construct showed good healing with a gap of 0.25 mm at 3 weeks and excellent healing with continuity of the repaired zone at 8 weeks. The medicated construct showed good healing with a gap of 0.75 mm at both 3 and 8 weeks, respectively. Less inflammatory cells and vascularization were observed with the medicated construct compared to that with the reinforced, medicated construct. The tubular braid showed

fair healing with a gap of 1.5 mm at 3 weeks and excellent healing with continuity of the repaired zone at 8 weeks. The modified Kessler suture showed good healing with a gap of 0.75 mm at 3 weeks and excellent healing with continuity of the repaired zone at 8 weeks.

The connective tissue surrounding the repair site was also investigated histologically. In the techniques using the AUP/PCL polymers with hyaluronic acid/naproxen, a larger number of macrophages and multinucleated foreign body giant cells were observed (Figure 8A) compared to that with the tubular braid and modified Kessler techniques (Figure 8B). Additional pan-macrophage CD 68 – immunohistochemical staining (Dako, clone KP1, 1/200 dilution) demonstrated stronger cytoplasmic granular staining in the macrophages and multinucleated giant cells in the technique using AUP/PCL polymers with hyaluronic acid/naproxen (Figure 8C) compared to the modified Kessler (Figure 8D).

	Week 3	Week 8
Reinforced, medicated construct	Good	Excellent
Medicated construct	Good	Good
Tubular braid	Fair	Excellent
Modified Kessler	Good	Excellent

Table 1. Grading scales qualitative histological examination of the repaired region

Figure 6. Longitudinal histologic sections of all four techniques at 3- and 8 weeks postoperatively. H&E staining. The region of interest is indicated at 12.5x magnification and enlarged at a 100x magnification. Single scalebar, 800 micron; double scalebar, 100 micron.

Figure 7. Longitudinal histological sections of all four techniques at 3- and 8 weeks postoperatively. Masson's trichrome staining. Region of interest is indicated at 12.5x magnification and enlarged at a 100x magnification. Single scalebar, 800 micron; double scalebar, 100 micron.

Figure 8. Illustration of the stronger macrophagic reaction in the connective tissue surrounding the repair in the medicated constructs compared to that in the modified Kessler control group (A) H&E staining, medicated construct demonstrating larger

presence of macrophages and multinucleated foreign body giant cells (B) H&E staining, modified Kessler showing fewer macrophages and no multinucleated foreign body giant cells (C) CD 68 staining, medicated construct: strong granular cytoplasmic CD68 staining in macrophages and multinucleated foreign body giant cells (multinucleated foreign body cells are marked in boxes) (D) CD 68 staining, modified Kessler with only punctate presence of macrophages. Magnification 200x, scalebar 50 micron.

For the reinforced medicated construct, the mean number of blood vessels on a transverse section was 16.9/mm² at 3 weeks and 41.8/mm² at 8 weeks. For the medicated construct (Fig. 9), the mean number of blood vessels was 14.3/mm² at 3 weeks and 23.7/mm² at 8 weeks. For the tubular braid, the mean number of blood vessels was 18.1/mm² at 3 weeks and 22.9/mm² at 8 weeks. For the modified Kessler, the mean number of blood vessels was 28.1/mm² at 3 weeks and 14.4/mm² at 8 weeks.

Figure 9. Example of transverse histologic section (medicated construct) stained with CD31 immunohistochemical staining. Black arrowhead illustrating one blood vessel. Scalebar 20 micron.

4. Discussion

In this *in vivo* study, biomechanical, macroscopic, and histological analyses were performed for four different techniques to repair flexor tendons. Based on previous studies, a rabbit tendon model was used as a model for human flexor tendons^{8,16}. The modified Kessler suture was used as the control group because it is the most used technique in biomechanical studies both in hand surgery and regenerative medicine studies using rabbit tendon models¹⁶.

In the biomechanical tests, the ultimate load to failure showed no statistically significant differences between the experimental techniques and the control group. This demonstrates that using a tubular braid as a repair strategy for tendon rupture has a strength comparable to that of the modified Kessler suture. Notably, the tubular braid with a smaller filament size (0.09 mm) used as a mechanical support in the reinforced medicated construct showed similar strength as that of the tubular braid in technique 3 (0.18 mm). Furthermore, the techniques constituting the AUP/PCL polymers containing hyaluronic acid and naproxen also exhibited comparable strength, which indicates that the medication release does not adversely influence the strength of the repair.

A significant difference was observed between the ultimate load to failure at 3 and 8 weeks. Evaluation of the graphs and post-hoc analysis indicates that the increase in strength of the medicated construct between 3 and 8 weeks may have caused this group difference. The reinforced medicated construct also increased in strength but failed to reach significance. Although one would expect the tendon repair complexes to become stronger over time, a previous study similar to ours failed to show significant differences between the postoperative periods¹.

Adhesion formation is one of the main causes of unsatisfactory postoperative results after flexor tendon surgery. It can lead to stiffness and pain, and sometimes necessitates a second surgery to release the repaired tendon. In this study, there were two distinct patterns of adhesion (figure 5). In the reinforced, medicated construct and the tubular braid techniques, adhesions were observed at the constructs' peripheral ends and not centrally where the actual repair zone was located. This is illustrated in figure 10. It can be hypothesized that these adhesions were provoked by multiple exposed interrupted suture knots, rather than by the construct itself. Conversely, in the medicated construct and the modified Kessler techniques, adhesions were observed more centrally, again at the suture knot. It is known that monofilament sutures require additional half-hitches compared to that with braided sutures because of their poorer loop-holding capability and inherently low coefficient of friction¹⁷. This creates longer and sharper knots that promote adhesion. In hindsight, an adaptation in the protocol using a continuous suture technique with only one suture knot or a (thin) multifilament suture that requires fewer half-hitches could potentially lead to a better evaluation of the anti-adhesion and anti-inflammatory effects of the medicated electrospun constructs.

Although the anti-adhesion and anti-inflammatory effects of the medicated electrospun constructs (techniques 1 and 2) were unfortunately confounded by the adhesion reaction on the suture knots, a trend towards a positive effect could still be assumed when comparing the complete absence of adhesions centrally in the reinforced medicated construct compared to the presence of some adhesions in the tubular braided technique.

Figure 10. Image showing adhesion peripherally (black arrowhead) and no adhesion centrally (white arrowhead) when using a reinforced tubular, medicated electrospun construct

The histological qualitative estimation of the repair using the four different techniques was graded from excellent to fair healing. Using a semi-quantitative scale is difficult because within a grade, some variation remains between the results because of the four evaluation criteria (gap formation, inflammation, vascularization, and the presence of newly formed collagen fibers). Inevitably, for example, between the samples of techniques 1, 2, and 4, all of which scored grade 2 at week 3, some variation was still observed in gap formation and the amount of inflammation.

In general, there seems to be an evolution from good/fair tendon healing at 3 weeks to excellent/good healing at 8 weeks. All repairs showed small to limited gap formation with the largest gap of 1.5 mm. This is smaller than the 2-mm limit for gap formation in human flexor tendon repair. In humans, a gap larger than 2 mm is considered a clinical failure and has been shown to result in inferior postoperative outcomes^{18,19}. Extrapolation must be executed carefully because of the difference in the diameters of the tendons.

Notably, a noticeable increase in macrophages and giant cells was observed in the techniques using electrospun polymers. Since it is unlikely that there would be an

inflammatory reaction against naproxen, either the AUP/PCL polymers or hyaluronic acid must be the causative agents. According to Zimmermann et al., a literature search revealed that in plastic surgery, where hyaluronic acid is commonly used, a foreign body reaction with macrophages and a few multinucleated giant cells is a physiological rather than pathological reaction after hyaluronic acid injections²⁰.

Another important consideration is the influence of tubular braids on vascularization. The braid exerts a grasping effect, which could theoretically have a negative influence on vascularization. On the other hand, when the braid relaxes, it may also cause a pumping effect that could stimulate blood circulation. Therefore, a vessel analysis was performed. Healthy vessels were found in all four techniques, including those using a braid. Moreover, techniques using a braid did not show the lowest number of vessels at 3 or 8 weeks. Although this does not suffice to predict long-term results and the analysis was done only on one section per technique, it is reassuring that after 8 weeks, vascularization did not seem to be affected.

Limitations

As in any *in vivo* study on animals, the animals' individual behavioral characteristics might cause differences in clinical outcomes. Fighting for dominance could have altered the results, even when the paw was immobilized. On the other hand, this immobilization could have provoked or reinforced adhesion formation, but was necessary to protect the repairs.

5. Conclusion

The three experimental surgical techniques: (i) a reinforced tubular, medicated electrospun construct, (ii) a tubular, medicated electrospun polymer without reinforcement, and (iii) a tubular braid, as such, showed similar strength as that of a

modified Kessler suture repair. Macroscopically, a significantly different adhesion pattern was observed at the suture knots, either centrally or peripherally, depending on the applied technique. Histological analysis showed good to excellent repair at the tendon repair site for all conditions assessed. Chinese finger trap configurations did not cause any adverse effects on the vascularization of the tendon repair.

Acknowledgements

I. Peeters would like to acknowledge the financial support of the UGent Bijzonder Onderzoeksfonds (BOF: Grant no: 01D28316). The work of N. Pien was supported by a Vanier Canada Graduate Scholarship. We would like to thank Frank Van Dael for his assistance during the project.

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