Biomechanical effect of the use of anti-adhesive membranes on reconstructed rabbit-model flexor tendons

Konstantinos Katakalos¹ and Dimitrios Kapoutsis^{a,2}

Abstract— The effects of anti-adhesive membranes on repair sites of injured tendons in animal models have been extensively studied especially in recent years. Our study focuses on the biomechanical influence of the anti-adhesive membrane Divide (Johnson-Johnson) on a tendon deficit that is reconstructed with an autologous tendon graft. Totally 14 tendon-specimens were tested under quasi-static tension with the INSTRON 5969 testing-rig. All specimens had been used as autografts to bridge a 3cm artificial tendon deficit in zone II of a rabbit model. Half of the specimens had been coated with the anti-adhesive membrane, whereas the rest had been not. As a result, 7 pairs of tendon specimens were investigated. All tendons had been surgically excised from the right forepaw of a rabbit. For each tendon-specimen the load-extension diagram was obtained. Results showed that after a 6 week post-operatively period, no statistically significant difference was detected in mechanical strength (maximum load before rupture) between tendon-grafts with the membrane and grafts without it (p=0.511>0.05). On the contrary, grafts coated with the membrane resulted in a significant increase of flexibility (greater maximum elongation before rupture, p=0.029<0.05)

Index Terms— biomechanical effect, flexibility, rabbit tendon autograft, tensile extension, tensile strength, zone II flexor tendon

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1 INTRODUCTION

EVELOPMNET of adhesions is known as one of the most frequently encountered problems following tendon repairs. [1],[2],[3],[4] Surgical techniques and postoperative rehabilitation programs have met great development. Despite all progress, formation of adhesions at repair sites remains a clinical difficulty restricting adjacent joints' movement. According to literature, our main goal in a flexor tendon repair, especially in zone II, is not only to achieve a sufficient degree of tensile strength at the repair site, but at the same time to preserve adequate tendon excursion which leads to good function (greater flexibility). Both parameters are served by reducing the formation of intra-synovial adhesions, restoring the gliding surface, hastening healing of the repair site, and following proper postoperative rehabilitation protocols. [5],[6],[7] Adhesion formation is encountered in random tendon injuries, surgically induced trauma, tendon ischemia, tendon gaps in the repair site and damage of the tendon sheath. [8]

Clinical and laboratory research has been conducted to prove that formation of adhesions can be limited by COX inhibitors, anabolic steroids, 5-FU, local injections of hyaluronic acid, hyaluronic acid sheaths, human amniotic fluid, antihista-

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Corresponding author: Dr. Konstantinos Katakalos Adjunct Lecturer, Dpt. of Civil Engineering Aristotle University of Thessaloniki University Campus, Thessaloniki, Greece, 54006 tel: +30 2310995653, fax: +30 2310995769 email: kkatakal@auth.gr mines, silicone and cellophane mechanical blocks, peritoneum, grafts, even tunica vaginalis in men. [3], [9] The film we tested, Divide (Mitek, Johnson-Johnson), is a sterile absorbable adhesion barrier prepared by the oxidation of regenerated cellulose, that has been used in gynaecological surgery since 1990 under the commercial name of Interceed.

The use of anti-adhesive membranes is crusial not to interfere with the process of the wound healing. [9] At the same time maximum repair strength and flexibility should not be affected. There is limited literature concerning the use of antiadhesive membranes coating a reconstructed rabbit flexor tendon gap in zone II.

In general, material properties of a tendon affect its ability to store and return elastic energy, resist damage, provide mechanical feedback and amplify or attenuate muscle power. The biomechanical properties of a tendon are well known to respond to a variety of stimuli. [10],[11],[12] The damaged tendons ideally need to maintain their initial biomechanical properties. This need is more difficult to fulfill when a tendon autograft is used to reconstruct a tendon deficit. Structural properties of a tendon are negatively affected by the development of adhesions. [13],[14],[15],[16]

The purpose of this study is to investigate the impact of application of Devide antiadhesive membrane on the biomechanical properties of an autologous tendon graft used to bridge a deficit in a rabbit flexor zone II model.

2 MATERIALS AND METHODS

Totally, two groups of 7 tendon-specimens were formed out of 10 rabbits. The author has performed all surgical procedures for the present study. We operated on ten (10) New Zealand White rabbits. Their average weight was 3.5 kg (3-4.2 kg). Ac-

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cording to the literature, the rabbit forepaw model for flexor tendon healing is the most popular among researchers and it has also been our model of choice. The approval of the local ethics committee was also obtained for this study.

2.1 Surgical Technique

After administration of 50mg/kg ketamine and 10mg/kg xylazine, we shaved and secured the right forelimb toes and draped the limb in a sterile fashion. We also applied a local block anesthesia by injecting xylocaine in the web spaces blocking the respective digital nerves, in a fashion quite similar to the way we operate on a human hand. A tourniquet was applied for less bleeding. Common FDP tendon was identified at a higher level, being deep to the superficial flexor tendon, and was transected to eliminate tension distally .The FDP tendons of the randomized two toes were recognized and dissected after two longitudinal skin incisions. We opened and released the A3 pulley, but we kept pulleys A2 and A4 intact. We also made every effort to handle FDS tendon atraumatically. After determining a zone corresponding to the human zone II, we used a scalpel to excise a piece of FDP approximately two (2) cm long creating a flexor tendon gap. The tendon deficits were immediately bridged with autologous tendon grafts. The piece of tendon excised from one flexor tendon to create the gap was used as a tendon graft in the other toe and vice versa. We stitched the graft at its ends with a 5-0 nonabsorbable suture using a modified Kessler technique, and in some cases we reinforced with a simple end to end running 6-0 non-absorbable suture. Care was taken to keep the graft gliding through the preserved pulleys. The reconstructed tendons were thoroughly washed out with normal saline. After bridging the tendon gaps, one tendon graft was coated with the anti-adhesive membrane Divide (Johnson-Johnson), whereas in the other toe the graft was not covered by the membrane. In this way each rabbit served as its own control. The allocation of the membrane to the one or the other toe also followed a randomization schedule. Skin was closed with a running 3-0 nylon suture, and the toes were dressed with sterile gauze and self adhesive wrap. Operated rabbits were freely allowed to resume activities. All animals were re-operated at six (6) weeks and the incorporated tendon grafts were excised (see Figure 1) and put to biomechanical testing. Three rabbits out of ten demonstrated toe necrosis during the post-operative course and were excluded from the subsequent study.

2.2 Biomechanical Testing

Biomechanical testing was conducted on 14 tendon specimens, obtained by 7 New Zealand White rabbits. The specimens formed 7 pairs. Each pair comprised of one tendon treated with anti-adhesive membrane Divide (Mitek,Johnson-Johnson) and one tendon which was not treated. All specimens were subjected to quasi-static tension utilizing the electric INSTRON model 5969 testing rig of Laboratory for Strength of Materials and Structures of Aristotle University of Thessaloniki. (A.U.Th.)

Each specimen was fixed to the grips with its midpoint approximately in the middle of the distance between the grips. The initial distance between the grips was kept constant and equal to 8.5 mm. The rate of the imposed displacement varied

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from 0.5 mm/sec to 1.0 mm/sec. Both load (in N) and extension-elongation (in mm) were recorded throughout the whole experiment utilizing the data acquisition system provided by Instron; as a result the load versus extension curve for each specimen is presented in this study.

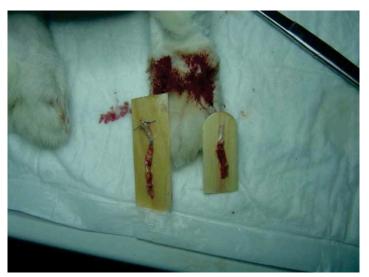


Figure 1. Tendon autologous grafts during surgery

Gripping is a major challenge faced during the tensile testing of tendons. [17],[18] Being soft and aqueous materials, tendons are prone to slipping and demonstrate premature failure during mechanical testing. Several gripping methods have been tested and evaluated in the past, including serrated jaw, sandpaper, frozen ends, and air-dried ends. It was found that by using the pneumatic grips with cardboard lining stress concentration at the grip-specimen interface reduced substantially. [18]



Figure 2. Failure at the mid-substance of the tendon due to the scholastic gripping

On the other hand, an analysis of specimens that failed at the grip-specimen interface versus those that failed at midsubstance shows that there was no significant difference in their tensile properties. During the present study the griping of all tendons was taken under consideration scholastically. As a result, we managed to avoid local slippage and failure of the International Journal of Scientific & Engineering Research, Volume 7, Issue 10, October-2016 ISSN 2229-5518

specimens near the grips. (see Figure 2)

3 RESULTS

2.3 Statistical Analysis

The experimental results were further processed statistically utilizing the program MINITAB.

Due to the fact that each pair of tendon grafts, one with or and one without the membrane, was taken from the same rabbit, the statistical analysis was done in pairs. In total, seven pairs underwent statistical study. The parameters to process are the maximum load, before failure and the extension. (recorded at the maximum load) The paired t-test method was implemented when the normal test Kolmogorov-Smirnov was satisfied. (p>0.05) The statistical analysis focused on answering the following question; at a time point of six weeks after surgery, are the biomechanical properties (load – flexibility) of tendon autografts influenced by the use of the anti-adhesive membrane?

The following paragraph presents the results obtained by the present study. Each tendon specimen in denoted with a number and the sign plus (+) or minus (-). The number depicts the code name of the rabbit from which the flexor tendon specimen was obtained. The sign minus (-) refers to an autograft tendon that was not covered with the anti-adhesive membrane whereas the sign (+) presents an autograft tendon that was covered after the surgery with the anti-adhesive membrane.

In total, 14 autgraft flexor tendon specimens were subjected to quasi-static tension. Half of the tendons had been covered with the anti-adhesive membrane Divide (marked with the sign +) whereas the other half was not. (marked with the sign -) We obtained the 14 tendon-specimens from 7 New Zealand White rabbits named as 10, 16, 18, 26, 28, 29, 30). From each rabbit one tendon had been covered with the membrane, and the other tendon had been not. This is the reason why the analysis of the results was done in pairs.

Table 1 presents the summary results obtained from the present study. Columns 1 to 4 present the results obtained by autograft tendons that were not covered with the antiadhesive membrane whereas columns 5 to 8 present the results of autograft tendons that were covered with the antiadhesive membrane. This table also depicts the rate (mm/sec) of the imposed tensile displacement, the maximum load before failure in Newton (N) and the tensile extension at the maximum load in millimeters (mm). The maximum load of autograft tendons without membrane varies from 6.53N to 40.89N with a mean equal to 22.03N.

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Autograft flexor tendon without membrane				experimental results Autograft flexor tendon with membrane			
Name	Rate (mm/sec)	max Load (N)	max Exten- sion (mm)	Name	Rate (mm/sec)	max Load (N)	max Exte sion (mn
10 (-)	0.5	15.07	0.88	10 (+)	0.5	16.33	1.12
16 (-)	0.5	22.71	1.68	16 (+)	0.5	27.50	1.91
18 (-)	0.5	21.24	0.84	18 (+)	0.5	21.19	1.29
26 (-)	1.0	6.53	0.39	26 (+)	1.0	17.46	1.92
28 (-)	1.0	20.17	0.26	28 (+)	1.0	37.02	1.24
29 (-)	1.0	40.89	0.65	29 (+)	1.0	38.64	0.62
30 (-)	1.0	27.61	0.59	30 (+)	1.0	15.87	0.76
	Mean	22.03	0.76		Mean	24.86	1.27

When the autograft tendon is covered with the membrane the maximum load varies between 15.87N and 38.64N with a mean equal to 24.86N. The variation of the ultimate load is explained by the fact that the tendon-specimens were obtained by live rabbits. Their weight, their attitude and their willingness of movement varied during curing. As a result it is more accurate to compare the recorded results in pairs of autograft tendons that were operated from the same rabbit. This principle has been also followed in the discussion of the extension at maximum load as well.

For tendon pairs 10, 16, 26 and 28 there is an increase of the ultimate load before failure that varies between 8.3% and 167.5%, whereas for tendon pairs 18, 29 and 30, the ultimate load before failure decreased by 0.2% to 42.5%. This observation depicts that the existence of the anti-adhesive membrane does not affect systematically the ultimate load. This variation

of the load is due to other parameters of rabbit's biology rather than the coating of the tendon with the anti-adhesive membrane.

As far as the extension at maximum load is concerned it can be extracted that for all autograft tendon pairs the extension was increased, except for specimens 29 (29-, 29+) where the extension at maximum load was decreased by 3.9%. For the rest six pairs the increase of the extension varies between 13.7% and 387.3%. By taking into account the average values of the recorded extension, the increase of the ultimate extension observed at the maximum load is 67.6%. Thus, coating the flexor tendon autograft, with the anti-adhesive membrane, results to an increase of the ultimate extension, (flexibility) at the time point of a 6-week period after the surgical intervention.

In the following figures 3a-g, the load-extension relationship is presented. As observed in these figures, the loadextension relationship is not similar among all autograft tendons, due to the fact that they were surgically excised from different rabbits. Despite this fact the average behavior is similar to a hyper-elastic behavior, which means that they are soft

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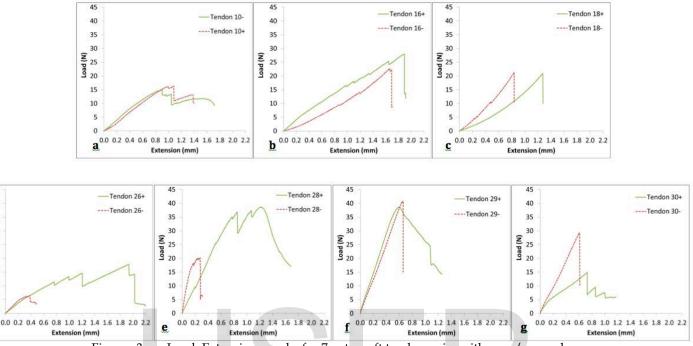
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Load

at the beginning (a small slope of the curve) and they become stiffer after a certain displacement. (increase of the slope of the curve)



Figures 3a-g. Load-Extension graphs for 7 autograft tendon pairs with or w/o membrane

A great variation of the load-extension graphs is demonstrated in figures 3a-g. The slope of the curves is not constant for all specimens, neither the ultimate load nor extension. This great variation, as discussed earlier, was attributed to the fact that the rabbits from which the tendon-specimens were excised were different to one another in terms of the conditions under which they grew. In other words, no living rabbit is exactly identical to another. This is the reason why the load-extension graphs are going to be discussed in pairs.



Figure 4. Typical mode of failure into the mid-substance of the autograft tendon

Autograft tendons acquired from rabbit 10, (specimen 10+ and 10-) behaved similarly when the displacement control extension was imposed on the specimen. This observation is valid for tendons acquired from rabbit 29 as well. The explanation of this observation is that for all four autograft tendon-specimens adhesions did not develop in such a way that they could affect the flexibility of the tendon. (see figure 4)

Autograft tendons taken from rabbits 18, 28 and 30 that were covered with the anti-adhesive membrane (18+, 28+, 30+) behaved with greater flexibility than the ones without the membrane. (18-, 28-, 30-) The observation indicates that the membrane affected the autograft tendon specimen by increasing the flexibility of the autograft tendon.

Figure 3d presents the pair of autograft flexor tendons of rabbit 26. The tendon covered with the membrane initially behaved in a similar way to the tendon that was not covered with the membrane (up to 0.39mm). After the point of fracture of tendon 26(-), the tendon 26(+) increased its strength by 3 times and its flexibility by almost 5 times. This observation is an extreme observation that also demonstrates the effect of the anti-adhesive membrane.

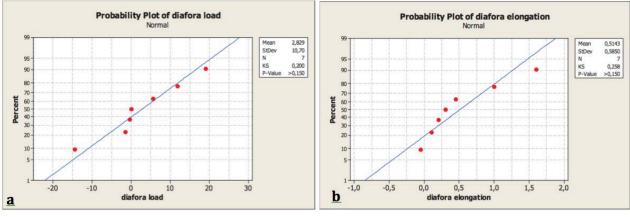
In general, many tendon-specimens showed an initial drop of the load. Further increase of the extension resulted in further increase of load. This observation indicates that a partial fracture of the autograft tendon does not result to the ultimate fracture of the tendon itself.

The aforementioned phenomenon comes together with the observed mode of failure. In the following figure 4, the mode of failure is presented. All specimens were fractured at the region of the autograft, which was placed very carefully between the grips. This was ensured visually by checking the sutures of the tendon after the test. No specimen failed at the sutures. The behavior of the sutures is not investigated in this study.

We impose three of the paired autograft tendon specimens to a tensile displacement control extension with a rate of 0.5 mm/sec and four pairs were extended with a rate of 1.0 mm/sec. From the graphs depicted in figures 3 it can be extracted that this difference of the imposed rate does not affect systematically the biomechanical properties of the tendons, with or without the membrane.

Due to the fact that we recorded a great variation among all acquired experimental results, a statistical investigation was considered mandatory. The statistical analysis was conducted utilizing the program MINITAB. As it was mentioned before the statistical analysis was done in pairs following the rational of the aforementioned presentation of the results. The parameters to be processed were the ultimate load and the extension at the ultimate load.

Figures 5a and 5b demonstrate the plots of the normal test Kolmogorov-Smirnov for both ultimate load (6a) and extension at ultimate load (6b).



Figures 5a-b. Normal test Kolmogorov-Smirnov for ultimate load and extension

The statistical analysis depicts that the normal test Kolmogorov-Smirnov was satisfied (p>0.05) for both ultimate load and extension at ultimate load. As a consequence, the t-paired statistical process was conducted for both investigated parameters.

The paired T-Test demonstrated that the ultimate load is not statistically affected from the use of the anti-adhesive membrane since the P-Value is equal to 0.511 >> 0.05.

On the other hand, the extension at the point of the ultimate load is statistically affected by the use of the anti-adhesive membrane due to the fact that the P-Value is equal to 0.029 < 0.05.

To sum up, the ultimate load of the flexor autografts is not influenced by the use of the anti-adhesive membrane, after a period of six weeks from the surgery. On the other hand, the flexibility of the flexor autografts increases when grafts are coated with the anti-adhesive membrane Divide. (Mitek, Johnson & Johnson)

4 DISCUSSION

The present study focuses on the biomechanical investigation of flexor autografts in zone II in a rabbit model in terms of tensile maximum load and extension at maximum load. The Instron 5969 testing rig of Laboratory for Strength of Materials and Structures of A.U.Th. was utilized to impose the tensile quasi-static displacement with a rate of 0.5 and 1.0 mm/sec. The specimens were obtained with a surgical procedure from 7 different New Zealand White rabbits. From each rabbit two specimens were acquired, one without the anti-adhesive membrane and one with it. The experimental investigation showed that the average maximum load is 13% higher for tendons grafts coated with membrane compared to tendon grafts without the membrane (controls) after a period of 6 weeks from initial surgery. The difference in ultimate load (24.86 N compared to 22.03 N) was not statistically significant. On the other hand the increase of the extension at maximum load of 67% (1.67mm compared with 0.76mm) was statistically significant. (p=0.029) As a result, the autograft tendons behaved with greater flexibility.

By extending the aforementioned rational to the clinical practice in humans, this could mean for example, that a 13% improvement in load increase of a tendon repair with a 70 N failure strength would represent a 9.1 N increase. This incremental increase in load resistance together with a 67% increase of the flexibility is sufficient enough to allow smoother active flexion and potentially tip the balance toward improved tendon excursion with smaller rupture and tenolysis rates.

The use of anti-adhesive membranes has been extensively studied in the past fact that demonstrates the big interest of the research community for the subject. According to Meier Burgisser et al [20] no type of anti-adhesive material (membranes, fluids, gels etc), tested up to now has proved its superiority. We should also stress the fact that, according to the author, all related research is about simple tendon lacerations repairs, and not reconstructions with tendon grafts. The present study focuses on the investigation of reconstructed tendon grafts.

In addition Menderes et al. [2] studied 30 rabbit specimens. From each rabbit, he acquired two tendons, one having been coated with the anti-adhesive membrane and one not. The tendon-specimens of Menderes study were obtained through a surgical process utilizing a simple tendon laceration repair. On the contrary, tendon-specimens in our study were excised from a reconstructed tendon gap where tendon autografts had been utilized to bridge the deficit. This fact makes the present study unique. Menderes' study showed that the use of anti-

adhesive membrane significantly decreased the development of adhesions. However, Menderes did not investigate the biomechanical properties of the repaired specimens. Our results prove that the flexibility of the deficit tendon was increased, due to the fact that the developed adhesions were decreased. Furthermore the study of Chen et al. [19] showed that simple tendon lacerations repairs coated with anti-adhesive membranes exhibit maximum loads similar to the specimens that were not covered with the membrane. Our investigation exhibit the same result but for tendon autografts. The influence of a membrane on the tendon in terms of flexibility was not studied in depth in the literature. In addition no study has investigated the influence of reconstructed tendon with grafts. [21] Our study focused exactly on this aspect of investigation. We measured that the use of anti-adhesive membranes resulted in an increase of the flexibility of the autograft tendon specimens that we investigated.

We acknowledge that the current study has some important limitations. First, the number of tendons tested is relatively limited. The investigation of 14 tendons is not sufficient to generalize our observations and results. Due to this fact the analysis was conducted in pairs of autograft tendons with or without membrane. Moreover, the biomechanical investigation focused on the autograft itself and not on the repair sites between autograft and original tendons. This limitation drove the study to focus on the behavior of the autograft itself with or without the membrane. We did not take under consideration the behavior of the repair sites.

It is also true that we did not measure cross-sectional area or perform cyclical loading of the autograft tendons. These types of measurements would further enlighten the biomechanical properties; nevertheless, maximum load and elongation are probably the most clinically relevant biomechanical parameters with the biggest clinical relevance and were therefore our focus of this proof of concept study in a flexor tendon autograft model.

We found significant variation among the 14 repaired tendons in both ultimate load and extension at ultimate load. These results add to an increasing body of evidence indicating that a tendon is a dynamic tissue with the potential to modulate material properties in response to mechanical demand. We analyzed and discussed all measurements in pairs of autograft tendons with or without the membrane. Each pair was obtained by the same rabbit-specimen.

5 CONCLUSIONS

The conclusive observation is summarized bellow:

The ultimate load of autograft flexor tendons is not influenced by the use of anti-adhesive membranes after a period of six weeks after surgery. On the other hand the flexibility (ability to extent) of autograft flexor tendons increases by utilizing the anti-adhesive membrane, possibly due to the fact that adhesions are limited.

Future studies are necessary to address all the aforementioned limitations and to further investigate the effect of antiadhesive membranes that could be used to coat flexor tendon autografts

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