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# Influence of suture material on biomechanical and histological indicators of Achilles tendon heeling in rabbits

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**ABSTRACT**

This study shows on a rabbit model *in vivo* the importance of suture material elasticity in Achilles tendon healing. Tendon reconstruction was performed on 30 rabbits divided into three groups of 10 animals, according to the suture material. Tendons were sutured by the Krackow suture. Steel wire as a stiff material, polypropylene as a plastic material, and polybutester as a material that shows elasticity and resistance to deformities were used. In all the animals the unoperated, contra lateral limb served as control. A servohidraulic jack was used to set a maximum loading capacity until the breaking point ($F_{max}$) and drawing ability (A). The breaking strength of tendons reconstructed by polybutester was not statistically different in comparison to the breaking strength of control tendons, but were significantly higher in comparison to tendons sutured by wire or polypropylene. Different histological staining methods were used to show the parameters of healing tendon tissue. The collagen fiber structure and arrangement, and the cellularity of tendon scar tissue sutured by polybutester are more similar to those of the control tendon. Achilles tendon suturing with polybutester is beneficial for tendon healing, so its clinical use is recommended.

**Key words:** Achilles tendon, suture material, breaking strength, polybutester

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## Introduction

Partial or complete Achilles tendon rupture usually occurs during the hyperextension of the tendon because of overstraining of the tissue or extensive ankle flexion, and is considered a sports injury (HOSEY et al., 1991). Achilles tendon rupture in animals usually occurs in a high jump, traffic accidents or fighting. One of the main requirements for the reconstruction of the tendon is to achieve adequate tensile strength. The proper choice of suture material is particularly important. The mechanical properties of the suture material should resemble the properties of the reconstructed tissue (BOOTHE, 1998). This study shows, on a rabbit model *in vivo,* the importance of material elasticity in Achilles tendon healing. The aim of this study was to establish whether, and to what extent, different mechanical properties of suture materials, particularly in respect to elasticity, influence the strength of the reconstructed Achilles tendon. It examines the influence of steel wire and polypropylene as the two most commonly used materials for tendon suturing, and polybutester, a less-known material, under the assumption that its mechanical properties are more suitable for the reconstruction of the tendon. Steel wire is a rigid material; polypropylene is a plastic material with moderately pronounced elongation, while polybutester is a material with marked elasticity and resistance to deformation (BERNSTEIN, 1988).

The purpose of this study was to compare the basic mechanical and histological indicators of tendon healing after Achilles tendon reconstruction with three frequently used suture materials.

## Materials and methods

*Animal model.* The study used 30 rabbits, Californian white females, weighing 370 ± 55 g, at around 5 months old. They were placed in standard rabbit cages, under the same conditions. Three groups of rabbits were formed according to the three suture materials. For the first group, stainless steel wire was used, for the second group polybutester (Vascufil®, Syneture), and for the third, polypropylene (Prolene®, Ethicon). The animals underwent surgery by the same surgeon. Twenty minutes preoperatively, cefazolin (15 mg/kg) was applied intramuscularly. The animals were anesthetized with intramuscular injections of ketamin hydrochlorid (40 mg/kg) and xylazine (3 mg/kg). The animals were kept anesthetized with intermittent intramuscular applications of ketamin and xylazine in half doses (GILL et al., 2004). After posteromedial skin incision, the Achilles tendons were dissected free from the surrounding tissue. The contra lateral, left leg, served as a control. Simulation of a rabbit's Achilles tendon rupture was made by tenotomy 2 cm proximal from the right tendon's vertex of calcaneus. Tendons were sutured by Krackow suture, suture material diameter USP 3-0 (KRACKOW et al., 1986). Marginal points of tissue entry for transverse parts of the suture were placed 1.5 cm away from the tenotomy.

Skin wounds were reconstructed by simple continuous polypropylene suture USP 4-0. The right leg was immobilized by synthetic cast bandages in mild plantar flexion of the tarsus for 3 weeks. Five weeks postoperatively the animals were examined clinically and were euthanized with pentobarbital 120 mg/kg i.v. (PNEUMATICOS et al., 2000).

*Biomechanical testing.* Tendon samples were stored in Ringer solution at -20 ºC (ASLAM and AFOKE, 2000). The stitches were removed so that the results of biomechanical testing were related solely to the tissue of tendons, without affecting the suturing materials (KANGAS et al., 2001). For better acceptance of the testing machine jaws, the calcaneus and muscle-tendon transition was wrapped in carbon thread, and then impregnated with two-component epoxy adhesive in the form of cubes. The length of the tendon examined was 4 cm from the insertion point of the calcaneus bone to the clip of the testing machine. During the testing of samples, their moisture was maintained by a spray of Ringer solution. Load tensile force was set by the speed of 100 millimeters per minute (O'BROIN et al., 1993). Servohidraulic jack, model 8511 (Instron, Massachusetts), was used to set a maximum loading capacity until the breaking point ($F_{max}$) and drawing ability (A). The drawing ability of tendons is expressed as the percentage increase in the length of the tendon before breaking, compared to the initial length of the tendon prior to testing.

*Histology testing.* Different histological staining methods were used to show components of the examined tissue of Achilles tendons, namely Hemalaun and eosin staining methods (HE), PAS-staining method, Pincus acid staining and Giemsa orcein, Alcian blue staining, and Alcian blue-PAS method for mucopolysaccharides. The monitored and assessed parameters of healing tendon tissue, or scar were: 1) Structure and collagen fiber orientation, 2) Concentration of cellular elements (cellularity), 3) Glycosaminoglycans content (GAG), 4) Elastic fiber content. For the first two parameters, the samples were stained with hemalaun and eosin, for the third parameter with Alcian blue and Alcian-PAS staining, and the fourth parameter with Pincus staining. From each of the four groups investigated, three areas of histological observations of tissue samples were randomly determined. Control tissue samples were taken away from the scar. A light microscope with 20x and 40x magnification was used (Leitz, Wetzlar, Germany). Observed parameters were interpreted according to the MOVIN (1998) gradation scale.

*Statistical metods.* The results were statistically processed using the analysis of variance method (ANOVA) to compare the biomechanical characteristics of the three groups of samples, and the differences were considered statistically significant if $P<0.05$. To test the difference between the control group, as well as the difference between the examined groups sutured by various suture materials, nonparametric Kruskal-Wallis ANOVA was used, since the samples were mutually independent and small. The difference between the test and control tendons of animals within the same group was established using the Wilcoxon test, since the samples were mutually dependent and small. To test the significance of the difference between the median of the examined groups, the Mann-Whitney U-test was used. Statistical analysis of the results was done using the computer program STATISTICA. Data were presented by median and corresponding range of minimum-maximum (PETZ, 1985).

**Results**

*Clinical findings.* In all rabbits, the satisfactory use of the limbs in all phases of movement was clinically evident. All tenotomized tendons healed, which was indicated by a thickened scar. Elongations of tenotomized tendons were noticed compared to contra lateral tendons in all groups.

*Biomechanical indicators of healing.*

*Breaking strength.* Breaking strength of control tendons 5 weeks postoperatively was 205 (170-260) Newton. Tendon group 2 (polybutester) endured significantly more power compared to tendon group 1 (wire) and group 3 (polypropylene), before breaking. Significant differences found between the various breaking forces of tendon groups sutured with different materials, as well as the fact that between the group sutured with polybutester and the control group there were no statistically significant differences (208N vs. 221N), indicates the importance of proper material selection for Achilles tendon suturing. The values of the maximum breaking force of the Achille's tendons of the test ($F_{max}$) and control ($F_{max}$k) groups are shown as median and corresponding minimum - maximum values in Table 1. For the purpose of obtaining more precise values, the tendon breaking forces of the test groups were defined as the difference between the breaking force of the test ($F_{max}$) versus the control tendons ($F_{max}$k) and designated as ($F_{max}$ dif.).

Table 1. Parallel display of breaking force values for control and test Achilles tendons in rabbits

| | Steel wire<br>Med (Min-Max)<br>n = 10 | Polybutester<br>Med (Min-Max)<br>n = 10 | Polypropylene<br>Med (Min-Max)<br>n = 10 | Kruskal-Wallis<br>ANOVA |
|---|---|---|---|---|
| $F_{max}$ (N) | 155 (131-183) | 208 (141-274) | 113 (77-177) | P = 0.0001*<br>H = 18.0264 |
| $F_{max}$k | 202 (170-245) | 221 (180-260) | 191 (180-230) | P = 0.2483<br>H = 2.7860 |
| $F_{max}$dif. | -57.50 (-85 do -22) | -12.50 (-39 do 14) | -72 (-140 do -23) | P = 0.0001*<br>H = 18.9472 |
| Wilcoxon Test | P = 0.0051* | P = 0.0593 | P = 0.0051* | |

Med (Min-Max) = median values (minimum-maximum); *Statistically significant values

In the Kruskal-Wallis variance test, the values of breaking strength between the control groups ($F_{max}k$) were not statistically significant, whilst the values of breaking strength between the groups of sutured tendons were statistically significant. Wilcoxon test showed statistically significant differences between the control and test Achilles tendons in groups 1 (wire sutured tendons) and 3 (polypropylene sutured tendons). In group 2 (polybutester sutured tendons) there were no statistically significant differences between control and test tendon groups.

*Drawing ability.* The data for the drawing ability of the operated Achilles tendon (A) and control (A control) groups are shown as the median and corresponding range of minimum-maximum in Table 2.

Table 2. Parallel display of drawing ability values for control and test Achilles tendons in rabbits. Drawing ability of the tendons is expressed in the percentage increase in length before breaking compared to prior to testing.

| | Steel wire<br>Med (Min-Max)<br>n = 10 | Polybutester<br>Med (Min-Max)<br>n = 10 | Polypropylene<br>Med (Min-Max)<br>n = 10 | Kruskal-Wallis<br>ANOVA |
|---|---|---|---|---|
| A (%) | 27 (17.80-35) | 29.15 (25.40-41) | 43 (20-49) | P = 0.0377*<br>H = 6.5590 |
| A control | 22.30 (15-25) | 21.50 (18-25) | 22 (14.40-31) | P = 0.9557<br>H = 0.0906 |
| Wilcoxon Test | P = 0.0125* | P = 0.0051* | P = 0.0051* | |

Med (Min-Max) = median values (minimum-maximum); * Statistically significant values

A significant statistical difference between the test groups was established by the Whitney-Mann U-test. Statistically significant differences appeared in the ‘A’ variables, between the groups sutured with wire and the groups sutured with polybutester, as well as between the groups sutured with polybutester and the groups sutured with polypropylene (WIRE vs. VASCUFIL P = 0.0004, VASCUFIL vs. PROLEN P = 0.0002). Drawing ability was significantly lower in the group of tendons sutured with polybutester compared to wire sutured tendons and polypropylene sutured tendons.

*Histological indicators of healing.*

*Structure and orientation of collagen fibers.* In all control samples of Achilles tendons stained with hemalaun and eosin, parallel bundles of dense collagen fibers were detected. Fiber flow was slightly wavy. Between the collagen fiber bundles, a small amount of loose connective tissue could be identified. In the experimental group of rabbits sutured with wire, the collagen fibers were thinner, and there was a noticeable loss of proper fiber alignment as they were laid down in different directions. In the experimental group sutured with polybutester, a more proper arrangement of collagen fibers was observed

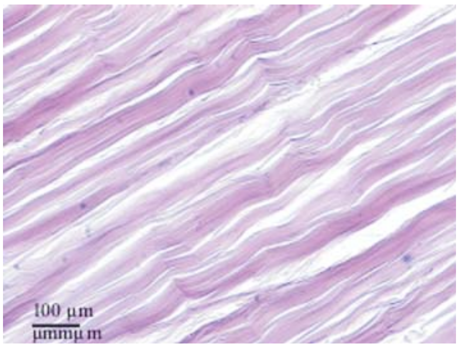


Fig. 1. Achilles tendon. Control rabbits group. Collagen bundles are parallel and closely situated. Fiber flow is slightly wavy. H&E, ×20.

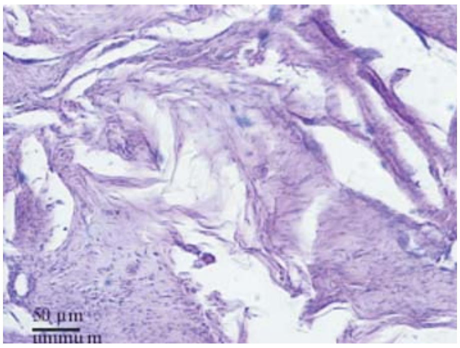


Fig. 2. Achilles tendon. Steel wire sutured rabbits group. Collagen fibers are thinner, disarranged in a different course. H&E, ×20.

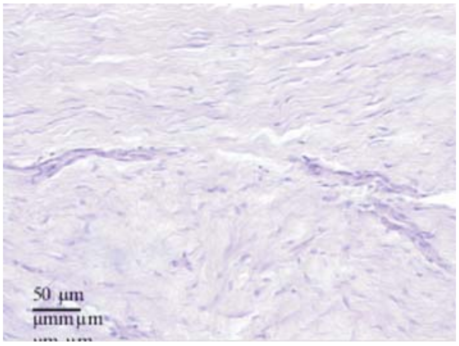


Fig. 3. Achilles tendon. Polybutester sutured rabbits group. Fewer cells can be seen than in tissue samples of other groups. By the appearance of the nuclei, cells mostly show the characteristics of fibrocytes. Fiber flow is less wavy. H&E, ×20.

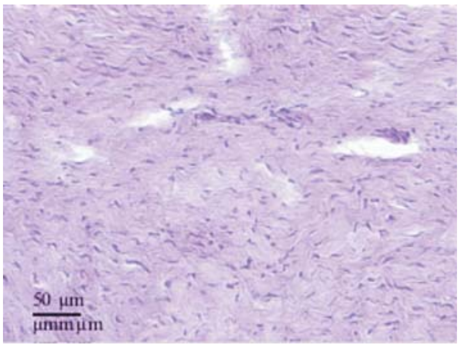


Fig. 4. Achilles tendon. Polypropylene sutured rabbits group. The number of cells with fibroblast characteristics is abundant. H&E, ×20.

than in the other experimental groups. Bundles of collagen fibers were mainly laid in a parallel arrangement. In histological samples from the polypropylene sutured rabbit group, the collagen fiber arrangement was very irregular, almost chaotic.

*The concentration of cellular elements.* In the control tissue samples, celullarity was expressed as the sporadic presence of tendocytes, flat in shape, and between fibers there were clearly visible elongated fibrocyte nuclei. In the samples of scarred tissue from all the groups, a significant increase was observed in the number of cells and fibroblasts in various stages of development, and the fibrocytes of oval and elongated shape. The samples of Achilles tendon in wire sutured rabbits had a significantly increased number of cells. Cell nuclei were oval to round, and by their characteristics they corresponded to fibroblasts. Other types of cells were not observed. In places of tendon healing, neovascularization was observed. In most samples of tendons sutured with polybutester, fewer cells were observed than in other tendon test sample groups. Neovascularization was also present. The appearance of cell nuclei generally showed characteristics of fibrocytes. Neovascularization was enhanced and the number of cells with fibroblast characteristics was high in tendons sutured with polypropylene.

*Content of glycosaminoglycans (GAG).* The Achilles tendon tissue samples of the control group rabbits stained by the PAS method showed a weak positive reaction, as did the Achilles tendon stained with Alcian blue. Samples of tendon sutured with wire, stained by the PAS method and with Alcian blue showed a somewhat stronger reaction in comparison to the control group, while dark blue staining was observed in samples stained by the Alcian blue (pH 2.5)-PAS method which indicated a large amount of glycosaminoglycans. Tissue reaction to the presence of neutral and acidic mucopoly substances in the group sutured with polybuster was stronger than in the control group, and glycosaminoglycans were present in large quantities. In comparison to the control group, the PAS reaction in rabbits sutured with polypropylene was more intense, as was the reaction to Alcian blue. A strong reaction was present in tissue sections stained by the Alcian blue (pH 2.5)-PAS method, which indicates that the Achilles tendons of the experimental group sutured with polypropylene had large amounts of glycosaminoglycans.

*Content of elastic fibers.* In rabbits where the tendon tissue was sutured with wire, the healing zone contained some elastic fibers. A large amount of elastic fibers was observed in scarred tendon tissue sutured with polybutester. Elastic fibers could barely be noticed in tendons of the experimental group sutured with polypropylene.

*Reactions to the suture material.* After 6 weeks, tissue around the suture had a thin fibrous envelope, a reflection of a mild fibrous reaction, with no signs of active inflammatory reaction. Fibrocytes in direct contact with the polypropylene, and especially with polybutester, were flat in shape, adhering directly to each other, forming a thin membrane-like mezotel. The surrounding tissue, in the cases of tendons sutured with wire and with polypropylene, was moderately disorganized in relation to the tendons sutured with polybutester.

## Discussion

The parameters that determined the quality of biomechanical tendon healing tested in this study were breaking strength and drawing ability. Measuring of the tendon cross-section is very demanding and unreliable because of the irregular scar tissue and differences in diameter of the scar tissue, so we determined maximum tensile force before tendon rupture instead of tissue strength. Measuring the breaking strength of the reconstructed tendons reflects the tensile strength of the newly scarred tissue collagen, but not the tensile strength of the suture, because it was previously cut off. This kind of test differs from previous similar tests (MASHADI and AMIS, 1992; O´BROIN et al., 1993), where the results were influenced by the suture material present, which does not give an objective result. The calcaneus region, with the tendonous muscle part, was wrapped in carbon fibers and then impregnated with two-component epoxide glue. This helped stop kneading of the attaching points, especially the calcaneus bone, but also the tendon fibers in the tendonous muscle area that were noticed in preliminary tests. Five weeks postoperative, the breaking strength of the control group tendons was 205 (170-260) Newton. ROBERTS et al. (1983) had a tearing force of 347 (240-489) N in similar tests. In our opinion, this confirms that tendons heal very slowly and that they need to be mechanically supported by the sutures. The significant statistical difference between breaking strength in the operated and control groups in this paper can be used to back this up. Significant differences found between the breaking forces of tendon groups sutured with different types of suture materials and the lack of significant differences between the breaking forces of tendons sutured with polybutester and the control group (208 vs. 221 N) show the importance of choosing the right suture material for Achilles tendon suturing. These results are contrary to the results of MASHADI and AMIS (1992) who did not find differences between polybutester and polyglyconate. We believe that polyglyconate (Maxon®) used in that research as a resorptive material with tensile strength from five weeks within the tissue, was an inadequate choice of suture material, as tendons need a great deal longer to heal, and during that healing period they need the mechanical support of non-resorptive suture material.

The main characteristic of polybutester, as opposed to wire and polypropylene, is elasticity. Biomechanical tests of the abdominal wall reconstructed with polybutester have shown greater tissue strength (RODEHEAVER et al., 1986). A common reason for unsuccessful tenorraphy is the suture material cutting in along collagen fibers (JANN et al., 1992). Our thesis is based upon the assumption that elasticity of suture material in tendon reconstruction will help conform tissue movement, to prevent tissue cut-off, just as in the abdominal wall reconstruction. All the three suture materials that were tested in this work are non-resorptive monofilaments, with tensile strength kept for years within the tissue and materials whose reactivity is minimal (confirmed histologically in this paper). We find the difference in elasticity and deformity to be the only possible reason for the different tearing forces of the Achilles tendons, established in this paper. When comparing polypropylene as a plastic material and polyvinyliden fluoride (Asflex®), a material with strongly expressed elasticity, WADA et al. (2001) also came to the conclusion that elastic material allows more pulling strength for tendons. We chose drawing ability as a measure of deformability, to form a complete view of scar tissue quality. Material with decreased drawing is more resistant to biomechanical deformations (FRANZ, 1998). The Achilles tendon is naturally exposed to frequent stretch loads. Unless the scar tissue is adequately resistant to deformations, a loss of structure will occur and permanent deformations can result, which affects Achilles tendon function (RODKEY et al., 1985). The results of our study show that polybutester sutures create a scar resistant to deformation due to tensile loads.

The results of histological healing parameters were different in the structure and organization of scar tissue collagen fibers, as well as the elastic fiber cell content, compared to the control group. The control group views showed parallel, dense collagen fibers. Scar tissue of all groups showed different degrees of accurate arrangement loss and focus in different directions. This was especially noticeable in tendons sutured with polypropylene. On Pincus dyed tissue specimens we saw a higher amount of elastic fibers in tendons sutured with polybutester and wire, compared to tendons sutured with polypropylene. Reaction to suture material was minimal in all specimen groups. In tendons sutured with polypropylene and polybutester we noticed a thin, mezotel-like fibrous sheath, which indicates absolute tissue tolerance to the suture material. The study by MASHADI and AMIS (1992) presented a similar finding, on chicken tendons sutured with polybutester 45 days post operative. SRUGI and ADAMSON (1972) believe that nylon is an ideal material for tendon suturing, due to the minimal tissue reaction. This paper, as well as MASHADI and AMIS (1992), confirms progress in new suture material technology with minimal tissue reaction, like polybutester. The tendon group sutured with polybutester showed better tissue organization, and also collagen fiber arrangement in the load direction. We presume that suture material elasticity allows interaction between the suture material and tissues, which results in the greater elasticity of scar tissue during healing. The better scar tissue collagen fiber arrangement sutured with polybutester confirms this. To confirm this noticeable difference with facts, immunohistochemical tests should be done and also quantitative measurements of histological parameters of healing using an electronic microscope, which could be the goal of further tests in this area of research.

## Conclusions

1. The breaking strength of tendons reconstructed with polybutester did not differ from the control tendons.
2. The breaking strength of tendons reconstructed with polybutester was significantly higher in comparison to the tendons sutured with steel wire or with polypropylene.
3. The collagen fiber structure and arrangement, as well as cellularity of tendon scar tissue sutured with polybutester were more similar to those of the control tendon.
4. The elasticity of the suture material is an important factor in tendon healing.

5. Achilles tendon suturing with polybutester is beneficial for tendon healing, so its clinical use is recommended.

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**SAŽETAK**

Ova studija na modelu kunića *in vivo* istražuje značenje elastičnosti šivaćega materijala u cijeljenju petne tetive. Šivanje tetiva izvedeno je na 30 kunića podijeljenih u 3 skupine po 10 životinja, s obzirom na upotrijebljen šivaći materijal. Uspoređeni su čelična žica kao krut materijal, polipropilen kao plastičan materijal i polibutester kao elastičan materijal otporan na deformacije. Servohidraulična kidalica upotrijebljena je za određivanje sile kidanja i istezljivosti tetiva. Sila kidanja tetiva rekonstruiranih polibutesterom nije se statistički značajno razlikovala od sila tetiva kontrolne skupine, ali je bila značajno veća u odnosu na sile kidanja tetiva sašivenih čeličnom žicom ili polipropilenom. Različite metode histološkoga bojenja rabljene su za prikazivanje pokazatelja cijeljenja tetiva. Građa kolagenih vlakana, uređenost i brojnost stanica ožiljkastoga tkiva tetiva sašivenih polibutesterom sličnija je tetivama kontrolne skupine. Šivanje tetive polibutesterom učinkovito je za njezino cijeljenje, te je stoga preporučljiv u kliničkoj uporabi.

**Ključne riječi:** petna tetiva, šivaći materijal, snaga kidanja, polibutester