Effect of Bovine Collagen Addition on Early Biomechanical Strength of Sutured Rabbit Achilles Tendon

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ABSTRACT

Introduction. Tendon rupture due to various causes is common issue in orthopaedics. Its management is often difficult and still debatable due to causing some complications such are re-rupture, adhesion, and gap formation. Suturing technique, early rehabilitation, and tissue engineering have been proposed to overcome these circumstances. Collagen scaffold is one of the tissue engineering technique which used in this research to find the improvement of tendon healing biomechanically, in order to have an early rehabilitation is more possible. The objective is this study to prove increase on Achilles tendon suture biomechanically after bovine tendon triple helix collagen strand on certain period.

Materials and methods. Rabbit Achilles tendons for model were cut then sutured with 2 strand modified Kessler technique. These rabbits were randomly selected into 3 groups: sutured only, sutured with collagen sponge on-lay, and sutured with collagen sponge inserted between stumps plus on-lay additions. The rabbits were sacrificed on day 10 afterwards. The tendons were tested on the sutured site by traction machine until 4 mm gap formed (tensile strength) then continued until totally ruptured (ultimate strength). The result is analyzed by Anova test.

Results. Tensile strength shows improvement on the collagen groups other than on sutured only ($p<0.05$). The ultimate strength among groups is not significantly different, either collagen groups or non collagen one ($p=0.689$; $p>0.05$).

Conclusions. Bovine collagen addition as scaffold shows better biomechanical result in early tensile strength of ruptured tendon.

Keywords: tendon rupture, tissue engineering, collagen scaffold, early rehabilitation, tensile strength

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Pengaruh Penambahan Bovine Collagen pada Kekuatan Biomekanis Dini dari Tendon Achilles Kelinci yang Dilakukan Tindakan Repair Tendon

ABSTRAK

Pendahuluan. Ruptur tendon oleh berbagai sebab merupakan kasus yang sering terjadi di bidang orthopaedi. Manajemen dari kejadian ini seringkali menyulitkan dan masih menjadi perdebatan karena seringnya terjadi komplikasi berupa ruptur ulang, adhesi, dan terbentuknya *gap.* Beberapa solusi diusulkan untuk mengatasi hal ini, seperti perbaikan teknik penjahitan, rehabilitasi dini, dan rekayasa jaringan. *Scaffold* kolagen merupakan salah satu teknik dari *tissue engineering* yang dalam penelitian ini diharapkan dapat meningkatkan kekuatan biomekanik pada awal penyembuhan, sehingga lebih memungkinkan dilakukan rehabilitasi dini.
Bahan dan cara kerja. Kami menggunakan tendon Achilles kelinci yang dipotong sebagai model. Kelinci ini kami bagi secara acak menjadi 3 kelompok: kelompok yang dijahit dengan teknik *Kessler modified 2 strand,* kelompok yang dijahit dengan teknik yang sama serta ditambahkan spon kolagen secara *on-lay*, dan kelompok yang dijahit dengan teknik yang sama serta ditambahkan spon kolagen secara *on-lay* juga di antara *stump* tendon. Kelinci kemudian dipelihara dan dikorbankan pada hari kesepuluh. Tendon kemudian diuji tarik dengan mesin dan di evaluasi gaya yang dibutuhkan sampai terjadi gap antara *stump* sampai 4 mm (*tensile strength*), kemudian dilanjutkan sampai benar benar terputus (*ultimate strength*). Hasil pengukuran dianalisis menggunakan tes anova.
Hasil. Hasil penelitian ini menunjukkan peningkatan kekuatan *tensile* pada kelompok yang diberikan kolagen dibandingkan dengan yang hanya dijahit ($p<0,05$). Perbandingan di antara kelompok yang menggunakan kolagen sendiri hanya didapatkan peningkatan yang tipis pada kelompok yang diberikan *on-lay* dan di antara *stump* dibandingkan dengan yang hanya diberikan secara *on-lay* saja. *Ultimate strength* yang terukur tidak menunjukan perbedaan yang signifikan antar kelompok ($p=0.689$; $p>.05$).
Simpulan. Penggunaan *bovine collagen* sebagai *scaffold* menunjukkan adanya peningkatan biomekanis dini, terutama pada kekuatan *tensile* dari tendon.

Kata kunci: ruptur tendon, *tissue engineering*, *collagen scaffold*, rehabilitasi dini, kekuatan *tensile*

Introduction

Tendon rupture due to various causes are common orthopedic cases which the management had troubled the surgeons due its complications (re-rupture, adhesion, and gap formation).[1-3] Suturing technique, early rehabilitation, and tissue engineering have been proposed to overcome these circumstances. Many research have been done to find a proper solution. Collagen scaffold as one of simple tissue engineering has been proposed to be one of solution. Collagen scaffold bridge the cells seeding and increase the affinity for growth hormone, plasma, and drugs.[4] Even the use of collagen scaffold as treatment for Achilles tendon repair still inconclusive.[5] We had held a preliminary study on collagen as scaffold (Collacure) for tendon healing, compared with non collagen group, which result in favor for collagen group. Triple helix collagen strand we have used in this research as scaffold seek the improvement of tendon healing biomechanically, in order to have an early rehabilitation is more possible. Early rehabilitation itself will improve tendon healing and also remodeling respectively.[6-8]

Whether triple helix collagen strand bovine tendon application will increase early biomechanical strength of sutured rabbit Achilles tendon remains unknown. It is the objective of this study to prove the increases the strength of Achilles tendon suture biomechanically after application of triple helix collagen strand bovine tendon in certain period. The result of this research might be used for clinical consideration in rupture tendon management.

Materials and methods

This is a true experimental study with *post test control group* design. This experiment has been granted ethical clearance by Ethical Clearance Team of Medical Faculty of Brawijaya University. This research was held at

Orthopedic and Traumatology Laboratory of Brawijaya University and Mechanical Laboratory of Malang Polytechnic Institute on September 11th to October 10th 2012. The population of this study are 5-6 month old rabbit (*Oryctolagus cuniculus*), weighted 2-3 kg. Sample size were determined using formula for experimental studies with 15% drop out assumption, yielding 22 sample.

The inclusion criteria for the sample were healthy, active rabbits, has normal lower extremity form and movement while the exclusion criteria were infected rabbits, not survived (death) during experiment, and unhealed tendon or re-ruptured during harvesting.

The rabbits were anesthetized, by using Ketamine 40mg/BW intra peritoneally, then by midline approach after disinfecting procedures with povidone iodine, the Achilles tendons were cut then sutured with 2 strand Kessler modified technique. These rabbits were randomly selected into 3 groups with different procedures: sutured only (Figure 1), sutured with collagen sponge on-lay addition (Figure 2), and sutured with collagen sponge inserted between stumps plus on-lay addition (Figure 3). These manipulate extremities were cast afterwards. The rabbits were sacrificed on day tenth afterwards. The tendons were tested on the sutured site by traction machine until 4 mm gap is formed (tensile strength) then continued until totally ruptured (ultimate strength).

The result from traction machine is analyzed by one way ANNOVA test on SPSS 16.0 program.

Results

We have 22 rabbits on procedures divided into 3 groups randomly, 7 rabbits in each group, except for the sutured only group which had 8 samples. Nineteen samples were included in the analysis while 1 rabbit died during experiment (suture only group), 1 rabbit got infection (collagen on-lay group), and the other one got tendon re-rupture.

Macroscopically there was little difference between collagen group with sutured only. Non collagen group have rough edges on sutured site while collagen group have smooth & neat edges, but not fully absorbed collagen sponge.

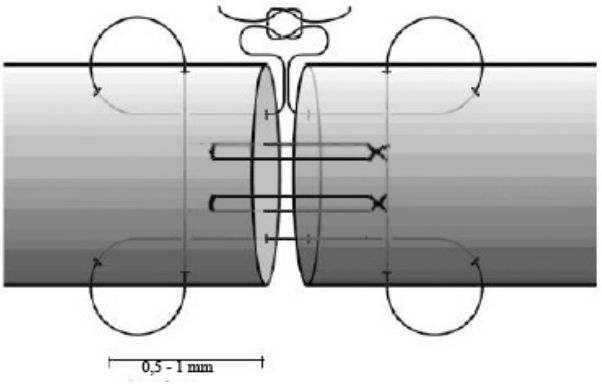

Figure 1.Kessler modified 2 strand tendon suture with epitenon running suture

The tensile strength for sutured only, sutured with collagen on-lay, and sutured with collagen on-lay and insertion were 1.06, 1.56, and 1.90 N/mm^2 respectively. The tensile load were 25.34, 32.7, and 40.88 N respectively. Both tensile load and tensile strength differed significantly among group (0.003 and 0.026 respectively).

The ultimate load for sutured only, sutured with collagen on-lay, and sutured with collagen on-lay and insertion were 42.51, 38.42, and 43.32 N while the ultimate strength were

1.77, 1.83, and 1.98 N/mm2 respectively. No statistical difference were found among group for ultimate load and ultimate strength (p=0.088 and p=0.659 respectively).

Discussions

Smoother and neat edges in collagen group sutured site shows better fibrous formation within the group.[9,10] Even though further investigation using histological analysis is needed. Tensile properties in non collagen group result in the same rate compared to other researches for Kessler modified 2 strand suturing technique, which exhibit the same standard for suturing technique we had. Tensile load to make a 2 mm gap in Kessler 2 strand technique in many research is 18.89–26.3 N, which is parallel to our result 25.34 N in mean.[11,12] Collagen addition gives better

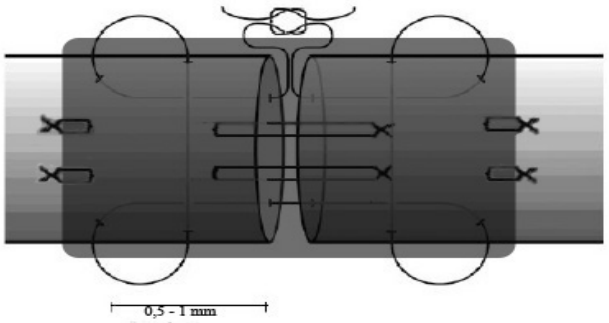

Figure 2. Kessler modified 2 strand tendon suture with epitenon running suture and collagen on-lay addition

healing biomechanically. It is due to the improvement on extrinsic and intrinsic healing by collagen inserted and on laid respectively.[13,15]

The positive results in tensile load for collagen supplementation groups does not followed by the ultimate properties measurement. Ultimate phenomenon showed by our result is parallel to other researches which Kessler 2 strand exhibit ultimate load 35 N.[11,12] This phenomenon stated that this property mostly is influenced by the suture strength.

The increase strength of biological properties give us suggestive assumption that this sutured tendon with collagen group will sustain load better than non collagen group. Early mobilization can be initialized. This means that rehabilitation program may start early. Early rehabilitation will improve tendon healing quality and prevent muscle weakness, tendon weakness, and joint compromise due to prolong immobilization.[14,15]

Conclusions

Tendon repair is still a controversial problem in orthopedic. Bovine collagen gives better biomechanical result in early tensile strength of ruptured tendon. Collagen scaffold improves the tensile strength of sutured tendon, allowing it to bear more stress/load and sustainable to early mobilization. Improvement of suture technique and material will increase ultimate load. Bovine collagen gives better biomechanical result in early tensile strength of ruptured tendon, but biological process is unknown and histopathological evaluation is needed. Evaluation by time interval will also sufficient to understand the process better. Further investigation is needed before this approach is clinically amenable.

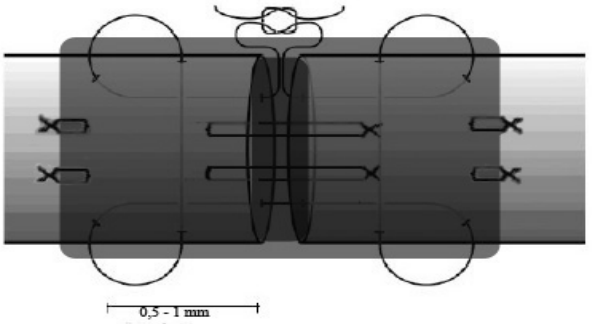

Figure 3. Kessler modified 2 strand tendon suture with epitenon running suture and collagen on-lay plus inserted addition

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