学 位 論 文 の 要 旨

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学 位 論 文 名　Effectiveness and Biocompatibility of a Novel Biological Adhesive Application for Repair of Meniscal Tear on the Avascular Zone

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論 文 内 容 の 要 旨

INTRODUCTION

The meniscus is categorized as fibrocartilage and intervenes between the femoral condyle and tibial plateau. Meniscus not only absorbs shocks, but also facilitates gliding and stabilizes the knee joint, while assisting the rotation and transmitting loads. The peripheral of the meniscus is attached to the joint capsule, and is vascularized in 10-25% of the region from the periphery by arborizing vessels. The remaining two-thirds of the meniscus is a free edge scanty of vessels, which is supplied from the synovium. Therefore, in case of meniscus injury, it is known that the region two-thirds from the free edge is difficult to heal spontaneously.

Surgical treatment methods for meniscal tear include rasping, suturing, partial menisectomy, allograft implantation, autograft implantation, and scaffold implantation. Currently, typical commercially available chemosynthetic biomedical adhesive are cyanoaccrylate adhesive, aldehyde-based adhesive, and fibrin-based adhesive. We contrived to use the novel biological adhesive, disuccinimidyl tartrate (DST) as a crosslinker and human serum albumin (HSA) as a hardener, for repairing longitudinal tear of the avascular zone of the meniscus, investigate the optimum usage, and evaluate the effectiveness and safety using animal models.

MATERIALS AND METHODS

Menisci in our *in vitro* study were collected from Japanese swine (24 months old). The quality of these menisci was strictly controlled (Shimane Meat Wholesale Co., Shimane, Japan). We used menisci of Japanese white rabbits (2 months old, body weight 2.2–2.4 kg;

KBT Oriental Co., Ltd., Saga, Japan) for the *in vivo* study.

To determine adequate concentrations of the DST-HSA adhesive, bonding strength was measured by setting the concentrations of DST at 0.05, 0.075, and 0.10 mmol, and 0.8 mg of HSA at 38, 40, and 42w/v% [0.1 M phosphate buffer solution (PBS, pH 6)]. Using porcine models, a longitudinal tear was made with a scalpel in the avascular zone of the meniscus at 5 mm from the joint capsule. Five minutes after applying the adhesive to the artificially torn region, bonding strength was measured using a tension tester (Instron 5565; Instron, Canton, Massachusetts, USA) providing the tensile load normal to the surface of repaired region. We compared the bonding strengths of the DST-HSA, cyanoaccrylate-based and fibrin-based adhesives. We applied these adhesive to the surface of the artificially torn porcine meniscus and measured the bonding strength. A total of 10 knees of 5 Japanese white rabbits were used, and longitudinal tears were made in the avascular zone of the lateral meniscus in both knees. The left knees were left untreated as control, whereas the right knees were treated applying the DST-HSA adhesive (DST 0.1 mmol, HSA 42 w/v%). Three months after the operation, the bonding strength was measured and histological findings were analyzed.

We used 2-0 braided polyester suture (Ethibond Excel®, Ethicon, Inc., Somerville, New Jersey, USA) for suturing the torn meniscus of the avascular zone of matured porcine. The bonding strength was compared among 3 groups: 1) sutured only (Suture Group), 2) sutured after applying the adhesive on the surface of torn region (Adhesive + Suture Group), and 3) sutured using the adhesive-soaked suture thread (Adhesive-soaked Suture Group).

Analysis of variance (ANOVA) was used to compare the bonding strength in each experiment. A p-value of less than 0.05 was considered to indicate a significant difference.

All the animal procedures were approved by the local committee for animal research of Shimane University(IZ 25-31).

RESULTS AND DISCUSSION

The bonding strength was compared among the DST-HSA adhesive (DST 0.1 mmol and HSA 42 w/v%) and two commercially available adhesive. The corresponding values were 767 $\pm 220 \times 10^{-3}$ N/mm^2 for the cyanoacrylate-based adhesive, $78 \pm 22 \times 10^{-3}$ N/mm^2 for the DST-HSA adhesive and $43 \pm 15 \times 10^{-3}$ N/mm^2 ($p<0.05$) for the fibrin-based adhesive. At 3 months after operation, the bonding strength of the treated knee was 2.08 N/mm^2, approximately 1.5 times higher than that of the untreated control knee. Hematoxyline-eosin stained samples at 3 months after operation showed no inflammation in the synovium where the adhesive had been applied, and crosslinking was seen in the ruptured meniscus, suggesting biological repair.

There was no statistically significant difference in bonding strength between the Suture Group (61 ± 5 N) and the Adhesive + Suture Group (60±8 N). There were statistically significant differences between the Adhesive-soaked Suture Group (77±6 N) and the other two

groups ($p < 0.05$). In the ruptured samples all failures were suture rupture under the knot.

Bonding strength of the DST-HSA adhesive was approximately two times greater than that of the fibrin-based adhesive in our study, and healing was histologically confirmed. Blood flow is present in only one-third of the meniscus, and the remaining two-thirds of the meniscus is the avascular zone, in which spontaneous healing is difficult biologically.

In cases of radial tear or horizontal tear of the meniscus, sometimes menisectomy may be the only choice of treatment, and there has been no established repairing method. Generally, to preserve the meniscus as much as possible, arthroscopic suturing of the meniscus is done for its longitudinal tear; however, the tensile load of the repaired site depends on the strength of the suture strands. Therefore, we proposed to improve treatment of the meniscus by application of a newly developed biological adhesive.

This adhesive is composed of DST as a crosslinker and HSA as the hardener, which combined active ester group with carboxyl group of tartrate. When DST and HSA are blended, the amino group of HSA and carboxylate ester of DST make an amide bond, and gelation occurs. When this blended adhesive is placed in collagen, amide bonding occurs with the amine in collagen, and results in crosslinking of collagens and biological bonding.

In our study, the combination of 42 w/v% of HSA and 0.1 mmol of DST yielded the highest bonding strength. In our experiments, the bonding strength of the DST-HSA adhesive was inferior to that of cyanoacrylate-based adhesive, but superior to that of fibrin-based adhesive.

This adhesive is a derivative of citrate, which exists in the human body, it provides high bonding strength, biocompatibility, and low toxicity. In our *in vivo* experiment using Japanese white rabbits, no inflammation was observed. Therefore, we believe our DST-HSA adhesive may be useful for clinical application to meniscus injury to preserve the meniscus in cases of horizontal tear or degenerative tear in the avascular zone.

In our study, the bonding strength of suturing using an adhesive-soaked suture strand was significantly greater than suturing after applying the adhesive on the surface of the lesion. We suggest that the DST-HSA adhesive remained in meniscal tissues and enforced bonding strength between the suture strand and collagen of meniscal tissues.

CONCLUSION

The newly developed DST-HSA adhesive is considered safe and may be effective in enforcement of bonding of avascular zone tear of the meniscus. If we can use this adhesive clinically, we may be able to keep the function of joints and avoid the procession of osteoarthritis in future.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

膝関節半月板は膝関節運動において重要な役割を有しているが、加齢や過重によって断裂を来たし膝関節障害の原因となる。かつて半月板断裂には半月板切除術が行われていたが、切除により荷重負荷が増強し変形性膝関節症を招来することから、半月板を温存する治療法が求められている。近年の関節鏡下手術の発達により、損傷半月板の縫合治療が可能となり、術後長期にわたる膝関節機能維持が得られるようになったが、半月板の無血行野での断裂では、現在でも縫合術による癒合修復が困難であり、治療上の課題となっている。申請者は半月板無血行野断裂に対して、新しい生体接着剤を用いた半月板修復治療法の有効性に関しての基礎実験を行った。使用した生体接着剤はクエン酸誘導体であるジスクシンイミジルタータレートを硬化成分に、ヒト血清アルブミンを接着成分として新たに開発した生体接着剤であり、コラーゲン同士のアミド結合による架橋によって接着させる作用がある。実験は、①ブタ半月板を用いて断裂モデルを作製し、試験薬と市販の医療用接着剤（シアノアクリレート系接着剤とフィブリン系接着剤）との接着強度を比較した。②ブタ断裂半月板に対し、a) 縫合単独、b) 縫合+断裂面への試験薬塗布、c) 試験薬浸漬縫合糸による縫合＋断裂面への試験薬塗布の３群での力学的強度を比較した。③日本白色家兎の外側半月板無血行野縦断裂モデルを作成し、断裂部への試験薬塗布６か月後の組織学的評価を行った。実験の結果、試験薬の接着強度はシアノアクリレート系接着剤に劣るものの、フィブリン系接着剤の1.8倍の強度が認められた。断裂半月板に対し、縫合に加えて断裂面および縫合糸に試験薬を浸漬させた群では縫合単独群の1.3倍の接着強度を得た。組織学的評価では、試験薬を塗布しない半月板は断裂のままであったが、塗布により断裂半月板の癒合が観察され、膝内の周囲組織に炎症等の有害所見を認めなかった。本研究は修復治療が困難な半月板無血行野断裂に対する生体接着剤を用いた新しい治療法の開発の基礎となりうる研究であり、高い臨床的価値を有する論文と評価した。

最終試験又は学力の確認の結果の要旨

申請者の研究は、膝関節疾患の治療上の課題である半月板無血行野断裂において、新しい生体接着剤の使用による半月板温存縫合手術の有効性を示し、臨床応用への可能性を期待させるものである。関連領域の学識も十分であり、学位授与に値すると判断した。（主査　秋山恭彦）

申請者は、半月板無血行野断裂の治療における、新しい生体接着剤を併用した縫合治療法の有用性を、綿密に計画された動物実験によって明らかにした。臨床応用が期待できる価値ある研究であり、関連領域の知識や考察力も十分であったので、学位授与に値すると判断した。

（副査　安井幸彦）

申請者は、整形外科分野の臨床上の課題である膝関節半月板損傷に対して、新規の生体接着剤を用いた治療法の有効性・安全性を動物実験により明らかにした。高い学術的価値と臨床応用への可能性を有する優れた研究で、関連分野の学識も十分であり、学位授与に値すると判断する。

（副査　織田禎二）

（備考）要旨は、それぞれ４００字程度とする。