EFIKASI PENGGUNAAN KOMPOSIT *FREEZE DRIED TENDON ALLOGRAFT*DAN SEL PUNCA MESENSIMAL DALAM REKONSTRUKSI DEFEK TENDONFLEKSOR Penelitian Eksperimental Pada Hewan Coba

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ABSTRACT

THE EFFICACY OF USING COMPOSITE FREEZE DRIED TENDON ALLOGRAFT AND MESENCHYMAL STEM CELL TO RECONSTRUCT FLEXOR TENDON DEFECT

Heri Suroto

Flexor tendon defect caused by acute or chronic ruptured should be filled with tendon graft. So far there was no functional results with a tendon allograft obtained to address the flexor tendon defect. This study aimed to regain the integrity and biomechanical properties of tendons in the flexor tendon defect connected by composite of freezed dried tendon allograft and bone marrow mesenchymal stem cells.

The design of this research was the randomized post test only control group. Experimental unit were male New Zealand White Rabbits. In these experimental animals were slaughtered their Flexor Carpi Ulnaris tendon right leg by 1 cm and then made a connection with reconstruction using autograft tendon (control group) and composite of freeze dried tendon allograft and bone marrow mesenchymal stem cells (treatment group). After 6 weeks FCU tendons that had been reconstructed were analyzed by macroscopic examination, histological, immunohistochemical, and biomechanical examination as well to assess the new tendon tissue regeneration.

The Reconstructed flexor tendon defect with composite freeze dried tendon allograft –mesenchymal stem cell in cross sectional examination are bigger than autograft tendon (7,18 mm^2 and 4,20 mm^2 , p = 0,000).The Scaffold degradation process that expressed by MMP-8 (p=0,000) are more active, and also these happened in new tendon regeneration process through the expression of Scleraxis and Collagen type 1(p=0,000). Reconstruction of the flexor tendon defect with composite freeze dried tendon allograft – bone marrow mesenchymal stem cell actually decreased in maximal load (88%), tensile strength (78,7%), modulus elasticity (72,66%) compare to tendon autograft, but the difference is not significant.

The conclusion of this research was composite freeze dried tendon allograft and mesenchymal stem cell can be used in tendon tissue engineering instead of autograft tendon.

Key words: Functional tendon, freeze dried allograft tendon , mesenchymal stem cell

SUMMARY

THE EFFICACY OF USING COMPOSITE FREEZE DRIED TENDON ALLOGRAFT AND MESENCHYMAL STEM CELL TO RECONSTRUCT FLEXOR TENDON DEFECT

Flexor tendon defect caused by acute or chronic ruptured should be filled with tendon graft. The first series in which free flexor tendon graft were used in the hand was reported by Lexer in 1912 (Boyer, et.al., 2002). He used grafts to repair flexor tendon after rupture, old laceration, infection and hopeless cases of ischaemic contracture. Bunnel (1918) preferred the Palmaris longus tendon as the donor graft. The surgical methods and results of free flexor tendon grafting had subsequently been modified and reviewed by various leaders in the field of hand surgery from all over the world: Pulvertaft (1956) in England, Graham (1947), Littler (1947), Boyes-Stark (1971) and White (1956) in United States, Rank dan Wakefield (1952) in Australia, Verdan (1972) in Switzerland and Tubiana (1973) in France, but the limited availability of donor tendon and the morbidity that developed from the harvesting make it necessary to consider the other alternative to use allograft tendon. So far there was no functional results with a tendon allograft obtained to address the flexor tendon defect.

Reconstruction of the flexor tendon defect continues to change along with the development to achieve better results. Autograft and allograft tendon have been used with various clinical success rates. Currently, biodegradable polymers have been used as a scaffold. This biodegradable materials such as collagen, chittin, polylactic acid (PLA) and polyglycolic acid (PGA) have been used in tendon repair, but there is no general principle that can explain and predict the incidence of adhesion, proliferation and spread of cell culture on different polymer surfaces. Because of the relatively avascular, tendon is the leading candidate in the replacement of the tissue. Previous experiments have been made to create a tendon in vitro, but less successful because of difficulties in forming a compatible construction, both mechanical and biological environment in vivo (Birk and Zycband, 1994).

This study aimed to regain the integrity and biomechanical properties of tendons in the flexor tendon defect connected by composite of freezed dried tendon allograft and bone marrow mesenchymal stem cells.

The design of this research was the randomized post test only control group. Experimental unit were male New Zealand White Rabbits. In these experimental animals were slaughtered their Flexor Carpi Ulnaris tendon right leg by 1 cm and then made a connection with reconstruction using autograft tendon (control group) and composite of freeze dried tendon allograft and bone marrow mesenchymal stem cells (treatment group). After 6 weeks FCU tendons that had been reconstructed were analyzed by macroscopic examination, histological, immunohistochemical, and biomechanical examination as well to assess the new tendon tissue regeneration.

The research proposal was approved by the Research Ethics Committee of Faculty of Veterinary Medicine, Airlangga University (Animal Care and Use Committee / ACUC) No: 063-KE on 21 August 2009. Data were analyzed using the Mann-Whitney test, t-test and *One Way Anova*.

The Results showed that flexor tendon defect reconstruction using composite freeze dried tendon allograft and mesenchymal stem cells had larger cross section surfaces compared with autograft tendon (7.18 mm2 and 4.20 mm2, p = 0.000). Results of biomechanical testing of the flexor tendon tissue defect reconstruction is a decline in the maximal load on the flexor tendon defect reconstruction with a tendon autograft and composite tendons (51.40 N + / - 15.99 and 45.30 N + / - 3.93, p = 0.432) as well as the tensile strength (13.19 N/mm^2 + / - 3.18 and 10.38 N/mm^2 +/ - 1.13, p = 101) and Modulus of Elasticity (17.63 Mpa +/ - 5.46 and 12.81 Mpa +/ - 2.19 .) In case tensile strain increased (91.89% +/ - 24.34 vs 93.69% +/ - 5.72, p = 876). But we did not find statistically significant differences in biomechanical properties of these tendons. Results Scaffold degradation process through the expression of MMP-8 showed that the flexor tendon defect reconstruction with freeze dried composite tendon allograft and stem cells mesenchymal greater MMP-8 activity was compared with tendon autograft and these differ significantly. The result of the synthesis of new tendon tissue through the expression Scleraxis and Collagen - 1 shows that the reconstruction of the flexor tendon defects with composite freeze dried tendon allograft and stem cells mesenchymal more active than tendon autograft and these differ significantly.

The conclusion of this research was the integrity and biomechanical properties of the rabbit flexor tendon defect reconstruction can be achieved by using composite freeze dried tendon allograft and stem cells mesensimal.

The new findings in this study was the use of composite freeze dried tendon allograft and mesensimal stem cells from bone marrow in the reconstruction of the flexor tendon defect.

RINGKASAN

EFIKASI PENGGUNAAN KOMPOSIT *FREEZE DRIED TENDON ALLOGRAFT* DAN SEL PUNCA MESENSIMAL DALAM REKONSTRUKSI DEFEK TENDON FLEKSOR

Adanya defek pada terputusnya tendon fleksor maka graf tendon semestinya dilakukan untuk mengembalikan keutuhan tendon tersebut. Seri *free graft* pada tendon fleksor pertama, yang digunakan pada tangan telah dilaporkan oleh Lexer tahun 1912 (Boyer *et al*., 2002). Penggunaan graf ini dilakukan pada putusnya tendon baik akut maupun kronis, pada kasus infeksi dan kontraktur iskemia. Bunnel (1918) telah menggunakan tendon palmaris longus sebagai graf donor. Metode pembedahan dan hasil dari *free graft* tendon fleksor telah dimodifikasi oleh pakar di bidang bedah tangan dari berbagai negara seperti: Pulvertaft (1956) dari Inggris, Graham (1947), Littler (1947), Boyes-Stark (1971) dan White (1956) dari Amerika Serikat, Rank dan Wakefield (1952) dari Australia, Verdan (1972) dari Swiss dan Tubiana (1973) dari Perancis, tetapi semuanya masih menggunakan donor tendon dari autograf. Keterbatasan dari donor tendon dan morbiditas yang dapat ditimbulkan dari pengambilannya, maka perlu dipikirkan alternatif lain yaitu tendon alograf. Sejauh ini belum didapatkan hasil yang fungsional pada penyambungan dengan tendon alograf untuk mengatasi defek tendon fleksor.

Rekonstruksi defek tendon fleksor terus mengalami perubahan seiring dengan perkembangan untuk mencapai hasil yang lebih baik. Tendon autograf dan alograf telah

digunakan dengan angka keberhasilan klinis yang masih bervariasi. Saat ini polimer *biodegradable* telah digunakan sebagai *scaffold.* Material *biodegradable* ini seperti kolagen, *chittin*, *polylactic acid* (PLA) dan *polyglycolic acid* (PGA) telah digunakan dalam reparasi tendon, namun belum ada prinsip umum yang bisa menjelaskan dan memprediksi kejadian adhesi, proliferasi dan penyebaran kultur sel pada permukaan polimer yang berbeda. Karena relatif avaskular maka tendon merupakan kandidat utama dalam penggantian jaringan. Percobaan sebelumnya telah dibuat untuk menciptakan tendon in vitro, tetapi kurang berhasil karena kesulitan dalam pembentukan konstruksi yang kompatibel baik secara mekanis maupun biologis dalam lingkungan in vivo (Birk dan Zycband, 1994).

Penelitian ini bertujuan untuk mendapatkan kembali keutuhan dan sifat biomekanik tendon pada defek tendon fleksor yang disambung dengan *freezed dried tendon allograft* yang telah ditanami sel punca mesensimal dari sumsum tulang.

Jenis penelitian adalah *randomized post test only control group design*. Unit eksperimen adalah kelinci *New Zealand White Rabbit* jantan. Pada hewan coba ini dilakukan pemotongan tendon Fleksor Carpi Ulnaris (FCU) kaki kanan sebesar 1 cm kemudian dilakukan rekonstruksi berupa penyambungan dengan menggunakan tendon autograf (kelompok kontrol) dan komposit *freeze dried tendon allograft* dan sel punca mesensimal dari sumsum tulang (kelompok perlakuan). Setelah 6 minggu dilakukan pengambilan tendon FCU yang telah direkonstruksi dan dianalisis dengan pemeriksaan makroskopis, histologis, imunohistokimia, dan biomekaniknya untuk menilai regenerasi jaringan tendon yang baru.

Proposal penelitian telah mendapatkan persetujuan laik etik dari Komisi Etik Penelitian Fakultas Kedokteran Hewan Universitas Airlangga (*Animal Care and Use Committee / ACUC*) No: 063-KE tanggal 21 Agustus 2009. Data hasil penelitian dianalisis dengan uji komparasi *Mann-Whitney* dan *T-test* serta *One Way Anova*.

Hasil rekonstruksi defek tendon fleksor dengan komposit *freeze dried tendon allograft* dan sel punca mesensimal lebih besar penampang melintangnya dibandingkan dengan tendon autograf (7,18 mm^2 dan 4,20 mm^2 , p = 0,000). Hasil uji biomekanik terhadap jaringan rekonstruksi defek tendon fleksor ini terjadi penurunan dalam hal *maximal load* pada rekonstruksi defek tendon fleksor dengan tendon autograf dan komposit tendon (51,40 N +/- 15,99 dan 45,30 N +/- 3,93, p=0,432) demikian juga halnya dengan *tensile strength* (13,19 N/mm^2 +/- 3,18 dan 10,38 N/mm^2 +/- 1,13, p=101) dan *Modulus Elastisitas* (17,63 Mpa +/- 5,46 dan 12,81 Mpa +/- 2,19). Dalam hal *tensile strain* terjadi peningkatan (91,89 % +/- 24,34 vs 93,69 % +/- 5,72, p=876). Secara statistik tidak didapatkan perbedaan yang bermakna pada sifat-sifat biomekanik tendon tersebut. Hasil proses degradasi scaffold melalui ekspresi MMP-8 menunjukkan bahwa rekonstruksi defek tendon fleksor dengan komposit *freeze dried tendon allograft* dan sel punca mesensimal lebih besar aktifitas MMP-8 nya dibandingkan dengan tendon autograf dan ini berbeda secara bermakna (p=0,000). Hasil proses sintesa jaringan tendon baru melalui ekspresi Scleraxis dan Kolagen - 1 menunjukkan bahwa rekonstruksi defek tendon fleksor dengan komposit *freeze dried tendon allograft* dan sel punca mesensimal lebih banyak jumlah Scleraxis dan Kolagen - 1 nya dibandingkan dengan tendon autograf dan ini berbeda secara bermakna (p=0,000 dan p=0,000).

Kesimpulan penelitian ini adalah terjadi kembali keutuhan dan sifat biomekanik tendon yang fungsional pada jaringan rekonstruksi defek tendon fleksor kelinci yang disambung dengan komposit *freeze dried tendon allograft* dan sel punca mesensimal.

Penemuan baru pada penelitian ini adalah penggunaan komposit *freeze dried tendon allograft* dan sel punca mesensimal dari sumsum tulang dalam rekonstruksi defek tendon fleksor dapat mengembalikan keutuhan dan sifat biomekanik tendon fleksor.