The Effect of Allogenic Bone Marrow on Integration Dev-Mesenchymal Stem Cell And Vascular Endothelial Growth Factor

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Abstract

Background: The integration of the tendon graft within the bone tunnel is one of the decisive success factors of the Anterior Cruciate Ligament (ACL) reconstruction. In ACL reconstruction that uses tendons as graft, the healing process that occurs in the bone tunnel is healing with fibrotic tissue.

Aim: This study aims to determine the role of bone marrow mesenchymal stem cells and vascular endothelial growth factor intraarticularly in improving the biomechanical strength.

Methods: This study uses experimental research design by using animal as a testing tool. The animals used in this study are male New Zealand white rabbits (Oryctolagus cuniculus), with the weight ranging of 2000 grams up to 3000 grams.

Results: From the experiment, all samples from both treatment and control groups were all evaluated and no post-operative complication was found. At the evaluation time of 3 weeks, the difference between the mean of maximum strain rate between the treatment group and the control group was not statistically significant ($p > 0.05$). At the evaluation time of 6 weeks, it was found that the difference in mean value of the maximum strain load force between the treatment groups versus the group was statistically significant.

Conclusions: From this study, it can be concluded that intraarticular BM-MSC and VEGF administration can increase the ultimate tension strength of postoperative graft tendon rosary ACL reconstruction.

Keywords: *Healing of bone tendon-tunnel, ACL reconstruction, Vascular Endothelial Growth Factor (VEGF), Bone Marrow Derived Mesenchymal Stem Cell (BM-MSC).*

Introduction

The process of widening the tunnel is an important concern in ACL reconstruction. This process occurs due to two things,s namely the existence of micro-motion and extravasation of synovial fluid into the bone tunnel[1]. The ACL post-operative rehabilitation program requires limited mobilization to provide sufficient opportunities for integrated graft within the bone tunnel[2]. One of the critical to the success of ACL reconstruction through the process of integration between tendon graft and bone tunnel[3].

Techniques used in ACL reconstruction to improve and improve integration between tendon graft and bone tunnel. The hope is, allowing the sufferer to start and undergo the rehabilitation process and ultimately speed up the chances of the sufferer to immediately return to the activity and return to the sporting activities[4]. Mesenchymal Stem Cell has been assessed to have the potential to improve the quality of integration between tendon graft and bone tunnel.

The ability of stem cells to differentiate and ease in the extraction process is one of the considerations in the use of mesenchymal stem cells in an attempt to enhance the integrity of the tendon graft bone tunnel. Several

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studies have mentioned a soft tissue graft augmentation technique using mesechymal stem cell histologically and biomechanically[5]. Histologically, the tissue formed at the border contact of the tendon graft surface and the bone wall of the bone tunnel resembles the healing of the bone-tendon-bone graft on the reconstruction of the ACL[5]. Another study mentions a histological picture of integration that resembles chondral enthesis of native ACL insertion[2].

In addition, it is biomechanically mentioned that the augmented graft tendon with mesenchymal stem cell has a failure limit on higher loads when compared with other augmentation methods[5]. This is due to the widening of the tunnel[4]. One theory has explained the influence of synovial fluid within the joints, in which the synovial fluid has the effect of inhibiting the process of forming the hematoma.

Bone marrow derived mesenchymal stem cells (BMSCs) are a new source of cells to help speed the healing and regeneration of new tissues. BMSCs are able to differentiate into osteoblasts that will form cartilages to form fibrocartilage tissue. Healing with fibrocartilage resembles healing in the bone-tendon-bone graft type[2]. Biomechanical studies that have been done also provide results of fibrocartilage healing on bone-tendon-bone graft better than the healing fibrocollagen on soft tissue graft[6]. Furthermore, histologically, fibrocartilage healing may provide an image resembling chondral enthesis in normal native ACL insertions[4].

One of the problems with the ACL reconstruction process is the occurrence of hypoxia in the automotive tendon tissue after harvesting or harvesting. A study in Japan has proven that VEGF can improve vascularization and improve the quality of fibroblast tissue integration[7]. In other side, VEGF was evaluated based expression identified through immunohistochemical staining[8]. Based on the description above, this study aims to determine the effect of intra articular bone marrow derived stem cells (BMSCs) and vascular endothelial growth factor (VEGF), to increase the biomechanical strength of the autograft tendon integrity in the bone tunnel in retaining the pull-out force ACL reconstructions.

Method

This study uses experimental research design by using animal as a testing tool. The animals used in this study are male New Zealand white rabbits (Oryctolagus cuniculus), with the weight ranging of 2000 grams up to 3000 grams. The study was conducted from June to August 2014 for the research procedure, while it takes one month (August 2014-September 2014) for the analysis of the results and the preparation of the research report. This study located at Tropical Disease Center (TDC) Airlangga University.

The anesthesia drugs used were a combination of xylazin with a concentration of 100mg / cc and ketamine, a minor surgical base set, consisting of a scalpel, tweezers, tissue scissors, skin retractor, scissors of yarn, and pean clamp, sewing threads consisting of ethibond 4.0 for graft fixation and monofilament absorbable syntetic measures 4.0 to cover the capsule and field operation. The research instrument used a ruler to measure elongation and translational distance (drawer), Autograph AG-10E engine (Shimadzu Corporation, Japan) to measure the forces required to create pullouts or ruptures from tendon graft.

After doing the research result of evaluation and biomechanical test collected then done analysis and statistical test. Data collected from result of evaluation from second time done recapitulation, tabulation, and statistical analysis. All data were analyzed using IBM SPSS v.21.0 for windows program (SPSS. Inc. Chicago. IL).

Results

Based on the results of data analysis obtained the results of posterior anterior translational distance measurement at various angles (Table 1).

Table 1. Measurements of posterior anterior translation at various angles and ultimate tension strength measurements and types of damage occurring in the tendon graft during evaluation periods 3 and 6 weeks.

Group	Unit Exp	Anterior-Posterior Translation (mm)			Ultimate Tension Strength	Type of Damage
		30°	60°	90°		
3-week control	K3.1	1	2	2	0.027	Rupture
	K3.2	3	2	2	0.012	Rupture
	K3.3	3	3	5	0.036	Rupture
	K3.4	3	3	3	0.010	Rupture
	K3.5	3	4	4	0.012	Rupture
	K3.6	2	4	5	0.021	Rupture
6-week control	K6.1	2	3	4	0.042	Rupture
	K6.2	3	4	3	0.038	Rupture
	K6.3	4	3	3	0.035	Rupture
	K6.4	2	3	4	0.041	Rupture
	K6.5	2	6	7	0.067	Rupture
	K6.6	1	4	3	0.050	Rupture
3-week treatment	P3.1	4	6	8	0.027	Rupture
	P3.2	1	2	3	0.012	Pullout
	P3.3	1	3	4	0.019	Rupture
	P3.4	1	2	5	0.027	Rupture
	P3.5	2	3	4	0.013	Rupture
	P3.6	2	4	6	0.012	Pullout
6-week treatment	P1.1	2	4	3	0.057	Rupture
	P1.2	2	3	2	0.075	Rupture
	P1.3	3	4	3	0.055	Rupture
	P1.4	2	2	3	0.083	Rupture
	P1.5	2	2	2	0.082	Rupture
	P1.6	2	3	2	0.067	Rupture

From the table 1, after the calculation of mean and standard deviation of anterior-posterior translation distance that occurred in each group both control and treatment group (Table 2). Here is result for table 2.

Table 2. The Mean Result of Posterior Anterior Translational Distance at Various Angles and Ultimate Tension Strenght.

Variable of anterior-posterior translation gap	**Group**			
	Control		**Treatment**	
Duration	3 weeks	6 weeks	3 weeks	6 weeks
Translation Angle				
30°	2.50 ±0.837	2.33 ±1.033	1.83 ±1.169	2.17±0.408
60°	3.00 ±0.894	3.83 ±1.169	3.33±1.506	3.00±0.894
90°	3.50 ±1.378	4.00 ±1.549	5.00±1.789	2.50±0.548
Variable of ultimate tension strength	Groups			
	Control		Treatment	
	3 week	6 week	3 week	6 week
	0.0197±0.010	0.046±0.012	0.018±0.007	0.0698±0.012

From that result, statistical analysis in both groups was based on two observation times (3 and 6 weeks). From the two groups, a comparative test was performed using Mann-Whitney test method. Normality test results using Shapiro-Wilk test showed the results p >0.05 indicating the data is not normally distributed. Therefore, the test used was Mann-Whitney test (Table 3).

Table 3. Data distribution normality test results for the mean of anterior-posterior translation at various angles and Shapiro-Wilk test results.

Normality Test					
	Group	**Kolmogorov-Smirnov***		**Shapiro-Wilk**	
		Statistic	**Sig.**	**Statistic**	**Sig.**
Translation 300	3 week control	0.392	0.004	0.701	0.006
	6 week control	0.293	0.117	0.915	0.473
	3 week control	0.277	0.168	0.773	0.033
	6 week treatment	0.492	0.000	0.496	0.000
Translation 600	3 week control	0.202	0.200*	0.853	0.167
	6 week treatment	0.277	0.168	0.773	0.033
	3 week treatment	0.254	0.200*	0.866	0.212
	6 week treatment	0.202	0.200*	0.853	0.167

Cont... **Table 3. Data distribution normality test results for the mean of anterior-posterior translation at various angles and Shapiro-Wilk test results.**

Translation 900	3 week control	0.195	0.200*	0.861	0.191
	6 week control	0.333	0.036	0.721	0.10
	3 week control	0.212	0.200*	0..933	0.607
	6 week	0.319	0.056	0.683	0.004
Pull-out strenght	3 week control	0.271	0.191	0.885	0.291
	6 week control	0.284	0.140	0.847	0.150
	3 week treatment	0.271	0.194	0.798	0.056
	6 week treatment	0.187	0.200*	0.893	0.337

a Lilliefors Significance Correction

*This a lower bound of the true significance

The result of Mann-Whitney test on 3 weeks postoperative evaluation was 0.21, 0.867 and 0.166 which mean significant with p> 0.05. Thus, it can be concluded that there was no significant difference between posterior anterior posterior translation between control group and treatment at angle 30, 60 or 90 degree (Table 4).

Table 4. Mann-Whitney test results for comparison of mean posterior anterior posterior translation at evaluation of time 3 weeks and 6 weeks

Test	3 week-evaluation			6 week-evaluation		
	Translation 300	**Translation 600**	**Translation 900**	**Translation 300**	**Translation 600**	**Translation 900**
Mann-Whitney Test	0.21	0.867	0.166	0.849	0.235	0.020

Evaluation of 3 weeks p >0.05

Evaluation of 6 weeks at 30^0 dan 60^0 p >0.05 and translation 90^0 p < 0.05

From table 4 there was a significant difference of AP translation distance at 90 degree knee angle between control and treatment group (significancy 0.020, p <0.05) (Table 4). The control group at 3 weeks evaluation period had a mean value of 0.0197, the control group with the evaluation period of 6 weeks had a mean value of 0.046 while the treatment group with 3 weeks evaluation had a mean value of 0.018 and in the treatment group with 6 weeks evaluation period had a mean value of 0.0698 (Table 2).

Shapiro-Wilk test results obtained significance value 0.291, 0.150, 0.058 and 0.337.Bleh because the value of p >0.05 it can be concluded that the data distribution for each group is normal, so for comparative test that can be done is by t-test unpaired (independent student t test).

For comparison group comparison and treatment at 3 weeks evaluation, the result of significancy was 0.801. Because p >0.05 it can be concluded that there was no significant difference between pullout strength / ultimate tension strength of control group and treatment during evaluation period 3 weeks.

Table 5. The result of independent test of T test comparing the ultimate value of tension strength between the two groups during the evaluation period of 3 weeks and 6 weeks

Variable	Group (N=6)					
	3 weeks			6 weeks		
	Control	Treatment	T-test	Control	Treatment	T-test
Pull-out strength	0.1967±0.10328	0.01833±0.07202	0.801	0.04550±0.011675	0.06983±0.012172	0.005

The biomechanical test was performed in 3 weeks postoperative control group and 6 tendon graft was ruptured on the intraarticular (midsubstance) part (100%) while the 3-week treatment group also obtained 4 graft tendon rupture on the intra-articular (midsubstance) (66.7%) and 2 graft tendons had pullout on the bone tunnel of the femur (33.3%).

Discussion

ACL reconstruction often uses a hamstring tendon as one of the tendon graft options[9]. Many studies have studied aspects of the use of hamstring tendons as grafts, ranging from techniques, biomechanical, histologic aspects as well as the results of this procedure[4].

The success of the ACL reconstruction procedure using a hamstring tendon as a tendon graft depends not only on the initial fixation when the graft mounting operation is performed, but on the biological integration of the longitudinal graft in the bone tunnel that determines long-term outcomes, but the factors affecting the biological integration of the tendon graft on the bone tunnel remains unclear. However, from a variety of materials used, there has been no research that proves the superiority of a material that significantly outweighs other additions[3].

Biomechanical testing is one of the most commonly used methods to study the effectiveness of a material used as an augmentation to accelerate or increase the integration strength of tendon graft in a bone tunnel[10]. There are different histologic features that occur in the mouth of the bone tunnel or the entrance and along the bone tunnel. In the study also mentioned that the position of the tendon graft insertion in the bone tunnel also has a role to play in the resulting force and work on the tendon graft. This is important given the various techniques and tunnel positions used in actual human ACL reconstruction[11].

One of the things that happen to the post-reconstruction tendon graft is the occurrence of reduction in tendon stiffness. This results from the effects of VEGF on the tendon graft which results in a decrease in the stiffness of the tendon graft itself. In the present study this phenomenon can be observed by the posterior anterior-posterior translation of the postoperative femur-graft-tibia complex compared between the control and treatment groups[12].

The results of this study differ from those shown by previous studies that mention significant differences in mean AP translation distances between control groups and treated groups in the form of VEGF[7]. But at the beginning of postoperative graft tendon histologic features obtained in the form of new vascular formation areas, infiltration of new cells and the formation of new collagen fibers that have not been arranged regularly[13]. The formation of new vascular tissues, irregular infiltration of cells and collagen fibers is a “weakness” of the tendon graft structure used[14]. In addition external VEGF applications also enzyme matrix metalloproteinase (MMP) by several types of cells[9]. The result of mean comparison of AP translation distance between control and treatment group in this study showed that there was no significant difference between the two groups except in the treatment group at 90 degree angle at 6 weeks evaluation[6].

This is consistent with the results of previous studies showing no significant difference between ultimate tension strength between the tendon groups receiving VEGF and the control group[7]. However, in this study for comparisons of 6 weeks evaluation results showed significant differences. In the control group, the tendon graft underwent ischemic and transient necrosis following harvesting, but after implantation, there was a normal healing process through fibroblast tissue[3].

Conclusion

From this research it can be concluded if there is no significant difference from AP translation distance that reflects the stiffness of the tendon graft between reconstruction of ordinary ACL and reconstruction ACL coupled with intraarticular BM-MSC and VEGF delivery.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

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