BIOMECHANIC STUDY OF GRAFT BONE TUNNEL MODEL IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION USING INTRATUNNEL ALLOGENIC BONE MARROW MESENCHYMAL STEM CELLS (BM-MSCs) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

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ABSTRACT

Successful anterior cruciate ligament (ACL) reconstruction using tendon graft requires good and rapid integration between the tendon graft and the bone tunnel. The strength of the tendon-bone tunnel graft in the initial phase is very important to facilitate aggressive rehabilitation and as early as possible to support rapid recovery to normal activities. The objective of this study was to determine ultimate tension strength (UTS) on the femoral tendon-bone tunnel graft model after reconstruction of anterior cruciate ligament (ACL) by administering allogenic bone marrow mesenchymal stemcells (BM-MSCs) and vascular endothelial growth factor (VEGF) intratunnel in experimental animals. The design of this research was Post-Test Only Control Group Design using 24 rabbits divided into treatment and control group. Biomechanical evaluation was done at week 3 and 6. Evaluation at week 3 found ultimate tension strength of treatment group significantly higher than control ($p < 0,05$). In the 6th week evaluation, Ultimate tension strength was found that the treatment group significantly higher than the control group ($p < 0.05$). Ultimate tension strength at week 3 did not differ significantly with week 6 ($p > 0.05$). Intravenous administration of BM-MSCs and VEGF on ACL reconstruction increased ultimate tension strength in graft-bone tunnel significantly since week 3. The study of Ferdiansis et al using BM-MSCs and VEGF intraarticular, only showed a significant increase in ultimate tension strength in graft-bone tunnel since week 6. Comparison of this method indicates acceleration in incorporation of tendon graft with bone tunnel on intratunnel method better thaninvitro intraarticular method.

Keywords : Anterior cruciate ligament, allogenic bone marrow mesenchymal stem cells, vascular endothelial growth factor and biomechanic study.

INTRODUCTION

Anterior Cruciate Ligament (ACL) reconstruction varies considerably in terms of surgical technique, graft source used, and the graft fixation method (Alpert et al., 2008). The main weakness of ACL reconstruction using soft tissue graft is the weak initial relationship of the tendon-bone interface. The power of graft-bone tunnel in the early phases becomes crucial to facilitate aggressive rehabilitation, so recovery processes including functional exercises, exercise and daily activities can be undertaken soon (Dong et al., 2012). Therefore, the success of ACL reconstruction

using a tendon graft requires good and rapid integration between the tendon graft and the bone tunnel(Dong et al., 2012; Baxter & Bach, 2010; Ferdiansis, 2014).

Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) are multipotent stem cells and have become an important cell source for engineering purposes in terms of tissue repair and cell therapy (Griffin et al., 2010). Several studies have shown the effects of several polypeptides such as Bone Morphogenetic Proteins (BMPs), and Growth Factors (GFs) such as Transforming Growth Factors (FGFs), Platelet-Derived Growth Factors (PDGF), and Epidermal Growth Factor (EGF) to the activation and regulation of proliferation and differentiation of bone tissue, fibrous tissue, and soft bone tissue (Milano et al., 2008; Dong et al., 2012; Ma et al., 2007; Zelle et al., 2005).

Another problem with post-reconstruction ACL healing process is the occurrence of hypoxia in the tendon tissue autograft. These conditions cause the occurrence of hypocellular and early degeneration conditions that cause a risk of rupture and elongation in the structure of the tendon graft. Study in Japan, VEGF has been shown to increase vascularization and improve the quality of fibroblast tissue integration between the tendon graft and bone tunnel (Ju et al., 2006; Reinert et al., 2013). Keep in mind also that synovial fluid contains enzymes that cause haematomas as early healing phases are difficult to form (Kaplan & Fu, 2004). Thus the provision of fibrin plugs on the articular side to block the entry of synovial fluid may have a positive effect on bone tunnel integration. In addition, using such plugs allows treatment no longer to be intraarticular, but may be given intratuously.

Based on that idea, this study was made to determine the role of Bone Marrow-derived Mesenchymal Stem Cells (BMSCs) + Vascular Endothelial Growth Factor (VEGF) intra-tunnel to biomechanical strength and acceleration of postcoon-reconstruction tendon-bone graft integration.

METHODS

This research was a true experimental laboratory study which samples were devided into control group and treatment group. The study design used randomized post test only controlled group design. In this study ACL reconstruction using hamstring autograft tendon which then divided into the control group and treatment as follows:

1. Control group (K1) performed ACL reconstruction with hamstring autograft tendon on graft-tunnel, given phosphate buffer saline intratunnel, but not given glue seal glue or injection of BMSCs + VEGF, this group will be tested biomechanics at week 3.
2. The control group (K2) performed ACL reconstruction with hamstring autograft tendon on the graft-tunnel, given intravenous saline phosphate buffer, but not given glue-seal fibrin and BMSCs + VEGF injection, the group will be tested biomechanically at week 6.

3. The second treatment group (P1) after ACL reconstruction with hamstring autograft tendon on the graft-tunnel, followed by fibrin glue seal at the end of the intraarticular tunnel, then injected BMSCs + VEGF on the intratunnel, the group will be tested biomechanically at week3.
4. The second treatment group (P2), after ACL reconstruction with hamstring autograft tendon on the graft-tunnel, followed by fibrin glue seal at the end of the intraarticular tunnel, then injected BMSCs + VEGF on the intratunnel, the group will be tested biomechanically at week to-6.

The results obtained were evaluated biomechanically at third week and sixth weeks after treatment with different sample units. The sample size of this study is 6, with the calculation of the number of test groups is 4 then the required sample size is 24 samples. The research was conducted at Stem Cell Research and Development Center of Airlangga University Surabaya. The study was conducted for 8 weeks,the first 2 weeks for culture process from BM-MSCs and 6 weeks later for treatment and evaluation process.

RESULTS

The ultimate tension strength evaluation showed that the mean of ultimate tension strength of control group and treatment at week 3 was 0.840 ± 0.3089 N and 4.340 ± 1.0188 N according to table 1 below.

Table 1. Evaluation Ultimate Tension Strength (N) at week 3

Group	No. Sample	Ultimate Tension Strength (N)	Explanation	x̄±SD
Control	C3.1	0,93	Pullout	1,025 ± 0,1415
	C3.2	1,12	Ruptur	
	C3.3	1,17	Pullout	
	C3.4	0,88	Pullout	
Treatment	T3.1	1,25	Ruptur	1,378 ± 0,1011
	T3.2	1,40	Ruptur	
	T3.3	1,32	Ruptur	
	T3.4	1,52	Pullout	
	T3.5	1,40	Ruptur	

From table 2 it was found that after 6 weeks evaluation, the mean ultimate tension strength in the control group was 1.025 ± 0.1415 N and 1.378 ± 0.1011 N in the treatment group.

Table 2. Evaluation Ultimate Tension Strength (N) at week 6

Group	No. Sample	Ultimate Tension Strength (N)	Explanation	x̄±SD
Control	C6.1	1,25	Pullout	1,280 ± 0,0474
	C6.2	1,28	Pullout	
	C6.3	1,34	Ruptur	
	C6.4	1,22	Pullout	
	C6.5	1,31	Ruptur	

Treatment	T6.1	1,29	Ruptur	1,4667 ± 0,1257
	T6.2	1,59	Pullout	
	T6.3	1,43	Ruptur	
	T6.4	1,36	Ruptur	
	T6.5	1,55	Ruptur	
	T6.6	1,58	Ruptur	

Table 3. Comparison of Ultimate Tension Strength of Control and Treatment Group on Week 3

Group	N	x̄±SD	p
Control	4	1.025 ± 0.1415	0,003
Treatment	5	1.378 ± 0.1011	

Table 3 shows that in the treatment group using BM-MSCs intratunnel had ultimate tension strength significantly greater than control group ($p < 0.05$).

Table 4. Comparison of Ultimate Tension Strength of Control and Treatment Groups at Week 6

Group	N	x̄±SD	p
Control	5	1.280 ± 0.0474	0,012
Treatment	6	1.467 ± 0.1256	

From table 4 found in the treatment group given intratunnel BM-MCs, after the sixth week the ultimate tension strength was greater than the control group and statistically significant ($p < 0.05$).

Table 5. Comparison of Ultimate Tension Strength Treatment Groups at 3 weeks and 6 weeks

Group	N	x̄±SD	p
3 weeks	5	1.378 ± 0.1011	0,236
6 weeks	6	1.467 ± 0.12	

When compared to the evaluation of ultimate tension strength of treatment group at week 3 and week 6, it can be seen from Table 4.6 that there was no significant difference between the two groups ($p > 0.05$).

DISCUSSION

ACL reconstruction surgery aims to restore knee biomechanical stability. The power of graft-bone tunnel in the early phases becomes crucial to facilitate aggressive rehabilitation, so recovery processes including functional exercises, exercise and daily activities can be undertaken soon (Dong et al., 2012). Therefore, the success of ACL reconstruction using tendon graft requires good and rapid integration between the tendon graft and the bone tunnel (Griffin et al., 2010).

The process of tissue formation between the tendon and bone is the most important stage at the beginning of the healing process. Therefore, it is important to determine how to heal the graft-tunnel as early as possible. In the healing process of the tendon-bone, a fibrovascular scar layer develops between the tendon and bone in the graft-tunnel interface. This layer will eventually arrange the fibers that are perpendicular to each other to resemble Sharpey's fiber. The presence and amount of these fibers is directly proportional to ultimate

tension strength.

Bone Marrow-Derived Mesenchymal Stem Cells (BMSCs) are multipotent stem cells and have become an important cell source for engineering purposes in terms of tissue repair and cell therapy (Griffin et al., 2010). Studies have shown that a bone tunnel with increasing amount of BMSCs can increase tendon-to-bone healing in rabbit models through the formation of perpendicular collagen fibers connecting the tendon to bone (resembling Sharpey's fibers) and an increase in the number of cartilage-like cells proliferation by week four (Dong et al., 2012; Milano et al., 2008; Ma et al., 2007; Kohno et al., 2007; HW et al., 2004).Given growth factor VEGF has been shown to increase vascularization and improve the quality of fibroblast tissue integration between the tendon graft and bone tunnel (Reinert et al., 2013).

This study was a true experimental laboratory study evaluating ultimate tension strength biomechanically on the ACL reconstruction tendon-bone tunnel graft treated with BM-MSCs and VEGF intratunnel by using glue seal fibrin to keep BM-MSCs and VEGF in the bone tunnel. Observations were made at week 3 and week 6 according to an evaluation performed by Tomita et al in 2001 (Tomita et al., 2001). The relative hypoxic conditions of the tendon graft result in tissue degradation. In this study the administration of BM-MSCs and VEGF is expected to promote neovascularization, fibroblast proliferation, collagen synthesis in bone tunnel (Deehan & Cawston, 2005).

The results of the comparative analysis using independent T test in this study showed significant differences in ultimate tension strength between the ACL reconstruction treatment group who were given BM-MSCs and VEGF intratunnel compared with the control group in both 3 weeks observation ($p < 0.05$) and week observation to 6 ($p < 0.05$). However, there was no significant difference between the 3rd week observation group and the 6th week observation ($p > 0.05$), although the results at week 6 (1.467 ± 0.12) showed higher ultimate tension strength compared to week 3 (1.378 ± 0.10).

The Ferdiansis et al study on ACL reconstructed models treated by BM-MSCs + VEGF intra-articular showed no significant difference between the 3rd week treatment with new controls with significant difference after week 6 when compared with control ($p < 0.05$) (Ferdiansis, 2014). While in this study, significant differences with controls can be demonstrated since week 3. This indirectly suggests that the intravenous method of administering BM-MSCs + VEGF will provide an acceleration in the integration of the tendon-bone tunnel graft when compared with intraarticular administration (Ferdiansis , 2014).

The results of this study is in accordance with Kanazawa research, which at week 2 and 4 the expression of type 1 collagen with chondroid coating was formed (Kanazawa et al., 2012). Where the tensile strength of the bone tunnel was aligned with the number of collagen type 1 and the chondroid layer.

CONCLUSION

In the biomechanics test on the tendo-bone tunnel graft model after anterior cruciate ligament reconstruction with intravenous administration of BM-MSCs and VEGF, it can be concluded as follows:

1. Ultimate tensile strength of 3rd week observational group treated by BM-MSCs and VEGF in intratular was significantly higher than control group.
2. The ultimate tensile strength of the 6th week observational group treated by BM-MSCs and VEGF by intratular was significantly higher than the control group.
3. There was no significant difference in ultimate tensile strength in week 3 and week 6 observation group.
4. This ultimate difference in tensile strength illustrates indirectly to the integration of the tendon-bone tunnel, ie, there is a significant difference in controls since the 3rd week after ACL reconstruction.

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