Sys Rev Pharm 2021;12(03):880-884
A multifaceted review journal in the field of pharmacy

The Effect of Stromal Vascular Fraction on Fracture Healing with Bone Defect: Experimental Study on Rattus Novergicus

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ABSTRACT

Introduction: Bone defect is a condition whereby the bone tissue cannot undergo a natural healing process caused by severe trauma with significant tissue loss, tumor, or irradiation. Bone defect is a challenge even for experienced Orthopedic surgeons. Bone tissue engineering (BTE) is one of the methods pursued to manage this problem. One of the examples of BTE is the application of stromal vascular fraction (SVF). This study aims to evaluate the effect of SVF application on bone defect healing evaluated by radiographic study.

Materials and Methods: This is an animal study involving 12 Wistar strain Rattus Norvegicus. They were divided into three groups: Negative control group (normal rats), Positive control group C2 (rats with bone defect without SVF and scaffold application), and Treatment group (rats with bone defect with SVF application). After 30 days, the rats were sacrificed, and the bone was evaluated radiographically.

Results: Callus formation is best observed radiographically in the treatment group. All comparisons of SVF and positive control group showed a significant difference ($p<0.05$).

Conclusion: Administration of SVF could aid bone defect healing through improving callus formation, marked by increased radiographic callus formation.

Keywords: Bone defect; Scaffold; Stromal vascular fraction; Tissue engineering

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INTRODUCTION

Fractures and segmental bone defects are a major cause of morbidity in patients and a source of high economic burden in health care. A severe bone defect is a condition in the bone which the bone cannot heal itself naturally.[1] Bone defects remain a challenge for orthopedic surgeons because they make fracture management complicated and required additional reconstructive procedures.[2] The normal bone healing process cannot resolve fractures with large defects on the bone. Therefore, autografts are the most preferred method for bone replacement.[3] BTE is an alternative to autografts which involves the integration of various components together. These various components are stem cells that are held together by a three-dimensional biomaterial framework that provides shape and provides initial mechanical strength, as well as molecular signals that induce differentiation of progenitor cells into osteoblastic phenotypes.[1]

The scaffold is one of the important components in tissue engineering that can stimulate the regeneration of tissue that is lost or damaged. From a mechanical point of view, its function is to bear external loads and shape the regenerated tissue over it. Meanwhile, from a biological point of view, these structures support the extracellular matrix and cell colonization development. A good scaffold for bone tissue engineering must be biocompatible, biodegradable, and osteoconductive.[4] Based on research conducted by Mahadhipta and Kamal, there are several types of scaffolds with known biocompatibility available in Indonesia, including bovine xenograft from BATAN, Bongros (HA), Perossal (nano-crystalline HA-CaSO4), Ostim (nano-crystalline HA), and local HA from Dr. Soetomo.[5][6] The combination of scaffold and Stromal Vascular Fraction (SVF) is a form of BTE that is still being studied a lot and is developing rapidly. SVF is a heterogeneous cell population derived from fat tissue obtained from minimal manipulation of the fat tissue itself. SVF consists of heterogeneous cells, mainly: Mesenchymal Stem Cells (MSCs), Hematopoietic Stem Cells (HSCs), T reg cells, pericyte cells, complex microvascular beds (fibroblasts, white blood cells, dendritic cells, intra-adventitial smooth muscular-like cells), and extracellular matrix.[7] SVF has several advantages over other biologically active materials. These advantages include (1) patient discomfort can be minimized when taking fat tissue, (2) contains a high volume ratio of stem cells, (3) tissue extraction is relatively easy and can be adjusted according to needs, (4) processing of fat tissue into Adipose-Derived Mesenchymal Stem Cells (ADMSCs), which then become SVF with high mesenchymal cells, can be carried out quickly, and (5) the multipotent cells contained in SVF can bind rapidly to scaffold material, proliferate rapidly, and can differentiate into osteogenic elements.[8]

The use of SVF and scaffold in the field of medicine and orthopedics is proven to be beneficial. SVF therapy has begun to be used in cases of burn injuries, nerve injuries, osteoarthritis, osteonecrosis, rheumatoid arthritis, Achilles tendon rupture, and also growth plate defects.[9][10][11][12] Studies related to the use of SVF and scaffold in the treatment of bone defects have been carried out several times. However, no studies have compared the effect of SVF alone when compared to a combination of SVF with several types of scaffolds. In this study, the researchers wanted to know the in vivo effect of SVF administration from fat tissue and the use of several types of scaffold on the healing process of bone defects which were assessed based on the area of callus formation.

MATERIAL AND METHODS

Study Design and Animal Model

The study design for this research was a laboratory experimental method with a randomized post-test-only control group. This study used a total of 12 male rats of the Rattus novergicus strain Wistar who were around six months old, healthy, active, and had no limb defects, and had not experienced any treatment or had not received any chemical intake, with an average bodyweight of around 200 - 250 g. And five adult male rats as a source of adipose tissue. Five adult male rats were sacrificed to obtain adipose tissue from epididymal and perirenal fat as a source of SVF. Then the other 12 male rats were randomly divided into three groups, namely: negative control group (without fractures and bone defects or treatment, n = 4), positive control group (samples that were not given treatment after being fractured with bone defects, n = 4), treatment group (samples that were given SVF after being fractured with bone defect, n = 4). Fractures surgery with bone defects was carried out by forming 5 mm of bone defect on the femur shaft using a rongeur kerrison, then fixed using a Plaster of Paris (POP). Previously, an anesthetic was administered by ketamine injection 100 mg/kgBW and xylazine hydrochloride 10 mg/kgBW intraperitoneally and antibiotics injection (cefazolin, 20 mg/kgBW) on the right leg. On the 30th day after the procedure, the radiological examination was carried out to assess the extent of the callus formation. The Ethics Committee of Universitas Brawijaya Faculty of Medicine has approved all animal protocols and all subsequent experiments were carried out according to the relevant guidelines and regulations.

Preparation of Stromal Vascular Fraction Suspension from Fat Tissue

Adipose tissue was collected from five male rats aged six weeks, washed with PBS (Phosphate-buffered saline solution; Sigma-Aldrich, Germany) containing 10% antibiotic-antimycotic mixture, then crushed with a knife into small fragments. Subsequently, it was immersed with 0.075% type IA collagenase solution (Sigma-Aldrich, Germany) and PBS for 30 minutes with the temperature at 37° Celsius. The digested tissue was then filtered with a 100 µm mesh filter (Sigma-Aldrich, Germany) and centrifuged at 1200 rpm for 10 minutes with the temperature at 20° Celsius and the supernatant formed was discarded. The result was a suspension containing heterogeneous cells with an estimate that every 1 gram of fat tissue has 2 X 10^6 cells.[7][13]

Radiological Analysis

The radiological examination was carried out on the 30th day after surgery by assessing the mean gray value. The Arbitrary units were measured quantitatively by assessing radiopacity using the Image J application by comparing the mean gray value of the bone defect region with the mean gray value in the area around the bone defect.

Statistical Analysis

Statistical analysis was carried out through comparative hypothesis testing with the following steps: data normality test, variant homogeneity test, and comparative test using One-way ANOVA. If the data obtained was not homogeneous by ANOVA, then a non-parametric analysis was carried out using the Kruskal Wallis method. All technical data processing results were computerized analyzed by using the Statistical Product and Service Solution (SPSS) software (IBM SPSS Statistics 20) with a significance level or a probability value of 0.05 (p = 0.05) and a confidence level of 95% (α = 0.05).

RESULTS

Result of Observation and Analysis of Callus Formation Area on Radiological Examination

Observation of callus formation was carried out by observing the radiological study of the treated femur. Callus was shown by the radiopaque image in the area of the bone defect. Radiological pictures from the negative control group, positive control group, and treatment group were shown in figure 1.

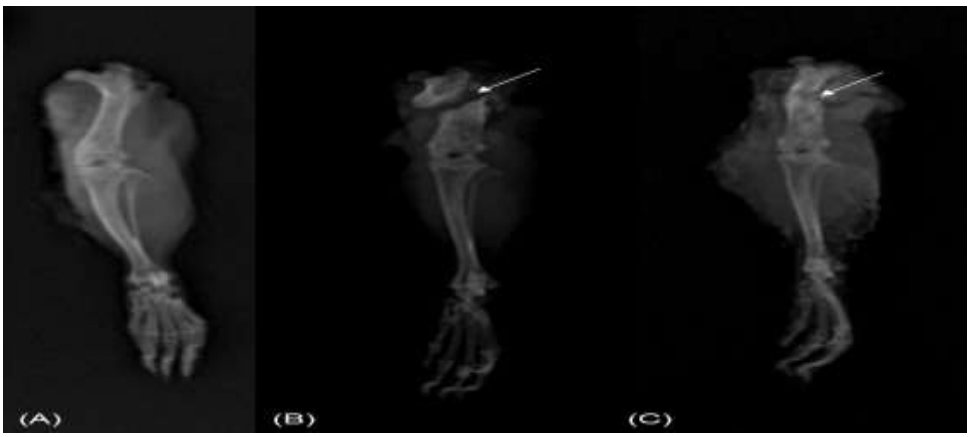


Figure 1: The radiographic image (a) Negative control group (b) Positive control group (c) Treatment group. The callus formation area is indicated with the white arrow.

Calculation of the callus area was carried out using the manual counting method, where the overall mean gray value was measured using the Image J application. The data will be averaged, and the data was presented in the following table.

Table 1: Data on callus formation area measurement results

Treatment Group	**Mean ± SD (*Mean Gray Value)***		
Negative control	89.725	±	17.89
Positive Control	62.9975	±	3.74835
Treatment group (SVF)	76.4075	±	4.09827

Before statistical analysis of callus formation area data was carried out, the data normality and variance homogeneity assumptions were tested. Furthermore, the normality test was carried out with the normally distributed data results, but the homogeneity test did not get homogeneous results. Based on the results of the normality and homogeneity test of the data, it can be concluded that the statistical testing process was carried out with a non-parametric approach using the Kruskal-Wallis test because the data was not homogeneous even though it was normally distributed. The non-parametric Kruskal-Wallis test was performed to determine the differences in SVF administration`s effect at the area of callus formation on radiological examination. The following table were the test results of SVF administration on the area of callus formation with the Kruskal-Wallis test.

Table 2: Comparative test of SVF administration to the area of callus formation with the kruskal-wallis test and post hoc test.

Treatment Group	**Mean ± SD**				**p-value**
K-Neg	89.725	±	17.89	bc	0.002
K-Pos	62.9975	±	3.74835	a	
K-P1 (SVF)	76.4075	±	4.09827	b	

Information : On average ± sd if it contains different letters, it means that there was a significant difference (p <0.05) and if it contains the same letters means that there was no significant difference (p > 0.05).

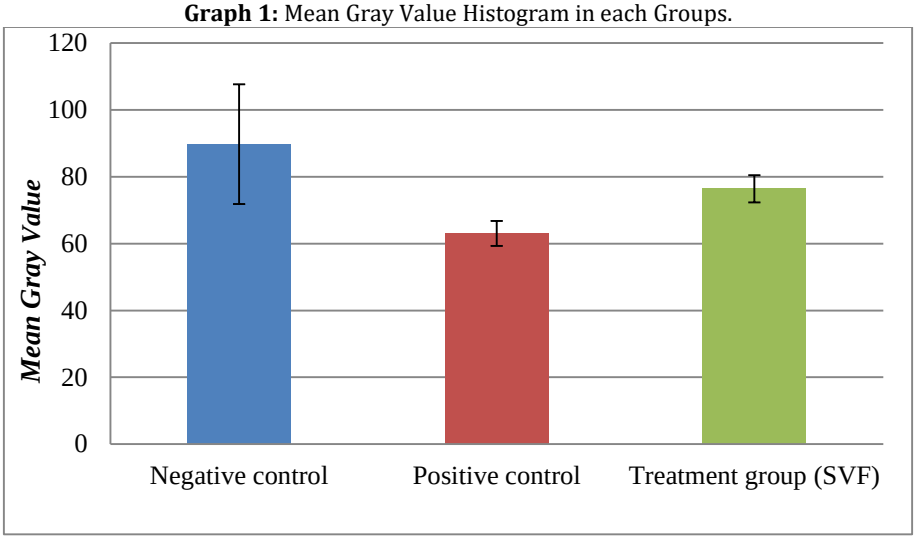
Based on the Kruskal-Wallis test analysis results, the p-value was 0.002, smaller than α = 0.05 (p <0.05). So, from this test, it can be concluded that there was a significant effect of SVF administration on the area of callus formation on radiological examination. Therefore, a post hoc test was needed to find out the differences in each treatment.

Table 3: Post hoc test, comparative test of SVF and scaffold administration to the area of callus formation on radiological examination.

Comparison		***p-value***
Negative control	Positive control	0.017
	Treatment group (SVF)	0.169
Positive control	Treatment group (SVF)	0.314

Based on the post hoc test results above, the comparison between groups was shown from the p-value. If the p-value is less than 0.05 (p <0.05), then the treatment groups had a significant difference in the mean value. The total mean gray value in each group was shown in the following histogram.

Graph 1: Mean Gray Value Histogram in each Groups.



DISCUSSION

This study was an in vivo study that aimed to evaluate the SVF administration`s effect on callus formation through radiological examination. On the 30th day after surgery, a radiological examination was performed to assess the extent of callus formation. Insilico and in vitro research on SVF used in this study have been previously researched by Sananta in 2019. From the results of Insilico research, it was found that several growth factors are known to play a role in osteoinduction and osteogenesis in SVF, such as Transforming Growth Factor β (TGF β), Insulin Growth Factor 1 (IGF 1), and Fibroblast Growth Factor 2 (FGF 2) which correspond to the growth factors needed by bones and cartilages.[12][14][15][16][17]

Effect of Stromal Vascular Fraction and Scaffold on Callus Formation in Rattus novergicus

Radiological examinations were carried out 30 days after surgery and treatment. X-Ray examination was performed to evaluate the fracture healing process by assessing the callus area at the fracture site quantitatively using the Image J application by calculating the mean gray values.

In this study, there was a significant effect on the area of callus formation with the administration of SVF ($p = 0.002$). The callus formation area in the K-Positive group had the lowest mean area of callus formations (62.9975 ± 3.74835). This is understandable as this group received no treatment. Note that the negative control group has a normal bone radiograph (not fractured) correlates with the highest mean gray value.

The results also showed that giving SVF to the treatment group provided a larger callus formation area than in the positive control group. This was in accordance with the theory, which states that SVF has various osteogenic components, such as ADMSCs, T-reg cells, pericyte cells, mast cells, complex microvascular beds, and the extracellular matrix. In addition, SVF also contains growth factors which in this case can help the osteogenesis process.[7] From the post hoc test, it was found that giving SVF to synthetic HA-Ca10(PO4)6(OH)2 scaffold can improve the process of remodeling and bone healing fracture compared to giving SVF alone. This is because the combination of SVF containing stem cells and osteogenic growth factors has a role in inducing osteogenic differentiation of ADMSCs and can increase the scaffold's osteoinductive and osteoconductive abilities. The osteogenic differentiation of ADMSCs is also influenced by several factors, including specific culture media, growth factors, platelet-rich plasma, alendronate, mechanical factors, surface topography, and chemical structure of various types of the scaffold.[18]

CONCLUSION

From the results of this study, we can conclude that SVF administration can improve and increase callus formation based on radiological examination in fracture-model rats with bone defects. Giving SVF will also increase osteogenesis in fracture model rats with bone defects.

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