Autologous microlobular fat combined with platelet-rich fibrin is associated with good fat graft viability

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Abstract. Fat grafting is popular in facial plastic reconstructive surgery. However, a fat graft shows unpredictable resorption. The addition of autologous platelet-rich fibrin (PRF) may enhance its viability. PRF has several growth factors, including vascular endothelial growth factor (VEGF), and these factors can improve fat graft viability by enhancing angiogenesis. We aimed to determine whether the use of PRF and the graft processing method influence fat graft viability (angiogenesis and VEGF expression). The study included New Zealand white rabbits. The rabbits were divided into the following groups: microlobular fat (A), microlobular fat with PRF (B), centrifuged fat (C), and centrifuged fat with PRF (D). After 30 days, biopsy was performed for all grafts. Hematoxylin and eosin staining and immunohistochemical staining for VEGF were performed. The angiogenesis areas in groups A to D were 112.67, 90.51, 84.48, and 110.52, respectively. The VEGF expressions in groups A to D were 241.33, 287.22, 212.89, and 231.44, respectively. Increased angiogenesis and VEGF expression can improve graft viability. PRF was found to be associated with increased VEGF expression, whereas microlobular fat was found to be associated with good angiogenesis. Thus, microlobular fat combined with PRF is recommended as an autologous graft.

1. Introduction

Presently, autologous fat grafts are being widely used for augmentation in reconstructive surgery. These grafts are easy to harvest and are present in large amounts. Prior to the era of autologous grafts, synthetic grafts, such as collagen and silicone grafts, were popular, but these synthetic grafts were absorbed quickly and caused body reactions. Additionally, studies have reported that these grafts can cause several complications, such as scar tissue, unwanted pigmentation, and skin thinning, and might increase the risk of malignancy [1,2].

The popularity of fat grafts increased in the late 19th century with the spread of liposuction. In early 1893, Neuber used free fat to fill a soft tissue defect. In 1909, autologous abdominal fat was used to fill defects in the malar and cheek areas. The use of autologous fat grafts increased because of the low risk of allergic reactions. However, fat reabsorption is a major problem in fat grafting. Many approaches have been developed to provide growth factors to fat grafts in order to increase their survival rate and viability [2].
Previous study reported that fat grafts have adipose-derived stem cells (ADSCs), which are capable of proliferation and differentiation [3]. However, fat grafts are usually absorbed up to 30%–50%, and thus, other materials are required to support graft viability [3,4].

Platelet-rich fibrin (PRF) includes platelets and contains several growth factors, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and epidermal growth factor. In fat tissue, PRF can stimulate angiogenesis that is necessary for maintaining fat graft viability [5–8]. VEGF, which is present in PRF, has several functions, and it acts as an endothelial cell mitogen, chemotactic agent, and inducer of vascular permeability. VEGF is considered as a unique growth factor because it has an effect on the wound healing cascade, including angiogenesis, epithelialization, and collagen deposition [9]. The present study aimed to determine whether the use of PRF and the graft processing method influence fat graft viability (angiogenesis and VEGF expression).

2. Methods
This experimental study was conducted in the laboratory of the Department of Pathology Anatomy, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital. The study was performed according to the guidelines published by the research ethics committee of Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital. The study included New Zealand white rabbits weighing 2.5–3.0 kg. The rabbits were divided into the following groups: microlobular fat graft (A), microlobular fat graft with PRF (B), centrifuged fat graft (C), and centrifuged fat graft with PRF (D). The autologous fat grafts were placed in the ears of the rabbits. There were 9 samples in each group. After 30 days, biopsy was performed for all grafts. The specimens were embedded in paraffin. Tissue sections were prepared, and hematoxylin and eosin staining and immunohistochemical staining for VEGF were performed.

2.1. Angiogenesis assessment
The angiogenesis area was examined under a light microscope at 100X magnification and was calculated as 400X magnification. The angiogenesis area was measured using ImageJ software (National Institutes of Health, Bethesda, MD).

2.2. VEGF assessment
VEGF expression was examined under a light microscope at 100X magnification and was calculated at 400X magnification. VEGF expression was measured by counting the number of VEGF-positive cells in five fields using the cell counter of ImageJ software.

3. Results
3.1. Angiogenesis
The angiogenesis areas in groups A to D were examined under light microscope (Figure 1), and calculated as 112.67, 90.51, 84.48, and 110.52, respectively (one-way ANOVA, p = 0.268). Regression analysis between adipocytes and angiogenesis showed that an increase in angiogenesis was proportional to an increase in fat amount ($r^2 = 0.72$; $p < 0.05$ for adipocyte = 679,880–3,022 [neo] + 0.015 [neo] [2]).
3.2. VEGF expression
The VEGF expressions in groups A to D were examined under light microscope (Figure 2), and calculated as 241.33, 287.22, 212.89, and 231.44, respectively. The VEGF expression was the highest in group B, but there was no statistical significance when compared to the expressions in the other groups (ANOVA test, $p = 0.124$). On comparing the 2 groups that received PRF (B and D groups), it was found that the VEGF expression was significantly higher in group B than in group D ($p = 0.036$, T-test).

4. Discussion
The present study found that platelet rich fibrin (PRF) and the fat graft processing method could influence fat graft viability. PRF has many growth factors, such as Vascular Endothelial Growth Factor (VEGF), Platelet-derived growth factor (PDGF), and epidermal growth factor. Topcu et al. showed that the addition of VEGF to the recipient area resulted in better fat graft viability owing to increased angiogenesis [10]. In the present study, PRF was used to provide autologous growth factors. A greater fat area was obtained in the microlobular groups, but there were no significant differences among the study groups. Regression analysis showed that an increase in angiogenesis was proportional to an increase in fat content. It was found that angiogenesis could predict 72% viability of a fat graft. Nishimura et al. [11] showed that VEGF levels significantly rise on day 7 after grafting. Fat necrosis is
known to occur until revascularization is accomplished. A previous study showed that endothelial cells have promotive effects on preadipocytes in a paracrine manner via VEGF or other factors [10].

The rabbit ear was used as a recipient graft bed model. This model has several advantages. The area has a relatively avascular plane and does not contain fat tissue. Thus, the only biopsied fat is the grafted fat. Owing to the low vascularity of the recipient fat bed, placement of a graft with a modified environment that has sufficient nutrition is important to maintain fat graft viability [10]. In the present study, 4 weeks after grafting, fat was identified and was found to be viable with vascular structures.

ADSCs in fat graft contained VEGF indicated by the amount of capillary and fat that may live [12]. Thus, VEGF plays an important role in the neovascularization of the recipient area. The group that received microlobular fat with PRF had the most number of VEGF-positive cells. Thus, the addition of PRF to a fat graft and the use of microlobular fat can help create an environment with growth factors that may improve graft viability.

5. Conclusion
Increased angiogenesis and VEGF expression can improve graft viability. PRF was found to be associated with increased VEGF expression, whereas microlobular fat was found to be associated with good angiogenesis. Thus, microlobular fat combined with PRF is recommended as an autologous graft.

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