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Effects of Lipokit® Centrifugation on Morphology and Resident Cells of Adipose Tissue

Efectos de la Centrifugación de Lipokit® sobre la Morfología y las Células Residentes del Tejido Adiposo

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DUMAN, S.; AKTAN, T. M.; CUCE, G.; CIHANTIMUR, B.; TOKAC, M. & AKBULUT, H. Effects of Lipokit® centrifugation on morphology and resident cells of adipose tissue. ***Int. J. Morphol., 31(1)***:64-69, 2013.

SUMMARY: The aim of adipose tissue engineering is creating autologus vascularized fat tissue to be used for practical soft tissue reconstruction in human clinic. Unfortunately, in practice, long-term results of fat transplantation are often untrustworthy and unreliable, to overcome this problem different many lipoinjection techniques developed in the last 20 years. Centrifuge is a fundamental step in the preparation of adipose tissue. We focused on some cell markers especially MSCs markers and histological structural properties after with lipokit centrifugation and without lipokit centrifugation of adipose tissue obtained by liposuction by this new technique. Adipose tissue was taken by liposuction and separates to two portions. One of them is centrifugated by Lipokit machine (C+) has a micro filter and the other is not (C-). After centrifugation smear slides and paraffin sections were prepared from these tissues. These slides were stained with H&E and Toluidine Blue. Paraffin sections were immunohistochemically stained with CD34, von Willebrand Factor, CD73, CD90 and CD105. Smear preparations showed a continuous three dimensional plasma membrane appearance of adipocytes. C+ and C- showed expression of CD34, von Willebrand Factor, CD73, CD90, CD105. C+ seems to have more free cells expressing than C-. While passing the filter of Lipokit, large adipocytes and connective tissue parts disintegrate and thus increases the surface area of lipoaspirate. Lipokit® machine release the group cells which are necessary for angiogenesis and they become more freely to construct angiogenesis.

KEYWORDS: Adipose tissue; Stem cell; Angiogenesis.

INTRODUCTION

Adipose tissue acts as a mechanical protective cushion for bone, nerve, blood vessel tissue and organs. Loss of this tissue may occur by traumatic wounds, deep burns, pressure ulcers and usually leads to a loss of aesthetics and function (Borzacchiello *et al.*, 2007). The aim of adipose tissue engineering is creating autolog vascularized fat tissue to be used for practical soft tissue reconsruction in human clinic (Hemmrich *et al*., 2008).

The first known case registered as autologous fat transplantation was made in 1893 by Neuber. Fat from the upper arm transferred to a wound showing pitting percent (Leong *et al*., 2005). It is difficult to create and build a functional adipose tissue. There should be a high microvascularite to ensure the viability of the transplanted or regenerated adipose tissue (Chiu *et al*., 2011). Many studies show 30-70% of transplantated autologous fat graft is resorbed within one year (Leong *et al*., 2005). The viability of the graft depends on enormous proliferation capacity of preadipocytes and again vascularization (Chan *et al.*, 2008).

Also adipose derived stem cells (ASCs) are available in adipose tissue (Kishi *et al.*, 2010; Ulicna *et al.*, 2010). ASCs *in vivo* are transformed into endothelial cells and they organize and develope angiogenesis. ASCs were found after the enzymatic parsing of stromal vascular fraction (SVF) in cultured cell populations. A large number of cells of human ASCs are being in perivascular tissues of adipose tissue (Kishi *et al.*).

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Preadipocytes and mesenchymal precursor cells have high proliferative potential and the capacity to refresh population of adipocyte tissue. If these local features locked open, it may contribute to probability of survival of the implanted graft. As an alternative strategy to maintain the viability of the graft, adipocytic precursor cell populations in tissue graft must be released (Leong *et al*.). Unfortunately, in practice, long-term results of fat transplantation are often untrustworthy and unreliable, to overcome this problem different many lipoinjection techniques developed in the last 20 years. However, in practice a standard procedure could not be adopted. There is no consensus about fat processing applications which can guarantee the maximum graft viability (Condé-Green *et al*., 2007).

A new device called Lipokit® processing of adipose tissue brings new parameters for tissue processing (weight and mesh during centrifugation) and has an advantage as excluding collagenase chemical treatment which needs extra enzyme inactivation steps so we focused on some cell markers especially MSCs markers and histological structural properties after lipokit centrifugation of adipose tissue obtained by liposuction by this new technique.

MATERIAL AND METHOD

Our study began after approvement of local ethic committee (Selcuk University, Meram Medicine Faculty). After regional anesthesia, light sedation and antiseptic cleaning of the skin with Polivinilpirolidon iodine solution, liposuction was performed on the lower abdomen of patients during operation performed at Estetik International- Bursa. The tumescent injection fluid consisting of 1:500 000 epinephrine in physiologic saline solution was given to the liposuction area and then liposuction was made with Lipokit® suction equipment that is a combined machine, shelter vacuum pump and centrifugation. Aspirated adipose tissue was collected into disposable 50 ml Fat Processing Unit (FPU) syringes. This syringe has a weight-mesh filtering (50-100 micron diameter).

Excess lipoaspirate material separates to two portions within 10 minutes at upward resting of FPU. These two parts are an upper densely adipose tissue section and at below a fluid portion composed of blood and tumescent liquid. The blood and tumescent liquid portion is poured out from FPU syringes. Lipoaspirate separated to two sections, one of them is prepared for lipokit process (C+) and the other lipoaspirate (C-) is prepared for rutin histologic process. For processing 4000 rpm is applied 8 minutes to these samples at the room temperature.

After centrifugation of the adipose tissue, three layers in FPU syringes can be seen, at the top of weight piston an oily liquid layer composed of triglycerides, the middle layer just below weight piston a yellow condensed adipose tissue layer and at the bottom a red layer composed mostly blood and processing liquids respectively. The top and bottom layers are discarded. Adipose tissue fraction in the middle layer was taken for our study (C+).

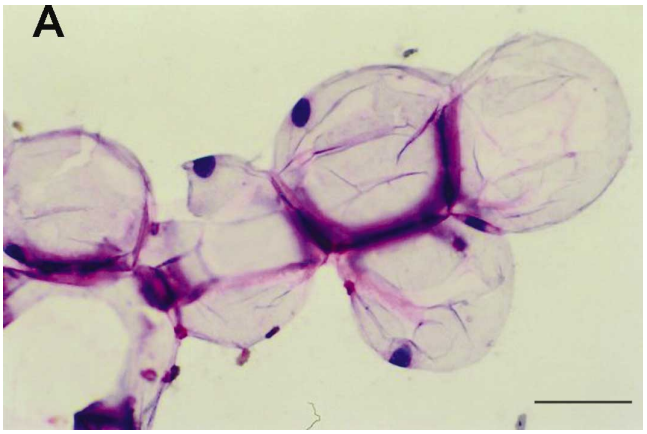
All histologic process and analyses were done in Department of Histology Embryology, Faculty of Meram Medicine, University of Selcuk. Smear slides were prepared immediately from C+ and C- and air dried at the operation room. These slides were transfered to the laboratory and stained with H&E and Toluidine Blue, in our staining technique especially no alcohol solution was used. A second histological technique on the samples of C+ and C- were done for immunohistochemical staining. For this aim adipose tissue fraction of C+ and C- were fixed 24 hours with Holland solution and after routine tissue processing, samples were embedded to paraffin blocks, 4 μm thin sections were prepared. Sections were immunohistochemically stained with Monoclonal Mouse Anti-Human CD34 class II (clone QBEnd 10, Dako, Denmark), Polyclonal Rabbit Anti-Human von Willebrand Factor (Dako, Denmark), CD105 (RB-9291-R7: Neomarker), CD73 (S2054: Epitomics) and CD90 (2694-1:Epitomics) were used. All microscopic inspections were done under light microscope and microphotographs were taken (Olmypus BH-2).

RESULTS

Microscopic examination of smear preparations stained with Toluidin blue and H&E stained tissue samples showed a continuous three dimensional plasma membrane appearance of adipocytes where nucleuses were clear with oval shaped and localized peripherally (Figs. 1A and 1B) Small adipocyte cell clusters were seen in C+ smear (Fig. 1A) and large intact adipocyte cell clusters were observed from C- (Fig. 1B).

Light microscopic observations done on paraffin sections stained with immunohistochemical stains revealed that mature adipocytes passed the filter mainly preserved their contours.

C+ fraction obtained by purification seems to have more free cells expressing vWF (Fig. 2A), CD34 (Fig. 3A), CD105 (Fig. 4), CD73 (Fig. 5A), CD90 (Fig. 5B) and according to the fraction of the C- (Figs. 2B and 3B). CD34, vWF, CD90, CD73 and CD105 immunostained cells can be



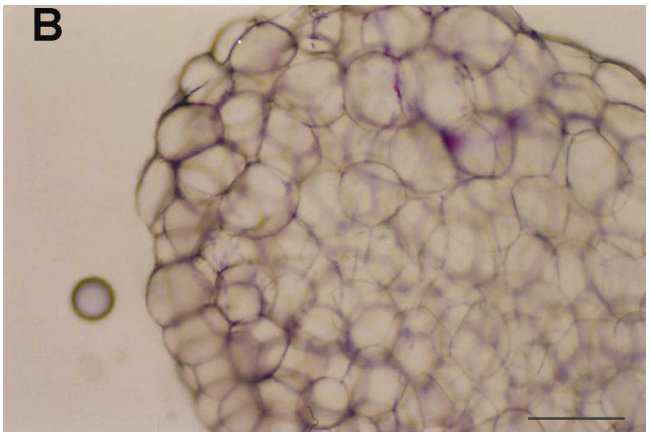
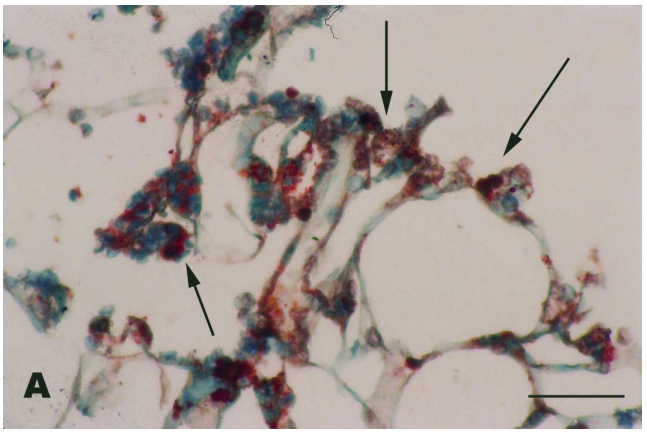


Fig 1. A). Smear preparation processed with C+, cells are observed in small groups. HE, bar represent 100 micron. B). Smear preparations, Toluidine Blue stained, and adipocytes are intact and large groups can be seen in C-. Bar represents 50 micron.



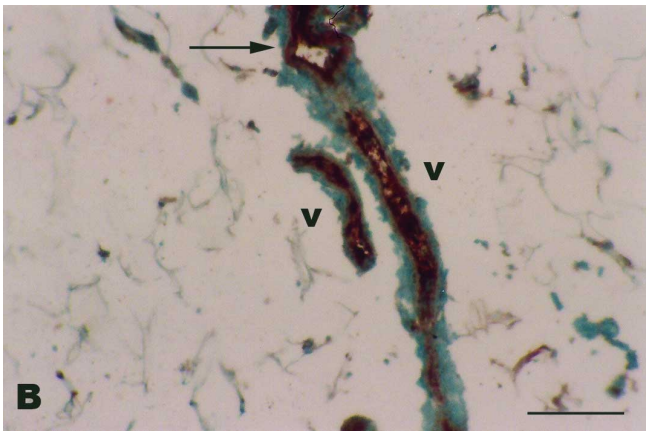
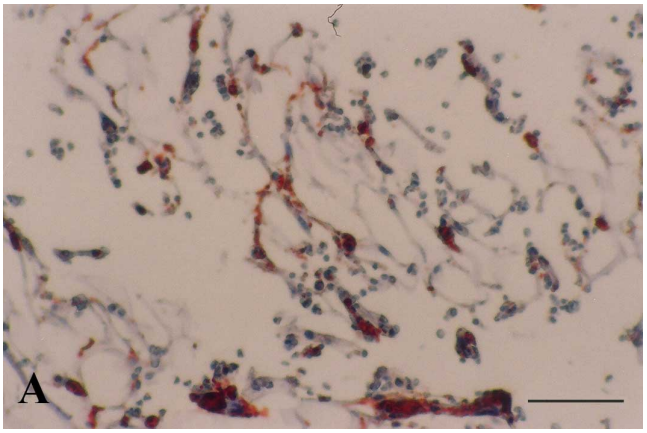


Fig 2. A). vWF positively stained cells were spread in tissue of C+, Bar represents 50 micron. B). vWF positively stained cells (arrow) were seen in vessels in C-, Bar represents 100 micron.



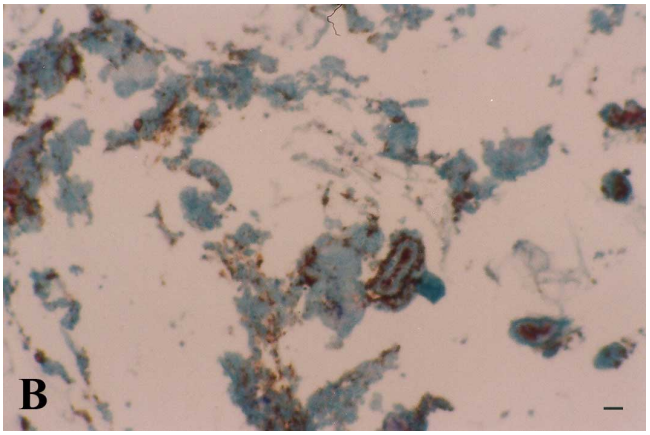


Fig. 3. A). In C+ CD34+ cells seen in fragmented vessels. Bars represent 100 micron. B). A rich population of CD34+ cells located around vessels in C-, bar represents 10 micron.

seen in fragmented SVF and in small vessels in C+ preparations.

The strongest expression belonged to CD105, expression of CD90 was normal and expression of CD73 was weak compared to CD105 and CD90. There was a significant difference between air-dried smears (Figs. 1A and 1B) and paraffin sections that adipocyte cell clusters were not seen in paraffin sections (Fig. 5B).

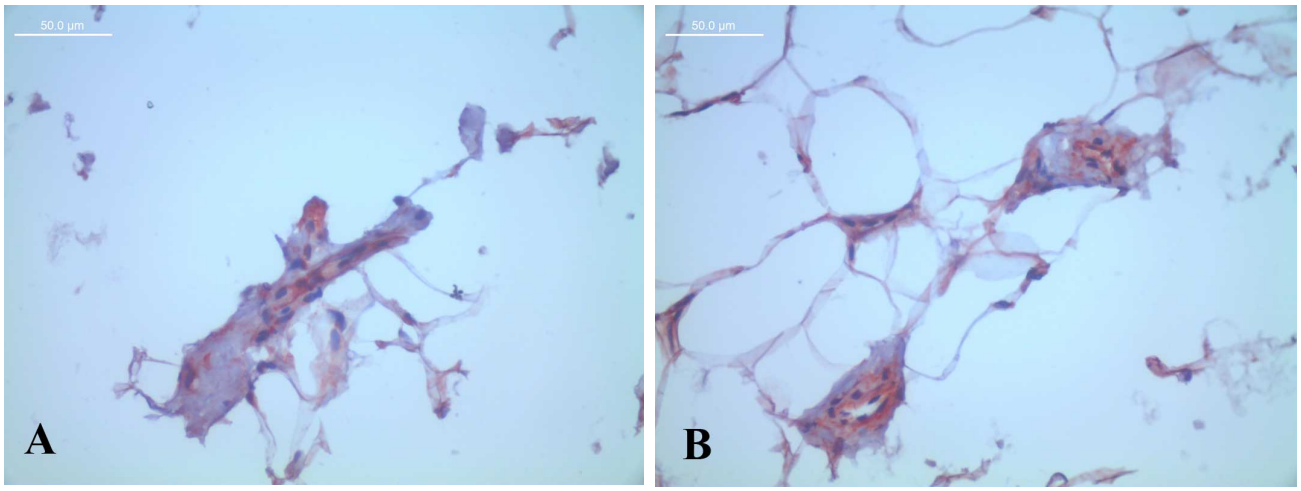


Fig. 4. CD105 expression can be seen in C+ in fragmented (A) and small vessels (B).

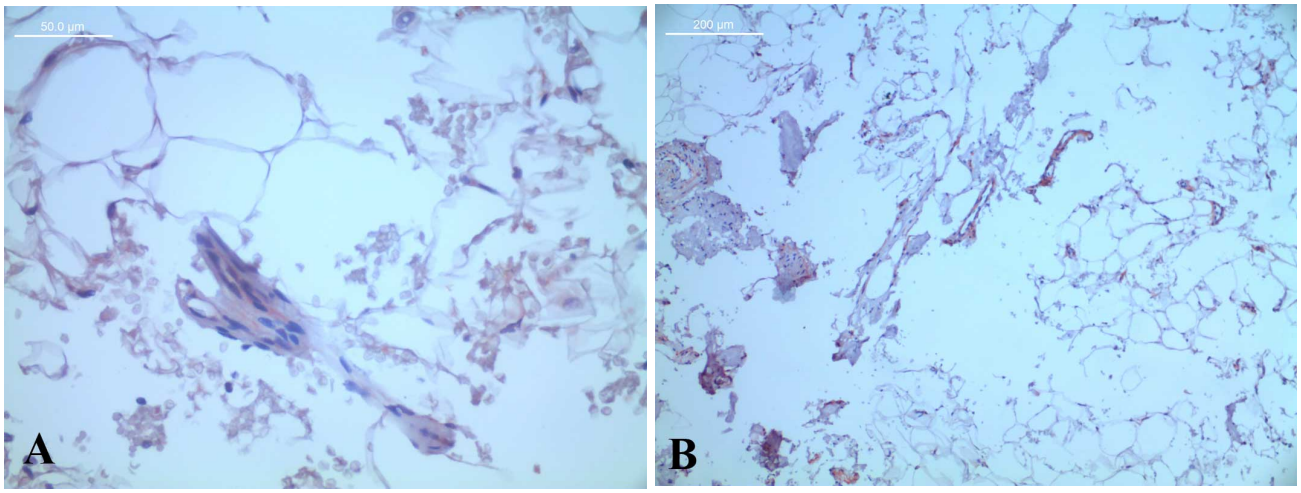


Fig. 5. A). CD73 expression in C+. B). CD90 expression in C+.

DISCUSSION

According to our search done on PubMed till October 2012, there was no any study using air dried fixed fresh processed adipose tissue smear preparation for histological examination. From the same samples of air-dried and tissue processed adipocyte cell clusters were seen only in smear preparations, this seems to be a result of absence of chemical hazardous materials like xylene and alcohol in rutin smear technique. By this observation we suggest researchers to use air-drying to obtain images more realistic, so to make more appropiate decisions.

SVF components of adipose tissue can give a different route that includes a variety of stem and precursor cells *in vitro* and *in vivo* (Ailhaud, 2006). ADSC's share many similarities to bone marrow mesenchymal stem cells (Locke *et al.*, 2009). Mesenchymal stem cells (MSCs) have unique properties: they exhibit transdifferentiation. MSCs must express at least CD105, CD73 and CD90 (Odabas *et al.*, 2008).

In our study, CD105, CD73 ve CD90 expressed in our preparations. It was reported that MSCs caused angiogenesis *in vitro* and *in vivo* with the angiogenic cytokines like vascular endotelial growth factor (VEGF1), Tranforming growth factor Beta (TGFb), Fibroblast growth factor (FGF), Angiopoetin-1 (Ang-1) (Ulicna *et al.*, 2009).

CD105 expression degrees after differentiation process *in vitro*. CD105 might be a key marker to follow the differentiation process of MSCs (Jin *et al*., 2009). Adipose

derived stem cells have the potential for differentiation to endothelial cell seeded in culture as a result of chemical and mechanical stimulation (Harris *et al*., 2010). We think that the strongest expression of endoglin in liposuction material may be coming from a process before the differentation. CD34+ cells have capacity to renew itself and use this capacity to produce and differentiate a variety of other stem cells (Pafumi *et al*., 2001). EPCs expresses CD34. Endothelial progenitor cells (EPCs) were determined as the identity of circulating cell population in peripheral blood. When EPCs are transplanted to animal models, these cells participated in neovascularization areas and angiogenesis seems to be organized and arranged by EPCs. In addition to the angiogenic effects of EPCs, they can serve as reparative cells and renew dysfunctional endothelium (Rehman *et al.*, 2004). Von Willebrand factor is known as a strong adhesive proteins involved in hemostasis and tissue injury. vWF stored in Weibel-Palade granules of endothelial cells. These organelles are specific to vWF. In humans, immunohistochemistry of VWF is used as a marker for angiogenesis(Baruch *et al*.). Adipose tissue has a high angiogenic capacity (Hemmrich *et al*.).

We examined the confirmed expression of markers which indicate the existence of the required cells for the formation of angiogenesis. According to the volume ratio, smaller grafts have a higher surface area and more advantageous than to large grafts. A large part of the graft is in contact with the graft bed. This event facilitates revascularization (Chan *et al*.). In our study, while passing the filter of Lipokit, large adipocytes and connective tissue parts disintegrate and thus increases the surface area of lipoaspirate.

Adipocytes produce and secrete a wide variety of bioactive substances. These substances called adipokines and include growth factors, cytokines and complement factors (Matsuzawa, 2005; Ginter & Simko, 2010). Our mesh diameter was between 50-100 micron, which is considered to disrupt the old-large adipocytes and disintegrate liposuctioned tissue. Disruption of adipocytes may expose large amounts of cytokins which will facilitate angiogenesis. Needed cellular integrity for angiogenesis were preserved in liposuction material that processed with related techniques.

In conclusion, Lipokit® is a specialized instrumentation for fat tissue harvest and release the group of cells which are necessary for angiogenesis and they become more freely to construct angiogenesis.

DUMAN, S.; AKTAN, T. M.; CUCE, G.; CIHANTIMUR, B.; TOKAC, M. & AKBULUT, H. Efectos de la centrifugación de Lipokit® sobre la morfología y las células residentes del tejido adiposo. ***Int. J. Morphol., 31(1)***:55-63, 2013.

RESUMEN: El objetivo de la ingeniería del tejido adiposo es la creación de tejido graso vascularizado autólogo para ser utilizado en clínica humana para la reconstrucción de tejido blando. Desafortunadamente en la práctica, los resultados a largo plazo del trasplante de grasa son poco fiables y no seguros; para superar este problema, se han desarrollado en diferentes países, en los últimos 20 años, variadas técnicas de lipoinyección. La centrifugación es un paso fundamental en la preparación del tejido adiposo. Nos hemos centrado en algunos marcadores, especialmente, de células precursoras mesenquimales y propiedades histológicas estructurales después de la centrifugación mediante Lipokit y sin la centrifugación por Lipokit del tejido adiposo obtenido mediante liposucción. El tejido adiposo fue tomado por liposucción y se separó en dos porciones. Una se centrifugó mediante el sistema Lipokit (C +), con un microfiltro y la otro no (C-). Después de centrifugación, muestras del frotis y secciones de parafina se prepararon a partir de estos tejidos. Los frotis se tiñeron con H&E y azul de toluidina. Las secciones de parafina se tiñeron inmunohistoquímicamente con CD34, factor de von Willebrand, CD73, CD90 y CD105. Las preparaciones de los frotis mostraron una apariencia tridimensional continua de la membrana plasmática de los adipocitos. Tanto en C+ y C- se observó la expresión de CD34, factor de von Willebrand, CD73, CD90 y CD105. En C+ parecen expresarse más células libres que en C-. Cuando se utilizó el filtro de Lipokit, los adipocitos grandes y partes del tejido conectivo se desintegraron, por lo tanto aumentó el área de superficie de lipoaspirado. El sistema Lipokit® libera los grupos celulares que son necesarios para la angiogénesis y se hacen más libres para promoverla.

PALABRAS CLAVE: Tejido adiposo; Células precursora; Angiogénesis.

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