

Use of Mechanically Isolated Stromal Vascular Fraction in Different Wound Types

Farklı Yara Tiplerinde Mekanik Yolla İzole Edilen Stromal Vasküler Fraksiyonun Kullanımı

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Öz

Yara iyileşmesi hemostaz, inflamasyon, proliferasyon ve maturasyon evrelerinden oluşan fizyolojik bir süreçtir. Bu iç içe geçmiş evrelerin herhangi bir aşamasında meydana gelen bozulma, klinisyenin karşısına için kronik bir yara olarak çıkar. Lipoaspiratın enzimatik veya mekanik sindiriminden sonra sulu fraksiyonun bir parçası olarak izole edilen stromal vasküler fraksiyon, aynı zamanda önemli bir mezenkimal kök hücre rezervidir. İçerdiği preadipositler, endotelial öncüler, immün hücreler, hematopoietik hücreler, fibroblastlar ve perisitler nedeniyle heterojen bir doku kokteyli olarak kabul edilir. Proanjyogenik, antiapoptotik, antifibrotik, immünomodülatör ve antiinflamatuvar aktivitelerinin yanı sıra izolasyondaki avantajları nedeniyle, stromal vasküler fraksiyon son zamanlarda mevcut yara tedavisinde önem kazanmıştır. SVF tek başına yara iyileşmesi için etkili olmasa da, uygun yara bakımı ve rekonstrüksiyon öncesi yara yatağı hazırlığı için yeterli hücre sayısı ve canlılığı sağlayan ve cerrahi başarısızlığı en aza indiren yeni ve etkili bir teknolojidir.

Anahtar Kelimeler: Kronik yara, stromal vasküler fraksiyon, yağ grefti, yara iyileşmesi

Abstract

Wound healing is a physiological process consisting of hemostasis, inflammation, proliferation, and maturation phases. The disruption that occurs at any stage of these intertwined phases is presented to the clinician as a chronic wound. The stromal vascular fraction, isolated as a part of the aqueous fraction after enzymatic or mechanical digestion of lipoaspirate, is an important mesenchymal stem cell reserve, as well as. It is considered a heterogeneous tissue cocktail due to the preadipocytes, endothelial precursors, immune cells, hematopoietic cells, fibroblasts and pericytes it contains. Because of its proangiogenic, antiapoptotic, antifibrotic, immunomodulatory, and anti-inflammatory activities, as well as its advantages in isolation, stromal vascular fraction has lately acquired prominence in current wound therapy. Although SVF alone is not effective for wound healing, it is a new and effective technology that provides adequate cell count and viability for proper wound care and wound bed preparation prior to reconstruction and to minimize surgical failure.

Key words: Chronic wound, fat grafting, stromal vascular fraction, wound healing

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INTRODUCTION

Wound healing is a physiological process consisting of hemostasis, inflammation, proliferation, and maturation phases. The disruption that occurs at any stage of these intertwined phases is presented to the clinician as a chronic wound. An ideal method could not be determined due to the high cost of chronic wound management, the inability of current therapeutic strategies to affect all healing phases alone, and potential adverse effects. After it was discovered in 2001 that stem and stromal cells could be obtained from adipose tissue, more research studies were designed about the use of this cell groups with regenerative properties (1). The stromal vascular fraction (SVF), isolated as a part of the aqueous fraction after enzymatic or mechanical digestion of lipoaspirate, is an important mesenchymal stem cell reserve, as well as. It is considered a heterogeneous tissue cocktail due to the preadipocytes, endothelial precursors, immune cells, hematopoietic cells, fibroblasts and pericytes it contains (2,3). SVF has two primary characteristics that distinguish it from mesenchymal stem cells in terms of tissue regeneration. The first of these is that thanks to the different cell components it contains it offers greater benefits in aspects such as immunomodulation, anti-inflammatory, and angiogenesis than compared to the application of stem cells alone, and the second is that SVF is easier to produce than stem cell isolation (4). Because of its proangiogenic, antiapoptotic, antifibrotic, immunomodulatory, and anti-inflammatory activities, as well as its advantages in isolation, SVF has lately acquired prominence in current wound therapy. The goal of our research was to present the outcomes of mechanically produced SVF in patients with different wounds caused by a variety of factors.

Application Procedure

Patients were operated in the operating room with spinal anesthesia with sedation or general anesthesia. Fat is harvested from any area of excess subcutaneous fat and/or areas of patient preference if sufficient fat is available. The patient who have malignancy, coagulation disorder, pregnancy and connective tissue disease is accepted unsuitable candidate for lipoaspirate. Tumescence anesthesia material, which is approximately 35% more than the amount of fat planned to be harvested, was given to the donor area from which lipoaspirate was to be harvested, and it was waited for an average of 5-10 minutes. Tumescence anesthesia includes 500 mL of Ringer lactate with 25 mg lidocaine and

1 vial of epinephrine [1:1000]. With a blunt cannula with maximum dimensions of 20 mm length, 3 mm diameter and 2 mm aspiration holes, lipoaspirate was transferred to the closed system bag as milli-fat. For the separation of Milli fat tumescence anesthesia in the bag, 60 cc of SF was added and suspended for 5 -7 minutes to ensure decantation. After separation, the tumescence anesthesia material was removed from the bag by means of a 3-way cock. The washed Milli fat remaining in the bag was reduced to micron level by mechanically refining with the help of knives in 2 separate Lipocube SVF black cubes, respectively. Autologous fat, which was reduced to micron degree, was separated in a patented piston tube with 4 separate concave gaskets (black piston injector) and Lipocube special software variable speed Celldrive (Centrifuge) device for 9 minutes. 'Stromal Vascular Matrix' was obtained by combining Extracellular Matrix and Stromal Vascular cell collection in the separated fat. The resulting Stromal Vascular Matrix was injected intralesional and perilesional to the wound of the patients

CASE

Case 1

A 62-year-old male patient was admitted to the wound care clinic with a tissue defect accompanied by ulcerated areas which is hyperemic and edematous on the left cruris diagnosed as a venous ulcer (Figure 1). Venous doppler ultrasound showed no thrombosis



Figure 1. The tissue defect accompanied by ulcerated areas which is hyperemic and edematous on the left cruris diagnosed as a venous ulcer

on the venous system. He has a history of diabetes mellitus, coronary artery disease and hypertension. At the time of admission, the patient's body temperature was 36.10C. The erythrocyte sedimentation rate was 55 mm/h and the CRP value was 31.6 mg/L. Revascularization was not considered necessary after the Ankle/Arm index was found to be 0.9. Debridement was performed, and cultures were taken. Upon the growth of *P. Aeuriginosa* in deep tissue culture, piperacilin/tazobactam was applied in accordance with the culture antibiogram, and antibiotic therapy was applied for 10 days. In the wound care follow-up process, Negative Pressure Wound Therapy was applied for 9 days. Fat-derived Mechanical Stromal Vascular Matrix application was applied to the patient whose granulation tissue progression slowed down during this period. After the operation patient had hyperbaric oxygen therapy for 20 sessions. Pre-procedure sedimentation rate was 32 mm/h and CRP was 9 mg/L. Wound dimensions were measured as 6 cm x 6 cm x 3 cm (108 cm³) during the application. After the SVM application, the follow-up was continued with the hydrocolloid-containing passive wound dressing and offloading. Photographing and wound care were performed at 1st, 3rd, and 6th week follow-ups. At the end of the sixth week, 96.7% success was achieved with a wound volume of 1.5 cm³ (Figure 2-3).

Case 2

A 43-year-old male patient was admitted to the wound care clinic with a detachment of the anterolateral thigh flap on the anterior and inferomedial side on the



Figure 2. Postoperative third week of venous ulcer on left medial malleolus



Figure 3. Postoperative sixth week of venous ulcer on left medial malleolus

postoperative third months. Tissue defect that was present on the previous operation was due to the car accident. There was no early complication during the follow-up. Patient was discharged 7th day of operation with no detachment or infection. Patient has no significant medical history. At the time of admission, the patient's body temperature was 36.40C. The CRP value was 32.4 mg/L. Debridement was performed, and cultures were taken. No significant growth of any bacteria in deep wound culture. On the wound care follow-up process, Negative Pressure Wound Therapy was applied for 9 days. Fat-derived Mechanical Stromal Vascular Matrix application was applied to the patient. After the SVM application, the follow-up was continued with the hydrocolloid-containing passive wound dressing and offloading. Photographing and wound care were performed at 1st, 3rd, and 6th week follow-ups. At the end of the sixth week, no more tissue defect between the flap and nearby tissue.

Case 3

A 47-year old man patient was admitted to the wound care clinic with a total necrosis of D1 and D2 at the level of metatarsophalangeal joint (Figure 4). An arterial doppler ultrasonography showed that the anterior tibial artery had biphasic flow pattern on the affected side. The patient consulted to the orthopedy and traumatology department. Orthopedy department planned a ray amputation. On postoperative 13th day, patient came to our clinic with detachment and bed-smell at the incision site. Debridement was performed, and cultures were taken. Upon the growth



Figure 4. The tissue defect on the level of D1 and D2 metatarsophalangeal joint

of *A. Baummani* in deep tissue culture, tigesiklin was applied in accordance with the culture antibiogram, and antibiotic therapy was applied for 18 days. At the end of the sixth week of SVM application, no more tissue defect between the flap and nearby tissue (Figure 5).

Case 4

A 62-year old man patient was admitted to the wound care clinic with a diabetic ulcer that is hyperemic, sniffy and purulent discharge on the dorsal surface of



Figure 5. Postoperative sixth week without no more tissue defect

the left foot. Peripheral pulses were palpable. Arterial and venous doppler ultrasonography showed no significant changes on the vascular system bilaterally. On the X-Ray; there was no sign of osteomyelitis or any kind of osseous pathologies. CRP value was 256 g/dL. Three sessions of debridement were performed. Upon the growth of *A. Baummani* and *P. Aeruginosa* in deep tissue culture, tigecycline and piperacillin/tazobactam were applied in accordance with the culture antibiogram, and antibiotic therapy was applied for 13 days. Fat-derived Mechanical Stromal Vascular Matrix application was applied to the patient. After the SVM application, on the postoperative 30th day; we reconstruct the tissue defect with the split-thickness skin graft.

DISCUSSION

Chronic wounds, which are difficult to heal and complex, cause patients to stay in the hospital for a long time periods and create a workload for the health workforce. Although wound healing follows the same process in all tissues, wounds due to different etiological reasons can become complicated. Even though progress has been achieved in chronic wound management with the developing technology, complex procedures are still required in the management of severe wounds treatments despite modern wound dressings (5). Since there is no method that can provide success in chronic wound management alone, many procedures are performed before reconstruction in order to optimize wound healing and achieve success in chronic wound management (6). The stromal vascular fraction included in these procedures is an application that is obtained from adipose tissue through several mechanisms and has a positive contribution to wound healing (8). Isolation methods that can be used to obtain SVF can be basically divided into three as enzymatic methods, automatic devices, and mechanical separation (9). Despite the fact that enzymatically generated lipoaspirate can contain around 100000-1300000/gr cells, its high cost and long manufacturing time (average 120 minutes) limit its application (7). In addition, it has been evaluated by the FDA within the scope of drug research since it is argued that the enzymatic SVF production causes deterioration of the original content and tissue integrity of the adipose tissue. On the other hand, automatic devices have an advantage over enzymatic isolation as they provide isolation in closed environment, limit contamination, and standardize in clinical practice. However, the cost of the devices

has been the major limitation of this method (8). Due to these disadvantages, mechanical methods such as shaking, vibration, centrifugation, and sonication have been tried in recent years to obtain SVF. SVF obtained via this way is called 'Tissue-like SVF' (10). They contain extracellular matrix fragments and cells because they are microfragmented adipose tissue. When different mechanical isolation methods were compared to enzymatic techniques, it was observed in the study by Tiryaki that, while the cell number in the SVF population obtained by mechanical methods was low, the cell division rate and type 1 collagen gene expression were higher in the SVF population isolated by mechanical methods (11). Increased type 1 collagen gene expression indicates the acceleration of collagen production and the secondary enhanced wound healing as a result. The physiological activities and functionality of cells are thought to be enhanced by cellular forces during mechanical isolation. The most significant advantage of this method is that it can be obtained easily and quickly even in the operating room, since no enzymes are used during mechanical isolation; nevertheless, obtaining fewer cells is seen as a disadvantage when compared to enzymatic methods. Banyard et al. suggested in a study that cell number is less important in SVF activity than previously thought, and that cell activity is more important (12). In addition, Fraser et al. determined that mesenchymal stem cells were 500 times more abundant in SVF than in bone marrow. Thus, it can be argued that SVF, which is easily obtained from adipose tissue, can be an alternative to bone marrow-derived mesenchymal stem cells (13). Another advantage regarding the use of SVF for wound healing is that it contains not only stem cells, but also a regulatory T cell (Treg), which is a cell of the immune system and has anti-inflammatory properties. According to another study, it was argued that there are more Tregs in the SVF compared to the peripheral regions, and that the anti-inflammatory effect to be obtained by SVF application can be increased in this way (14). There is not enough information on when it should be used in the chronic wound method, however in our study, it was preferred to be applied after showing the absence of microbial growth and osteomyelitis in the wound culture. Furthermore, it would be appropriate to use it in chronic wound management after correcting the pathologies that need to be treated depending on the etiology. Although SVF alone is not effective for wound healing, it is a new and effective technology that provides adequate cell count and viability for

proper wound care and wound bed preparation prior to reconstruction and to minimize surgical failure.

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