CellR4 2015; 3 (5): e1675

Subcutaneous reconstruction of hand dorsum and fingers for late sequelae of burn scars using adipose-derived stromal vascular fraction (SVF)

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Keywords: Stromal vascular fraction, Mesenchymal stem cells, Burn sequelae, Skin fibrosis, Angiogenesis.

Abstract

Background: **Burn scar-derived skin fibrosis has functional and aesthetic sequelae for patients, still pose therapeutic challenges. This is specially pronounced in the hands where contractures limit function. Despite the fact that fat grafts have shown good results in partially restoring function and appearance, limitations still persist.**

Objectives: **The aim of this case report is to show the functional and aesthetic effects of the application of adipose-derived stromal vascular fraction in the treatment of burn-related late sequelae affecting skin of the hand dorsum and the fingers' function.**

Patients and Methods: **The patient received intra-articular and subcutaneous injections of adipose-derived autologous stromal vascular fraction. Muscle strength, range of motion, functional, dexterity and occupational statuses were assessed continuously before and after surgery (6 week and 6, 12 and 24 months follow-ups). Color power Doppler ultrasound imaging was performed at 4 months after SVF application to visualize small vessel presence and their associated density at the infiltrated locations.**

Results: **No local/systemic complications were evidenced with the procedure. As early as 6 weeks (and extended throughout the follow-up period), all joints regained full and painless range of motion, handwriting was restored, as well as daily activities. Elasticity and color of the skin were improved. Ultrasound examination evidenced the presence of an intricate network of vessels surrounding each injected MCP joint and throughout the dermis of the dorsal surface of the treated hand.**

Conclusion: **Having observed physical changes in scarred skin and functional improvement in hand/fingers after the application of SVF, we propose that this technique can constitute a novel and minimally invasive means to alleviate the effects of scarring in these patients.**

Introduction

Burn scar-derived fibrosis continues to be a reconstructive challenge. No region of the human body is more sensitive to subcutaneous fibrosis than the hand. Despite the most accurate and timely treat-

ment, late scar contractures continue to be a challenge for function. Histological studies of fat grafts have documented changes in texture and appearance with signs of regeneration characterized by neovascularization, increased collagen content, and dermal hyperplasia[1-3]. This results in better flexibility, color, and texture with improvement in contractures. However, the subcutaneous space in a burn injury constitutes a hostile environment for a fat graft, often compromising the ultimate clinical result. Hence, an optimal strategy would be to introduce into that space elements capable of inducing new blood vessels formation while assuring volume restoration and and tissue regeneration.

Mesenchymal Stem Cells (MSCs) constitute a cell population present in all tissues, including fat, organized at the perivascular space[4-7]. As part of their trophic activities[8], they produce angiogenic factors such as VEGF (vascular endothelial growth factor) and HGH (hepatocyte growth factor), postulated to enhace graft survival[9,10].

Today there exist various technologies that permit the processing of fat in order to obtain the stromal vascular fraction (SVF), which constitutes the cellular component where MSCs and other cell types are included. These techniques have, as a common factor, an enzymatic digestion of adipose tissue using one form or another of collagenase, followed by centrifugation. SVF obtained in this manner constitutes an autologous vascular element of the patient's own fat and, as a consequence, could be readministered back into the patient following a similar logic as if it were an autotransfusion of blood or other hemoderivatives (e.g., platelet rich plasma)[11].

The effect of adding SVF to a conventional fat graft is to potentiate its survival by means of induction of new blood vessels generation. Examples of this include treatment of facial lipodystrophy, breast and buttock reconstruction, and therapy for chronic ulcers[12-14]. Whether administered as an SVF-enhanced fat graft or as a solution of SVF placed either subcutaneously or intra-articularly the goal is to alter the biology of the recipient tissue.

Case report

A 58 year-old right-handed female journalist presented to the APROQUEN Foundation for treatment for late sequelae of burn scars injuries in her right upper extremity involving the dorsum of her right hand, web spaces and fingers, resulting in functional limitations in her wrist not observed in elbow and shoulder (Figure 1 and Table 1). Injuries were sustained 2 years before for which she received local treatment, with subsequent severe contractures of the right hand and forearm involving hyperextension of her wrist, fingers, and limited flexion of the metacarpophalangeal (MCP), proximal interphalangeal (PIP), and distal interphalangeal (DIP) joints (Table 2). Over the following 4 years she underwent a total of 10 reconstructive procedures, including releases with split thickness skin grafts and z-plasties, recovering some degree of mobility (documented by her hand therapist) but still presenting functional deficits that significantly limited her daily activities. The skin of the dorsum of the hand was tight with numerous scar contractures. Composite flexion was incomplete and painful. Tightness of the web space

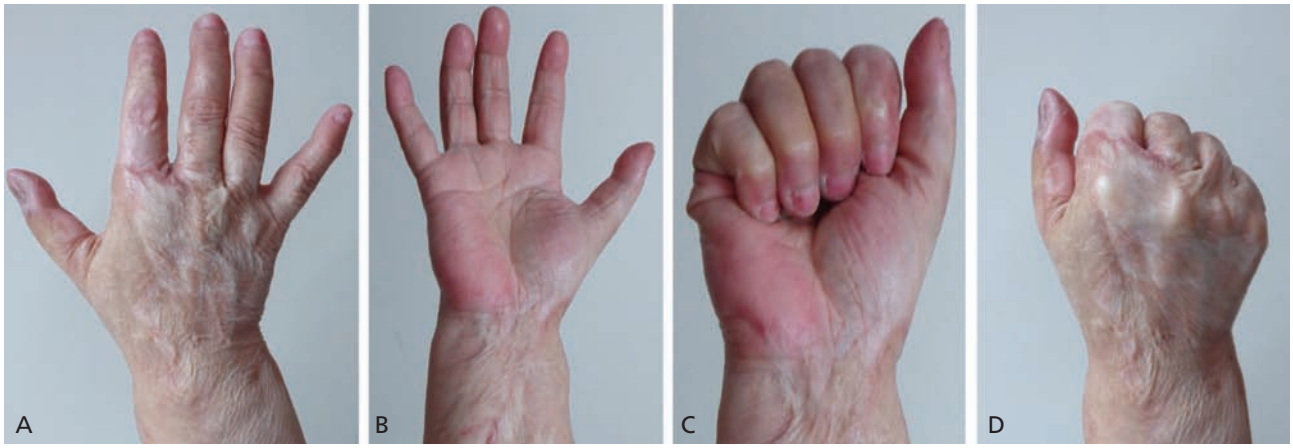


Figure 1. Pre-operatively right hand function: *A*, Abduction dorsal; *B*, Abduction palmar showing limitation of the thumb; *C*, Fist and composite digit flexion (palmar) showing inability to achieve full closure; *D*, Fist dorsal showing extensive scarring (Carstens M. et al).

Table 1. Upper right extremity functional assessment: Joints range of motion (articular amplitude) and muscle strength (1 to 5) evaluated in the upper right extremity at the time of treatment and subsequent follow-ups. N = Normal; L = Limited.

SEGMENT	Initial evaluation		6 weeks follow-up		6, 12, 24 months follow-ups	
	Articular amplitude	Muscle strength	Articular amplitude	Muscle strength	Articular amplitude	Muscle strength
Shoulder						
Flexion	N	N	N	N	N	N
Extension	N	N	N	N	N	N
Abduction	N	N	N	N	N	N
Horizontal adduction	N	N	N	N	N	N
Horizontal abduction	N	N	N	N	N	N
External rotation	N	N	N	N	N	N
Internal rotation	N	N	N	N	N	N
Elbow						
Flexion	N	N	N	N	N	N
Extension	N	N	N	N	N	N
Pronation	N	N	N	N	N	N
Supination	N	N	N	N	N	N
Wrist						
Flexion	N	N	N	N	N	N
Extension	N	N	N	N	N	N
Fist	L	3/5	N	3+/5	N	4/5

particularly on either side of the middle finger limited finger abduction. Scarring around the base of the thumb resulted in a complete inability to perform opposition (Figure 1 and Table 2). Functionally, her handwriting was severely limited as she was not able to properly grasp a pen. In addition, her use of the keyboard for writing reports was restricted to two fingers. Other routine activities, such as embroidery were similarly limited (Table 3).

As a result of incomplete functional recovery, 1 year later the patient was declared candidate for a cell-based therapy approach, thus receiving local injections of fat-derived stromal vascular fraction (SVF) containing autologous mesenchymal stem cells (MSCs). Immediately after the cell count, 2 cc of saline containing a total of 6 x 10^6 SVF-derived cells were divided between and injected into each of the 4 MCP joints affected. The dorsum of the hand received a total of 20 cc of autologous fat enriched with a total of 2.7 x 10^6 SVF-derived cells as a series of injections into the subcutaneous space. The patient tolerated the procedure without issues, and no local or systemic complications were evidenced thereafter.

Table 2. Right hand's joints functional assessment: Range of motion of digits joints (MCP = Metacarpophalangeal; PIP = Proximal Interphalangeal; DIP = Distal Interphalangeal), opposition of the thumb to each one of the digits, and digit flexion measured as the distance from fingertip to the proximal palmar crease (in cm), evaluated at the time of treatment and subsequent follow-ups. Ach = Achieved.

	Initial evaluation				6 weeks follow-up				6, 12, 24 months follow-ups			
	Index	Middle	Ring	Small	Index	Middle	Ring	Small	Index	Middle	Ring	Small
MCP	70°	70°	70°	70°	90°	90°	90°	90°	90°	90°	90°	90°
PIP	50°	50°	50°	50°	100°	100°	100°	100°	100°	100°	100°	100°
DIP	45°	45°	45°	45°	90°	90°	90°	90°	90°	90°	90°	90°
Thumb opposition to	NO	NO	NO	NO	YES	YES	YES	YES	YES	YES	YES	YES
Digit flexion	2.5	3	3.5	3	Ach.	Ach.	Ach.	Ach.	Ach.	Ach.	Ach.	Ach.

Table 3. Right hand's function and dexterity: Evaluation of various daily activities performed with the hands (dexterity), evaluated at the time of treatment and subsequent follow-ups.

Functional test/symptoms	Initial evaluation	6 weeks follow-up	6, 12, 24 months follow-ups
Fingers muscle strength	3+/5	4/5	4/5
Key pinch	Not achieved	Achieved with difficulty	Achieved without difficulty
Pencil pinch	Not achieved	Achieved with difficulty	Achieved without difficulty
Cylinder pinch	Achieved without difficulty	Achieved without difficulty	Achieved without difficulty
Carry briefcase	Achieved with difficulty	Achieved without difficulty	Achieved without difficulty
Adduction pinch	Not achieved	Achieved without difficulty	Achieved without difficulty
Computer writing (typing)	Unable	No limitations	No limitations
Hand writing (with a pen)	Unable	No limitations	No limitations
Pain and numbness	With mobility	Absent	Absent

At six (6) weeks post-op, and corroborated at 6, 12 and 24 months, all joints had achieved a full and painless range of motion, including composite flexion of MCP, PIP and DIP, and opposition of the thumb (Tables 1 and 2). The patient's handwriting was restored, as well as the use of the computer keyboard and the embroidery activities, all without difficulties (Table 3). The extensor tendon gliding was improved, as was the elasticity and color of the skin over the dorsum (Figure 2). Point of care ultrasound examinations of the patient's hands were performed 4 months after SVF application to compare the vascularity of the subcutaneous tissues of the right hand with the uninjured left hand. The presence of a new network of vessels surrounding each injected MCP joint and throughout the dermis of the dorsal surface of the treated hand was readily documented (Figure 3, first MCP).

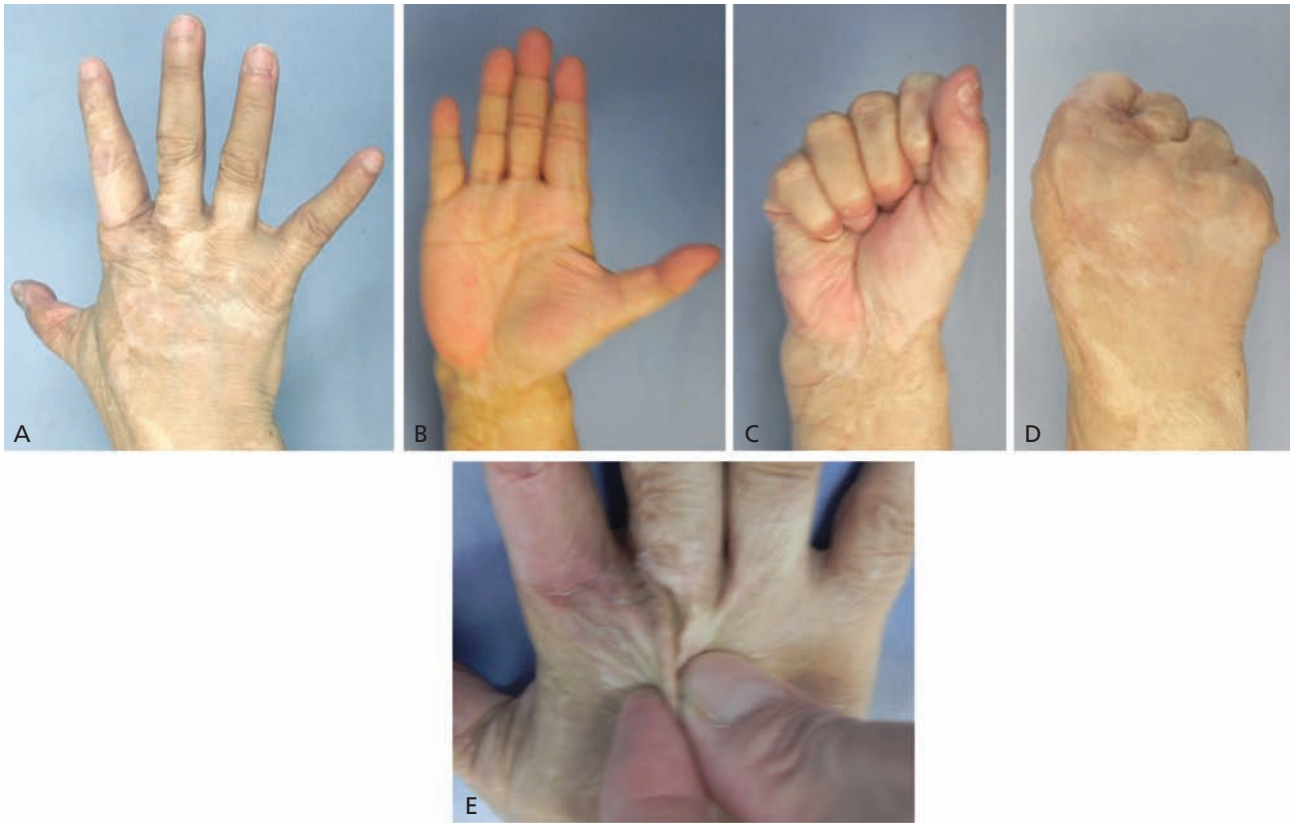


Figure 2. Post-operatively right hand function: *A,* Abduction dorsal showing increase in webspaces; *B,* Abduction palmar demonstrating dramatic increase in first webspace; *C,* Fist and composite digit flexion (palmar) showing the ability to achieve closure into the palm and the position of the thumb, which is now in contact with the index finger. Patient now can oppose the thumb into all positions and has effect key pinch and chuck pinch. *D,* Fist dorsal exhibiting extensive dorsal scarring softened, with a more elastic skin; E) Pinch test view shows dramatic changes in elasticity of the hand dorsum skin. Post op series was taken at 6 weeks. No appreciable changes seen in subsequent follow-ups (Carstens M. et al).

Patients and methods

The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national, Universidad Nacional Autonóma de Nicaragua – León) and with the Helsinki Declaration of 1975, as revised in 2000.

Obtaining of fat-derived stromal vascular fraction (SVF)

Under short general anesthesia, 108 cc of dry fat were harvested from subcutaneous fat directly into a sterile processing canister (GID SVF-1, Louisville, CO, USA). The lipoaspirate was washed three times to remove red cells and fat oil. Approximately 125 ml of Lactated Ringer's solution was added to the adipose tissue with collagenase enzyme (Worthington CLS-1, Lakewood, NJ, USA) at a concentration of 200 CDU/ml of total volume. The mixture was dissociated for 40 minutes in an incubated shaker table at 38ºC and at 150 RPM. After dissociation, the mixture was centrifuged for 10 minutes at 800x gravity. The resulting concentrated SVF at the bottom of the device was removed using a 6-inch needle. Ten microliters (µl) of SVF were taken from the final suspension and submitted for differential staining. Two samples were then passed through an image cytometer (ADAM MC, Portmouth, NH, USA) for cell counting and viability assessment. More than 4 x 10^7 total mononuclear cells (MNC) were obtained, equivalent to an average of 5 x 10^5 MNC per gram of dry fat. The total time to process the SVF cells was 70 minutes.

Physical and occupational therapy evaluation

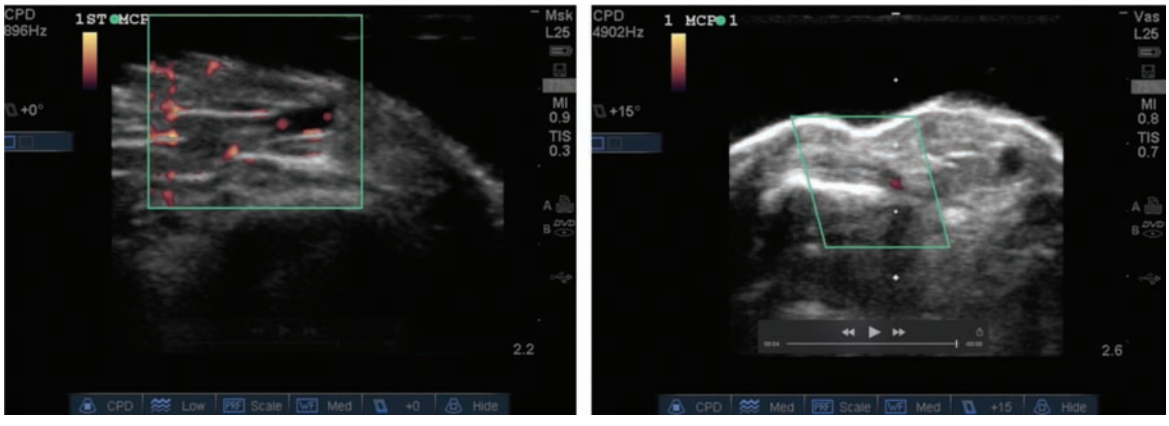
Muscle strength (functional muscle test of Daniels), range of motion, functional, dexterity and occupational statuses were assessed continuously before and after surgery (6 week and 6, 12 and 24 months follow-ups).

Color Power Doppler (CPD) Imaging

Using a linear, high frequency 10-5 MHz ultrasound probe, images were recorded through a water bath to prevent tissue distortion. Emphasis was placed on the MCP joints and the dorsal dermal tissues. Color Power Doppler imaging was performed to visualize small vessel presence and their associated density.

Discussion

The use of fat grafts for subcuteneous reconstruction has been part of the plastic surgery armamentarium for over 100 years. The advent of lipoaspiration in the 1980's facilitated the collection of fat, giving impulse to renewed efforts to inject the harvested tissue. Nonetheless, clinical experience had to mature because the techniques for processing such fat grafts were at first very rudimentary, with consequent poor results[15]. At the beginning of a fat grafting procedure the graft must acquire its nutrition by diffusion until vessels from the surrounding tissue can infiltrate it with new ramifications. In large grafts, the resulting vascular insufficiency causes necrosis of the central part of the tissue that is manifested during the first 6 months as a progressive atrophy (*i.e.:* resorption) with loss



A B

Figure 3. High frequency ultrasound power Doppler image of the dorsal first metacarpophalangeal joint: *A,* Right (treated) hand shows increased vascularity and evidence of branching neovascularization throughout the thickness of the dermis. Zero degree angle interrogation and 896Hz frequency is sufficient to demonstrate the marked vascular activity. Images were recorded through a water bath medium to avoid compression of microvessels and dermal structures. Images obtained via SonoSite M Turbo with 5-10MHz linear probe. *B,* Left (untreated) first metacarpophalangeal joint obtained through a water bath to avoid tissue distortion. Increased frequency (4902 Hz) and 15 degree interrogation angle was necessary to detect any vascular signal. (Carstens M. et al).

of adipocytes, fibrosis and the production of cysts[16]. To minimize these volume losses, Coleman developed less traumatic techniques for harvest, processing, and injection with results proven to be reliable and durable[17,18]. This methodology, called "lipostructure", distributes the fat graft via microdispersal, drop-by-drop, in different planes, using multiple entry sites[7].

Many reports now document the utility of fat grafts to re-establish subcutaneous volume in the face[19-22], in the breast[23,24], in congenital subcutaneous defects[25], and in skin ulcers[26]. As lipostructure in its various forms has been incorporated into plastic surgery, many have noted regenerative changes in skin previously damaged by radiation, scars, or burns as a response to subcutaneous fat grafting[1-3,27,28]. The key to understanding the mechanisms involved in this therapeutic phenomenon lies in the existence of Mesenchymal Stem Cells (MSCs) within the adipose tissue as first reported by Zuk et al[29,30] at the University of Pittsburgh. The fat is considered a structural connective tissue, composed by accumulations of adipocytes interspersed within a framework of stroma. This stromal component includes a reticular fiber network, vasculature (typically small caliber vessels) and its associated cells. Therefore, after processing the fat with digesting enzymes (*e.g.:* collagenase), the cellular component of the stroma can be released. This stromal vascular fraction (SVF) contains differentiated cells such as monocytes/macrophages, leucocytes, fibroblasts, pericytes, and immature adipocytes, as well as undifferentiated cells such as endothelial progenitors and MSCs[31]. These undifferentiated cells within the SVF constitute the biologically active component of a fat graft, since they are capable of inducing the formation of new blood vessels while exerting other trophic effects[8].

An extensive and growing body of literature exists today that documents the therapeutic effects of these cells after recognizing injured sites characterized by active inflammation[6,32-34]. These effects are related with immunomodulatory and trophic mechanisms, all exerted through the paracrine secretion of growth factors and citokines by MSCs to the local envionment. The resulting multi-signaling cascades promote the establishment of a regenerative environment by limiting inflammatory-mediated tissue scarring/fibrosis while inducing angiogenesis and survival/multiplication of local cells[8,35-37]. During cutaneous wound healing, special mechanisms have been described that account for the regenerative effects of MSCs[38]. They include: 1) Modulation of the phenotype and function of T-cells and macrophages; 2) Neutralization of reactive oxygen species locally; 3) Secretion of anti-fibrotic factors that modulate the production of wound healing-associated fibroblasts; 4) Enhancing the function of dermal fibroblasts while reducing their myofibroblastic convertion; 5) Promoting angiogenesis and vascular stability; and 6) Potentially differentiating directly into skin resident cells such as keratinocytes and dermal fibroblasts.

Interestingly, various proposed mechanisms of MSCs that are active during acute situations seem to be also present in chronic scenarios such as burn-derived sequelae, also called hypertrophic scarring (HTS). In particular, and based on our imaging assessments, the formation of new vascular structures constitutes a predominant phenomenon. It is well established that MSCs express and secrete angiogenic factors such as basic VEGF-A, HGF and FGF, which enhance proliferation, migration and differentiation of endothelial cell progenitors[39,40]. In addition to this inductive effect, MSCs-secreted factors also promote vascular stability and vasoprotection[41-43]. It has been hypothesized that this angiogenic promoting capability of MSCs is facilitated by their perivascular origin, which is adopted back during the regenerative period after homing to injured sites[6], and when the vascular remodeling process takes place[44,45].

On the other hand, it is well established that HTS (*i.e.:* fibrosis) presents abnormal characteristics of the ECM structure and function including increased matrix degradation, formation and consequently turnover[46]. This turnover and the associated remodeling is directly affected by the availability of local fibroblasts to proliferate, migrate and secrete the ECM. Various mechanisms and signaling cascades have implications in this tissue balance, all exerting anti-scarring effects. Interestingly, and as expected, MSCs participate in all these mechanisms via their paracrine activities. For instance, MSCs suppress fibroblast proliferation and reduce skin fibrosis via the secretion and activity of TGF-β3[47]. In this work, Wu et al demonstrated *in vitro* and *in vivo* that TGF-β3 prevents collagen accumulation and myofibroblast proliferation and differentiation in pro-fibrotic conditions. In addition, MSCs affect dermal fibroblast migration in a dose-dependent manner[48]. Moreover, it has been demonstrated that MSCs can prevent HTS via the secretion of TSG-6 (TNF-α-stimulated gene/protein 6) involving MSCs apoptosis via caspase-3 activation[49]. This mechanism suggests that the regulatory effect of MSCs on inflammation and the subsequent scarring process involves their temporal presence at the injured

site, where they secrete TSG-6 induced by apoptotic signals. Interestingly, the authors demonstrate that through inhibition of caspase-3-mediated MSCs apoptosis, the anti-inflammatory/anti-scarring effect of MSCs is hampered. Finally, it has been demonstrated that HTS fibroblasts have a reduced collagenase activity through matrix metalloproteinase 1 (MMP-1)[50,51], resulting in impaired remodeling. Lozito et al demonstrated that MSCs from various sources including fat are capable of activating exogenous proMMP-2 and proMMP-13 into their degradative form[52]. These MMPs target various ECM components including different collagens, aggrecan, laminin, perlecan, tenascin, fibronectin and elastin[53], thus promoting a remodeling state.

Adipose tissue processed in the manner of Coleman has a firm place in the treatment of burns[54]. SVF has the demonstrated capacity to enhace graft survival and to produce regenerative factors[55-57]. The importance of SVF therapy in hand burns is that it can improve the quality of cutaneous coverage and articular flexibility in established burn injuries. The technique is simple and relatively non-invasive. Therefore, MSC-based treatments offer the possibility that a similar protocol, applied to the hand within 3 weeks of grafting, might perhaps prevent the process of secondary scar contracture from initiating.

Finally, ultrasound tissue imaging has the advantages of being non-invasive, inexpensive, rapid, and accurate. Use of Color Power Doppler (CPD) allows for greater detection and increased sensitivity to low flow states such as arteriole and capillary neovascularization. CPD is very sensitive to the presence of microvascular flow because it detects Doppler shift at a high resolution. The image generated is a monochromatic demonstration of vascularity without information on velocity or direction of flow. In contrast, color or pulse wave Doppler is dependent on detection of a mean Doppler shift that is only present in higher velocity laminar vascular flow[58,59].

Conclusions

We report successful treatment of late sequelae of burn scars to the dorsum of the right hand using adipose-derived MSCs administered as intra-articular SVF and as SVF-enhanced fat graft. Not only was the skin coverage improved but extensor tendon gliding was enhanced and periarticular structures were improved all the way along the digits – *i.e.:* although only the MPS were injected, function in all the PIPs and the DIPs was restored to normal. The time course was very rapid with definitive changes in range of motion as early as 3 weeks and confirmatory values at 6 weeks. At one and two years, no relapse is present with the patient maintaining a high level of functionality using her hand.

Having previously observed physical changes in scarred skin (increased elasticity, enhanced articular flexibility) after the application of SVF we propose that this technique can constitute a novel and minimally invasive means to alleviate the effects of scarring in these patients. In such cases a logical treatment would consist of introducing SVF into the affected zone in one of two forms: (1) SVF enriched fat graft under the skin; and (2) direct injection of SVF.

This case also demonstrates the importance of ultrasound for documenting vascular changes in this type of reconstruction. These findings evidence histologic improvements in the subcutaneous space in an inexpensive and non-invasive way. It is recommended that future studies of this kind take advantage of ultrasound to document clinical progress.

Statement of Interests:

Authors' declaration of personal interests:
(i) Michael H. Carstens and Diego Correa have served as consultants for the GID Group. No other individuals have reported interest.

Declaration of funding interests:

Devices and enzymes for this study were donated by the GID Group/GID Américas. Costs for radiologic studies were donated by the Hospital Metropolitano Vivian Pellas, Managua, Nicaragua.

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