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Treatment of late sequelae of burn scar fibrosis with adipose-derived stromal vascular fraction (SVF) cells: a case series

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Abstract

We present an uncontrolled longitudinal study of five patients with sequelae of burn scar fibrosis of the hand and upper extremity, with a minimum of one year post-burn therapy, treated with local injection of non-expanded autologous, adipose-derived stromal vascular fraction (SVF) cells. The aims of this study was to determine the safety and the efficacy of the treatment, analyze pain and pruritis, flexibility and hardness, before and after the intervention. Target sites for administration of SVF cells were finger and web space contractures. Response to treatment was evaluated by analyzing clinical and physical parameters. We scored the scars with the modified Vancouver Scar Scale (VSS), range of motion and by measurements of scar hardness using a Durometer® and scar elasticity using the Courage-Khazaka cutometer®. All patients demonstrated clinical improvement (increased flexibility, scar thickness, range of motion). Of a total of 192 treatment zones, 113 were symptomatic and, of these, 97/113 (85%) demonstrated a positive clinical response. Scar hardness was assessed in 32 zones with 27 (84%) zones responsive. Net elasticity measurements improved in 81% of treatment zones. This series highlights the utility of non-expanded, adipose-derived heterogenous SVF cells population processed at the point-of-care, for the treatment of established burn scars refractory to further physical therapy to achieve enhanced functionality.

Introduction

The face and hands are frequent targets of burn injuries. Both possess pliable skin cover well-disposed to scar contractures, the sequelae affect both appearance and function. Subcutaneous fat injection for burn scar management has been shown to promote regeneration with increased collagen content, neovascularization, and dermal hyperplasia[1]. Better flexibility, texture and color have been documented in these studies. But the subcutaneous environment of the burn scar is a hostile territory for such grafts; reabsorption is variable, and the reliability of improved clinical outcomes disappointing. Beginning in 1992 Coleman demonstrated an improvement in the technique leading to greater survival of fat grafts[2]. The use of fat injection for radiodermatitis was reported by Rigotti et al in 1997[3]. Klinger's group applied fat injection to burn scars with similar outcomes[4,5]. Recent results from Klinger's group in 694 patients using autologous fat grafting for scars[6] demonstrated consistent reduction of pain and increased elasticity as measured by the Patient and Observer Scar Assessment Score (SOSAS)[7] and by durometry, a technique using a hand-held device to quantitate scar hardness[8].

Adipose tissue is a reservoir for mesenchymal stem cells in great numbers[9,10]. These can be readily obtained from lipoaspirate after digestion and centrifugation as a subpopulation of the stromal vascular fraction (SVF)[11,12], the characteristics of which have been previously reviewed[13,14]. SVF cells stimulate neovascularization via angiogenic factors such as VEGF, IGF-1, PDGF-B and HGF[15].

The addition of SVF to fat graft is postulated to enhance graft survival[16]. SVF obtained by such techniques constitute the autologous stromal and vascular elements of the patient's own adipose tissue layer and can be re-administered as an autologous tissue graft. SVF-enhanced fat grafts have been used for facial atrophy, soft tissue reconstruction and chronic wounds[17,18].

Previous experience by our group with 10-year sequelae of hand burns using intra-articular SVF in the metacarpal joints and SVF-enhanced fat grafting to the dorsum demonstrated significant changes in mobility and skin quality as early as three weeks[19]. Imaging using vascular ultrasound showed multiple new vessels in the periarticular space, as a manifestation of SVF-induced neoangiogenesis. The clinical results were consistent with a substantial (and relatively rapid) resolution of subcutaneous fibrosis in the target tissue. We attribute these finding to the anti-fibrotic effects exerted by the SVF cells at multiple levels of an established pathologic state.

Background

Fibrosis is a common *final outcome* pathway of severe burn injury. It results from a remodeling of the extracellular matrix (ECM) and fibrous tissue with the formation of scar tissue and skin dysfunction. The principal causes of fibrosis are chronic inflammation, tissue hypoxia, and an altered balance between accumulation and degradation of the ECM.

Fibrosis is initiated as the result of tissue injury; it involves a number of well-known mechanisms. Implantation of stromal vascular fraction (SVF) cells that include mesenchymal stem cells (MSCs) can potentially address four major components of the fibrotic process: immunomodulation, TGF-B1 signaling, tissue ischemia, and ECM remodeling[20].

Immunomodulation. The immunosurpressive properties of MSCs are well-known[21] and affect T-cells, B-cells, macrophages, and cytokines. We hypothesize that the sum total of these effects is to reduce the inflammatory state of the microenvironment, permitting resident cell survival and tissue regeneration[22-24].

TGF-Beta1 signaling. This pathway involves activation of receptors that lead to the production of profibrotic cells, such as myofibroblasts and (2) the induction of transformation processes that convert epithelium and endothelium to mesenchymal cells that synthesize ECM. The overall effect of SVF is to change the ratioTGF-*B*1/TGF-*B*3 from a high (profibrotic) state to a low (antifibrotic) state[25-28].

Reduced hypoxia and oxidative stress. Hypoxia and oxidative stress in fibrotic tissues involve reactive species of oxygen and nitrogen (ROS, RNS) which stimulate the production of TGF-B1 (with all its downstream profibrotic effects) and concomitant apoptosis of cells. SVF produces factors that improve tissue oxygenation and reduce the apoptotic effect of TGF-*B*1. They have been shown to increase neovascularization in liver fibrosis and myocardial infarction[27,31].

Remodeling of the extracellular matrix. MSCs, acting through a paracrine mechanism, decrease the concentration of collagen by increasing the concentrations of MMPs and repressing the expression of tissue inhibitors of matrix metalloproteinases (TIMPs)[32]. This effect may explain the ability of infused SVF to ameliorate or resolve subcutaneous fibrosis in burn scars.

Study objectives

With the above factors in mind, we decided to investigate the effects of SVF implantation for adult patients with established burn scar contractures of one year of age or more. These scars were all *refractory to further physical therapy* and represented the end-point for these unfortunate patients. The goal was to assess if improvement in the biologic characteristics of the burned skin could be achieved via transplantation of SVF cells in terms of qualitative clinical assessment of the burn scars, as well as quantitation of hardness and elasticity in the treatment zones. Accordingly, observations were made in 5 patients with mature burn scars (greater than 1 year of evolution) before and after the intervention, without a control group.

Materials and methods

Study population

Ethics

The prospective, uncontrolled longitudinal case series was conducted at the Asociación Pro Niños Quemados de Nicaragua (APROQUEN) burn center from 2014 to 2016. Observations It was approved by the Medical Ethics Committee of the National Autonomous University of Nicaragua and by the Ministry of Health of Nicaragua (MINSA).

The procedures were performed free-of-charge and were carried out in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national, Universidad Nacional Autónoma de Nicaragua – Leon) and the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all participants in accordance with standards of MINSA and the World Health Organization, and included consent to publish this study in all formats.

Inclusion/exclusion criteria

Five adult patients were selected from the records of the rehabilitation service for the APROQUEN Burn Foundation (Asociación Pro Niños Quemados de Nicaragua). They were admitted to the study for persistent functional deficits resulting from burn scars to the hand and, in one case, to the face. All scars were greater than 1 year of age after completion of reconstruction. Exclusion criteria included: age less than 18 years, use of steroids or immunosuppressive drugs, substance abuse and difficulty with transportation to return for follow-up.

Surgical procedures

SVF preparation

The SVF cells were obtained after enzymatic digestion of surgically harvested adipose tissue. Liposuction of subcutaneous fat was performed from the flanks and abdomen with the yield of dry fat per case ranging from 250 to 350 cc. The lipoaspirate was collected directly into a sterile tissue-processing canister (GID SVF-1, Louisville, CO, USA) for tissue dissociation and processing under closed conditions at all times and following manufacturer's instructions. It was first washed three times with sterile Lactated Ringer's Solution inside the canister to remove red cells and residual oils, and then dissociated with GMP-grade collagenase (GIDzyme, GID, Louisville, CO, USA) in 125 ml of Lactated Ringer's Solution, at a concentration of 200 CDU/ml of total volume. The mixture was dissociated for 40 minutes by placing the canister inside an incubated benchtop orbital shaker (MaxQ 4450, Fisher Scientific) at 38 °C and 150 rpm. After dissociation, human serum albumin was added (Baxter, 2.5% solution v/v) and then centrifuged (Sorvall ST40, Fisher Scientific) for 10 minutes at 800 g. The resulting SVF cell pellet at the bottom of the device was removed using a 6-inch #14 gauge spinal needle (Abocath-T, Hospira, Sligo, Ireland) connected to a 20 ml syringe with 15 ml Hartmann solution. Ten microliters of SVF were taken from the final suspension and submitted for differential staining. Two samples were then passed through an image cytometer (ADAM MC, Portsmouth, NH, USA) for counting and to assess cell viability. The cell suspension was then administered. Individual applications are detailed under each case summary.

SVF administration

The SVF cells were delivered by subcutaneous injection into the subcutaneous tissues subjacent to the burn scars. A #19 gauge needle on a 3 cc Leurlock syringe was used to avoid trauma to the cells. Although each patient varied in the total number of cells obtained after processing, we attempted to use the same clinical volume per surface area in all treatment zones.

Methods of scar assessment

Intake consisted of choice of treatment sites, photography, qualitative and quantitative evaluation of the burn scars. Patients were evaluated clinically on a monthly basis. Measurements with durometry and cutometry were performed at three months and six months. In this report, the pre-op and final values are reported.

Modified Vancouver Scar Scale

Treatment areas were determined for each patient, marked with respect to anatomic landmarks, and photographed. Scars were evaluated by a single physical therapist with 25 years of experience. The Vancouver Scar Scale (VSS) has been used for burn scar assessment and consists of four parameters[33]. *Flexibility* (pliability), *height* (thickness), *vascularization* and *pigmentation*. The Scar pliability is measured by wrinkling the scar into a fold. Height/thickness is measured directly. Vascularization and pigmentation are measured visually. The various scores ranged from zero (normal) to a defined end point of pathology. A modified VSS, as defined by Nedelec, provides additional scoring for *pruritis*, *pain*, and *satisfaction*[34]. Patient data are listed in Table 1. Data for individual parameters of the scars, presented in Tables 2-10, compare the pre-operative values with the final post-operative measurements taken at six months.

Articular range of motion

Articular range of motion was measured using a goniometer, with values measured in angular degrees. Specific occupational deficits were annotated[35,36].

Methods of measurement

All measurements were performed in the same room with constant temperature by our chief therapist. Burn garments were removed where applicable. Patients were rested for 30 minutes with the study site exposed.

Measurement of scar hardness

The durometer (Rex Gauge Durometer, type 00; REX GAUGE, Buffalo Grove, IL, USA) is a spring-loaded instrument designed to measure hardness of materials[8]. The 00 type is best suited for soft substances, such as animal tissue (skin). This device has been previously used for measuring skin hardness in scleroderma[37,38,39] and in scars[6]. The device has a 5 mm diameter round head containing a spring supported sensor. It is supported without tension, positioned perpendicular to the scar, and left is placed for 10 seconds. Each point was measured three times and the average was calculated. Between each reading the durometer was set to zero. Durometer values are dimension-less and range between 0 and 100. Durometer readings have been used with lipodermatosclerosis[8] in which increasing severity of skin disease was associated with higher durometer readings ($p < 0.01$).

Measurement of scar elasticity

The viscoelastic properties of the treatment areas were assessed with the Cutometer SEM 580® (Courage-Khazaka Electronics GmbH, Germany)[40]. This device has been used extensively in dermatologic studies (burns, scleroderma. cosmetology); a recent summary of the literature is available[41]. The device measures a series of parameters by pulling the skin using a negative pressure into a probe, available diameters of which are 2, 4, 6, and 8 mm.

The technique involves placing the probe over the skin, taking care to balance it without exerting pressure. A complete cycle consists of two seconds of negative pressure of 450 m bars followed by two seconds of relaxation. An optical system measures the depth of penetration of the skin using the diminution in intensity of an infrared light beam. Changes in skin deformation in response to this vertical traction are recorded into a computer. The device measures skin deformation with an accuracy of 0.10 mm. A schematic cutometer plot (deformation curve, or strain-time curve) is depicted in Supplemental Figure 1.

For our measurements the strain-time curve (Mode 1) was used with the application of 450 mbar load for 2 sec (on-time) followed by a relaxation time of 2 sec (off-time). Each measurement session generated four color-coded curves, each of which generated a series of measured and calculated parameters. There are two directly *measured parameters* (R0 and R8) and six *calculated parameters* (R2, R5, R6, R7, R9, and U_E). More details are available in the online manual (www.courage-khazaka.de). Parameter values were averaged for each session. Although, in many instances, the readings taken at 3 months showed a progression toward the final 6-month result, we report here the values taken at the pre-op session (0 months) and at the final session (6 months).

Fig. 1. Rex Gauge durometer®. The pressure sensitive pin is seen at the base of the instrument. The instrument has a manual calibration and reset button at the top of the dial with measurements from 0 to 100.

Untoward events

Patients were monitored in the post-operative period for infection, seroma, and tissue necrosis.

Statistical analysis

Durometer, cutometer, and VSS data were recorded as pre-op and post-op values per patient per treatment zone. Post-op values were obtained at 6-month follow-up. The measurements for each metric were compared using the paired data two-tailed *t*-test. All data from all patients for each metric were combined and no correlation between zones between patients was assumed, keeping the data paired for each patient for each zone. Significance was as follows: (1) alpha < or = 0.05 was significant; (2) alpha < or = 0.01 was very significant, and (3) alpha < or = 0.001 was considered extremely significant.

Results

Patient demographics and clinical case summaries (Table 1)

Note: data from VSS, durometer and cutometer given as separate tables (below).

The study enrolled 3 females and 2 males with ages ranging from 18 to 37 years. The burns scars were very mature. The time between the burn injury and treatment with SVF ranged from 2.5 years to 15 years with an average of 10.3 years, patient #2 skewing the mean. The patients were, in general, lean, with total fat harvested averaging 120 cc (one patient produced 260 cc) Viability index ranged from 62% to 97% (average 82%) but it did not appear to correlate with age. Although the oldest patient had the lowest value (62%), the youngest patient was well below the mean. However, the number are so small that valid conclusions cannot be reached.

CLINICAL CASE SUMMARIES:

VSS DATA GIVEN IN TABLES BELOW

Case 1

Treatment areas

Zone 1: left hand, dorsum, in plane with 3rd finger
Zone 2a: left hand, thumb, metacarpophalangeal joint (MP), ulnar border
Zone 3b: left hand, thumb, MP, radial border
Zone 3: left forearm distal 1/3, palmar

Scar analysis showed grade 2 (moderate) hyperpigmentation in each of the four zones. Pigmentation *normalized in all zones*. Zones 1-3 were flexible to minimal resistance whereas zone 4 (distal volar forearm, radial aspect) had grade 2 flexibility (yielding to pressure); all zones normalized. Zone 1 had grade 2 pruritis which normalized. Pain at level 5 was present in zone 4 which normalized. Hardness changed significantly in zones 1, 3, and 4. Zone 2. located over the thumb metacarpal, did not respond, perhaps because the scar was tethered to the underlying bone.

Preoperatively, the patient had difficulty carrying cases with pain and itching. Painful wrist extension accompanied by tension of the anterior volar forearm. Tension along the dorsum of the index finger on making a fist (composite flexion). Flexion of the index finger was painful. Fine pinch was painful. Limitation of carrying heavy objects and with fine pinch. Decrease sensibility dorsum of the hand.

Wrist extension no longer was burning or painful. No tension noted in the forearm with wrist extension. Full composite flexion of the hand without difficulty and without tension along the dorsum of the left index finger. Flexion of the index finger without pain. Fine pinch without pain.

Table 1. Patient demographics (3.1).

	#1	#2	#3	#4	#5
Age (years)	37	18	26	23	26
Sex	Female	female	male	female	male
Burn age	5 years	15 years	2.5 years	5 years	6 years
# zones	4	6	8	8	6
Fat (cc)	120 cc	120 cc	260 cc	120 cc	
Total cells	$46x10^6$	$155x10^6$	$124x10^6$	$115x10^6$	
Viability %	62%	76%	85%	80%	85%
viable cells per gm fat	$237x10^5$	$982x10^5$	$405x10^5$	$767x10^5$	
cell dose					
dose/zone					

Case 2 (Table 2)
Treatment areas (note both hands are included in the mexometer studies)
Zone 1: right hand, palmar, MP at index finger
Zone 2: right hand, palm, zone 2 flexor at middle finger
Zone 3: right hand, palm, MP at little finger
Zone 4: left hand, dorsal extensor, MP of thumb
Zone 5: left hand, dorsum, 1st interdigital space
Zone 6: left forearm, distal 1/3, ulnar border, 4 figerbreadths (FBs) from palmar crease

Hypopigmentation was present in the palmar right hand (zones 1-2 and the left forearm (zone 6) but not in the dorsum of the left hand. Hypopigmentation *improved* in zones 1-2. Flexibility improved in all six zones. Little change in scar hardness, perhaps due to proximity of measurement zones to underlying osseous structures.

Patient had the following articulations with preop limitation of flexion, all of which normalized by three months (red) or six months (blue).

Pre-op the patient had upon finger extension, palmar tension corresponding to finger IV and V.

Patient achieved full finger extension without limitations. Residual alight tension in the dorsum of the hand upon flexion of distal phalanx.

Case 3
Treatment areas
Right hand
Zone 1: dorsum of distal 1/3 1st metacarpal
Zone 2: dorsum of 2nd webspace
Zone 3: dorsum of 3rd metacarpal
Zone 4: dorsum of 3rd webspace

Left hand
Zone 1: dorsum of distal 1/3 metacarpal
Zone 2: dorsum of 2nd webspace
Zone 3: dorsum of 3rd webspace
Zone 4: dorsum of 4th webspace

Hypopigmentation in zones 1, 3, 5, 8 was unchanged at 6 months (remaining zones were normal). Across-the-board normalization of grade 1 flexibility. Appreciable change in hardness in zones 2, 6, and 8. Measurements of melanin dropped in all zones except 5.

Patient has tension dorsum of fingers on making a fist. Interdigital spaces 2-3 and 6-8 show limitation of abduction.

Able to achieve full composite flexion without tension in dorsum of the fingers. No changes in abduction of the affected zones.

Case 4
Treatment zones
Zone 1: frontal midline
Zone 2: glabellar midline
Zone 3: nasolabial fold (lip/cheek fold), right
Zone 3: nasolabial fold (lip/cheek fold), left
Zone 5: lip/chin junction, right
Zone 6: lip/chin junction, left
Zone 7: mandibular angle, right
Zone 8: mandibular angle, left

All zones normalized pigmentation zone 2. Zone 1, 3 pruritic but normalized. All zones normalized flexibility, except zones 3, 5. All zones responded in hardness, especially zone 7. Patient had moderate (grade 2) hypopigmentation in all zones except zone 6. Across-the-board decreases in melanin.

Case 5
Treatment zones
Zone 1: right hand, thumb, MP
Zone 2: right wrist, radial border
Zone 3: right distal 1/3 radius, radial border
Zone 4: left hand, thumb, MP
Zone 5: left wrist, radial border
Zone 6: left distal 1/3 radial border
Zones 4-6 hypervascular but normalized. Pigmentation change all zones. Quite inflexible; one-category change in zones 1, 4-6. Zones 2, 4 decreased hardness. Significant drop in melanin except zone 6.

Had difficulty opposition with dorsal tension of index fingers in both hands. This completely resolved.

Table 2. Prange of motion, case 2.

	RIGHT HAND					LEFT HAND				
Joint	**1st**	**2nd**	**3rd**	**4th**	**5th**	**1st**	**2nd**	**3rd**	**4th**	**5th**
MP	40 N	90 N				45 N	NA	NA	NA	NA
PIP		100 N				NA	NA	NA	NA	NA
DIP		70 N	85 N			75 N	NA	NA	NA	NA

Table 3. VSS – Pigmentation.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	2B	0	1A	0	1A	1A	2B	0	2A	1A
Zone 2	2B	0	1A	0	0	0	2B	0	3B	2B
Zone 3	2B	0	3B	1B	2A	1A	2B	0	3B	2B
Zone 4	2B	0	2B	2B	0	0	2B	0	2A	1A
Zone 5			2B	2B	1A	1A	2B	0	2B	1B
Zone 6			1A	1A	0	0	2B	0	2A	1A
Zone 7					0	0	2B	0		
Zone 8					1A	1A	2B	0		
Total	8		10		5		16		14	
Average per zone	2		1.7		1.25		2.0		2.3	

Vancouver scar scale: scores by parameter, aggregate analysis

Note: in all tables below values given are pre-op > 6-month post-op

Vascularity

Hypervascularity was present in all six zones in one of the five patients. His scars were also the most symptomatic. Zones 1-3 were grade 1 and did not respond. Zones 4-6 were grade 3; at six months two of these active zones were responsive to SVF therapy.

Pigmentation (Table 3)

A total of 32 zones were tested, 4 were normal with abnormal pigmentation present in 28. Of these, 22/28 zones (78.6%) responded to treatment by improving at least 1 category; 6/22 were unresponsive. 14/22 responder zones (63.6%) achieved normal pigmentation at six months.

Cases 1 and 4 were the most responsive, showing a change in two categories in 12/12 zones. Case 5 was uniformly responsive as well, with a one-category color change in 6/6 zones. The degree of color change in this particular patient was mitigated by the thickness of his scars. Case 3 was least responsive with 0/4 pigmented zones demonstrating a change in color. Using colorimetry in this same patient, considerable changes in pigmentation were seen.

Hypopigmentation involving a total of 10 zones was present preop in 4/5 patients. In 6/10 the hypopigmentation improved by one grade response to SVF of skin with hypopoigmentation versus hyperpigmented skin.

In the table below, those zones with abnormal pigment are scored and the average obtained. Note: if a zone had normal pigmentation it was not counted.

Flexibility (Table 4)

29/32 treatment zone demonstrated some limitation of flexibility prior to treatment. 29/29 (100%) responded with 16/29 (77%) normalizing and the remainder decreasing by one category. This is a single-treatment protocol. It would be of interest in the case of thicker scars to see if multiple applications of SVF would exert a serial additive effect. The limited response of scars in patient 5 can be attributed to mechanical difficulties in diffusion of paracrine factors

Table 4. VSS – Flexibility.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	1	0	2	1	2	0	1	0	4	3
Zone 2	1	0	3	1	0	0	1	0	4	4
Zone 3	1	0	2	1	1	0	1	1	4	4
Zone 4	2	0	3	2	0	0	1	0	4	3
Zone 5			2	1	1	0	0	0	4	3
Zone 6			2	1	4	0	2	0	4	3
Zone 7					4	0	1`	0		
Zone 8					4	0	1	0		

Table 5. VSS – Thickness.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	1	0	0	0	0	0	1	0	1	1
Zone 2	1	0	0	0	0	0	1	0	2	2
Zone 3	1	0	1	0	0	0	1	1	2	1
Zone 4	0.5	0	1	0	0	0	1	0	3	0
Zone 5			1	0	0	0	1	0	3	2
Zone 6			0	0	0	0	1	0	3	2
Zone 7					0	0	1`	0		
Zone 8					0	0	1	0		

Thickness (Table 5)

In these patients, with a total of 21 treatment zones were symptomatic, of which 19 responded (90.4%) responded to treatment. Patients with minimal to mild thickening of the scars achieved reduction in height to normal. Thick scars were unresponsive.

Pruritus (Table 6)

16/32 zones were symptomatic; of these 15/16 (94%) responded to treatment. Three patients had moderate itching (grade 2) in a total of 12 zones, all of which resolved. The most severely affected patient had grade 3-4 itching in all six zones with resolution in one, and a decrease of two grades in the remainder. All pruritic responded to treatment, with complete resolution of itching in 10/15 (67%) zones.

Pain (Table 7)

13 zones in three patients were painful, with quantitation using the Visual Analog Scale (VAS), graded 0-10. 13/13 (100%) painful zones responded to treatment, all of the responders demonstrated a decrease in value at 3 months, with 9/12 responder zones (75%) becoming pain-free by six months. Two patients had grade 5 pain in a single zone which resolved at 6 months. The last patient had pain in five of six zones, three of which were grade 5 and normalized.

Table 6. VSS – Pruritis.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	2	0	0	0	2	0	1	0	4	0
Zone 2	0	0	0	0	0	0	1	0	4	2
Zone 3	0	0	0	0	0	0	1	1	4	2
Zone 4	0	0	0	0	2	0	1	0	3	1
Zone 5			0	0	0	0	0	0	3	1
Zone 6			0	0	0	0	2	0	3	1
Zone 7					0	0	1`	0		
Zone 8					0	0	1	0		

Table 7. VSS – Pain.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	0	0	0	0	2	0	0	0	10	6
Zone 2	0	0	0	0	0	0	0	0	10	8
Zone 3	0	0	0	0	1	0	0	0	6	6
Zone 4	5	0	0	0	0	0	0	0	5	0
Zone 5			0	0	1	0	0	0	5	0
Zone 6			0	0	4	0	0	0	5	0
Zone 7					4	0	0`	0		
Zone 8					4	0	0	0		

Table 8. VSS – Satisfaction.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	6	9	7	10	8	9	6	8	4	3
Zone 2	6	9	7	10	8	9	6	7	4	4
Zone 3	6	9	7	10	8	9	6	10	4	4
Zone 4	6	9	7	10	8	9	6	10	4	3
Zone 5			7	10	8	9	6	7	4	3
Zone 6			7	10	8	9	6	7	4	3
Zone 7					8	10	6	10		
Zone 8					8	9	6	10		

Satisfaction (Table 8)

All patient reported improved satisfaction with the scars. This parameter is complex because it reflects the patient's overall experience of the scar process, from the time of burn (score 0-1) to final reconstructive stead-state. Most patients reported consistent numbers across all their zones; this lack of individualization may reflect the patients' overall appreciation of his condition, rather than individualizing to individual treatment zones. Because this value is so subjective, we did NOT include it in the total VSS score.

Total modified Vancouver scar scale per zone (Tables 9, 10)

NOT including satisfaction, total VSS for all zones, and average. Please note: numbers do NOT include satisfaction as this was a global assessment, and not for each individual treatment zone. Maximum score 27 (very symptomatic), minimum score 0 (asymptomatic).

Of a total number of 192 zones in these six categories (vascular, pigmentation flexibility. height, pruritis and pain), 113 were symptomatic. Of these symptomatic zones, 97/113 (85.8%) demonstrated a positive clinical response. When the response rate per VSS category was compared, a remarkable pattern was observed: pain 13/13 (100%), pruritus 15/16 (93.8%), flexibility 27/29 (93%), thickness 19/21 (92%), pigmentation 23/28 (82%), and vascularity 2/6 (33%). Vascularity was the least common category, being documented in only one case, precisely the patient with the thickest and most reactive scars. Excluding the category of vascularity, the clinical response rate increases 95/107 (88.8%).

Table 9. VSS – Total score per zone.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	6	0	3	1	7	1	7	0	18	12
Zone 2	4	0	4	1	2	0	4	0	24	19
Zone 3	4	0	7	2.5	5	1	7	1	20	16
Zone 4	9.5	0	5	2.5	0	0	5	0	17	7
Zone 5			5	3.5	4	1	10.5	0	20	9
Zone 6			3	3	4	0	6	0	20	11
Zone 7					4	0	5`	0		
Zone 8					6	1	4	0		

Table 10. VSS – Clincal response per symptomatic zone.

	#1	#2	#3	#4	#5	Total	% +
Vascular					2/6	2/6	33%
Pigment	4/4	3/6	1/4	8/8	5/6	21/28	75%
Thickness	4/4	3/3		8/8	5/6	19/21	92%
Flexibility	4/4	6/6	6/6	7/7	4/6	27/29	93%
Pruritis	1/1		2/2	6/7	6/6	15/16	93.8%
Pain	1/1		6/6		6/6	13/13	100%
						97/113	
						85.8%	

Table 11. Durometry – Total score per zone.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	23	5	29	27	41	40	35	25	32	32
Zone 2	19	18	25	25	20	13	27	24	63	45
Zone 3	21	11	28	27	25	25	15	14	41	39
Zone 4	20	7	28	28	20	17	17	15	45	30
Zone 5			29	28	27	19	17	14	42	34
Zone 6			30	29	16	13	20	11	40	30
Zone 7					16	16	30	18		
Zone 8					29	18	20	14		

The data below were significant as measured by the two-tailed test to a value of $p<0.0001$ (see statistics summary below).

VSS scores: points per zone per patient (Table 9)
VSS scores: clinical response per symptomatic zone per VSS parameter (Table 10)
Scar hardness – durometer® (Table 11)

Individual patient data is given in the table below with 3 measurements: pre-op, 3 months and 6 months. Scars ranged in initial hardness from 15-63. There were a total of 6 outlier zones of hardness > 35. Average hardness was consistent within each patient (#1 = 22, #2 = 28, #3 = 24, #4 = 20.5, #5 = 34.6)

Of the 32 total zones, 27 (84%) were responsive, demonstrating a decrease in hardness; and 5 zones (16%) were non-responsive. Because each clinical case is different, the concept of averaging the percent change across the board is non-valid. On a per-patient basis however, we see the following average changes (i.e. decrease in hardness): patient 1 (34%), patient 2 (2.8%), patient 3 (17.4%), patient 4 (27.9%), and patient 5 (14.2%). What is clear is that all patients responded with decrease hardness in ≥ 2/3 of treatment zones. The data below were significant as measured by the two-tailed test to a value of $p<0.0001$ (see statistics summary below).

Scar elasticity: **cutometer**® case-by-case, aggregate (Tables 12-17)
Blue = value consistent with increased elasticity
Red = value inconsistent with increased elasticity
Note: numbers ≥ 1 indicate very dry (very elastic) skin

Case 1 All zones (4/4) were responsive. 15/20 (75%) of the parameters were consistent. Figure in black is the percent change

Case 2 In the right hand zones #1-#3 showed 7/15 parameters consistent, 3/15 neutral, and 5/15 inconsistent. In the left hand zones #4-#6 showed 6/15 consistent, 2/15 neutral and 7/15 inconsistent. Elasticity parameters very erratic. 12/30 zones (40%) were inconsistent.

Case 3 Zones 1-3 were from the right hand; 4-6 from the left hand. 36/40 (90%) of the treatment zones were consistent. High R5 and R7 values consistent with dry skin.

Case 4 33/40 (82.5%) of the treatment zones were consistent

Case 5 Treatment zones 1-3 involved the right extremity; treatment zones 4-6 involved the left upper extremity. 21/30 (70%) zones showed changes consistent with increased elasticity. The right hand was more uniform, having 14/15 zones consistent, versus 6/15 on the left. 22/30 (77%) zones consistent.

Table 12. Scar elasticity – Case 1.

	R0 PRE	R0 POST	R2 PRE	R2 POST	R5 PRE	R5 POST	R7 PRE	R7 POST	R9 PRE	R9 POST
Zone 1	0.395	0.379	90.4	93.6	58.7	80.0	47.7	62.3	0.007	0.015
Zone 2	0.648	0.346	66.2	94.0	69.2	80.1	59.9	64.3	0.005	0.008
Zone 3	0.313	0.294	84.9	92.9	60.4	78.6	40.5	59.0	0.009	0.007
Zone 4	0.54	0.410	99.0	94.3	94.8	70.7	56.9	77.1	0.007	0.005

Response rate per parameter

In decreasing order R5 and R7 > R0 > R2 > R9.

Note: all patients demonstrated responses consistent with increased elasticity.

Untoward events

No complications were seen in this study. In particular, despite the injection of fluid beneath the scars, no skin necrosis occurred.

Table 13. Scar elasticity – Case 2.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	0.442	0.388	93.1	96.9	72.3	91.0	54	65.5	0.007	0.0
Zone 2	0.286	0.619	94.1	94.3	86.	67.5	59.8	51.1	0.009	0.010
Zone 3	0.247	0.283	93.2	97.4	99.4	105.4	67.5	71.0	0.006	0.007
Zone 4	0.231	0.209	93.2	91.7	1.01	89.3	81.0	66.8	0.012	0.005
Zone 5	0.361	0.209	96.8	95.2	1.12	98.3	81.0	66.8	-.006	0.005
Zone 6	0.287	0.287	96.5	96.6	95.2	98.3	56.9	71.5	0.009	0.007

Table 14. Scar elasticity – Case 3.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	0.470	0.207	89.3	95.8	47.9	1.38	31.2	71.9	0.037	0.013
Zone 2	0.934	0.476	99.6	92.6	55.0	1.156	56.3	62.8	0.029	0.013
Zone 3	0.215	0.462	90.4	98.0	62.8	76.9	46.6	52.9	0.008	0.009
Zone 4	0.907	0.462	90.5	98.0	39.3	76.9	31.4	52.9	0.006	0.009
Zone 5	0.622	0.439	95.4	97.6	63.1	94.4	49.8	59.6	0.013	0.013
Zone 6	0.487	0.366	93.7	97.6	64.6	94.4	50.5	59.6	0.010	0.005
Zone 7	0.380	0.366	93.0	96.9	62.8	94.7	41.8	63.2	0.011	0.005
Zone 8	0.168	0.366	87.7	96.9	75.2	94.7	38.5	63.2	0.006	0.005

Table 15. Scar elasticity – Case 4.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	0.420	0.539	81.9	89.2	60.0	69.5	41.7	48.9	0.048	0.006
Zone 2	1.167	0.381	93.39	93.3	32.9	91.3	27.2	55.7	0.025	0.002
Zone 3	1.101	0.524	95.4	96.5	54.3	90.0	46.9	67.7	0.015	0.009
Zone 4	0.491	0.453	90.2	95.5	60.0	85.6	51.3	65.6	0.018	0.005
Zone 5	0.645	0.440	95.7	95.5	55.1	82.6	46.2	65.6	0.008	0.012
Zone 6	0.534	0.364	93.5	95.4	65.9	86.4	52.1	65.1	0.010	0.005
Zone 7	0.501	0.343	94.7	92.3	74.1	67.5	56.6	50.5	0.005	0.008
Zone 8	0.488	0.254	83.7	95.7	51.7	1.158	40.9	77.4	0.014	0.008

Table 16. Scar elasticity – Case 5.

	#1 PRE	#1 POST	#2 PRE	#2 POST	#3 PRE	#3 POST	#4 PRE	#4 POST	$5 PRE	#5 POST
Zone 1	0.215	0.158	85.6	94.3	0.85	1.334	53.1	74.3	0.014	0.009
Zone 2	0.566	0.208	96.5	92.3	63.5	96.0	48.5	62.1	0.016	0.011
Zone 3	0.269	0.173	87.3	93.8	82.8	1.14	56.4	71.8	0.018	0.012
Zone 4	0.366	0.199	91.8	100	77.1	1.24	57.0	71.8	0.009	0.001
Zone 5	0.244	0.341	92.6	92.4	1.06	0.77	66.9	55.7	0.009	0.011
Zone 6	0.059	0.176	93.8	87.8	69.6	82.0	54.7	54.5	0.009	0.010

Table 17. Scar elasticity – Response rate per parameter.

	#1	#2	#3	#4	#5	Total, %
R0	3/4	3/6	6/8	7/8	5/6	23/32 71.8%
R2	3/4	2/6	7/8	6/8	3/6	20/32 62.5%
R5	3/4	3/6	8/8	7/8	5/6	26/32 81.25%
R7	4/4	3/6	8/8	7/8	5/6	26/32 81.25%
R9	2/4	2/6	5/8	6/8	5/6	14/32 43.75%
TOTAL	15/20	13/30	34/40	33/40	23/30	
Response rate	75%	43%	85%	82.5%	76%	

Statistical analysis (Table 18)

Statistical analysis of the durometer, cutometer, and VSS data are given below as preop and post opt values per patient per treatment zone.

Modified Vancouver scar scale, total score per patient. Caveats: (1) very small number of patients, (2) Individual zones cannot be compared among patients.

Note that the sensitivity of the cutometer parameters to treatment varied from most responsive to least responsive: R7 > R5 > R2 > R0.

Discussion

The purpose of this study was to determine the safety and efficacy of SVF cells infused into mature burn scars. The surgical procedure of SVF procurement and processing was well tolerated by all the patients. There were no untoward events in the postoperative period.

Patients' subjective evaluation describes their burn scars as softer, smoother, and less symptomatic. When present, functional limitations, such as range-of-motion or maximum oral opening, were improved or normalized without exception.

Changes in the inflammatory state of the scars were reflected in reduced hypervascularity, pruritis, and pain. A remodeling of the extracellular matrix characterized by increased hydration, better oxygenation and reduction in fibrosis. These were accurately reflected by the Vancouver scar scale. Of 97 clinically affected zones, 84 (87%) had a positive clinical response to treatment. In 4 of 6 clinical categories the response rate per affected zone exceeded 90%. Quantitation of melanocyte count in the dermal-epidermal layer would be of interest to compare the histologic response to SVF of skin with hypopoigmentation versus hyperpigmented skin. Thick scars were not responsive. This may relate to the ability of SVF-produced paracrine factors to diffuse through the scar and to the inherent limitations of injecting volume into the scar itself. This phenomenon may be due to paracrine factor modulation of inflammatory factors and/or the function of the local immune response. This is a single-treatment protocol. It would be of interest in the case of thicker scars to see if these would respond to multiple doses of SVF.

Quantifiable changes in hardness and elasticity were observed using the durometer and cutometer. All measurements were statistically significant. Durometer readings were very sensitive to change in the treated skin. Although they could not be correlated with any single clinical parameter, the findings are in agreement with the patients' appreciation of their results. Cutometer data detected changes in the physical parameters of skin elasticity that were statistically significant. These findings are in agreement with published data that correlate cutometer readings with observed changes in the

Table 18. Statistical analysis.

	VSS score	Durom	Cutom R0	Cutom R2	Cutom R5	Cutom R7	Cutom R9
Mean, 0 months	8.016	32.531	0.4818	94.356	70.847	51.188	0.01300
Mean, 6 months	3.177	22.129	0.3453	94.461	92.784	63.413	0.008281
Mean diff	4.839	10.313	0.1366	-3.284	-21.938	-12.225	0.0045719
Mean S.D.	2.761	28.408	0.2322	6.4354	26.447	12.637	0.009949
95% C.I. of	3.826	0.07	0.05	-5.64	-31.47	-16.78	0.001132
difference score	5.851	20.55	0.22	-0.93	-12.40	-7.67	0/008306
Signif. two-tailed	$p<0.001$ extremely significant	$p<0.0485$ significant	$p<0.0023$ extremely signficant	$p<0.0078$ very significant	$p<0.0001$ extremely significant	$p<0.0001$ extremely signficant	$p<0.0116$ extremely significant

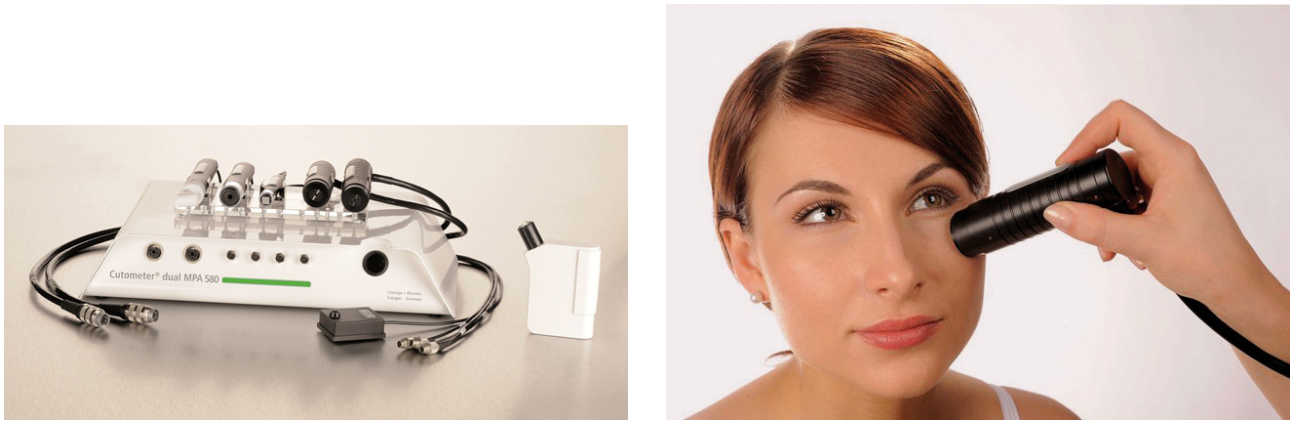

Fig. 2. Courage-Khazaka cutometer® MPA 580 — with the handpieces stored on top. Measurements are taken using the cylindrical handpiece applied to the skin. The handpiece has a suction port at the tip into which the skin is withdrawn and released. The device is connected to a PC with data entered automatically into a program to analyze the curves produced.

modified Vancouver scar scale[43]. Much work remains to further correlate tissue biology and the R parameters. Using this technology, it may be possible to demonstrate a beneficial effect of SVF treatment at earlier points in skin injury such as the amelioration or prevention of fibrosis, thus simplifying post burn rehabilitation.

Implications

This study represents the first application in humans of adipose-derived SVF cells for the management of burn scar fibrosis. The positive clinical responses for all parameters are consistent with the antifibrotic properties of MSCs seen in multiple other tissues[20] and indicate that this approach may be applicable to a wide variety of wound healing scenarios. Surgical incision characterized by scar hypertrophy, such as for median sternotomy or Ceasarean section might be treated with injection immediately after closure. Radiation therapy could make use of SVF either prior to therapy or as a postoperative treatment for radiation fibrosis. The prominent anti-inflammatory effects of SFV lend themself as a treatment for symptomatic keloids, or as a means of forestalling the formation of same. Burn units may find use for SFV at the earliest stages of wound care, or shortly after skin grafting. As SVF undergoes further clinical analysis, much will be learned about the biology of wound healing, with the ultimate goal to achieve control over the process itself.

Limitations

This study has recognizable limitations. It involves a small number of patients. It is not controlled and not randomized. In addition, the patients returned to the burn center infrequently due to logistical difficulties, hence collection of intermediate data points was limited. Facilities for cell culture that would enable standardization of cell count per cc of injectate were not available. Each patient had a different number of viable cells per gram of dry fat. The oldest patient was 37, so no observations can be made regarding decline in SVF cell populations with age [although this has been reported in the literature][44]. Thus, the number of cells transplanted in each patient varied widely.

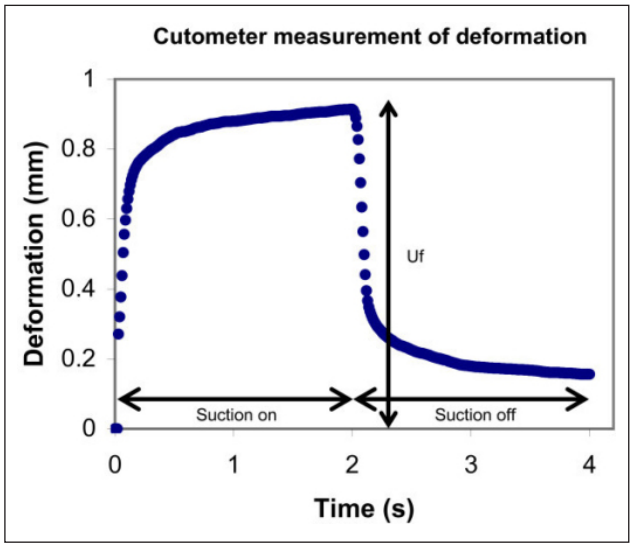

Fig. 3. Clinical measurement of skin elasticity using Courage-Khazaka cutometer showing skin recovery from deformation. Four superimposed deformation curves are averaged and parameters measured.

Supplemental Figure 1. Viscoelastic properties of skin seen in deformation (strain) versus time curve.
U_E is immediate distention (skin extensibility).
U_V is the *delayed distention* due to viscoelasticity of the skin.
U_R is the *immediate retraction* of the skin (after removing the vacuum).
U_F is the *final deformation* of the skin.
U_A is the *resilient distention*.
U_V/U_E is the *viscoelastic ratio*.
U_F is the *gross elasticity* or relative elastic recovery.
U_R/U_E is the *net elasticity*.
U_A/U_F is the *biological elasticity*.

THE STRAIN-TIME CURVE GENERATES THE FOLLOWING PARAMETERS:
R0 = U_F: *extension*; *total deviation of the skin*. R0 represents the firmness of the skin (passive behavior to force**).** Lower the R0 value, higher the firmness. **Result = distance in mm**
R2 = U_A/U_F = R8/R0: ***gross elasticity*** (the resistance versus the ability to return to normal). **Result = %**
R5 = U_R vs. U_E: ***net elasticity*** (elastic portion of the suction part versus the elastic portion of the relaxation part). **Result = %** The closer the value comes to 1 (100%), the more elastic. Note, very thin dry skin can exceed 1.
R6 = U_V/U_E = $(R0-U_E)/U_E$: ***viscoelastic*** component of total elasticity. The lower the value, the higher the elasticity. **Result = %**
R7 = U_R/U_F = U_R/R0 portion of the elasticity compared with the complete curve. The closer to 1 (100%) the more elastic the curve. **Result = %**
R8 = U_A: ***overall elasticity*** (complete relax with the suction is cut off). The closer UA is to zero the greater the ability of the skin to return to its original state. **Result = distance in mm**
R9 = R3 - R0: ***tiring effect*** on the skin after repeated suction. The smaller the R9, the smaller the tiring effect
$\mathbf{U_E}$ = (R7xR0)/R5 "elasticity". Note this is a calculated value, not shown by the device.
Note that the literature uses mostly R0, R2, R5, R7, and R9.

No generalizations can be drawn between cell "dose" and clinical outcome. Some elderly patients had high counts while others did not. All open wounds responded, even with the lowest of counts. Future studies with cultured cells and controlled dosing in animal models can prove be useful to answer these questions.

Conclusions

The harvesting, processing and implantation of adipose-derived SVF cells is simple, fast, safe, and less expensive than other cell-based therapies involving culture expansion and characterization. Processing the SVF cells in the OR (point-of-care) took approximately 60 minutes.

We conclude that implantation of adipose-derived SVF cells represents a biologically rational point-of-care cell-based therapy for symptomatic patients with established burn scars, offering the possibility of relief from pruritus and pain, and improving stiffness and range of motion. Use of this intervention early in the course of burn reconstruction may offer the possibility for reduction in primary fibrosis, with concomitant improvement the appearance and function of scars.

Future clinical studies are needed to further refine our understanding of SVF cells therapy in many areas: (1) to define minimal cell concentrations to achieve a clinical response; (2) to elaborate mechanisms of action using histochemistry and biophysical measurements; (3) to examine clinical outcomes of implementing SVF therapy at earlier points in the burn care cycle.

Disclosure of Potential Conflicts of Interest

Michael Carstens consults for the GID Group, in which he holds stock options. GID Group, inc. is a provider of products and methods to process regenerative cells and adult stem cells from fat (adipose) and bone tissue – including the GID SVF-1 device utilized in this study.

Declaration of Funding Interests

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