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Treating Elderly Patient with Full-Thickness Tear and Contusion with MEBT/MEBO : A Case Report

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【**Abstract**】　An elderly patient with extensive full-thickness tear with contusion in right forearm was given medical instructions to treat at home with Most Exposed Burn Therapy/Moist Exposed Burn Ointment (MEBT/MEBO). After 13 days of treatment, the wound healed completely without obvious hypertrophic scar or abnormal pigmentation. Sensory and motor function recovered well.

【**Key Words**】　MEBT/MEBO; Elderly; Full-thickness tear; Contusion; Therapeutic effect

Full-thickness tear is often caused by the tangential forces acting on the skin, or the skin being squeezed. It often accompanies by subcutaneous soft tissue and muscle damage, even bone and internal organ damage.[1] In recent years, with the gradually increased aging population in China, the elderly patients with full-thickness tear are also increasing every year. Whether those patients can receive early effective treatment can directly affect their prognosis. In this study, Moist Exposed Burn Therapy/Moist Exposed Burn Ointment (MEBT/MEBO) was applied on an elderly patient with extensive full-thickness tear and contusion with good result.

1. Patient data

A 97-year-old male was admitted with an extensive full-thickness tear of right forearm with contusion due to accidental fall from bed. The patient was bedridden for a long time and stated with no previous medical history of hypertension, diabetes, heart disease, etc. The full-thickness tear was noted on the right posterior forearm and on the elbow, with wound area of 20cm*9cm and 6cm*5cm. The wound base was bright red, peri-wound was blue and purple due to contusion (Figure 1-2). Clinical diagnosis was full-thickness tear of right forearm with contusion.

After admitted to the hospital, MEBO application instructions were given to his family: Apply MEBO (ShanTou MEBO Pharmaceutical CO, LTD, China) evenly on wound at 1mm thickness; cover with MEBO impregnated gauze, and secure with sterile gauze; change dressing twice a day.

When the wound has no obvious exudates, only cover with MEBO impregnated gauze and change dressing 4 times during the day; apply MEBO once at night, cover with MEBO gauze and secure with sterile gauze.

To avoid compression on the wound surface, patient is instructed to lie on his back, elevate his affected limb with two 5cm-thick cotton pads placed under his elbow and wrist respectively. After 13 days of treatment, the wound was healed completely without obvious hypertrophic scar and abnormal pigmentation. (Figure 3-8).

Figure1 - 2 : Before treatment   **Figure3:** Day 3   **Figure 4:** Day 5

Figure5: Day 7   **Figure 6:** Day 9   **Figure 7:** Day 11   **Figure 8:** Day 13
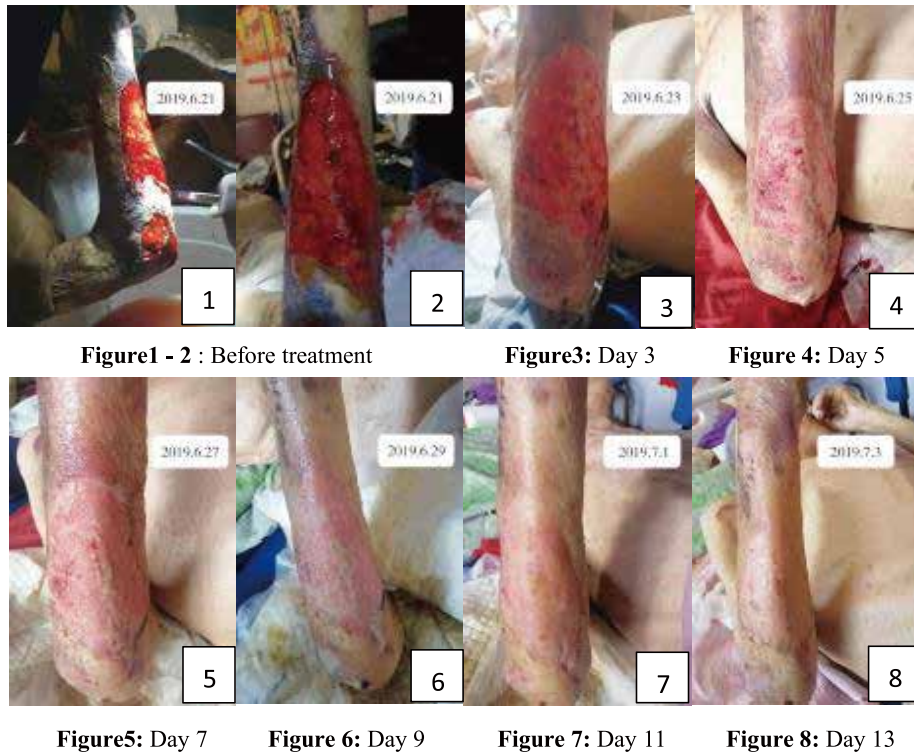
2. Discussion

It is common for the elderly to have thin skin, skin atrophy, reduced skin elasticity, and capillary fragility, which make them vulnerable to skin injuries. Once the skin is injured, skin's ability to defend against pathogenic microorganisms reduces, and its ability to heal also deceases.[2] Without proper treatments, wound infections tend to occur, and skin injury can be aggravated, which can delay wound healing and affect the recovery of skin function. In severe cases, it can also lead to toxemia and sepsis, and endanger the lives of patients. Literatures showed that skin grafting and flap transplantation are generally used for treating these types of wounds, but there are limitations: medical specialties are needed; preconditions for operations are required; more antibiotics may be needed after surgery to prevent infection; flaps necrosis tend to happen; prolonged treatment courses can occur; surgeries are generally expensive and painful.

Studies have shown that MEBT/MEBO is effective in the treatment of burns, traumatic and chronic wounds, and it is easy to apply. Therefore, in this study, the elderly patient was instructed to apply MEBT/MEBO at home and the result showed desired outcome with wound healed completely.

This study showed that MEBT/MEBO can significantly promote wound healing in elderly patients with full-thickness tear and contusion. Possible mechanism of actions: MEBO can activate the Potential Regenerative Cells (PRCs) in the wound, and promote PRCs to proliferate and differentiate into different layers of tissue cells therefore promote wound healing.[3] β-sitosterol, baicalin, berberine and other ingredients contained in MEBO can change the environment suitable for bacteria growth, inhibit bacteria activity and toxicity, and effectively prevent wound infections.[4] MEBO is rich in phytosterol, oleic acid, linoleic acid which can provide sufficient exogenous nutrients for the regeneration and repair of wound.[5] The active components contained in MEBO can inhibit the excessive proliferation and differentiation of fibroblasts, promote the growth of epithelial cells and fibroblasts with a ratio of 1:4, so as to reduce the formation of scar and prevent scar contracture caused by myofibroblast contraction.[6-8]

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Efficacy of Moist Exposed Burn Ointment Combined with Vacuum Sealing Drainage in Treating Thermal Crush Injury

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【Abstract】 Objective To evaluate clinical efficacy of Moist Exposed Burn Ointment (MEBO) and vacuum sealing drainage (VSD) in treating thermal crush injury.

Methods 81 patients with thermal crush injuries in upper limbs, admitted to Department of Burns, Wounds and Ulcers & Plastic Surgery of the Second People's Hospital of Dezhou City from June 2013 to March 2018, were divided with random number table into treatment group (n=42) and control group (n=39). Treatment group, control group were treated with MEBO and VSD, Mupirocin ointment and VSD respectively. Wound healing time was compared between two groups. Results All wounds in two groups completely healed. Wound healing time of treatment group, control group was 38.79±5.48 days, 56.62±6.97 days respectively, t=12. 850, p=0. 000.

Conclusion Applying MEBO and VSD in thermal crush injury treatment could promote wound healing and shorten wound healing time.

【Key Words】 MEBO; Mupirocin ointment; Vacuum sealing drainage; Thermal crush injury; Wound; Clinical efficacy

Most of thermal crush injuries are caused by hot machines at work. Upper limbs are mostly injured with III to IV degree of burn, often complicated with fractures, nerve and blood vessel injuries. Without timely or proper treatment, dysfunction of upper limbs or hands even amputation may occur. Lately, Moist Exposed Burn Ointment (MEBO), Mupirocin ointment, and vacuum sealing drainage (VSD) have demonstrated significant clinical efficacy in treating acute and chronic wounds such as burns, pressure ulcers, and diabetic foot ulcers.[1] In order to evaluate their clinical efficacy and to seek a more effective method for treating thermal crush injuries, the study enrolled 81 patients with upper limbs thermal crush injury from June 2013 to March 2018, and treated them with VSD combined with MEBO or with Mupirocin ointment.

1. Clinical data

1.1 Baseline data

81 patients (43 males and 38 females; average age: 42.23±2.37 years old) with upper limbs thermal crush injuries were enrolled at the Department of Burns, Wounds & Ulcers and Plastic Surgery of the Second People's Hospital of Dezhou City from June 2013 to March 2018. Among them, there were hot machine crush injury (n=79) and motorcycle engine burn (n=2); simple

thermal crush (n=73), fracture (n=8), subcutaneous tissue injury (n=43), tendon injury (n=20), and bone injury (n=18).

With random number table, patients were randomly divided into treatment group (n=42) and control group (n=39). The baseline data of the two groups was compared, such as gender, age, cause of injury, degree of injury, etc., p> 0.05 (Table 1). This study was approved by the Ethics Committee of the Second People's Hospital of Dezhou City and and all patients signed consent forms.

Table 1   Baseline data

Group	Cases (n)	Gender (n)		Age (year, $\bar{x}\pm s$)	Cause of injury (n)		Complications (n)		Degree of injury (n)		
		M	F		Machine	Engine	None	Fracture	Subcutaneous layer	Tendon	Bone
Treatment group	42	24	18	42.25±2.13	41	1	37	5	21	11	10
Control group	39	19	20	42.49±2.67	38	1	36	3	22	9	8
χ^2/t value		0.576		0.449	0.003		0.403		0.335		
p value		0.448		0.655	0.958		0.525		0.846		

1.2 Inclusion and exclusion criteria

Inclusion criteria: (1) clinically diagnosed thermal crush injury; (2) admitted within 1 day after injury without previous informal treatment; (3) compliant and cooperative; (4) informed about this study and consent forms obtained.

Exclusion criteria: (1) diabetes, severe cardiovascular and cerebrovascular diseases, liver and kidney dysfunction or hematological diseases; (2) long-term use of glucocorticoids; (3) contraindications for VSD; (4) allergic to medication used in this study.

2. Methods

2.1 Treatment methods

Treatment group: given systemic treatment including intravenous medicine to improve microcirculation, and broad-spectrum antibiotics to prevent infection, later changed to sensitive antibiotics based on results of bacterial culture of wound secretions and drug sensitivity tests. After removing the eschar and obvious necrotic tissue (deep fascia was incised to decompress

wounds with necrotic tendon and fascia, fractures were internally fixed with Kirschner wire or steel plate screw, and injured limbs were externally fixed with braces), MEBO was applied at 1-2 mm thickness, followed by MEBO impregnated gauze and sterile gauze. Dressing was changed daily. After necrotic tissue was liquefied, wound was covered with VSD dressing, connected with VSD (-60 to -40 kPa or -450 to -300 mmHg) for 7 days; after removing VSD, continue dressing change with MEBO. If there was still much necrotic tissue in the wound after 7 days, VSD and MEBO were repeatedly applied until the granulation tissue was pink and even, then split-thickness skin grafting was conducted to close the wound.

Control group: systemic treatment was same as that in treatment group. Mupirocin ointment was applied at 1-2mm thickness and dressing was changed daily. After necrotic tissue was completely separated from normal tissue, wound was covered with VSD dressing, connected with VSD (-60 to -40 kPa or -450 to -300 mmHg) for 7 days; after removing VSD, continue to apply mupirocin ointment. If there was still much necrotic tissue in the wound after 7 days, VSD and mupirocin ointment were applied repeatedly until the granulation tissue was pink and even, and then split-thickness skin grafting was conducted to close the wound.

2. 2 Statistical processing

SPSS 17.0 statistical software was used for data analysis. The measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$), using t-test; the count data was expressed as percentage, using chi-squared test, p <0.05 is statistically significant.

3. Results

3.1 Treatment results

Wounds were completely healed in both groups and data were compared between treatment group and control group: healing time: 38.79±5.48 days vs. 56.62±16.97 days, t=12.850, p=0.000. Healing time of wounds deep to subcutaneous layer: 24.23±1.97 days vs. 42.37±2.48 days, t=26.477, p=0.000; healing time of wounds deep to tendon: 39.17±2.17 days vs. 63.49±3.26 days, t=19.973, p=0.000; healing time of wounds deep to bones: 71.56±2.79 days vs. 90.18±3.41 days, t=12.074, p=0.000.

3.2 Patient case

A 19-year-old female patient, right forearm thermal crush injury caused by a hot machine shaft crushing for 30 minutes. Examination on admission: right forearm and right hand was III-IV degree burn (3.5% TBSA), complicated with embolism of the distal radial artery, damaged median nerve in the wrist, injured radial nerve and ulnar nerve; mostly damaged distal flexor tendon and palmar aponeurosis, partial necrosis of scaphoid and hamate bones, partial loss of hand sensation and impairment of finger movement. Clinical diagnosis: III-IV degree thermal crush injury (3.5%

TBSA) of right forearm and right hand; right median nerve, radial nerve and ulnar nerve injury.

After admission, patient was given intravenous medication to improve microcirculation, broad-spectrum antibiotics to prevent infection, and other systemic treatment. Wounds were treated with MEBO daily. After 12 days, necrotic tissue started to separate from normal tissue. After removing necrotic tissue under brachial plexus anesthesia, wound was covered with VSD dressing, and connected with VSD (-60 to -40 kPa or -450 to -300 mmHg) for continuous 7 days. After removing VSD, MEBO was applied daily for 7 days. The combined treatment of MEBO and VSD each for 7 days was repeated twice until the granulation tissue was pink and even, and then split-thickness skin grafting was conducted to close the wound. Wound healed completely after 84 days (Figure 1-6). Patient was discharged from the hospital. At 1 year follow-up, superficial scar was noted, limb numbness relieved and finger movement improved significantly.

Figure 1-2: Second day of treatment; **Figure 3:** Before first time VSD treatment

Figure 4: Before second time VSD treatment; **Figure 5-6:** At 1 year follow-up, superficial scar was noted.

4. Discussion

Thermal crush wounds are often leather-like and covered with eschar. Topical medications are often applied after surgical debridement, but surgical debridement may aggravate damage to blood vessels, nerves and other tissues, prolong wound healing time, and affect healing effect. Lately, VSD has been widely used in treating acute and chronic wounds such as burns, trauma, and pressure ulcers with good clinical outcomes. Its mechanism is to isolate the external environment, promote the clearance of wound secretion and necrotic tissue, and prevent infection,[2] it also could provide a moist environment for the wound, improve local microcirculation,[3] promote the growth of granulation tissue[4]; reduce dressing change frequency and pain during dressing changes.

Most of the thermal crush wounds are severe deep injuries,[5] often complicated with infection, severe inflammation, and edema of granulation tissue,[6] if not treated properly, they tend to become infectious refractory wounds.[7] VSD could compress edema tissue with negative pressure, accelerate edema resolution, promote the formation of new blood vessels in the wound, and improve local microcirculation. It could also promote the discharge of lactic acid and other substances in the exudate, prevent prolonged inflammatory phase caused by the accumulation of lactic acid; inhibit the growth and reproduction of bacteria, reduce wound infection and promote wound healing.[8-10]

In this study, wounds of both groups completely healed, which showed that VSD combined with topical medication could promote thermal crush wound healing, but different topical medication has different efficacy. Studies have shown that mupirocin ointment could only control infections caused by Gram-positive cocci and has no effect in promoting granulation tissue growth. MEBO could enable necrotic tissue to undergo a series of biochemical reactions , so the necrotic tissue could be liquefied and removed without damaging normal tissue. At the same time, a protective membrane is formed on the wound surface to prevent the invasion of external pathogens. The wound surface is kept in a physiologically moist environment to improve local microcirculation and promote wound healing.

In addition, MEBO is rich in various nutrients which could provide sufficient nutrition for wound repair and promote wound healing.[11] Therefore, in this study, wound healing time of MEBO combined with VSD group was significantly shorter, $p < 0.05$.

In summary, MEBO combined with VSD in treating thermal crush wounds could promote wound healing, shorten healing time, and achieve significant clinical efficacy.

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Ultrapulsed Fractional CO_2 Laser Combined with Moist Exposed Burn Ointment in Treating Traumatic Scars: A Clinical Efficacy Analysis

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【 Abstract 】 **Objective** To evaluate the clinical efficacy of ultrapulsed fractional CO_2 laser combined with Moist Exposed Burn Ointment (MEBO) in treating traumatic scars. **Methods** 62 patients with traumatic scars, admitted to the Department of Plastic Surgery and Cosmetology of Zhumadian Central Hospital from March 2016 to January 2019, were divided with the random number table into treatment group (n=31) and control group (n=31). Both groups were treated with ultrapulsed fractional CO_2 laser, combined with MEBO (treatment group) or mometasone furoate cream (control group). Vancouver Scar Scale (VSS) score, clinical efficacy and adverse reaction were evaluated. **Results** After treatment, results were compared between treatment group vs. control group, VSS score (4.43 ± 2.01 vs. 6.57 ± 1.94, t= 4.265, p= 0.000), total effective rate (90.32% vs. 74.19%, z= -2.181, p= 0.029), and adverse reaction rate (16.13% vs. 38.71%, x^2=3.971, p=0.046). **Conclusion** Ultrapulsed fractional CO_2 laser combined with MEBO could improve the color and texture of traumatic scars, therefore increase treatment efficacy.

【Key words】 Ultrapulsed fractional CO_2 laser; MEBO; Traumatic scar; Efficacy

Traumatic scars are pathological changes in the appearance and structure of tissues caused by burns, trauma, surgery, etc., which might not only affect the skin appearance of patients, but also cause serious dysfunction. Ulcers, infections, and canceration may also tend to occur to endanger the lives of patients.[1] At present, the main methods in treating scars are laser, surgery, pressure, local drug injection, radiotherapy, cryotherapy, etc. In particular, laser treatment has achieved relatively good results in recent years, but with difficulty to treat postoperative wounds.[2,3] In this study, in order to further increase clinical efficacy in treating traumatic scars, MEBO or mometasone furoate cream was applied after laser treatment to compare.

1. Clinical data

1.1 Baseline data

62 patients with traumatic scars, admitted to the Department of Plastic Surgery and Cosmetology of Zhumadian Central Hospital from March 2016 to January 2019, were divided into treatment group (n=31) and control group (n=31) by using random number table. Treatment group: 18 males

and 13 females, average age is 32.73±20.51 years old, types of scar: scald (n=7), abrasion (n=8), surgical scar (n=13), and others (n=3); control group: 12 males and 19 females, average age is 29.46±18.37 years old, types of scar: scald (n=10), abrasion (n=5), surgical scar (n=9), and others (n=7). Chi-squared test was used to compare the distribution of gender and scar type between the two group: χ^2=2.325, 3.549; p=0.127, 0.314. T-test was used to compare age (t=0.661, p=0.511). This study was approved by the Ethics Committee of Zhumadian Central Hospital, Zhumadian City, and all patients signed consent forms.

1.2 Inclusion and exclusion criteria

Inclusion criteria: diagnosed as traumatic scars[4]; informed about this study and voluntarily signed consent form.

Exclusion criteria: present with underlying conditions: other skin or immune system diseases, mental diseases or poor compliance, during pregnancy or lactation, scar diathesis.

2.   Methods

2.1 Treatment methods

Treatment group: compound lidocaine cream (Tongfang Pharmaceutical Co., Ltd.) was applied on the scar, followed by ultrapulsed CO_2 fractional laser treatment after anesthesia. The laser energy (25-60mJ), pulse duration (0.5-2.5ms), and frequency (40-60Hz) were set according to the patient's skin color, wrinkle depth, skin thickness, scar area, etc. The laser energy was increased step by step according to patient's tolerance level. MEBO (Shantou MEBO Pharmaceutical Co., Ltd.) was applied evenly on the scar surface after laser treatment and dressing was changed every 4-6 hours till the wound healed.

Control group: after laser treatment (same procedure as treatment group), mometasone furoate cream (Zhejiang Xianju Pharmaceutical Co., Ltd.) was applied evenly on the scar surface every 4-6 hours for 3 days.

2.2 Measurement indicators and evaluation standards

Before and after treatment, VSS was used to assess scars from 4 aspects: vascularity, pigmentation, pliability and height. With a total score of 15, higher score indicates more severity of the scar.[5,6]
Clinical efficacy was classified as: healed (scar improvement index> 75%), significantly effective (50%-75%), effective (25%-50%), ineffective (≤25%). Total efficacy rate= (number of healed cases+significantly effective cases+effective cases)/total number of cases*100%; scar improvement index= (VSS score before treatment– VSS score after treatment)/ VSS score before treatment*100%. Adverse reactions such as erythema, hyperpigmentation and wound infection were closely monitored during treatment.

2.3 Statistical processing

SPSS 22.0 statistical software was used to conduct statistical processing. Count data was expressed as percentage, using chi-square test or rank sum test; measurement data was expressed as mean± standard deviation ($\overline{x}$±s), using t-test; p<0.05.

3. Results

Before treatment, VSS scores were comparable between the two groups (p> 0.05). After treatment, compared to control group, treatment group had a significant lower VSS score, and a significant lower incidence of adverse reaction, both p< 0.5. (Table 1)

Table 1 Comparison between two groups

Group	Cases (n)	Scar score (Points, $\overline{x}$±s)		Clinical efficacy (n, %)					Adverse reaction (n, %)			
		Before	After	H	SE	E	I	TER	Erythema	Hyper-pigmentation	Infection	IR
Treatment group	31	12.51± 2.62	4.43± 2.01	12	10	6	3	90.32	2	2	1	16.13
Control group	31	11.84± 2.43	6.57± 1.94	7	6	10	8	74.19	6	3	3	38.71
t/z/x² value		1.044	4.265	-2.181					3.971			
p value		0.301	0.000	0.029					0.046			

Note: H: healed; SE: significantly effective; E: effective; I: ineffective; TER: total effective rate; IR: incidence rate

4. Discussion

Traumatic scar, a common complication after trauma, could lead to superficial scar, hypertrophic scar, pitting scar, atrophic scar, contracture scar, and keloid because of different severity and area of injuries, infection, treatment time and methods, etc. It often occurs among the young and middle-aged, and affects the patient's skin appearance and may even cause dysfunction. Because of the rapid social development and increased requirement of cosmetic appearance, scar prevention has been an hot topic in clinical researches.[7]

Ultrapulsed fractional CO_2 laser has brought revolutionary changes to laser treatment of scar as well as lead to a new understanding of the wound healing mechanism after laser treatment. It utilizes a specific laser frequency to generate a micro-treatment zone on the scar surface and the skin around scar remains intact, which is beneficial to wound repair and could reduce adverse reactions. Some researchers pointed out that ultrapulsed fractional CO_2 laser could stimulate certain cells and blood vessels in the micro-treatment zone, enable them to react, thereby influence

the production of various cytokines and growth factors, and promote scar repair. Other researchers stated that the ablative fractional laser heats the dermis layer to certain extent to balance the collagen synthesis and collagen dissolution, thereby improve the texture of scars.[8] MEBO contains active ingredients such as radix scutellariae, cortex phellodendri, and coptis chinensis.[9] It could activate potential regenerative cells in the tissue and convert them into stem cells. These stem cells further proliferate and differentiate into tissue cells of different layers, therefore in situ regenerate and repair the wound. MEBO could also inhibit excessive differentiation and proliferation of fibroblasts, prevent excessive synthesis and secretion of collagen mucopolysaccharide, and control epithelial cells and fibroblasts as 1:4 during the early stage of wound healing, thus reduce the formation of scar.[10] In addition, the inflammatory response on wound could stimulate the production of pigment cells in the basal layer of the epidermis, which could aggravate the hyperpigmentation of the scar surface. The active ingredients in MEBO could inhibit the production and release of inflammatory mediators such as tumor necrosis factor-α and interleukin-6, thus reduce local and systemic inflammatory reactions which could lead to hyperpigmentation.[11] To sum up, compared to control group, treatment group had a significant lower VSS score ($p<0.05$), which indicates better clinical efficacy, and treatment group also had less adverse reactions ($p<0.05$). While combined with ultrapulsed fractional CO_2 laser, MEBO demonstrated better clinical efficacy than mometasone furoate cream in repairing wound and preventing scars.

In summary, ultrapulsed fractional CO_2 laser combined with MEBO could improve the color and texture of traumatic scars, therefore increase treatment efficacy, also MEBO showed higher safety profile with less incidence of adverse reactions.

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Treating Ischemic Foot Gangrene in Uremic Patients with Moist Exposed Burn Ointment

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【Abstract】 **Objective** To evaluate clinical efficacy of Moist Exposed Burn Ointment (MEBO) in treating ischemic foot gangrene in uremic patients. **Methods** 19 uremic patients with ischemic foot gangrene were admitted to the Department of Integrated Chinese and Western Internal Medicine, Central Hospital of Zhuanghe City from January 2017 to December 2018. MEBO was applied as a topical treatment in addition to systemic treatments. Wound healing time, healing outcome and vascular endothelial growth factor (VEGF) level in wound before and after treatment were evaluated. **Results** After 21 days of treatment, 8 patients were completely cured and 11 patients demonstrated significant effect. All wounds healed in 29.25±7.32 days. VEGF significantly increased on 2nd day of the treatment (t= -7.036, p=0.000). **Conclusion** MEBO could promote healing of ischemic foot gangrene in uremic patients, which may be related to its role in increasing VEGF in wound.

【Key words】 MEBO; Uremia; Ischemic foot gangrene; Wound; Therapeutic effect

Uremic patients generally have poor nutritional status, peripheral circulation, mobility and sensations,[1] which makes distal limbs (especially feet) prone to ischemic gangrene. Improper treatment of ischemic gangrene could lead to amputation or even endanger the patient's life. Previous studies have shown that Moist Exposed Burn Ointment (MEBO) could shorten healing time, improve healing outcome, reduce amputation rate and increase cure rate of chronic refractory wound, such as pressure ulcers and diabetic foot ulcers. In this study, MEBO was applied on ischemic foot gangrene in 19 uremic patients, while systemic treatments were given to reduce inflammation, improve microcirculation and enhance nutritional status, aiming to further evaluate its efficacy and explore its mechanism of actions.

1. Clinical data

19 uremic patients (13 males and 6 females) with ischemic foot gangrene who were admitted to the Department of Integrated Chinese and Western Internal Medicine, Central Hospital of Zhuanghe City from January 2017 to December 2018, were enrolled in the study. Average age (65.34±8.38) years old, course of uremia (6.33±2.21) years, course of foot gangrene (4.38±3.79)

months, hemoglobin (88.63±7.21) g/L, serum creatinine (420.84±19.34) μmol/L. All patients presented with unilateral foot gangrene, left foot gangrene (n=7), right foot gangrene (n=12). Underlying conditions: atherosclerotic coronary artery disease (n=15), hypertension (n=15), diabetes (n=13), chronic obstructive pulmonary disease (n=1), undergoing hemodialysis (n=15) and peritoneal dialysis (n=4).

2. Methods

2.1 Systemic treatment

Systemic treatments were given to reduce inflammation, regulate blood glucose level and control blood pressure. Beraprost Sodium Tablets (PO), Shuxuetong (IV) or Papaverine Hydrochloride Injection (IV) was given to improve microcirculation. Erythropoietin Injection (IV) was given to treat anemia. Electrolytes, blood lipids, and creatinine level were closely monitored and regulated. Hemodialysis or peritoneal dialysis was continued based on patient's condition. Interventional or surgical treatments were performed according to ischemia severity. Patients were instructed to have a low-protein, high-fiber diet (diabetic or hypertensive patients to have diabetic diet or low-fat low-sodium diet).

2.2 Wound treatment

Apply MEBO (ShanTou MEBO Pharmaceutical Co., Ltd, China) onto the wound; cover with MEBO impregnated gauze; secure with 3-5 layers of sterile gauze; change dressing once a day.

2.3 Measurement indicator and evaluation criteria

Vascular endothelial growth factor (VEGF) level in wound was tested by immunohistochemistry before treatment and on the 2nd day of treatment: granulation tissue of wound base was taken and dehydrated, embedded in paraffin and cut into thin sections; sections were mounted onto microscope slides and baked at 60 ℃ for 5 hours, processed with xylene for 5 min*3 times; isopropyl alcohol for 5 min*3 times; processed respectively with 96%, 90%, 80%, 70%, 50% ethanol for 3 min each; rinsed with double distilled water for 3 min*3 times; rinsed with hydrogen peroxide for 10 to 15 min; rinsed with phosphate buffered saline (PBS) for 5 min; antigen retrieval was performed by microwave heating, then sections were rinsed with PBS for 5 min *3 times, and sealed with 50 μL non-immune animal serum.

After removing the serum, sections were added with 50 μL of primary antibody, incubated for 60 min at room temperature and rinsed with PBS for 5 min*3 times; added with 50 μL of secondary antibody, incubated for 20 min at room temperature and rinsed with PBS for 5 min*3 times; added with avidin-biotin-peroxidase complex 50 μL, incubated at room temperature for 20 to 30 min and rinsed with PBS for 5 min*3 times.

Sections were stained with 3,3'-Diaminobenzidine (DAB) solution for 3 to 10 min; rinsed with double distilled water for 1 min*2 times; counterstained with hematoxylin for 30 s to 1 min, and washed with tap water for 30 min and then mounted with coverslips. Microscopic images were captured and Image-Pro Plus 6.0 image analysis software was used for quantitative analysis of the positive expression of VEGF.

After 21 days of treatment, the wound healing outcomes were evaluated and classified as follows. Completely effective: the wound is completely healed; significantly effective: more than 50% reduction in wound surface area; effective: 20% to 50% reduction in wound surface area; ineffective: less than 20% reduction in wound surface area. Wound surface area reduction rate = (initial wound surface area - residual wound surface area) / initial wound surface area*100%.[2]

2.4 Statistical process

SPSS 19.0 was used for statistical analysis. The measurement data was expressed as mean ±standard deviation ($\overline{x}$±s). t-test was used, $p<0.05$ is statistically significant.

3. Results

3.1 Treatment results

No adverse reactions occurred among patients during treatment. After 21 days of treatment, out of 19 patients, 8 were cured, and 11 demonstrated significant effect. All wounds healed completely. The wound healing time was 29.25±7.32 days. No patients had recurrence within 6 month follow-up.

Before treatment, VEGF level in the wound was 17.88±4.01. On the 2nd day of treatment, the VEGF level increased to 25.22±2.79. T-test was used to compare VEGF level before and after treatment (t = -7.036, p= 0.000). This showed that MEBO can increase VEGF level in wounds.

3.2 Case report

A 65-year-old male patient had a non-healing wound for 3 months after toe amputation in another hospital due to gangrene of 2nd and 3rd toes of right foot. After amputation, wound did not heal and gradually expanded, and tissue became black and necrotic. Patient had a history of uremia and hypertension for many years, and had been undergoing regular hemodialysis and taking Nifedipine Extended-release tablets. Physical examination: 2nd and 3rd toes of right foot were absent, with an irregular wound surface area about 3 cm*3 cm. Necrotic tissue and bone exposure were noted (Figure 1), with odor and pain. Pulse of dorsalis pedis artery of both feet and posterior tibial artery could not be palpated, popliteal pulse was weak. WBCs $12.4*10^9$/L, hemoglobin 89g/L, serum creatinine 423 μ mol/L. Bacterial culture results of wound exudate showed Klebsiella pneumoniae infection. Computed tomography angiography (CTA) results showed

arteriosclerosis and occlusion of both lower extremities. Clinical diagnosis: partial gangrene of right foot, uremia, hypertension.

After admission, the patient was given symptomatic treatments such as regular hemodialysis, anti-hypertensive medication, anticoagulants, and balloon angioplasty to treat arterial occlusion of right lower extremity. MEBO was evenly applied on wound after thorough debridement, followed by MEBO impregnated gauze, and 3 to 5 layers of sterile gauze. Dressing was changed once a day.

After 21 days of treatment, the wound surface area was significantly reduced (Figure 2). VEGF level in the wound before treatment was 16.00, and significantly increased to 27.00 on the 2nd day of treatment. The VEGF status before and after treatment is shown in Figure 3-4.
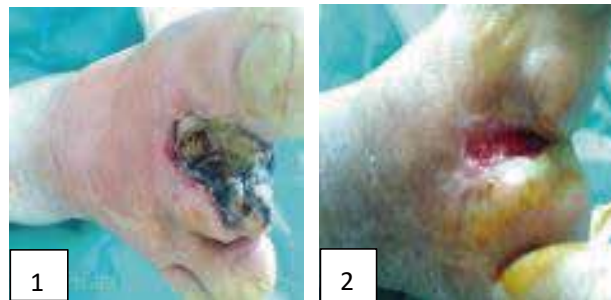
Figure 1: On admission; **Figure 2:** 2nd day of treatment
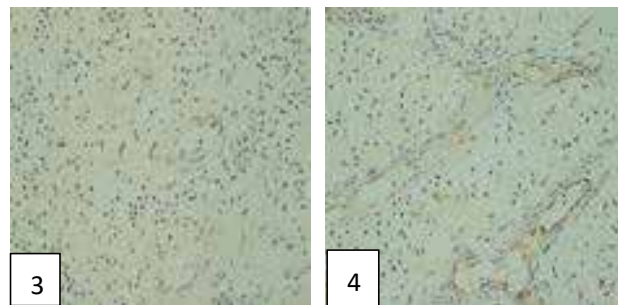
Figure 3: VEGF expression before treatment (immunohistochemistry, × 100);
Figure 4: VEGF expression on the 2nd day of treatment (immunohistochemistry, × 100)

4. Discussion

Epidemiological studies have shown that the annual incidence rate of uremia in China is about 187/1,000,000 with an increasing trend.[3] Due to inadequate secretion of erythropoietin and poor blood circulation, uremic patients are susceptible to distal limb (especially feet) gangrene because of ischemia and hypoxia, and the wound is prone to enlarge and difficult to heal. Studies have shown that although interventional treatment, bypass surgery, and carotid endarterectomy could improve blood circulation of such patients, the treatment of gangrene is still difficult.[4-5]

Clinical studies have shown that the active ingredients contained in MEBO could assist wound necrotic tissue in undergoing a series of biochemical reactions such as hydrolysis, enzymolysis, rancidification, saponification, esterification, and lipidation. MEBO could liquefy and remove the necrotic tissue without damaging normal tissue, and provide physiologically moist environment to promote the regeneration of tissue.[6-8] Ingredients such as β-sitosterol, baicalin, berberine can activate the Potential Regenerative Cells (PRCs) in the wound, and promote PRCs to proliferate and differentiate into different layers of tissue cells, therefore promote in-situ regeneration and repair of wound.[9-10] The active ingredients contained in MEBO can increase VEGF level in wound, thereby promote proliferation and differentiation of vascular endothelial cells and accelerate wound healing.[11]

In this study, MEBO was applied on ischemic foot gangrene of 19 uremic patients while systemic treatments were given to reduce inflammation, improve microcirculation, enhance nutritional status, etc. During treatment, no obvious adverse reactions occurred in any patients. After 21 days of treatment, 8 patients were cured, and 11 patients demonstrated significant effect; all wounds healed completely with wound healing time 29.25 ± 7.32 days; VEGF level in the wound on the 2nd day of treatment was significantly higher than that before treatment ($p<0.05$), which is consistent with the results of previous researches.[11]

In summary, applying MEBO to treat ischemic foot gangrene of uremic patients could effectively promote wound healing, its mechanism of actions may be related to its role in increasing VEGF level in wound. Due to small sample size and the absence of control group in this study, further studies such as multicenter randomized controlled trails with large sample sizes are needed.

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Functional Testing of a Skin Topical Formulation *In Vivo*: Objective and Quantitative Evaluation in Human Skin Scarring Using a Double-Blind Volunteer Study with Sequential Punch Biopsies

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Objective: Many topicals claim an efficacious role in skin scar management with limited evidence. Our aim is to present a clear format for functional testing of a skin scarring ointment, using noninvasive and invasive measurements, categorizing findings under the physiological, structural, and mechanical parameters of a scar.

Approach: A double-blinded, randomized volunteer research study of 45 subjects receiving an ointment composing of natural ingredients against a widely used antiscarring topical used as a positive control with temporal sequential punch biopsies (up to 16 weeks) was evaluated using noninvasive quantitative devices and validated by gene and protein studies.

Results: Outcome measures included physiological, mechanical, and structural features of scars. Significant non-invasive findings included an increase in skin hydration ($p < 0.05$) at week (W) 4, 8, and 12, and elasticity (W16; $p = 0.009$). These findings were validated by immunohistochemistry (IHC) and quantitative real-time PCR (qRT-PCR). Hyaluronic acid IHC (W4 $p = 0.014$, W12 $p = 0.034$, and W16 $p = 0.042$), qRT-PCR (W16 $p = 0.049$); Collagen I (W16 $p = 0.034$, and 0.049) IHC and qRT-PCR, respectively. Collagen III qRT-PCR (W12 $p = 0.035$, and W16 $p = 0.32$); elastin IHC (W12 $p = 0.044$); and fibronectin IHC (W4 $p = 0.009$, W12 $p = 0.038$, and W16 p = 0.026).

Innovation: Utilizing this model allows for quantitative, objective evaluation of any topical, where previously there has been a paucity of relevant methods to evaluate their effect.

Conclusions: The positive effect of a topical formulation with an unknown mechanism of action on early cutaneous scar maturation over progressive sequential time points is now evidenced using noninvasive and invasive techniques with the findings categorized on the basis of scarring parameters.

Keywords: skin, scars, skin scarring, clinical trial, topicals, MEBO Scar, scar ointments

INTRODUCTION

DESPITE THE ABUNDANCE of topical formulations on the market, the majority are ranked only as category 4, (data from case series), according to the Oxford (United Kingdom) Centre for Evidence-Based Medicine, Levels of Evidence (Supplementary Table S1).[1] The perceived lack of relevant methods for objective and quantitative

evaluation of topicals for skin scarring, may provide an explanation for the apparent limited level 1 or 2 evidence-based studies to date.

In March 2013, the sale of cosmetic products, which had been tested on animals, was banned in the European Union.[2] Even though animal models were widely used, they were not without their own disadvantages, principally, the structural differences to human skin.[3,4] *Ex vivo* organ culture models provide a good alternative to animal models,[5,6] but their limited viability does not allow for longer term evaluation.[7,8] In addition, in many human skin clinical trials, the evidence is poor due to low patient numbers, poor randomization and blinding, short follow-up periods, disparity in anatomical scar sites, and most notably, the lack of objective and consistent outcome measures.[1]

CLINICAL PROBLEM ADDRESSED

A recent study observed some positive clinical effects when MEBO Scar ointment (Julphar Pharma, UAE) was applied to surgical scars, showing improvement in scar appearance and symptomatic relief.[9] However, no objective and quantifiable method was applied to evidence these findings, as is the case with many topicals.

Taking all the above factors into account, an *in vivo*, randomized, blinded research study was devised, comparing this ointment against a positive control. Both noninvasive and invasive quantitative data were obtained in this model. The noninvasive devices measured the evolving scar features, subdivided into the structural, mechanical, and physiological properties of a skin scar. The results were then used to guide relevant subsequent confirmatory gene and protein studies.

MATERIALS AND METHODS

Volunteer study design

The primary outcome of this study, to assess the role of a topical formulation on the cosmetic appearance of skin scarring, has been registered on the ISRCTN registry (ISRCTN16551998). An independent statistician from the University of Manchester determined sample size and randomization. It was advised that with 40 subjects (based on previous studies by our group where $n = 20$), the study will have 80% power to detect effect sizes of 0.454 between treatment and control topical arms, based on comparing within-subject differences between the two topicals using a paired *t*-test at a two-sided 5% significance level. Forty-five healthy volunteer subjects were recruited and followed up (between August 1st, 2016, and May 31st, 2017). Recruitment

was through ethically approved advertisement on the University of Manchester intranet and volunteer pages. All subjects were screened by the health care professional conducting the trial and any with a medical history of relevance or keloid scarring was excluded (for patient demographics and full inclusion and exclusion criteria, please see Supplementary Tables S2 and S3). All subjects signed a written consent form (UREC ref 16098) in accordance with Declaration of Helsinki principles, and consent was rechecked verbally at each visit.

The subjects were divided into four groups—representing a temporal sequential time point: 4 (40 subjects), 8 (30 subjects), 12 (20 subjects), and 16 (10 subjects) weeks. After the initial screening appointment on "Day 0," a 5 mm punch biopsy (using a punch biopsy kit) was taken under local anesthetic from both upper inner arms to create a uniform scar. Each subject was seen on a fortnightly basis, where all measurements were undertaken, including baseline values on "Day 0": normal (healthy, nonscarred) skin before injury. No measurements were taken immediately after injury, as the primary focus of this trial was the effect of a topical on scarring, as opposed to wound healing. On the "Day 14" appointment, the first objective measurements of the scars were taken and subjects were given both treatment and control topicals with clear instructions on how exactly to apply them. The subjects were blinded as to which arm received the treatment and control formulations. At the final appointment, they received a 6 mm punch biopsy over the scar site. At that time point, the subject exited the study. All appointments were held at South Manchester University Hospital, part of the Manchester University NHS Foundation Trust (Manchester, United Kingdom). A flowchart summarizing the methodology of this clinical trial and analysis thereafter is shown in Fig. 1a.

Tissue samples

All skin samples were appropriately and anonymously labeled with the participants' study ID and stored according to HTA (United Kingdom) guidelines. Biopsies were bisected, half were stored in formalin and the other half in RNA later and stored at –80°C.

Treatment and control topical formulations

MEBO Scar ointment (Julphar Pharma), made from natural ingredients, which was originally formulated and developed in China, a derivative of MEBO™ (Moist Exposed Burn Ointment) (Julphar Pharma), had previously been found to increase vascular endothelial growth factor and fibroblast growth factor expression, affecting angiogenesis and providing anti-inflammatory properties.[9–13] This
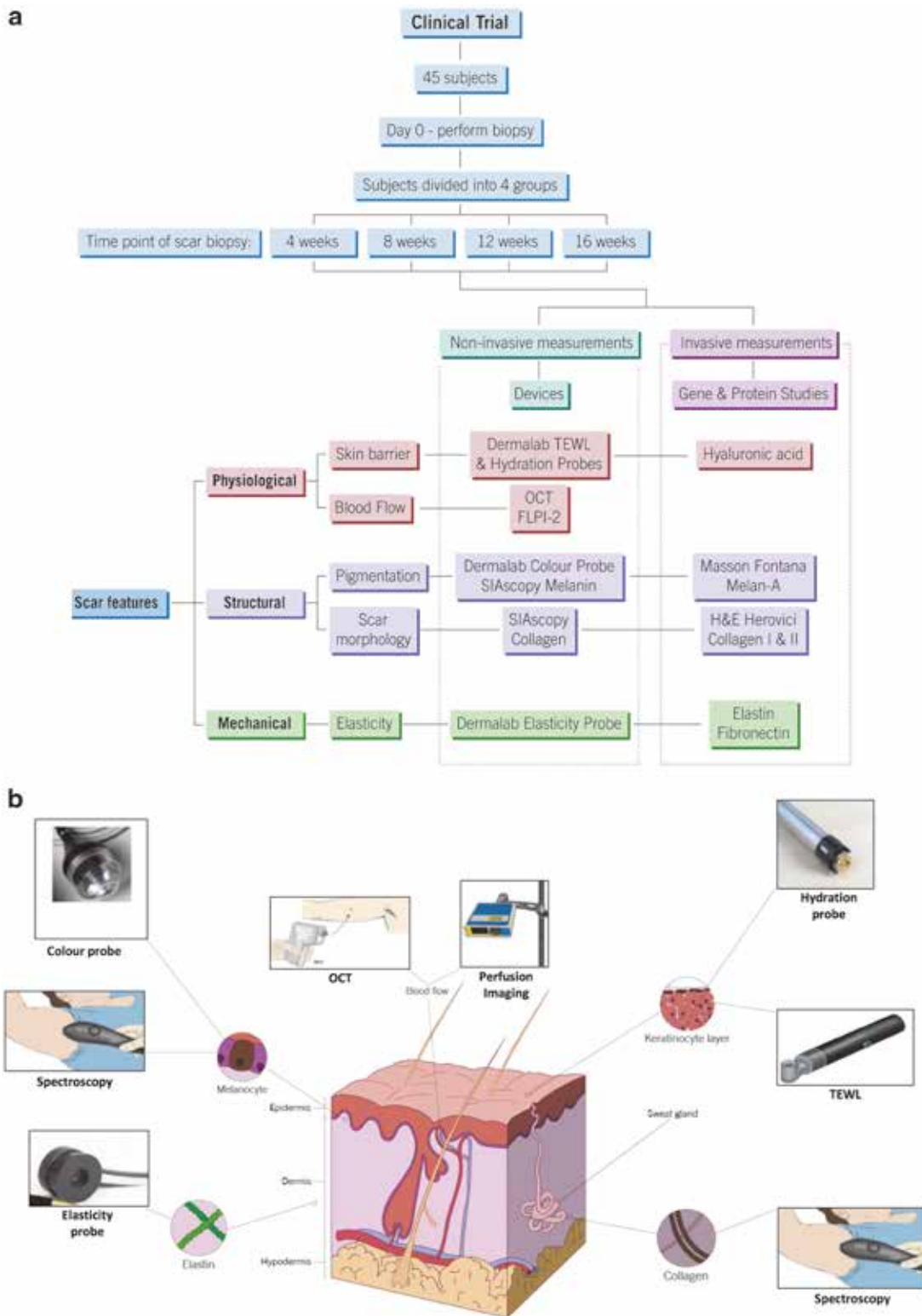
Figure 1. Flow chart and noninvasive devices. **(a)** Summarizes the methodological approach to this study. The format of the clinical trial is in *blue*. The findings of both noninvasive and invasive measurements were used to analyze the physiological, structural, and mechanical features of the scar, allowing for thorough assessment of wound healing. **(b)** The use of noninvasive devices and their anatomical targets within the skin/scar, which allow for assessment of the parameters in 1a. OCT, optical coherence tomography; TEWL, transepidermal water loss.

ointment is comprised of sesame, cactus, and beeswax and claims to contain the following "components": linoleic acid and tyramine, which are thought to improve cosmesis of skin scars.[9] All ingredients are existing and approved cosmetic raw materials. For the purpose of our study, this ointment was considered the "treatment" topical for evaluation. The positive "control" topical was kelocote® (Sinclair Pharma, United Kingdom), a widely used 100% silicone-based gel topical used principally to prevent abnormal scarring[14] and improve symptoms of hypertrophic and keloid scars.[15] This trial therefore pursues a noninferiority design, to assess whether there is any clinical benefit to using this experimental treatment (MEBO Scar) in comparison to a silicone-based topical with proven efficacy.

In view of dominant-handedness, the treatment and control formulations were randomized to each arm. The randomization was carried out by an independent medical statistician at the University of Manchester in nQuery Advisor 7.0 using a computer-generated permuted block design with mixed block sizes and random seed, and sent to a different member of the research team (not conducting the trial), who ensured correct dispensing and labeling of topicals and separated them into bags labeled "left arm" and "right arm," ensuring neither the participant nor the health care professional conducting the trial knew which formulation was in each tube.

Devices

The use of measurement devices in a clinical study setting has many advantages, in that some can provide noninvasive and objective quantitative measurements. These devices have been shown to make measurements that span all three phases of cutaneous wound healing, thus providing continual analysis of the physiological, structural, and mechanical parameters, which can be used to define a skin scar. Where the mode of action for a topical is unknown, treatment response to hypothesized improvements in the properties of the scar can be monitored by such noninvasive devices. In this case, three unique skin properties, (1) skin barrier function, and blood flow (physiological), (2) skin thickness and pigmentation (structural), as well as (3) elasticity (mechanical), were analyzed in a quantitative manner (Fig. 1b).

Another advantage of the noninvasive devices specifically selected for this study is that they are multifunctional; the Vivosight optical coherence tomography (OCT) (Michelson Diagnostics, United Kingdom) provides information on the skin architecture and blood vessel formation throughout all phases of wound healing,[16] generating high-resolution "near" real-time (<1 s) infra-red images (with a resolution of 10 μm) of the skin. The Moor full-field laser perfusion imager (FLPI-2) (Moor Instruments, United Kingdom) also gives high-resolution images, which provide real-time color-contrast images of changes in vascular perfusion[17] (hemoglobin flux) in skin microcirculation at a thickness of 1 mm and capillary diameters up to 10 μm, with flow rates of $0.01–10\,\mathrm{mm\,s^{-1}}$. Spectrophotometric intracutaneous analysis (SIAscopy) (Medx Health, Canada) provides quantitative readings of hemoglobin, melanin, and collagen content, using reflected light when the probe is placed on the skin over an area measuring 12×12 mm at a depth of 2 mm and wavelength between 400 and 1,000 nm.[18] The Dermalab system (Cortex Technology, Denmark), is a multiprobe device that measures a variety of quantitative measurement functions, including levels of hydration, color, erythema, elasticity, and transepidermal water loss (TEWL).[19] In addition, a subjective scale, in the form of a participant diary, was also used to measure reported levels of pain, itching, and redness of the scar on a scale of 1–10 for the duration of the study. Noninvasive data were analyzed using a Wilcoxon signed-rank test.

Histology and immunohistochemistry

Samples were formalin fixed and processed, and then slides were prepared from wax blocks using a microtome (Leica, United Kingdom) set at 5 μm. Slides were dewaxed and rehydrated in xylene and ethanol (Supplementary Tables S2 and S3 for specific stains). Analysis of histological data was carried out using Definiens Tissue Studio (Germany) and using a paired *t*-test.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was used to evaluate whether specific gene targets were also upregulated in the scar samples. RNA was extracted from normal and scarred skin samples stored in RNA later using QIAgen's (Germany) RNEasy mini kit with beta-mercaptoethanol. The RNA quantity and quality were measured using a Nanodrop and converted to cDNA using Roche (Switzerland) EvoScript Universal cDNA master kit. Resultant cDNA was diluted and stored at –20°C until use for qRT-PCR, using a Roche LightCycler 480 and primers and probes from Sigma-Aldrich (United Kingdom). The delta-delta Ct method was used for analysis and a paired *t*-test.

RESULTS

The design of this study allowed for the evaluation of a topical formulation by assessment of its

functional effect on the physiological, structural, and mechanical features of a skin scar. The non-invasive measurements were subsequently validated by relevant histology, immunohistochemistry (IHC), and qRT-PCR studies. There were 40 subjects in the group for the shortest time period (4 weeks); 10 subjects exited at this (and every subsequent) time point, leaving 30 subjects in the week 8 group, 20 subjects in the week 12 group, and finally 10 subjects in the week 16 group.

Physiological

Assessment of skin barrier function. Measurements of hydration and TEWL using the Dermalab System (Cortex Technology) gave an assessment of the skin barrier on normal and scarred skin of each participant. Hydration of the scar in the treatment group was increased significantly from the point of application at week 2 (new scar formation) and through to week 12 ($p < 0.05$) (Fig. 2a). TEWL decreased as hydration increased in both treatment and control groups, but with no statistical significance between the groups (Fig. 2b.). Hydration of the scar did not show any significant difference between both treatment and control groups compared to normal as opposed to scarred skin; however, with regard to TEWL, at week 16, there was a significant ($p = 0.05$) improvement in the treatment group versus control compared to normal skin. Hyaluronic acid was used to assess hydration in the formalin-fixed samples and showed significantly higher levels in the scars that received the treatment ointment compared to the control, week 4 $p = 0.014$, week 12 $p = 0.039$, and week 16 $p = 0.042$ (Fig. 2c, d). At week 16, there was also a significant fold increase in hyaluronic acid levels shown by qRT-PCR, $p = 0.049$ (Fig. 2e).

Blood flow through the scar. OCT (Michelson Diagnostics) and FLPI-2, (Moor Instruments) were used to measure blood flow. OCT was used to measure blood flow at 0.15, 0.3, and 0.5 mm depth (Fig. 3a). Both treatment and positive control topical formulations demonstrated a sharp increase in blood flow at week 2, reflecting increased levels of angiogenesis, which subsequently decreased, but did not return to baseline levels (Fig. 3b); (no significant difference in blood flow was found between treatment and control in either scarred [from week 2] or normal skin [day 0]). FLPI-2 blood flow results were also raised between 2 and 4 weeks, and then decreased slowly, before increasing again from 12 weeks in both arms (Fig. 3c), reflecting a similar trend to OCT at 0.15 mm (Fig. 3d).

Structural

Pigmentation. SIAscopy (Medx Health) and colorimetry from Dermalab (Cortex Technology) were used to assess pigmentation. SIAscopy demonstrated an increase in melanin in both topicals from day 14 to week 16; however, at week 12, there was a significant difference between treatment and control arms ($p = 0.025$) when comparing scarred skin only (from week 2) (Fig. 3e). The Dermalab colorimetry values showed an increase in pigmentation and no significant difference between treatment and control in either scarred skin or comparing the scars to normal skin (Fig. 3f). To test the validity between the results for pigmentation from the two different devices, the trend in the results was assessed using Pearson's correlation: for each time point (except week 2), and between treatment and control groups, there was a strong correlation (>0.7) (Supplementary Fig. S1), therefore validating the results obtained from each device. Staining for pigmentation using Masson Fontana and Melan-A did not show any significant difference between treatment and control topical across any time point.

Scar morphology. SIAscopy (Medx Health) was used to assess collagen content. An expectant reduction in collagen following scar creation was demonstrated in both arms, which then increased over time at the start of the remodeling phase of wound healing. There was an increase in collagen in both arms; in the treatment arm, the trend showed an 11.2% increase in collagen by week 16 from baseline compared to 6.2% in the control arm, although this was not significant when comparing the difference between the two groups from either week 2 or baseline (day 0) (Fig. 4a). Immunostaining for collagen I was significant at week 16, $p = 0.034$ (Fig. 4b, c), reflected in the qRT-PCR results, $p = 0.049$ (Fig. 4d). Collagen III intensity (Fig. 4e, f) was higher in the control arm, which had a significant fold increase in qRT-PCR at weeks 12 and 16 ($p = 0.035$, and 0.032, respectively) (Fig. 4g). The ratio of Collagen I to III in the treatment arm was also significant at week 16 ($p = 0.048$), as seen by Herovici staining (Fig. 5a, b).

Mechanical

Elasticity. Noninvasive measurements of elasticity were taken with the Dermalab (Cortex Technology) system. A significant increase ($p < 0.05$) in elasticity in the control group compared to treatment group at week 2 from day 0 is evident in Fig. 6a, although the variation in day 0 values for both arms may suggest a technical or operator-
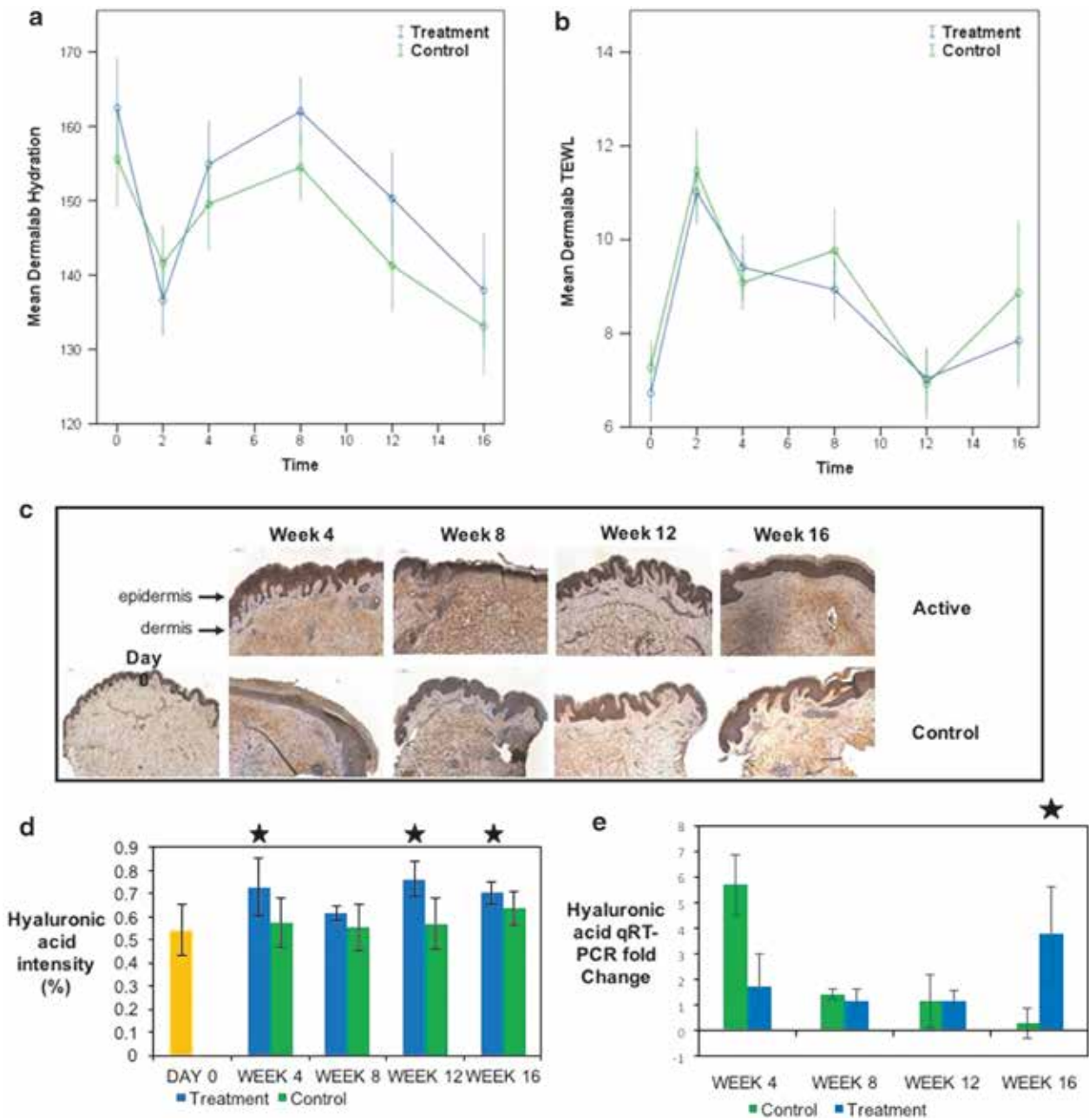
Figure 2. Assessment of the skin barrier. **(a)** Shows the increase in hydration, which is much greater in the treatment arm compared to the control and was significant in weeks 4–12 ($p < 0.05$). **(b)** (TEWL) results (which correlate with hydration). **(c)** IHC staining for hyaluronic acid. **(d)** The intensity of staining over sequential time points with $p < 0.05$ at weeks 4 ($p = 0.014$), 12 ($p = 0.039$), and 16 ($p = 0.042$), and in qRT-PCR **(e)** at week 16 ($p = 0.049$) (significant results marked with a *star*). There were 40 subjects in the group for the shortest time period (4 weeks); 10 subjects exited at this (and every subsequent) time point, leaving 30 subjects in the week 8 group, 20 subjects in the week 12 group, and finally, 10 subjects in the week 16 group. *Yellow* = normal skin (day 0). IHC, immuno-histochemistry; qRT-PCR, quantitative real-time PCR.

dependent error at this data point, and thus a potential limitation of these results. Elasticity is decreased in both arms, but at week 16, there was a significant increase in the treatment group ($p = 0.009$) compared to control (Fig. 6a) from week 2. IHC stains for elastin (Fig. 6b, c) showed an increase at week 12, $p = 0.044$ (no significance in qPCR

(Fig. 6d), and fibronectin (Fig. 6e, f) (IHC week 4, $p = 0.009$, week 12, $p = 0.038$, week 16, $p = 0.026$) for the treatment group.

Subjective

All subjects completed a "patient diary" using a numerical scale (1–10) to describe their symptoms
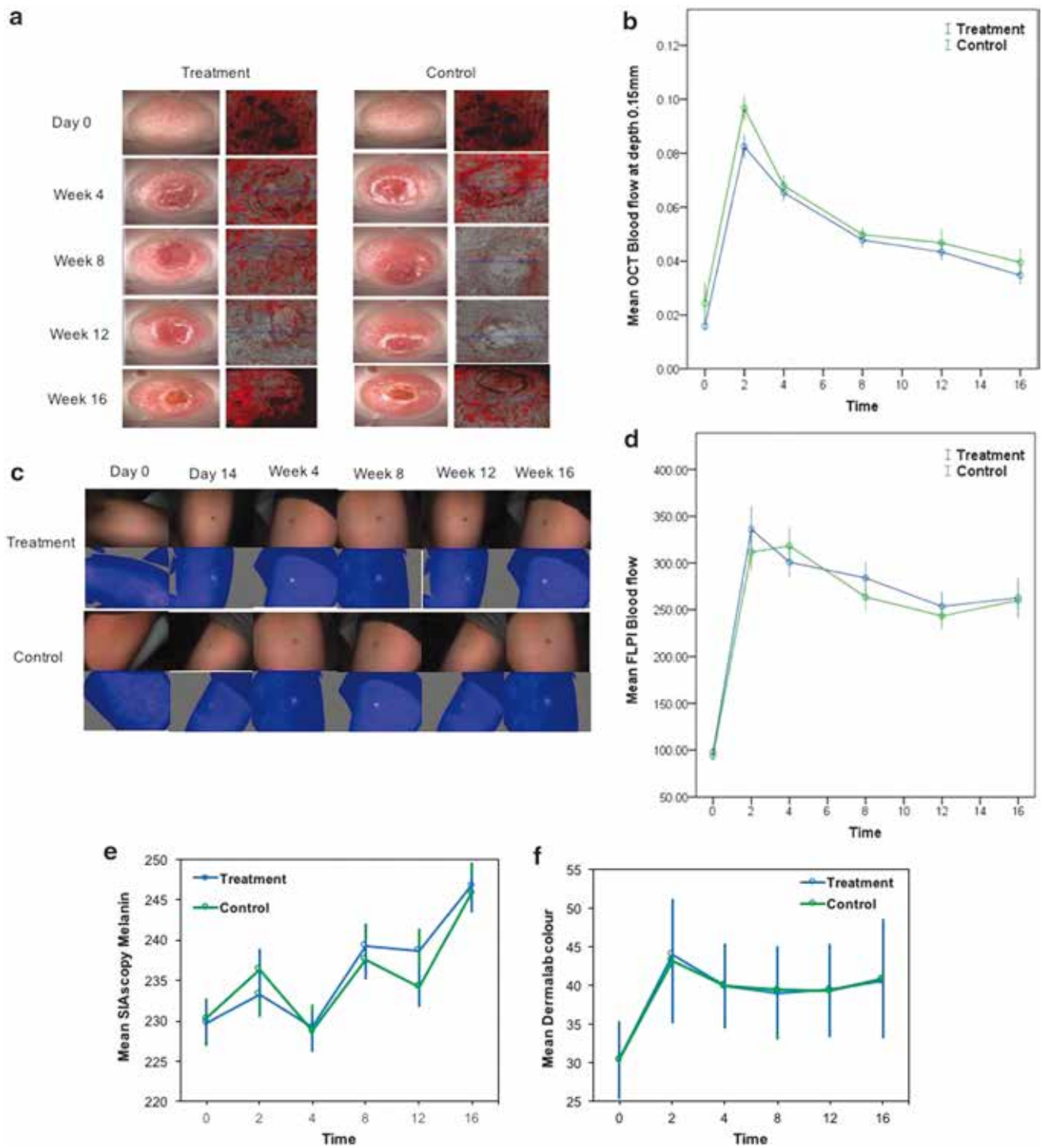
Figure 3. Assessment of cutaneous blood flow during healing and scar maturation. **(a)** Pictures from dynamic OCT in both arms. **(b)** OCT results at 0.15 mm depth. **(c)** FLPI-2 pictures from both arms. **(d)** FLPI-2 results. The treatment and control arms displayed a similar pattern with both devices. A sharp increase in blood flow from baseline is evident in both OCT and FLPI, which does decrease slowly, but returns to baseline by week 16. **(e)** Pigmentation results using SIAscopy continue to rise by week 16 in both arms, with a decrease at weeks 4 and 12. **(f)** Colorimetry values rise at week 2 and then plateau, but do not return to baseline values by week 16. There were 40 subjects in the group for the shortest time period (4 weeks), 10 subjects exited at this (and every subsequent) time point, leaving 30 subjects in the week 8 group, 20 subjects in the week 12 group, and finally, 10 subjects in the week 16 group. FLPI, full-field laser perfusion imager; SIAscopy, spectrophotometric intracutaneous analysis.
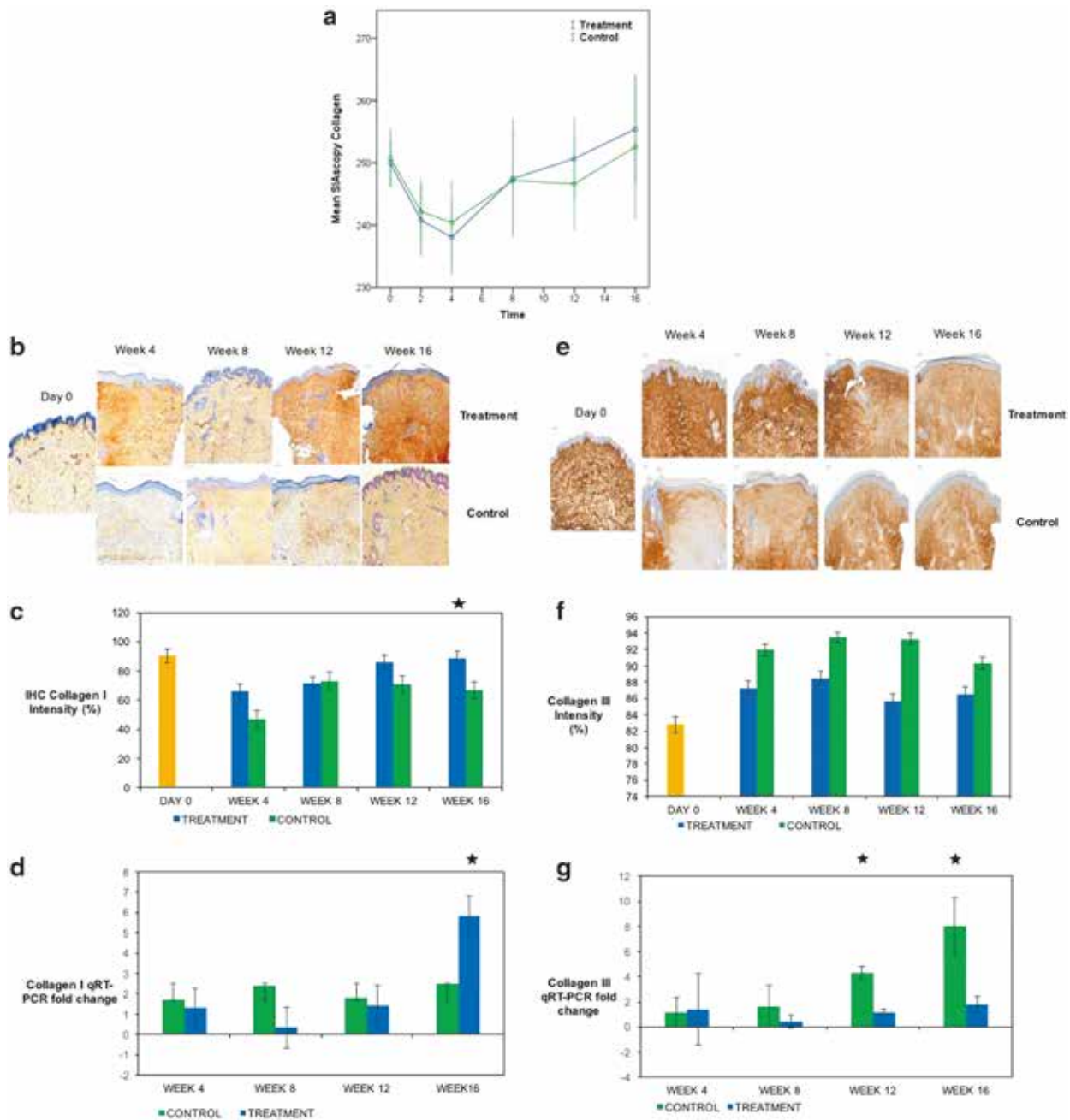
Figure 4. Assessment of collagen during healing and scar maturation. **(a)** SIAscopy demonstrates after an initial decrease in collagen following the biopsy, collagen continues to rise in both treatment and control arms. **(b)** Images for IHC staining for collagen I. **(c)** IHC intensity of collagen I in treatment and control arms, significant at week 16 ($p = 0.034$) between both groups. **(d)** qRT-PCR for collagen I in treatment versus control arms, significant at week 16 ($p = 0.049$). **(e)** Intensity of collagen III IHC stain. **(f)** Staining for Collagen III, matching trend in collagen III q-RT PCR **(g)**, significant at weeks 12 ($p = 0.035$) and 16 ($p = 0.032$), treatment versus control. There were 40 subjects in the group for the shortest time period (4 weeks), 10 subjects exited at this (and every subsequent) time point, leaving 30 subjects in the week 8 group, 20 subjects in the week 12 group, and finally, 10 subjects in the week 16 group. *Yellow* = normal (healthy non-scarred) skin (day 0). *Star* = significant *p* value at time point indicated.

of pain, redness, and itching. The trend showed a mean value of "10" (the maximum score allocation for each symptom) between weeks 2 and 4, and a mean value of "0" (*i.e.*, pain, redness, or itching) from weeks 12 to 16. This trend was identified in both arms, with no significance between treatment and control, implying that within the subjective data, the length of time, rather than the topical applied, was the principal factor improving the symptomatic features of skin scarring.
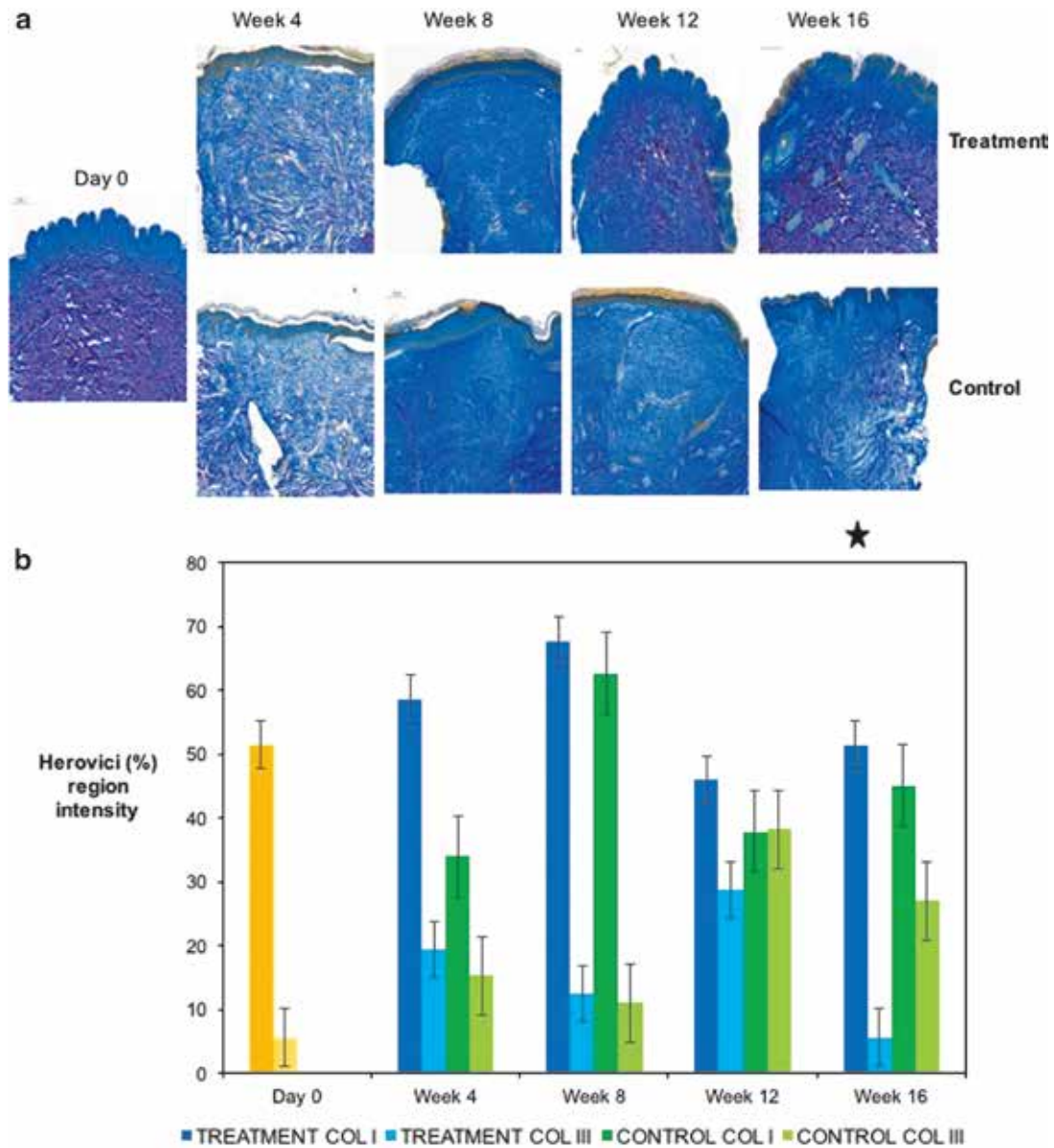
Figure 5. Herovici staining during healing and scar maturation. **(a)** Images of Herovici staining in both arms. **(b)** Ratio of collagen I to III (significant in the treatment arm at week 16 $p = 0.048$). *Yellow* = normal (healthy non-scarred) skin (day 0). *Star* = significant p value at time point indicated.

DISCUSSION

Despite the number of topical formulations, which exists commercially and purported to improve skin scarring, the evidence to support their claims remains poor and unconvincing, and cannot be substantiated.[20] Thus, there is a need for an improved quantitative approach and relevant tools for functional evaluation of the effects of topical formulations, which exist for skin scar management.

Previous clinical studies[9–13] had observed positive improvement in scars after application of the treatment topical; however, these results were based on case series and reports of patients with a variety of scars of different ages and from different anatomical sites. In addition, these reports had limited quantifiable data of scar outcome and absence of comprehensive noninvasive measurements, and lacked the use of subjective visual analogue scar scales.[9–13] Using this study design, we demonstrate how positive clinical outcomes observed in practice can easily be validated in an objective and quantifiable manner using a randomized blinded design with a positive control.

Quantitative changes were observed in both noninvasive and invasive approaches to our study. The treatment topical showed evidence of retaining
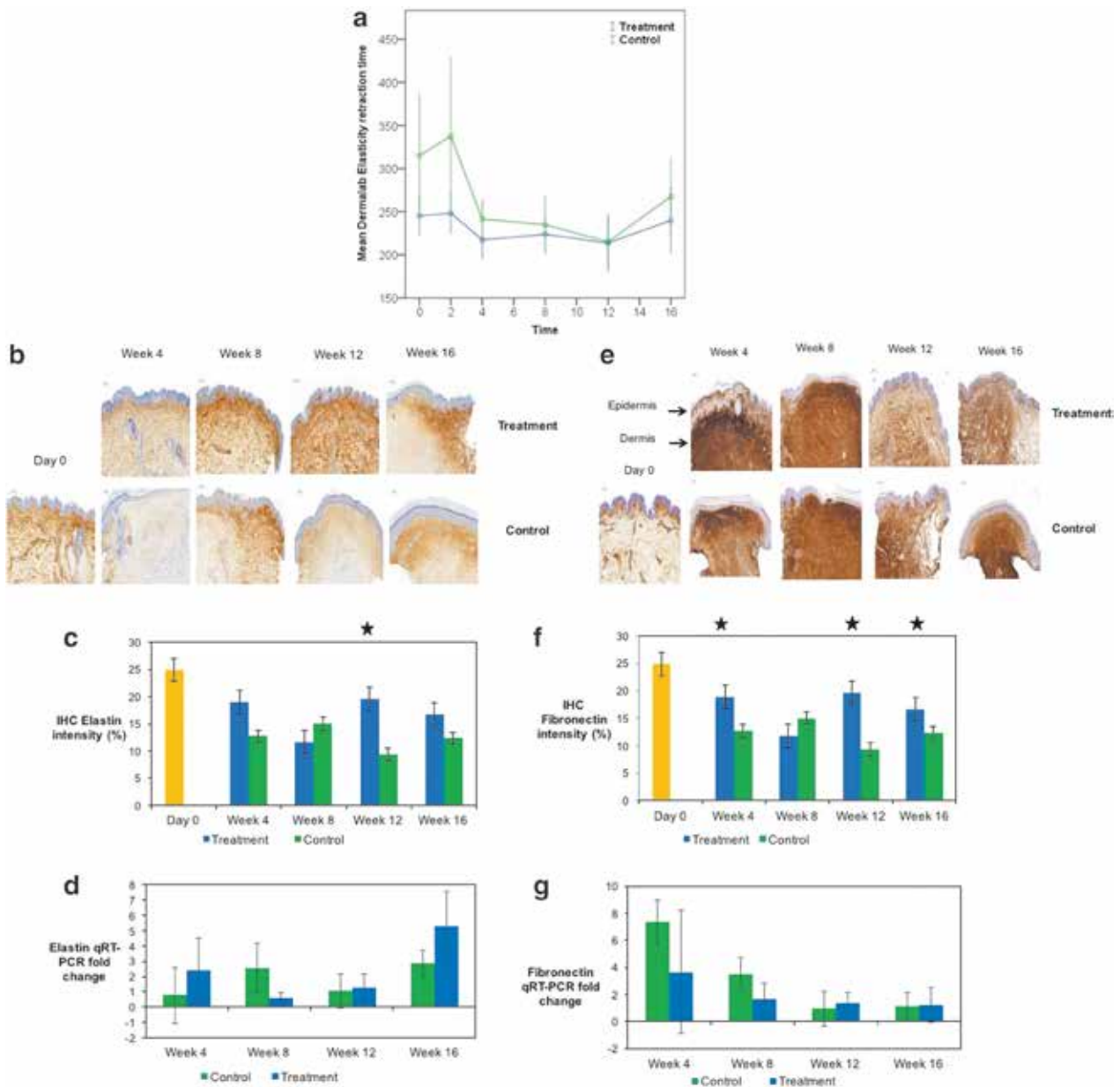
Figure 6. Assessment of elasticity during healing and scar maturation. **(a)** Dermalab elasticity results significantly increased in treatment arm ($p = 0.009$) at week 16. **(b)** IHC elastin staining in both arms. **(c)** Elastin intensity significantly increased at week 12 ($p = 0.044$) in treatment arm versus control. **(d)** qRT-PCR fold change in elasticity. **(e)** Fibronectin intensity significantly increased in the treatment arm compared to the control arm at weeks 4 ($p = 0.009$), 12 ($p = 0.038$), and 16 ($p = 0.026$). **(f)** Comparison of fibronectin staining in both arms. **(g)** qRT-PCR fold change of fibronectin in both arms. There were 40 subjects in the group for the shortest time period (4 weeks), 10 subjects exited at this (and every subsequent) time point, leaving 30 subjects in the week 8 group, 20 subjects in the week 12 group, and finally, 10 subjects in the week 16 group. *Yellow* = normal (healthy non-scarred) skin (day 0). *Star* = significant *p* value at time point indicated.

moisture, with significantly less compromise to the skin barrier in the scars that received it compared to the control from weeks 4 to 12. Evidence of increased hyaluronic acid levels in dermis was also demonstrated by IHC, corroborated by upregulated gene expression as shown by qRT-PCR in the treated skin samples. In addition, this study demonstrated the response within the structural components of the scars, as the treated arm demonstrated a higher

collagen content by SIAscopy measurements, of which a larger proportion was mature (type I) collagen evidenced by Herovici staining. However, there was a lack of reduction of inflammation or pigmentation with regard to physiological and structural parameters, respectively, in the noninvasive or invasive data. Finally, within mechanical parameters, elasticity was increased in both the treated and control arms, although to a significant

level in the treated arm by week 16. IHC supported this finding, with significantly increased elastin also by week 16, and fibronectin, at weeks 4, 8, and 16.

In this case, it would be prudent to imply that the best indicators of scarring assessment were structural changes, and physiological improvements in hydration. This methodology, however, allows for other parameters to be evidenced. For example, with a topical claiming to possess anti-inflammatory properties, the primary focus would be on the physiological features of a scar, specifically changes in erythema and blood flow. Previous claims and case series reports are therefore invaluable to this methodology as they can guide the investigator on the best indicators for scarring, and have the added advantage of building a picture of clinical effects through noninvasive data before the commitment of invasive investigations. In addition, having a statistically adequate number of human volunteers was an advantage. Volunteers could then receive sequential skin biopsies and undergo continual monitoring of the observed effect of the treatment topical against the placebo with 4-week increments over a total period of 16 weeks.

There are some limitations to this approach, namely, the unavoidable use of a positive control, that is, a topical with a different base in the absence of knowing the key active component/s of the treatment topical. It would also have been useful to extend the study period beyond 16 weeks to assess whether longer term, the observed findings would have persisted and/or if topical would have affected redness and pigmentation. However, compliance remains the major issue with longer-term studies. Technical errors both machine and operator dependent may also occur, as is the case when looking at the noninvasive data for elasticity. On day 0, both arms are comparing normal "healthy" skin, so variability at this point is a potential limitation of using the Dermalab system for measurements in elasticity.

This clinical research study demonstrates how using noninvasive quantifiable measures and invasive techniques, including biopsy-derived gene and protein studies, can objectively evaluate the clinical effects of any topical formulation, in human skin scarring.

INNOVATION

There is a paucity of relevant methods for topical evaluation: sequential time points, as opposed to static evaluation of wound healing, is one advantage, in addition to using devices. There is no single device that can measure all parameters.[21] Many provide data on multiple features, strengthening validity of

> **KEY FINDINGS**
>
> - The benefit of this double-blinded, randomized trial using sequential biopsies in human skin allows assessment of wound healing over time, and evaluates the impact of topical application of wound healing and scarring.
> - Noninvasive measurements are used to guide gene and protein studies and outcome measures documented under the physiological, structural, and mechanical properties of scars.
> - Meboscar™ demonstrated an increase in elasticity and hydration, and mature collagen formation compared to a positive control, supported by IHC and qRT-PCR data.
> - This model provides objective and quantitative evaluation of a topical for skin scarring, despite an unknown mechanism of action.

data. IHC and qRT-PCR corroborate noninvasive findings with positive upregulation of protein and gene expression, respectively, for each significant finding observed clinically, presenting the findings under physiological, structural, and mechanical parameters. MEBO Scar ointment demonstrates how this methodology can provide significant findings even when active compounds within a topical are unknown.

AUTHOR DISCLOSURE AND GHOSTWRITING

R.A.K. is an employee of Julphar Gulf Pharmaceutical Industries, UAE. For R.B., M.B., P.F., and A.B., no competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

SUPPLEMENTARY MATERIAL

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3
Supplementary Figure S1

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Abbreviations and Acronyms

FLPI-2 = full-field laser perfusion imaging
IHC = immunohistochemistry
OCT = optical coherence tomography
qRT-PCR = quantitative real-time PCR
SIAscopy = spectrophotometric intracutaneous analysis
TEWL = transepidermal water loss

Scientific Research Publishing

Clinical Observations on the Effects of a Dietary Supplement (GI Regenerate™) on Patients' Gastrointestinal Symptoms and Quality of Life Assessments

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Abstract

Background: Different treatments have been developed and used to control symptoms and improve quality of life in patients with digestive diseases and disorders. Although the use of drugs or alternative approaches has improved symptom severity in some but not all patients, often these improvements were not sustainable. **Objectives:** An open label clinical study was initiated to determine if oral capsules containing a dietary supplement of herbs and oils (GI Regenerate™) could reduce self-reported gastrointestinal symptoms and improve quality of life (QOL) indicators in patients with gastrointestinal conditions. **Methods:** Participants included 50 patients (40 females and 10 males) of mean age of 51.1 ± 12.7 years (range, 24 - 77 years) with a diagnosis of a gastrointestinal disorder or gastrointestinal symptoms. These patients consumed five soft-gels containing the test supplement 30 minutes before each meal for 90 days. Symptoms were evaluated by medical staff, and patient health status was self-reported using a validated quality of life questionnaire (Quality of Life Digestive Survey) designed for functional digestive disorders. Exit interviews (Patient Global Impression of Change, PGIC) were conducted by the medical staff. **Results:** Participants in the study responded with improved symptom severities and QOL scores to the test dietary supplement within the 90 day period; most improvements occurred within 20 days on the test dietary supplement. By the end of the study there were significant overall global improvements in the symptoms and QOL health surveys (p = 0.0183),

with significant improvements in symptom discomfort (p = 0.0004), daily activities (p = 0.029) and anxiety (p = 0.018). In contrast, there were insignificant improvements in diet (p = 0.398), sleep (p = 0.136), health perception (p = 0.686), coping with the disease (p = 0.309) and impact of stress (p = 0.785). Using the PGIC exit interview that measured each patient's impression of overall global change in symptoms and QOL these data also indicated overall significant improvements in symptoms and in satisfaction with the test supplement (moderately better improvements in symptoms and QOL or score of 4.8 ± 0.169, p < 0.0001). There were no significant differences in the responses between males and females, and no significant differences between older (>50 years) versus younger (<50 years) subjects. There were also no safety issues that arose during the trial. **Conclusions:** The GI Regenerate™-natural dietary supplement safely and significantly reduced gastrointestinal symptoms and improved quality of life in subjects with a broad spectrum of gastrointestinal disorders and symptoms.

Keywords

Gastrointestinal Symptoms, Quality of Life, Dietary Supplement, Digestive Disorders, Herbal Remedies, Dietary Oils

1. Introduction

There is a rather large burden to the United States population of morbidity, mortality and cost due to gastrointestinal (GI), liver and pancreatic diseases and disorders [1] [2], and this appears to be true in other nations as well [3]. With its aging population, the United States faces an increasing prevalence of digestive diseases over time [2]. This is likely to result in an overall worsening of the productivity and quality of life (QOL) in the aging population [4] [5].

Different treatments have been developed and used to control symptoms and improve QOL in digestive diseases and disorders [6] [7]. Among the pharmaceutical treatments that are commonly used, such as corticosteroids, aminosalicylates, antibiotics and immunosuppressive drugs, improvements in symptoms have been found, but not in every patient, and often these improvements are not sustainable. Also, the drugs that are often prescribed can have adverse effects in some patients. Thus complementary or alternative medicine approaches have been used to avoid the adverse effects of drug treatments and improve treatment outcomes [6] [7].

Among the alternative medical approaches to the treatment of GI diseases and disorders is the use of herbal combinations, and this has proved beneficial for many patients [8] [9] [10]. There is a rich history that goes back thousands of years of using single and multiple herbal formulations to treat digestive diseases and disorders [8] [10]. In the United States, a large proportion of patients with digestive disorders have tried some form of herbal treatment [10] [11]. The most commonly used herbal treatments for digestive diseases and disorders in the US

have their origin in traditional Chinese medicine [10].

One such combination of herbs and oils that has been used for years in China to treat digestive disorders has been utilized in the current study. This same combination dietary supplement has had different names (GIC, MEBO Gastrointestinal Capsule, Dr. Xu's GI Formula, or more recently GI Regenerate™), and it has been the subject of several scientific and clinical studies in China. These studies include: survival and growth promotion of intestinal and stomach epithelial cells [12] [13], clinical treatment studies on ulcerative colitis [14], gastroesophageal reflux disease [15], gastric ulcers [16], peptic ulcers [17], and repair of gastrointestinal damage due to ethanol [18] or infection [19].

Using a validated QOL questionnaire for functional digestive disorders [20] and patient global impression of change scores (PGIC) taken during exit interviews by contributing physicians this same dietary supplement formulation, or GI Regenerate™, has been examined for its use in treating digestive disorders and symptoms in North American patients.

2. Materials and Methods

2.1. Materials

GI Regenerate™ is a patented natural supplement containing a mixture of herbal ingredients and edible oils. It contains stigmasterol, campesterol, beta-sitosterol, chalinosterol, clionasterol, brassicasterol, alpha-spinasterol, daucosterol, desmosterol, poriferasterol and an edible wax [21]. This base mixture was placed (250 mg each) into soft gel capsules. The natural dietary supplement used in the clinical study was provided by MEBO Life Sciences, Brea, California.

2.2. Methods

An open label, independent Institutional Review Board (IRB)-approved study was initiated using subjects recruited from Southern California with formally diagnosed digestive disorders and diseases, such as ulcerative colitis, gastritis, esophagitis, gastroesophageal reflux disease (GERD), Crohn's disease, irritable bowel syndrome (IBS), or other digestive disorders. The study recruitment was limited to patients attending the Center for New Medicine, Irvine, California who volunteered for the study. The number of subjects was determined by the number of patients who volunteered and could be adequately scheduled, examined and treated by available staff during the trial period of January 2019 to January 2020.

The 40 females and 10 males recruited to the study presented with a variety of signs and symptoms related to digestive disorders (Table 1). Exclusionary criteria included subjects who were taking immunosuppressive drugs, or had cognitive impairment, or were pregnant, lactating or below the age of 18 years. Each subject was directed to take 5 capsules of GI Regenerate™ 30 min before meals 3X per day for the 90-day study period. Participants were advised not to change any of their daily medications, diet or routine during the study.

Table 1. Diagnoses/symptoms of subjects in the clinical study.

Diagnosis/Symptom*	N
Female Subjects (Total)	40
Abdominal bloating	26
Abdominal pain	22
Celiac disease	2
Constipation	25
Crohn's disease	3
Diarrhea	23
Fatigue	14
Flatulence	9
Food allergy	7
Gastric pain	3
Gastritis	10
GERD	17
IBS	16
Intestinal malabsorption	3
Nausea	10
Obesity	1
Regurgitation	4
Ulcerative colitis	7
Male Subjects (Total)	10
Abdominal Bloating	2
Abdominal pain	6
Celiac disease	-
Constipation	7
Crohn's disease	1
Diarrhea	9
Esophagitis	1
Fatigue	3
Flatulence	2
Food Allergy	3
Gastric pain	6
Gastritis	-
GERD	6
IBS	1
Intestinal malabsorption	-
Nausea	1
Obesity	1
Regurgitation	1
Ulcerative colitis	1

*Subjects may have more than one diagnosis and have multiple symptoms. IBS, irritable bowel syndrome; GERD, gastroesophageal reflux disease.

Study subjects were monitored at various times using a validated patient questionnaire for functional digestive disorders and QOL (Appendix Figure 1 in reference [20]). The data were normalized to baseline and analyzed as: a) Overall Global Scores; and subsets of data were normalized to baseline and analyzed as: b) Daily Activities Scores, c) Symptom Discomfort Scores, d) Anxiety Scores, e) Diet Scores, f) Sleep Scores, g) Coping with Disease Scores, h) Health Perception Scores, and i) Stress Impact Scores [20].

Subjects were also subjected to exit examination and surveys conducted by professional staff physicians of the Center for New Medicine of Irvine, California. In this (PGIC) analysis participants were asked whether their overall changes in symptom severity and QOL were very much better (score of 6), moderately better (score of 5), a little better (score of 4), no change (score of 3), a little worse (score of 2), moderately worse (score of 1) or very much worse (score of 0) (**Appendix Figure A1** of this paper). The mean satisfaction scores were determined and analyzed statistically.

2.3. Statistical Analysis

For statistical analysis we used generalized estimating equations (GEE) for the regression parameters as introduced by Liang and Zeger as a method for estimation of regression model parameters when dealing with correlated data [22] [23]. Generalized estimating equations (GEE) are a convenient and general approach to the analysis of several kinds of correlated data. The main advantage of GEE resides in the unbiased estimation of population-averaged regression coefficients despite possible misspecification of the correlation structure. Our longitudinal research was aimed at describing the marginal expectations of the outcome as a function of the predictors [24].

The objective of analyses that we have done and performed were to examine: (1) whether the QOL scores differed over the study time points; (2) whether the QOL scores differed over the study time points between males and females; and (3) whether the QOL scores differed over the study time points between age < 50 and age ≥ 50. Data were analyzed with significance defined as $p < 0.05$ and presented as mean data with 95% confidence levels.

The exit survey (PGIC) was conducted with 28 subjects, and satisfaction scores were calculated and analyzed by a one-sided, one sample t-test. In this analysis a significant overall improvement in exit scores would be a composite satisfaction score greater than 3.0. All of the statistical analyses were performed independently by the Statistical Unit of the Division of General Medicine, Department of Medicine, University of California, Irvine.

2.4. Safety Issues

The safety of patients was carefully monitored during the trial. Any issues of adverse reactions to the test supplement were carefully recorded and monitored during the trial. Potential changes in blood chemistry were monitored each

month during the clinical study using the NutrEval™ diagnostic blood evaluation panel (Genova Diagnostics, Asheville, NC). In this panel standard blood chemistry and a panel of blood levels of antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, and amino acids were monitored at the beginning and each subsequent month during the trial period.

3. Results

3.1. Participants in the Study

There were 50 participants in the IRB-approved clinical study (40 females, 10 males). They had a mean age of 51.1 ± 12.7 years (range, 24 - 77 years) and presented with a diagnosis of a gastrointestinal disorder. A summary of the participants and their presentation with a variety of digestive disorders and diseases (with multiple gastrointestinal symptoms) is summarized in **Table 1**.

### 3.2. Quality of Life Determinations

Using the validated digestive disorders questionnaire of Chassany, *et al.* [20] patients were examined for their responses in each survey category every 10 days during the 90-day test period (**Figure 1**). After the 90-day period, the analyzed results of the study indicated that there were significant overall global improvements in the health surveys (p = 0.0183) (**Figure 1(a)**), with significant improvements in symptom discomfort (p = 0.0004) (**Figure 1(b)**), daily activities (p = 0.029) (**Figure 1(c)**) and anxiety (p = 0.018) (**Figure 1(d)**). In contrast, there were insignificant improvements in diet (p = 0.398) (**Figure 1(e)**), sleep (p = 0.136) (**Figure 1(f)**), health perception (p = 0.686) (**Figure 1(g)**), coping with the disease (p = 0.309) (**Figure 1(h)**) and impact of stress (p = 0.785) (**Figure 1(i)**). Most health response improvements over baseline occurred within 20 days from initiating the dietary supplement (**Figures 1(a)-(d)**).

Based on the results from the GEE models, regression parameters indicated that the improvements in overall global symptoms and QOL scores were consistent and occurred with a low degree of variance. The estimated changes from baseline of the eight dimension scores are shown in **Table 2**. The dimension scores included: daily activities (DA), anxiety (AN), diet (DI), sleep (SL), discomfort (DT), health perceptions (HP), coping with disease (CD), and impact of stress (IS). In addition, two overall measures, the estimated change from baseline in the global score (GS) and an alternative scoring of the global score (Alt GS) are also displayed. The Table illustrates the low degree of variance in estimated changes from baseline in dimension scores and global scores over the 10 survey time points.

3.3. Exit Interviews

Exit interviews (PGIC) with each participant were conducted by the clinical study physicians (**Appendix Figure A1**). The exit interviews indicated that the
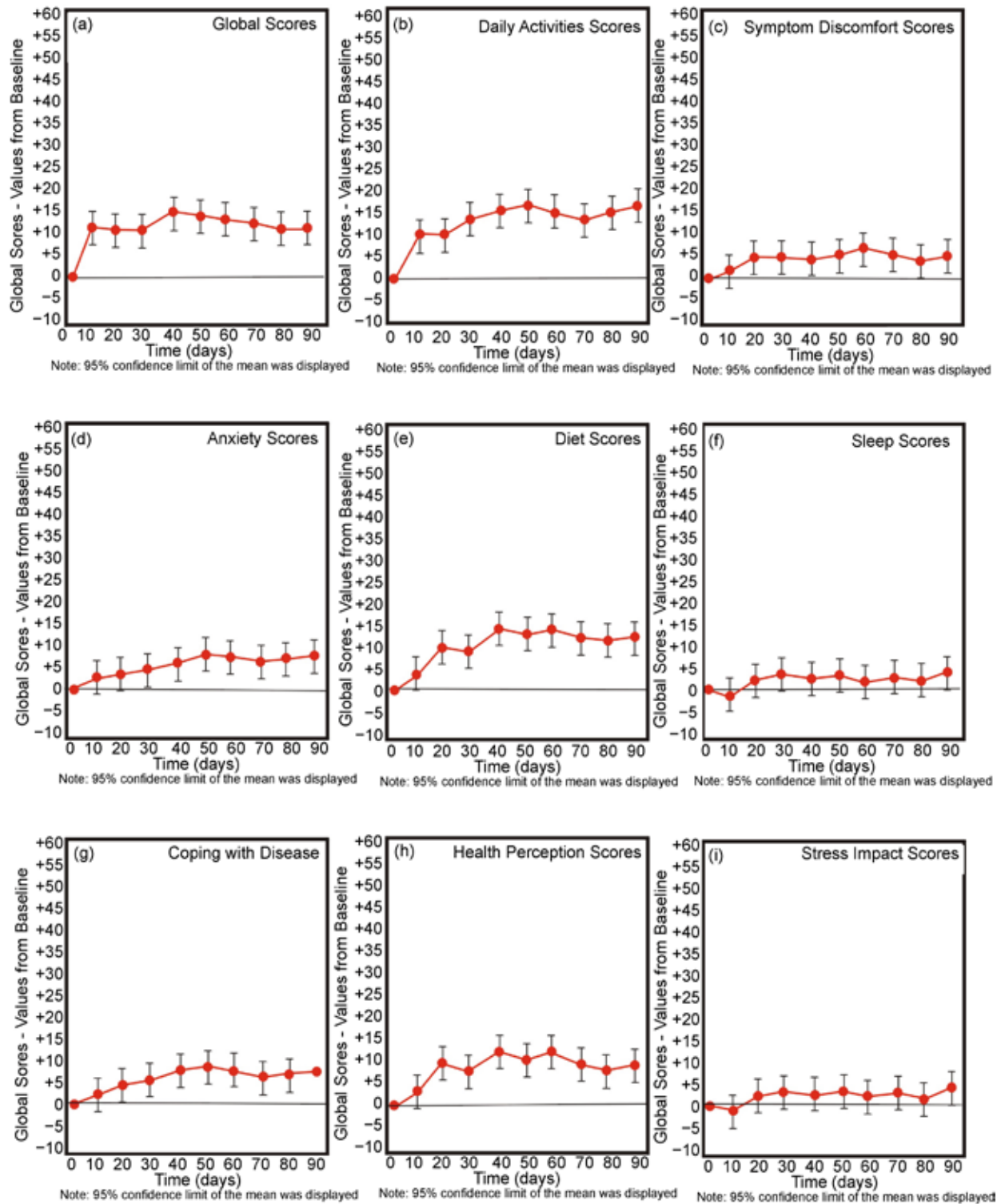
Figure 1. Digestive disorders questionnaire results. Combined results of the study (Global Scores) and subparts of the study over a 90-day period are presented. Results indicate normalized scores (mean scores minus baseline scores; brackets indicate 95% confidence levels of the means). Improvements in normalized scores are indicated by increases in normalized score values presented in the figure. Panels indicate Combined Global Scores (a), Daily Activities Scores (b), Symptom Discomfort Scores (c), Anxiety Scores (d), Diet Scores (e), Sleep Scores (f), Coping with Disease Scores (g), Health Perception Scores (h), Stress Impact Scores (i).

Table 2. The estimated changes from baseline of the dimension score at each survey time from the GEE models.

	The Estimated Changes from Baseline of the Dimension Scores at Each Study–Time during Survey									
Survey time point (days)	DA Estimates (95%CI)	AN Estimates (95%CI)	DI Estimates (95%CI)	SL Estimates (95%CI)	DT Estimates (95%CI)	HP Estimates (95%CI)	CD Estimates (95%CI)	IS Estimates (95%CI)	GS Estimates (95%CI)	Alt GS Estimates (95%CI)
0	0.02 (−0.28, 0.31)	0.02 (−0.2, 0.24)	0.11 (−0.18, 0.4)	−0.06 (−0.28, 0.16)	−0.05 (−0.2, 0.11)	−0.02 (−0.13, 0.08)	−0.05 (−0.27, 0.17)	−0.01 (−0.13, 0.12)	−0.02 (−0.17, 0.12)	−0.02 (−0.17, 0.12)
10	12.53 (6.57, 18.5)	9.79 (4.98, 14.61)	2.6 (−1.54, 6.75)	1.85 (−1.36, 5.06)	3.7 (−0.52, 7.92)	−1 (−4.54, 2.54)	3.62 (−4.06, 11.3)	−1.56 (−6.86, 3.73)	4.71 (2.1, 7.31)	4.66 (2.2, 7.11)
20	11.22 (5.53, 16.91)	9.8 (4.36, 15.24)	5.11 (−0.08, 10.3)	4.68 (0.31, 9.05)	10.65 (5.79, 15.51)	1.46 (−3.25, 6.18)	9.69 (2.91, 16.47)	−1.53 (−6, 2.94)	7.49 (4.15, 10.83)	7.19 (3.96, 10.42)
30	11.27 (4.75, 17.79)	12.19 (5.91, 18.47)	5.54 (0.01, 11.08)	5.42 (0.22, 10.61)	10.12 (5, 15.24)	3.11 (−0.95, 7.17)	5.31 (−1.45, 12.06)	−1.09 (−7.1, 4.91)	7.53 (3.89, 11.18)	7.4 (3.91, 10.89)
40	14.55 (8.52, 20.57)	14.64 (8.99, 20.28)	5.03 (−0.92, 10.97)	7.25 (1.87, 12.64)	15.85 (11.05, 20.66)	2.89 (−1.45, 7.22)	8.54 (1.72, 15.36)	−1.74 (−7.59, 4.11)	9.8 (5.9, 13.7)	9.74 (6.05, 13.43)
50	13.71 (7.13, 20.28)	16.91 (10.22, 23.61)	6.94 (0.69, 13.2)	9.81 (4.62, 15.01)	14.67 (10.08, 19.27)	3.53 (−1.04, 8.1)	10.98 (2.58, 19.38)	2.74 (−3.61, 9.09)	10.94 (6.88, 15)	10.65 (6.78, 14.51)
60	13.14 (6.75, 19.54)	16.03 (9.16, 22.9)	8.76 (2.54, 14.98)	9.68 (3.99, 15.36)	17.12 (11.48, 22.75)	1.42 (−2.98, 5.82)	8.78 (0.7, 16.87)	2.3 (−3.4, 8)	10.73 (6.75, 14.7)	10.74 (6.84, 14.63)
70	12.64 (6.81, 18.48)	14.51 (6.65, 22.37)	7.12 (−0.04, 14.28)	8.12 (2.29, 13.96)	14.16 (8.34, 19.99)	2.09 (−2.55, 6.72)	10.48 (1.7, 19.26)	1.27 (−5.69, 8.24)	9.89 (5.11, 14.68)	9.66 (5.11, 14.2)
80	10.93 (4.52, 17.34)	16.35 (7.66, 25.04)	6.31 (0.37, 12.24)	8.98 (3.78, 14.17)	13.11 (7.09, 19.13)	0.9 (−3.64, 5.44)	9.55 (2.11, 17)	0.74 (−7.84, 9.31)	9.44 (5.25, 13.64)	9.02 (4.87, 13.16)
90	10.88 (3.16, 18.6)	17.25 (8.84, 25.66)	7.13 (0.7, 13.55)	10.15 (4.11, 16.18)	13.83 (7.31, 20.35)	3.9 (−1.07, 8.87)	8.08 (−1.73, 17.88)	2.6 (−4.73, 9.94)	10.19 (5.66, 14.72)	9.83 (5.42, 14.24)

Abbreviations: DA, daily acitivites; AN, anxiety scores; DI, diet scores; SL, sleep scores; DT, symptom discomfort scores; HP, health perception scores; CD, coping with disease scores; IS, impact scores; GS, global scores; Alt GS, alternate global scores; CI, confidence intervals.

patients' impression of overall global change in symptoms and QOL showed significant improvements in satisfaction with the test supplement (moderately better improvements in symptoms and QOL, or a score of 4.8 ± 0.169, p < 0.0001).

3.4. Safety of the Study

There were no safety issues that came up during the clinical trial. In support of this the NutrEval™ diagnostic blood evaluation panels showed no significant changes in blood chemistry and levels of blood antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, and amino acids during the study.

4. Discussion

The dietary test supplement used in the present clinical study (now called GI Regenerate™) has been used for years in China and other countries to treat patients with a variety of gastrointestinal disorders and diseases [14]-[19]. These clinical studies were dependent on this dietary supplement repairing gastrointestinal damage. To demonstrate the effects of the test dietary supplement on stimulating gastrointestinal epithelial cell survival, growth and regeneration, some

experimental studies were initiated. After excision and *in vitro* culture of organ explants of murine stomach and intestinal tissues in medium containing fetal bovine serum, addition of the test dietary supplement was shown to stimulate epithelial cell survival, growth and differentiation, whereas the cells in explant cultures without the dietary test supplement began to die and never formed viable cell colonies [12] [13].

Consistent with the findings in China on the clinical benefits of using the oral test dietary supplement to treat ulcerative colitis [14], gastroesophageal reflux disease [15], gastric ulcers [16], peptic ulcers [17], GERD [23] and gastrointestinal damage due to ethanol [18] or infection [19] we found that North American patients with a variety of gastrointestinal disorders and symptoms (Table 1) responded positively to the test dietary supplement GI Regenerate™. These positive responses were collected using the validated digestive disorders questionnaire of Chassany, *et al.* [20] over a 90-day period. The results indicated that male and female patients with IBS, GERD, Crohn's disease, celiac disease, ulcerative colitis, gastritis, and digestive symptoms, such as abdominal bloating and pain, gastric pain, constipation, diarrhea, fatigue, flatulence, nausea, regurgitation and food allergies and malabsorption, improved significantly during the test period (p = 0.0183), with significant QOL improvements in symptom discomfort (p = 0.0004), daily activities (p = 0.029) and anxiety (p = 0.018).

Our results using the validated digestive disorders questionnaire were confirmed in the PGIC exit surveys where patients indicated moderately better improvements in symptoms and QOL (p < 0.0001) at the end of the study. Thus we have confirmed the benefits of taking oral capsules of GI Regenerate™ found in previous studies on the improvements in gastrointestinal symptoms in patients with digestive disorders and diseases [14]-[19].

There were no safety concerns that came up during the trial. Patients did not report issues with the GI Regenerate™ oral supplement, and blood chemistry analyses every month during the trial on every subject using the NutrEval™ diagnostic blood evaluation panel did not indicate any abnormalities in levels of blood antioxidants, vitamins, minerals, essential fatty acids, probiotics, pancreatic enzymes, or amino acids during the study.   Thus we concluded that the GI Regenerate™ oral supplement was safe and effective for use in treating gastrointestinal symptoms in early adults to the elderly.

Although the results of our clinical study were positive and generally significant statistically, there were obvious limitations of the trial. First, we note that although the numbers of females in our study were sufficient, we had less access to male patients. Thus the numbers of males in our study (10) were much lower than the numbers of females (40). Future studies should contain more balanced numbers of males and females. Also, the study was a preliminary open label study, not a robust, randomized, controlled clinical trial. There are few evidence-based clinical studies using randomized clinical trials on the use of Chinese dietary herbal supplements to treat digestive disorders [25] [26]. The results

presented here should stimulate the organization of a randomized, controlled clinical trial using GI Regenerate™ to test for improvements in symptoms in patients with digestive disorders and diseases.

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Disclosures

Garth L. Nicolson and Robert Settineri are part-time research consultants to Allergy Research Group, Inc., Naturally Plus USA, Inc. and Nutritional Therapeutics, Inc. There are no other disclosures.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Patient Name:
Subject Number:
Email:
Phone number:
Exit date:

Please choose the response below that best describes the overall change in your symptoms and quality of life since you started taking the study supplement.

- ❏ Very Much Better
- ❏ Moderately Better
- ❏ A Little Better
- ❏ No change
- ❏ A Little Worse
- ❏ Moderately Worse
- ❏ Very much Worse

Patient Signature: _____

Date: _____

Figure A1. Patient's Global Impression of Change (PGIC).