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● *Original Contribution*

EXTRACORPOREAL SHOCK WAVE THERAPY: AN EMERGING TREATMENT MODALITY FOR RETRACTING SCARS OF THE HANDS

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Abstract—Prolonged and abnormal scarring after trauma, burns and surgical procedures often results in a pathologic scar. We evaluated the efficacy of unfocused shock wave treatment, alone or in combination with manual therapy, on retracting scars on the hands. Scar appearance was assessed by means of the modified Vancouver Scar Scale; functional hand mobility was evaluated using a range-of-motion scale, whereas a visual analogue score was implemented for detecting any improvements in referred pain. Additionally, biopsy specimens were collected for clinico-pathologic correlation. For each active treatment group, statistically significant improvements in modified Vancouver Scar Scale were recorded as early as five treatment sessions and confirmed 2 wk after the last treatment session. Analogous results were observed when assessing pain and range of movement. Histopathological examination revealed significant increases in dermal fibroblasts in each active treatment group, as well as in neoangiogenetic response and type-I collagen concentration. (E-mail: saggini@unich.it) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Scarring, Retracting scar, Regeneration, Resolution, Collagen, ESWT, Histopathologic features.**

INTRODUCTION

The cutaneous dermis is a specialized connective tissue consisting of a collagen-rich fibrous network embedded in a ground substance matrix. The proteoglycan-rich matrix is key to skin viscous quality at low loads. On the other hand, the main fibrous constituents of the dermis, namely collagen and elastin, provide structural stiffness and elasticity (Lanir 1981; Smith et al. 1982).

By definition, cutaneous scars develop by means of wound healing through a combined process of regeneration and replacement of the dermal tissue with fibrous tissue. Several sources of damage to the reticular dermis and subcutis may lead to cutaneous scarring, including burns, abrasions, lacerations and surgery. Abnormal wound healing is often characterized by a protraction of the healing process over time, with wounds appearing to be "stuck" in the inflammatory and proliferative phases, which predisposes to excessive accumulation of collagen and pathologic scarring (Roques 2013). Pathologic scars are classified into atrophic and hypertrophic scars, with the latter being further divided into simple hypertrophic scars and keloids (Roques 2013).

Both loco-regional and systemic factors appear to be able to promote pathologic scarring: wound features (*i.e.*, healing by primary or secondary intention, scar orientation, source of primary damage and anatomic site), extent of bleeding, presence of hematoma and/or serum collection, superimposing infection, innervation deficits, constitutional characteristics (*i.e.*, age, gender and race), coexisting administration of drugs (such as corticosteroids, antimetabolite agents or immunosuppressive drugs), disorders of blood supply, lack of nutritional factors and endocrine factors (such as presence of glucose intolerance), among others (Widgerow 2011).

Once formed, pathologic scars may be subject to several changes, including regression, keloid formation, neoplastic degeneration and retraction. Retracting scars are characterized by collagen fibers in a cord-like

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disposition exerting significant traction on surrounding healthy tissues; ensuing functional limitation is especially prominent when scars occur secondarily to deep burns in the neck area or in proximity of appendicular joints. Indeed, retracting scars may cause significant functional deficit (Slemp and Kirschner 2006; Wollstein et al. 2012).

Multiple sources of dermal damage may induce deposition of new collagen through activation of dermal fibroblasts, which are mesenchymal cells that play a critical role in wound healing. Morphologic features of fibroblasts include a spindle-shaped cytoplasm with a central elliptic nucleus and inconspicuous nucleoli. Functional activation induces profound morphologic changes in fibroblasts, including a significant expansion of the rough endoplasmic reticulum as well as expression of different surface markers. Fibroblasts are capable of secreting the precursors of extracellular matrix components, including basal substance, collagen, glycosaminoglycans, reticular and elastic fibers and glycoproteins (Darby et al. 2014).

The circulating precursors derived from bone marrow are an additional source of cells involved in the metabolism of the extracellular matrix and wound healing. Bone marrow–derived circulating precursors may include fXIIIa-positive dendritic cells and CD34-positive fibrocytes. The fXIIIa-positive cells were the first subtypes of cutaneous dendritic cells to be recognized. Antibodies against fXIIIa detect a sub-set of dermal dendritic cells and resident macrophages in the dermis; a higher density of fXIIIa-positive cells in the dermis has been described in a number of metabolic disorders of the extracellular matrix (Yokoyama and Muto 2006), including morphea and systemic scleroderma. Although the origin of CD34-positive cells in the dermis is still considered controversial, CD34-positive fibrocytes appear to result from circulating hematopoietic progenitor cells. It is thought that CD34-positive dermal fibrocytes play a significant role in several conditions associated with excessive collagen deposition and fibrosis, such as nephrogenic systemic fibrosis, scleroderma and graft versus host disease (Oh et al. 2011).

Injury can affect the skin's structure and composition, thereby greatly influencing the biomechanics and directionality of the resulting scar tissue. The characteristics of scars are a result of altered structure and composition in the dermis. Scars typically have fewer blood vessels supplying the denser connective tissue, which is less elastic. A significant difference between normal tissue and scar tissue seems to lie in the orientation of the fibrous matrix. Human scar tissue is characterized by greater collagen density, with larger fibers exhibiting increased alignment compared to normal tissue, although such alignment is not exactly parallel to the skin. Further structural differences between scars and normal tissue include a different ratio of collagen types and a loss of normal hair follicles and sweat glands in scars. Type I and III collagen are formed in human skin in a higher proportion relative to other types and are maintained in a fixed proportion relative to one another in normal skin tissue. However, in human scar tissue, as a result of age or injury, there is alteration in the abundance of type I and III collagen as well as their proportions to one another. Recently, both the abundance and balance of type I and III collagen have received considerable research attention (Feng et al. 2001; Garner et al. 1993; Ghahary et al. 1996; Guan et al. 1997; Guo et al. 2002; Hurley et al. 1993; Ichiki et al. 1997; Kennedy et al. 1995; Linares 1996; Liu et al. 2001; Lu 2003; Shah et al. 1995; Tan et al. 1993; Tang et al. 2004; Thomas et al. 1995; Wan et al. 2001; Wang et al. 1999; Wu et al. 2000; Yin 1999; Zhou et al. 1997).

In healthy human skin, type I and III collagen have relatively substantial roles during collagen formation, comprising 80%–85% and 10%–15% of human skin, respectively (Riita et al. 2002). Newly developed scars undergo a maturation process, with type III collagen being gradually replaced by type I collagen so as to restore normal type I-to-III ratio (which is approximately 5:1). Despite the fact that physiologic healing has been extensively studied, much less is known about the causes and the pathogenetic mechanisms of pathologic scarring (Liu et al. 2001). Collagen is a keystone of skin formation and repair, playing a crucial role in the maintenance of skin tensility and elasticity. Variations in content and ratio provide the basis for hypertrophic scar formation. Collagen fibers within scar dermis show a reduced resistance potential, being only 70% of that of normal skin.

Possible treatment of pathologic, retracting scars currently includes several options, such as intra-lesional corticosteroids, cryotherapy, dermabrasion, excision and scar revision surgery, laser therapy and radiation therapy; likewise, prophylactic strategies may include variable combinations of compression therapy, silicone gel and oral supplements such as flavonoids. Nonetheless, retractive scarring is characterized by a complex etiology related to both local and systemic factors, and the efficacy rate of available treatments is still far from satisfactory. As a consequence, treatment of pathologic scars often requires lengthy and expensive procedures, posing the need for clinical studies aimed at the development of novel therapeutic strategies for pathologic scarring (Faga et al. 2013; Rabello et al. 2014).

The list of emerging therapies for retracting scars currently features, among others, intra-lesional injections of interferon, controlled enzymatic debridement and stem-cell infusion (Bush et al. 2010; Jalali and Bayat 2007; Prado et al. 2005; Reish and Eriksson 2008; Williams and Barbul 2003), as well as extracorporeal

shock wave therapy (ESWT). ESWT was successfully introduced approximately 30 y ago in the urologic and gastroenterologic fields, there known as lithotripsy. Over the past decade, ESWT has been shown to produce promising results for the treatment of various diseases of the musculoskeletal system. The Food and Drug Administration has approved ESWT for the treatment of chronic plantar fasciitis (2000) and chronic lateral epicondylitis (2003).

According to available literature, stimulation of connective tissues with ESWT seem to induce the expression of several endogenous growth factors (*i.e.*, epidermal growth factor, insulin-like growth factor-1 and vascular endothelial growth factor) and promote the production of nitric oxide, favoring angiogenesis and exerting an advantageous effect in the healing of fractures, ulcers and other complex lesions (Saggini et al. 2008, 2013; Wang et al. 2014).

The efficacy of manual myofascial therapy has been described in the available literature for detaching adhesions and reducing soft tissue contractures; it would seem that manual myofascial therapy is also capable of restoring the correct local blood perfusion of the tissue as well as reducing pain. Manual myofascial therapy may be associated with non-invasive technological devices capable of coupling pressure, aspiration and an effective draining activity. Such mechanical action is able to detach scars' deep adherences so as to promote tissue regeneration by stimulating fibroblasts (Leffler et al. 2010; Molnar et al. 2004; Roques 2006; Saggini et al. 2014; Watson et al. 1999).

The I-Coone system (I-Tech Industries s.r.l., Bologna, Italy) is an assisted massage device that produces a positive rolling pressure in association with the application of negative pressure so as to promote drainage of vacuolar and alveolar micro-structures within the dermis and subcutaneous tissue. According to the available literature, I-Coone system seems to be effective in the treatment of several connective tissue diseases, including fibrosing conditions, pathologic scars and burns.

The aim of the present study was to evaluate the efficacy of unfocused shock wave treatment of retracting scars of the hands, alone or in combination with manual therapy; clinical appearance of the scar, functional improvement of the hand, subjective pain and morphologic features at the histopathologic level were recorded for the assessment of ESWT efficacy.

MATERIALS AND METHODS

Patients were informed about the procedures and purpose of the study and they were required to give written informed consent before participating. Each of the study procedures was performed at the Department of Plastic and Reconstructive Surgery of "Sapienza" University in Rome; it was approved by the local ethics committee, and performed in accordance with the 1964 Declaration of Helsinki.

Seventy patients aged between 20 and 65 y were included in this study between November 2012 and May 2013. Selected patients had a history of hand surgery resulting in painful, retracting scarring of at least 1-mo duration. Exclusion criteria included evidence of arrhythmias, pacemaker implantation, coagulopathies, tumors, pregnancy, growth cartilage, local acute inflammation or exposed bone (Fig. 1).

Selected patients were randomly divided into four groups (group A to D).

- **Group A** (30 patients) was administered treatment with unfocused ESWT alone, at a frequency of two sessions per wk for 5 wk. Group A consisted of individuals with (Group A-II, 15 patients) or without (Group A-I, 15 patients) surgery-induced complex regional pain syndrome (CRPS) of the hand. CRPS is a chronic disease of the limbs that features a combination of disabling pain, swelling, vasomotor instability, abnormal sweating and/or impaired motor function. The post-operative course of hand surgery appears to be frequently complicated by CRPS. No specific diagnostic test is currently known for CRPS, with diagnosis relying on clinical history, clinical examination and instrumental support.
- **Group B** (15 patients with no evidence of CRPS) was treated with a combination of ESWT and manual myofascial therapy at a frequency of two sessions per wk for 5 wk.
- **Group C** (15 patients with no evidence of CRPS) received ESWT treatment associated with manual myofascial therapy and local treatment with I-Coone system at a frequency of two sessions per wk for 5 wk.
- **Group D** (control group, 10 patients with no evidence of CRPS) did not receive any treatment.

Treatment protocol consisted of 10 unfocused ESWT (Dermagold, MTS, Europe GmbH, Constance, Germany) sessions, administered twice per wk. Focal depth of shock waves was 49 mm with a total energy applied for each impulse of 0.13 mJ/mm^2; treatment frequency was 6 Hz and duration of each session was 1.5 min (500 pulses for session per 360 impulses/min), with a focus where the scar was most hypertrophic and painful.

Pictures of treated areas were taken at a distance of 10 cm before each treatment session in order to monitor the morphologic evolution of scars; a digital camera with a resolution of more than 5 megapixels and macrofunction was used.

Scar assessment and evaluation was performed before the beginning of the treatment cycle (T0), after

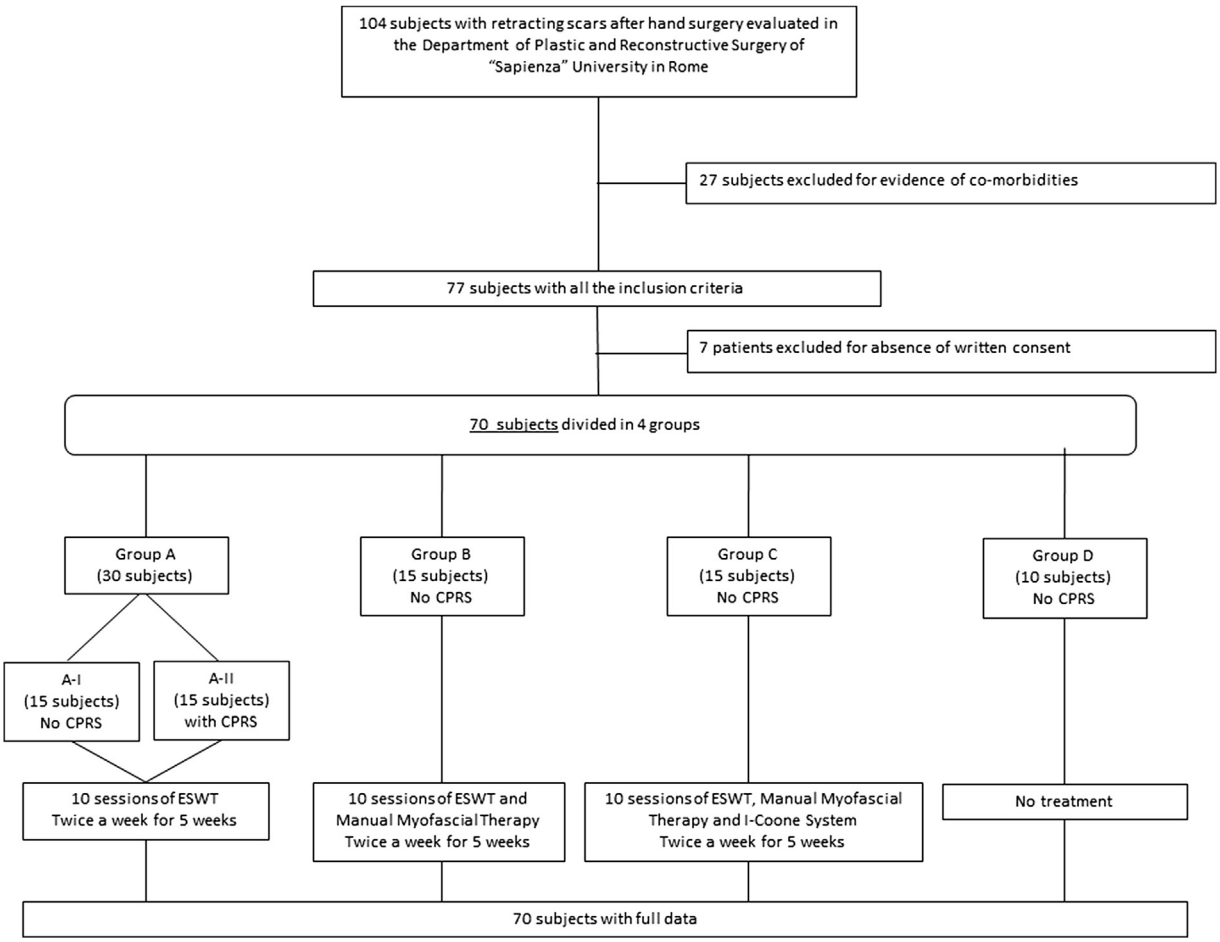

Fig. 1. Flow chart of the study.

the fifth treatment session (T1) and at 2 wk after the end of the 10th treatment session (T2).

Scar height (cm), pigmentation, vascularization and pain were assessed using the modified Vancouver Scar Scale (moVSS; Baryza and Baryza 1995; Durani et al. 2009), an observer-dependent scale based on the macroscopic appearance of scarring.

Compared with the original VSS scale, the moVSS is a numerical scale ranging from 0 to 14, with 0 corresponding to normal healthy skin. Four scar features are assessed by the moVSS, including height (ranging from 0 to 4), pliability (ranging from 0 to 4), vascularity (ranging from 0 to 3) and pigmentation (ranging from 0 to 3). At each scar site, a numerical value is assigned for each of the above-mentioned features, based on a comparison with the individual's normal skin.

Rating of subjective pain was performed using the Visual Analogue Scale (VAS); the VAS score is a simple tool commonly used for the evaluation of changes in pain intensity, with values ranging from 0 (no pain) to 10 (worst pain ever). Deficit in passive mobility because of scar-related contractures was evaluated using a range-of-motion (ROM) score ranging from a minimum of 0 to a maximum of 100.

After obtaining written informed consent from patients, biopsy specimens were taken from pathologic scars treated with unfocused ESWT at T0 and T2. A 3-mm, wedge-shaped incisional biopsy was collected from treated areas, perpendicular to clinically palpable scars, and sent for tissue processing and staining. Post-treatment biopsy specimens were taken adjacent to pre-treatment biopsy sites. Control bioptic specimens (Group D) of pathologic scars were collected from patients with a previous diagnosis of cutaneous neoplasm undergoing margins re-excision, selecting anatomic sites similar to those biopsied in the treatment group.

Tissue samples were fixed in 4% formalin for 24 h, embedded in paraffin and sectioned by microtome (Leica RM 2245; Leica Biosystems Nussloch GmbH, Nußloch, Germany). Five-μm sections were cut from biopsy specimens and stained with hematoxylin-eosin and picrosirius red to assess the structure of the skin, dermal thickness and collagen morphology. The picrosirius red has high affinity for the collagen fibers because of chemical

interaction between the sulfonic acid groups and bases with the consequent parallel arrangement of the dye molecules along the axis of the collagen fibers. The picric acid prevents the non-specific staining of the non-collagen structures. Biopsies of the groups treated with ESWT and control groups were independently assessed under a conventional light microscope by two observers; in each sample, the orientation of the collagen fibers was classified as parallel and non-parallel to the overlying epidermis.

Immunohistochemical analysis was performed assessing the expression of the following antigens: factor XIIIa (fXIIIa), CD34 and CD31. Sections for immunohistochemistry were treated with a citrate buffer and ethylenediaminetetraacetic acid at a high temperature. The endogenous peroxidase activity was inhibited by immersion in methanol containing 3% hydrogen peroxide. Primary antibodies against CD34, CD31 (Novocastra, Leica Biosystems Nussloch GmbH) and fXIIIa (Abcam, Nussloch Epitomics, Burlingame, CA, USA) were applied to the sections and then incubated overnight at 4 C°.

Revelation was made with the EnVision system (Dako, Carpinteria, CA) according to the manufacturer's instructions. Diaminobenzidine was selected as chromogen, and sections were then counter-stained with hematoxylin and mounted. Negative control was obtained by replacing primary antibodies with non-immune serum; no immunostaining was ever observed. Positive controls (samples of dermatofibroma for fXIIIa, hemangioma for CD31 and CD34) were labeled as expected. For each sample, the number of fXIIIa-positive dendrocytes and CD34-positive fibrocytes was evaluated, as well as the density of CD31-positive vessels.

Statistical differences between mean values before and after rehabilitation period were tested for significance in each treatment group using Wilcoxon signed-rank test for paired observations for each of scar's characteristics and total score of moVSS, and the Mann-Whitney U test was used for ROM values and VAS score. Fischer's protected least significant difference test was employed for *post hoc* analysis. The minimum level of statistical significance was set at $p < 0.05$.

RESULTS

At T0, no statistically significant difference was recorded among study groups (*i.e.*, groups A, B, C, and D) with regard to moVSS, VAS or ROM (Mann-Whitney U test, $p > 0.05$).

For each active treatment group (*i.e.*, groups A, B, and C), statistically significant improvements in moVSS were recorded as early as five treatment sessions (T1; $p < 0.05$); such improving trends in moVSS were confirmed at T2 ($p < 0.05$). Analogous results were observed when assessing individual moVSS components (*i.e.*, pliability, vascularity, pigmentation and height) for each active treatment group ($p < 0.05$). No statistically significant improvement was recorded for the control group (group D) in either total moVSS or any of the individual moVSS components at T1 or T2 ($p > 0.05$; Figs. 2a–d, 3, 4, 5).

With regard to pain, for each active treatment group (*i.e.*, group A, B and C), statistically significant improvements in VAS were observed as early as at T1 ($p < 0.05$); improvements in VAS were confirmed at T2 ($p < 0.05$). Analogous results were observed when assessing patients with or without CRPS within group A ($p < 0.05$). No statistically significant improvement in reported pain was recorded for the control group (group D) at either T1 or T2 ($p > 0.05$; Fig. 6).

Statistically significant improvements in passive ROM at T2 ($p < 0.05$) were recorded for groups A and B, but not for group C or the control group (group D).

At T2, significantly different changes in ROM were noted in the comparison between groups A and B and group D (Mann-Whitney U test, $p < 0.05$), but not between group C and group D; on the contrary, for each other variable assessed (*i.e.*, moVSS and VAS), statistically significant improvements were recorded between active treatment groups (*i.e.*, groups A, B and C) and the control group (group D; Mann-Whitney U test, $p < 0.05$; Fig. 7).

Histopathological examination revealed significant increase in dermal fibroblasts in each active treatment group (*i.e.*, groups A, B and C), as well as in neoangiogenetic response and type-I collagen concentration; likewise, in each active treatment group, significant qualitative improvement of dermal collagen was observed, with a finer and more fibrillar appearance. Staining with picrosirius red indicated that treatment with ESWT resulted in a collagen fiber arrangement parallel to the skin surface and replacement of type III collagen with type I collagen (thus restoring the physiologic relationship between type I and III collagens). Immunohistochemical comparison of pre- and post-ESWT treatment biopsies revealed that administration of a shock wave regimen resulted in a significant increase in infiltrating fXIIIa-positive fibrocytes, CD34 dermal expression and CD31-positive small vessels. By contrast, no significant changes were observed in the control group (group D) by either hematoxylin-eosin, picrosirius red or immunohistochemical staining (Figs. 8 and 9).

DISCUSSION

In the present study, unfocused shock waves (*i.e.*, ESWT) were indicated to be an effective therapeutic modality for the treatment of painful, retracting scars of

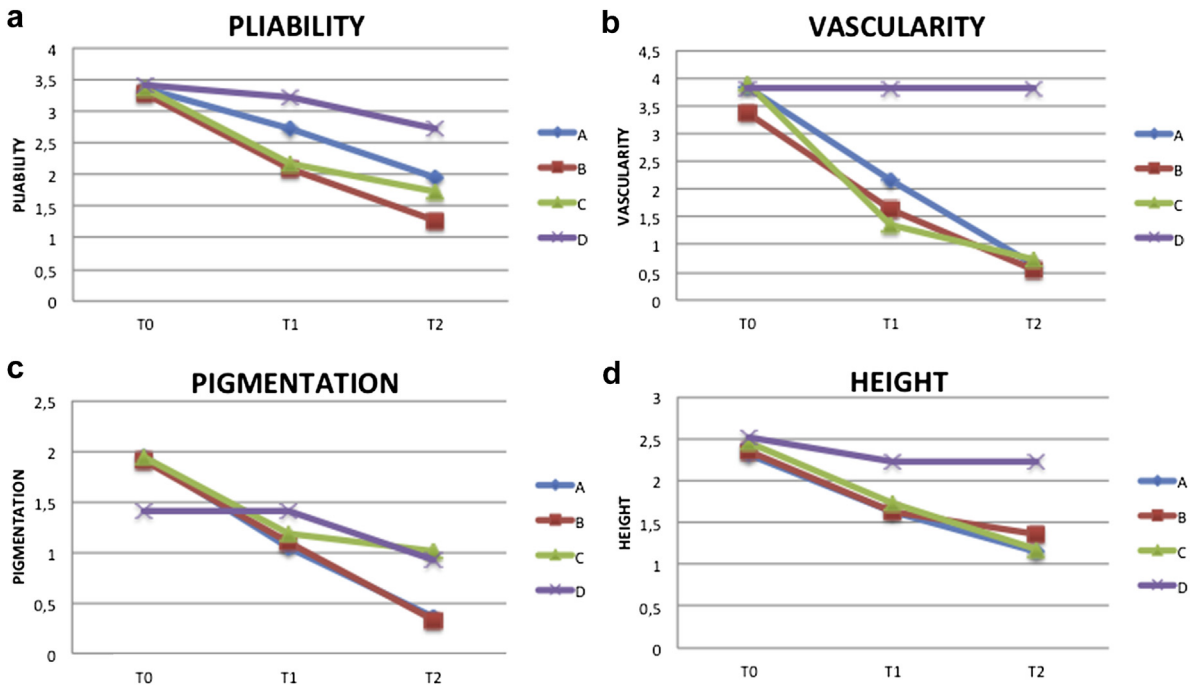

Fig. 2. (a) The figure shows reduction in modified Vancouver Scar Scale (moVSS) pliability in the four main groups of treatment; in particular, Group B shows a greater reduction than the other treatment groups, but all treatment groups demonstrated greater reduction than group D (control). (b) MoVSS reduction in vascularity in the four main groups of treatment, specifically the trend of the three treatment groups (groups A, B and C) was similar, while the control (group D) showed no changes. (c) Pigmentation in the four main groups of treatment. The most significant reduction is seen in groups A and B; it is lower in group C and not significant in group D. (d) Reduction in moVSS height in the four main groups of treatment; the three treatment groups (group A, B and C) had a similar pattern, whereas group D had no changes.

the hands (Kasuya and Tokura 2014; Wang et al. 2011). The effectiveness of ESWT on retractive scarring was evaluated in multiple respects, including clinical appearance of scars, motion function of underlying joints and subjective pain. In particular, scar appearance was assessed by means of the moVSS score, which is based on scar height, pliability, pigmentation and vascularity; functionally valuable changes in hand mobility were evaluated using a ROM scale, and a VAS score was implemented for detecting any improvements in referred pain.

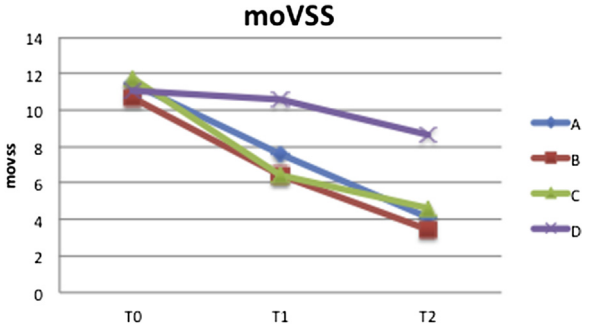

Fig. 3. Modified Vancouver Scar Scale (MoVSS) total score in the four main groups of treatment reflects the trend of the parameters taken into account separately, highlighting the overall improvement in the three groups A, B and C as compared with control group D.

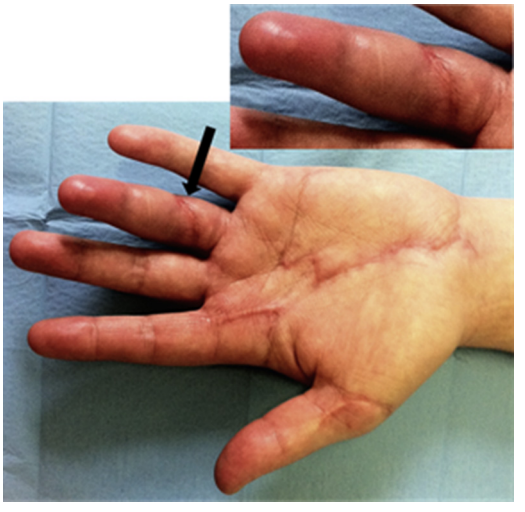

Fig. 4. Retracting scar before treatment with extracorporeal shock wave therapy (ESWT), group A (T1). Height of scar was 3 (range, 0 to 4), pliability was 4 (range, 0 to 4) that define the retraction of scar, vascularity was 3 (range, 0 to 3), and pigmentation was 3 (range, 0 to 3).

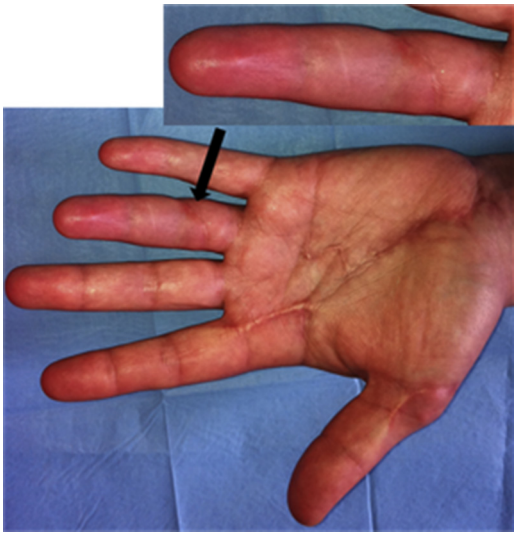

Fig. 5. Retracting scar after 5 wk of treatment with extracorporeal shock wave therapy (ESWT), group A (T2). Height of scar was 1 (range, 0 to 4), pliability was 2 (range, 0 to 4) that define the retraction of scar, vascularity was 1 (range, 0 to 3), and pigmentation was 1 (range, 0 to 3).

Administration of ESWT resulted in clinically meaningful improvements in height, pliability, pigmentation and vascularity of treated scars as early as at T1 (*i.e.*, after five treatment sessions at a rate of two sessions per wk); these improving trends were maintained to the end of treatment cycle (*i.e.*, at T2 after 10 treatment sessions), being observed irrespective of whether ESWT had been delivered in association with manual myofascial therapy and/or local treatment with I-Coone system.

Analogous results were recorded with regard to subjective pain; importantly, comparable improvements in reported pain were observed in individuals both with

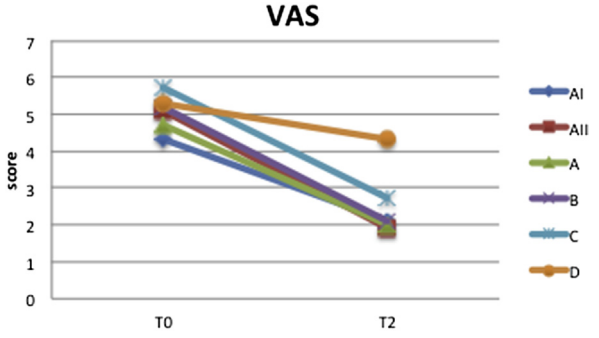

Fig. 6. Statistically significant improvements in visual analogue scale (VAS) were observed as early as at T1 ($p < 0.05$); improvements in VAS were confirmed at T2 in groups B and C. Analogous results were observed when assessing patients with or without complex regional pain syndrome (CRPS) within group A. No statistically significant improvement in reported pain was recorded for the control group (group D).

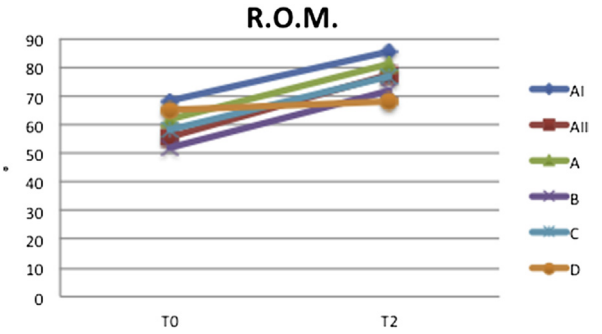

Fig. 7. Statistically significant improvements in passive range-of-motion (ROM) at T2 were recorded for groups A and B, but not for group C or the control group (group D). At T2, significantly different changes in ROM were noted in the comparison between groups A and B and group D, but not in that between group C and group D.

and without CRPS. The post-operative course of the hand is often complicated by CRPS, a chronic syndrome characterized by a combination of impairing pain, swelling, vasomotor instability, pathologic sweating and/or deficits in motor function. Coping with CRPS frequently proves to be psychologically taxing for affected patients, as they may become unable to perform jobs or daily care tasks and come to depend upon assistance from family members or caregivers. In addition to physical limitations, patients suffering from CRPS may also be affected with regard to memory, ability to concentrate and self-esteem, leading to a sizable decrease in quality of life. The data of the present study, showing that ESWT efficacy in ameliorating hand scar–related pain was maintained even in the presence of CRPS, corroborate ESWT's status as an emerging treatment modality for complex, difficult-to-treat scarring. Furthermore, we believe that the role of ESWT in treating scar-associated CRPS deserves additional studies in the future.

Patients developing retracting scarring in the vicinity of hand joints are known to be at risk for impairing reduction in the hand's range of motion. The results from our study appear to suggest that treatment of retracting hand scars with ESWT will allow meaningful amelioration in passive ROM in the majority of patients, although such improvement tends to occur at a slower pace compared to effects on scar clinical appearance and reported pain. Failure to reproduce such beneficial effects when the combination of ESWT and manual myofascial therapy was associated with local treatment with I-Coone system is baffling and difficult to explain; further studies may be warranted in this regard.

As a secondary goal, our study attempted to correlate clinical improvement induced by ESWT with morphologic changes at the histopathologic level (Darby et al. 2014; Frairia and Berta 2012).

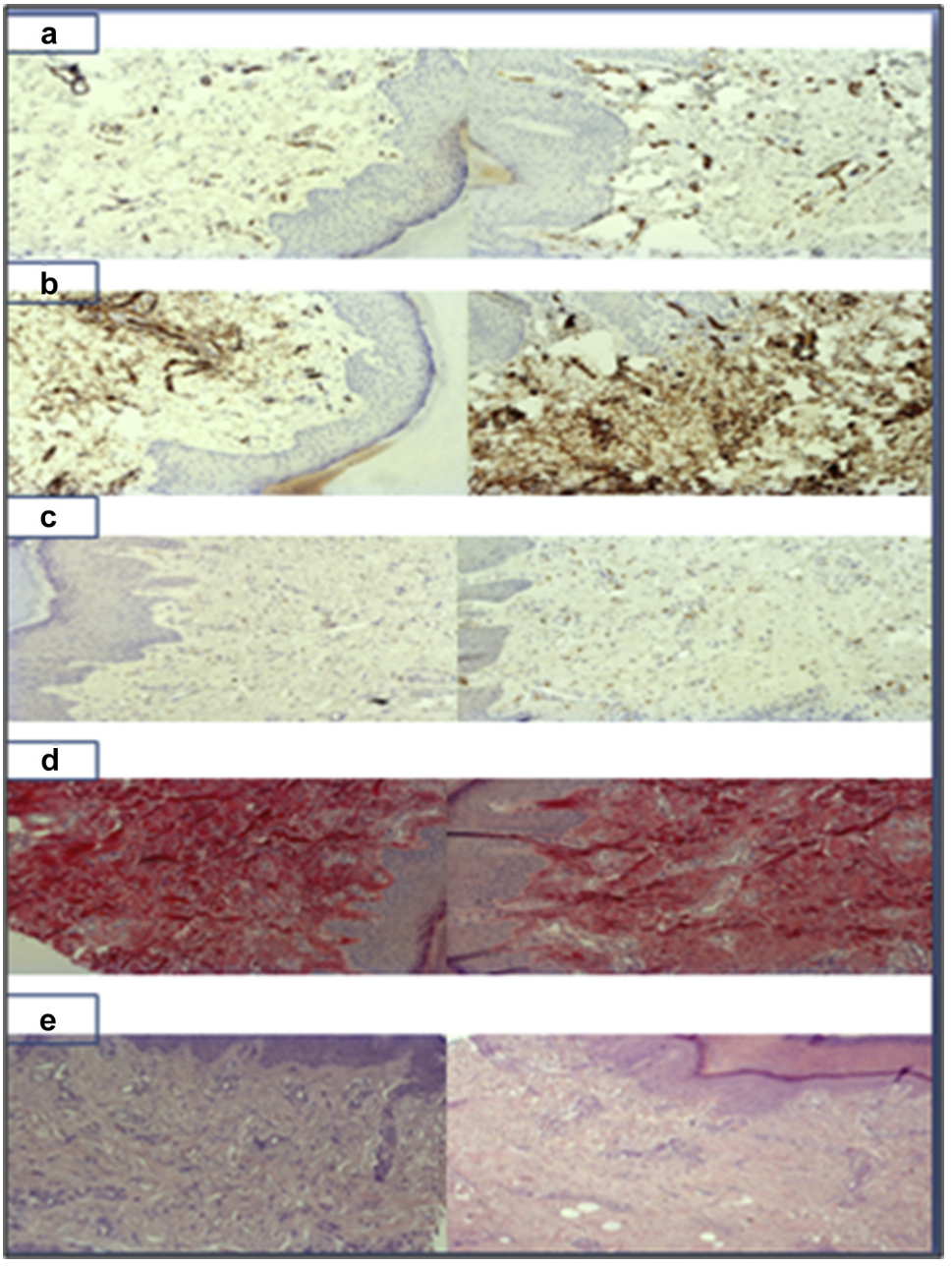

Fig. 8. Biopsy specimens taken from patients in the group treated with extracorporeal shock wave therapy (ESWT). (a) Immunohistochemical staining of fibroblasts and angiogenesis (right, before the treatment; left, after treatment) for FXIII reveals a significant increase in FXIII+ dendrocytes after treatment with ESWT. (b) Immunohistochemical staining for CD34 of fibroblasts, collagen type I and angiogenesis (right, before the treatment; left, after treatment) shows a significant increase in CD34+ dendrocytes and CD34+ vessels after treatment with ESWT. (c) Immunohistochemical staining for CD31 of angiogenesis (right, before the treatment; left, after treatment) demonstrates a significant increase in CD31+ vessels after treatment with ESWT. (d) Picrosirius red (right, before the treatment; left, after treatment) staining reveals modified arrangement of collagen fibers after treatment with ESWT, parallel to the epidermis, and increased collagen type I to type III ratio. (e) Hematoxylin-eosin (right, before the treatment; left, after treatment) highlights significant improvement of dermal collagen after treatment with ESWT, showing a thinner and more fibrillary collagen.

Histopathological examination with different histochemical (*i.e.*, hematoxylin-eosin and picrosirius red) and immunohistochemical (fXIIIa, CD31 and CD34) stains showed that treatment of scar tissue with ESWT resulted in a significant increase in dermal fibroblasts, small vessel density, type-I-to-type-III collagen ratio, and number of fXIIIa- and CD34-positive cells.

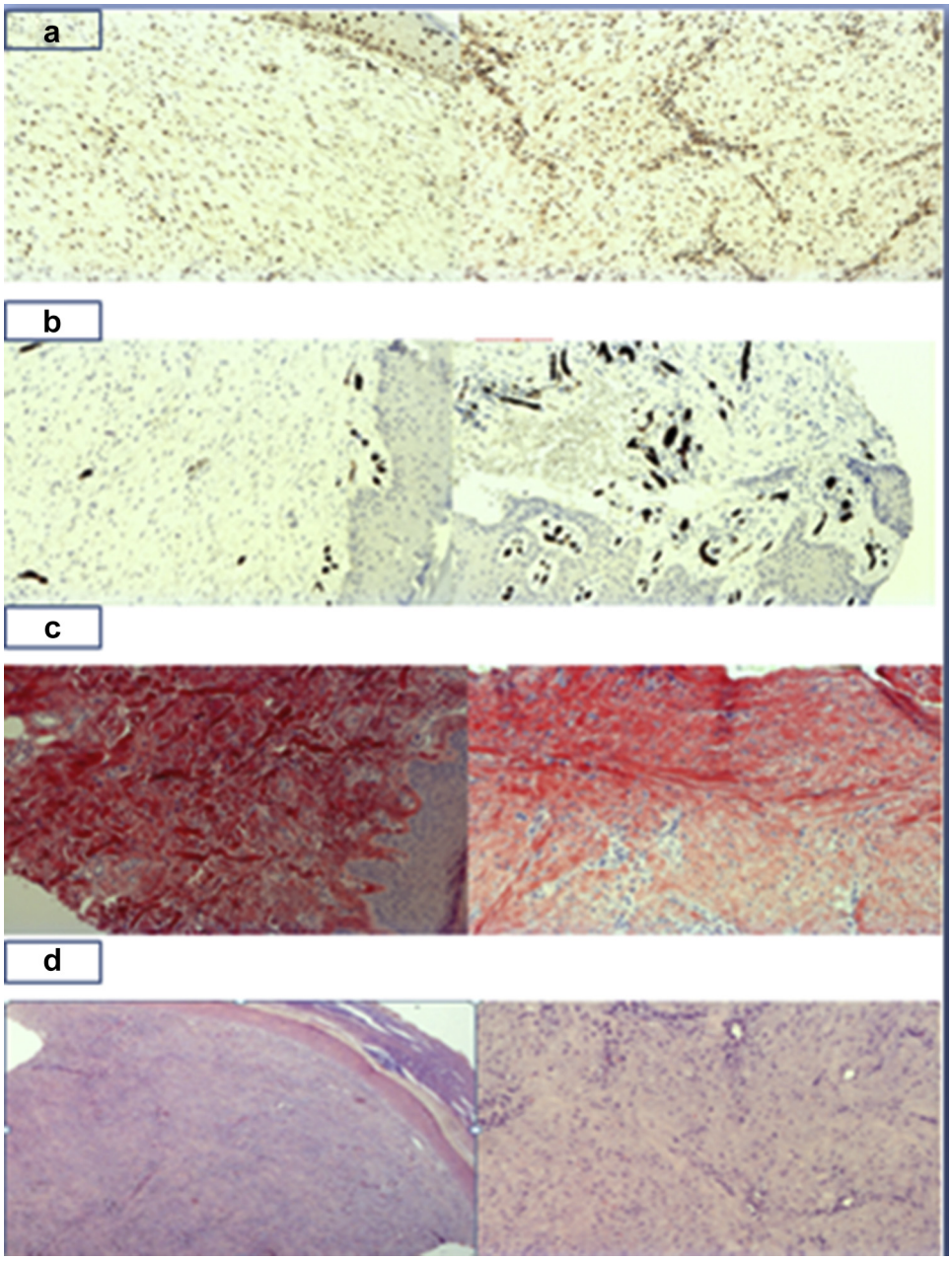

Fig. 9. Biopsy specimens taken from patients in the control group (group D). (a) Immunohistochemical staining of fibroblasts and angiogenesis (right, before the treatment; left, after treatment) for FXIII shows similar FXIII expression in the control group (Group D). (b) Immunohistochemical staining for CD34 of fibroblasts, collagen type I and angiogenesis (right, before the treatment; left, after treatment) shows no change in CD34 expression in the control group (Group D). (c) Picrosirius red (right, before the treatment; left, after treatment) staining reveals no modified arrangement of collagen fibers in the control group (Group D). (d) Hematoxylin-eosin (right, before the treatment; left, after treatment) highlights no significant improvement of dermal collagen (Group D).

The results from our study seem to confirm that shock wave treatment is capable of inducing an increase in the number of activated fibroblasts, CD34-positive fibrocytes and fXIIIa-positive dendritic cells; this process is thought to lead to the deposition of new collagen, characterized by thinner collagen fascicles and parallel orientation to the dermo-epidermal junction. Such a process would explain the strong correlation observed between such histologic features and scar macroscopic appearance in treated patients. Additionally, shock wave therapy may be regarded as playing a significant role in the increase in CD31-positive vessel density in

the dermis of treated patients, allowing an improved tissue metabolism (Reichenberger et al. 2012).

With regard to skin wounds, Kuo et al. (2009) demonstrated that proper administration of ESWT to wound tissue may be able to reduce the inflammatory response, with consequent reduction in the number of infiltrating leukocytes and oxygen free radical production. It may also promote fibroblasts hyperplasia and dermal neoangiogenesis while reducing the number of apoptotic cells. It has also been shown that, at the skin level, therapy with ESWT may be capable of promoting endothelial reorganization, connective tissue deposition and re-epithelialization; such effects seem to be linked to the release of specific growth factors (such as transforming growth factor $\beta 1$; Fioramonti et al. 2012; Kuo et al. 2009; Wang et al. 2014).

Additionally, it is currently believed that administration of ESWT to wounded skin leads to an early response with conditioning of sympathetic nerve endings and opening of the capillary bed (wash-out effect), followed by a secondary response occurring after some days from treatment characterized by true neoangiogenesis and extensive cleaning of treated tissues with removal of inflammatory mediators (Fioramonti et al. 2014; Henry and Garner 2003; Kuo et al. 2009; Meirer et al. 2005; Saggini et al. 2008, 2013; Wang et al. 2014). Furthermore, ESWT may also be capable of inducing an analgesic effect; indeed, tissue levels of substance P have been shown to increase within the first 24 h after the cutaneous application of shock waves and progressively decrease in the subsequent 6 wk. It appears that the typical course of perceived pain (*i.e.*, intense after the first application but gradually decreasing after subsequent applications of shock waves) is paralleled at the histologic level by the progressive degeneration of nerve fibers originating from small, ATF3-positive neurons (Maier et al. 2000).

In conclusion, our study supports the role of ESWT as an emerging option for the treatment of painful, retracting hand scars; administration of ESWT appears to result in significant improvements in scar clinical appearance, hand mobility and subjective pain. Clinical data were mirrored by histologic changes in connective tissue appearance, scar vascularization and density of fXIIIa- and CD34-positive cells. We believe that our data warrant further studies comparing ESWT to traditional treatment modalities for retractive scarring.

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