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ORIGINAL ARTICLE

Prospective Randomized Phase II Trial of Accelerated Reepithelialization of Superficial Second-Degree Burn Wounds Using Extracorporeal Shock Wave Therapy [AQ1]

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Background: As extracorporeal shock wave therapy (ESWT) can enhance healing of skin graft donor sites, this study focused on shock wave effects in burn wounds.

Methods: A predefined cohort of 50 patients (6 with incomplete data or lost to follow-up) with acute second-degree burns from a larger study of 100 patients were randomly assigned between December 2006 and December 2007 to receive standard therapy (burn wound debridement/topical antiseptic therapy) with (n = 22) or without (n = 22) defocused ESWT (100 impulses/cm^2 at 0.1 mJ/mm^2) applied once to the study burn, after debridement. Randomization sequence was computer-generated, and patients were blinded to treatment allocation. The primary endpoint, time to complete burn wound epithelialization, was determined by independent, blinded-observer. A worst case scenario was applied to the missing cases to rule out the impact of withdrawal bias.

Results: Patient characteristics across the 2 study groups were balanced ($P > 0.05$) except for older age (53 ± 17 vs. 38 ± 13 years, $P = 0.002$) in the ESWT group. Mean time to complete (>95%) epithelialization (CE) for patients that did and did not undergo ESWT was 9.6 ± 1.7 and 12.5 ± 2.2 days, respectively ($P < 0.0005$). When age (continuous variable) and treatment group (binary) were examined in a linear regression model to control the baseline age imbalance, time to CE, age was not significant ($P = 0.33$) and treatment group retained significance ($P < 0.0005$). Statistical significance ($P = 0.001$) was retained when ESWT cases with missing follow-up were assigned the longest time to CE and when controls with missing follow-up were assigned the shortest time to CE.

Conclusions: In this randomized phase II study, application of a single defocused shock wave treatment to the superficial second-degree burn wound after debridement/topical antiseptic therapy significantly accelerated epithelialization. This finding warrants confirmation in a larger phase III trial (ClinicalTrials.gov identifier: NCT01242423).

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The shockwave device used in this trial is an investigational device currently used in TRT LLC-sponsored FDA trials under the trade name DermaGold.

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All study patients were enrolled in an IRB-approved clinical trial at Unfallkrankenhaus Berlin, Zentrum für Schwerbrandverletzte mit Plastischer Chirurgie, Berlin, Germany.

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Advances in the treatment of the thermally injured patient including timing and extent of fluid resuscitation, early burn wound excision and grafting, topical antibiotic and biosynthetic therapy, application of skin substitutes, and critical care in burn centers of excellence have impacted significantly the clinical outcomes of these patients. Nature and intensity of treatment is directed according to burn wound location, extent, and depth. Most superficial-thickness burns heal within 2 weeks of injury and are managed effectively with debridement, topical antiseptic therapy, and biologic or nonbiologic dressings. These measures reduce contamination, decrease insensible fluid losses, improve pain, and accelerate reepithelialization. Many research efforts have focused on accelerating partial-thickness burn wound healing through the use of growth factors and other topical treatment modalities.[1,2]

The noninvasive modality, extracorporeal shock wave therapy (ESWT), may improve perfusion and accelerate epithelialization in burn wounds; however, few studies have addressed the clinical utility of this approach.[3,4] We have recently shown in an exploratory phase II randomized trial that defocused ESWT applied immediately after split-thickness harvest of skin graft accelerates graft donor site epithelialization.[5] The current phase II randomized trial was conducted to determine if similar accelerated reepithelialization could be attained on burn wounds through the single application of shock waves after superficial-thickness burn wound debridement.

METHODS

This report complies with the reporting standards established by the revised Consolidated Standards of Reporting Trials (CONSORT) consensus statement.[6]

Participants

A prospective randomized phase II clinical trial was conducted from December 2006 to December 2007, which was approved by the Charité Berlin Ethics Committee, under authorization number EA/160/06. During the study period, 100 patients were enrolled, 50 patients with donor sites treated with standard of practice with or without ESWT, and 50 patients with superficial second-degree burns to this study who provided informed consent. There were no refusals to enroll in the study among those approached for study participation. Once eligibility was confirmed, study patients were assigned randomly to 1 of 2 study groups. The control group underwent burn wound debridement of devitalized skin (epidermis) and ruptured blisters and daily antiseptic dressing changes involving application of topical nonadherent silicone mesh (Mepitel) and antiseptic gel (Polyhexanide/Octenidine) until complete epithelialization, according to institutional standards of practice. The shock wave group underwent the same treatment in addition to a single application of unfocused shock wave therapy to the study burn. Eligible patients were nonpregnant women or men, between 18 and 80 years of age, capable of providing informed consent. Eligible patients were also those with second-degree burns (superficial second degree: involving epidermis

and extending into dermis). Determination of burn wound depth was established on clinical grounds on the basis of the color and burn wound appearance, capillary refill, and sensation. Superficial second-degree burns appear erythematous, possibly blistered, have capillary refill, and are sensate to pin prick testing. Laser doppler imaging was not used in this study to determine burn wound depth. First, second degree deep dermal, and third-degree burn wounds were excluded from study as were patients with insulin-requiring diabetes mellitus, dialysis dependent renal failure, ongoing systemic therapy for malignancy, systemic dermatologic disease, ongoing corticosteroid therapy, and active drug abuse. Six of the 50 study patients enrolled were excluded from final analysis because of incomplete data or loss to follow-up. There are 44 evaluable patients, who were blinded to treatment allocation, and analyzed on an intent-to-treat basis.

Shock Wave Administration

After study burn wound debridement shock wave therapy was administered as a single treatment within 24 hours of superficial second-degree burn wound debridement to patients randomized to the ESWT intervention arm of the study. The shock waves were delivered to the superficial second-degree burn wound as a single treatment. The administered shock wave dose was 100 impulses/cm^2 (according to burn wound surface area) using an energy flux density of 0.1 mJ/mm^2, administered at 20 seconds/cm^2. Sterile ultrasound conducting gel (Lavaseptgel) was applied to the burn wound surface. A sterile plastic protective film was placed over the wound. Ultrasound gel was then applied onto the drape as a coupling media. Unfocused shock waves were applied through the conducting gel and sterile film directly to the debrided superficial second-degree burn wound, using the OW180C DermaGold [MTS Europe GmbH, Tissue Regeneration Technologies, LLC, Woodstock, GA, which is a certified medical device in Europe (TÜV Rheinland CE 1275)].

Primary Outcome (Burn Wound Epithelialization) Assessment

Study participants that have provided informed consent to participate in this clinical trial were followed in-hospital daily until discharge and were evaluated 12 weeks after hospital discharge in outpatient clinic. Complete burn wound healing was defined as more than 95% reepithelialization. Study patients were monitored carefully during the follow-up period for cardiac, neurological, dermal, thermal, or allergic reactions or adverse events.

Objective

The principal aim of this study was to determine if a single application of defocused ESWT to a superficial second-degree burn wound can accelerate reepithelialization over our current standard of practice. The prospective hypothesis tested (H_0) was: there is no difference in time to complete reepithelialization between ESWT and control; versus (H_1), there is a reduction in time to complete reepithelialization for ESWT versus control.

Outcomes

The primary outcome variable was time to complete burn wound healing ($\geq$95% reepithelialization).

Sample Size

The sample size was prospectively set at 50 patients to detect a reduction in mean time to 95% reepithelialization consistent with a mean 2-day reduction for ESWT and a 2- to 2.5-day standard deviation (SD) for the time to 95% reepithelialization; no formal power calculation was originally performed. On the basis of the actual study data, the current study had more than 80% power to detect a 0.85 effect size for the treatment differences in mean time to healing of 2 days between the 2 study arms using a 2-sided test with 5% type I error.

Randomization and Determination of Primary Study Endpoint

Patients were randomized in a 1:1 ratio using Rancode 3.6 Professional (IDV, Gauting, Germany) to undergo superficial second-degree burn wound therapy in accordance with institutional standards specified above or the same treatment with a single, unfocused shock wave treatment at the aforesaid parameters. All patients were treated as randomized. Randomization was achieved through a computerized randomization system (without stratification) based on random number generation. The randomization sequence was concealed until study group assignment. Study participants were blinded to treatment assignment. The primary endpoint, time to complete wound reepithelialization, was determined by an independent, blinded-observer. This observer is a highly trained professional, senior plastic surgeon, with experience in complex burn and wound care. Serial digital images of study wounds were reviewed by an expert in wound care, blinded to treatment group assignment, who determined completeness of study wound epithelialization. Both independent reviewers were given the photos of the study wounds to assess the time to complete reepithelialization. The interclass correlation was 0.986 for the time to 95% reepithelialization.

Statistical Methods

Summary statistics were obtained using established methods. Categorical variables between groups were compared using a 2-sided Fisher exact test. Continuous baseline data were presented as means and standard deviations (mean $\pm$ SD) with medians and ranges for each treatment group and compared using an unpaired *t* test or a Wilcoxon rank sum test. The primary outcome variable in this study was time to superficial second-degree burn wound reepithelialization, which was defined as time from initial debridement/application of ESWT to the first documentation of complete study superficial second-degree burn wound healing ($\geq$95% reepithelialization). Mean time to burn wound epithelialization ($\pm$ SD) was compared between study groups according to an unpaired *t* test; analysis of covariance was performed using age (continuous variable) and treatment group (binary) together as independent predictors of the dependent variable, time to complete epithelialization, to control for the age imbalance in comparing treatment groups. Statistical analysis was performed using JMP(v8) and SAS software (JMP and SAS, Cary, NC). A 2-sided $P \leq 0.05$ was considered significant.

RESULTS

Patients

Between December 2006 and December 2007, 50 study patients were enrolled and provided informed consent. No patient approached for study participation refused to enroll in the study. Study patients were randomly assigned to undergo standard institutional treatment of the superficial second-degree burn wound consisting of debridement of devitalized skin (epidermis) and ruptured blisters and daily antiseptic dressing changes or the same treatment in addition to a single application of unfocused ESWT (100 impulses/cm^2) to the study second-degree burn. All patients were treated as randomized. All patients of the ESWT group were treated within 24 hours of admission to the burn unit.

Not all study patients were available for final analysis. Six of the 50 study patients were excluded from final analysis because of incomplete data (1 patient) or loss to follow up (5 patients), leaving 44 evaluable patients, who were analyzed on an intent-to-treat basis

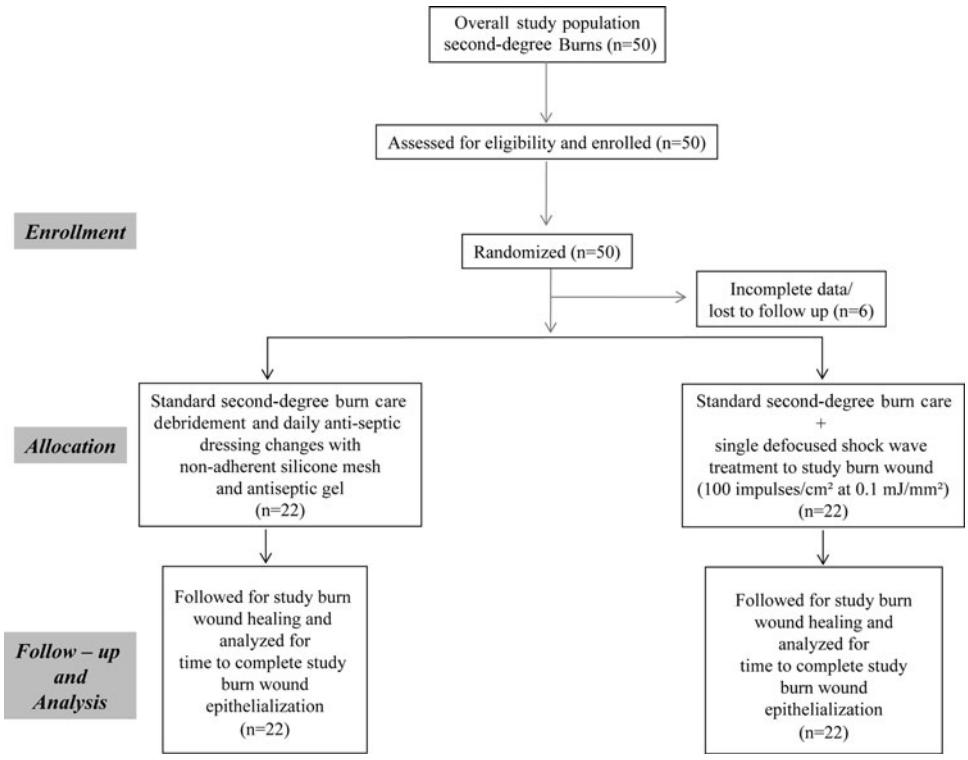

FIGURE 1. Flow chart of participants through study phases.

(Fig. 1). Baseline demographic characteristics of the 44 evaluable study patients are shown in Table 1. Patients were predominantly men with median total body surface area (TBSA) involved by thermal injury of 3% to 4%. Involved anatomic location by burn was mostly the extremity and face, and the majority (95%) of patients underwent only a single burn wound procedure; when 2 patients underwent a second wound procedure, the worst wound was used as the index wound for the primary efficacy analysis. Patient characteristics across the 2 study groups were balanced ($P > 0.05$) except for older age (53 ± 17 years vs. 38 ± 13 years, $P = 0.002$) in the ESWT group.

Toxicities

There were no reported cardiac, neurological, dermal, or allergic reactions. Clinically apparent burn wound infection developed in 9% and 14% of ESWT and control patients, respectively ($P = 0.99$). Postburn bacteremia and other nosocomial infections occurred in 8% and 16% of patients, respectively, and did not differ significantly between study groups.

Primary Outcome Assessment

All superficial second-degree burn wounds healed over a mean period of 11.0 ± 2.4 days. Mean time to complete second-degree burn wound epithelialization for patients that did and did not undergo ESWT was 9.6 ± 1.7 and 12.5 ± 2.2 days, respectively ($P < 0.0005$; Fig. 2). The proportion of patients with epithelialization on each study day is shown in Figure 3; 100% of shock wave treated patients healed completely by day 13, whereas 68% of patients in the control group demonstrated 100% epithelialization of the study wound.

Figure 4 shows time to complete second-degree burn wound epithelialization as a function of study patient age and suggests benefit of ESWT relative to controls in the older age groups. When age (continuous variable) and treatment group (binary) were examined in a linear regression model together as independent predictors of the dependent variable, time to complete (>95%) epithelialization, age was not significant ($P = 0.33$) and treatment group retained significance ($P < 0.0005$).

When all 50 enrolled study patients are analyzed by imputing the missing data and by assuming the worst case scenario for the patients with incomplete data in terms of time to healing for each study group the mean difference in time to complete (>95%) second-degree burn wound epithelialization is 2.0 days [95% confidence interval (CI), 0.7–3.2], and remains significantly more rapid in the intervention arm of the study (ESWT: 10.0 ± 1.9 vs. control: 12.0 ± 2.5 days; $P = 0.003$); the healing time was set to 13 days for those ESWT patients with missing data and was set to 8 days for the control group subjects.

DISCUSSION

The current randomized phase II clinical trial was conducted to determine if a single application of low-energy defocused shock wave therapy within 24 hours of superficial second-degree-degree burn and after debridement/topical antiseptic therapy can significantly accelerate burn wound epithelialization compared to our current standard of practice. ESWT in this study was associated with significantly reduced time to complete superficial second-degree burn wound healing.[5] Patients receiving shock wave therapy showed significantly reduced mean time to complete (>95%) second-degree burn wound epithelialization (9.6 ± 1.7 vs. 12.5 ± 2.2 days; $P < 0.0005$) compared to the control group without ESWT. These results are similar to our recent finding of enhanced healing of skin graft donor sites treated with ESWT in a separate randomized phase II clinical trial.

The current trial adds to the mounting clinical evidence supporting the hypothesis that a biomechanical stimulus can exert clinically relevant, favorable outcomes in terms of tissue repair and

TABLE 1. Distribution of Study Patient Characteristics Across the 2 Study Groups

Characteristic	ESWT n = 22	Control n = 22	*P*	Total n = 44
Sex (Male), n (%)	14 (64%)	18 (82%)	0.31	32 (73%)
Age (years), mean ± SD	52.5 ± 16.6	37.5 ± 13.3	0.002	45.0 ± 16.7
Cardiac or peripheral vascular disease, n (%)	8 (36%)	3 (14%)	0.16	11 (25%)
Antecedent immunosuppression, n (%)	1 (5%)	2 (9%)	0.99	3 (7%)
TBSA (%), median (range)	3% (1%–8%)	4% (1%–50%)	0.37	4% (1%–50%)
Degree of study burn, n (%)			0.99	
Superficial second degree	22 (100%)	22 (100%)		44 (100%)
Mechanism of burn, n (%)			0.52	
Flame	8 (36%)	11 (50%)		19 (43%)
Scald	8 (36%)	8 (36%)		16 (36%)
Explosion flame	2 (9%)	2 (9%)		4 (9%)
Contact burn	3 (14%)	0 (0%)		3 (7%)
Electrical	1 (5%)	1 (5%)		2 (5%)
Location of burn, n (%)			0.64	
Extremity	15 (68%)	11 (50%)		26 (59%)
Extremity and face	4 (18%)	6 (27%)		10 (23%)
Extremity and face and trunk	1 (5%)	2 (9%)		3 (7%)
Extremity and trunk	1 (5%)	2 (9%)		3 (7%)
Entire anterior body surface	0 (0%)	1 (5%)		1 (2%)
Trunk	1 (5%)	0 (0%)		1 (2%)
Trauma in addition to burn, n (%)			0.99	
No	19 (86%)	19 (86%)		38 (86%)
Yes, blast	2 (9%)	2 (9%)		4 (9%)
Yes, motor vehicle/cycle accident	1 (5%)	1 (5%)		2 (5%)
Presence of inhalational injury, n (%)	2 (9%)	3 (14%)	0.99	5 (11%)
Need for mechanical ventilation, n (%)	2 (9%)	3 (14%)	0.99	5 (11%)
Need for ICU care, n (%)	4 (18%)	6 (27%)	0.72	10 (23%)
Need for blood product transfusion, n (%)	1 (5%)	1 (5%)	0.99	2 (5%)
Need for escharotomy/fasciotomy, n (%)	0 (0%)	1 (5%)	0.99	1 (2%)
Number of burn wound, median (range)	1 (1–1)	1 (1–3)	0.49	1 (1–3)
1, n (%)	22 (100%)	20 (91%)		42 (95%)
≥ 2, n (%)	0 (0%)	2 (9%)		2 (5%)
Presence of burn wound infection, n (%)	2 (9%)	3 (14%)	0.99	5 (11%)
Presence of other hospital acquired infection, n (%)	2 (9%)	5 (23%)	0.41	7 (16%)
Postburn bacteremia, n (%)	2/20 (10%)	1/20 (5%)	0.99	3/40 (8%)
Use of systemic antibiotics, n (%)	6 (27%)	3 (14%)	0.46	9 (20%)
Time from burn to study treatment (hrs), median (range)	6.5 (2.0–72.0)	6.8 (3.0–50.0)	0.93	6.5 (2.0–72.0)
Time to CE of study second-degree burn wound (days), mean ± SD	9.6 ± 1.7	12.5 ± 2.2	< 0.0005	11.0 ± 2.4

Of the predefined cohort of 50 patients, 6 had incomplete data or were lost to follow-up. Statistical significance ($P = 0.001$) was retained when ESWT cases with missing follow-up were assigned the longest time to CE and when controls with missing follow-up were assigned the shortest time to CE. All patients of the ESWT group were treated within 24 hours of admission to the burn unit.

regeneration. Cellular mechanotransduction has been demonstrated in vitro and in animal models.[7,8] One modality presently in clinical use that applies this principle of mechanotransduction is negative pressure wound therapy, which when applied to a wound bed, has been in association with wound bed neovascularization, increased granulation, and epithelial cell proliferation, indicative of accelerated tissue regeneration.[9,10]

Further progress pursuant to accelerated tissue repair and regeneration through mechanotransduction has been made through the application of noninvasive treatment modalities such as ultrasound and ESWT.[5,11–13] Experimental studies by our group and others of ESWT in wounds demonstrated shock wave-mediated proangiogenic and antiinflammatory effects in both ischemic tissues and acute burns.[14–18] One of the earliest reported studies demonstrating the positive effects of ESWT on wound healing was by Haupt et al in 1990, which showed a significant reduction in the time for reepithelialization when low energy shock waves were applied to partial thickness wounds in a porcine model, which coincided with significantly increased vascularisation of the upper dermis and thicker layer of the newly formed epithelial cells covering the wound.[19] These promising findings were replicated in subsequent animal model experiments of tissue ischemia.

The positive effect of shock wave therapy on ischemic skin flap survival was demonstrated in a rat model of ischemic epigastric skin flaps. ESWT was shown to significantly improve epigastric skin flap survival through reduction of areas of necrotic zones; these findings were associated with enhanced growth factor expression.[20] Recent work by Takahiro et al extended these findings to improved functional recovery in shock wave treated ischemic tissue.[21] Takahiro et al studied the effect of ESWT on ischemia-induced myocardial dysfunction in a porcine model. The authors report that ESWT of the ischemic myocardium was associated with complete recovery of left ventricular ejection fraction and regional myocardial blood flow restoration to the ischemic region within 4 weeks of ESWT.[21] These results were confirmed in humans by Fukumoto et al who successfully applied ESWT to humans with severe coronary artery disease and associated with reduced myocardial ischemia.[22] The suggested beneficial clinical effects of ESWT seem to extend beyond

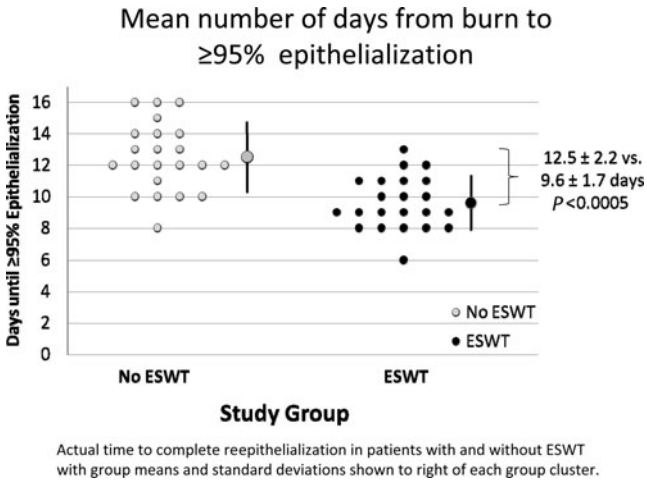

FIGURE 2. Mean number of days from burn to ≥95% epithelialization.

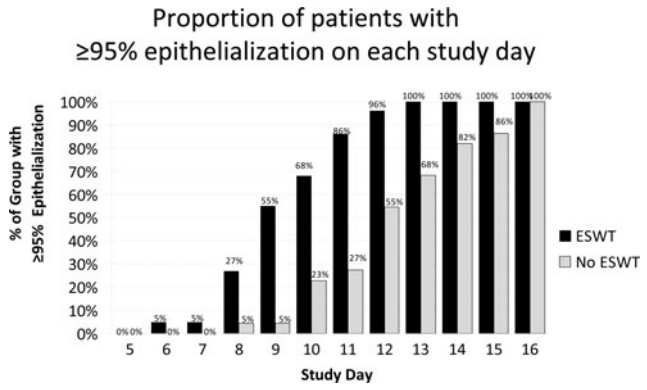

FIGURE 3. Proportion of patients with ≥95% epithelialization on each study day.

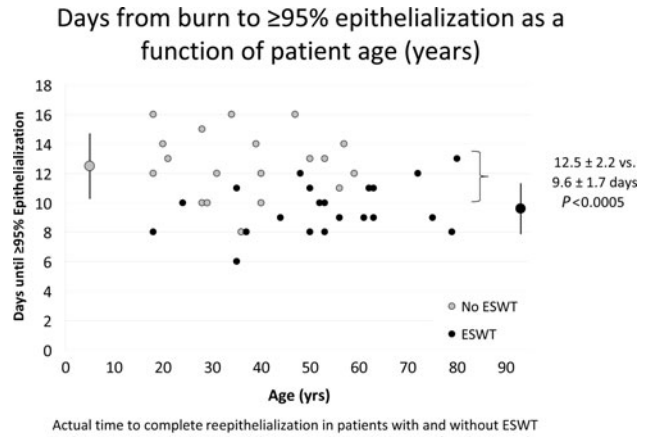

FIGURE 4. Days from burn to ≥95% epithelialization as a function of patient age (years).

neovascularisation, modulation of inflammation, and functional ischemic tissue recovery. The findings reported by Gerdesmeyer et al point to a bactericidal effect of ESWT.[23]

Encouraged by these findings we had previously conducted a phase II trial to assess the feasibility and safety of ESWT for acute and chronic soft-tissue wounds. In a population of 208 patients with complicated wounds we identified complete healing in 156 (75%) patients undergoing a treatment protocol consisting of wound bed preparation (debridement), outpatient, low-energy, defocused shock wave therapy (100–1000 shocks at 0.1 mJ/mm^2, according to wound size, every 1–2 weeks over mean 3 treatments), and moist dressings. No treatment-related toxicity or infection became evident during the course of study, and no treated wound deteriorated with shock wave therapy.[13] Mean time to complete healing (100% epithelialization) varied between groups according to type of wound treated but was most rapid in burns and those with disturbed postoperative wound healing. We concluded after this study that further testing of a wound treatment strategy incorporating low-energy defocused shock waves was safe and feasible, particularly in acute traumatic burns and wounds.

We then conducted a phase II trial in patients with burns requiring skin grafting. Patients were randomized to receive standard topical therapy [nonadherent silicone mesh (Mepitel) and antiseptic gel (Polyhexanide/Octenidine)] to skin graft donor sites with or without low-energy defocused ESWT (100 impulses/cm^2 at 0.1 mJ/mm^2) applied once to the donor site, immediately after skin harvest.[5] Mean time to complete graft donor site epithelialization for patients undergoing ESWT was significantly reduced compared to controls (13.9 $\pm$ 2.0 vs. 16.7 $\pm$ 2.0 days; $P = 0.0001$). A single application of low-energy defocused shock wave therapy to the graft donor site immediately after skin graft harvest may be clinically useful as it can significantly accelerate donor site epithelialization. Future studies will assess the impact on donor site pain, patient symptom distress, and quality of life.

The current phase II randomized trial was conducted to determine if similar accelerated reepithelialization could be attained through the single application of shock waves after superficial second-degree burn wound debridement. This is the largest clinical trial published to date in patients with superficial second-degree burns treated with ESWT. Arno et al reported their initial experience with shock wave therapy in 15 patients with deep partial/full thickness burns.[23] In that study 2 shock wave therapy sessions were applied to the deep partial/full thickness burns on the third and fifth day after injury by using low-energy defocused ESWT (500 impulses at 0.15 mJ/mm^2).[23] Of all treated burns, 80% healed uneventfully before 3 weeks; as many as 15% required surgical debridement and grafting. In our study, 100% of shock wave treated patients with less severe superficial second-degree burns healed within 2 weeks after a single shock wave application. Importantly Arno et al noted significantly enhanced burn wound perfusion with laser doppler imaging after the first shock wave treatment, findings concordant with prior mechanistic studies demonstraing a proangiogenic effect of low-energy defocused shock waves.

Because ESWT as a treatment for acute and chronic soft tissue wounds is in its early clinical investigational stages, the precise mechanism of action has yet to be precisely defined. A number of hypotheses have been proposed as to the mechanism in experimental studies. Fukumoto et al suggests that cellular permeability changes account partly for the positive effects of shock waves in a model of cardiac dysfunction induced by ischemia.[22] This hypothesis is also supported by Takahiro et al, whose work group studied the effects of ESWT on pathologically altered coronary arteries.[21] Gotte et al showed that low energy extracorporeal shock waves stimulate a rapid increase in neuronal nitric oxide synthase (nNOS) activity and basal nitric oxide

(NO) production in a rat glioma cell line C6.[24] In addition, the treatment of C6 cells with ESWT blocks the decrease of nNOS activity and NO production induced by a mixture of lipopolysaccharides (LPS), interferon-γ (IFN-γ) plus tumour necrosis factor-α (TNF-α). Shock wave treatments also downregulate NF-κB activation and NF-κB-dependent gene expression, including inducible NOS and TNF-α in this C6 cell line model.[24,25] These findings point to a putative molecular mechanism of antiinflammatory action and a role of substance P. Another hypothesis regarding the mechanism of actions suggested by Mariotto et al, assumes a direct NO-triggered effect, without consecutive release of neurotransmitters, based on findings in human umbilical vein endothelial cells of shock wave stimulated tyrosine-dephosphorylation of endothelial nitric oxide synthase, ensuing increase in NO production, and downregulation of NF-κB activation.[26]

Wang et al showed that shock waves could enhance growth of rat femur derived bone-marrow osteoprogenitor cells through tumor growth factor beta 1 (TGF-β1) induction, and further demonstrated the potential of shock waves to stimulate differentiation of mesenchymal progenitor cells in human umbilical cord blood into osteogenic cell lineage through superoxide-mediated TGF-β1 production.[27,28] This same group of investigators demonstrated a systemic effect on circulating growth factors in a patient population undergoing ESWT for orthopedic nonunion.[29] Patients whose nonunited fractures healed after ESWT treatment had significantly higher serum NO, TGF-β1, vascular endothelial growth factor (VEGF), and bone morphogenetic protein 2 (BMP-2) levels after treatment than those with persistent nonunion.[29] Thus, animal work and preliminary human experimental data points to a complex, multifactorial mechanism of therapeutic shock waves; however, that ESWT has an effect on biological tissue based on the current level of scientific knowledge is incontrovertible.

This study demonstrated a clinically important effect of low-energy defocused shock waves in superficial second-degree burns; however, the mechanism of action was not studied. Further laser doppler imaging and burn wound histology, which could have been illustrative, were not utilized in this study. Although this study is further limited by modest sample size and lack of long-term follow-up, the difference in time to complete burn site healing was highly significant in favor of the shock wave treated group. Statistical significance in favor of shock wave therapy was maintained when imputing missing data by assuming the worst case scenario for the shock wave group and the best case scenario for the control group patients with incomplete data or lost to follow-up. Although not assessed in this study, quality of life outcome measures should be assessed in future clinical studies to include assessment of pain, symptom distress, and profile of mood state over a longer period of follow-up than studied herein.

Our analyses show that the ESWT advantage persisted when also controlling for age as a baseline covariate. We chose to perform this additional analysis to simply address the age imbalance between treatment groups. Regarding the study power, we considered a 2-day mean difference between treatments to be clinically meaningful, which could be detected with $>80\%$ power for the observed SD (2 days when pooled across treatment groups). We also note that the sample size and SD remain adequate after accounting for the loss of a degree of freedom for including age as a baseline covariate. Thus, we are confident that the study is adequately powered for a 2-sided hypothesis test of superiority with a 5% type I error.

CONCLUSIONS

In this randomized phase II clinical trial application of a single defocused shock wave treatment to the superficial second-degree burn wound after debridement significantly accelerates reepithelialization at the treated site. The conclusion of ESWT superiority remains when accounting for a worst case scenario and a baseline age imbalance. ESWT superiority warrants confirmation in a larger prospective randomized clinical trial. ESWT may prove to be a feasible, noninvasive, safe, and cost-effective method to enhance the healing of both skin graft donor sites and superficial second-degree burns.

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REFERENCES

1. Brown GL, Nanney LB, Griffen J, et al. Enhancement of wound healing by topical treatment with epidermal growth factor. *N Engl J Med.* 1989;321:76–79.
2. Cribbs RK, Luquette MH, Besner GE. Acceleration of partial-thickness burn wound healing with topical application of heparin-binding EGF-like growth factor (HB-EGF). *J Burn Care Rehabil.* 1998;19:95–101.
3. Arnó A, García O, Hernán I, et al. Extracorporeal shock waves, a new non-surgical method to treat severe burns. *Burns*. 2010;36(6):844–849.
4. Meirer R, Kamelger FS, Piza-Katzer H. Shock wave therapy: an innovative treatment method for partial thickness burns. *Burns.* 2005;31(7):921–922.
5. Ottomann C, Hartmann B, Tyler J, et al. Prospective randomized trial of accelerated re-epithelization of skin graft donor sites using extracorporeal shock wave therapy. *J Am Coll Surg.* 2010;211(3):361–367.
6. Moher D, Schulz KF, Altman DG. CONSORT. The CONSORT statement: revised recommendations for improving the quality of reports of parallel group randomized trials. *BMC Med Res Methodol.* 2001;1:2.
7. Ingber DE. Cellular mechnotransduction: putting all the pieces together. *FASEB J*. 2006;20:811–827.
8. Pietramaggiori G, Liu P, Scherer SS, et al. Tensile forces stimulate vascular remodeling and epidermal cell proliferation in living skin. *Ann Surg.* 2007;246(5):896–902.
9. Saxena V, Hwang CW, Huang S, et al. Vacuum-assisted closure: microdeformations of wounds and cell proliferation. *Plast Reconstr Surg*. 2004;114:1086–1096; discussion 1097–1098.
10. Argenta LC, Morykwas MJ, Marks MW, et al. Vacuum-assisted closure: state of clinic art. *Plast Reconstr Surg*. 2006;117(7 Suppl):127S–142S.
11. Ennis WJ, Formann P, Mozen N, et al. Ultrasound therapy for recalcitrant diabetic foot ulcers: results of a randomized, double-blind, controlled, multicenter trial. *Ostomy Wound Manage*. 2005;51:24–39.
12. Ennis WJ, Lee C, Meneses P. A biochemical approach to wound healing through the use of modalities. *Clin Dermatol.* 2007;25:63–72.
13. Schaden W, Thiele R, Kölpl C, et al. Shock wave therapy for acute and chronic soft tissue wounds: a feasibility study. *J Surg Res.* 2007 Nov;143(1):1–12.
14. Huemer GM, Meirer R, Gurunluoglu R, et al. Comparison of the effectiveness of gene therapy with transforming growth factor-beta or extracorporal shock wave therapy to reduce ischemic necrosis in an epigastric skin flap model in rats. *Wound Repair Regen*. 2005;13:262–268.
15. Kuo YR, Wu WS, Hsieh YL, et al. Extracorporeal shock wave enhanced extended skin flap tissue survival via increase of topical blood perfusion and associated suppression of tissue pro-inflammation. *J Surg Res*. 2007;143(2):385–392.
16. Aicher A, Heeschen C, Sasaki K, et al. Low-energy shock wave for enhancing recruitment of endothelial progenitor cells: a new modality to increase efficacy of cell therapy in chronic hind limb ischemia. *Circulation*. 2006;114(25):2823–2830.
17. Davis TA, Stojadinovic A, Anam K, et al. Extracorporeal shock wave therapy suppresses the early proinflammatory immune response to a severe cutaneous burn injury. *Int Wound J.* 2009 Feb;6(1):11–21.
18. Stojadinovic A, Elster EA, Anam K, et al. Angiogenic response to extracorporeal shock wave treatment in murine skin isografts. *Angiogenesis.* 2008;11(4):369–368.

19. Haupt G, Chvapil M. Effect of shock waves on the healing of partial-thickness wounds in piglets. *J Surg Res.* 1990;49:45–48.
20. Meirer R, Kamelger FS, Huemer GM, et al. Extracorporeal shock wave may enhance skin flap survival in an animal model. *Br J Plast Surg.* 2005;58:53–57.
21. Nishida T, Shimokawa H, Oi K, et al. Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia induced myocardial dysfunction in pigs in vivo. *Circulation.* 2004;110:3055–3061.
[AQ6] 22. Fukumoto Y, Ito A, Uwatoku T, et al. Extracoporeal cardiac shockwave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. *Coron Artery Dis*. 2005;17:63–70.
23. Arnó A, Garcia I, Hernan J, et al. Extracorporal shock waves, a new non-surgical method to treat severe burns. *Burns.* 2010 Sep;36(6):844–849.
24. Gotte G, Amelio E, Russo S, et al. Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. *FEBS Lett.* 2002 June 5;520(1–3):153–155.
25. Ciampa A, de Prati E, Amelio E, et al. Nitric oxide mediates anti-inflammatory action of extracorporeal shock waves. *FEBS Lett.* 2005 Dec 19;579(30):6839–6845.
26. Mariotto S, Cavalieri E, Amelio E, et al. Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. *Nitric Oxide* 2005;12:89–96.
27. Wang FS, Yang KD, Chen RF, et al. Extracorporeal shockwave promotes growth and differentiation of bone-marrow stromal cells towards osteoprogenitors associated with induction of TGF-beta1. *J Bone Joint Surg Br*. 2002;84:457–461.
28. Wang FS, Yang KD, Wang CJ, et al. Shockwave stimulates oxygen radical-mediated osteogenesis of the mesenchymal cells from human umbilical cord blood. *J Bone Miner Res.* 2004 Jun;19(6):973–982.
29. Wang CJ, Yang KD, Ko JY, et al. The effects of shockwave on bone healing and systemic concentrations of nitric oxide (NO), TGF-beta1, VEGF and BMP-2 in long bone non-unions. *Nitric Oxide.* 2009 Jun;20(4):298–303.

Queries to Author

Title: Prospective Randomized Phase II Trial of Accelerated Reepithelialization of Superficial Second-Degree Burn Wounds Using Extracorporeal Shock Wave Therapy
Author: Christian Ottomann, Alexander Stojadinovic, Philip T. Lavin, Francis H. Gannon, Michael H. Heggeness, Richard Thiele, Wolfgang Schaden, Bernd Hartmann

[AQ1]: Please check whether the change in the title is OK
[AQ2]: Please provide the expansion of "NF-κB" for clarity.
[AQ3]: Please check whether the expansion "endothelial nitric oxide synthase" for eNOS is correct.
[AQ4]: Please check whether the expansion "bone morphogenetic protein 2" for BMP-2 is correct or not.
[AQ5]: References 12, 20, and 22 have been modified as per PubMed. Please check and verify.
[AQ6]: The author names have in References 21, 23, and 27 have been updated as per PubMed. Please check and verify.