Preventing Scars after Injury with Partial Irreversible Electroporation

Alexander Golberg[1,2], Martin Villiger[3], Saiqa Khan[4], Kyle P. Quinn[5], William C.Y. Lo[3,6], Brett E. Bouma[3,6], Martin C. Mihm, Jr.[7], William G. Austen, Jr.[4] and Martin L. Yarmush[1,8]

Preventing the formation of hypertrophic scars, especially those that are a result of major trauma or burns, would have enormous impact in the fields of regenerative and trauma medicine. In this report, we introduce a noninvasive method to prevent scarring based on nonthermal partial irreversible electroporation. Contact burn injuries in rats were treated with varying treatment parameters to optimize the treatment protocol. Scar surface area and structural properties of the scar were assessed with histology and non-invasive, longitudinal imaging with polarization-sensitive optical coherence tomography. We found that partial irreversible electroporation using 200 pulses of 250 V and 70 μs duration, delivered at 3 Hz every 20 days during a total of five therapy sessions after the initial burn injury, resulted in a 57.9% reduction of the scar area compared with untreated scars and structural features approaching those of normal skin. Unlike humans, rats do not develop hypertrophic scars. Therefore, the use of a rat animal model is the limiting factor of this work.

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INTRODUCTION

Wound care costs the U.S. health care system more than $20 billion each year, and care required to combat skin scarring represents an additional $12 billion burden (Sen et al., 2009). Hypertrophic scarring (HTS) after trauma and burn injury remains a major clinical challenge that leads to physical, aesthetic, functional, psychological, and social stresses in thousands of patients (Sen et al., 2009). Current data show that alterations in coagulation, inflammation, angiogenesis, fibroplasia, contraction, remodeling, and mechanical tension correlate with the formation of HTS (Aarabi et al., 2007; DiPietro, 2013; Rees et al., 2015; Wong et al., 2011). As yet, however, the mechanisms that induce HTS are not well understood. This gap in knowledge leads to limited clinical success in therapeutic procedures (Leventhal et al., 2006). Various techniques such as surgical excision, intralesional steroid or interferon injection, cryotherapy, laser therapy, electron-beam irradiation, mechanical compression dressing, silicone sheet application, and combinations thereof have been tested to treat scars over the years (Leventhal et al., 2006; Mofikoya et al., 2007; Rabello et al., 2014). Despite these efforts, a recent metareview shows that there are only modest improvements in the healing outcomes among all these treatments (Leventhal et al., 2006). In a parallel vein, encouraging results for tissue regeneration have been obtained using pulsed electric fields for tissue ablation in a procedure known as irreversible electroporation (IRE) (Davalos et al., 2005). IRE ablates tissue with short, pulsed electric fields that cause irreversible damage to cells by increasing the permeability of their cell membranes but spare the neighboring extracellular matrix, large blood vessels, and other accessory structures (Charpentier et al., 2010; Golberg et al., 2015a; Phillips et al., 2010; Rubinsky et al., 2007).

Inspired by recent clinical reports on the potential reduction of fibrosis in the liver after IRE instead of radiofrequency ablation (Narayanan, 2011; Rubinsky et al., 2007), and on combined bleomycin and electroporation treatment of HTS and keloids in humans (Manca et al., 2013), we set out to test the hypothesis that partial IRE (pIRE) ablation of the cells in the remodeling scar reduces scarring and diverts the wound-healing process toward scarless regeneration. This hypothesis is based on the assumption that pIRE prevents delayed myofibroblast apoptosis (Robson, 2003) and reduces the number of fibroblasts in the wound, thus creating more room for the secreted collagen to organize normally. However, pIRE of the skin is challenging, because this treatment may also lead to abnormal wound healing, contractions, and increased scarring, or even chronic wounds, if inappropriate parameters are used.

In previous work with healthy, normal skin and liver tissue, we showed that IRE preserves the extracellular matrix but

[1]*Center for Engineering in Medicine, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, and the Shriners Burns Hospital, Boston, Massachusetts, USA;* [2]*Porter School of Environmental Studies, Tel Aviv University, Tel Aviv, Israel;* [3]*Wellman Center for Photomedicine, Massachusetts General Hospital and Harvard Medical School, 50 Blossom Street, Boston, Massachusetts, USA;* [4]*Department of Surgery, Division of Plastic and Reconstructive Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA;* [5]*Department of Biomedical Engineering, University of Arkansas, Fayetteville, Arkansas, USA;* [6]*Harvard-Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge, Massachusetts, USA;* [7]*Department of Dermatology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; and* [8]*Department of Biomedical Engineering, Rutgers University, Piscataway, NJ*

Correspondence: Alexander Golberg, Center for Engineering in Medicine, Department of Surgery, Massachusetts General Hospital, Harvard Medical School, and the Shriners Burns Hospital, Boston, Massachusetts, USA and Porter School of Environmental Studies, Tel Aviv University, Tel Aviv, Israel. E-mail: agolberg@tauex.tau.ac.il

Abbreviations: DOP, degree of polarization; HTS, hypertrophic scarring; IRE, irreversible electroporation; pIRE, partial irreversible electroporation; PS-OCT, polarization-sensitive optical coherence tomography; ROI, region of interest

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completely ablates the cells, and leads to scarless regeneration with the formation of organ appendages (Golberg et al., 2013b, 2015a). No scar or tumor formation has been observed during the 6 months of these studies. We have also developed pIRE protocols for rejuvenation of skin (Golberg et al., 2015b). In that work we showed that a single application of pIRE to normal skin in rats triggers the secretion of new collagen, the formation of additional capillaries and the proliferation of the epidermis and increases the total metabolic activity in the treated area (Golberg et al., 2015b). In addition, we also have developed methods to control fibroblast cell populations using intermittently delivered pIRE in cell cultures (Golberg et al., 2013a) and to disinfect third-degree burns with complete IRE of the infecting bacteria (Golberg et al., 2014). Here, we present a protocol for a therapeutic procedure based on intermittently delivering pIRE to burn wounds to prevent scarring.

pIRE is defined through multiple parameters such as (i) electric field strength, (ii) pulse duration, (iii) pulse number, (iv) pulse frequency, and (v) frequency of treatment delivery, which all have to be optimized to improve the therapy result. In our previous work, using a Taguchi robust experimental design approach we determined the electric field strength and the number of pulses to be the most important pIRE parameters for skin rejuvenation (Golberg et al., 2015b). Accordingly, we limited the number of tested parameters in the present study to (i) electric field strength, (ii) number of pulses, and (iii) frequency of treatment delivery. To evaluate the impact of each of these parameters and to efficiently optimize the therapy protocol, we used Taguchi LS9 orthogonal arrays for the design of experiment (Rao et al., 2008). Taguchi arrays determine the impact of individual parameters on the overall outcome of complex processes, even when several parameters are involved simultaneously (Rao et al., 2008), and are a convenient tool for biomedical engineering.

To assess the effect of pIRE on scar formation, we treated third-degree burn injuries in rats for 3 months. We used the Taguchi robust experimental design for screening and optimizing pIRE parameters. In this study we tested the applied voltages of 125 V, 250 V, and 500 V; number of pulses of 200, 400, and 800; and therapy delivery every 10 days, 20 days, or 30 days after the third-degree burn injury. The tested output parameters were scar surface area, collagen density and collagen fiber directional variance assessed by histology, and skin optical properties as detected with in vivo imaging with polarization-sensitive optical coherence tomography (PS-OCT) (Lo et al., 2016).

RESULTS

The experimental scheme and treatment schedules are shown in Figure 1. We applied the first electric field treatment (Figure 1b and c) immediately after generating the third-degree burns. Supplementary Figure S1 online presents burn injuries in equally treated validation animals at up to 1 week after the injury to show full-thickness burns. The treatments were delivered at the specified time intervals (Figure 1c) for 3 months, and the wounds were left to heal for an additional 3 months after the delivery of the last treatment. Nine combinations of the possible pIRE parameters were tested (Supplementary Table S1 online). The wound healing was monitored by visual inspection, digital photography, and with PS-OCT (Lo et al., 2016) for the entire 6 months after the burn injury.

To evaluate the impact of pIRE on the wound healing, we measured the scar surface area (Figure 2, and see Supplementary Figure S2 online) and assessed features from the analysis of PS-OCT (Figure 3, and see Supplementary

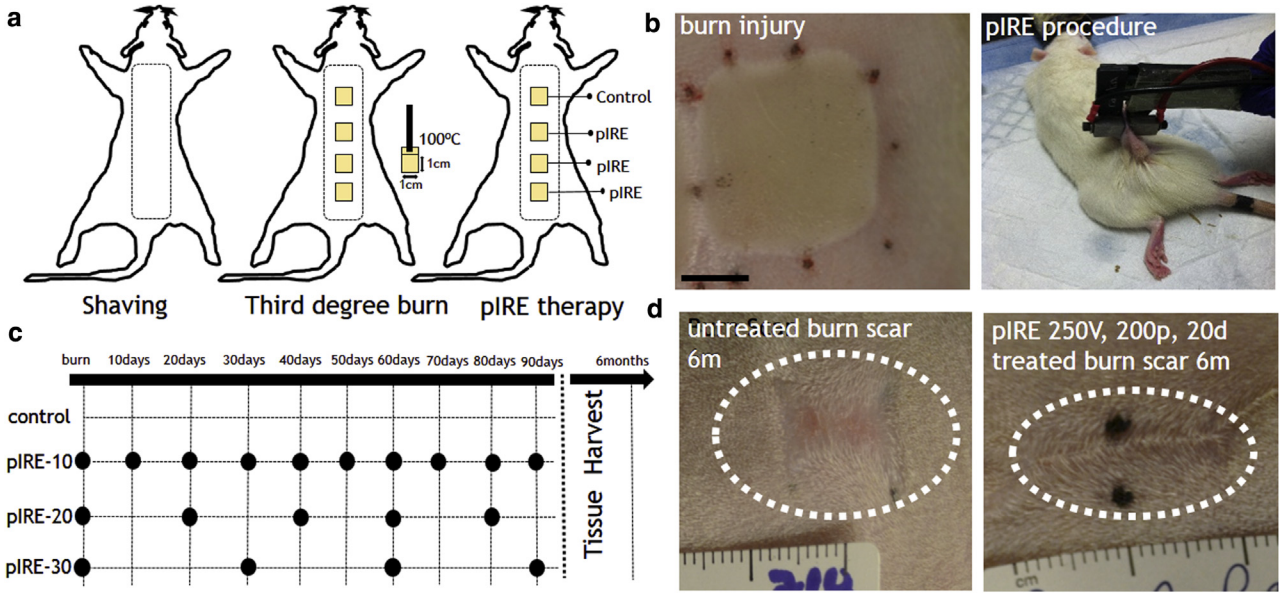

Figure 1. Partial irreversible electroporation of third-degree burns. (**a**) Schematic representation of third-degree burns on the dorsal skin of Sprague-Dawley rats. (**b**) Left panel (scale bar = 5 mm) shows the third-degree burn directly after the application of preheated brass blocks (≥95 °C) for 10 seconds. Right panel shows the pIRE procedure. (**c**) pIRE treatment schedule. (**d**) Left panel shows a typical scar resulting from untreated wound healing 6 months after the third-degree burn (n = 9). Right panel shows the wound-healing outcome on a treated lesion on the same animal after pIRE with 250 V, 200 pulses, 70 µs, 3 Hz delivered every 20 days five times after the injury. d, days; m, months; pIRE, partial irreversible electroporation.