Local Probiotic Therapy with *Lactobacillus plantarum* Mitigates Scar Formation in Rabbits after Burn Injury and Infection

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Abstract

Background: Infection is the most common complication in burn-injured patients and is believed to contribute to the hypertrophic scarring frequently observed in such injury. *Pseudomonas aeruginosa* is a common pathogen in burn wound infection. We examined the effect of local probiotic therapy with *Lactobacillus plantarum* on the severity of the scarring following burn wounding and infection with *P. aeruginosa* in a rabbit model.

Methods: Full-thickness burn wounds were inoculated with control vehicle or *L. plantarum*; wounds were then challenged with bioluminescent *P. aeruginosa*. The time course of the ensuing infection was monitored by quantification of the emitted light. After allowing wounds to contract to near completion, they were harvested and analyzed for markers of scar formation.

Results: Application of *L. plantarum* curtailed both the severity and the length of the pseudomonal infection. Probiotic therapy significantly reduced both Type I collagen mRNA concentrations and total collagen protein accumulation in infected wounds, consistent with reduced scarring. Surprisingly, the probiotic showed a nearly equivalent effect in uninfected wounds. Masson's trichrome staining confirmed these findings histologically.

Conclusions: *Lactobacillus plantarum* shows exciting potential as a therapeutic agent to both counteract burn wound infection and to alleviate scarring even in the absence of infection.

BURN INJURY TO THE SKIN evokes a cascade of events resulting in progressive deepening of the zone of injury, which is worsened by infection. The most cited reason for death in burned patients is infection [1,2], noted as the most common complication in patients of all ages by the American Burn Association. Burn injuries destroy the physical skin barrier that normally prevents the invasion of microorganisms, thereby providing novel sites for bacterial colonization, potentially leading to invasive infection and even sepsis [3]. Large-area burns are especially prone to infection, with the most common organisms being *Pseudomonas aeruginosa* and *Staphylococcus aureus* [4]. Recent studies describe the emergence of multi-drug-resistant strains of bacteria that pose a greater risk of sepsis and death [2,4–6], and fungal infections also are a major concern [6]. It has also been recognized that burn wounds play host to bacterial biofilms, communities of bacteria (or fungi) residing within an organized extracellular matrix, complicating further the attempt to manage burn wound infection, as biofilms make the or-

ganisms inside them highly resistant to conventional antibiotics [7,8].

The management of burn injuries to limit infection and resurface the wound currently relies on several core strategies: Deeper injuries usually require surgical excision with skin replacement through autografts, temporary dressings, or skin substitutes. Systemic antibiotics are sometimes used adjunctively, although some practitioners refrain from this practice until evidence of invasion or sepsis appears for fear of selecting resistant micro-organisms. Topical antimicrobial agents, especially silver-containing compounds such as silver sulfadiazine, have been the mainstay of local wound therapy for more than 40 years [9]. However, recent reports have noted multiple drawbacks to the use of topical silver as an anti-microbial agent: Development of resistance (including multi-drug resistance) by induction of an efflux mechanism (including in *P. aeruginosa*), local and systemic toxicity to host tissues (e.g., renal toxicity, hepatotoxicity), and argyria and dyschromia of the skin. In addition, some

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reports have questioned the clinical and cost efficacy of topical silver [10].

It has long been recognized that infected wounds of all sorts heal with greater scar and contracture than clean wounds, presumably because of the greater inflammatory stimulus elicited by pathogenic bacteria driving fibrosis [11]. Hypertrophic scar and contracture are especially problematic in the aftermath of burn injury, afflicting as many as 60% of burn-injured patients, restricting movement and function and having obvious psychological and social costs [12–14]. A recent investigation found that colonization of a burn wound by pathogenic bacteria correlates with the development of hypertrophic scarring [11]. This observation led us to consider whether reduction of pathogenic bacteria in a burn model would result in less scarring. Rather than resorting to conventional antibiotics, however, we investigated the utility of local probiotic therapy for this purpose.

Probiotics, that is, the application of living microorganisms for the benefit of the host, has already achieved clinical utility in multiple scenarios, including the treatment of adult and neonatal digestive tract diseases, female urogenital tract conditions, and so on [15]. Usually, the probiotic agent is some kind of lactic acid bacterium, often *Lactobacillus* or *Bifidobacterium*, and usually the probiotic bacteriotherapy is administered as an ingestible agent. There are as yet few reports of local or topical delivery of probiotics. In this study, we investigated the effect of local delivery of *Lactobacillus plantarum* to a burn wound on the subsequent scarring in both the presence and absence of the pathogen *P. aeruginosa*.

Materials and Methods

Materials

Pseudomonas aeruginosa strain Xen41, obtained from Caliper Life Sciences (Hopkinton, MA), was derived from the parental strain *P. aeruginosa* PA01 and was grown in standard Luria broth. This bacterium possesses a single copy of the *Photorhabdus luminescens luxCDABE* operon. *Lactobacillus plantarum* ATCC strain 10241 (American Type Culture Collection, Manassas, VA) was grown in MRS broth.

Methods

Skin Burn Wounds. The experimental design and the treatment of the animals were approved by the Institutional Animal Care and Use Committee of the Allegheny–Singer Research Institute. The dorsums of male Dutch Belted rabbits (3 months of age weighing approximately 3 kg) were depilated, and ketamine (25 mg/kg) and xylazine (1 mg/kg) were injected intramuscularly to induce sedation. The shaved dorsums were cleaned and sterilized with Betadine solution before a heated brass block was applied. These blocks (2.54-cm diameter) were maintained at a temperature of 100°C and applied for 30 secs to create dermal burn wounds. Wound depth was examined by hematoxylin and eosin staining to assess the depth of the damage to the following skin elements: Hair follicles, epithelium, connective tissue collagen, and blood vessels. Histologic examination confirmed that the animals sustained full-thickness dermal burns. Burn wounds were left undisturbed for five days; on the fifth day, the eschar was inoculated with 3×10^8 colony-forming units (CFU) of

Lactobacillus, and after six h, the burned areas were challenged with 4×10^7 CFU of *Pseudomonas*.

Experimental protocol. Each rabbit had four burn wounds created on its dorsum. The first wound served as a control and did not receive any treatment (vehicle only); the second and fourth wounds were inoculated with 3×10^8 CFU of *Lactobacillus*, an organism not known to cause significant pathology in human beings; and the third and fourth wounds were challenged six h later with 4×10^7 CFU of *Pseudomonas*. The four burn wound conditions therefore were: (1) Burn wound only; (2) *L. plantarum* only; (3) *P. aeruginosa* only; (4) *L. plantarum* + *P. aeruginosa*. The course of ensuing infection was monitored longitudinally and non-invasively by quantitation of the light emitted from the wounds by scanning the rabbits with the Xenogen imaging system (Caliper Life Sciences) twice/week for just over one month. No rabbits succumbed to their injuries or infection. The burn wounds were allowed to contract to near completion (approximately five weeks) before the resulting scars were excised and stored in RNALater or either fixed in 10% Formalin or frozen for hydroxyproline analysis. These scarred, contracted tissues were subjected to molecular and histologic analyses as described below.

RNA extraction. Total RNA was extracted from control unwounded skin and from each wound type using the RNeasy Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions after homogenization and an on-column DNase treatment step. The quantity and quality of RNA was determined by measuring the optical density 260/280 ratio using an ND-1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE) and by capillary electrophoresis using an Agilent 2100 BioAnalyzer (Santa Clara, CA). The RNA with a RIN value >6.0 was used for further analyses.

Histologic examination. Wound/scar tissues were harvested at 35 days. The tissues were fixed in 10% neutral buffered Formalin for subsequent histologic evaluation. Paraffin sections were stained with Masson's trichrome to determine their collagen content and Picrosirius red for assessing collagen alignment to evaluate the degree of fibroplasia and matrix deposition following standard protocols.

Collagen content. Histologic sections on glass slides were stained with Masson's trichrome to assess collagen content, as described by Yates et al. [16]. Collagen content was measured using MetaMorph analysis (Molecular Devices, Sunnyvale, CA) as previously described [17]. In brief, wound/scar samples from control and infected tissues were concomitantly stained and compared; at all times, the color settings in the Meta-Morph software were maintained between the samples to compare the blue- and red-stained areas. The final output was integrated intensity based on total area and staining intensity of individual pixels.

Collagen alignment. Picrosirius red staining was used to assess collagen alignment in terms of thickness (cross-sectional area), and the arrangement in terms of the length of the collagen scars was analyzed quantitatively using MetaMorph as previously described [18]. Polarization microscopy

of tightly packed thick and long fibrils of bright red-orange intense birefringence were interpreted as type I collagen, and thin short loose fibrils with yellow-green birefringence were interpreted as Type III collagen.

Hydroxyproline analysis. Total collagen content was measured using the hydroxyproline assay. Wound/scar samples were dried for 24 h at 110°C followed by hydrolysis with 6N HCl for 24 h following Woessner's method [19]. Hydroxyproline in the hydrolysates was assessed calorimetrically at 560 nm for the presence of p-dimethylaminobenzaldehyde. Results are expressed as mcg of hydroxyproline per g of tissue.

Quantitative Real-Time Reverse Transcriptase–Polymerase Chain Reaction (qRT-PCR). Real-time RT-PCR was performed on 60 ng of total RNA isolated from control and experimental wound/scar tissues, as well as from unwounded skin. Primers and Taqman probes for type I collagen were designed using Primer Express Software (Applied Biosystems, Foster City, CA). Forward and reverse primers were purchased from Integrated DNA Technologies (Coralville, IA), and fluorocoupled Taqman probes were purchased from Applied Biosystems. The RT reaction (using reverse primer) and subsequent real-time PCR assays were performed as previously described [20–23]. Using the comparative critical cycle (Ct) method and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control, the expression of the target genes was normalized, and the relative abundance was calculated. Data were analyzed using 7900 HT SDS software version 2.1 provided by Applied Biosystems. Primer and probe sequences for type I collagen and GAPDH are provided in Table 1.

Statistical analysis

Statistical analysis was performed using the Student t-test with a p value <0.05 being considered significant.

Results

Probiotic therapy with Lactobacillus plantarum reduced the intensity and duration of pseudomonal infection

Burn wound infection was monitored by biophotonic imaging for five weeks; all sites appeared to clear the infection in the course of some two to three weeks. We found that a single inoculum of *L. plantarum* significantly inhibited the ability of *P. aeruginosa* to establish and maintain an infection, as evidenced by the less emitted bioluminescent signal beginning from 24 h post-infection (Fig. 1). The peak inten-

sity of the infection was reduced by ~37%, and the time until clearance of infection (that is, until emitted bioluminescence was essentially indistinguishable from baseline) was shortened by some three days.

Probiotic therapy inhibited the injury-induced accumulation of type I collagen mRNA

Excessive deposition of type I collagen is perhaps the single most important hallmark of scar formation; we therefore examined the effect of our probiotic treatment on expression of type I collagen mRNA in injured and control wounds and skin. We found a marked increase in type I collagen message in burn alone (4.07 ± 0.31 mcg/g vs. control unwounded skin) with a similar increase in burns infected with *P. aeruginosa* (3.77 ± 0.44 mcg/g). Remarkably, a single treatment with probiotic *L. plantarum* inhibited this increase by ~50% in all wounds, whether infected or not (Fig. 2).

Probiotic therapy inhibited the injury-induced accumulation of total collagen protein

Total tissue hydroxyproline, reflecting total collagen protein content, was assayed directly in the four wound types as well as in unwounded control skin. Burn injury alone resulted in a modest but significant increase in total tissue hydroxyproline, with a similar increase in infected burn wounds (Fig. 3). Remarkably, as with type I collagen mRNA, a single probiotic treatment reversed this increase and actually resulted in significantly less total collagen than in even control unwounded skin. This effect occurred equally in both infected and uninfected wounds.

Direct histochemical visualization and quantitation of collagen deposition confirmed the inhibitory effect of probiotic therapy with Lactobacillus plantarum

In addition to molecular measures of tissue collagen content as above, we directly examined the pattern and intensity of collagen deposition after burn injury and infection by Masson's trichrome staining of tissue sections. The distribution and quantity of deposited collagen was then summated into a single representative value using the Meta-Morph software program as previously described [17].

Figure 4A shows representative sections from our four experimental conditions. It is readily apparent that wounds infected with *P. aeruginosa* deposited the densest collagen matrix (panel 3), and that this collagen accumulation is markedly decreased by a single probiotic treatment (panel 4). Wounds treated with *L. plantarum* alone (panel 2) show no

TABLE 1. PRIMERS AND PROBES DESIGNED USING PRIMER EXPRESS® SOFTWARE V 2.0 AND PROVIDED BY APPLIED BIOSYSTEMS

Gene	Primer Sequence	TaqMan Probe
Rabbit type I collagen	F=GGCCCAACCTGAAAACATCTC R=AAACTGGGTGCCACCATTGA	5'-6' FAM-CTCCAAGGCCAAGAAGCAT GTCTGGT-TAMRA-3'
Rabbit GAPDH	F=ACAACCTCTCTCAAGATTGTCTAGCAA R=CCGAAGTGGTCGTGGATGA	5'-6' FAM-CTGCACCACCAACTGCTTA GCCCC-TAMRA-3'

F=forward; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; R=reverse.

FIG. 1. Biophotonic profile of burn wounds showing reduction in bioluminescence with *L. plantarum* administration. (A) Representative biophotonic images of an animal carrying the four wound conditions: burn only control (labelled C, upper left); burn + *L. plantarum* (labelled L, upper right); burn + *P. aeruginosa* (labelled P, lower left); and burn + *L. plantarum* + *P. aeruginosa* (labelled P + L, lower right). Days post-infection (PI) are indicated. (B) Time course of the emitted light from infected and probiotic-treated wounds. Data are shown as mean total flux [photons/s] $\pm$ standard error. Data are derived from eight independent experiments. Color image is available at www.liebertpub.com/sur

grossly elevated collagen deposition beyond burn injury alone (panel 1) and indeed appear to show less.

These impressions were borne out by formal Meta-Morph evaluation (Figure 4B). When compared with control unwounded skin, both burn injury and burned and infected tissues demonstrated markedly higher Meta-Morph scores. Again, probiotic treatment significantly reduced those scores in both infected and uninfected wounds, consistent with our earlier molecular results that probiotics have a mitigating effect on collagen accumulation even in the absence of pseudomonal super-infection.

Probiotic therapy alters the type of collagen alignment after burn injury

Whereas scirrhous wound healing in the adult mammal typically proceeds with high deposition of type I collagen, in healing fetal wounds, type III collagen predominates and is associated with the diminished or absent scar observed therein. We examined the relative abundance of type III versus type I collagen in our experimental conditions by Picrosirius red staining of histologic sections. Burn-injured and infected wounds evinced the presence of abundant orange and red fibers with a thicker apparent pattern of collagen alignment, consistent with the predominant occurrence of mature type I collagen (Fig. 5, panels 1 and 2). In contrast, *L. plantarum*-treated wounds showed a markedly greater rela-

tive abundance of thinner green fibrils consistent with greater amounts of immature type III collagen (Fig. 5, panels 3 and 4), indicating that probiotic therapy can modulate, not only the quantity, but also the type of collagen synthesized in response to burn injury and infection.

Discussion

Scarring has long been recognized as a particularly problematic sequela of burn injury, and it is increasingly appreciated that the presence of micro-organisms in the burn wound contributes to this phenomenon [11]. Early excision and grafting has lessened but not eliminated this problem, and to date, there is no specific adjunctive therapy that can be applied at the time of burn injury that has proved efficacious in reducing scarring in its aftermath.

Our results show that even a single administration of the probiotic bacterium *L. plantarum* into the healing burn can help to counteract infection by a pathogen, in this case, *P. aeruginosa*. We also have observed (in a mouse model of burn wound-induced pseudomonal sepsis) that locally administered *L. plantarum* can rescue the animal from septicemia and death (Argenta et al., manuscript under review). The mechanism by which *Lactobacillus* exerts these beneficial effects is as yet unclear, but there are multiple possibilities. For example, the presence of lactic acid bacteria may result in acidification of the surrounding microenvironment,

FIG. 2. Type I collagen mRNA was significantly reduced in *L. plantarum*-treated wounds. Quantitative RT-PCR for type I collagen message was performed on RNA extracted from the four wound conditions as well as unwounded skin. Results are expressed as relative abundance compared with unwounded skin, set to a baseline of 1. Burn wound alone and burn wound infected with *P. aeruginosa* showed similar marked increases in collagen message accumulation (to approximately four-fold). In contrast, wounds treated with *L. plantarum* showed only a two-fold elevation compared with control skin. Values are means $\pm$ standard error of three independent studies performed in triplicate. Statistical analysis was performed using the Student t-test. $P < 0.05$ was considered significant.

inhibiting pseudomonal growth. We believe this is unlikely to be the explanation, as *Pseudomonas* thrives in the airways of cystic fibrosis patients, which are relatively acidic [24]. Moreover, topical treatment of burn-wounded patients with a polylactic acid-acetic acid dressing did not decrease the resident bacterial bioburden, and *Pseudomonas* frequently remained present [25]. It also is possible that factors elaborated by *Lactobacillus* interfere with pseudomonal physiology directly, and there is some evidence that *Lactobacillus* can interfere specifically with *Pseudomonas*' capacity to form biofilm and to produce its quorum-sensing molecules [26]. Alternatively, *Lactobacillus* may be having an effect on the host physiology, which secondarily renders the animal more capable of fending off the super-infecting pathogen.

Although the anti-pathogenic effects of probiotic bacteria have been described previously, we believe this is the first demonstration that probiotic bacteriotherapy can mitigate scar formation, as evidenced by the consistently lower amounts of collagen accumulation noted in our probiotic-treated wounds. All methods employed showed that probiotic therapy was able to inhibit the increase in collagen deposition markedly in the setting of *Pseudomonas* infection after burn injury; modest differences in the quantitation of this inhibition are likely attributable to variances in the parameters and sensitivities of the various techniques.

A more remarkable and somewhat unexpected finding is that application of probiotic bacteria even in the absence of a super-infecting pathogen attenuates the collagen deposition spurred by burn injury. This is again evident at the RNA, protein, and tissue levels. Multiple possibilities suggest themselves as to how this may be accomplished. First, al-

FIG. 3. Tissue hydroxyproline concentrations were significantly lower in *L. plantarum*-treated wounds. Total tissue hydroxyproline, reflecting total collagen protein content, was determined in the four wound conditions as well as in unwounded skin. Burn wounding alone and burn wounds infected with *P. aeruginosa* showed a modest increase in total hydroxyproline. Probiotic treatment with *L. plantarum* resulted in markedly less hydroxyproline accumulation, even less than in control skin. Data shown are the mean $\pm$ standard error of four independent studies performed in triplicate. Statistical analysis was performed using the Student t-test.

though no specific pathogen was introduced into the burn only or burn + *L. plantarum* wounds, it may be that other incidental (nonbioluminescent) bacteria did in fact inhabit those wounds and that *L. plantarum* is acting against them even as it does against *Pseudomonas*, thereby lessening bacterial stimulation of inflammation and fibrosis. More intriguingly, it may be that *Lactobacillus* is able to suppress independently and directly the inflammatory and fibrotic pathways activated by burn injury. There is an emerging body of evidence to support this idea. For example, lactobacilli in models of colitis inhibit the production of inflammatory mediators such as tumor necrosis factor (TNF)- α and interleukin (IL)-6 [27,28]. Other investigators have demonstrated that lactobacilli can suppress secretion of Th1, Th2, and Th17 cytokines by various immune cell types directly [29]. What seems clear is that *Lactobacillus* itself is not eliciting any harmful inflammatory or fibrotic effects in the host tissues. This is borne out in our mouse burn-sepsis model, where even *Lactobacillus* translocated to the liver failed to elicit any increase in TNF- α or IL-6 or IL-10 (Argenta et al., manuscript under review). These observations augur well for the potential safety and tolerability of locally or topically applied *Lactobacillus* as a clinical therapy.

We also observed that the type of collagen being deposited in probiotic-treated wounds is comparatively higher in the type III vs. the Type I isotype. It is unclear whether this reflects greater type III collagen accumulation or is apparent because of a relative decrease in type I collagen or both. Given that the total collagen content appears to be markedly decreased, and with the background that type III collagen is favored in the regenerative healing of fetal wounds, we postulate that the relative abundance of type III collagen here

FIG. 4. Histochemical demonstration of reduced collagen deposition in *L. plantarum*-treated wounds. **(A)** Representative cross-sections stained with Masson's trichrome of the four wound conditions at day 35 post-injury. Wounds treated with probiotic bacteria displayed less and less-dense collagen deposition compared with control burn wounds and, especially, compared with wounds infected with *Pseudomonas* (panel 3). (Original magnification $\times 20$). 1 = Burn wound only; 2 = burn wound + *L. plantarum*; 3 = burn wound + *P. aeruginosa*; 4 = burn wound + *L. plantarum* + *P. aeruginosa*. **(B)** Meta-Morph quantitation of collagen patterns observed histochemically. Analysis confirms the probiotic mitigation of collagen deposition after both burn injury and injury with pseudomonal super-infection. Data are shown as mean $\pm$ standard error of three independent studies. Statistical analysis was performed using the Student t-test. Color image is available at www.liebertpub.com/sur

presages a better long-term outcome vis-à-vis scar than if type I collagen were predominant. The actual importance of type I and type III collagens to hypertrophic scar formation in patients after a burn remains uncertain, with some studies reporting that type III collagen is a lesser component of the total collagen burden (e.g., [30]), whereas others have reported more type III collagen in hypertrophic scars than in

normal skin [31], observations that are not necessarily contradictory.

Our overall observations suggest that probiotic bacteriotherapy may someday prove a useful adjunct to the management of burn wounds independent of infection, but the study naturally has limitations. The rabbit model used heals its skin wounds primarily by contraction because of an

FIG. 5. Type III collagen was increased in *L. plantarum*-treated wounds. Representative cross-sections stained with Picrosirius red of the four wound conditions at post-injury day 35. A readily observable difference is observed in wounds treated with *L. plantarum*, which featured a much greener overall appearance, consistent with a higher relative abundance of type III collagen. Greater type I collagen (dark orange and red staining) was present in wounds that did not receive *L. plantarum*. (Original magnification $\times 20$). 1=Burn wound only; 2=burn wound + *L. plantarum*; 3=burn wound + *P. aeruginosa*; 4=burn wound + *L. plantarum* + *P. aeruginosa*. Color image is available at www.liebertpub.com/sur

underlying panniculus carnosus muscle; human skin in contrast lacks such a structure (except in the face) and heals with less pronounced contraction. As a practical matter, scar formation and collagen accumulation were allowed to proceed for only five weeks before analysis, whereas clinically, scar maturation and the emergence of hypertrophic scar occurs over a longer time. Our model introduces both probiotic and pathogenic bacteria into an eschar in place, whereas clinically, we would expect eschar to be debrided and probiotic agent to be applied topically. Although *L. plantarum* appears efficacious against *P. aeruginosa*, it remains to be seen whether similar activity can be demonstrated against other burn wound pathogens. This concern is lessened by observations that other probiotic lactobacilli counteract other burn wound pathogens (e.g., *S. aureus* [32]). It may be that ultimately a “cocktail” of probiotic bacteria will be more effective than any single agent.

Topical probiotic therapy for burn wounds has been reported once. Peral et al. applied *L. plantarum*-soaked sponges to a cohort of patients with smaller (<15%) total body surface area burns and compared outcomes with a similar cohort of patients treated with silver sulfadiazine [33]. Although the limited numbers of patients did not allow the results to reach statistical significance, the authors noted no overall differences in the rate of healing, nor in the bacterial counts recovered from wound biopsies between these two patient

populations. Importantly, the use of *L. plantarum* was not associated with any adverse outcomes, including no differences in acceptance of subsequent skin grafts. The authors did not monitor for scar outcome, but these encouraging initial observations underscore the potential for probiotic therapy in this setting.

A lingering concern is that *Lactobacillus* administered to a functionally immunocompromised burn patient may become a source of infection. *Lactobacillus* spp. have been implicated in bacteremia/sepsis in patients with compromised immune systems for other reasons (e.g., bone marrow transplant [34] or treatment with steroids and infliximab [35]). However, oral probiotics given to patients with large total body surface area burns (40%–70%) significantly decreased the number of deaths compared with patients from whom probiotics were withheld [36]; topical probiotics may have the same beneficial effects.

We suggest that locally applied probiotic therapy may offer a counterintuitive but attractive alternative to standard therapies for burn wound care. It is inexpensive, easy to apply, and thus far appears to pose little risk to the patient, although its real safety has yet to be tested rigorously. Probiotic bacteria can act against a range of pathogens simultaneously, including drug-resistant bacteria and fungi, and are unlikely to spur conventional antibiotic resistance. Their beneficial effects may extend beyond simple

infection control to scar mitigation. Further studies will undoubtedly clarify some of the operative mechanisms of action and may lead the way to adoption of local/topical probiotics in clinical practice.

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Author Disclosure Statement

The authors have no conflict of interest with regard to this manuscript.

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