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Purified Type I Collagen Wound Matrix Improves Chronic Wound Healing in Patients with Recessive Dystrophic Epidermolysis Bullosa

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Abstract: **Recessive dystrophic epidermolysis bullosa is a severe genetic blistering skin condition resulting in chronic wounds. Nonhealing wounds were treated over 8 weeks using a reconstituted natural purified type I collagen skin substitute. Chronic wounds were defined as nonhealing wounds present for longer than 6 months. For each patient, two chronic wounds were identified and randomized into a control or treatment group. Both groups received standard-of-care wound dressings. The treatment group received an additional type I collagen skin substitute. Wound size was measured at baseline and weeks 1, 4, and 8. Pain, pruritus, and burning and stinging were assessed. Wound cultures were obtained at baseline and thereafter as was considered clinically relevant. Ten subjects were enrolled; seven completed the study. Six subjects showed a positive response to the type I collagen skin substitute. Three subjects demonstrated full wound reepithelialization. Wounds treated using the collagen skin substitute showed statistically significantly greater improvement. Average scores for pruritus and pain decreased significantly. Reconstituted natural purified type I collagen skin substitutes improved the healing of chronic wounds and may be a valuable addition to the epidermolysis bullosa wound care arsenal.**

Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin disease that results in severe skin fragility. Individuals with RDEB have mutations in the *COL7A1* gene, leading to the low or absent expression of type VII collagen, a protein important for the structural integrity of skin. Because of skin fragility, individuals with RDEB often develop chronic nonhealing wounds (1). There is a paucity of effective and convenient treatment options for these wounds (2,3).

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TABLE 1. *Selected Collagen-Containing Skin Substitutes*

Product	Manufacturer	Description
Acellular products		
Helicoll	Encoll (Fremont, CA)	Purified type I bovine collagen
Integra	Integra LifeSciences (Plainsboro, NJ)	Bovine collagen and chondroitin-6-sulfate
Promogran	Systagenix (Quincy, MA)	Bovine collagen and oxidized regenerated cellulose
Oasis	Healthpoint (Fort Worth, TX)	Porcine small intestinal submucosa collagen matrix
Living cell–based products		
Dermagraft	Shire Regenerative Medicine (La Jolla, CA)	Foreskin-derived fibroblasts grown on degradable scaffold
Apligraf	Organogenesis (Canton, MA)	Bilayer with bovine type I collagen and foreskin-derived keratinocytes and fibroblasts

Various types of collagen-containing wound dressings have been used successfully to improve the healing of chronic wounds (Table 1). Living cell–based wound dressings such as Apligraf (Organogenesis, Canton, MA) and Dermagraft (Shire Regenerative Medicine, La Jolla, CA) are effective in patients with epidermolysis bullosa (EB) (4–6), but are expensive and require application by a medical professional in a clinical or surgical setting. Acellular dressings with collagen derived from a variety of sources have been shown to treat chronic wounds effectively (7–11). To our knowledge, only Integra (Integra LifeSciences, Plainsboro, NJ), a bilayer matrix wound dressing consisting of acellular bovine collagen and chondroitin-6-sulfate, has been reported to treat EB wounds (12), but it has not been assessed in a formal clinical trial.

Helicoll (Encoll, Fremont, CA) is a single-layer skin substitute consisting of an acellular matrix of purified bovine type I collagen. Prior studies have shown that Helicoll improves wound healing for donor sites for split-thickness skin grafts, skin burns, chronic venous ulcers, and diabetic foot ulcers (13). Furthermore, Helicoll may reduce pain at treated sites (14). In this study we investigated whether this type I collagen skin substitute improves the healing of chronic RDEB wounds.

MATERIALS AND METHODS

The Stanford Institutional Review Board approved this clinical trial (protocol 24915), which was conducted in accordance with the principles of the Declaration of Helsinki. The study was listed on clinicaltrials.gov (NCT01716169). Informed consent (or assent as applicable) was obtained for all subjects before any study-related procedures were performed.

At baseline, after a routine history and physical examination, target chronic wounds were selected. A wound was considered chronic if it was present and had been nonhealing for 6 months or more. For the first four subjects, one chronic wound was selected to receive Helicoll. In the last six subjects, two chronic wounds were selected; one wound was randomized to receive Helicoll and the other received standard treatment. Standard treatment consisted of the subject's routine wound dressing regimen (Table 2). Target wounds of similar size and duration were selected for each patient. Neither subjects nor investigators were blinded to the study treatment received.

Inclusion criteria included age 7 years or older and a diagnosis of RDEB. Subjects were excluded if they had sensitivity to bovine products or if target wounds showed clinical signs of infection or had a history of malignancy.

The primary outcome measure was wound size measurement as assessed using the SilhouetteStar (ARANZ Medical, Christchurch, New Zealand), a device that uses lasers to capture wound areas accurately without touching the skin. Before Helicoll application, levels of pain (15), pruritus (16), and burning and stinging (17) were assessed using a visual analog scale for each wound. Moreover, investigators assessed wound characteristics and duration and asked about previous wound treatments. Wound cultures were obtained and processed at the Stanford Hospital Clinical Laboratory. Helicoll was then applied to the target wound according to the manufacturer's instructions, followed by a contact layer of nonadhering wound dressing (Adaptic, Systagenix, Quincy, MA, or Mepitel, Mölnlycke Health Care US, Norcross, GA) and then sterile gauze moistened with sterile saline. Immediately after application, levels of wound pain, pruritus, and burning and stinging were again measured. Subjects were instructed to change the outermost gauze layer daily and moisten with sterile saline as needed. Helicoll was reapplied weekly.

Subjects were seen for follow-up in an ambulatory pediatric dermatology clinic at weeks 1, 4, and 8. If the wound healed completely before the scheduled study visit, the subject was seen sooner. At the follow-up

TABLE 2. *Demographic Characteristics and Wound Dimensions*

Demographic characteristics					Wound dimensions, cm^2 (% decrease from baseline)			
Patient	Age/sex	Wound location	Wound duration	Treatment	Baseline	Week 1	Week 4	Week 8
H01	8/Female	Left lateral elbow	>6 months	Helicoll	13.7	2.7 (80.3)	0.0 (100)	N/A
H02	24/Male	Left anterior lower leg	1 year	Helicoll	3.4	3.1 (8.8)	3.1 (8.8)	2.6 (23.5)
H03	18/Female	Right forearm superior	>6 months	Standard (Mepilex)	10.4	N/A*	N/A	N/A
		Right forearm inferior	>6 months	Helicoll	23.3	N/A*	N/A	N/A
H04	24/Female	Left posterior thigh	>6 months	Helicoll	18.0	4.6 (74.4)	1.6 (91)†	N/A
H05	16/Male	Left anterior superior upper arm	1.5 years	Helicoll	6.2	5.5 (11.3)	0.6 (90.3)	0.7 (88.7)†
		Left posterior upper arm	1.5 years	Standard (Mepilex, petrolatum gauze)	7.3	7.0 (4.1)	9.0 (–23.3)	8.4 (–15.1)
H06	18/Male	Central chest	4–5 years	Helicoll	30.0	17.0 (43.3)	8.2 (76.7)	3.3 (89)
		Left medial shoulder	4–5 years	Standard (Vaseline gauze)	12.8	7.8 (39.1)	0.7 (94.5)	8.8 (31.3)
H07	15/Male	Left lower back	6 years	Helicoll	ND‡	ND	ND	ND
		Right lower back	6 years	Standard (Vaseline gauze)	ND‡	ND	ND	ND
H08	8/Female	Right neck	3 years	Standard (Mepilex, Mepitel)	11.1	N/A*	N/A	N/A
		Left neck	1 year	Helicoll	9.8	N/A*	N/A	N/A
H09	12/Male	Left anterior lower leg	1 year	Helicoll	104.5	69.3 (33.7)	52.0 (50.2)	51.4 (50.8)
		Right posterior lower leg	1 year	Standard (Mepilex, Mepitel)	64.3	53.5 (16.8)	44.1 (31.4)	59.1 (8.1)
H10	13/Female	Left thigh superior	>1 year	Helicoll	2.8	3.8 (−35.7)§	N/A	N/A
		Left thigh inferior	>1 year	Standard (Mepilex)	0.7	0.3 (57.1)	N/A	N/A

ND, not done; N/A, not applicable (study visit did not occur).
*Subject did not return for follow-up visits.
†According to subject, wound healed completely and then reopened.
‡Unable to obtain accurate wound dimensions because of location.
§Wound infected; subject discontinued from study.

visits, target wound size, wound pain, pruritus, and burning and stinging were assessed. Wound cultures were obtained as needed. Adverse events and changes to concomitant medications were also elicited.

Wilcoxon signed-rank and rank sum tests were performed. All tests were two-sided, with $p < 0.05$ considered statistically significant.

RESULTS

Of the 10 subjects who consented (Table 2), 3 did not complete the study (Fig. 1). Subject H03 terminated the study early because she preferred more frequent dressing changes. Subject H08 was lost to follow-up because of unrelated health problems. Subject H07 was removed from the final analysis because of inconsistent application of the type I collagen skin substitute to the treated site. Subject H10 developed a wound infection midway through the study and study participation was terminated early for systemic treatment, although we included subject H10 in our analysis.

Six of seven (85.7%) evaluable subjects showed a positive response to type I collagen treatment. Three subjects (42.8%) (H01, H04, H05) had complete wound reepithelialization (Fig. 1), although two of these wounds reopened after type I collagen treatment was stopped. Three subjects (H02, H06, H09) demonstrated a marked decrease in wound size but did not achieve complete wound reepithelialization (Tables 2 and 3). Three of four (75%) control wounds exhibited no significant healing. According to the Wilcoxon signed-rank test, the percentage improvement was significant for wounds treated with type I collagen skin substitute ($p = 0.03$) and the percentage improvement was not significant for wounds treated with standard dressings ($p > 0.99$).

The average ratings for pruritus and pain were significantly lower immediately after application of the type I collagen skin substitute (Table 4). Before application, the average pruritus score was 1.28 and the average pain score was 1.33. After application, the pruritus score decreased to 0.39 ($p = 0.004$) and pain score decreased to 0.67 ($p = 0.004$). Ratings for burning and stinging in the group receiving type I collagen decreased as well, but did not reach statistical significance. Control wounds demonstrated no change in scores.

The most common adverse events were pain during dressing changes ($n = 5$), pruritus at a target wound

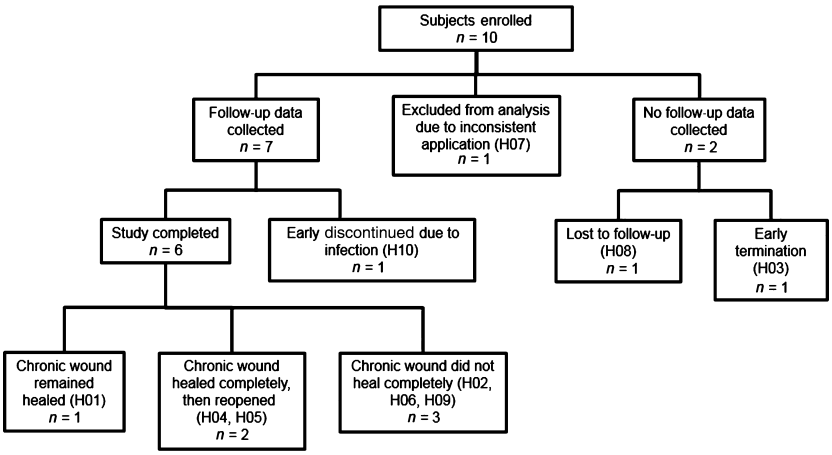

Figure 1. Subject enrollment algorithm.

(n = 4), and foul-smelling exudate (n = 2). Pain during dressing changes was most often due to the Adaptic contact layer or the sterile gauze outer layer sticking to the wound and not related to the type I collagen skin substitute. After these adverse events, the contact layer was changed from Adaptic to Mepitel, which helped according to patient feedback.

Microbiology studies revealed greater bacterial burden of different organisms in most of the treatment group than in the control group (Table 5). Although two subjects developed wounds with foul-smelling exudate (H02 and H03), neither reported fever, surrounding erythema, or pain. Despite greater bacterial load demonstrated by wound cultures, we concluded that the exudative wounds were not infected and had greater bacterial colonization.

No serious adverse events were reported.

DISCUSSION

Helicoll, a reconstituted natural purified bovine type I collagen skin substitute, significantly improved healing of chronic wounds in individuals with RDEB. It was well tolerated, convenient, and easy to apply in the medical office or at home, without any significant side effects. Type I collagen skin substitutes have been reported to promote wound healing by creating a scaffolding for keratinocyte migration. Type I collagen may also decrease the activity of collagenase and matrix metalloproteinases and may act as an anti-inflammatory agent by binding proinflammatory cytokines (7,18).

Most (85.7%) evaluable subjects saw improvement, and 42.8% had complete reepithelialization. Our results were similar to those of the Apligraf study, which showed that 44% of chronic EB wounds had complete healing after 6 weeks (4). A systematic analysis of multiple collagen products used for diabetic foot ulcers revealed that 58% of collagen-treated wounds healed completely (7), although the rate of epithelialization ranged from 0% to 100%. The variability in rates of epithelialization indicates a need for additional clinical research on the efficacy of collagen skin substitutes.

TABLE 3. *Percentage Improvement*

	Helicoll	Standard
	Mean ± standard deviation	
Baseline to week 1	30.9 ± 40.4, n = 7	29.3 ± 23.5, n = 4
Baseline to week 4	69.5 ± 34.4, n = 3	34.2 ± 58.9, n = 3
Baseline to week 8	63.0 ± 31.9, n = 3	8.1 ± 23.2, n = 3

In our study, two of the three chronic wounds that healed completely with the type I collagen treatment redeveloped after treatment was stopped. We believe that the reopening of the wound is likely due to the chronic nature of RDEB. Before treatment, these wounds were consistently nonhealing. Minor trauma to individuals with RDEB can cause significant damage to their skin. To prevent future wounding, correction of the underlying genetic defect would be required (19).

Pruritus can exacerbate RDEB wounds; scratching can impair wound healing and cause new wounds (2,20). Current treatments for pruritus in RDEB wounds are limited and are a clinically unmet need (20). We found that wounds treated with the type I collagen skin substitute exhibited a significant decrease in pain and pruritus immediately after wound dressing application. Anecdotally, many

TABLE 4. *Average Pruritus, Pain, and Burning and Stinging*

	Pruritus		Pain		Burning and stinging	
	Score (0–4)	p-Value	Score (0–5)	p-Value	Score (0–3)	p-Value
Before treatment	1.28	0.004	1.33	0.004	0.67	0.22
After treatment	0.39		0.67		0.33	

TABLE 5. *Target Wound Bacterial Colonization*

Helicoll			Standard treatment		
Patient	Load	Species	Patient	Load	Species
Increased bacterial load					
H02	2+ to 3+	*Staphylococccus aureus*	H05	0 to 1+	Diphtheroids
H02	0 to 4+	Beta *Streptococcus*	H05	0 to 1+	Beta *Streptococcus*
H04	1+ to 2+	*S. aureus*	H06	0 to 3+	Coagulase-negative *Staphylococcus*
H04	0 to 1+	Mixed skin flora			
H05	0 to 3+	Diphtheroids			
H05	1+ to 3+	*Pseudomonas aeruginosa*			
H05	Rare to 3+	Proteus			
H06	2+ to 3+	*S. aureus*			
H06	0 to 3+	Coagulase-negative *Staphylococcus*			
H07	0 to 3+	Gram-negative rods			
H07	0 to 2+	Coagulase-negative *Staphylococcus*			
H10	0 to 2+	Mixed skin flora			
H10	1+ to 3+	*P. aeruginosa*			
Decreased bacterial load					
H02	3+ to 1+	*S. aureus*	H05	2+ to 1+	Proteus
H02	2+ to 0	Mixed skin flora	H05	2+ to 1+	*P. aeruginosa*
H05	2+ to 0	Mixed gram-positive organisms	H05	2+ to 0	Mixed gram-positive organisms
H07	2+ to 1+	*S. aureus*	H06	4+ to 3+	*S. aureus*
No change					
H10	2+	*S. aureus*	H07	2+	*S. aureus*

No comparison available: H01, H09, H10 (standard treatment).

subjects described the type I collagen dressings as "cold," and this sensation may be responsible for the immediate pruritus relief. Cooling temperatures have been described as helpful for pruritus because of their activation of transient receptor potential melastatin channels (21,22), although we cannot exclude a direct mechanism.

Pruritus at wound sites was noted at later time points. We speculate that this pruritus was secondary to effective wound healing (20). Healing wounds in individuals with EB have been described as significantly more pruritic than nonwounded skin (23).

In wounds that received type I collagen, we observed greater bacterial colonization, with no impairment of wound healing. Bacterial colonization of wounds in individuals with EB is common and not necessarily indicative of infection (24–26). Bacterial colonization may have increased because the frequency of dressing changes at the target wound was decreased to weekly. One subject (H10) developed a superficial wound infection on a treated wound that responded to oral antibiotics; the underlying cause of the infection was unclear.

There were several limitations to this study. The sample size was small because of the rarity of RDEB. We amended the protocol to add a second wound per patient to serve as a control wound, so the first four subjects do not have a comparison wound. We did not assess hemoglobin levels. Severe anemia can lead to poorer wound healing (2). A patient or investigator bias may exist because the study was not blinded. Although we attempted to match treatment and control wounds of similar sizes, wound size was not formally standardized. Each patient self-reported the duration of their wounds, which may not have been accurate.

In individuals with RDEB, reconstituted natural purified type I collagen skin substitute helped the healing of chronic wounds and decreased wound pain and pruritus at the time of application, but larger controlled studies of collagen dressings in EB wounds

are necessary. This skin substitute provides another option in our EB wound care arsenal.

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