TREATMENT WITH NOVEL HYBENX® ROOT CANAL CLEANSER SUGGESTS BIOFILMS BLOCKED HEALING OF HUMAN WOUNDS: CASE SERIES

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Pathogenic biofilms are considered a potential major obstacle to healing chronic wounds. The six-month evaluation period in this study assessed the effect of a single treatment of the HYBENX® (HYB) Root Canal Cleanser on chronic wounds, especially its ease of use, safety, potential for shortening duration of Inflammatory Phase, and promotion of granulation. HYB gel was applied to the wound bed and periwound skin for 10 seconds. Gel was removed by cotton gauze pads and low pressure saline rinsing. Standard wound dressings based on wound etiology, location, and exudate characteristics were applied. Duration of these seven HYB-treated wound cases (two pressure ulcers, one surgical wound, four lower extremity ulcers—a venous reflux-associated calf ulcer, one diabetic foot ulcer, and two animal-induced wounds—brown recluse spider and bovine bite) ranged from 12 days to 10 years. Three cases had sinus tracts. After HYB application, sinus tract closure occurred at 7, 16, and 21 days. The 10-year recalcitrant wound from brown recluse spider bite healed in 97 days. Diabetic foot ulcer responded to HYB treatment plus additional strategies and healed in six months. These data support the hypotheses that pathogenic biofilm actively prevents the healing of chronic wounds, and biofilm can be disrupted with a single HYB treatment.

Chronic wounds present an increasing challenge to healthcare systems worldwide. Non-healing wounds have a disproportionately high treatment cost and are associated with high morbidity. In certain populations —notably the increasingly large diabetic population — such wounds can dramatically increase the amputation rate and mortality risk (1).

Key to meeting the challenge of chronic wounds is our emerging understanding of the complexity of the wound healing process (2). Necrotic tissue, slough, wound bioburden (3), and insufficient host molecular and cellular components (e.g. growth factors, protease inhibitors, extracellular matrix, angiogenic factors, antioxidants, oxygen saturation) can disrupt the normal healing process (2). Furthermore, the roles of the human microbiome— microorganisms which live on or within the human body— and of pathogenic bacteria and fungi in the non-healing of chronic wounds remain controversial (4-6). Bacteria in particular, but also a number of fungal species are now known to thrive on wounds in a polymicrobial community called "biofilm" (7) instead of a free-floating infection of a single species (5). Biofilms detected by scanning electron microscope were significantly more common in chronic wounds (60%) than acute wounds (6%) (3, 8).

Biofilm communities are highly resistant to host immune defenses and to conventional wound treatment modalities, including antiseptic cleansers and topical and systemic antibiotic therapies, for several key reasons (9, 10). Biofilm organisms exist in multiple "zones" within a complex extracellular

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polymeric substance (EPS) composed of proteins, polysaccharides and glycoproteins (5). EPS is strongly adherent to host tissue, contains channels for nutrients and waste removal, and can stimulate inflammation (5). Biofilm organisms comprise about 20% of the biofilm, and have various levels of metabolic activity and distinct roles, depending on their location (10, 11). For example, bacteria near the wound bed have very low metabolic activity and thus are very tolerant of many antimicrobials (11). Furthermore, biofilm bacteria are also capable of sharing resistance-conferring genes through direct, inter-species mRNA transfer inside the anatomic confines of the biofilm matrix. Due to the increasing prevalence of resistant organisms in the community, the risk of refractory, highly resistant biofilm populations has become a reality (12).

Such biofilm communities are known to alter wound conditions to favor the proliferation and success of the biofilm organisms, which subverts several wound conditions compatible with healing (5). An 11-clinician panel from the wound therapeutics research community strongly agree that biofilms play a major role in an increasing number of complex non-healing, chronic wound cases (13). Furthermore, they suggest that clinicians should consider different treatment paradigms that can lead to finding cost-effective, safe methods for removing biofilm in non-healing wounds (4). Disruption and removal of dead tissue, biomass, and biofilm from the wound bed is currently undertaken by various types of debridement.

Debridement methods include autolytic, biosurgery (e.g. larval therapy), enzymatic, hydrosurgical, mechanical, surgical, and ultrasonic (3). Each debridement method has specific advantages and disadvantages. The choice of debridement technique depends on the patient's wound type, most effective debridement method, anatomical site of wound, amount of necrotic tissue, patient's comorbidities, and preference of the patient (adherence, accessibility) (3). A recent position document suggests that management of biofilms may need to include debridement, cleansing, and antimicrobial treatment at regular intervals to prevent reformation (14).

Despite use of debridement methods and standard wound management, unsuccessful healing of many chronic wounds, especially diabetic foot ulcers, are associated with amputation rates as high as 21% (15). Since the standard of care did not promote healing in many chronic wounds, we hypothesized that the current modalities for removing biofilm were not sufficient in all chronic wound cases and that biofilms still hindered the progression of wound healing. In our search for efficacious agents against biofilms, we noted a substantial similarity of biofilm-related complications in non-healing wounds with biofilm-associated oral pathologies, including dental abscesses, plaque, and periodontitis (15).

HYBENX® (HYB) Root Canal Cleanser is an FDA-cleared dental device, and is used to cleanse infections from the root canal. The same product, marketed as HYBENX® Oral Tissue Decontaminant in Europe and Canada as a class I medical device, is used as an oral adjunctive debridement agent to treat biofilm-associated oral pathologies (16-19). HYB is a hygroscopic and denaturing sulphur-containing solution that denatures, desiccates, and coagulates the biofilm matrix and microbes (20). It can induce a protective layer of denatured, coagulated tissue debris over the ulcer wound bed which subsequently dissolves (19, 20). Furthermore, in a prospective, randomized trial, patients with recurrent aphthous stomatitis who received a single HYB application on the oral ulcer showed a significantly greater decrease in pain score on days 1 and 2 than those treated with multiple SaliCept applications (20). The single HYB application healed the oral ulcer in 50% of the patients with recurrent aphthous stomatitis by day 8, similar to the 44% healing rate of patients treated with multiple SaliCept applications (20). Both treatments were deemed safe as the adverse events appeared unrelated to treatment (20) .

Thus, we hypothesized that HYB used in dental offices to cleanse root canals in the US and to remove biofilms from teeth, tooth pulp, and periodontal tissues in Europe and Canada provides a distinct mode of action and may improve efficacy in at least some chronic wounds in our patients. Safety data were considered carefully, and no evidence was identified that might raise concern

over patient or clinician safety in the use of HYB as a topical treatment modality for human wounds (20, 21). Use in the oral cavity on oral lesions (20) would be expected to have at least as high uptake as an application to wounds located on extremities. Clearly, a series of investigations in patients with chronic wounds represents an opportunity to compliantly utilize an approved modality in an off-label application that could possibly improve the rate of healing chronic wounds.

In June of 2014, my team began a series of investigations with HYB as a treatment modality for use in chronic wound therapy in a hospital-based outpatient clinic population. These investigations were motivated by a need for improved strategies for addressing biofilm-related challenges in wound healing, promising results of HYB in treating biofilm-associated oral diseases, and the poor outcomes associated with standard modalities in chronic wounds.

MATERIALS AND METHODS

Patients

Patients with chronic wounds of varying etiologies were offered the opportunity to receive an application of the FDA-approved oral decontaminant, HYB. Over a six-month period, HYB was applied to wounds of varying etiologies in more than 30 patients, and observations were made in respect to changes in wound characteristics, including granulation tissue quality, velocity, and coverage, bioburden characteristics, epithelialization, wound drainage, periwound skin, and patient experience.

HYBENX® application

HYB gel was obtained from Epien Medical, Inc. (St. Paul, Minnesota). Our application strategy was simple. Loose debris was removed manually, and the application area was dried as much as possible. A mild topical anesthetic (EMLA cream, LET (lidocaine, epinephrine and tetracaine gel), or viscous lidocaine) was applied to the wound bed and surrounding edge. HYB gel was applied to the center of the wound bed from a 3cc syringe, and quickly spread with a gloved finger over the full wound bed and a variable width of periwound skin (range: 0.5 to 5 cm from wound bed). After 10 seconds, the HYB gel was wiped off with cotton gauze pads and was followed by low pressure saline rinsing of residual product. Total application and removal time was less than one minute. Following HYB application, standard wound dressings were applied as appropriate, with dressing selection based on wound etiology, location, and exudate characteristics. Follow-up of the patients' wounds were carried out at standard intervals.

RESULTS

We chose to present seven HYB-treated wound cases (two pressure ulcers, one surgical wound, four lower extremity ulcers—a venous reflux-associated calf ulcer, one diabetic foot ulcer, and two animal-induced wounds—brown recluse spider and bovine bite) for the following reasons. The cases represented the broad range of applications for which HYB was found to be useful. Secondly, the photographic documentation in these cases was effective in visually demonstrating the typical changes in wound bed characteristics seen following HYB application.

Patient comfort was not a significant issue as long as common methods for mild topical anesthesia were used prior to HYB treatment. Application of mild topical anesthesia successfully prevented the brief discomfort that occurred during application, and the following 2 minutes. Generally, HYB application was very well tolerated, and any discomfort was minor and brief (< 5 min).

Pressure ulcers

Case 1. A 40-year-old morbidly obese, non-diabetic male presented with a pressure ulcer on his left buttock (Fig. 1A). Prior care before referral to our clinic (neosporin, duoderm) had failed to maintain or stimulate the healing process of the pressure ulcer over a 60-day period. The wound was initially characterized by essentially 100% fibrin and slough coverage. After a single HYB application, which was associated with brief, minor pain, topical therapy was continued (duoderm), along with a continuation of offloading measures that had already been instituted. At the one-week follow up visit, substantial improvement in granulation coverage was noted (Fig. 1B). On the first and second weekly

post-application visit, a clear improvement in proliferative activity was noted (Fig. 1B, C). The wound went on to complete closure within 49 days of HYB application in spite of sub-optimal offloading due to the patient's occupational circumstances (Fig. 1D). The patient also reported that he had noticed significant improvement in wound pain following HYB application. No excisional debridement was required following HYB application.

Case 2. A 33-year-old female with spina-bifida, severe scoliosis and paraplegia presented with a stage IV pressure ulcer at the thoracolumbar spine area. It measured 7.6cm x 7.5cm x 5cm (depth) in size and had a 4 cm deep sinus tract which extended to lumbar spine bone, with evident osteomyelitis (Fig. 2A). The healing process had not been adequate over the prior 10 months despite the following treatments: supine and seated offloading, intensive nutritional support, serial debridement, and application of various dressings, which included Hydrofera Blue, Calcium Alginate with Silver, Silvasorb, Medihoney Alginate, Santyl, and negative pressure wound therapy (NPWT) with granufoam sponge, depending on the concurrent wound characteristics. Treatment involved a single HYB application. The patient was also treated with culture-directed antibiotic therapy for osteomyelitis, offloading measures, aggressive nutritional support, Hydrofera Blue, NPWT, Medihoney Gel, Santyl, Fibracol, Prisma, Endoform, 10 applications of Grafix Core, Iodosorb,

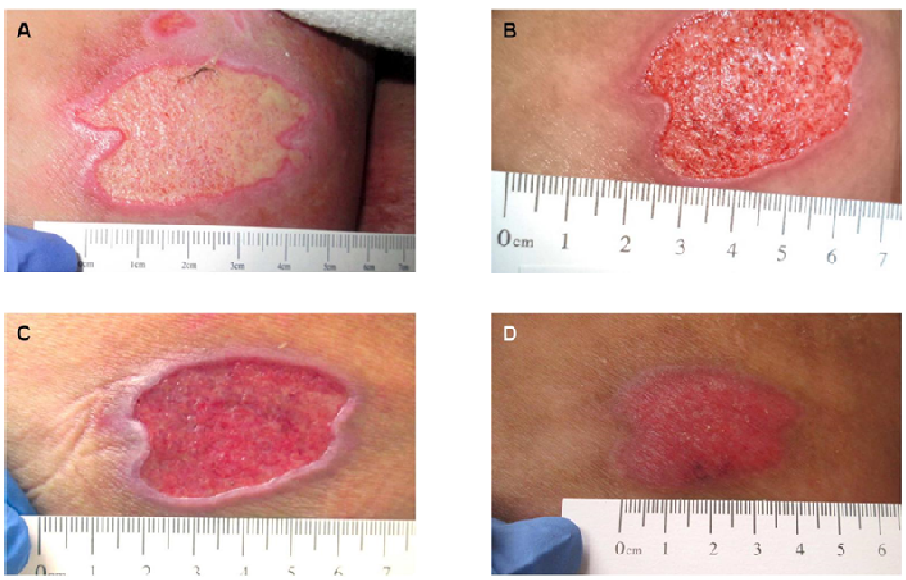

Fig. 1. *Pressure ulcer on left buttock of case 1, a 40-year-old morbidly obese, non-diabetic male.* ***A****) Wound prior to HYB application.* ***B****) One week post-HYB, note granulation coverage.* ***C****) Two-week visit.* ***D****) 49 days post HYB.*

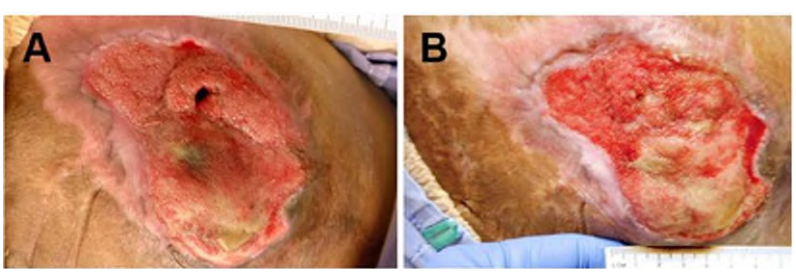

Fig. 2. *Stage IV pressure ulcer in case 2, a 33-year-old female with spina-bifida, severe scoliosis and paraplegia.* ***A****) Wound prior to HYB application.* ***B****) One month, note closure of sinus tract.*

and Medihoney Colloid. Of note, resolution of a previously worsening, 4 cm deep sinus tract to bone was noted within 7 days of HYB application. Over the subsequent three weeks, granulation coverage improved, and edge characteristics became more favorable to closure (Fig. 2B). The sinus tract remained closed. After 16 months, the wound depth has decreased to 0.2cm and closure was at 78%. The patient continues to await arrangements for plastics closure. Clinically, rapid resolution of the deep sinus tract was felt to be largely due to effects of HYB application; i.e. eradication of sinus tract biofilm, and stimulation of granulation tissue.

Surgical wound

Case 3. A 72-year-old female presented with a non-healing surgical T-spine wound at four days after diagnosis of wound dehiscence (surgical closure 21 days prior). The wound bed initially showed a paucity of granulation, heavy slough, and very poor edge characteristics (Fig. 3A). The patient was also diagnosed with severe protein calorie malnutrition, chronic kidney disease, and hyperlipidemia. Treatment involved nutritional repletion and a single HYB application followed by packing with Aquacel Ag ribbon every other day for 6 weeks, which was followed by five sequential Grafix Core Amniotic

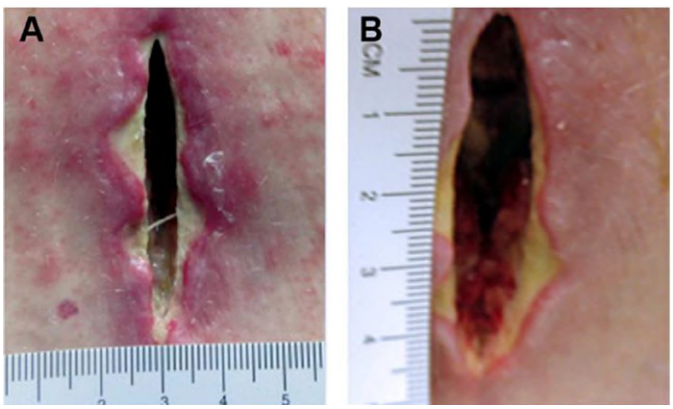

Fig. 3. *Non-healing surgical T-spine wound in case 3, a 73-year-old female.* ***A****) Wound prior to HYB application.* ***B****) Two weeks post-HYB treatment.*

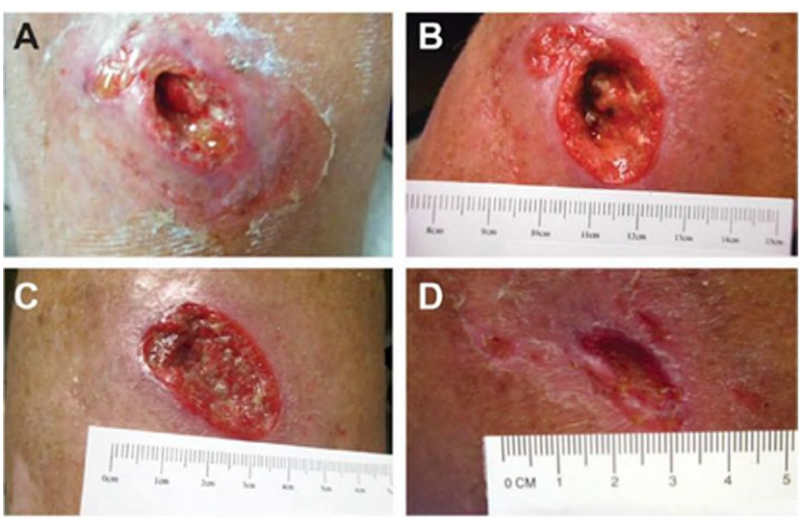

Fig. 4. *Fracture-induced lower extremity ulcer in case 4, a 62-year-old female with venous insufficiency.* ***A****) Wound prior to HYB application.* ***B****) One week post-HYB treatment, note granulation coverage.* ***C****) Three week visit, note closure of sinus tract.* ***D****) 90 days.*

Allograft applications. The wound bed showed a very rapid transition to proliferative status to nearly 100% granulation within two weeks (Fig. 3B). The wound had healed (complete closure) by 77 days.

Lower extremity ulcers

Case 4. A 62-year-old female presented with a calf ulcer and 2.5 cm sinus tract post tibial plateau fracture. The ulcer was at the site of a hematoma associated with the injury, and had been present for 45 days. The patient had a history of venous reflux and prior treatments (Hydrofera Blue) (Fig. 4A). The patient received the described HYB treatment, and Hydrofera Blue alternating with Iodosorb wound dressings were applied. After one week, the wound showed significant improvement in granulation (Fig. 4B). At three weeks post-HYB, sinus tract closure was achieved and granulation coverage was nearly 100% (Fig. 4C). Full healing was apparent at 90 days post-HYB (Fig. 4D). The extended course was chiefly due to the patient's incipient venous insufficiency.

Case 5. A 72-year-old male presented a diabetic foot ulcer, overlying a 1st metatarsophalangeal (MTP) gouty tophus, of three months duration (Fig. 5A). The patient has Type II Diabetes, gout, and secondary lymphedema. Prior treatments had included two Hydrofera Blue applications for one week, then Iodosorb three times per week (TIW) until HYB application. After a single HYB

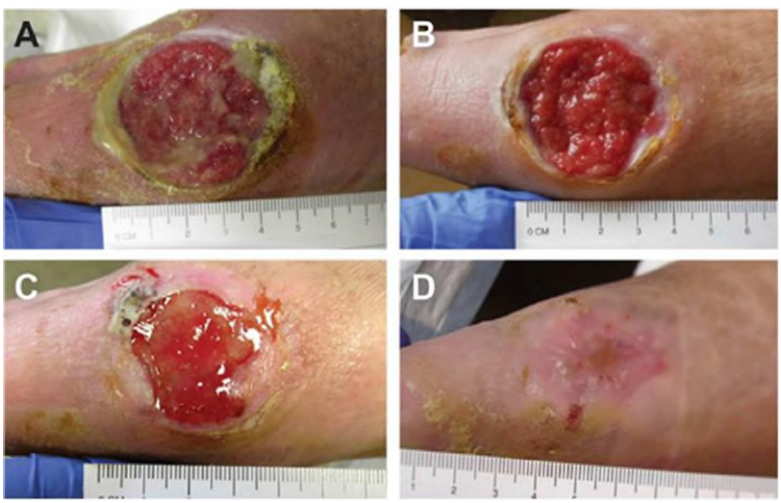

Fig. 5. *Diabetic foot ulcer in case 5, a 72-year-old male with type II diabetes, gout, and secondary lymphedema.* ***A****) Wound prior to HYB application.* ***B****) 14 days, note granulation coverage.* ***C****) 22 days.* ***D****) Six months.*

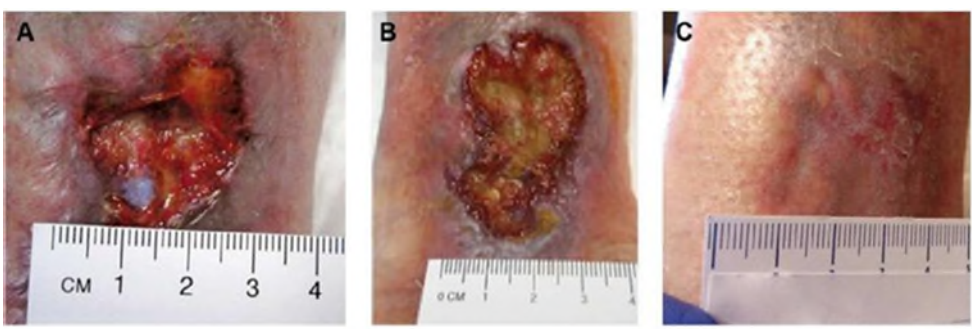

Fig. 6. *Brown recluse spider induced ulcer of 10-year duration in case 6, a 59-year-old female.* ***A****) Wound prior to HYB application.* ***B****) Three weeks post-HYB.* ***C****) 97 days post-HYB.*

application, the patient received the following treatments listed in sequential order: Iodosorb (TIW for three weeks), Endoform (TIW for three weeks), Hydrofera Blue (TIW for four weeks), Prisma (TIW for one week), Calcium Alginate with Ag for two weeks, and Fibricol for two weeks. The patient was also treated with NPWT, hyperbaric oxygen therapy (2.0 ATA for 90 minutes, 54 treatments), live-cell placental allograft (Grafix Core), aggressive diabetes management, and compression dressings. Improved granulation coverage and quality, and improved edge characteristics were observed at 14 days (Fig. 5B). Epithelialization was very active at the wound edge at 22 days post-application (Fig. 5C). Final closure was noted after six months (Fig. 5D). Note the diabetic foot ulcer quickly progressed to 100% granulation and showed strong improvement in edge characteristics following HYB application, which has been a typical finding.

Animal-induced wounds in lower extremities

Case 6. A 59-year-old female with chronic recurrent right medial calf ulceration at the site of self-reported "Brown Recluse Spider Envenomation" (Fig. 6A). The ulcer had been present for 10 years at the time of initial evaluation, and prior treatments in our facility had included Medihoney Alginate weekly for 4 weeks, followed by Hydrofera Blue for one week. Patient had evident venous reflux, which was at least partially responsible for non-healing. After a single HYB application, the wound was treated with Hydrofera Blue alternating with Iodosorb, followed by every-other-weekly application of EpiFix cryopreserved amniotic allograft. At three weeks post-HYB application, drainage had significantly diminished, wound depth was reduced, and better edge integration was noted (Fig. 6B). Complete healing occurred on day 97 (Fig. 6C), despite the more than 10-year duration of the ulcer prior to initiating HYB treatment.

Case 7. A 62-year-old female presented with a non-healing bovine bite wound on her right thigh of two-month duration (Fig. 7A). Comorbidities included hypertension, hyperlipidemia, chronic obstructive pulmonary disease, and obesity. Prior treatments included 23 days of topical therapy with mupirocin ointment prescribed by the patient's primary physician, with no improvement. A debridement was performed on the initial visit, one week prior to HYB application. Treatment involved a single HYB application along with NPWT and ciprofloxacin. In this case, rapid improvement was observed in granulation coverage, and more notably, rapid closure of a 4 cm sinus tract in 16 days (Fig. 7B). NPWT was clearly helpful, but closure rate was much greater than would be expected with NPWT alone, as the wound showed 99% closure in 14 days after HYB application (Fig. 7B). The patient moved and was lost to follow-up after the 14-day visit.

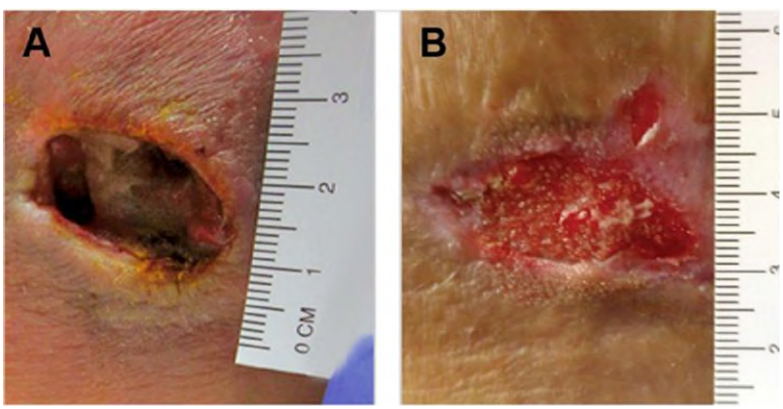

Fig. 7. *Bovine bite induced ulcer of two-month duration on case 7, a 62-year-old female.* ***A)*** *Wound prior to HYB application.* ***B)*** *Two weeks post-HYB treatment.*

DISCUSSION

The three principal challenges in wound care are to shift a wound as rapidly as possible from the Inflammatory Phase of wound healing to the Proliferation Phase, to maintain appropriate proliferative characteristics through obliteration of dead space, and to finally reach 100% epithelialization. In our hospital-based wound clinic population, we frequently manage chronic wounds, such as lower extremity venous ulcers and diabetic foot ulcers of more than one to two years duration. These patients have generally undergone extensive prior treatment with various wound care modalities, including debridement, topical therapies, specialty dressings, multiple courses of antibiotic therapy, prior attempts at revascularization, and compression modalities. One possible explanation for recalcitrant chronic wounds is the presence of a pathogenic biofilm that is not sufficiently controlled by the provided debridement and antimicrobial therapies (4, 8, 22). Biofilms on decubitus ulcers, diabetic foot ulcers, non-healing surgical wounds, and venous leg ulcers contained multiple bacterial and fungal species that differed only slightly among wound types and patient demographics (23).

Recently, an 11-clinician panel from the wound therapeutics research community provided clinical recommendations for detection and treatment of biofilm in various types of non-healing chronic wounds, including dehisced surgical wounds, diabetic foot ulcers, pressure ulcers, and venous leg ulcers (4). Mechanical debridement was strongly recommended for non-healing burns, diabetic foot ulcers, pressure ulcers, and venous leg ulcers and weakly recommended for dehisced surgical wounds (4). They strongly recommended the use of antimicrobial dressings for non-healing burns, diabetic foot ulcers, pressure ulcers, and dehisced surgical wounds and weakly recommended them for venous leg ulcers (4). The panel did not indicate specific products, which may vary in efficacy for different types of non-healing wounds, because the shared evidence and experience was not sufficient to support specific recommendations (4). The panel suggests that clinicians should consider different treatment paradigms that include finding cost-effective, safe methods for removing biofilm in other disease states and assessing the methods in non-healing wounds (4), which encourages case studies of adjunctive debridement agents.

The purpose of this six-month evaluation period of the HYB Root Canal Cleanser was to assess its effect on chronic wounds, especially its ease of use and safety, and the evident duration of the Inflammatory Phase and shift to Proliferation Phase. The seven presented cases indicated that a single HYB application reduced the need for subsequent extensive debridement and facilitated a more rapid transition to proliferative characteristics and closure than any other single modality. It also improved overall comfort of the patients, similar to HYB-treated patients with aphthous stomatitis (20, 24) or patients with abscesses (19).

We have found HYB treatment helpful in wounds of various etiologies, including abscess wounds, arterial ulcers, complicated traumatic wounds, diabetic foot ulcers, pressure ulcers, surgical dehiscence and non-healing surgical wounds, venous ulcers, vasculitic ulcers, and wounds related to osteomyelitis with exposed bone.

By utilizing HYB after the initial debridement and diagnostic procedures, we often noted a rapid transition to 100% granulation coverage. These data are consistent with the complete resolution of inflammation in the HYB-treated periodontal abscesses in all five cases within 16 to 30 days (19). Inflammatory characteristics, including periwound inflammation, excessive wound drainage, and inflammatory debris accumulation resolved relatively quickly in these seven cases after HYB application. As in HYB-treated oral biofilm-related pathologies (19), anti-inflammatory mechanisms of HYB probably included the denaturation, desiccation, and removal of the biofilm bacterial populations and the polysaccharide matrix, which harbors pro-inflammatory cytokines and matrix metalloproteases (MMPs). MMPs are proteases known to be deleterious to stable fibroblast proliferation and collagen deposition, and overproduction of these enzymes is typical of wounds in the inflammatory state.

Edge characteristics have also consistently improved. Edge transition is an important determinant of wound bed proliferation, and ultimately epithelialization. We found that HYB

application is associated with more favorable transition characteristics from periwound to wound bed, favoring a saucer-like characteristic most compatible with rapid wound closure.

No deleterious effects were observed on the wound bed or periwound skin characteristics, such as hypersensitivity, necrosis, diminished granulation velocity, or reduced keratinocyte migration, in agreement with patients treated with HYB for aphthous stomatitis (20). These findings agree with abundant dental experience with the product (25), where HYB has been well tolerated, and induced less side effects in oral applications in humans than in controls (21), and veterinary medicine. In contrast to the pain induced by surgical debridement, patient comfort has not been a significant issue, as long as common methods for mild topical anesthesia are used prior to HYB application. Generally, HYB application is very well tolerated, and any discomfort has typically been quite brief. In a prospective study of patients with oral lesions, HYB treatment significantly reduced pain from aphthous stomatitis on days 3 to 6 but not the full study (days 1-6) (24). Mild discomfort had resolved by day 3 in HYB-treated patients with abscesses (19).

Our observations in this patient population has established HYB as a clear breakthrough in clinical wound treatment: its use in our experience has been associated with relatively rapid progression to 100% granulation tissue and subsequent stable closure in patients who had previously failed to heal in spite of standard-of-care treatment.

It is the opinion of our team that our successes with HYB are most likely due to its unique efficacy in addressing a fundamental impediment to wound healing common to all problem wounds; namely, the presence of complex biofilms. Conventional approaches to eradication of biofilm have often failed *in vitro* and *in vivo*, for the following reasons:

- Resistance of biofilm organisms to standard culture techniques utilized to identify free-living (planktonic state) organisms;
- Sharing of antibiotic resistance genes between biofilm species;
- Self-shielding of biofilm organisms through the production of extracellular polymeric substance (EPS), a matrix of high-molecular-weight saccharide polymers that shields biofilm organisms from conventional attack by other cleansing agents, antiseptics, and antibiotics;
- Physical attachment of the biofilm matrix to host tissue by the EPS matrix, rendering biofilm *physically difficult to remove.*

In contrast, HYB represents a true innovation, as it consistently destroys both the biofilm matrix and biofilm organisms in a definitive biophysical manner: denaturation and desiccation (20). Development of bacterial resistance is considered impossible. Denaturation and desiccation destroys biofilm matrix components structurally and causes the bacteria and fungi to osmotically implode, while the application of HYB appeared to leave the host tissue, including fibroblasts and the developing collagen matrix, unharmed. We feel that this is a fundamentally sound approach to biofilm management that is universally applicable to the biofilm problem in wound healing.

The role of pathogenic biofilm in chronic wound healing has been controversial. The HYB-induced denaturation and removal of biofilm from non-healing wounds stimulated steady progression to granulation and epithelialization in the wound. Based on currently available evidence, we feel it is likely that the HYBENX® Root Canal Cleanser will become a standard treatment modality in wound care. These data strongly support the hypothesis that pathogenic biofilms play an active role in preventing the healing of chronic wounds.

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REFERENCES

1. Sen CK, Gordillo, GM Roy S, et al. Human skin wounds: a major and snowballing threat to public

health and the economy. Wound Repair Regen 2009; 17:763-71.
2. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle) 2015; 4:560-82.
3. Percival SL, Suleman L. Slough and biofilm: removal of barriers to wound healing by desloughing. J Wound Care 2015; 24:498, 500-493, 506-410.
4. Bianchi T, Wolcott RD, Peghetti A, et al. Recommendations for the management of biofilm: a consensus document. J Wound Care 2016; 25:305-17.
5. Percival SL, McCarty SM, Lipsky B. Biofilms and wounds: an overview of the evidence. adv wound care (New Rochelle) 2015; 4:373-81.
6. Withycombe C, Purdy KJ, Maddocks SE. Micro-management: curbing chronic wound infection. Mol Oral Microbiol 2017; 32:263-74.
7. Kalan L, Loesche M, Hodkinson BP, et al. Redefining the chronic-wound microbiome: fungal communities are prevalent, dynamic, and associated with delayed healing. MBio 2016; 7:
8. James GA, Swogger E, Wolcott R, et al. Biofilms in chronic wounds. Wound Repair Regen 2008; 16:37-44.
9. Hoiby N, Bjarnsholt T, Moser C, et al. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin Microbiol Infect 2015; 21Suppl 1:S1-25.
10. Maci MD, Rojo-Molinero E, Oliver A. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin Microbiol Infect 2014; 20:981-990.
11. Sun F, Qu F, Ling Y, et al. Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies. Future Microbiol 2013; 8:877-86.
12. Visavadia BG, Honeysett J, Danford MH. Manuka honey dressing: An effective treatment for chronic wound infections. Br J Oral Maxillofac Surg 2008; 46:55-56.
13. Bianchi T, Peghetti A, Pomponio G. Biofilm: it is time for consensus. J Wound Care 2015; 24:291.
14. Societies, WUoWH. Management of Biofilm. In: Proceedings of the Wounds International. Florence 2016.
15. Remington AC, Hernandez-Boussard T, Warstadt NM, et al. Analyzing treatment aggressiveness and identifying high-risk patients in diabetic foot ulcer return to care. Wound Repair Regen 2016; 24:731-36.
16. Lombardo G, Pardo A, Fiorino A, Corrocher G, Urbani G. Full-mouth ultrasonic debridement in combination with HYBENX® Oral Tissue Decontaminant as an initial approach to the treatment of chronic periodontitis. Quintessence International 2012; 28:5-14.
17. Lauritano, D, Girardi, A, Carinci, F. The efficacy of HYBENX(R) oral tissue decontaminant for periodontal disease treatment: a case series study. Int J Advances in Case Rep 2015; 2:405-8.
18. Bracke J, Basara M, Savord E, Dunaway A, Watkins M. Pilot evaluation of a simple adjunctive method for improved removal of oral biofilm during conventional scaling and root planing therapy. J Biol Regul Homeost Agents 2015; 29:6-9.
19. Pini-Prato G, Magnani C, Rotundo R. Treatment of acute periodontal abscesses using the biofilm decontamination approach: A case report study. Int J Periodontics Restorative Dent 2016; 36:55-63.
20. Porter SR, Al-Johani K, Fedele S, Moles DR. Randomised controlled trial of the efficacy of HybenX in the symptomatic treatment of recurrent aphthous stomatitis. Oral Dis 2009; 15:155-61.
21. Lombardo G, Signoretto C, Corrocher G, et al. A topical desiccant agent in association with ultrasonic debridement in the initial treatment of chronic periodontitis: a clinical and microbiological study. New Microbiol 2015; 38:393-407.
22. Gompelman M, van Asten SA, Peters EJ. Update on the Role of infection and biofilms in wound healing: pathophysiology and treatment. Plast Reconstr Surg 2016; 138:61S-70S.
23. Wolcott RD, Hanson JD, Rees EJ, et al. Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. Wound Repair Regen 2016; 24:163-74.
24. Lauritano D, Petruzzi M, Nardi GM, et al. Single application of a dessicating agent in the treatment of recurrent aphthous stomatitis. J Biol Regul Homeost Agents 2015; 29:59-66.
25. Aletaha D, Bingham CO, 3rd, Tanaka Y, et al. Efficacy and safety of sirukumab in patients with

active rheumatoid arthritis refractory to anti-TNF therapy (SIRROUND-T): a randomised, double-blind, placebo-controlled, parallel-group, multinational, phase 3 study. Lancet 2017;