Title

Prolotherapy: Potential for the Treatment of Chronic Wounds?

Journal

Advances in Wound Care, 8(4)

ISSN

2162-1918

Authors

Siadat, Amir Hossein

Isseroff, Roslyn Rivkah

Publication Date

2019-04-01

DOI

10.1089/wound.2018.0866

Peer reviewed

Prolotherapy: Potential for the Treatment of Chronic Wounds?

Amir Hossein Siadat[1] and Roslyn Rivkah Isseroff[2,*]

[1]Department of Dermatology, Isfahan University of Medical Sciences, Isfahan, Iran.
[2]Department of Dermatology, University of California, Davis, Sacramento, California.

Roslyn Rivkah Isseroff, MD

Submitted for publication September 17, 2018. Accepted November 2, 2018.

*Correspondence: Department of Dermatology, University of California, Davis, 3301 C St., Sacramento, CA 95816
(e-mail: rrisseroff@ucdavis.edu).

Significance: Chronic skin ulcers, including venous, diabetic, and pressure ulcers, constitute a major health care burden, affecting 2–6 million people in the United States alone, with projected increases in incidence owing to the aging population and rising epidemic of diabetes. The ulcers are often accompanied by pain. Standard of care fails to heal ~50% of diabetic foot ulcers and 25% of venous leg ulcers. Even advanced therapies do not heal >60%. Thus there is an unmet need for novel therapies that promote healing and also address the concomitant pain issue.

Recent Advances: Prolotherapy involves injection of small amounts of an irritant material to the site of degenerated or painful joints, ligaments, and tendons. Multiple irritants are reported to be efficacious, but the focus here is on dextrose prolotherapy. *In vitro* and *in vivo* studies support translation to clinical use. Concentrations as low as 5% dextrose have resulted in production of growth factors that have critical roles in repair. Numerous clinical trials report pro-reparative effects of dextrose prolotherapy in joint diseases, tendon, and ligament damage, and for painful musculoskeletal issues. However, most of the studies have limitations that result in low-quality evidence.

Critical Issues: The preclinical data support a role for dextrose prolotherapy in promoting tissue repair that is required for healing chronic wounds and ameliorating the associated pain. Critical issues include provision of evidence of efficacy in human chronic wounds. Another potential obstacle is limitation of reimbursement by third-party payers for a therapy with as yet limited evidence.

Future Directions: Preclinical studies in models of chronic wounds would support clinical translation. As dextrose prolotherapy has some mechanistic similarities to already approved honey therapies, it may have a shortened pathway for clinical translation. The gold standard for widespread adoption would be a well-designed clinical trial.

Keywords: prolotherapy, chronic wounds, diabetic ulcer, wound healing

SCOPE AND SIGNIFICANCE

Chronic skin ulcers, including venous, diabetic, and pressure ulcers, constitute a major health care burden, affecting 2–6 million people in the United States alone[1] with projected increases in incidence owing to the aging population and the increase in incidence of diabetes.[2] The ulcers are often accompanied by pain. Standard of care fails to heal ~50% of diabetic foot ulcers (DFUs) and 25% of venous leg ulcers.[3] Even advanced therapies do not heal ~60%, at best.[4] Thus, there is an unmet need for novel therapies that

DOI: 10.1089/wound.2018.0866

promote healing and also address the concomitant pain issue.

TRANSLATIONAL RELEVANCE

Preclinical studies have demonstrated proreparative effects of high-dextrose solutions. These include generation of growth factors critical for tissue repair such as platelet-derived growth factors (PDGFs), transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), basic fibroblast growth factor (b-FGF), insulin-like growth factor (IGF), and connective tissue growth factor (CTGF). *In vivo* studies have reported increased tendon and ligament repair after hypertonic dextrose injections. Thus these effects have the potential of translation to improved healing in skin wounds.

CLINICAL RELEVANCE

Prolotherapy is an approach to treatment of painful musculoskeletal issues and for treating injury of tendons, ligaments, and joints.[5] Although there are many reports of clinical success using these methods, the quality of the evidence is variable. Dextrose prolotherapy has many mechanistic analogies to honey therapy for wounds, a therapy that is indeed supported by evidence. Therefore, if good quality evidence can be generated for prolotherapy, it may prove to be another approach for improving healing while concomitantly decreasing pain.

BACKGROUND

Prolotherapy, Definition

The term prolotherapy was coined by Dr. George Hackett in 1956 as an approach for healing damaged ligaments and tendons, and derives from the Latin "proles," offspring or progeny and the English "therapy."[6] The medical definition of the prolotherapy in the *Merriam-Webster Dictionary* is "an alternative therapy for treating musculoskeletal pain that involves injecting an irritant substance (as dextrose, also known as D-glucose) into a ligament or tendon to promote the growth of new tissue,"[7] although some practitioners object to the "alternative" appelation.[8] Multiple agents are used in prolotherapy, some classified as irritants (such as phenol), some as chemoattractants (commonly sodium morrhuate), and others as osmotic agents (commonly dextrose). This review will focus only on dextrose prolotherapy, because of its potential mechanistic analogy to the already approved and widely clinically used, honey in wound therapy.

Clinical Uses

Since its introduction, perhaps as early as Hippocrates,[9] injections of irritants into tissues has been increasingly used to treat various musculoskeletal injuries including osteoarthritis, musculoskeletal pain, low back pain, refractory lateral epicondylosis and joint pain, and laxity.[10]

Clinical Evidence

There are numerous clinical trials proclaiming the efficacy of prolotherapy for treatment of the painful musculoskeletal issues.[11–13] For example, prolotherapy for patients with lateral epicondylitis, using a solution with a final concentration of 10% dextrose along with other components, administered at weeks 0, 4, and 8, showed significant improvement in the outcomes of pain and isometric strength, compared with placebo (saline)-treated patients.[11] Prolotherapy has also been reported to improve outcomes in knee osteoarthritis. In a systematic review with meta-analysis, prolotherapy was found to be more effective than exercise alone for significantly improving outcomes as measured by standardized scales for pain and functionality.[13] Chronic rotator cuff injury is yet another musculoskeletal entity that has been addressed with prolotherapy, with reported significant improvement in the outcomes of pain and functionality as measured by the Western Ontario Rotator Cuff and the Shoulder Pain and Disability Indices. In one study of prolotherapy for rotator cuff injury, injection of 4 mL of a solution with a final concentration of 22% dextrose to the bursa and 20 mL of a solution with final dextrose concentration of 13.5% to the surrounding soft tissue containing resulted in excellent or good outcomes reported in 92.9% (of 57 total treated) patients compared with 56.8% improvement in the group that only had physiotherapy.[12] Another recent review of evidence concluded that hypertonic dextrose prolotherapy is effective for treatment of diverse musculoskeletal entities including tendinopathies, knee and finger joint osteoarthrosis, and spinal/pelvic pain because of ligament dysfunction.[10]

However, there are also reports that show no, or limited, efficacy, especially when prolotherapy is used for chronic low back pain. A 2007 Cochrane review of the evidence concluded that prolotherapy is not effective when used alone for this indication, but may provide improvement when combined with other modalities and interventions.[5] The review did, however, point out some more promising evidence for efficacy in lumbar pain caused by disc disease.[14] As prolotherapy is associated with some adverse events, mostly transient increases in pain

and stiffness, similar to those seen with other needle injuries to the spine,[15] patients and physicians should consider these risks in weighing the potential benefits.

Technique

Prolotherapy involves the injection of aliquots of the prolotherapy solution to painful joints, ligaments, tendons, and joint spaces. These injections are performed in several sessions, usually every 2–6 weeks, and from three to six or more treatments.[16] Although different agents are used in prolotherapy, hyperosmolar dextrose is among the most common. The usual concentration ranges from 12.5% to 25%. Dextrose (D-glucose) is water soluble and a normal constituent of the blood chemistry. Concentrations of 15% dextrose are most often used for periarticular injections (tendon and ligament attachment) and 25% is used for intraarticular injections; saline and 1% lidocaine, to decrease pain associated with the injection, are typically used as co-injected diluents. The size of the needle is selected by the target tissue and the smallest needle that can reach the target tissue is selected.[16]

Proposed Mechanism of Action

Although the exact mechanism of prolotherapy is not clear, proponents of the technique believe that the injection of hypertonic dextrose causes cell dehydration and osmotic rupture at the injection site that leads to local tissue injury that subsequently induces granulocyte and macrophage migration to the site, with release of the growth factors and collagen deposition, and with final result of the new connective tissue formation, joint stability, and reduction in pain and dysfunction.[10] *In vitro* studies have shown that even concentrations as low as 5% dextrose have resulted in production of a number of growth factors critical for tissue repair. Some of these growth factors include PDGF, TGF-β, EGF, b-FGF, IGF-1, and CTGF.[17–19] High glucose can increase the production of the PDGF in cultured cells[20,21] and thus may contribute to reparative effects of prolotherapy.

Multiple studies have examined the effects of increased levels of glucose on cells in culture, as a model for hyperglycemic effects of diabetes. Thus most reports are focused on the deleterious effects of high glucose for cellular processes involved in wound healing.[22–25] However, pro-reparative effects have been reported as well. For example, the elevated reactive oxygen environment induced by cultivation in high-glucose medium induces the migration of mesenchymal stem cells,[26] which presumably would improve their recruitment to the wound site, improve healing. Human keratinocytes respond to cultivation in high-glucose medium with decreased migration,[27] but increase in differentiation-related proteins of filaggrin and claudin.[28] Increased generation of pro-reparative growth factors, as noted above, is also seen with cultivation in high-glucose medium.

A recent study, evaluated the effects of dextrose (and another prolotherapy agent phenol–glycerine–glucose [P2G], which is a combination of phenol, glucose, and glycerin) on cultured tenocytes, examining expression of several key markers that are essential for tendon development and inflammation. They found that both dextrose (50%) and P2G independently upregulated key pro-inflammatory markers including interleukin 8 (IL-8), cyclooxygenase-2, and prostaglandin E2, although collagen type I and TGF-β expression were decreased, perhaps because these were measured only at 24 h after the dextrose treatment.[29] The authors concluded that dextrose prolotherapy may work by triggering an inflammatory response, with later induction of collagen synthesis.

At the tissue level, one study showed significantly larger cross-sectional area of injured medial collateral rat ligaments injected with 15% dextrose compared with noninjured and injured saline-injected controls in the rat models.[30] Yet, even in *in vivo* models there is variability in response, limiting translational ability. In (noninjured) rat medial collateral ligaments, injection with P2G or sodium morrhuate resulted in a localized increase in inflammatory cells ($CD43^+$, $ED1^+$, and $ED2^+$ cells) but this inflammatory response was variable and not uniformly different from those of control saline injections or needlestick procedures.[31] One could argue that the needlestick trauma alone may be sufficient to induce the required inflammatory response, at least in this model.

Indications and Contraindications

Prolotherapy has been used to treat pain in many chronic conditions including knee and finger joint osteoarthritis,[7] tendinopathy,[31] lateral epicondylosis,[6] Osgood–Schlatter disease,[5] rotator cuff tendinopathy,[8] hip adductor tendinopathy,[31] Achilles tendinopathy, and palntarfasciopathy.[32]

Overall, prolotherapy seems to be safe and few adverse effects have been reported.[15] Active rheumatologic disorders, joint or skin infections, allergy to corn, and use of immunosuppressive are regarded as absolute contraindications of the dextrose prolotherapy. Acute fractures, acute gout, bleeding disorders, and use of anticoagulants are considered as relative contraindications.[32]

Chronic or Nonhealing Ulcers

One then might propose that prolotherapy could be a reasonable approach for chronic skin ulcers: nonhealing wounds with various etiologies, including venous leg/diabetic foot/pressure, and ischemic ulcers. Both tissue repair and pain, a common, although frequently overlooked, complaint in chronic wound patients[33] might be addressed by prolotherapy. The standard treatment for chronic ulcers depends on their underlying pathology and includes offloading, control of blood glucose, foot care education and revascularization techniques for DFUs, compression for venous ulcers, and infection control, surgical or chemical debridement, and moist healing environment for chronic wounds of any etiology[34] Nevertheless, standard of care fails to heal about 50% of DFUs[3] and 25% of venous leg ulcers.[35,36] When standard therapies fail to heal a chronic ulcer, advanced treatments are considered. Some of the advanced treatments include collagen products, biological dressings, biological skin equivalents, cultured keratinocytes, PDGF, platelet-rich plasma (PRP), silver products, intermittent pneumatic compression therapy, negative pressure wound therapy, electrical stimulation, hyperbaric oxygen, topical oxygen, and ozone.[34,37]

Here, we review the basic features of the wound healing with respect to where in the process prolotherapy might have pro-reparative effects and thus have potential as an adjunctive treatment for chronic cutaneous wounds.

Wound Repair Process

The normal process of the wound healing goes through three overlapping steps with hemostasis/inflammatory, proliferative, and remodeling phases. Multiple cytokines participate in the inflammatory and proliferative processes, including PDGF, FGF, tumor necrosis factor alpha (TNF-α), EGF, TGF-β granulocyte-monocyte colony-stimulating factor, among others that modulate the recruitment and proliferation of the cells involved in repair: fibroblasts, endothelial and epithelial cells, macrophages, neutrophils, and lymphocytes (reviewed in Gould *et al*.[37]). The remodeling phase is characterized by replacement of the provisional wound matrix that is primarily collagen type III by collagen type I, and cross-linking collagen fibers to increase the strength of the healed.[37]

In chronic cutaneous wounds, the ulcer repair is arrested in the inflammatory phase with altered extracellular matrix, decreased growth factors, senescent cells, and increased activity of matrix proteases.[37–40] Prolotherapy, with the changes it induces in tissues, might address some of the abnormalities that stall healing in chronic wounds.

Analgesic Effects

About 60% of the patients with venous leg ulcer report pain, and the same may apply to other chronic wounds.[41] Inattention to management of pain in chronic ulcers may contribute to increased health care costs and depression in affected patients.[41] Prolotherapy is reported to decrease pain in randomized controlled trials of patients with a number of different musculoskeletal conditions.[42–44] The mechanism underpinning the reduction in pain is not clear. In another randomized clinical trial, Maniquis-Smigel *et al.* have shown that 5% dextrose epidural injection resulted in >50% pain reduction in 84% of patients with chronic low back pain suggesting a pain-specific neurogenic effect.[45] The authors concluded that although the onset of 5% dextrose analgesia was comparable with fentanyl and morphine, the duration of analgesia was even longer for dextrose. No complication regarding use of 5% dextrose was reported. In this and another randomized trial demonstrating efficacy of dextrose epidural injection for chronic back pain,[46] the authors posit that dextrose can directly alter sensorineural pain perception; however, no mechanism is elucidated in these studies. Other investigators have reported that subcutaneous and peripheral nerve infiltration of dextrose and mannitol has the same analgesic effect in patients with chronic pain.[47] They demonstrate in a small trial that a topical cream containing 30% mannitol can mitigate experimental pain induced by capsaicin activation of receptor potential vanilloid type-1 (TRPV1) pain receptor.[47] Thus, although the mechanism remains unclear, they propose that mannitol or dextrose decreases TRPV1-mediated pain signaling. Although mechanistically unclear, pain reduction reported with dextrose prolotherapy may translate to pain relief in patients with chronic ulcers.

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

The Possible Role of the Prolotherapy in the Wound

Upregulation of growth factors. As *in vitro* studies have shown that cultivation of cells in high-glucose culture medium can increase PDGF expression, this may be one mechanism by which prolotherapy can improve healing in chronic wounds. PDGF has multiple pro-reparative effects in skin wounds, including promotion of angiogenesis, fibroblast proliferation, extracellular matrix

production (reviewed in Barrientos *et al*[48]), and, in fact, is the only growth factor approved by the Food and Drug Administration for clinic use to improve healing of diabetic chronic wounds (Becaplermin gel).[49]

Likewise, TGF-β, involved in all steps of wound healing including inflammation, angiogenesis, fibroblast proliferation, collagen synthesis, matrix deposition, and remodeling, and wound reepithelialization[48] is present in the normally healing wounds,[50] and conversely, signaling through this pathway is diminished in chronic wounds.[39] As TGF-β expression is also upregulated by high glucose,[17,19,20] another possible mechanism for potential of prolotherapy to enhance wound healing would be through increasing TGF-β expression and activity.

Upregulation of other pro-reparative growth factors by prolotherapy could also potentially contribute to improved healing of chronic wounds. EGF, yet another growth factor induced by dextrose prolotherapy,[18] also has multiple pro-reparative functions and improves healing in some animal wound models of impaired healing. b-FGF is yet another pro-reparative growth factor[48] that is produced during prolotherapy[21] and thus, the upregulation of both EGF and b-FGF by prolotherapy could potentially improve healing in chronic wounds. Other growth factors upregulated by high glucose include IGF-1[19] and CTGF,[51] both pro-reparative and with known contributions to *in vivo* wound healing models.[52,53]

Matrix effects. Although proponents of prolotherapy suggest that there are direct effects on collagen synthesis, the evidence to support this claim is quite limited. A few studies demonstrate upregulation of matrix in response to dextrose prolotherapy or *in vitro* cultivation with high concentrations of glucose. Collagen expression is increased after exposure of patellar tendon fibroblasts to the prolotherapy agents P2G or dextrose[54] and thus may contribute to tissue regeneration within a cutaneous wound. Collagen type I synthesis is also increased in high-glucose cultivation of renal fibroblasts, in a TGF-β-mediated pathway.[55] Changes in the cartilage matrix protein aggrecan is reported in chondrocytes cultured in high glucose,[56] and in patients who have received intraarticular injections of 12.5% dextrose, an increase in cellularity and fibrous components of articular cartilage has been observed.[57] Additional laboratory and clinical studies are needed to document potential effects of dextrose prolotherapy on extracellular matrix components.

Prolotherapy Analogy to Honey?

In 1981, Knutson *et al.* reported the use of granulated sugar and povidone–iodine in patients with wounds, burns, and ulcers, and noted improved healing that they ascribed to a reduction in bacterial contamination, and rapid debridement of eschar.[58] Another study found *in vitro* antimicrobial efficacy in three types of granulated sugar against 18 Gram-negative and Gram-positive bacteria, and in a pilot clinical study, treated 22 patients with sloughy or necrotic wounds with granulated sugar for 21 days, with the outcome of improved debridement, decreased pain, and malodor, although not in decreased time to healing.[59] The concentrations of glucose within the commonly used prolotherapy solutions do not reach the concentration that would be present when granulated sugar is directly applied to a wound; nevertheless, the antimicrobial activity of these solutions *in vitro* have not been reported, and may be worthwhile investigating.

Quite a number of animal studies have demonstrated improved healing of skin wounds with topical application of honey.[60,61] Its beneficial effect on healing has been ascribed to its antibacterial effects either through the generation of hydrogen peroxide (H_2O_2)[62] or by antibacterial effects of flavinoids present in some types of honey (notably Manuka honey).[63–65] H_2O_2 generated by topical application of honey in the wound may also signal for reparative pathways.[66,67] Multiple honey-containing topical creams and dressings are commercially available for wound therapy, although Cochrane review of the evidence of their efficacy supports its use only in burn wounds.[68] Honey is composed of water (<20%), sugars (most commonly fructose, $\sim$76%), and a host of other components,[67] and although it is tempting to propose that some of the effects of dextrose prolotherapy are mediated by generation of H_2O_2 in the injected tissues, there are no studies to evaluate this possibility. It may be that the use of honey for wound repair and its mechanisms could be considered a model for treatment of cutaneous wounds using prolotherapy, but laboratory studies will be needed to support this possibility.

Reported Clinical Use for Skin Wound Healing

There are only three reports of clinical use of high dextrose solutions to improve skin healing, and a few reporting use for cosmetic skin indications. Subcutaneous injection of 20% glucose solution was reported to improve dermal thickness (visual analysis) in atrophic scars with results lasting for up to 2 months,[69] a finding that has been extended to cosmetic applications of improving skin laxity in the aging face.[70,71] Topical applica-

tion of 50% glucose solution, in combination with negative pressure therapy is reported to hasten wound bed granulation tissue formation in *Pseudomonas aeruginosa*–infected chronic wounds.[72] A similar protocol of topical administration of 50% glucose solution is reported to have a small but statistically significant, improvement in the rate of healing of stage III pressure ulcers.[73]

Insurance Coverage

Currently Medicare and Medicaid do not cover prolotherapy,[74] as it is currently considered an experimental procedure. Likewise, major insurers follow the Medicare and Medicaid guidelines, and also do not provide benefit coverage for prolotherapy.[75,76] Lack of coverage will present a formidable obstacle to potential clinical implementation for wound therapy. There are at the time of this writing, 13 ongoing clinical trials examining the efficacy of dextrose prolotherapy for musculoskeletal indications.* If they generate high-quality evidence, insurance and Medicare/Medicaid coverage may follow.

TAKE HOME MESSAGES

- Prolotherapy is widely used to treat musculoskeletal injuries, and there are multiple clinical trials, of variable quality, showing efficacy for these indications.
- The *in vitro* evidence supports the pro-reparative role of this therapy, in that it can induce generation of growth factors.
- The *in vivo* evidence demonstrates that dextrose prolotherapy induces a limited inflammatory response that progresses to reparative responses.
- These findings suggest that dextrose prolotherapy may analogously improve healing in chronic wounds.
- Obstacles include lack of third-party insurer coverage and lack of clinical evidence in the skin wound healing area.
- Clinical trials are suggested to determine if this is a viable alternative therapy for chronic wounds.

FUTURE DIRECTIONS

To generate definitive evidence to support the potential of prolotherapy to improve healing of chronic wounds clinical trials will need to be performed, not only with different prolotherapy agents, but also with different concentrations of the tested agents. Since the agents are not likely to be able to be patented for this indication, trials would not be supported by industry, and it would fall to federal agencies to fund this research. In doing this, a low cost and effective therapy for disease that has limited effective options might be developed.

SUMMARY

Dextrose prolotherapy is widely used to treat many musculoskeletal injuries. This review examines the preclinical and clinical evidence to evaluate whether the mechanisms that support its use for musculoskeletal injury can be applied to improve healing in the sustained injury of chronic cutaneous wounds. *In vitro* evidence supports the use of high-glucose cultivation to address the decreased proliferative capacity of senescent fibroblasts in the wound that exhibit decreased responsivity to growth factors such as PDGF.[37] There is good evidence of increased growth factor generation in response to *in vitro* cultivation in high glucose. Other cellular mechanisms, including some modulation of the extracellular matrix could conceivably contribute to healing in the chronic wound. Clinical trials of dextrose prolotherapy for musculoskeletal indications, although of variable quality, find pro-reparative responses and decrease in pain in treated patients. As chronic wounds have such a high socioeconomic cost for both the health system and the patients with limited therapies, evidence suggests that dextrose prolotherapy could be a useful adjunct for patients with chronic wounds. The injection of the dextrose in the wound periphery and base is hypothesized to trigger release of the growth factors that may accelerate the process of wound healing. These hypotheses can only be tested by rigorous clinical trials.

ACKNOWLEDGMENTS AND FUNDING SOURCES

No funding sources to acknowledge.

AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The authors listed expressly wrote the content of this article. No ghostwriters were used to write this article.

ABOUT THE AUTHORS

Amir Hosein Siadat, MD, is an Associate Professor of Dermatology at the Isfahan University of Medical Sciences, as well as the Research Manager in Skin Diseases and Leishmaniasis Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. His interests are in immunodermatology, and the potential of stem cells in the treatment of human diseases. He is the author of 93 dermatology/basic science articles. He co-authored

*https://clinicaltrials.gov/ct2/results?cond=&term=prolotherapy&cntry=&state=&city=&dist=

this work while on mini-sabbatical and observership at the University of California, Davis. **Roslyn Rivkah Isseroff, MD,** is a Professor of Dermatology at the University of California, Davis. Her research and interests are in tissue repair and regeneration and her clinical expertise is in healing chronic wounds. She has authored over 130 skin biology publications. She co-directs the Wound/Ulcer clinic where multiple investigator-initiated, industry-sponsored, and/or federally funded clinical trials for drugs and devices to improve healing of chronic wounds are carried out.

REFERENCES

1. Frykberg RG, Banks J. Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle) 2015;4:560–582.
2. 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;17:763–771.
3. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. Healing diabetic neuropathic foot ulcers: are we getting better? Diabet Med 2005;22:172–176.
4. Lavery LA, Fulmer J, Shebetka KA, et al. The efficacy and safety of Grafix((R)) for the treatment of chronic diabetic foot ulcers: results of a multicentre, controlled, randomised, blinded, clinical trial. Int Wound J 2014;11:554–560.
5. Dagenais S, Yelland MJ, Del Mar C, Schoene ML. Prolotherapy injections for chronic low-back pain. Cochrane Database Syst Rev 2007:(2);CD004059.
6. Hackett G, Hemwall GA, Montgomery GA. Ligament and Tendon Relaxation, 5th ed. Oak Brook, IL: Charles C. Thomas, Ltd., 2008.
7. Merriam-Webster, Medical Dictionary. Prolotherapy. 2018. www.merriam-webster.com/medical/prolotherapy (last accessed June 29, 2018).
8. Wikipedia. Talk:Prolotherapy. 2018. https://en.wikipedia.org/wiki/Talk%3AProlotherapy (last accessed August 20, 2018).
9. Hippocratic Writings. Penguin Classics, UK, 1978. Lloyd GER (ed.), Chadwick J (Trans.), London, United Kingdom.
10. Hauser RA, Lackner JB, Steilen-Matias D, Harris DK. A systematic review of dextrose prolotherapy for chronic musculoskeletal pain. Clin Med Insights Arthritis Musculoskelet Disord 2016;9:139–159.
11. Scarpone M, Rabago DP, Zgierska A, Arbogast G, Snell E. The efficacy of prolotherapy for lateral epicondylosis: a pilot study. Clin J Sport Med 2008;18:248–254.
12. Seven MM, Ersen O, Akpancar S, et al. Effectiveness of prolotherapy in the treatment of chronic rotator cuff lesions. Orthop Traumatol Surg Res 2017;103:427–433.
13. Sit RW, Chung V, Reeves KD, et al. Hypertonic dextrose injections (prolotherapy) in the treatment of symptomatic knee osteoarthritis: a systematic review and meta-analysis. Sci Rep 2016;6:25247.
14. Klein RG, Eek BC, O'Neill CW, Elin C, Mooney V, Derby RR. Biochemical injection treatment for discogenic low back pain: a pilot study. Spine J 2003;3:220–226.
15. Dagenais S, Ogunseitan O, Haldeman S, Wooley JR, Newcomb RL. Side effects and adverse events related to intraligamentous injection of sclerosing solutions (prolotherapy) for back and neck pain: a survey of practitioners. Arch Phys Med Rehabil 2006;87:909–913.
16. Rabago D, Slattengren A, Zgierska A. Prolotherapy in primary care practice. Prim Care 2010;37:65–80.
17. Oh JH, Ha H, Yu MR, Lee HB. Sequential effects of high glucose on mesangial cell transforming growth factor-beta 1 and fibronectin synthesis. Kidney Int 1998;54:1872–1878.
18. Fukuda K, Kawata S, Inui Y, et al. High concentration of glucose increases mitogenic responsiveness to heparin-binding epidermal growth factor-like growth factor in rat vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1997;17:1962–1968.
19. Pugliese G, Pricci F, Locuratolo N, et al. Increased activity of the insulin-like growth factor system in mesangial cells cultured in high glucose conditions. Relation to glucose-enhanced extracellular matrix production. Diabetologia 1996;39:775–784.
20. Di Paolo S, Gesualdo L, Ranieri E, Grandaliano G, Schena FP. High glucose concentration induces the overexpression of transforming growth factor-beta through the activation of a platelet-derived growth factor loop in human mesangial cells. Am J Pathol 1996;149:2095–2106.
21. Ohgi S, Johnson PW. Glucose modulates growth of gingival fibroblasts and periodontal ligament cells: correlation with expression of basic fibroblast growth factor. J Periodontal Res 1996;31:579–588.
22. Wu Y, Quan Y, Liu Y, et al. Hyperglycaemia inhibits REG3A expression to exacerbate TLR3-mediated skin inflammation in diabetes. Nat Commun 2016;7:13393.
23. Lan CC, Wu CS, Huang SM, Wu IH, Chen GS. High-glucose environment enhanced oxidative stress and increased interleukin-8 secretion from keratinocytes: new insights into impaired diabetic wound healing. Diabetes 2013;62:2530–2538.
24. Wong SL, Demers M, Martinod K, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. Nat Med 2015;21:815–819.
25. Pavlou S, Lindsay J, Ingram R, Xu H, Chen M. Sustained high glucose exposure sensitizes macrophage responses to cytokine stimuli but reduces their phagocytic activity. BMC Immunol 2018;19:24.
26. Oh JY, Choi GE, Lee HJ, et al. High glucose-induced reactive oxygen species stimulates human mesenchymal stem cell migration through snail and EZH2-dependent E-cadherin repression. Cell Physiol Biochem 2018;46:1749–1767.
27. Lan CC, Wu CS, Kuo HY, Huang SM, Chen GS. Hyperglycaemic conditions hamper keratinocyte locomotion via sequential inhibition of distinct pathways: new insights on poor wound closure in patients with diabetes. Br J Dermatol 2009;160:1206–1214.
28. Yamada K, Matsushita K, Wang J, Kanekura T. Topical glucose induces claudin-1 and filaggrin expression in a mouse model of atopic dermatitis and in keratinocyte culture, exerting anti-inflammatory effects by repairing skin barrier function. Acta Derm Venereol 2018;98:19–25.
29. Ekwueme EC, Mohiuddin M, Yarborough JA, et al. Prolotherapy induces an inflammatory response in human tenocytes in vitro. Clin Orthop Relat Res 2017;475:2117–2127.
30. Jensen K. Healing Response of Knee Ligaments to Prolotherapy in a Rat Model. Madison, WI: Biomedical Engineering, University of Wisconsin, Madison, 2006.
31. Jensen KT, Rabago DP, Best TM, Patterson JJ, Vanderby R, Jr. Early inflammatory response of knee ligaments to prolotherapy in a rat model. J Orthop Res 2008;26:816–823.
32. Rabago D, Nourani B. Prolotherapy for osteoarthritis and tendinopathy: a descriptive review. Curr Rheumatol Rep 2017;19:34.
33. Iqbal A, Jan A, Wajid MA, Tariq S. Management of chronic non-healing wounds by hirudotherapy. World J Plast Surg 2017;6:9–17.
34. Greer N, Foman N, Dorrian J, et al. Advanced Wound Care Therapies for Non-Healing Diabetic, Venous, and Arterial Ulcers: A Systematic Review. Department of Veterans Affairs; Washington, DC, 2012. Available from http://www.ncbi.nlm.nih.gov/books/NBK132238/
35. O'Meara S, Cullum NA, Nelson EA. Compression for venous leg ulcers. Cochrane Database Syst Rev 2009:CD000265.
36. Marston WA, Carlin RE, Passman MA, Farber MA, Keagy BA. Healing rates and cost efficacy of outpatient compression treatment for leg ulcers associated with venous insufficiency. J Vasc Surg 1999;30:491–498.
37. Gould L, Abadir P, Brem H, et al. Chronic wound repair and healing in older adults: current status and future research. J Am Geriatr Soc 2015;63:427–438.

38. Panuncialman J, Falanga V. The science of wound bed preparation. Surg Clin North Am 2009;89: 611–626.

39. Pastar I, Stojadinovic O, Krzyzanowska A, et al. Attenuation of the transforming growth factor beta-signaling pathway in chronic venous ulcers. Mol Med 2010;16:92–101.

40. Krishnaswamy VR, Mintz D, Sagi I. Matrix metalloproteinases: the sculptors of chronic cutaneous wounds. Biochim Biophys Acta 2017;1864: 2220–2227.

41. Price P, Fogh K, Glynn C, Krasner DL, Osterbrink J, Sibbald RG. Managing painful chronic wounds: the Wound Pain Management Model. Int Wound J 2007;4(Suppl 1):4–15.

42. Refai H. Long-term therapeutic effects of dextrose prolotherapy in patients with hypermobility of the temporomandibular joint: a single-arm study with 1–4 years' follow up. Br J Oral Maxillofac Surg 2017;55:465–470.

43. Wilkinson HA. Injection therapy for enthesopathies causing axial spine pain and the "failed back syndrome": a single blinded, randomized and cross-over study. Pain Physician 2005;8:167–173.

44. Farpour HR, Fereydooni F. Comparative effectiveness of intra-articular prolotherapy versus periarticular prolotherapy on pain reduction and improving function in patients with knee osteoarthritis: a randomized clinical trial. Electron Physician 2017;9:5663–5669.

45. Maniquis-Smigel L, Dean Reeves K, Jeffrey Rosen H, et al. Short term analgesic effects of 5% dextrose epidural injections for chronic low back pain: a randomized controlled trial. Anesth Pain Med 2017;7:e42550.

46. Maniquis-Smigel L, Reeves KD, Rosen HJ, et al. Analgesic effect and potential cumulative benefit from caudal epidural D5W in consecutive participants with chronic low-back and buttock/leg pain. J Altern Complement Med 2018;24:1189–1196.

47. Bertrand H, Kyriazis M, Reeves KD, Lyftogt J, Rabago D. topical mannitol reduces capsaicin-induced pain: results of a pilot-level, double-blind, randomized controlled trial. PM R 2015;7:1111–1117.

48. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16: 585–601.

49. Center for Drug Evaluation and Research USFaDA. Product approval information—licensing action. 1997. www.accessdata.fda.gov/drugsatfda_docs/appletter/1997/becaomj121697l.htm (last accessed June 29, 2018).

50. Penn JW, Grobbelaar AO, Rolfe KJ. The role of the TGF-beta family in wound healing, burns and scarring: a review. Int J Burns Trauma 2012;2:18–28.

51. Murphy M, Godson C, Cannon S, et al. Suppression subtractive hybridization identifies high glucose levels as a stimulus for expression of connective tissue growth factor and other genes in human mesangial cells. J Biol Chem 1999;274:5830–5834.

52. Achar RA, Silva TC, Achar E, Martines RB, Machado JL. Use of insulin-like growth factor in the healing of open wounds in diabetic and non-diabetic rats. Acta Cir Bras 2014;29:125–131.

53. Henshaw FR, Boughton P, Lo L, McLennan SV, Twigg SM. Topically applied connective tissue growth factor/CCN2 improves diabetic preclinical cutaneous wound healing: potential role for CTGF in human diabetic foot ulcer healing. J Diabetes Res 2015;2015:236238.

54. Freeman JW, Empson YM, Ekwueme EC, Paynter DM, Brolinson PG. Effect of prolotherapy on cellular proliferation and collagen deposition in MC3T3-E1 and patellar tendon fibroblast populations. Transl Res 2011;158:132–139. pii: E34. doi: 10.3390/jfb9020034.

55. Han DC, Isono M, Hoffman BB, Ziyadeh FN. High glucose stimulates proliferation and collagen type I synthesis in renal cortical fibroblasts: mediation by autocrine activation of TGF-beta. J Am Soc Nephrol 1999;10:1891–1899.

56. Wu TJ, Fong YC, Lin CY, Huang YL, Tang CH. Glucose enhances aggrecan expression in chondrocytes via the PKCalpha/p38-miR141–3p signaling pathway. J Cell Physiol 2018;233:6878–6887.

57. Topol GA, Podesta LA, Reeves KD, et al. Chondrogenic effect of intra-articular hypertonic-dextrose (prolotherapy) in severe knee osteoarthritis. PM R 2016;8:1072–1082.

58. Knutson RA, Merbitz LA, Creekmore MA, Snipes HG. Use of sugar and povidone-iodine to enhance wound healing: five year's experience. South Med J 1981;74:1329–1335.

59. Murandu M, Webber MA, Simms MH, Dealey C. Use of granulated sugar therapy in the management of sloughy or necrotic wounds: a pilot study. J Wound Care 2011;20:206, 208, 210 passim.

60. El-Kased RF, Amer RI, Attia D, Elmazar MM. Honey-based hydrogel: in vitro and comparative In vivo evaluation for burn wound healing. Sci Rep 2017;7:9692.

61. Vaghardoost R, Mousavi Majd SG, Tebyanian H, et al. The healing effect of sesame oil, camphor and honey on second degree burn wounds in rat. World J Plast Surg 2018;7:67–71.

62. Irish J, Blair S, Carter DA. The antibacterial activity of honey derived from Australian flora. PLoS One 2011;6:e18229.

63. Viuda-Martos M, Ruiz-Navajas Y, Fernandez-Lopez J, Perez-Alvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci 2008;73:R117–R124.

64. Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. Mol Nutr Food Res 2008;52:483–489.

65. Alvarez-Suarez JM, Gasparrini M, Forbes-Hernandez TY, Mazzoni L, Giampieri F. The composition and biological activity of honey: a focus on manuka honey. Foods 2014;3:420–432.

66. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic Biol Med 1995;18:775–794.

67. Martinotti S, Ranzato E. Honey, wound repair and regenerative medicine. J Funct Biomater 2018;9.

68. Jull AB, Rodgers A, Walker N. Honey as a topical treatment for wounds. Cochrane Database Syst Rev 2008:CD005083.

69. Kim H, Yoo KH, Zheng Z, Cho SB. Pressure- and dose-controlled transcutaneous pneumatic injection of hypertonic glucose solution for the treatment of atrophic skin disorders. J Cosmet Laser Ther 2017;19:479–484.

70. Kwon HH, Choi SC, Park KH, Jung JY. A novel combination regimen with intense focused ultrasound and pressure- and dose-controlled transcutaneous pneumatic injection of hypertonic glucose solution for lifting and tightening of the aging face. J Cosmet Dermatol 2018;17:373–379.

71. Mashiko T, Abo Y, Kuno S, Yoshimura K. A novel facial rejuvenation treatment using pneumatic injection of non-cross-linked hyaluronic acid and hypertonic glucose solution. Dermatol Surg 2015; 41:755–758.

72. Zhao JC, Xian CJ, Yu JA, Shi K, Hong L. Hypertonic glucose combined with negative pressure wound therapy to prepare wounds with *Pseudomonas aeruginosa* infection for skin grafting: a report of 3 cases. Ostomy Wound Manage 2015; 61:28–44.

73. Zhou DP, Lu LQ, Mao XL. Insulin and hyperosmotic glucose solution external used for treating pressure sore [in Chinese]. Hunan Yi Ke Da Xue Xue Bao 2001;26:475–476.

74. Centers for Medicare and Medicaid Services. Decision memo for prolotherapy for chronic low back pain (CAG00045N). 1999. www.cms.gov/medicare-coverage-database/details/nca-decision-memo.aspx?NCAId=68&fromdb=true (last accessed June 29, 2018).

75. United Healthcare. Prolotherapy for musculotherapy indications. 2018. www.uhcprovider.com/.../prolotherapy-musculoskeletal-indications.pdf (last accessed June 29, 2018).

76. Premera Blue Cross. Medical policy—2.01.26 prolotherapy. 2018. www.premera.com/medicalpolicies/2.01.26.pdf (last accessed June 29, 2018).

ABBREVIATIONS AND ACRONYMS

b-FGF = basic fibroblast growth factor
CTGF = connective tissue growth factor
DFU = diabetic foot ulcer
EGF = epidermal growth factor
H_2O_2 = hydrogen peroxide
IGF = insulin-like growth factor
P2G = phenol–glycerine–glucose
PDGF = platelet-derived growth factor
TGF-β = transforming growth factor-beta
TRPV1 = receptor potential vanilloid type-1