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ORIGINAL ARTICLE

Treatment of chronic diabetic lower leg ulcers with activated protein C: a randomised placebo-controlled, double-blind pilot clinical trial

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Abstract

Lower leg ulcers are a serious and long-term complication in patients with diabetes and pose a major health concern because of the increasing number of patients diagnosed with diabetes each year. This study sought to evaluate the clinical benefit of topical activated protein C (APC) on chronic lower leg ulcers in patients with diabetes. Twelve patients were randomly assigned to receive either APC ($N = 6$) or physiological saline (placebo; $N = 6$) in a randomised, placebo-controlled, double-blind pilot clinical trial. Treatment was administered topically, twice weekly for 6 weeks with final follow-up at 20 weeks. Wound area was significantly reduced to $34{\cdot}8 \pm 16{\cdot}4\%$ of week 0 levels at 20 weeks in APC-treated wounds ($p = 0{\cdot}01$). At 20 weeks, three APC-treated wounds had completely healed, compared to one saline-treated wound. Full-thickness wound edge skin biopsies showed reduced inflammatory cell infiltration and increased vascular proliferation following APC treatment. Patient stress scores were also significantly reduced following APC treatment ($p < 0{\cdot}05$), demonstrating improved patient quality of life as assessed by the Cardiff Wound Impact Questionnaire. This pilot trial suggests that APC is a safe topical agent for healing chronic lower leg ulcers in patients with diabetes and provides supporting evidence for a larger clinical trial.

Introduction

Impaired skin wound healing in patients with diabetes is associated with a prolonged inflammatory reaction and delayed granulation tissue formation, angiogenesis and re-epithelialisation. The current standard care for lower leg ulcers consists of debridement, daily moist dressing changes and off-loading (1). A number of topical treatments and recombinant growth factors have been trialled and shows potential for healing ulcers in patients with diabetes, including recombinant human vascular endothelial growth factor (VEGF) (2–4), hepatocyte growth factor (5), basic fibroblast

Key messages

- successful wound healing depends on inflammation, granulation tissue formation, angiogenesis and re-epithelialisation
- the aim of this study is to determine whether activated protein C can improve wound healing of diabetic lower leg ulcers
- activated protein C significantly reduced wound area and volume, improved wound appearance and reduced patient stress associated with impaired wound healing

growth factor (5,6), epidermal growth factor (7,8) and platelet-derived growth factor (PDGF)-BB (9,10). PDGF-BB is the only growth factor to be FDA approved (Regranex® Gel, Healthpoint Biotherapeutics; Houston, TX), although it is not widely used and comes with a 'black box' warning because of an increased risk of mortality secondary to malignancy in patients treated with three or more tubes (11).

Activated protein C (APC) is a natural anti-coagulant activated from its zymogen, protein C, by thrombin and thrombomodulin. Once activated, APC can engage the endothelial protein C receptor (EPCR) and elicit anti-inflammatory, angiogenic, anti-apoptotic and re-epithelisation effects that may promote wound healing in lower leg ulcers in patients with diabetes (12–17).

Our open-label pilot study previously showed that APC reduces inflammation, enhances granulation tissue formation and promotes re-epithelialisation in a small cohort of patients with chronic wounds of varying aetiology (12). In another open-label pilot trial of non-healing orthopaedic wounds, combined APC and topical negative pressure treatment increased granulation tissue, and reduced wound area and depth (17). Following treatment, wounds completely re-epithelialised or provided sufficient closure to support surgical intervention.

This study was designed to assess the efficacy, safety and tolerability of topical APC on lower leg ulcers in patients with diabetes in a small randomised, placebo-controlled, double-blind pilot trial.

Patients and methods

Patients

The primary criteria for this study included established diagnosis of diabetes mellitus in accordance with the criteria of the American Diabetes Association (18) and the presence of a lower leg ulcer despite at least 6 months of standard wound care. Ulcers were defined as diabetic or venous, with ischaemic ulcers excluded by ankle brachial index. Individual patient and wound demographics are shown in Table 1. This study was approved by the Northern Sydney Health Human Research Ethics Committee (#0611-218M) and performed in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Treatment protocol and assessment

Recombinant human APC (Drotrecogin alfa activated or Xigris® (Eli Lilly; Indianapolis, IN; 400 µg/ml in sterile water) or physiological saline was applied topically beneath a sterile, occlusive polyurethane adhesive film (Tegaderm®, 3M; Maplewood, MN) as previously described (12). Treatment was applied twice weekly for 6 weeks, with post-treatment follow-ups at weeks 8, 12 and 20. An outline of the treatment and follow-up protocol is depicted in Table 2. Wound area was assessed at each treatment and follow-up visit with a digital measuring device (Visitrak, Smith and Nephew; North Ryde, Australia) as previously described (12). The primary outcome measure was percentage change in wound area at 20 weeks. The 20-week assessment period was chosen as the end point for wound healing or non-healing as it has previously been used to assess treatment of ulcers in patients with diabetes using topical PDGF gel (9,10) or topical platelet-derived wound healing formula (also called CT-102 activated platelet supernatant) (19).

Secondary outcomes included: (i) proportion of patients achieving >30% reduction in wound area at 2 weeks, (ii) clinical assessment of wound appearance including erythema and exudate utilising standard Bates-Jensen wound scores at week 0, treatment and follow-up visits, (iii) plasma functional protein C levels at weeks 1 and 8, (iv) serum glycated haemoglobin (HbA1C), C-reactive protein (CRP) and plasma coagulation parameters, international normalised ratio (INR) and activated partial thromboplastin time (APTT) at week 0 and week 8, (v) histological analysis of skin biopsies at week 0 and week 8 and (vi) quality-of-life questionnaire using the Cardiff Wound Impact Questionnaire at week 0 and each treatment visit.

Histology

Four micrometer sections of 3-mm skin punch biopsies from the wound edge were processed for histological analysis. Anatomical pathology of haematoxylin and eosin sections was reviewed by a dermatopathologist independent of the trial and blinded to each case.

Statistics

Data between treatment groups and time points was analysed by Fisher exact test, paired or unpaired parametric Student t-test, non-parametric Mann–Whitney U-test, or repeated measures analysis of variance (ANOVA) with Newman-Keuls post-hoc analysis as appropriate. Statistical analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software; San Diego, CA) and p-value $<0{\cdot}05$ was considered as statistical significant.

Results

No significant differences were observed between saline- and APC-treated patients with respect to sex, age, wound aetiology, wound duration or baseline wound area and volume. Wounds treated with saline and APC at week 0 and week 20 are shown in Figure 1A. Overall, three APC-treated wounds completely healed by week 20, compared to one wound in the saline-treated group. The mean wound area was significantly reduced in the APC-treated group at week 20 compared to week 0 (immediately before treatment began, $p = 0{\cdot}01$), while there was no significant difference in the saline-treated group ($p = 0{\cdot}74$) (Figure 1B). Lower leg ulcers in patients with diabetes treated with APC showed early reduction in wound size at week 2 of >15% in three patients and >30% in two patients (Figure 1C). In saline-treated group, two patients demonstrated >15% reduction and one patient >30% at week 2.

During the trial, the mean wound volume for the saline-treated group significantly decreased to $82{\cdot}6 \pm 0{\cdot}24\%$ of week 0 levels at week 4 ($p < 0{\cdot}05$) but returned to near week 0 levels by week 6 (Figure 2). In the APC-treated group, wound

Table 1 Individual demographic data for patients receiving topical saline or activated protein C (APC)

Sex/age (years)	Wound duration (months)	Wound location /aetiology	Other wound care management
Saline			
M/59	36	Left lateral malleolus/neurotrophic	Three times/week polyurethane foam dressing and medical adhesive tape, monthly debridement
M/47	24	Right lateral foot/neurotrophic	Three times/week nanocrystalline silver-coated polyethylene net dressing, polyurethane foam dressing, weekly debridement
M/55	12	Left plantar calcaneal foot/neurotrophic	Weekly debridement, absorbent dressing with strike through barrier, oral cephalexin for cellulitis
M/73	48	Left posterior heel margin/neurotrophic	Angioplasty, weekly debridement, capillary dressing, absorbent dressing with strike through barrier
F/31	7	Left plantar foot/venous	Weekly debridement, polyurethane foam dressing, CAM walker
F/90	6	Right medial malleolus/venous	Cadexomer iodine and gauze
APC			
M/66	48	Right plantar heel/neurotrophic	Two times/week debridement, nanocrystalline silver-coated polyethylene net dressing, polyethylene dressing, medical adhesive tape
M/82	12	Left retrocalcaneal foot/neurotrophic	Three times/week polyurethane foam dressing, medical adhesive tape, weekly debridement
F/74	7	Right lateral dorsal foot/venous	Angioplasty, weekly debridement, cadexomer iodine dressing, polyurethane foam dressing
M/65	12	Right lateral plantar foot/venous	Two times/week debridement, cadexomer iodine dressing, polyurethane foam dressing
M/75	36	Left medial malleolus/venous	Weekly debridement and polyurethane foam dressing with compression stockings
M/78	36	Left lateral lower leg/neurotrophic	Weekly debridement, povidone iodine/polyethylene glycol viscose net and absorbent dressing with strike through barrier

Table 2 Outline of treatment and follow-up protocol

Visit no.	Week no.	Clinical procedures (in order)
0	0	Pre-treatment: include or exclude according to criteria, obtain consent, physical examination, wound swab, skin biopsy, serum haematology and biochemistry
1	1	Treatment visits: wound history, Bates-Jensen tool, pain score, Cardiff Wound Impact Questionnaire, photograph wound, wound analysis with digital measuring device, debride, apply saline/activated protein C
2	2	
3	3	
4	4	
5	5	
6	6	
7	8	Follow-up visit: wound swab, skin biopsy, serum haematology and biochemistry
8	12	Follow-up visits: Cardiff Wound Impact Questionnaire, photograph wound, wound analysis with digital measuring device
9	20	

volume was significantly reduced to $73{\cdot}6 \pm 2{\cdot}2\%$ at week 2 ($p < 0{\cdot}05$) and continued to decrease, reaching $42{\cdot}5 \pm 7{\cdot}8\%$ of week 0 levels at week 6 ($p < 0{\cdot}001$; Figure 2). At the final treatment (week 6), the mean wound volume between the saline- and APC-treated groups was significantly different ($p = 0{\cdot}03$).

Bates-Jensen wound scores provide a numerical indicator of wound health or degeneration assessing wound erythema, exudate, granulation tissue and re-epithelialisation. Lower scores indicate healthier wounds, with a score of 13 indicating an intact healed wound. Mean scores for wounds at week 1 were $29{\cdot}0 \pm 1{\cdot}8$ and $26{\cdot}5 \pm 2{\cdot}5$ for saline- and APC-treated groups, respectively, indicating mild-moderate wound severity (Figure 3). Over the entire trial period (week 1 to week 20), there was no significant difference in mean scores for saline- or APC-treated groups. However, when only the treatment period (week 1 to week 6) was considered, there was a significant decrease in the APC-treated group from weeks 4–6 reaching $21{\cdot}7 \pm 2{\cdot}0$ at week 6 ($p < 0{\cdot}01$; Figure 3). The mean score for the saline-treated group at week 6 was $26{\cdot}0 \pm 1{\cdot}4$ and did not differ significantly from the APC-treated group. The increase in mean score for the APC-group at week 12 was due to one patient displaying wound deterioration (score of 45).

Anatomical pathology of the biopsies at week 0 showed epidermal acanthosis and hyperkeratosis, and/or dermal neutrophilic debris and inflammatory cell infiltrate in 7 of 12 wounds (4/6 APC-treatment group and 3/6 saline-treatment group). At week 8, biopsies in the saline-treated group displayed no change (2/6; 33%), reduced inflammatory cell infiltration (1/6; 17%) and increased vascular proliferation (1/6; 17%). Meanwhile, one patient displayed increased inflammatory cell infiltration with reduced vascular proliferation (1/6; 17%), and another patient displayed increased acanthosis and hyperkeratosis (1/6; 17%). APC-treated group biopsies showed no change (1/6; 17%), reduced inflammatory cell infiltration (3/6; 50%) and increased vascular proliferation (2/6; 33%).

The Cardiff Wound Impact Questionnaire was used to assess the impact of chronic lower leg ulcers on patient quality of life and tolerability of the trial from week 0 to week 20. There were no significant differences in Cardiff Wound Impact

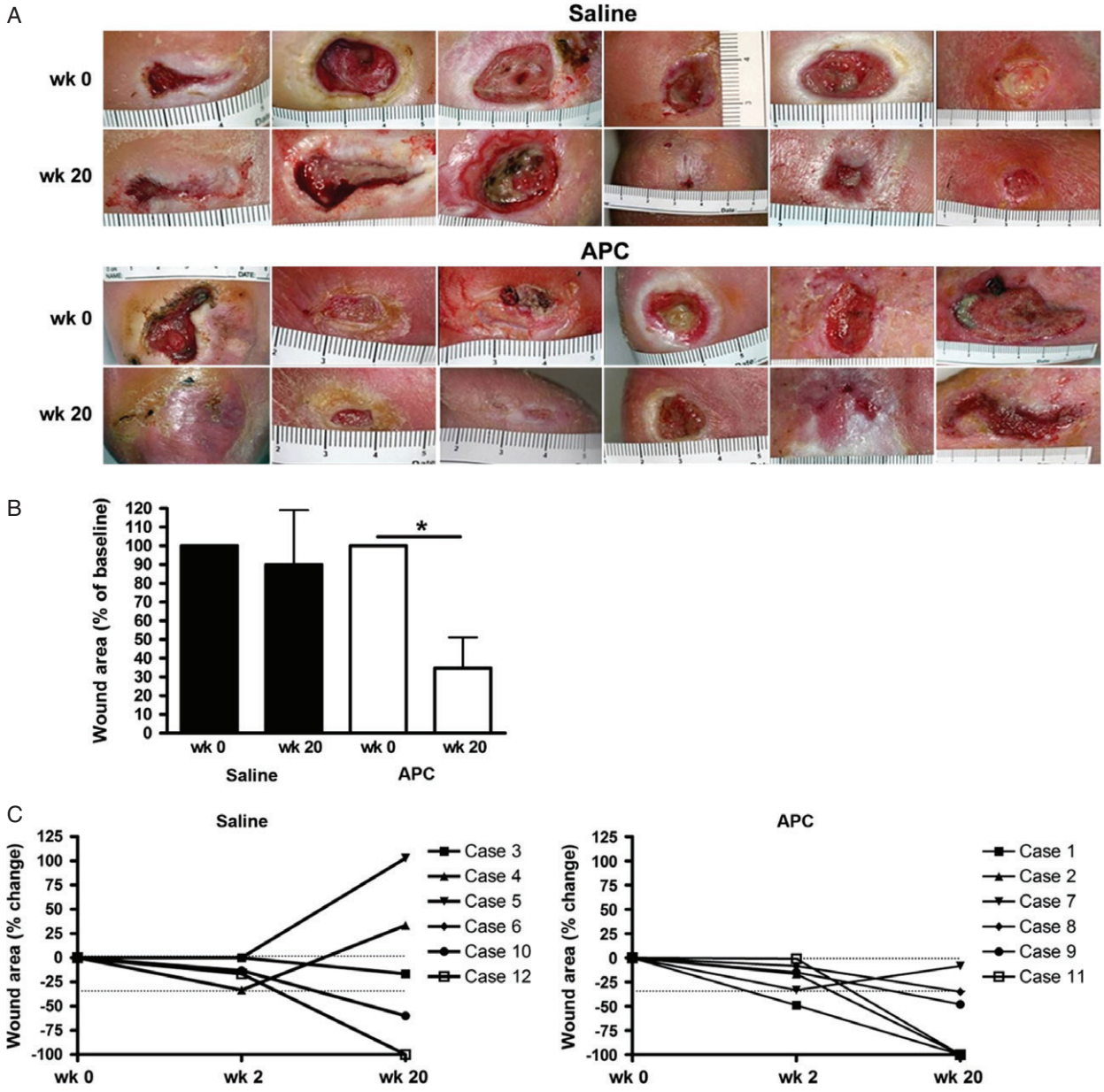

Figure 1 Photographs (A) and mean percentage change in wound area at week 20 (B) for saline- and activated protein C-treated groups. Data expressed as mean ± standard error of the mean. $*p < 0{\cdot}05$, paired Student *t*-test. Ruler scale is in centimetre. (C) Percentage change in wound area indicating the improvement (negative) or deterioration (positive) at week 2 and week 20 compared to week 0 measurements. Dotted line shows 30% reduction (−30%) in wound size, with −100% denoting wound closure.

scores between saline- and APC-treated groups at week 0 or week 20 (Figure 4). At week 20, APC-treated patients had significantly reduced stress associated with physical symptoms and daily living ($p = 0{\cdot}04$) and social life when compared to week 0 ($p = 0{\cdot}02$; Figure 4). Physical symptoms and daily living experience of APC-treated patients also showed a trend towards improvement ($p = 0{\cdot}06$). At the same time point, saline-treated patients had no significant changes in quality of life compared to week 0 (Figure 4).

Clinical signs assessed for safety, included body temperature, wound erythema, exudate and infection, were normal in both saline- and APC-treated groups. Laboratory parameters including serum functional protein C, HbA1c, CRP levels, and plasma coagulation parameters INR and APTT, did not differ significantly between treatment groups or time points.

Discussion

The chronic wound environment in patients with diabetes is complex and involves pathogenic changes in the epidermis and dermis, including inflammatory cell infiltration, neuropathy, impaired granulation tissue formation, angiogenesis and re-epithelisation. Ideal treatments should have a wide-ranging effect that targets multiple cell populations and

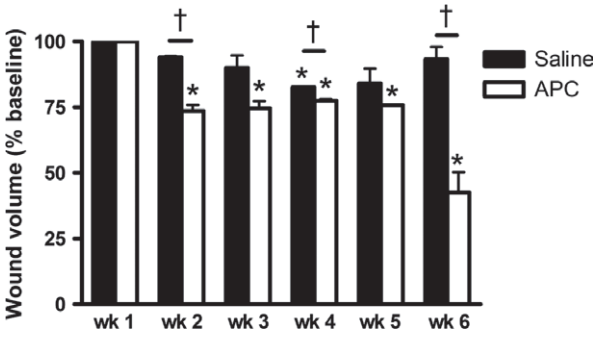

Figure 2 Wound volumes for saline- and activated protein C-treated groups. Data expressed as mean ± standard error of the mean. $^{*}p < 0{\cdot}05$, repeated-measures analysis of variance with Newman-Keuls post-hoc analysis; $^{\dagger}p < 0{\cdot}05$, unpaired Student *t*-test.

healing processes. APC has a number of wound healing properties including reduction of inflammatory cytokines, tumour necrosis factor-α, vascular cell adhesion molecule-1 and endothelial selectin and inhibition of neutrophil infiltration causing suppression of inflammation (16,20,21); increase in the production of angiogenic factors VEGF, matrix metalloproteinase-2 and Tie2, which promotes angiogenesis and creates stable blood vessels (13,16,22); stimulation of keratinocyte proliferation and stabilisation of epidermal barrier, required for re-epithelialisation (13,14,23). These effects are largely mediated through EPCR, with PC and EPCR expression by keratinocytes evident in the skin of healthy individuals (13,23) and in patients with diabetes (12).

Prior to its withdrawal from the market in late 2011, APC (marketed as Xigris® by Eli Lilly) had only been used clinically for the treatment of severe sepsis in adult patients with high mortality risk (24). Evidence is now emerging that APC is a potential treatment for chronic wounds of varying aetiologies (12,17). In this study, APC reduced inflammatory cell infiltration, increased vascular proliferation and significantly reduced the wound size and volume of lower leg ulcers in patients with diabetes. Decreasing volumes of APC injected into the wound space as a measure of wound volume and a decrease in Bates-Jensen scores over the treatment period were consistent with the observed improved wound healing by APC. Notably, our data suggests that APC improves wound health during the treatment period. However, the effect is not residual once APC treatment is terminated.

Further consideration for safety in our study was the effect of topical exogenous APC on plasma PC levels and coagulation parameters. Patients with diabetes have abnormalities of the coagulant system associated with a hyper-coagulable state (25). Plasma PC, and routine plasma coagulation parameters, INR and APTT were normal. Thus, topical APC treatment in our study did not exacerbate the hyper-coagulable state of patients with diabetes. Overall, the results show that APC treatment of lower leg ulcers in patients with diabetes improves wound healing, with a subsequent improvement in patient quality of life and no deleterious effect on patient safety.

The presence of unhealed lower leg ulcers in patients with diabetes can reduce their experience of daily and social activities compared to those with healed ulcers (26). The Cardiff Wound Impact Schedule has been validated in demonstrating the impact of chronic lower leg ulcers on patient health-related quality of life (26,27). Patients whose wounds were treated with APC reported less stress associated with physical symptoms and daily living, and social life compared to those treated with saline.

The method of application had the advantage of allowing the treatment to be in contact with the wound for 24 hours. However, this method was not ideal as it kept the wound in a wet environment which tended to cause maceration of local

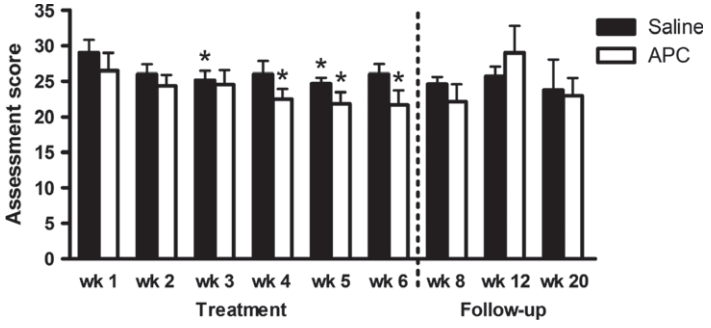

Figure 3 Bates-Jensen assessment scores for saline- and activated protein C-treated groups. Data expressed as mean ± standard error of the mean, where lower scores indicate wound improvement. $^{*}p < 0{\cdot}05$, repeated-measures analysis of variance with Newman-Keuls post-hoc analysis.

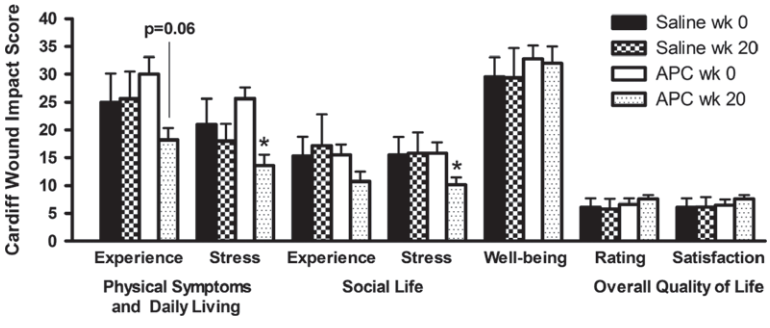

Figure 4 Cardiff Wound Impact Schedule scores from saline- and activated protein C-treated groups at week 1 and week 20. Data expressed as mean ± standard error of the mean, where lower scores indicate improved quality of life. $^{*}p < 0{\cdot}05$, paired Student *t*-test.

tissue. Future studies should investigate alternative application methods. Another limitation of this study was the small sample size. While a larger clinical trial is clearly required, this pilot study indicates that bi-weekly application of topical APC is a potentially effective, safe and well-tolerated treatment for chronic lower leg ulcers in patients with diabetes. By promoting wound healing, APC may reduce the number of lower extremity amputations in the population with diabetes thereby improving patient health-related quality of life.

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