

Greetings everyone;

Continuing with our theme on debridement, this week's article titled "[Effect of sharp debridement using curette on recalcitrant non-healing venous leg ulcers: A concurrently controlled, prospective cohort study](#)" was published in "Wound Repair And Regeneration " The International Journal of Tissue and Regeneration by Dr. DEAN WILLIAMS, MBBS, FRCS(Eng), FRCS (Gen Surg) and colleagues.

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Sincerely;

G.S. Dhillon MD PhD

**"Effect of sharp debridement using curette on
recalcitrant non-healing venous leg ulcers:
A concurrently controlled, prospective cohort study"**



Effect of sharp debridement using curette on recalcitrant nonhealing venous leg ulcers: A concurrently controlled, prospective cohort study

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The objective of this study was to evaluate the effect of sharp debridement on the progression of recalcitrant chronic venous leg ulcers (CVLU) and to assess the feasibility of performing this procedure in an outpatient setting. We performed a prospective study of 55 CVLU (53 patients) over a 12-month period. The study group, which underwent debridement, contained 28 CVLU whose wound beds had slough, nonviable tissue, and no granulation tissue. The control group was 27 CVLU with minimal (15–20%) granulation tissue, but no slough or nonviable tissue. Treatments were otherwise similar. Age, body mass index, mean ulcer surface area (MSA) and mean ulcer duration were comparable in both groups. Ulcer measurements were taken at 4 weeks before debridement, at the time of debridement, and 4 and 20 weeks post-debridement. There was no change in the MSA from 4 weeks before to the time of debridement in either group. At 4 weeks post-debridement, the study ulcers showed a 6 cm² reduction in the MSA vs. a 1 cm² reduction in controls ($P = 0.02$). By week 20 post-debridement, the study ulcers achieved a 7.4 cm² reduction in the MSA vs. an increase of 1.3 cm² in controls ($P = 0.008$). Between weeks 8 and 20 post-debridement, 16% of study ulcers vs. 4.3% of control ulcers achieved complete healing. Infection rates and antimicrobial usage were similar. We conclude that sharp debridement is effective in stimulating healing of recalcitrant CVLU. It is safe, well tolerated, and can be performed in an outpatient setting. (**WOUND REP REG 2005; 13:131–137**)

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ABPI	Ankle-brachial pressure index
BMI	Body mass index
CVLU	Chronic venous leg ulcer
MSA	Mean ulcer surface area

Despite advances in molecular biology and tissue-engineering, and a repertoire of other therapeutic options, venous leg ulceration remains a significant problem in the elderly. This problem is likely to worsen further with the predicted increase in the aged population. The estimated prevalence of active leg ulceration in Europe is at least 0.1–0.3%, with more than 1% of the population being affected at some time in their lives,^{1,2} about 70% of such leg ulcers are caused by venous disease.¹

Although the majority of simple venous ulcers respond to appropriate standard treatment, some ulcers are refractory to all conventional forms of therapy, and become chronic venous leg ulcers (CVLU).

The classical features of CVLU include a wound bed containing slough and nonviable tissue and the failure to develop and sustain healthy granulation tissue, an essential prerequisite for reepithelialization. The cause for nonhealing in such wounds is multifactorial, including the presence of nonviable tissue, an altered cellular and biochemical environment, the presence of pathogenic bacteria, and excess exudate that further worsens with infection. All these factors are detrimental for healing.^{3,4}

Debridement of the amorphous material from the wound bed of chronic ulcers has been used for many years to enhance healing. Various forms have been described: surgical, sharp, autolytic, enzymatic, mechanical, and biosurgical.⁵ Among them, surgical and sharp debridements are the quickest way to remove the nonviable, unhealthy material from the wound beds. Surgical debridement is performed under regional or general anesthesia when extensive excision of nonviable tissue is required. Sharp debridement, using a scalpel or curette, is performed to remove lesser amounts of devitalized, unhealthy tissue from chronic wounds, usually but not always with the aid of topical anesthesia.

The role of sharp debridement in the management of CVLU has never been fully evaluated. Likewise, there is no study to date that identifies the group of CVLU that might benefit from this form of debridement. The aims of this study were to evaluate the effectiveness of sharp debridement in combination with standard treatment regimens in the management of nonhealing CVLU and to assess whether it was appropriate and safe to perform the procedure in an outpatient setting.

MATERIALS AND METHODS

The study was a concurrently controlled, prospective parallel study over a 12-month period, consisting of two patient cohorts, comparing debridement and standard treatment vs. standard treatment alone in the management of nonhealing CVLU. All patients with static ulcers referred to a specialist wound center were considered for entry into the study. The duration of the study period was 24 weeks (4 weeks prior to debridement and 20 weeks post-debridement). Due to the existing evidence on the efficacy of debridement in chronic wounds,^{6,7} it was deemed clinically inappropriate to randomize patients with ulcers containing slough and nonviable tissue to a treatment option where debridement would not be part of their care. Hence a nonrandomized design was used.

Patient selection

Patients were included or excluded according to the criteria presented in Table 1. Venous disease was diag-

nosed clinically by history and examination, and was sometimes supplemented by duplex imaging. Patients with gross varicosities, characteristic skin changes (lipodermatosclerosis), and an ulcer, which appeared classically venous, were not subjected to duplex imaging. Duplex imaging was performed to confirm the presence of venous disease when the diagnosis was ambivalent and could not be made clinically. Significant arterial disease was excluded by palpation of both foot pulses and an ankle-brachial pressure index (ABPI) of 0.8 or greater. Patients with loss of one or both foot pulses or on whom it was not possible to perform an accurate ABPI measurement underwent arterial duplex imaging to exclude significant arterial disease.

Patients were recruited from the three weekly clinics in the study center. The patients in the study group, containing 26 patients (28 ulcers), were selected by virtue of their ulcer bed characteristics (see below). The control group, containing 27 patients whose wounds were not debrided, was selected from the patient attendance list using systematic sampling.

Patient assessment

Baseline patient characteristics including age, ulcer duration, and body mass indices (BMI) were recorded as well as initial mean ulcer surface area (MSA) and the state of the wound bed. All relevant past medical history including diabetes mellitus, neoplasia and connective tissue diseases were documented. Relevant drug history including steroidal and nonsteroidal anti-inflammatory medications was noted.

Criteria for debridement

The selection of ulcers for debridement was based on the appearance and state of the wound bed. The study group had nonhealing ulcers with slough and nonviable tissue in the wound beds but no granulation tissue. Ulcers that were clinically infected were not debrided during that visit. Ulcers in the control group were selected systematically and had wound beds with 15–20% granulation tissue, but no slough or nonviable tissue.

Debridement and post-debridement management

Debridement was performed by a single operator, using a sharp circular curette (size 4 or 7; Stiefel Laboratories Ltd., Buckinghamshire, UK) with the patient in a reclining or supine position. Debridement was performed avoiding the sensitive ulcer edges, and was aimed to remove the slough, nonviable tissue, and any avascular fibrous tissue down to the vascular base. In all patients, debridement was commenced without topical anesthesia and the procedure abandoned if the patient experienced pain or discomfort. Where the procedure was

Table 1. Inclusion and exclusion criteria

Inclusion criteria

- Ulcers of > 3 months duration
- Ulcers refractory to conventional treatment
- Ulcers with absent granulation tissue or the presence of nonviable tissue
- Ulcers containing yellow/white slough with or without fibrous/scar tissue
- Ulcers with copious amount of exudate
- Venous disease confirmed clinically and/or by color flow duplex imaging (CDI)
- No evidence of peripheral vascular disease, either on clinical examination or on CDI

Exclusion criteria

- ABPI < 0.8
- Patients with small (<2.5 cm²) or very large ulcers (> 100 cm²)
- Ulcers which were clinically infected with or without microbiological confirmation
- Ulcers associated with mixed etiology (e.g., arterio-venous ulcers)
- Ulcers secondary to systemic causes such as diabetes mellitus, connective tissue diseases (e.g., rheumatoid arthritis) and metabolic diseases
- Suspicion of malignancy within the ulcer
- Patients with concurrent unrelated malignancy

abandoned, a topical local anesthetic cream (Emla[®], combination of lidocaine [lignocaine] and prilocaine; AstraZeneca, London, UK) was applied to the wound for 30–45 minutes at the patient's repeat visit to complete debridement. Caution was exerted with patients taking anticoagulants. All but one patient (two episodes) had a single episode of debridement. A calcium alginate dressing (e.g., Kaltostat[®]; ConvaTec, Skillman, NJ) was applied to control the blood loss after debridement. Simple analgesics were prescribed for pain relief.

General ulcer management

There were no differences in the forms of compression or the types of dressings used between the groups. Four-layer bandage system was the most commonly used system in both groups, although alternative forms of compression such as short stretch bandages and tubular bandages (tubigrip) were employed if patients could not tolerate the four-layer system. Non- or low-adherence dressings were used in both groups and the frequency of dressing changes was similar.

Ulcer measurements

The ulcer measurements were performed at 4 weeks prior to debridement (−4), at debridement (0), and at 4 (+4) and 20 weeks (+20) post-debridement in the study group and at similar points in the controls. Ulcer areas were calculated using maximum length and width measurements using disposable measuring tapes complemented by digital area analysis using acetates. Irregular shaped ulcers were measured using a “Mavis” (Measurement of Area and Volume Instrument; University of Glamorgan, Ponty Pridd, UK) digital camera.⁸ All the ulcer measurements and images were taken by nursing staff in the unit.

Follow-up

All patients were followed up for a minimum of 20 weeks after debridement. Any complications, including pain, hemorrhage, and infection, were documented.

The use of systemic and topical antimicrobials was also recorded.

Statistical analysis

Appropriate summary statistics were used to describe the sample at baseline and comparisons were made using Chi Square and Student's *t*-test. MSA and rate of change were calculated using mixed ANOVA, with post hoc analysis assuming nonhomogenous variance. Two-tailed alpha was set at 0.05.

RESULTS

Fifty-three patients with 55 ulcers were considered suitable initially and were entered into the study. Twenty-six patients with 28 ulcers underwent debridement and 27 patients acted as controls. One patient contributed two ulcers (different limbs) on separate occasions to both the study and control groups. Two patients (two ulcers) from the debridement group had to be subsequently excluded: one patient was diagnosed as having leukemia during the later stages of follow-up and the second patient did not comply with the recommended compression. Hence a total of 24 patients (26 ulcers) from the study group and 27 patients from the control group were included in the analysis. Wound measurements at week 20 were not obtained in two patients from the study group.

The sex ratio, mean age of patients, BMI, and mean ulcer duration were very similar for both groups (Table 2). Six patients from the study group and seven from the control group had taken nonsteroidal anti-inflammatory drugs during the study period ($P=0.21$). One patient from both groups had systemic steroids (one short course of oral prednisolone for bronchial asthma) during the study period. Among the 26 patients who had wound debridement, 24 patients tolerated the procedure without topical anesthesia. There was no

Table 2. Baseline patient characteristics

Parameter	Study group	Control group	P-value
n	24	27	
Mean age in years (SD)*	74.5 (8.5)	71.1 (13.5)	0.28
BMI mean (kg ⁻²) (SD)	29.2 (4.4)	27.7 (4.5)	0.22
Sex			
Male	10 (41%)	13 (48%)	0.48
Female	14 (59%)	14 (52%)	
Mean ulcer duration in years** (SD)	3.3 (3.7)	3.7 (3.4)	0.65
NSAID usage [‡]	6 (25%)	7 (27%)	0.21

*Standard deviation.

**Duration of ulcer prior to study period.

[‡]Nonsteroidal antiinflammatory drug usage at any point during study period.

post-debridement hemorrhage. In all patients, pain after debridement was controlled with simple analgesics.

The initial MSA of the ulcers, measured at -4 weeks, was 20.2 cm² in the study group and 21.2 cm² in the control group ($P=0.86$). At 0 weeks, the MSA of the ulcers was 19.6 cm² and 20.8 cm² in the study and control groups, respectively ($P=0.83$), and the change in MSA between -4 weeks and 0 weeks was not statistically significant ($P=0.92$; Table 3). From 0 weeks to +4 weeks, the ulcers in the study group achieved a 6 cm² (19.6 cm²-13.6 cm²) reduction in the MSA compared to a 1 cm² reduction (20.8 cm²-19.8 cm²) in the control group ($P=0.02$). By week +20, the MSA of the study ulcers reduced further and achieved a 7.4 cm² total reduction in the MSA compared to an increase of 1.3 cm² in controls during the same period ($P=0.008$; Table 3). The reduction in the MSA between the groups over the entire study period did not achieve statistical significance (ANOVA; $F_{3,49}=3.03$, $P=0.13$) (Table 4; Figure D).

Five ulcers from both groups healed completely within the 24-week study period. Of these, one ulcer (3.8%) from the study group and four ulcers (14.8%) from the control group healed within 12 weeks of entering the study. However, four ulcers (16%) in the study group and only one ulcer (4.3%) in the control group healed between weeks 12 and 24.

There were eight clinically diagnosed episodes of wound infection in the study group in the first 4 weeks after debridement compared to 10 in the control group

over the same period. The use of antimicrobials, both systemic and topical, was similar in both groups ($P=0.48$; Table 5).

DISCUSSION

Although the majority of simple venous leg ulcers respond to a combination of rest, graduated compression, dressings, and care of the surrounding skin, some ulcers are refractory to all established forms of treatment and progress to CVLU. Various treatment modalities such as autologous full-thickness punch grafts,⁹ cultured autologous and allogeneic keratinocyte sheets,¹⁰ stabilized hydrogen peroxide cream¹¹ (Crystacide®, Bradley Pharmaceuticals, Fairfield, NJ), oral micronized purified flavanoid fraction (Daflon 500 mg, Laboratory Servier, Orleans, France)¹² and fascial pedunculated rotation flaps¹³ have been attempted to treat CVLU, but none of them are currently established in routine clinical practice.

Advances in molecular biology have resulted in the development of several tissue-engineered skin substitutes aimed to treat chronic ulcers.¹⁴ However, such devices are not effective in infected ulcers, wounds containing slough and nonviable tissue, and/or wounds with a devitalized wound bed.¹⁵ Complete excision of the ulcer with surrounding lipodermatosclerosis and covering of the area with meshed split-skin graft (shave therapy)¹⁶ is an established method to treat CVLU. However, this procedure requires general or

Table 3. Change in MSA: Comparison between debrided (study) group and control group

	Change in MSA (cm ²)		
	Study group	Control group	P-value
n (ulcers)	26	27	
-4 to 0 weeks	-0.6*	-0.4	0.92
0 to +4 weeks	-6.0	-1.0	0.02
0 to +20 weeks	-7.4	+1.3**	0.008

*Decrease in MSA.

**Increase in MSA.

Table 4. MSA of wounds during the entire study period

Time	MSA in cm ² (SD)*		
	Study group	Control group	P-value
n (ulcers)	26	27	
-4 weeks	20.2 (20.4)	21.2 (19.9)	0.86
0 weeks	19.6 (19.2)	20.8 (21.1)	0.83
+4 weeks	13.6 (15.3)	19.8 (22.7)	0.25
+20 weeks	12.2 (16.5)	22.1 (27.0)	0.13

*Standard deviation.

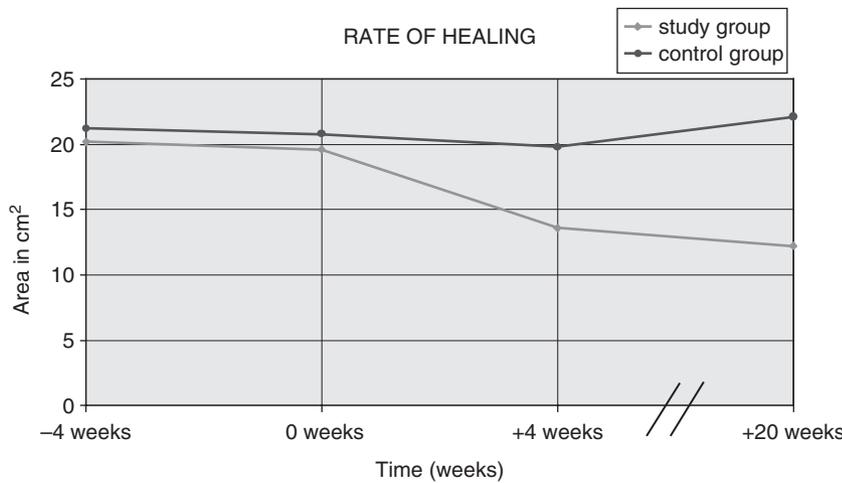


FIGURE 1. Rate of healing of the two patient groups over the 24 weeks of the study.

regional anesthesia and results in the creation of a new wound (graft donor site) with associated risks such as infection. In addition, the graft take is variable with the possibility of complete graft loss.

Chronic ulcers, unlike acute ulcers, seldom follow the normal pattern of repair. Several factors such as the alterations and imbalance in the actions of cytokines, growth factors, and cellular and extracellular elements have been purported to contribute to the nonhealing nature of chronic ulcers.¹⁷⁻²⁰ In addition, there is accumulation of nonviable tissue and slough, and excess exudate, all of which encourage bacterial colonization and prevent healing.^{21,22} Furthermore, in chronic ulcers, when bacteria proliferate, they form microcolonies that attach to the wound bed and secrete a glycocalyx or “biofilm” that helps to protect them from the action of antimicrobial agents.²³

The role of debridement, therefore, is to efface the wound bed of excess exudate, expunge nonviable material and slough, and disassemble or dislodge bacterial colonies (biofilms). Although various forms of debridement have been advocated to enhance healing, none has gained universal acceptance. Randomized controlled trials are lacking and there is insufficient evidence at present to advocate any one form of debridement in the management of CVLU.²⁴

Surgical debridement is not a new technique and historical texts show that ancient civilizations often made surgical changes to the wound bed.²⁵ It is the most rapid way of removing the nonviable, unhealthy

material from the wound. Surgical debridement is performed in the operating room, usually under regional or general anesthetic, when there is a need for extensive debridement to remove necrotic tissues, and if the patient is septic.²⁶ Sharp debridement, on the other hand, can be performed at the patient’s bedside, in the home or clinic, using a sharp instrument such as a scalpel²⁷ or curette.²⁸ Although all forms of debridement aim to remove the detrimental agents from the wound bed, sharp debridement, in addition, creates acute wounds, which not only improves the vascularity of the ulcer bed, but also stimulates an acute wound healing response. Once hemostasis is secured, neutrophils and macrophages are attracted to the wound site. The action of these cells, in addition to secreting growth factors and enhancing inflammation, is to phagocytize bacteria (sharp debridement helps to break down bacterial colonies) and nonviable tissue present within the wound. The cytokines and growth factors released into the ulcer bed after debridement can act more effectively in an exudate- and slough-free environment. These features distinguish sharp from other forms of debridement.

In this study, although the overall reduction in MSA between the groups failed to reach statistical significance over the 20-week follow-up period, sharp debridement was clearly effective in initiating the healing process in the study group, as observed by the 6 cm² reduction in the MSA achieved by ulcers within 4 weeks of debridement compared to 1 cm² reduction in controls. The patient’s ulcers also continued to show a decrease in MSA until week + 20, achieving a 7.4 cm² decrease from the point of debridement compared to the 1.3 cm² increase observed in controls (*P* = 0.008) during the same period.

The patients in the study group had nonhealing ulcers whose wound beds had slough and nonviable tissue, but no granulation tissue. The patients in the control group also contained nonhealing ulcers but their wound beds had 15–20% granulation tissue (but

Table 5. Infection rates and antimicrobial usage 4 weeks following debridement

	Study group	Control group	<i>P</i> -value
n (ulcers)	26	27	
Infection*	8 (31%)	10 (37%)	0.77
Antimicrobial usage**	15 (58%)	13 (48%)	0.49

*Clinical infection with or without microbiological confirmation.

**Use of topical or oral antimicrobials for wound infection or concurrent use for other indication.

no slough or nonviable tissue) and hence were expected to progress toward healing. Indeed, four ulcers from this group (compared to one from the study group) achieved total healing in the first half of the study period (-4 weeks to +8 weeks). However, the remaining ulcers failed to show any improvement in the rate of healing despite having healthier wound beds compared to the study ulcers, and only one ulcer healed between weeks +8 and +20. On the contrary, four ulcers from the study group achieved total healing between +8 and +20 weeks, suggesting that debridement not only acts as a useful trigger in initiating the healing process, but could also help in achieving complete healing.

These results must be treated with caution, however, as some key limitations must be borne in mind. This is an ethically difficult area in which to conduct randomized clinical trials, and as such, a nonrandomized method was used in this study. The consequence of a less rigorous design is that the patient groups are less homogenous than expected, which may explain some of the variability in response to treatment. In addition, the sample size is relatively small, although the findings are sufficiently interesting to justify further work in this area.

One of the concerns regarding sharp debridement is the creation of acute wounds that might provide new portals of entry for pathogenic microorganisms into the wound, thus increasing the risk of infection. Our study, however, does not support this claim. The infection rate in the study group after 4 weeks following debridement was, in fact, less than in the control group during the same period.

This study shows that sharp debridement using a curette could be performed effectively in an outpatient setting. The procedure is well tolerated by patients and only two patients in this study required topical anesthesia. Anesthesia may not be required if the procedure is performed carefully, avoiding the sensitive skin edges; if required, a topical anesthetic cream such as Emla[®] applied 30 minutes prior to debridement is effective.²⁹ The time to debride an ulcer is approximately 5 minutes and hence not more than that needed for cleansing slough and exudate from the wound bed. Post-debridement bleeding is minimal and is easily controlled by the application of gentle local pressure and a calcium alginate dressing (e.g., Kaltostat[®]). Although none of the patients in our study had significant post-debridement hemorrhage, the procedure should not be taken lightly as there is a clear risk of hemorrhage from underlying vessels. This is particularly significant in the gaiter region of the leg because of its close proximity to the long saphenous vein. The ability to deal with and manage such injuries is therefore imperative.

Because this study was intended to be a pilot study, only wounds with slough, nonviable tissue, and absent

granulation tissue were debrided, and they advanced toward healing. The control group, devoid of slough and nonviable tissue but containing 15–20% granulation tissue, although expected to heal failed to show progress. It is hence conceivable that ulcers in this group would have also improved if subjected to debridement. The authors therefore recommend that clinicians debate how patients with different wound bed characteristics could be randomized in the future in trials involving debriding agents. Because our study has shown sharp debridement to be useful in stimulating healing, the ethics of not debriding a CVLU with slough and nonviable tissue is an obvious concern that needs to be addressed when designing such trials.

In conclusion, sharp debridement, in combination with the standard venous ulcer treatment regimen, is an effective method to expedite healing of intractable CVLU, refractory to conventional treatment alone. The procedure is safe, well tolerated, and can be performed in an outpatient setting. Randomized clinical trials are needed to further substantiate the results observed in this study.

REFERENCES

1. Callam MJ, Ruckley CV, Harper DR, Dale JJ. Chronic ulceration of the leg: extent of the problem and provision of care. *BMJ* 1985;290:1855–6.
2. Nelzen O, Bergqvist D, Lindhagen A. The prevalence of chronic lower-limb ulceration has been underestimated: results of a validated population questionnaire. *Br J Surg* 1996;83:255–8.
3. Falanga V. Classifications for wound bed preparation and stimulation of chronic wounds. *Wound Rep Reg* 2000;8:347–52.
4. Edlich RF, Panek PH, Rodeheaver GT, Turnbull VG, Kurtz LD, Edgerton MT. Physical and chemical configuration of sutures in the development of surgical infection. *Ann Surg* 1973;177:679–88.
5. Schultz GS, Sibbald RG, Falanga V, Ayello EA, Dowsett C, Harding K, Romanelli M, Stacey MC, Teot L, Vanscheidt W. Wound bed preparation: a systematic approach to wound management. *Wound Rep Reg* 2003;11 (Suppl. 1):S1–S28.
6. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg* 1996;183:61–4.
7. Drager E, Winter H. Surgical debridement versus enzymatic debridement. In: Baharestani M, Gottrup F, Holstein P, Vanscheidt W, editors. *The clinical relevance of debridement*. Berlin: Springer-Verlag, 1999:59–71.
8. Jones BF, Plassman P. An instrument to measure the dimensions of skin wounds. *IEEE Transact Biomed Engineer* 1995;42:464–70.
9. Mol MA, Nanninga PB, van Eendenburg JP, Westerhof W, Mekkes JR, van Ginkel CJ. Grafting of venous leg ulcers. An intra-individual comparison between cultured skin equivalents and full-thickness skin punch grafts. *J Am Acad Dermatol* 1991;24:77–82.
10. Lindgren C, Marcusson JA, Toftgard R. Treatment of venous leg ulcers with cryopreserved cultured allogenic keratinocytes: a prospective open controlled study. *Br J Dermatol* 1998;139:271–5.
11. Belcaro G, Cesarone MR, Nicolaidis AN, Geroulakos G, Di Renzo A, Milani M, Ricci A, Brandolini R, Dugall M, Ruffini I, Cornelli U, Griffin M. Improvement of microcirculation and healing of venous hypertension and ulcers with Crystacide. Evaluation of free radicals, laser Doppler flux and PO₂. A prospective-randomized-controlled study. *Angiology* 2003;54:325–30.

12. Coleridge Smith PD. From skin disorders to venous leg ulcers. pathophysiology and efficacy of Daflon 500 mg in ulcer healing. *Angiology* 2003;54 (Suppl. 1):S45-50.
13. Montisci R, Ruju C, Massoni G, Sanfilippo R, Brotzu G. Chronic venous ulcer treatment with fascial pedunculated graft. *J Cardiovasc Surg (Torino)* 2003;44:249-53.
14. Falanga V, Sabolinski M. A bilayered living skin construct (Apligraf) accelerates complete closure of hard-to-heal venous ulcers. *Wound Rep Reg* 1999;7:201-7.
15. Brem H, Balledux J, Sukkarieh T, Carson P, Falanga V. Healing of venous ulcers of long term duration with a bilayered living skin substitute: results from a general surgery and dermatology department. *Dermatol Surg* 2001;27:915-9.
16. Schmeller W, Gaber Y, Gehl HB. Shave therapy is a simple, effective treatment of persistent venous leg ulcers. *J Am Acad Dermatol* 1998;39 (2 Part 1):232-8.
17. Barone EJ, Yager DR, Pozez AL, Olutoye OO, Crossland MC, Diegelmann RF, Cohen IK. Interleukin-1 alpha and collagenase activity are elevated in chronic wounds. *Plast Reconstr Surg* 1998;102:1023-7.
18. Harris IR, Yee KC, Walters CE, Cunliffe WJ, Kearney JN, Wood EJ, Ingham E. Cytokine and protease levels in healing and non-healing chronic venous leg ulcers. *Exper Dermatol* 1995;4:342-9.
19. Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, Murphy G, Schultz G. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Rep Reg* 1999;7:442-52.
20. Yager DR, Zhang LY, Liang HX, Diegelmann RF, Cohen IK. Wound fluids from human pressure ulcers contain elevated matrix metalloproteinase levels and activity compared to surgical wound fluids. *J Invest Dermatol* 1996;107:743-8.
21. Falanga V. Wound bed preparation and the role of enzymes: a case for multiple actions of therapeutic agents. *Wounds* 2002;14:47-57.
22. Falabella AF, Carson P, Eaglstein WH, Falanga V. The safety and efficacy of a proteolytic ointment in the treatment of chronic ulcers of the lower extremity. *J Am Acad Dermatol* 1998;39: 737-40.
23. Davey ME, O'Toole GA. Microbial biofilms: from ecology and molecular genetics. *Microbiol Mol Biol Rev* 2000;64:847-67.
24. Bradley M, Cullum N, Sheldon T. The debridement of chronic wounds: a systematic review. *Health Technol Assess* 1999; 3 (17 Part 1):iii-iv, 1-78.
25. Lyons AS, Petrucelli RJ. In: Abrams HA, editor. *Medicine: an illustrated history*. New York: Aberdale Press, 1987:7-21.
26. Sieggreen MY, Makelbust J. Debridement choices and challenges. *Adv Wound Care* 1997;10:32-7.
27. Baharestani M. The clinical relevance of debridement. In: Baharestani M, Gottrup F, Holstein P, Vanscheidt W, editors. *The clinical relevance of debridement*. Berlin: Springer-Verlag, 1999:3.
28. Higgins KR, Ashry HR. Wound dressings and topical agents. *Clin Podiatr Med Surg* 1995;12:31-40.
29. Rosenthal D, Murphy F, Gottschalk R, Baxter M, Lycka B, Nevin K. Use of topical anaesthetic cream to reduce pain during sharp debridement of chronic leg ulcers. *J Wound Care* 2001;10: 503-5.