The LeucoPatch System for the management of hard-to-heal diabetic foot ulcers in the UK, Denmakr and Sweden: An observer-blinded, randomised controlled trial

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Research in context

Evidence before this study.

Successive systematic reviews performed by the International Working Group of the Diabetic Foot and others have failed to show consistent cost effective benefit of any topical therapy to accelerate healing of foot ulcers in patients with diabetes and the majority of published studies have significant methodological weaknesses. Nevertheless, a number of studies have suggested the potential benefit of blood-derived products even though the results have hitherto been inconsistent.

Added value of this study

This study is, to our knowledge, the first large randomised observer blind controlled trial of the use of multi-layered patches comprising autologous leucocytes, platelets and fibrin generated by the bedside and without adding any reagents. The effect of the patches was compared with good usual care in people with diabetic foot ulcers which were not healing despite good usual care. The study was conducted to a high standard as recommended in recent guidelines.

In the intention-to-treat analysis the addition of the autologous multi-layer patch to usual good standard care was associated with a significant 1.58 fold increase in the proportion of wounds confirmed healed. There were no significant differences in the number of adverse events, in particular the number of patients who developed anaemia.

Implications of all the available evidence

In people with diabetes complicated by foot ulcers which are not healing despite best standard of care, this new bedside treatment has the potential to significantly accelerate wound healing.

Abstract

Background: The LeucoPatch® device uses bedside centrifugation without additional reagents to generate a disc comprising autologous leucocytes, platelets and fibrin which is applied to the surface of the wound. The aim of the study was to test the effectiveness of LeucoPatch on the healing of hard-to-heal foot ulcers in people with diabetes.

Methods: This was a multicentre, international, observer-blind, randomised controlled trial of 595 people with diabetes and a foot ulcer who consented to participate. After a 4 week run-in-period those with a reduction in ulcer area of < 50% were randomised to either pre-specified good standard care alone or care supplemented by weekly application of LeucoPatch. The primary outcome was percentage of ulcers healed within 20 weeks, defined as complete epithelialisation confirmed by an observer blind to randomisation group and maintained for four weeks.

Findings: 269 people were randomised; mean age 62 years, 82% male and 83% Type 2 diabetes. In the intervention group 34·1% (n=45/132) of ulcers healed within 20 weeks vs. 21·6% (n=29/134) of the controls (OR 1·58, 95% CI 1·06 - 2·35; p= 0·0235) by intention-to-treat analysis. Time to healing was shorter in the intervention group (p=0·0246). No difference in adverse events was seen between groups.

Interpretation: The use of LeucoPatch is associated with significant enhancement of healing of hard-to heal foot ulcers in people with diabetes.

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Trial registration: International Standard Randomised Controlled Trial, ISRCTN 27665670. ClinicalTrials.gov, NCT02224742. Registered on 5 July 2013.

Background

Diabetic foot ulcers (DFUs) are common and present a major source of disability, distress and cost. Healing is often delayed for many months and limb loss through amputation is common. The incidence of new ulceration after healing is approximately 40% at 12 months and DFUs therefore present a major financial cost to patients, their families and health care services.[1,2] A principal cause of the problem is the absence of treatments for which there is evidence of effectiveness and this relates to a large extent to the quality of available research, which is mostly of poor design.[3]
It follows that trials to document the effectiveness of treatments for this complex clinical problem should conform to defined criteria for trial design and reporting.[4] To that end, it is necessary that the evaluation of any treatment should be undertaken in a population which has already been shown to be responding poorly to good standard care (ie. 'hard to heal' ulcers) and should be based on a comparison of the effect of the treatment being tested with contemporaneous controls in an appropriately blinded randomised trial.

One possible treatment option for non-healing ulcers is the use of platelet-rich plasma or platelet-rich fibrin, which may promote healing in DFUs judged to be 'hard to heal' through the release of cytokines and growth factors involved in tissue repair, angiogenesis and inflammation.[3,5-8] While the use of platelet preparations is not new, it has been associated with only inconsistent evidence of benefit [3,9,10]. However, the recent development of the capacity to produce multi-layered patches comprising autologous leucocytes, platelets and fibrin by the bedside and without adding any reagents (Leucopatch®, Reapplix ApS, Birkerød, Denmark) (Figure1) has introduced a possible new option. [11,12] Two pilot studies, of which one included participants with hard-to heal diabetic foot ulcers only, have reported beneficial effects on ulcer healing, without raising any safety issues.[13,14]

We now report the outcome of a randomised controlled observer-blinded trial aimed to determine whether the application of LeucoPatch, when used in addition to usual care in a multidisciplinary specialist diabetes foot clinic setting, is superior to usual care alone in healing of hard-to heal DFUs which are not infected at the time of randomisation.

Methods

Trial Design

The trial protocol and rationale has previously been published.[15] This was a multicentre, multinational, observer-blind, randomised controlled trial, undertaken in 32 centres with specialist diabetic foot clinics in the UK, Denmark, and Sweden. After providing written informed consent, all participants were entered into a 4 week run-in period before randomisation 1:1 to either the intervention plus usual care arm or usual care alone arm. The intervention period of 20 weeks was followed by a 6 week observation period. The study was performed in compliance with the regulatory requirements of the three countries, in accordance with the ethical principles of the Declaration of Helsinki and recommendations for Good Clinical Practice. The study was approved by the National Research Ethics Committee West Midlands - Birmingham South (reference 13/WM/0202) and by the R&D departments of the participating NHS trusts (UK), the ethics committee for Region Midtjylland, Committee 1, Denmark (Reference 1-10-72-99-13) (Denmark) and by the Regionala Etikprovningsnamnden, Lund, Sweden (Reference 2013/6). This trial is registered with Clinicaltrials.gov (NCT02224742) and ISRCTN 27665670.

Study setting and participants

Participants were people aged 18 years and over who had diabetes according to WHO criteria complicated by one or more foot ulcers and a baseline HbA_{1c} of $\leq$108 mmol/mol. Ulcers were situated below the level of the malleoli, but excluded ulcers confined solely to the interdigital clefts because of the difficulty in measurement and in placing a patch directly on the wound. All ulcers

were “hard to heal” meaning that the cross-sectional area decreased by less than 50%, and the cross-sectional area of the index ulcer was ≥50 and ≤1000 mm^2 at the end of the 4 week run-in period.[16,17] At baseline, the index ulcer was clinically non-infected according to the criteria of the Infectious Diseases Society of America (IDSA)[18] and either the ankle-brachial index (ABPI) of the affected limb was between 0·50 and 1·40 or the dorsalis pedis pulse and/or the tibialis posterior pulse was palpable. Participants had to have the capacity to understand study procedures, and to provide written informed consent.

Participants were not eligible for inclusion if any of the following applied: cross-sectional area of the index ulcer had increased by ≥25% or had decreased by >50% during the 4 week run-in period, or was either smaller than 50 mm^2 or larger than 1000 mm^2 at the end of that time. They were similarly ineligible if there were clinical signs of infection of the index ulcer or other reason to suspect that infection was present at randomisation, if a revascularisation procedure in the affected limb was planned, or had been undertaken within the 4 weeks prior to the baseline visit, if the foot ulcer had been treated with growth factors, stem cells or an equivalent preparation within the 8 weeks prior to the baseline visit or if there was a need for continued use of negative pressure wound therapy. Participants were also not eligible if their haemoglobin concentration was <105 g/L at screening, they had sickle-cell anaemia, haemophilia, thrombocytopenia (<$100x10^9$/L) or any other clinically significant blood dyscrasia, if there was known potential infectivity of blood products, including known HIV and hepatitis, if they were on renal dialysis or had an estimated GFR (based on cystatin C or serum creatinine) of <20 ml/min/1·73m^2, if they were on current treatment with cytotoxic drugs or with systemically administered glucocorticoids or other immunosuppressants, if they were unlikely to comply with the need for weekly visits because of other planned activity, if they had been in another interventional clinical foot ulcer-healing trial within the 4 weeks prior to the baseline visit, if they had been previously randomised to the current study or if the investigator judged that they did not have the capacity to understand the study procedures or provide written informed consent.

Patients were recruited from and were managed in one of 32 specialist diabetic foot clinics in 3 countries (UK, Denmark, Sweden).

Randomisation and blinding

Participants who were eligible following the run-in period, were randomly assigned to either usual care plus intervention or usual care alone. The computer-generated, web based, randomisation code using permuted blocks of randomly varying size (2, 4 and 6) was created by the Nottingham Clinical Trials Unit (CTU). Trial participants were allocated with equal probability to each treatment arm with stratification by centre, and by ulcer area $\leq 100mm^2$ versus $>100mm^2$.

Clinical investigators assessing outcomes were masked to group assignment throughout the study duration, as was the study statistician before the clinical database had been cleaned and locked. Participants, care givers and site investigators were not blind to the treatment allocation. In the event of a disagreement between site investigators and the blinded clinical primary outcome assessor, or if a blinded assessment was not done or was delayed beyond the permitted window described in the protocol, a blinded adjudication committee reviewed the digital images.

Procedures

Clinical investigators were instructed to manage all eligible ulcers with the best available standard of usual care, including offloading, according to International Working Group of the Diabetic Foot (IWGDF) guidelines either alone (during the run-in period and those in the control arm) or in addition to the intervention (during the 20 week active treatment phase if in the intervention arm).[21] Basic demographics, medical history and eligibility criteria were assessed at baseline. Thereafter, assessment of wound characteristics, wound size by acetate tracing (area assessed at a later date using Image J[20] by a single blinded assessor), digital images of the ulcer taken post debridement, active medication including antibiotic prescriptions, type of offloading used (classified into 9 types), adverse device effects, serious adverse events and adverse device events were recorded at every visit. Participant visits were scheduled every 2 weeks during the run-in period, and weekly during the intervention period. Should the index ulcer have healed during the intervention period, the participants were seen again at 2 weeks and 4 weeks post healing, with a blinded assessment of healing both at the point of healing and at the 4 weeks post healing visit. A detailed description of study procedures is provided in the previously published protocol paper.[15]

The study intervention

The active intervention was the application of a LeucoPatch patch directly to the wound which was performed at the bed-side in the clinical centres. Each week a patch was produced by drawing 18 mL of the patient's venous blood into a LeucoPatch device (Reapplix ApS, Birkerød, Denmark), which was then transferred to a LeucoPatch Centrifuge (Reapplix Aps; Birkerød, Denmark) and spun for 20 minutes according to an automatic and pre-specified programme. The final three layered LeucoPatch (Figure 1) was then removed from the device using aseptic precautions, cut into appropriate size if necessary, and applied onto the ulcer with the leucocyte side adjacent to the surface of the ulcer, before being covered by a low adherent, knitted viscose rayon primary dressing (Tricotex®, Smith & Nephew, London, UK) and a protective secondary dressing. Participants with an ulcer area larger than 5 cm^2 had two patches prepared and applied. New patches were made and applied on a weekly basis until healing or the end of the study.

Outcomes

The primary outcome was the number (%) of ulcers that healed within 20 weeks following randomisation. Healing was assessed following any necessary debridement and was defined as complete epithelialisation without drainage, and which was maintained for four weeks. Healing was confirmed both at the start and the end of the four week period by an appropriately trained observer who was blind to randomisation group. The date of healing was defined as that at which the ulcer was first noted by the clinical researcher and confirmed by an observer who was blind to the randomisation group.

Secondary ulcer-related outcomes included time to healing in those that healed within the 20 week active intervention period, the proportion of healed ulcers at 12 and 26 weeks, the change in ulcer area at 4, 12, 16, 20 and 26 weeks (as compared to week 0), was assessed from digital images of acetate tracings using Image J,[20] the incidence of secondary infection, the number of days of systemic

antibiotic therapy administered for infection of the foot ulcer during the 20 weeks from randomisation. Secondary patient-related outcomes also included the incidence of major (above ankle) amputation affecting the target limb by 12, 20 and 26 weeks, the incidence of major amputation affecting the contralateral limb by 26 weeks, the incidence of minor (below ankle) amputation affecting the target limb by 12, 20 and 26 weeks, the incidence of minor amputation affecting the contralateral limb by 26 weeks, and incidence of new anaemia, defined as haemoglobin level below <105 g/L (6.5 mmol/L) and decreased more than 10 % compared to baseline.

Statistical analysis

Previous non-controlled LeucoPatch outcome data suggested a healing rate (intention to treat) of 54% during a 20 week follow-up period, in a cohort of patients selected by a less than 40 % ulcer area reduction during a 2 week run-in-period.[14] The incidence of healing in a matched control group as well as in placebo/control groups in other diabetic foot studies with inclusion and exclusion criteria similar to those used in the present study was between 27 and 32% at 20 weeks follow-up, although some authors have reported healing rates below 10%.[3] A sample size for comparing two proportions with Fleiss continuity correction, based on alpha = 0·05 and beta = 80% and with an incidence of healing in the control group of 30% and with an improvement of 18 percentage points (i.e. to 48%) in the treatment group gave a sample size of 250 evaluable patients. To allow for 30% drop out a sample size of 350 randomised participants was estimated. A pre-planned interim analysis of the primary endpoint was carried out by an independent statistician, not otherwise involved in the study, when 140 patients had completed 20 week follow-up. The purpose was to check for non-futility, establish the drop-out rates and, if necessary, recalculate the sample size. The result of the interim analysis was only conveyed to the independent Data Monitoring Committee (DMC). On the advice of the DMC the Trial Steering Committee (TSC) suggested an adjustment to the sample size to from 350 to 260 patients based on fewer drop outs than anticipated, and further, on the basis of a slight imbalance in the numbers randomised to each group, to 269. The interim analysis meant that the level of significance, for the primary endpoint, was reduced to 0·04.

Statistical analyses were conducted with SAS EG 7.1. All analyses were performed on both the intention-to-treat (ITT) population and the per-protocol (PP) populations. The ITT population included all randomised patients from whom any post randomisation data were collected. The PP population included all ITT patients without major protocol deviations and who were treated with the allocated dressing for at least four weeks, was used for a confirmatory sensitivity analysis. Safety endpoints were analysed using the safety population which consisted of all randomised patients. The difference between the two populations amounted to 3 patients who were randomised, but never had a followup visit.

The primary analysis was carried out by a Chi-square test, as well as by logistic regression with healed/not healed as outcome and treatment and other explanatory variable. Pertinent variables, such as wound area at baseline, ulcer depth, duration of wound, APBI and country, were included exploratively, but only wound size at baseline and duration of wound (less than, or more than 1 year) ended up as a covariates.

Time (measured in days) to healing was analysed by regression analysis of survival data based on the Cox proportional hazards model, with a censor variable indicating whether the subject was healed or not. A graphical illustration is presented with Kaplan-Meier curves.

All other proportions were analysed by Chi-square tests, except where the low numbers, necessitated the use of Fishers Exact test (e.g. amputations, AEs and SAEs). Total number of days of antibiotic therapy was analysed by a t-test and pain reduction was analysed by repeated measures analysis.

A p-value <0·05 was considered as statistically significant in all other analyses except the Primary Endpoint analysis.

Role of the funders and sponsor

The study was sponsored by Nottingham University Hospitals NHS Trust, Nottingham UK, and was funded by Reapplix ApS, Birkerød, Denmark. The funder had no role in study design, study

performance, data collection, data analyses, data interpretation, or writing of the report. The statistician (JJ) had full access to all data in the study. The chief investigators (FG, WJ, LT, ML) had full access to all data after the database was locked and had final responsibility for the decision to submit the results for publication.

Results

The first patient was consented in August 2013 and the last in May 2017. Altogether 595 people with diabetes were consented and 269 randomised, 55% of participants failed run-in, the main reason for this being change in ulcer area of more than 50% over 4 weeks (Figure 2). 137 participants were randomised to usual care and 132 participants to LeucoPatch in addition to usual care, of these 134 and 132, respectively were included in the ITT-population. Patients were recruited from 32 specialist centres; 22 in the UK, 3 in Sweden and 7 in Denmark. The mean number of patients consented was 18·5 (range 1- 78), and the mean number of patients randomised 8·4 (range 0-31) per centre.
The baseline characteristics were well balanced between treatment groups (Table 1). The mean age of participants was 61·9 (SD 1·6) years, 81·6 % male, and 83·5 % had Type 2 diabetes. The median duration of diabetes was 16 (IQR 10-23) years and the median HbA_{1c} 66 (IQR 55-77) mmol/mol. The majority of index limbs were neuropathic with 85·3 % of participants being unable to feel a 10-g monofilament at ≥ 2 out of 3 pre-specified sites on the affected foot. The majority of ulcers were greater than 100 mm^2 (74·4%) and superficial (86·5 %). Only 9 ulcers (3·4 %) extended to bone. The majority of the ulcers were situated on the forefoot (77·8 %). The 2 groups were well matched in terms of the types of offloading used throughout the study.

Within 20 weeks 45 (34 %) of index ulcers in the LeucoPatch group had healed vs. 29 (22 %) in the usual care group (p=0·029) giving an unadjusted odds-ratio of 1·58 (96% CI 1·04 - 2·40, p=0·024) for healing in the ITT population. On 6 occasions the decision that an ulcer had healed was made by the blinded assessment committee on the basis of the digital images. On each occasion the committee agreed that the ulcer was healed. In the PP population, healing within 20 weeks was 44 (39%) in the

intervention group vs 28 (26%) in the usual care group (OR 1·47 (96% CI 0·98 -2·23, p=0·049). Healing incidence at 12 and 26 weeks is shown in Table 2. The median time to healing in those who healed was 72 (IQR 56-103) days in the LeucoPatch group vs. 84 (IQR 64-98) days in the usual care group (Figure 3)(p=0·0343). The change in ulcer area from baseline is given in Figure 4.

Diabetic foot infection occurred in 51 participants in the LeucoPatch group and 63 in the usual care group (p=0·21). No differences in minor or major amputations between groups could be seen after 12, 20 or 26 weeks (Table 2). No difference in change of pain score between the groups could be identified during the follow-up period (Table 2).

The incidence of new anaemia was not significantly different between groups (9·8 % vs. 8·2 %). Overall there were no differences in adverse events between groups (Table 2).
The country of recruitment did not significantly affect the results.

Discussion

This multicentre, observer-blinded, randomised controlled trial found a significantly higher incidence of healing within 20 weeks (unadjusted OR 1·58) in those receiving LeucoPatch applications for hard to heal DFUs when compared to good quality standard care. There was also a significantly higher rate of decrease in ulcer area in the intervention group as well as a reduced time to healing in those that healed. There was no difference in the incidence of either major or minor amputation and none in the incidence of any adverse events or serious adverse events. In particular, there was no increased incidence of anaemia in the intervention group – even in those with reduced GFR – despite the need for weekly venesection. There was also no significant difference between groups in the incidence of either the number of episodes of clinical infection or of antibiotic use, even though a difference may have been expected because of the leucocytes contained within the application.

The main strength of the study was that the design and conduct of this study fulfilled the exacting requirements specified for work in this field.[4] The study population was appropriate in that it was designed to focus on those with hard-to-heal ulcers – which is the group for which new treatments are most needed. All investigators were instructed to manage participants according to the principles of good standard care using pre-specified criteria and this was reinforced at regular investigator meetings. The groups were well-matched. Recruitment was to target and retention was very high, with few drop-outs.

The main weakness regarding study design and conduct was that it was not possible to blind either the participant or the clinical researcher. The use of sham venepuncture was rejected as being unethical, but assessment of the primary outcome was undertaken by an independent and blinded observer and backed up with digital imaging. The recruited population was representative of a hard to heal population and this is reflected in the low overall incidence of healing in the non-intervention group. Nevertheless, an element of selection is evident in that the mean age was slightly less than anticipated (approximately 62 years as opposed to the expected 67 years) and presumably reflects the need for participants to attend each week for up to 5 months. There was also a high proportion of males (approximately 82% instead of the expected 67%) but this is now recognised as being a typical feature of large trials in this field. The overall incidence of healing was lower than anticipated, and lower than that observed in the pilot studies, but is likely to reflect the more rigid selection of a defined hard-to-heal population. It is also possible that the low healing rate reflected a poor standard of usual care, and this this might have been different between the 2 groups. We feel that this is unlikely as the low healing rate in those already preselected as being "hard to heal" following a 4 week run-in period was similar to that seen in the series of Coerper et al.[17] Additionally the 2 groups were well matched in terms of their baseline characteristics, their offloading strategies, and similar numbers were revascularised during the 26 week follow-up, and so we feel that a differential standard of care is not the explanation for the additional benefit seen on healing in the intervention group.
It is of interest that the odds ratio for healing in the intervention group was, however, very similar to that which was the basis of the sample size calculation, being 1·58. It is acknowledged that the

number of patients screened who were not eligible may suggest that the patient population was not representative of patients seen in a specialist diabetic foot clinic. We felt that for this protocol, although we were including hard to heal ulcers, we had to exclude those with little chance of healing within the 20 weeks of the study (for example very large ulcers, those with severe ischaemia, and those with severe renal disease) as their data had little chance to contribute to the final results. The median number of ulcers consented per centre was, however, similar to or even slightly higher than a recently published RCT of another product designed to accelerate wound healing in patients with neuro-ischaemia.[21]

Thus, this study has demonstrated the apparent effectiveness of this new intervention in the management of people with hard to heal DFUs. It adds to the increasing number of studies that have reported benefit from the use of platelets and platelet-derived application to the surface of the chronic wound. Such benefit could be mediated through any of a number of mechanisms relating to the process of inflammation and tissue repair. But in addition to delivering living, autologous platelets to the wound surface, LeucoPatch also delivers living autologous neutrophils and macrophages and could, therefore, confer additional advantages. Even so, no difference was observed between groups in the apparent incidence of episodes of wound infection.

The production of LeucoPatch patches is simple and quick and easily undertaken during the course of routine clinical practice. Weekly application was used for this definitive study. It is of interest, however, that the analysis of the PP population, ie those who attended and/or had LeucoPatch treatment on a weekly basis for most of the required visits resulted until healing, showed a similar improvement in healing at 20 weeks. It is possible, therefore, that the treatment may not need continuing until full healing and could be discontinued earlier even though this possibility has not been tested with this protocol. It is also possible that LeucoPatch will be effective in other types of DFU (including those that are not so hard to heal) but this has also not been assessed in this study. Whilst not directly tested in this study the low numbers of drop outs from the study protocol suggests that patients find this an acceptable treatment strategy.

Whereas reviews of the published literature on wound care products for DFUs have repeatedly emphasised the urgent need for trials of better quality, this is the third relatively robust RCT to be reported in the last 12 months: the others being a study of a dressing product which has an action on the activity of matrix metalloproteinases (sucrose octasulphate dressing)[21] and a dressing which releases intradermal nitrix oxide (ProNox1).[22] While these three approaches represent contrasting modes of action, and include different types of ulcer with different durations and different healing criteria, all three trials reported an almost identical figure for unadjusted OR: approximately 1·5 - 1·6.

In summary, this trial demonstrates a clinical and statistically significant benefit associated with the use of weekly application if autologous immune cell/fibrin/platelet patches (LeucoPatch) in a population of people with hard-to-heal diabetic foot ulcers. The treatment was without apparent adverse event, specifically without evidence of new onset anaemia. It is possible that this treatment might also be of benefit in other types of DFU but this has not been studied.

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Data Monitoring Committee (Independent Members): Prof Sally Marshall, Newcastle University; Volkert Dirk Sierma, University of Copenhagen; Jane Lewis, Cardiff and Vale University Health Board / Cardiff Metropolitan University; Trine Vestergaard, Statcon ApS

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LT and ML have received research support from ReApplix. FG, WJ, DF, SE, DW, JJ, EH have no conflicts to declare.

Authors contributions:
The study protocol was written and approved by FG,WJ, LT, JJ, DF and ML. FG, WJ, LT and ML were the national Chief Investigators of the study. DW, EH and SE coordinated the running of the trial and data collection. JJ performed the statistical analysis. FG, WJ, LT and ML interpreted the data with JJ. The manuscript was written by FG, WJ, LT, JJ and ML but all the authors have approved the final version.

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LEGENDS

Table 1. Baseline clinical characteristics

Data are given as median (IQR) or number of participants (%).

Table 2. Primary secondary and safety study outcomes

Data are given for important primary and secondary outcomes for patients allocated to intention-to-treat population in people randomised to usual care alone (N=134) or usual care and Leucopatch plus usual care (N=132). For the pre protocol analyses the population was usual care alone (N=107) and Leucopatch plus usual care (N=114).

Figure 1. Schematic of a LeucoPatch showing the 3 layers

Figure 2. Consort Diagram

Figure 3. Time to healing

Kaplan-Meyer survival curve showing proportion of ulcers remaining unhealed, with healing defined as complete epithelialisation without any drainage sustained for at least 4 weeks, in the intention-to-treat population in people randomised to usual care alone (134) or usual care and Leucopatch (132).

Figure 4. Ulcer area reduction