ORIGINAL ARTICLE

A pilot multi-centre prospective randomised controlled trial of RECELL for the treatment of venous leg ulcers

Paul D. Hayes[1] | Keith G. Harding[2] | Susan M. Johnson[3] |
Charles McCollum[4] | Luc Téot[5] | Kevin Mercer[6] | David Russell[7]

[1]St John's Innovation Centre, Cambridge, UK

[2]Clinical Innovation Cardiff, Cardiff, Wales

[3]Doncaster Royal Infirmary, Doncaster, UK

[4]Wythenshawe Hospital, Manchester, UK

[5]Lapeyronie Health Facility, Montpellier, France

[6]Bradford Royal Infirmary, Bradford, UK

[7]Leeds General Infirmary, Leeds, UK

Correspondence
Paul D. Hayes, MD, FRCS, Consultant Vascular Surgeon, St John's Innovation Centre, Cambridge CB2 0QQ, UK.
Email: paul.hayes@addenbrookes.nhs.uk

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Abstract

Venous leg ulcers (VLUs) have a significant impact on approximately 3% of the adult population worldwide, with a mean NHS wound care cost of £7600 per VLU over 12 months. The standard care for VLUs is compression therapy, with a significant number of ulcers failing to heal with this treatment, especially with wound size being a risk factor for non-healing. This multicentre, prospective, randomised trial evaluated the safety and effectiveness of autologous skin cell suspension (ASCS) combined with compression therapy compared with standard compression alone (Control) for the treatment of VLUs. Incidence of complete wound closure at 14 weeks, donor site closure, pain, Health-Related Quality of Life (HRQoL), satisfaction, and safety were assessed in 52 patients. At Week 14, VLUs treated with ASCS + compression had a statistically greater decrease in ulcer area compared with the Control (8.94 cm^2 versus 1.23 cm^2, $P = .0143$). This finding was largely driven by ulcers >10 to 80 cm^2 in size, as these ulcers had a higher mean percentage of reepithelialization at 14 weeks (ASCS + compression: 69.97% and Control: 11.07%, $P = .0480$). Additionally, subjects treated with ASCS + compression experienced a decrease in pain and an increase in HRQoL compared with the Control. This study indicates that application of ASCS + compression accelerates healing in large venous ulcers.

KEYWORDS
autologous skin cell suspension, compression therapy, health-related quality of life, RECELL, venous leg ulcers

1 | INTRODUCTION

Venous leg ulcerations are the most common type of chronic lower extremity wounds arising from venous valve incompetence and calf muscle pump insufficiency, which impairs the normal processes for the maintenance and healing of skin. Subsequent to intervention aimed at correction of the venous insufficiency, ulcerated areas of tissue can continue to be unhealed for extended periods of time.[1-3] Symptoms of venous leg ulcers (VLUs) include pain, exudate, and infection with significant effects on activity, mobility, hygiene, choice of clothes and footwear, sleep, and relationships. Health-Related Quality of Life (HRQoL) is impacted by the presence of VLUs, principally because of pain and the associated limited mobility and reliance on daily visits from health care providers

for dressing changes. Patients' HRQoL is also affected by levels of exudate and whether the wound is infected.[4-6]

The treatment of VLUs results in a considerable cost to the health care system. VLUs have a prevalence approximated as high as 0.3% of the population in the United Kingdom and 3% of the adult population worldwide.[2,7-16] Annual cost incurred by the United Kingdom's National Health Service in managing chronic wounds was estimated to be £5.3 billion, with each VLU costing on average £7600 per 12 months.[17] Furthermore, it has been demonstrated that the annual cost of managing an unhealed VLU is four to five times more than that of managing a healed VLU (£3000 per healed VLU and £13 500 per unhealed VLU).[18,19]

Limb compression is considered standard of care for VLUs, with the application of compression bandaging to maintain 30 to 40 mmHg of pressure. However, compression alone does not always heal the wound and success rates vary between 30% and 60% with 24 weeks of therapy.[20,21] In recent reviews, it has been demonstrated that autografting may improve the rate of wound closure, with split-thickness skin grafts being an option for surgeons wishing to close long-standing open wounds.[1,22] These reviews also concluded that further research is needed to assess whether other forms of autografts incorporating tissue engineering concepts may increase the incidence of ulcer healing.

Applications of autologous skin cell suspension (ASCS) have long been used in burn care for purposes of epidermal regeneration in deep partial-thickness and full-thickness injuries.[23-28] More recently, ASCS has been used to benefit patients who have ulcers of various aetiologies, including VLUs and diabetic foot ulcers.[29-34] Authors have observed that use of ASCS appears to restart the healing process and have hypothesized that the introduction of a population of healthy, disaggregated cells may modulate the wound environment and provide a source of healthy cells to stimulate healing of a wound that has been static for months.

ASCS can be prepared in the clinical setting using the RECELL Autologous Cell Harvesting Device (AVITA Medical, Valencia, CA). Using this device, both enzymatic and mechanical processes are used to release skin cells from a small (1.5–2.0 cm^2), thin skin sample (0.15–0.20 mm). The resulting suspension, containing keratinocytes, fibroblasts, and melanocytes, allows dispersion of single cells across the surface of the wound bed.[35] In addition to the placement of skin cells where they are needed, the disaggregation process may induce changes normally associated with loss of contact inhibition, thereby activating cellular processes associated with wound healing.[36]

Clinicians evaluating the use of ASCS in their treatment algorithms for chronic wounds have demonstrated favourable results[29-34]; however, a prospective randomised study evaluating the use of the cell suspension in VLUs compared with standard compression therapy has not been conducted to date. Therefore, the objective of this pilot study was to evaluate the effectiveness of the addition of ASCS to standard compression therapy in comparison with compression therapy alone. Effectiveness measurements evaluated included healing outcomes, pain, subject and physician satisfaction, and disease-specific HRQoL in patients with chronic VLUs compared with outcomes obtained with standard compression therapy alone. Safety and tolerability of the ASCS in VLUs was also assessed by capturing the occurrence of adverse events (AEs) as well as local reactions including allergic response to components of ASCS or infection at the wound site.

Key Messages

- incomplete healing of venous leg ulcers (VLUs) impacts a patient's quality of life in relation to treatment efficacy, physical functioning, and psychosocial functioning
- fifty-two subjects were enrolled in a multicentre, prospective, randomised, controlled trial to assess VLU wound closure after treatment with autologous skin cell suspension and compression therapy, or compression therapy alone
- patients with larger VLUs treated with ASCS + compression therapy had accelerated healing, reduced pain, and higher health-related quality of life scores than compression therapy alone

2 | METHODS

This was a multicentre, prospective, randomised controlled clinical trial designed to evaluate the preliminary effectiveness and safety associated with the use of ASCS for the treatment of chronic leg ulcers associated with venous insufficiency. The clinical benefit of the addition of ASCS treatment to standard practice (debridement and cleansing) and multi-layer compression bandages (Profore, Smith and Nephew, London, UK) was compared with standard practice (debridement and cleansing) and multi-layer compression bandages (Profore) without ASCS treatment. This study was registered at clinicaltrials.gov under number: NCT01743053.

Subjects with an open leg ulceration were recruited from six sites in England and one site in France between July 2013 and November 2015. Ulcers were to have been present for at least 4 weeks and present with an area of

between 2 and 80 cm^2, with no exposed tendon or bone. All subjects had a clinical assessment and ankle-brachial pressure index (ABPI) measurements to confirm eligibility (ABPI < 0.8) and the study only included patients with confirmed, managed venous reflux according to the clinician's judgement, and an ulcer defined as a 6 using the comprehensive CEAP (Clinical, Aetiology, Anatomy, and Pathophysiology) classification system. Prior ulcer treatments included compression therapy, antibiotics, debridement, dressings, negative pressure therapy, autografting, and compression. Subjects were assigned a treatment group (ASCS + compression or compression only) based on the randomisation schedule created by the statistician. To ensure comparability, wounds were pre-specified and stratified, prior to randomisation, into two groups by wound area (Group 1: ulcers 2 to 10 cm^2 and Group 2: ulcers > 10 to 80 cm^2). Randomisation occurred in the Electronic Data Capture system following the site's selection of 'Group 1—small wound' or 'Group 2—large wound'.

All sites participating had ethics committee approval prior to the start of the study and an ethics committee-approved informed consent form was given to each participant before any study-specific procedures were conducted. After consenting to study participation, subjects entered a two-week run-in period of standardised dressing and compression therapy to confirm selection of patients whose care has been appropriate and had neither a rapidly healing nor rapidly deteriorating ulcer (30% change in wound size). Subjects completing the run-in period with their index ulcer unchanged and meeting all other eligibility requirements were randomised and treated according to group assignment. Visits were scheduled for 1 week and then every second week after the initial treatment. Subjects were followed in nurse-led clinics or surgical units and assessed prospectively. Dressings were changed in accordance with patient needs and standard care as well as at each follow-up visit. Study follow-up lasted for 14 weeks.

Subjects in both groups received debridement of their index ulcer, and those randomised to the treatment group also received application of ASCS prepared using RECELL Autologous Cell Harvesting Device in accordance with the manufacturer's instructions for use. The donor site location was selected using clinical judgement and similar considerations as for autograft harvesting. Typically, donor sites were located on the lower extremity or abdomen. When located on the lower extremity, 17 of the donor sites were ipsilateral and 9 were contralateral to the ulcer. Briefly, under local anaesthetic, a small (2 cm^2) thin (0.15–0.20 mm) skin sample was taken and incubated in a proprietary enzyme formulation. After 15 to 20 minutes of enzyme exposure, the skin sample was rinsed in a buffer solution and then manually

scraped until both the epidermis and dermis were fully dissociated. The cellular material scraped from the skin sample was suspended in approximately 2.5 mL of buffer solution, drawn up and filtered, yielding approximately 2 mL of cell suspension that was then applied to the prepared wound bed by dripping from a syringe, followed immediately by placement of non-adherent Telfa Clear (Covidien, Dublin, Ireland) wound dressing. The donor sites were treated according to the discretion of the clinician. Subjects received a second ASCS treatment at the 6-week visit, unless the index ulcer had achieved ≥85% reepithelialisation or had improved at least 15% from the prior visit.

After randomisation, subjects were treated according to group assignment and study participation lasted for 14 weeks. The primary effectiveness endpoint of the study was to evaluate the incidence of complete wound closure at Week 12 post-treatment. The surgeon-investigator determined healing status by visual assessment, with complete wound closure being defined as full reepithelialisation without drainage on two consecutive visits. Subject and investigator blinding was not practical, because treatment with ACSC required taking a donor skin sample. Reduction in wound area was also recorded at each time point.

Wound area was quantified using the Eykona Wound Measurement System (Fuel3d, Greenfield, NC). Donor sites were assessed for closure after skin sample harvesting. Ulcer pain was evaluated by patient self-report on a 10-point, numeric scale where 1 represents no pain, and 10 represents the worst imaginable pain. Patient and physician ratings of overall satisfaction were also captured on a 10-point scale. The Charing Cross Venous Leg Ulcer Questionnaire (CCVLUQ)[37] was used at each visit, excluding the Week 1 follow-up, to assess disease-specific HRQoL for the subjects.

Safety and tolerability of the ASCS treatment was confirmed based on the occurrence of local reactions including but not necessarily limited to: wound infections not present at baseline or previous assessments, worsened ulceration, local allergic response, or other treatment-related AEs requiring subsequent surgical intervention.

2.1 | Statistical analysis

A total of 65 subjects were planned for enrolment in this study to obtain 30 completed subjects in each group followed through 14 weeks. While this was intended as a pilot study, the sample size was considered adequate for this purpose based on several considerations. With a sample size of 30 subjects per arm, the study would have 80%

power to obtain a significant treatment difference on a two-sided, alpha equal to 0.05, Fisher's Exact test if the control response rate was 40% and the active response rate was 78%.

Furthermore, based on a sample size of 30 active subjects, the study would have greater than an 80% chance to detect at least one medically significant AE if the true rate an event occurs in subjects was at least 5.3%. Second, for common events, the sample size of 30 in both treatment groups provided at least an 80% chance on each event to indicate the correct trend between groups, if the true difference in rates was at least 14% higher in the ASCS + compression group.

Independent analyses were conducted by FGK Clinical Research, Munich, Germany. All statistical analyses were performed using SAS 9.3 TS Level 1M1 (SAS Institute Inc., Cary, NC). A $P < .05$ was considered significant. Incidence of healing, ulcer duration, smoking, and comorbidities were analysed by Fisher's exact test. Subject age, body-mass index (BMI), and wound duration were evaluated using the Wilcoxon Test (Two-sided). Gender was evaluated using the Pearson Chi-Square test. Time to first closure was calculated using Kaplan Meier survival analysis and Cox regression. Wound size, pain and HRQoL scores for the treatment and control groups were compared using an analysis of variance (ANOVA). Data analyses were stratified according to initial ulcer size.

3 | RESULTS

3.1 | Subject and wound characteristics

Fifty-two subjects were recruited to tertiary care providers and were enrolled in the study at seven investigative sites. Recruitment of eligible patients was challenging and the original sample size of 65 subjects was not met. Often patients that were cared for at nurse-led treatment centres and were not referred to tertiary care providers. Another limitation was the exclusion of infected wounds and wounds over 80 cm² in area.

Of the 52 subjects, 45 subjects completed follow-up to Week 14 (Full analysis set: FAS) (Figure 1). Of these subjects, 21 received ASCS + compression and 24 received compression only. Reasons for subjects lost to follow-up included withdrawal of consent, patient non-compliance, and AEs. Of the completed subjects, 13 were deemed per protocol (PP Population in which subjects completed the study without major protocol deviations) for the ASCS + compression group and 20 were deemed per protocol for the compression group. Protocol deviations included three subjects lost to follow-up, three subjects discontinued early for AE, one subject withdrew consent, four subjects with second treatment of ASCS not being applied because of protocol deviations.

Table 1 summarises patient demographics and comorbidities and is presented for the 52 subjects enrolled.

FIGURE 1 CONSORT diagram. Eighty-four subjects were consented and screened for eligibility into the study. Thirty-two subjects were excluded because of eligibility criteria not met, rapid healing during the 2-week screening period, or infection of the ulcer designated as the study ulcer. Of the 52 subjects meeting eligibility, 26 were randomised to receive the autologous skin cell suspension (ASCS) and compression (ASCS + compression) and 26 were randomised to receive compression only. Over the 14-week study period, seven subjects were lost to follow-up with five subjects in the ASCS + compression group and two subjects in the compression group. Analysis was performed on the Full Analysis Population (ASCS + compression = 26, Compression = 26) or the Per Protocol Population (ASCS + compression = 13, Compression = 20)
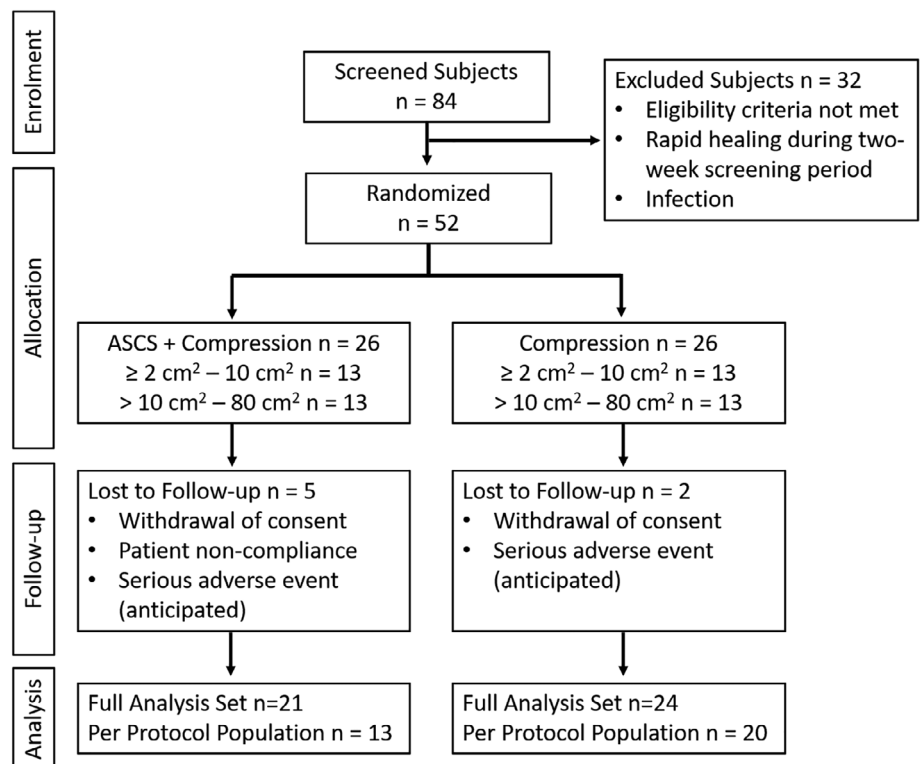
The mean age for the subject population was 69.7 years and 71.2% were male with a mean BMI of 29.65. A statistical difference was observed between the mean age between groups ($P = .0086$); however, this difference can be explained by the enrolment of one younger patient aged 21 years, randomised to the ASCS + compression group. Additionally, a statistical difference was noted for smokers with 23.1% of the subjects in the ASCS + compression group indicating current smoking status, whereas no

smokers were randomly allocated to the compression group ($P = .0226$).

Subjects enrolled in the study had comorbidities consisting of coronary and peripheral artery disease, diabetes, as well as other chronic diseases and conditions including those affecting the musculoskeletal, gastrointestinal, and cardiovascular systems. No statistical differences were found between any of the pre-mentioned comorbidities between the ASCS + compression and compression groups. The number of ulcers per subject was found to be statistically different between treatment groups; however, only one ulcer was included as a study ulcer.

The ulcer size at baseline ranged from 1.66 to 43.03 cm^2 (Table 2). The mean ulcer area was 5.22 cm^2 for the Group 1 cohort (ASCS + compression: 5.41 cm^2 and compression: 5.01 cm^2) and 22.40 cm^2 for the Group 2 cohort (ASCS + compression: 23.81 cm^2 and compression: 21.09 cm^2) with no statistical differences found between groups. The mean ulcer duration was 181.3 weeks (median: 82 weeks) for the patient population with no statistical difference observed between the two groups.

3.2 | Venous leg ulcer healing outcomes

At Week 12, for the primary analysis population (FAS), the incidence of ulcer closure was observed for 23.1% of ulcers treated with ASCS + compression versus 15.4% for ulcers treated with compression only (Table 3). When stratifying the ulcers by size, it was found at Week 12 for Group 1 30.8% of ulcers treated with ASCS + compression had ulcer closure and 25.0% of ulcers treated with compression had closure. For larger (Group 2) ulcers, 15.4% and 7.1% had wound closure for ASCS + compression versus compression, respectively. Although no statistical difference was observed, the trend suggests a greater percentage of ulcers healed at Week 12 when treated with ASCS + compression over compression alone, with Group 2 ulcers having a greater percentage difference. When evaluating the incidence of closure at Week 12 for the PP analysis population, a larger difference exists between

TABLE 1 Subject demographics and comorbidities

Full analysis set	Total	ASCS + Compression	Compression
Age (years) Mean ± SD Range	69.7 ± 15.2 21.0–90.0	64.2 ± 16.5 21.0–80.0	75.2 ± 11.66 42.0–90.0
Sex (% male)	71.2%	76.9%	65.4%
BMI Mean ± SD Range	29.65 ± 8.43 17.0–58.0	30.17 ± 9.10 17.0–58.0	29.12 ± 7.83 20.70–51.9
Smoking (%, regularly)	11.5%	23.1%	0.0%
Coronary artery disease (%)	9.6%	7.7%	11.5%
Peripheral artery disease (%)	15.4%	26.9%	3.8%
Peripheral neuropathy (%)	0.0%	0.0%	3.8%
Type I diabetes (%)	0.0%	0.0%	3.8%
Type II diabetes (%)	11.5%	15.4%	7.7%
Other chronic disease(s) or conditions (%)	59.6%	69.2%	50.0%
Number of ulcers (%) 1 2 3 or more	46.2% 26.9% 26.9%	38.5% 50.0% 11.5%	53.8% 3.8% 42.3%

Abbreviations: ASCS, autologous skin cell suspension; BMI, body-mass index.

TABLE 2 Wound characteristics

FAS	Total N = 50	ASCS + Compression N = 25	Compression N = 25	Statistics P-value
Ulcer size (cm^2) at baseline Mean ± SD Range	13.81 ± 11.33 1.66–43.03	14.25 ± 11.94 1.66–43.03	13.37 ± 10.91 1.75–39.54	.4594
Ulcer duration (weeks) Mean ± SD Range	181.3 ± 269.4 6.0–1560.0	156.1 ± 302.4 14.0–1560.0	206.6 ± 235.3 6.0–850.0	.2883

Abbreviations: ASCS, autologous skin cell suspension; FAS, full analysis set.

TABLE 3 Incidence of ulcer closure at Week 12

	ASCS + Compression	Compression	Statistics P-value
FAS	N = 26	N = 26	
All ulcers (cm^2)	23.1%	15.4%	.7265
Group 1 (2-10 cm^2)	30.8%	25.0%	1.0000
Group 2 (>10-80 cm^2)	15.4%	7.1%	.5956
PP	N = 13	N = 20	
All ulcers (cm^2)	38.5%	15.0%	.2134

Abbreviations: ASCS, autologous skin cell suspension; FAS, full analysis set.

TABLE 4 Mean change in wound size at Week 14

FAS	ASCS + Compression N = 21	Compression N = 24	Statistics P-value
All ulcers (cm^2)	8.94 ± 9.79	1.23 ± 8.22	.0143
Group 1 (2-10 cm^2) (cm^2)	3.24 ± 2.94	3.78 ± 2.18	.6336
Group 2 (>10 to 80 cm^2) (cm^2)	16.07 ± 10.81	−2.28 ± 11.94	.0061*

* indicates statistical difference ($P < .05$).

Abbreviations: ASCS, autologous skin cell suspension; FAS, full analysis set.

ulcers treated with ASCS + compression (38.5%) versus compression alone (15.0%) than occurred for the FAS; however, these data should be cautiously interpreted because of the small sample size evaluated.

In addition to incidence of closure at Week 12, time to first closure was calculated using Kaplan Meier survival analysis and Cox regression. For Group 2, the mean time to first ulcer closure was 43.0 days in the ASCS + compression group versus 84.0 days in the compression only group; however, no significant difference in time to first ulcer closure was observed, likely because of the small sample size.

The mean absolute change in wound area from baseline was evaluated using the Eykona Wound Measurement System. At Week 14, a statistical difference existed in the mean absolute reduction in wound area for ulcers treated with ASCS + compression (8.94 ± 9.79 cm^2) versus those treated with compression only (1.23 ± 8.22 cm^2) ($P = .0143$) (Table 4). When stratifying the ulcers by size, the difference in mean change in wound size at Week 14 was statistically different between the two groups ($P = .0061$) for Group 2, whereas no statistical difference existed between the groups for Group 1 ($P = .6336$) (Table 4). In the ASCS + compression group, a general decrease in mean absolute change in wound size was noted over time for all ulcers (Figure 2). Particularly in the case of the large ulcers, the ASCS + compression group demonstrated a consistent decrease in wound size over time, while the Control group cycled between decreased and increased wound size from week to week and the mean area was larger at Week 14 than reported at baseline. Furthermore, Group 2 ulcers treated with ASCS + compression had a higher mean percentage of reepithelialization at 14 weeks compared with ulcers treated with compression only (69.97% and 11.07%, respectively), with a statistical difference observed at Week 14 ($P = .0480$). No statistical difference was observed for Group 1 in terms of reepithelialization at Week 14.

3.3 | Healing of donor site

By Week 6, 85% of the subjects had complete healing of the donor site. Potential reasons for delayed healing include infection or other underlying health conditions which also delayed treatment site healing.

3.4 | Pain

The absolute change in pain ratings from baseline as measured by the subject reported measurement of pain was compared for ASCS + compression and compression only treatment groups. At 2-weeks post-treatment/baseline measurement, ASCS + compression treated subjects reported a statistically significant decrease in pain rating ($P = .017$) compared with the non-ASCS treated subjects (Figure 3). When stratifying by wound size, a larger difference was detected for the smaller Group 1 wounds; however, no statistical differences were observed. At all other time points evaluated, no statistical differences were observed between the treatment groups.

3.5 | Health-related quality of life

The absolute change in all HRQoL ratings as measured by the Charing Cross Quality of Life questionnaire (CCVLUQ, cosmesis, domestic activity, social interaction, and emotional status) were compared between ASCS + compression and Control groups at each visit. A consistent trend of improvement in all aspects of the CCVLUQ was reported by subjects receiving ASCS + compression compared with the Control group (Figure 4). The differences between the groups in all sections emerged by 4 weeks and reached statistical significance by 8 weeks for
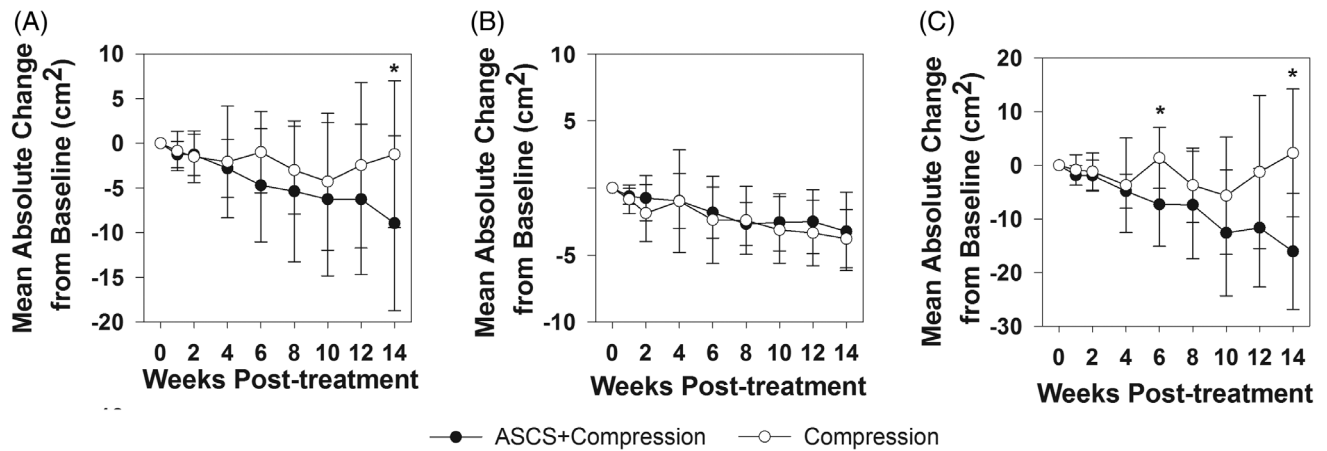
FIGURE 2 Mean wound area reduction. At each visit, the size of the ulcers was measured using the Eykona Wound Measurement System. Wound area reduction was calculated relative to the baseline wound size (dotted line at 0 cm²). Mean values are presented for autologous skin cell suspension (ASCS) + compression (solid black circles) and compression alone (white circles). A statistical difference was found at Week 14 between ASCS + compression and compression alone for all ulcers ($P = .0143$) (A). A comparison of ASCS + compression and compression only stratified by ulcer size was also evaluated for small ulcers of size 2 to 10 cm² (B) and large ulcers >10 to 80 cm² (C) with a statistical difference being observed at Week 6 and Week 14 between the two treatment groups for the large cohort (Week 6 $P = .0224$ and Week 14 $P = .0061$). * indicates statistical difference
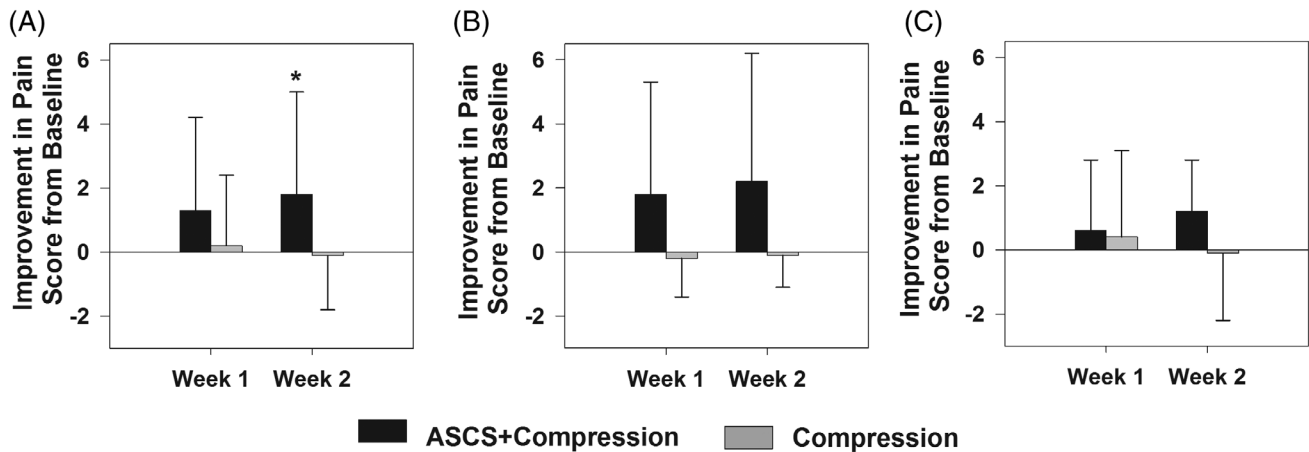

FIGURE 3 Self-reported pain scores. Subjects reported pain using a visual analogue scale and the absolute change from baseline for self-reported pain was calculated. Mean values are presented for autologous skin cell suspension (ASCS) + compression (black bar) and compression alone (grey bar) at Week 1 and Week 2 follow-up visits. A decrease in pain scores for subjects receiving ASCS + compression was found for all ulcers at Week 2 ($P = .0170$) (A). No statistical differences were found for small ulcers (2-10 cm²) (B) or large ulcers >10 to 80 cm² (C). * indicates statistical difference

cosmesis ($P = .0145$) and 14 weeks for emotional well-being ($P = .0439$). At Week 14, for both the smaller Group 1 and larger Group 2 ulcers, all subjects receiving ASCS + compression reported greater HRQoL ratings than those receiving compression alone. The total CCVLUQ score improved 6.2 points in Group 1 and 10.8 points in Group 2 for ulcers treated with ASCS + compression compared with control treatment.

3.6 | Subjects' and physician satisfaction

While there was no significant difference in satisfaction ratings, the ASCS + compression group consistently had slightly higher satisfaction scores in both the subject and physician ratings for all wounds. At Week 14, the mean subjects' satisfaction for all wounds was 8.8 ± 1.5 for the ASCS + compression group compared with 7.6 ± 2.4 for the compression group ($P = .0841$). For physician

FIGURE 4 Health-related quality of life. The absolute change from baseline in Health-Related Quality of Life (HRQoL) from baseline as assessed by the Charing Cross VLU (CCVLUQ) Quality of life questionnaire. Treatment with autologous skin cell suspension (ASCS) (solid circles) consistently resulted in improvements in all areas of the CCVLUQ and reached statistical significance for emotional status by 14 weeks after treatment with ASCS + compression compared with compression alone (P = .0439) and cosmesis at 8 weeks after treatment with ASCS + compression compared with compression alone (P = .0145). *Indicates statistical difference
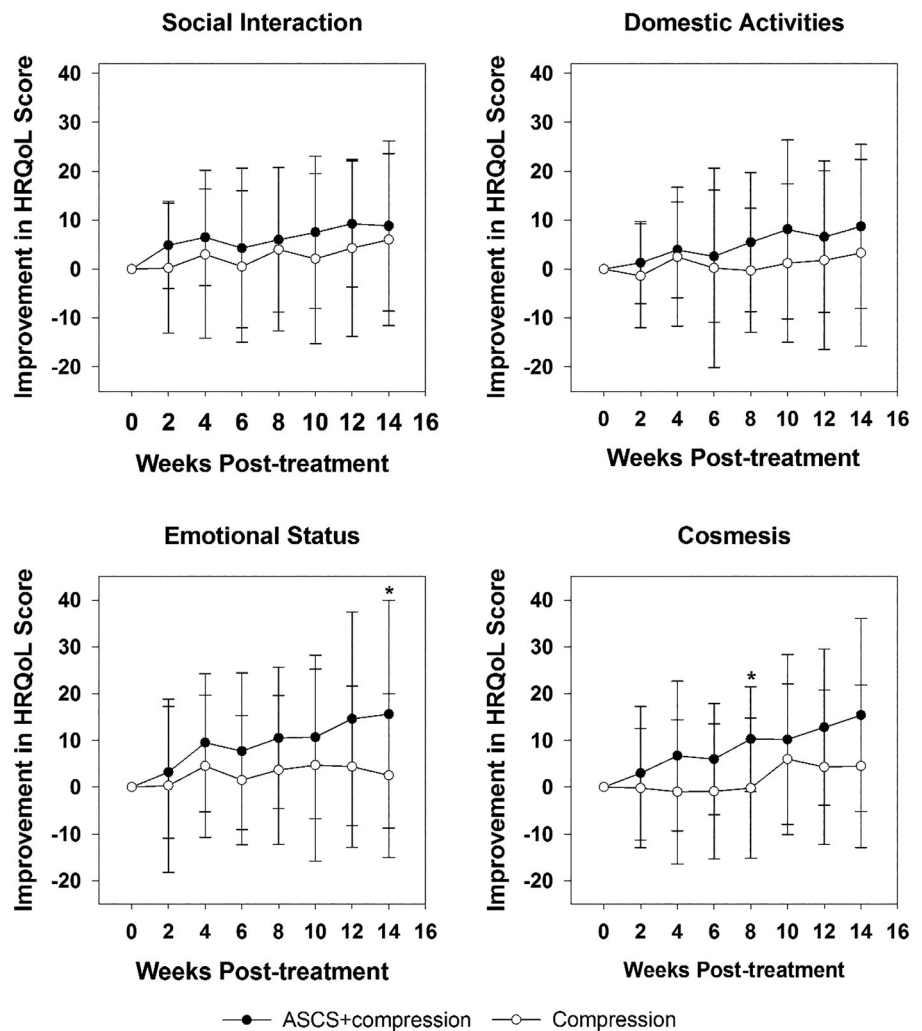
satisfaction, the mean value at Week 14 was 8.6 ± 1.6 for the ASCS + compression group compared with 7.8 ± 1.7 for the compression group (P = .1491).

3.7 | Safety

A similar percentage of subjects had AEs between the treatment groups (17 subjects in the ASCS + compression group and 16 in the compression group). In the ASCS + compression group, a total of 10 treatment-related AEs occurred in 7 subjects, with the majority being wound infection (80%), one of which had an infection located at the donor site. Additional AEs noted included post-procedure bleeding and maceration of the peri-ulcer skin.

Ten serious adverse events (SAEs) were recorded in six subjects. These SAEs were unrelated to the device and included exacerbation of existing unrelated conditions requiring hospital stay, hospitalisation for a fall, and pneumonia. Of these SAEs, two subjects in the

ASCS + compression group reported five SAEs and four subjects in the compression group reported five SAEs. In this study, infections rates were similar between the two study groups. By week 14, four subjects in the compression-only group had mild or moderate infection versus no observable infection in the treatment group.

3.8 | Representative case example

A 76-year-old female with a 28.12 cm^2 VLU was unresponsive to previous treatments including surgical debridement, absorption dressings, calcium alginate dressings, systemic antibiotics, and compression dressings. She was randomised to the ASCS + compression treatment group. Wound measurement imaging displayed the wound at run-in Week 2 (Figure 5A), baseline visit Week 0 (Figure 5B), and Week 1 through Week 6 (Figure 5C-E). At Week 6, the subject's ulcer was 56% open and she received a second ASCS treatment (Time 0, Figure 5F).
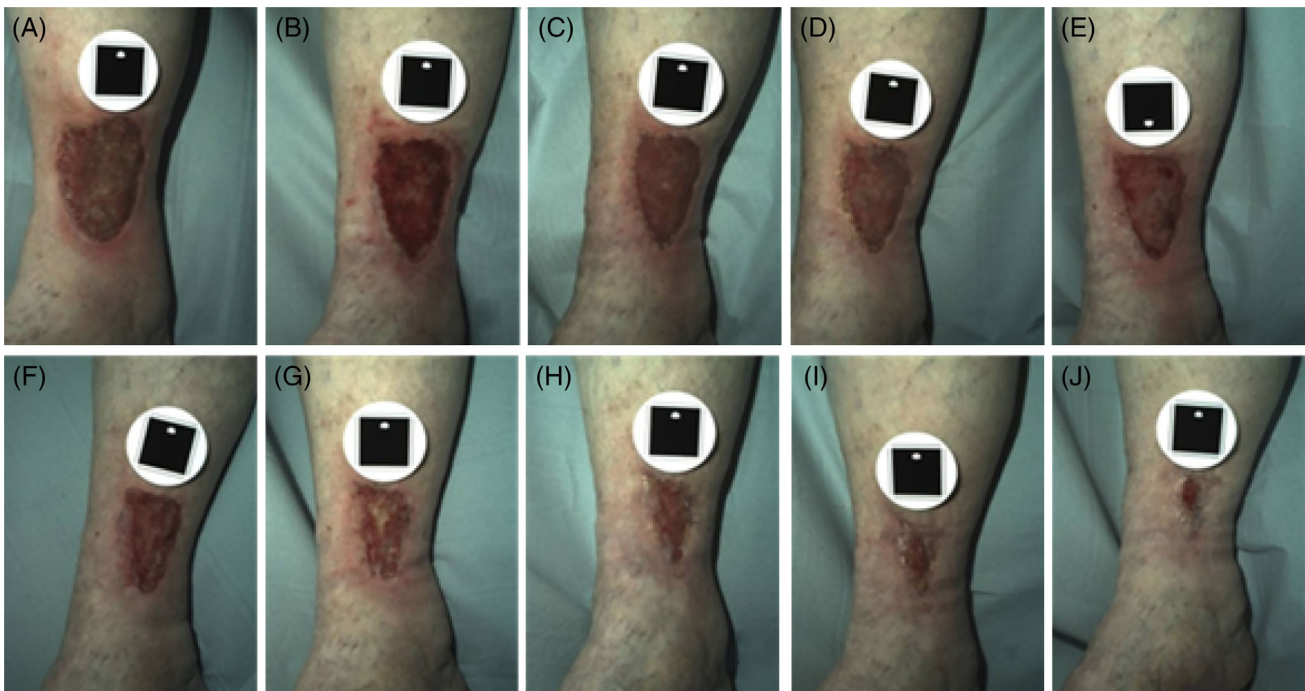
FIGURE 5 Healing progression of ulcer treated with autologous skin cell suspension (ASCS) + compression. A 76-year-old female with a venous leg ulcer that was unresponsive to previous surgical debridement, absorption dressings, calcium alginate dressings, systemic antibiotics, and compression dressings. Images were captured at each study visit using the Eykona wound measurement system. Run-In Week 2 (A), Baseline visit Week 0 (28.12 cm^2) (B), Week 1 (84% open) (C), Week 2 (79% open) (D), Week 4 (77% open) (E), Week 6, Time 0 for second ACSC treatment (56% open) (F), Week 8 (38% open) (G), Week 10 (23% open) (H), Week 12 (9% open) (I), and Week 14 (2% open) (J)

From Weeks 8 to12 (Figure 5G-I), the ulcer continued to heal, and at the last visit, the ulcer was only 2% open (Week 14, Figure 5J).

4 | DISCUSSION

Chronic venous disease is a relatively common and debilitating condition. Patients with venous disease may experience pain and lower extremity heaviness that usually worsens as the day progresses. Clinical signs include mild venous dilation, varicose veins, swelling, skin changes, and ulceration. Conventional treatment for venous ulcerations includes compression therapy but often these wounds remain unhealed and have significant impact on the well-being and HRQoL of the patient. The use of cellular therapies for the treatment of chronic wounds has been reported as an adjunctive therapy to compression therapy, aimed at modulating the environment to stimulate healing.[1,22,38]

In this study, the clinical effectiveness of ASCS, in combination with compression therapy was evaluated for the treatment of VLUs compared with compression therapy alone. The cellular suspension was generated using the RECELL Autologous Cell Harvesting Device, which allowed the clinician to process a small skin sample from the subject at the point-of-care for immediate application to the VLU without the need for laboratory cultivation.

The initial hypothesis of this study was that the incidence of ulcer wound healing at Week 12 would be superior for ASCS + compression. While closure was higher for the ASCS + compression group (33.3%) compared with the Control group (15.0%), it was not statistically significant ($P = .34$) at Week 12. This was likely owing to the study being underpowered because of slow enrolment and multiple protocol deviations. Nevertheless, a statistically significant change was observed in wound size from baseline at Week 14 for the Group two cohort (ulcers > 10 to 80 cm^2) treated with ASCS + compression compared with compression alone. Analysis of the data suggests these sized wounds treated with ASCS + compression exhibited a steady improvement in wound healing over time compared with the Control group, which had an inconsistent pattern of healing. In addition to size reduction, the mean percentage of reepithelialization at 14 weeks was 69.97% versus 11.07%, with a statistical difference observed at Week 14 detected between treatment groups for ulcers >10 to 80 cm^2 in size. These findings suggest that the >10 to 80 cm^2 ulcers receiving the cell suspension were on a healing

trajectory, whereas the wounds treated with compression-alone remained static. This finding is promising, as a review by Margolis et al identified that a VLU larger than 10 cm^2 and that has been present for more than 12 months has only a 22% chance of healing at 24 weeks.[39] Furthermore, it should also be noted that within the ASCS + compression group, there were subjects who qualified for re-application of the cellular suspension that did not receive the second application at Week 6 as described by the protocol, and this deviation may have impacted observed healing outcomes in the FAS. When evaluating the per protocol (PP) population, larger differences were observed, as presented in the incidence of healing at Week 12.

VLUs are a known cause of significant pain that impacts HRQoL,[4] with the cost of pain management associated with VLUs constituting a significant part of health service budgets.[17] Findings from this study indicate a decrease in patient reported pain for those treated with ASCS + compression, which was statistically different at Week 2 from subjects receiving compression only. Despite no reported differences in healing rate in Group 1 (ulcers 2-10 cm^2), this group reported significant improvements in pain. Furthermore, in all areas of the HRQoL, ASCS + compression treated subjects reported improvements over those not receiving the cell suspension. Although subjects were not blinded to treatment allocation, HRQoL and pain is a subjective assessment of their ulcer post-treatment relative to the baseline ulcer rather than a within-subject direct comparison with the control treatment. The total CCVLUQ score improved in both groups for ulcers treated with ASCS + compression compared with control treatment. The improvement in HRQoL of life may be associated with wound healing in these previously unresponsive wounds and the associated decrease in pain. In addition, while not captured statistically, many patients noticed decreased exudate levels, allowing for less dressing changes which positively impacted their quality of life.

The complexity of the wound and healing capacity is known to be impacted by age, smoking, comorbidities, as well as wound size, and chronicity of the wound.[40-42] Post hoc analyses were conducted for parameters with statistical significance between treatment groups (age, smoking), as well as those not reaching statistical significance individually, but were of clinical importance and may have a combined effect on the results of the trial. Results of logistic regression analysis on incidence of ulcer closure, Cox regression analysis for time to ulcer closure, and ANCOVAs for change in pain, wound size, CCVLUQ score, and satisfaction were conducted at Week 12 between treatment groups and no further statistical differences were

found; however, it should be noted that the sample sizes in this study were limited.

Use of ASCS appears to augment outcomes relative to what can be expected for conventional compression therapy for ulcers >10 to 80 cm^2 in area, and while harvesting a small skin sample is required, a donor site 1/80th of the size of the ulcer being treated is a lower-risk prospect than the harvesting of skin for an autograft matching the size of the ulcer treated or half as with a 2:1 meshed ratio. Relative to the other alternative of cultured epithelial autografts, ASCS is available without the time, logistics, and costs associated with laboratory culturing as the ASCS can be produced in approximately 30 minutes at point-of-care, while the ulcer is being prepared.

In conclusion, the results from this study indicate that application of ASCS, as prepared using the RECELL Autologous Cell Harvesting Device, appears to be a safe and an effective approach for treatment of VLUs when combined with compression therapy, specifically for ulcers >10 to 80 cm^2 in area. In addition to the healing potential, the reduction in pain and improvement in HRQoL may have implications on the overall reduction of costs associated with the treatment of VLUs regardless of ulcer size. Future work is warranted to study the effect of ASCS + compression on wounds evaluating the healing outcomes and the impact that the decrease in wound size has on pain and cost effectiveness of the treatment.

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