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Enhanced chronic wound healing with autologous conditioned serum dressing in a randomized prospective clinical trial

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Abstract:	Aims: In this study, we aimed to assess both the efficacy and tolerability of autologous conditioned serum (ACS) as an innovative wound dressing in the local management of chronic wounds. Materials and Methods : In this single-blinded randomized controlled trial, a total of 30 patients with chronic wound were randomly assigned either to receive 3 weeks of ACS or to receive normal saline dressings. The treatment was applied once a week and the latest assessment was planned after passing three weeks from the first ACS application. Results : Analysis of wound assessment's data displayed statistically significant differences for wound surface area and Pressure Ulcer Scale for Healing scores (PUSH: area score, exudate, and tissue) in the participants underwent ACS dressing, but not in the normal saline group from baseline up to the end of the study. There were statistically significant differences in the changes in the wound surface area on the third week (-6.4±2.69 vs. +0.4±2.52 cm2), changes in the area score at week 3 (-2.2±1.08 vs. +0.2±0.86), exudate at week 2 (-1.2±0.70 vs. +0.0±0.45) and week 3 (-1.3±0.72 vs. -0.1±0.63), tissue at week 2 (-1.1±0.35 vs. +0.0±0.53) and week 3 (-1.8±0.65 vs. -0.1±0.63) and changes in the PUSH total score at week 1 (-1.6±0.98 vs.

	+0.4±1.22), week 2 (-3.2±0.86 vs. +0.4±0.98) and week 3 (-5.3±1.17 vs. -0.0±1.33) between ACS and saline groups, respectively. Conclusions : This trial for the first time revealed a significant decrease in wound surface area as well as a considerable improvement in chronic wound healing by ACS dressing.
Suggested Reviewers:	
Response to Reviewers:	Editors Journal of Wound Care Dear Dr. Negin Shamsian Enclosed is the revised version of manuscript entitled “Enhanced chronic wound healing with autologous conditioned serum dressing in a randomized prospective clinical trial”. Reviewers' comments: Reviewer #1: 1. 2.1: "Patients with chronic wounds referred to outpatient clinics were continuously recruited in the study using the non-probability convenience sampling method in terms of the eligibility criteria." This still does not make sense. To randomized patients, one has to use a randomization algorithm. The simplest is A or B. If you do not use a randomization algorithm you have a cohort study, not a randomized study. Author response: I completely agree with respected reviewer. Patients were continuously recruited in the study using the non-probability convenience sampling method and then were randomly allocated into the two groups using a simple randomization method through computer-generated random numbers. Accordingly, this was done with the allocation ratio of 1:1 using opaque sealed envelopes comprising indicators of groups 1 and 2, in order to conceal the allocation process. In 2.3 you say" The subjects with and without diabetes who met the eligibility criteria of the study were randomly allocated into the two groups using a simple randomization method." Ok, now tell us what you did exactly. Author response: The subjects were randomly allocated into the two groups using a simple randomization method through computer-generated random numbers. Accordingly, this was done by an independent statistician with the allocation ratio of 1:1 using opaque sealed envelopes comprising indicators of groups 1 and 2, in order to conceal the allocation process. In this regard, envelope 1 referred to ACS dressings, while envelope 2 indicated normal saline dressings (page 8). 2. 2.3: "When a participant entered the study, non-recognizable data were logged on the secure system." This needs rewriting as it does not make sense. Author response: I completely agree with respected reviewer. We omitted the sentence. 3. 2.3: "This study was an open-label trial. It was not probable…" possible instead of probable. Author response: I completely agree with respected reviewer. Revised and highlighted. 4. 2.3: "however, the investigators providing the treatment were different from those performing wound evaluations and those who were responsible for clinical tests." If, in fact, the evaluating physicians did not know the assignments of the patients, and could not tell the assignment by looking at the wound after dressings were removed, then these individuals were blinded, and this should be stated. Author response: I completely agree with respected reviewer. Revised and highlighted (page 8-9). 5. 2:5: "Additionally, both wound's size and appearance were estimated using the Pressure Ulcer Scale for Healing (PUSH). As I stated before this tool was developed for pressure injuries (pressure ulcers) and is not suitable for other wound types. If this is part of your outcomes, then you need to state that this is a major limitation of the study in the Discussion, in the last paragraph, where you discuss the limitations of the study. Author response: I completely agree with respected reviewer. We stated this as a major limitation of the study (page 17). 6. 2.6: "Because of the absence of drop-out during the trial, the analysis was performed based on the intent-to-treat methodology." I would hope the analysis would be intent-to-treat regardless of subjects that dropped out! Please delete the first phrase. Author response: I completely agree with respected reviewer. The sentence was omitted.

7. 3. "Primarily, 35 patients with chronic wound were evaluated in terms of the eligibility criteria, of whom 30 participants (11 cases with the surgical ulcer, 7 cases with a pressure ulcer, 7 cases with burn ulcer, and 5 cases with diabetic wounds) met the inclusion criteria and started the study. I would say something like: Thirty-five participants were screened and 30 patients were randomized; having met all the inclusion and exclusion criteria." In table 1 you describe the wound types, so you do not need to mention the wound types in this sentence.
Author response: I completely agree with respected reviewer. Revised.
9. 3. "At baseline, no differences were found in terms of wound surface area…" The results described in this long paragraph would be better understood if the results were presented in a table. Then you can summarize the highlights of the results with reference to the table. I would divide Table 1 into 2 smaller tables: patient-related variables, and wound-related variables.
Author response: I completely agree with respected reviewer. Revised.
10. The figures need far better resolution, and the Y axis still needs labeling.
Author response: I completely agree with respected reviewer. Revised.
11. CONSORT file: Item 2b: this is stated on page 8 not page 4. Items 14a/b: stated as found on P11 but there are no dates in the text. Please go through the checklist more thoroughly.
Author response: I completely agree with respected reviewer. Revised.

Reviewer #2:
- Re the types of chronic wounds included, could you provide more detail on the 'surgical ulcers' - are these dehisced surgical wounds, or failed grafts, or surgical wounds complicated by infection?
Author response: I completely agree with respected reviewer. 'surgical ulcers' were dehisced surgical wounds which was stated in Table 2.
Although you have provided some treatment details for the pressure injuries and diabetic foot ulcers, some details on the treatment for the other types of wounds is also needed. The accepted international classifications for the pressure injuries (Stage) and foot ulcers (SINBAD, or Texas) would also be useful.
Author response: I completely agree with respected reviewer. Some details were provided (page 9)
- what methods were used for debridement?
Sharp debridement with scalpel till pinpoint bleeding form in depth of the lesions, was applied weekly for as long as needed, in order to eliminate as much non-viable tissues as possible using the same way for all the patients under local anaesthesia (page 9).
- it's unclear what type of dressing the ACS intervention was inserted into? was this gauze?
Author response: the ACS-soaked gauze dressing was located on the surface of the wound bed and also the activated ACS was injected into the border of the wound by a trained physician (page 10).
- in the abstract and various places your aim is stated as investigating the 'efficiency of the intervention, later you have 'efficacy', which is what I suspect you mean, as there is no efficiency evaluation in the article, instead you have looked at efficacy
Author response: I completely agree with respected reviewer. Revised.
- there are more than four classes/types of chronic wounds, these may be the commonest ones but need to indicate others exist (as your wounds would then not be included)
Author response: I completely agree with respected reviewer. Revised.
- wound care methods should be evidence based, not based on traditional treatments. The most important treatment is to address the underlying cause of the ulcer, in addition to wound bed treatments
Author response: I completely agree with respected reviewer. We provided references for each stage of management.
PUSH scores should be reported as a total score, not by individual items of the score. A reference is needed to support its use in multiple types of wounds
Author response: We reported the PUSH total score. The use of PUSH in all types of wounds is a limitation of this trial which is stated in the limitation section (page 17).
- the systematic review reference for negative pressure is outdated and the newest review should be referenced
Author response: I completely agree with respected reviewer. Reference 18-19.
- your sample size calculation methods needs a description on what the mean variable was you used to calculate - mean ulcer area difference?

	Author response: Considering the mean ulcer area difference (55.5 and 72.1) and SD (21.6 and 19.9) derived from a previous study by Bansal 42, an alpha value of 0.05, power of 80 %, and 40% reduction in wound volume, the number of patients needed for each group was estimated as 14 using G-Power version 3.1. Moreover, considering a dropout rate of 10% and a 1:1 allocation ratio, the sample size was calculated as 30 (15 per arm). - your comment on p.10 on intention to treat methods demonstrates lack of understanding of this method, which is about analysing all participant data according to their randomised groups, whether or not they received the full intervention (if not, this could be due to multiple reasons apart from drop outs) Author response: I completely agree with respected reviewer. Revised. - adverse events should encompass all events, not just rash or oedema Author response: No adverse events comprising rash or edema or any other side effect were described in either treatment group throughout the 3-week follow up period. - the lack of adequate follow-up time could be added to the limitations Author response: Lack of adequate follow-up time is other limitation of this study (page: 17) - I note that the original trial protocol in the clinical trial registry has differing methods to this report, could you explain the issues you had requiring changes? Author response: the original trial protocol in the clinical trial registry was updated. - some of the references are still very old, and could be updated e.g. updated IWGDF 2019 guidelines for diabetic foot Author response: I completely agree with respected reviewer. Updated. - some language/grammar errors e.g. p.1 should be assessments' , p.2 .. . is rising fast globally, if the natural wound ...., due to lack of .... p.3 'a great request' needs rewording, p.4 'depressing' should be dressing ... etc. Author response: I completely agree with respected reviewer. Revised. Yours sincerely
Additional Information:	
Question	**Response**
Please enter the word count of your manuscript **excluding references and tables**	4185

Title: Enhanced chronic wound healing with autologous conditioned serum dressing in a randomized prospective clinical trial

Running head: Autologous conditioned serum for chronic wound healing

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Abstract

Aims: In this study, we aimed to assess both the efficacy and tolerability of autologous conditioned serum (ACS) as an innovative wound dressing in the local management of chronic wounds.

Materials and Methods: In this single-blinded randomized controlled trial, a total of 30 patients with chronic wound were randomly assigned either to receive 3 weeks of ACS or to receive normal saline dressings. The treatment was applied once a week and the latest assessment was planned after passing three weeks from the first ACS application.

Results: Analysis of wound assessment's data displayed statistically significant differences for wound surface area and Pressure Ulcer Scale for Healing scores (PUSH: area score, exudate, and tissue) in the participants underwent ACS dressing, but not in the normal saline group from baseline up to the end of the study. There were statistically significant differences in the changes in the wound surface area on the third week (-6.4±2.69 vs. +0.4±2.52 cm2), changes in the area score at week 3 (-2.2±1.08 vs. +0.2±0.86), exudate at week 2 (-1.2±0.70 vs. +0.0±0.45) and week 3 (-1.3±0.72 vs. -0.1±0.63), tissue at week 2 (-1.1±0.35 vs. +0.0±0.53) and week 3 (-1.8±0.65 vs. -0.1±0.63) and changes in the PUSH total score at week 1 (-1.6±0.98 vs. +0.4±1.22), week 2 (-3.2±0.86 vs. +0.4±0.98) and week 3 (-5.3±1.17 vs. -0.0±1.33) between ACS and saline groups, respectively.

Conclusions: This trial for the first time revealed a significant decrease in wound surface area as well as a considerable improvement in chronic wound healing by ACS dressing.

Trial registration: The trial has been registered at the Iranian Clinical Trial Registry database (No. IRCT20100720004422N7).

Keywords: Wound, Autologous conditioned serum, Pressure ulcer scale for healing

1. Introduction

Chronic wounds are included in the most common complaints of patients referred to general and vascular surgeons, orthopedists, infectious disease specialists, and dermatologists. Chronic disorders, including diabetes mellitus (DM), cardiovascular diseases (CVD), hypoxia, malignancy, and immunosuppression, local vascular disease, infection, and repeated trauma are the common causes of chronic wounds.[1] The prevalence rate for non-healing chronic wounds is between 1 and 2% of the general population and 8.5% of the elders in industrialized countries. [2-4] The burden of managing chronic wounds is globally rising fast, because of growing health care costs, an aging population, and drastic increase in diabetes and obesity prevalence. [5] Besides the physical, emotional, and social perspectives, costly medical treatments also make a great financial load on the health system.[6]

The wound healing process is a dynamic response to damage, which has three stages as follows: inflammation (2–5 days), proliferation (3–14 days), and maturation (3 weeks to 2 years).[7] It requires an interaction among different cell types, building proteins, growth factors, and proteins.[8] However, if natural wound healing process is disrupted, the wound can become chronic due to lacks of growth factors and cytokines that play a role in the wound healing process.[9] Chronic ulcers are lesions that do not usually heal within 3 months due to some underlying pathological conditions and they also indicate an imbalance between chronic traumatic factors and poor restorative responses. [10] They are categorized into the following four classes: pressure ulcers (PUs), diabetic ulcers, venous ulcers, and arterial insufficiency ulcers. [11] Wound care methods are a series of traditional treatments, including debridement followed by

wound dressings and application of topical treatment agents, which are often slow and time-consuming processes.[9] The idea of preparing the chronic wound bed to support re-epithelialization of wounds has been used in the treatment of different wounds for decades. Of note, a common approach to conform a better preparation is DIME approach (debridement of nonviable tissue, Inflammation and Infection management, moisture control, and environmental and epithelialization valuation).[12, 13] The conventional debridement is one of the main procedures in preparing wound beds as bacteria and toxins often condense in necrotic tissue.[14] Removing the necrotic tissue can also decrease the bacteria load, abnormal cells, and local edema, as well as regularizing the microenvironment of the surface of the wound.[15] The production and promotion of modern dressings are done according to the therapeutic concept of the wet environment, which have frequent benefits in comparison with the traditional dressing methods.[16] The most commonly applied modern wound dressings in clinical practice are hydrogels, hydrocolloid, alginates, foams, and films.[17] The application of therapeutic agents consist of growth factors and antimicrobial drugs, which principally emphasize on stimulating healing procedure and preventing infection, plays a crucial role in the management of all types of wounds. Therefore, a great request still exists in discovering new therapeutic drugs for performing topical treatment. Nowadays, modern methods are used for wounds healing. Notably, negative pressure wound therapy has displayed greater medical efficacy in the treatment of diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs) compared to the standard wound therapy.[18, 19] Other innovative modalities comprise of bioengineered skin substitutes, extracellular matrix proteins, hyperbaric oxygen (HBO2) therapy, ultrasound, and regenerative therapy.[9, 20]

Autologous conditioned serum (ACS) is an experimental medical procedure in which a patient's personal blood is extracted, manipulated, and then injected into his/her body as an anti-

inflammatory drug. [21] ACS is exclusively obtained from the subject's blood, and because it is cell-free, basically varies from platelet-rich plasma (PRP), which is the alternative autologous blood therapy. [22] ACS's efficiency is attributed to high concentrations of IL-1 receptor antagonist (IL-1ra), anti-inflammatory cytokines (IL-4, IL-10, and IL-13), and growth factors (eg, TGF-b, IGF1), which differentiate ACS from PRP. Growth factors and cytokines are suggested to be used to improve the healing process of soft tissue and skin. ACS contains more growth factors than PRP, so it seems to be able to have better effects on the wound healing process.[23] In addition, it is noteworthy that PRP, whole blood, and ACS, are promising upcoming and new treatment modalities. [22]

Several studies have confirmed the beneficial effects of individual growth factors, e.g. platelet-derived growth factors (PDGFs), fibroblast growth factors (FGFs), and granulocyte-macrophage colony stimulating factor (GM-CSF) on wound healing process in animal models as well as in humans.[24-27] However, the efficacy of ACS, as a representative of biological treatment with multiple growth factors besides IL-1ra and anti-inflammatory cytokines, in the management of chronic wounds has not been verified yet. Therefore, in this trial, we aimed to determine the efficacy of ACS wound dressing in the healing of chronic wounds with different etiologies.

2. Methods

2.1. Study design and setting

This was an open-label, parallel-group, randomized controlled trial performed to determine the efficacy and safety of ACS wound dressing in comparison with normal saline dressing, as a control, in the treatment of chronic wounds. The current study was conducted in two university

affiliated to outpatient clinics (Shohada and Imam Reza) between February 2019 and March 2020. Patients with chronic wounds referred to outpatient clinics were continuously recruited in the study using the non-probability convenience sampling method in terms of the eligibility criteria.

2.2. Study sample

The subjects with all types of chronic wounds and the following characteristics were included in this trial: both sexes aged between 18 and 80 years old, classified as grade I or II based on wound depth (dermis as grade I, subcutaneous tissue as grade II) [28], duration more than three months, and willingness to participate in the study. Participants were excluded if they were smoking; had wound with bacterial, viral or fungal infection according to Gardner et al.'s [29]wound infection criteria; had any coagulation disorders or platelet conditions; had severe vascular disorders; taking systemic steroid-containing medications; using corticosteroid ointment near the wound area, or were unable to collaborate with the indispensable dealings during the trial. None of the participants were pregnant or in the breastfeeding period.

Each one of the included patients received information on the study purpose and intervention and then signed the informed consent form before beginning the trial. The study was performed in terms of the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.154). The trial has been registered at the Iranian Clinical Trial Registry database (No. IRCT20100720004422N7). As well, the CONSORT guidelines were monitored and the CONSORT flowchart has been provided.

Baseline demographic characteristics of the subjects (including age and sex), anthropometric indices, and clinical findings were assessed at the baseline. Data on the wound (the type and size of wound) were investigated and documented before the start of the trial. Moreover, the participants' weight and height were measured using standard scales (Seca 813 digital scale and Seca 206 roll-up measuring tape, respectively). Body mass index (BMI) was calculated by dividing the weight (per kg) by the square of height (per m^2). [30]

Medical documents of the participants were obtained from the previous hospital admissions and then studied by a member of investigation team who was unaware of the trial arms, to evaluate any complication related to wound.

2.3. Assignment of interventions

The subjects with and without diabetes who met the eligibility criteria of the study were randomly allocated into the two groups using a simple randomization method through computer-generated random numbers. Accordingly, this was done by an independent statistician with the allocation ratio of 1:1 using opaque sealed envelopes comprising indicators of groups 1 and 2, in order to conceal the allocation process. In this regard, envelope 1 referred to ACS dressings, while envelope 2 indicated normal saline dressings.

This study was an open-label trial. It was not possible to blind the participants to the treatment. Furthermore, the treating physicians were not blinded to the treatment; however, the investigators providing the treatment were different from those performing wound evaluations and those who were responsible for clinical tests. Moreover, the statistician who executed all the statistical analyses was blinded to the study groups' allocation. These investigators did not know

the assignments of the patients, and could not tell the assignment by looking at the wound after dressings were removed.

2.4. Interventions

At this stage, wounds were washed, debrided, and evaluated by a physician. Thereafter, the related laboratory tests were done to confirm that they were not infected and were appropriate for the ACS treatment. Sharp debridement with scalpel till pinpoint bleeding form in depth of the lesions, was applied weekly for as long as needed, in order to eliminate as much non-viable tissues as possible using the same way for all the patients under local anaesthesia, if any participant was feeling pain. [31] Physiologic saline then was used at pressures for eliminating microorganisms without distressing the tissue. Pressure off-loading (removable walker cast) was also prescribed in terms of the guidelines by the treating physician depending on clinical necessity, and wound appearance and position in diabetic foot ulcers.[32, 33] Subsequently, primary treatment was applied, including glycemic control. EmsiG AM30 Air Mattress was prescribed for pressure relief in those patients with pressure wound who were repositioned every 2 hours on the mattress.[34] The treatment protocol was determined based on the site, ischemia, neuropathy, bacterial infection, and depth (SINBAD) score. The scoring system is easy to apply in routine clinical practice.[35] Additionally, nutrition assessment was performed by checking hemoglobin and albumin levels once a week. In smokers, they were requested to quit smoking at least 4 weeks before the treatment. [36]

Group 1: Dressing contained ACS

ACS was prepared in a sterile situation using the method demonstrated in references.[37, 38] Thirty-five milliliters of each participant's own blood was drawn from the antecubital vein under sterile protections, transferred to six polypropylene syringes (5 ml) containing glass beads, and incubated for 6 hours at 37°C. These tubes were then centrifuged on a table-top centrifuge for 15 minutes at 1500 rpm and serum was aspirated. Thereafter, the ACS-soaked gauze dressing was located on the surface of the wound bed and also the activated ACS was injected into the border of the wound by a trained physician. Finally, dressing and injection processes were applied at baseline and changed once a week for a period of 3 weeks. This period was selected based on the results of some previous studies.[39, 40]

Group 2: Dressing contained normal saline (Control)

The patients in the control group were all managed with normal saline solution. Standard sterile cotton gauze was soaked in normal saline and then used directly to the bed of the wound. Correspondingly, it was changed once a week directed by the investigating physician for a period of 3 weeks.

All the steps were performed under sterile condition for all the participants. Wound's assessment was performed every 48 h by the handling physician for any adverse wound consideration.

2.5. Main outcome measures

The main outcome measures were wound's surface size and wound healing. Wounds' surface sizes were measured by a one-centimeter flexible grid, which is a standard measurement for wound size. [41, 42] Two-dimensional evaluations were also applied by determining its linear

dimension; for instance, a rectangle (length × width), a circle (diameter × diameter) or an oval (maximum diameter × maximum diameter perpendicular to the first measurement). [42]

Additionally, both wound's size and appearance were estimated using the Pressure Ulcer Scale for Healing (PUSH). Accordingly, the PUSH is a rapid and reliable measure tool used to screen the alteration in pressure ulcer status over time as well as chronic leg ulcers in the clinical setting. The PUSH includes three parameters and subscales as follows:

- *surface area of the wound*, spans both the maximum length (vertical) and the maximum width (horizontal), in square centimeters. To obtain the wound's surface area, two measures are multiplied.

- *exudate amount present in the wound*, measured after removing the wound dressing and before putting any agent on it. It can be categorized as none, light, moderate, and heavy, which match with the scores of 0 to 3.

- *tissue type of the wound bed*, considered as the most prevalent types of tissue in the wound area, determined as follows: necrotic tissue (eschar), black, brown or tan tissue that tightly coheres to the bed of the wound or edges of the ulcer and may be either tighter or weaker than circumambient skin; *slough*, the tissue (yellow or white) that adheres to the bed of ulcer in strings or thick masses or is mucinous; *granulation tissue*, pink or beefy red color tissue with a glossy, wet, and granular look; *epithelial tissue*, for superficial wounds, new pink or glossy tissue (skin) that develops from the margins or as isles on the surface of ulcer; and *closed/resurfaced wound*, the wound is entirely enclosed with epithelium. These tissues are scored as 0 (closed wound), 1 (epithelial tissue), 2 (granulation tissue), 3 (slough), and 4 (necrotic tissue).

All the evaluations were performed at baseline, 1 week, 2 weeks, and 3 weeks after the beginning of the trial.

The safety parameters of ACS over 3 weeks were examined by the analysis of adverse events (AEs) at each study follow up.

2.6. Statistical analysis

Considering the mean ulcer area difference (55.5 and 72.1) and SD (21.6 and 19.9) derived from a previous study by Bansal [43], an alpha value of 0.05, power of 80 %, and 40% reduction in wound volume, the number of patients needed for each group was estimated as 14 using G-Power version 3.1. Moreover, considering a dropout rate of 10% and a 1:1 allocation ratio, the sample size was calculated as 30 (15 per arm).

All the statistical analyses were performed by SPSS software Version 17.0 (SPSS Inc., Chicago, IL, USA). The obtained data were provided as mean ± standard deviation (SD) and frequency counts (n, %). Kolmogorov-Smirnov and Shapiro-Wilks tests were applied to examine normal distribution of the data. Between-group comparisons of baseline variables were also performed using the Student t-test for continuous variables with a normal distribution, Mann-Whitney U test for continuous variables with no normal distribution, and Fisher exact test was applied for discrete variables. To assess within group changes and between group differences, two-way mixed ANOVA test (time [within subject] * group [between subjects]) besides the sidak posthoc as adjustment procedure were applied. The patients were assessed at baseline (week 0), week 1, week 2, and week 3. We illustrated effect size in terms of Cohen's d for outcome measures. In this regard, the effect sizes of 0.2, 0.5, and 0.8 were labeled as small, medium, and large, respectively. [44] A p-value of 0.05 or below was considered as statistically significant.

3. Results

Thirty-five participants were screened and 30 patients were randomized; having met all the inclusion and exclusion criteria and all of them completed the study and involved in the final analysis (Figure 1). Fifteen participants were randomized to ACS dressing group and 15 participants were enrolled into the normal saline dressing group. All the participants' demographic characteristics are shown in detail in Table 1. Their baseline demographic and wound characteristics were similar between the two groups except for weight that the participants in the control group had higher weight, with no difference in BMI between the groups.

There was a significant interaction among the time points (0, week 1, week 2, and week 3) serving as the within-group factor and group (ACS dressing vs. normal saline dressing as the control) as the between-group factor regarding the study outcomes (wound surface size, $p < 0.001$; area score, $p= 0.003$; exudate, $p= 0.01$ and tissue: $p= 0.008$). Based on the Cohen's d values, the results denoted to large effect size for the study outcomes ($d=2.36$, $d=3.01$, $d=1.77$ and, $d = 1.97$ for wound surface size, area score, exudate, and tissue, respectively). So, we analyzed the difference between the study groups at each level of the time factor.

At baseline, there were no differences in wound surface area and area, exudate, tissue and total scores between the ACS group and the control group (Table 2). Wound surface area and PUSH area, exudate, tissue and total scores decreased significantly in ACS treated group after 3 weeks (-6.4 ± 0.40, $p<0.001$; -2.2 ± 1.08, $p<0.001$; -1.3 ± 0.72, $p<0.001$, -1.8 ± 0.65, $p<0.001$, and -5.3 ± 1.17, $p=0.001$, respectively). There were no significant differences in control group concerning

wound surface area and PUSH area, exudate, tissue and total scores during the study period (p=0.150, p=0.069, p=0.463, p=0.572 and p=0.926 respectively). The result of mixed anova test showed that the differences in wound surface area and PUSH area, exudate, tissue and total scores in ACS treated group were significantly higher than control group (p=0.006, p=0.005, p<0.001, p<0.001 and p<0.001 respectively). However, there was no complete wound healing in any of the trial groups.

No adverse events comprising rash or edema or any other side effect were described in either treatment group throughout the 3-week follow up period.

4. Discussion

The results of this study demonstrate that three weeks of ACS dressing resulted in the reduced wound surface and the improved wound healing in grades 1 and 2 of chronic wounds based on the PUSH scale.

The traditional treatments of chronic wounds are disappointing because of their long duration, extensive trauma, great costs, and unsatisfactory outcomes. The current improvements in the fields of biomaterials may play key roles in chronic wound healing process.

In chronic wounds, tissue restoration is stopped in the inflammatory phase leading to pathologic inflammation and causing blockage of the beginning of advanced steps of healing process. [45] ACS was initially defined to advance muscle renewal in an animal model of muscle contusion [46] and to provide anti-inflammatory properties in carpal osteoarthritis in horses [47] as well as in human subjects with knee osteoarthritis in a clinical trial. [48] ACS is derived by the incubation of

venous whole blood at approximately 37°C. Afterward, this persuades the secretion of anti-inflammatory cytokines. [49] Kerscher et al. in their study established the efficacy and safety of micro-needling with ACS in improving of cutaneous elasticity and skin firmness in female patients with the reduced facial skin elasticity. [50]

Blood products contain growth factors, the capability of which is suggested to advance the healing procedure in chronic damages and to increase repairing speed in both acute and chronic wounds. [51] ACS is principally interesting because it is a derivative of the subject's own blood. [52, 53] Accordingly, this reflects an exceptional safety that consequently diminishes the adverse effects and any cost of construction.

To the best of our knowledge, this study is the first trial performed to evaluate the beneficial effect of ACS on the healing process of chronic superficial wounds. Reducing the area of the wound is considered as a good criterion for assessing the extent of healing. In the present study, wound surface area decreased from 10.9±5.52 cm^2 at baseline to 4.5±3.31 cm^2 after 3 weeks of ACS dressing (mean difference: -6.4±0.40 cm^2, $p<0.001$). Subsequently, this led to a 2.2±1.08 point decrease in area score at the third 3 week. While in the saline dressing group, wound surface area increased with a mean difference of +0.4±2.52 cm^2; however, it was not statistically significant. Wound area usually decreases due to wound healing and connective tissue deposition during the healing process. The contractile phenomenon that pulls the epidermal layer towards each other at the wound surface, reduces the area, and increases wound healing is the presence of both active fibroblasts and myofibroblasts in the bud tissue of granulation wounds. [54] In the participants underwent ACS dressing, exudate and tissue scores decreased as 1.3±0.72 and

1.8±0.65 points in 3 weeks, in comparison with 0.1±0.63 decreases of both scores in the saline group ($p<0.001$), respectively.

However, numerous investigations have established the promising effects of individual growth factors on the wound healing process. The transforming growth factor beta (TGF-b) superfamily is known as an essential mediator of tissue renovation. This multifunctional growth factor can provide pleiotropic properties during wound healing process by adjusting cell reproduction and immigration, differentiation, extracellular matrix construction, and immune regulation. [55] Of note, chronic, refractory wounds may also have an actual or practical insufficiency of TGF-b action. As well, some previous studies have shown the beneficial impact of exogenous IGF-I on the wound healing process, especially in combination with other growth factors. [56, 57] Furthermore, liposome-mediated IGF-I gene transfer was found to have the ability of enhancing the pathophysiology of a skin injury.[58, 59] However, there are some experiments of recombinant growth factors and ACS application conducted to improve the tendon healing process in an animal model with variable finding. [60-62]

Cytokines such as IL-1Ra and growth factors such as TGF-β and IGF-1 have a short half-life after exogenous utilization. [60, 63] However, wound healing may be improved not only by the direct connection of both the cytokines and growth factors to the receptors of the cell surface, but also by the incitement of endogenous construction of growth factors because of secondary properties.[64, 65] So, the impact of ACS can possibly be improved by several sequential injections as seen in the present trial. [66]

Chronic wounds impose a great burden on the affected patients. They cause pain, dysfunction, infections, and financial expenses, and frequently lead to sepsis or amputations. Population aging, obesity, and diabetes are quickly growing in most regions of the world, and simultaneously, the prevalence rates of non-healing pressure, venous, and diabetic wounds are increasing. [67] Therefore, this highlights the importance of investing in the expansion of wound management sciences as a multidisciplinary field. The complexity of chronic wounds has delayed proposing novel pharmacological approaches as alternates to change the wound parameters. Therefore, dressings are the mainstay of wound management, despite a few clinical evidences. [17] However, there is a great potential in the field of exogenous growth factors and cytokines.

The current research could have some implications for the care of patients with chronic wounds for paramedics, nurses, surgeons, and other physicians caring these patients. The novelty of our investigation lies in our findings for the efficacious management of chronic wounds using the method of ACS dressing. This investigation can be considered as the basis for more trials with greater sample size to evaluate the superiority of ACS over traditional dressings in chronic.

As well, there were some potential limitations in the trial that should be considered. One of thelimitations was the unavoidable un-blinded design of the trial, which can introduce observer bias. [68] To minimize this bias, a single-blind trial was applied, where the individuals evaluating wounds were not aware of the type of the treatment that was being applied. Another major limitation of our study is using PUSH for estimating the wound's size and appearance. This tool was developed primarily for pressure injuries (pressure ulcers) and is not suitable for other wound types. Additionally, the sample size for this trial was small and may have been

underpowered to assess the efficacy of the treatment on each type of wound. Lack of adequate follow-up time is other limitation of this study. So, this trial can be supposed as a pilot study. Future studies (power size calculated) by including more participants and stratifying wounds of different ethnologies are warranted to attest to the validity of this trial.

5. Conclusion

In conclusion, it was indicated that ACS dressing for three weeks can provide an effective and safe figure in chronic wounds. This can significantly reduce wound surface area and improve the healing process according to the PUSH index in a safe manner, which is likely ascribed to high concentrations of growth factors and anti-inflammatory cytokines.

Declarations

Ethics approval and consent to participate

All participants were provided written informed consent and endorsement has been acknowledged from the Ethics Committee of the Research Vice-Chancellor of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.154). Patients' personal data about patients were conserved in a database to keep patients' security. The study was also registered in the clinical trial registry under number IRCT20100720004422N7 code (https://www.irct.ir/trial/41444).

Authors' contributions

Sh.Gh., S.K.Sh. and A.P contributed to conception and design of the study and data analysis and interpretation. N.D., F.E., M.Y. and B.P.Kh. collected all data and contributed to data interpretation. N.D. prepared the first draft of the manuscript. N.D., Sh.D., and R.Y. revised the

first draft of the manuscript and prepared the final draft of manuscript. All authors read and approved the final manuscript.

Competing interests

All authors declare that they have no competing interests.

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Figure 1 Flow Diagram of Study Participants

Figure 2 Trends in Main Study Outcomes from the Beginning to Last Follow-up of Patients in the Study Groups: 2a. Wound surface area; 2b. PUSH area score; 2c. PUSH exodate score; 3. PUSH tissue score

Table 1 Patient-related Characteristic of the Study Participants

Variable	ACS group (n=15)	NS group (n=15)	P-Value
Age (yr)	53.2±12.53	56.3±10.77	0.469‡
Sex			
Male	13 (86.7%)	12 (80%)	0.775†
Female	2 (13.3%)	3 (20%)	
Weight (kg)	72.4±6.22	78.6±5.85	0.009‡
Height (cm)	167.3±5.48	169.6±5.59	0.271‡
BMI (kg/m^2)	25.9±3.30	27.4±2.98	0.220‡
Hemoglobin (g/dL)	12.5±2.37	11.9±2.89	0.158‡
Albumin (g/dL)	2.4±0.68	2.6±0.54	0.091†
HbA1C	6.5±0.76	6.7±0.92	0.507†

Data are reported as means ± standard deviations, or n (%). BMI, body mass index

† P obtained from Chi-Square test, ‡P obtained from Independent samples t-test.

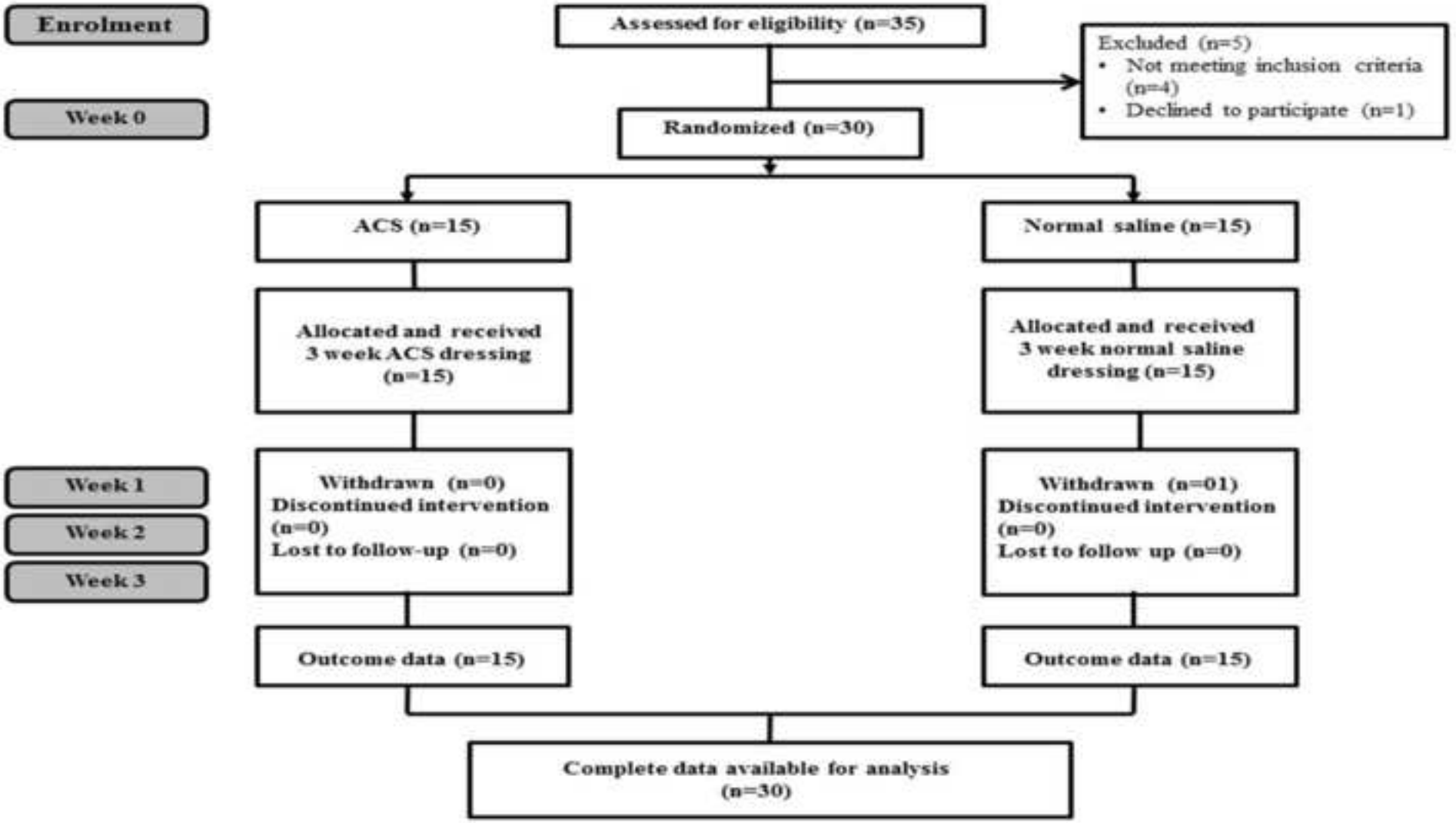
Table 2 Wound-related Characteristic of the Study Participants

Variable	ACS group (n=15)	NS group (n=15)	P-Value
Duration (month)	2.8±0.56	3.±0.70	0.345‡
Location			
Leg	5 (33.3%)	4 (26.7%)	0.389†
Scalp	6 (40.0%)	4 (26.7%)	
Buttock	2 (13.3%)	3 (20.0%)	
Heel	1 (6.7%)	2 (13.3%)	
Thigh	1 (6.7%)	2 (13.3%)	
Type			
Diabetic Wound	3 (20.0%)	2 (13.3%)	0.389†
Pressure Wound	6 (40.0%)	5 (33.3%)	
Dehisced Surgical Wound	3 (20.0%)	4 (26.7%)	
Burn Wound	3 (20.0%)	4 (26.7%)	
Wound Surface Area (cm^2)	10.9±5.52	9.0±3.86	0.436‡
PUSH Area Score	7.8±1.01	7.3±0.97	0.250‡
PUSH Exodate Score	1.3±1.72	1.0±0.70	0.345‡
PUSH Tissue Score	2.9±0.45	2.5±0.51	0.089‡
PUSH Total Score	12.0±1.83	10.9±1.53	0.081‡

Data are reported as means ± standard deviations, or n(%).

† P obtained from Chi-Square test, ‡P obtained from Independent samples t-test.

Flow Diagram of Study Participants



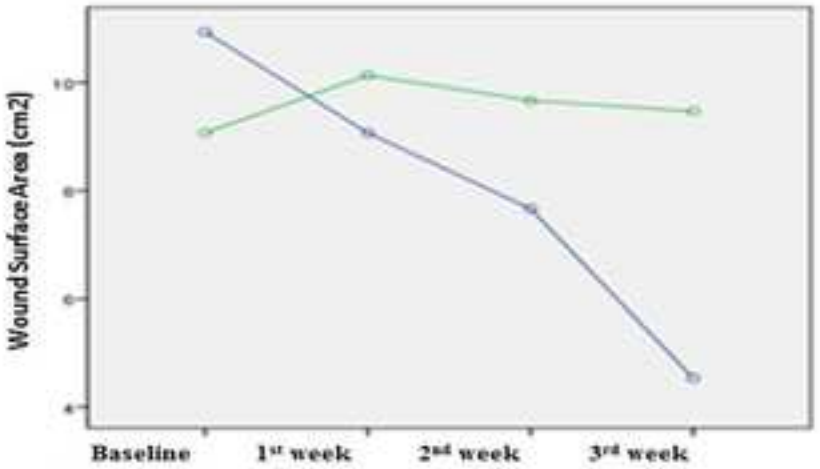


Fig 2a

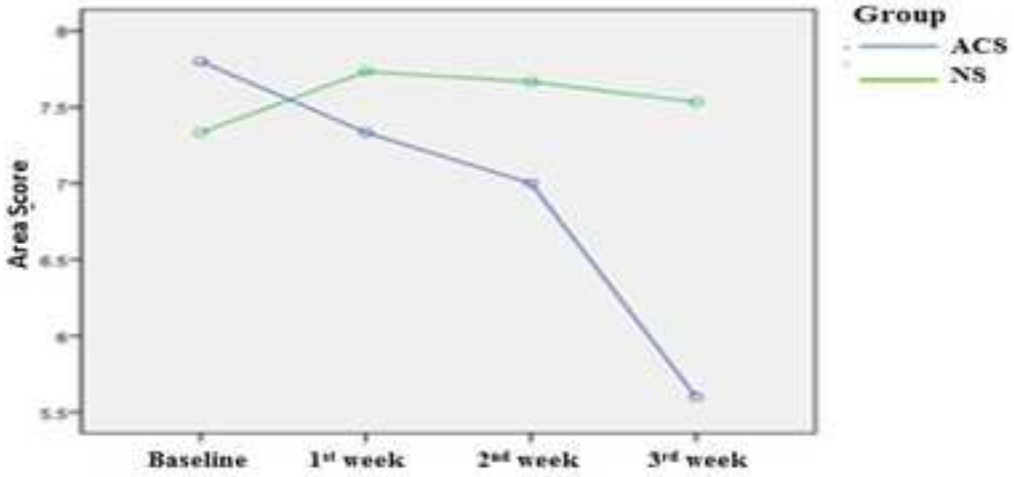


Fig 2b

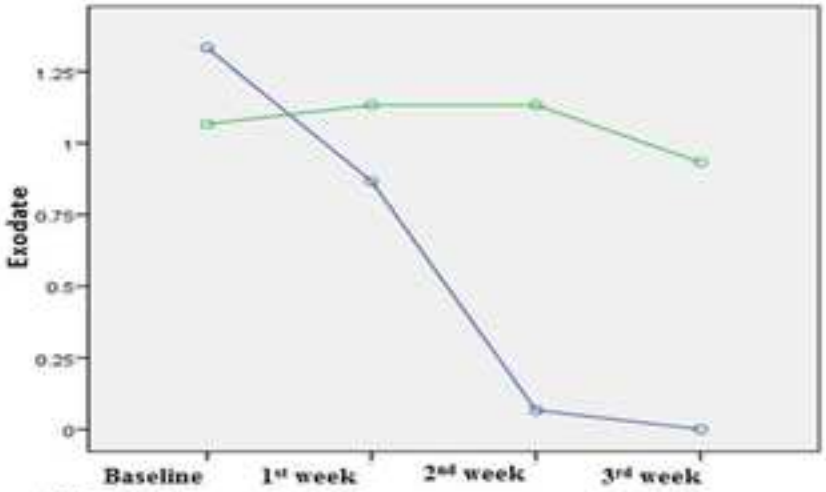


Fig 2c

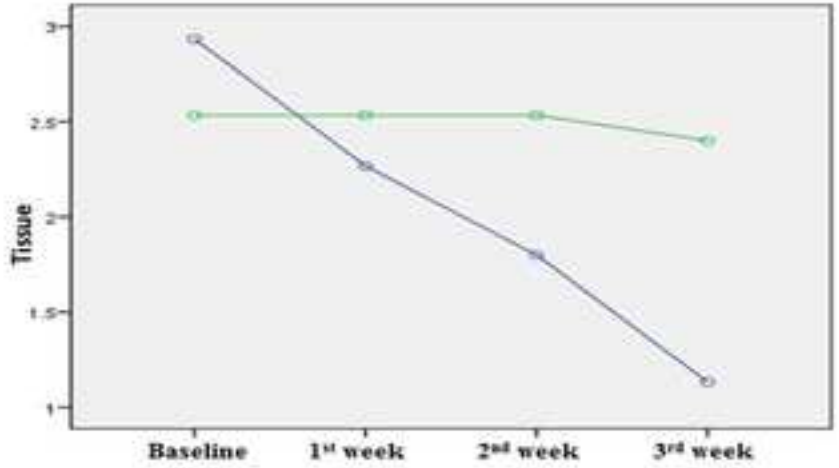
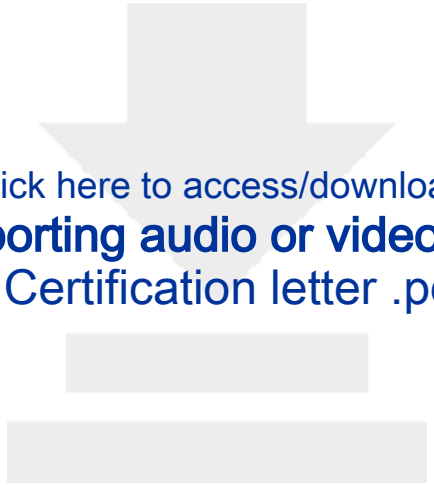


Fig 2d



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