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An exploratory clinical trial for combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: a study protocol

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Abstract

Background

Chronic skin ulcers such as diabetic ulcers, venous leg ulcers, and pressure ulcers are intractable and increasing in prevalence, and are a costly problem in health care. We have developed combination therapy with a gelatin sheet that is capable of the sustained release of PRP (platelet-rich plasma). The objective of this study is to investigate the safety and efficacy of autologous PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.

Methods and analysis

Thirty patients with chronic skin ulcers that have not healed by conventional therapy for at least 1 month are being recruited. Patients will be applied with PRP after debridement, and the wound will be covered with a hydrocolloid dressing or a gelatin sheet. The efficacy is evaluated according to the time from the beginning of PRP application to secondary healing or the day on which wound closure is achieved by a relatively simple surgical procedure such as skin grafting or suturing. Patients will be followed up until 6 weeks after application to observe the adverse events related to the application of PRP and dressings. This study has been designed to address and compare the safety and efficacy of PRP covered with a hydrocolloid dressing and with a gelatin sheet. If successful, this combination therapy may be an alternative to bioengineered skin substitutes containing living cells and lead to substantial progress in the management of chronic skin ulcers.

Ethics and dissemination

This protocol was approved by the Institutional Review Board of Kansai Medical University (KMUNo.0649-1). The findings of this trial will be disseminated through peer-reviewed journals and national and international scientific meetings and will also be disseminated to patients.

Trial registration number: UMIN000015689

Strengths and limitations of this study

- This protocol will provide new evidence evaluate the efficacy of PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.
- This study is a historical control study.
- Only subgroups are randomized and the small number of study may affect generalizability.

INTRODUCTION

Chronic skin ulcers caused by diabetes mellitus, venous insufficiency, pressure sores, collagen disease, trauma, or radiation are an intractable and costly problem in health care.[1-3] In particular, diabetic foot ulcers and venous leg ulcers are frequent and costly complications of their underlying diseases. The prevalence of diabetic foot ulcers ranges from 4% to 10% among diabetes patients and the lifetime incidence is reported to be as high as 25%. [2,3] It has been reported that venous leg ulcers recurred in 72% of cases and the recurrence rate after skin grafting was 48% within one year.[4] With the development of tissue engineering, bioengineered skin substitutes and genetically derived growth factors have progressed greatly in recent years in the treatment of chronic skin ulcers.[5-7] however, there are still issues that remain to be resolved in the treatment of those ulcers.

Platelet-rich plasma (PRP) is blood plasma enriched with platelets. Autologous PRP is a source of autologous growth factors such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF). [8,9] PRP has been used clinically in a variety of treatments of bone defects, tendon injuries, cartilage injuries, cosmetic surgery, and chronic ulcers.[10] Regarding the efficacy of PRP in the treatment of chronic wounds, PRP has been described as improving healing rates compared with standard care in many reports.[11, 12] On the other hand, no major difference was found in the healing outcome of leg ulcers between PRP treatment groups and control groups in other studies.[11, 12]

A drug delivery system (DDS) is technology for enhancing the biological activity of growth factors that is very short in vivo half-life after application. Biodegradable hydrogels of gelatin with an isoelectric point (IEP) of 5.0 have been designed and developed for the sustained release of positively charged growth factors such as basic fibroblast growth factor (bFGF), PDGF-BB, and TGF-β1.[13-15] PDGF-BB and TGF-β are major growth factors involved in PRP for improving the wound healing process, and enhanced angiogenesis by gelatin microspheres impregnated with PRP has been reported.[13] Gelatin hydrogel sheet has also been used for the sustained

system of growth factors and can be applied on wounds directly as a wound dressing.[16] The wound healing effect of PRP covered with this sheet is expected to be enhanced by the sustained growth factors from the gelatin sheet on the basis of its biodegradation after application.

Here, we designed an exploratory clinical study to investigate the safety and efficacy of PRP application covered with our gelatin sheet compared with PRP covered with a hydrocolloid dressing in the treatment of chronic skin ulcers. In addition, we estimated the contents of PDGF-BB, TGFβ1, and VEGF in PRP and compared these contents and wound healing in association with the gelatin sheet.

MATERIALS AND ANALYSIS

Primary objective

The objective of this study is to evaluate the safety and efficacy of the administration of PRP in the treatment of chronic skin ulcers that are not expected to heal using conventional treatments. The safety and efficacy of PRP covered with the gelatin sheet and PRP covered with hydrocolloid dressing will also be evaluated.

Methods and design

Open-label, non-randomized, controlled clinical trial

Design

This study is a prospective cohort study using historical data as a control. A group with PRP application covered with a gelatin sheet and a group with PRP application covered with a hydrocolloid dressing have been set. Patients will be randomized to the gelatin sheet subgroup or the hydrocolloid dressing subgroup. Randomization-based comparison between dose groups can achieve significant improvements in accuracy and a lack of bias. This comparison can provide useful information for designing and conducting future trials.

Setting and participants

This study is being conducted at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital. Patients with chronic skin ulcers are referred by physicians and also identified through a number of wound care clinics in Osaka Prefecture and surrounding prefectures.

Inclusion criteria

1. Patients aged 20 years or older at informed consent
2. Presence of chronic skin ulcers as below:
   - Not healing for at least 1 month with conventional treatments
   - Not expected to respond to conventional treatments
   - Expected to be closed by autologous skin grafting or suturing after PRP treatment
3. Written informed consent

Exclusion criteria

1. Patients showing unstable hemodynamics due to ischemic heart disease, or impaired blood coagulation as in clotting disorders
2. Patients taking anticoagulants or antithrombotics judged by the investigator or sub-investigator to be inappropriate as subjects since the drugs may affect the clotting system
3. Have any of the following systemic diseases:
   - Uncontrolled diabetes mellitus (defined by HbA1c ≥10%) according to the latest laboratory data obtained within 28 days before registration)
   - Requiring continued use of oral corticosteroid therapy (>20 mg/day prednisolone equivalent)

- A history of malignant tumor with a disease-free interval of 3 years or less

4. Patients who are or may be pregnant
5. Other patients judged by the investigator or sub-investigator to be inappropriate as subjects of this study

Analysis of subgroups

In this study, patients will be to either the gelatin sheet subgroup or the hydrocolloid dressing subgroup at a ratio of 1:1 without stratification. Randomization will be performed by assigning random numbers from random number tables to the treatment conditions.

Interventions

Preparations of PRP

PRP is prepared using the Magellan Autologous Platelet Separator System (Medtronic Inc., Minneapolis, MN) and its basic disposable kit. PRP is prepared by sampling blood using the 60-ml syringe of the basic disposable kit filled with an anticoagulant and separating and concentrating platelets to 3-fold or more compared with that in peripheral blood using the Magellan Autologous Platelet Separator System. The obtained PRP is activated as described in our previous study [8,9]. Briefly, a 1:1 (v/v) mixture of 0.5 M $CaCl_2$ (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and autologous thrombin is prepared in advance as an activator. A 10:1 (v/v) mixture of PRP and the activator is incubated for 5 min at room temperature. Platelets of prepared PRP are counted and the concentrations of growth factors (TGF-β1, PDGF-BB, and VEGF) in activated PRP are also quantified. If platelets cannot be separated and concentrated 3-fold or more compared with that in peripheral blood, the study is discontinued.

Preparation of the gelatin sheet

Acidic gelatin with an isoelectric point of 5.0 (Nitta Gelatin, Osaka, Japan) is to be used in this

study. Gelatin hydrogel sheets of 12x 3 cm in size were prepared at the Department of Biomaterial, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University[16]. Briefly, 40 g of aqueous gelatin solution (5wt%) preheated at 40°C was mixed with 80 µl of aqueous glutaraldehyde (GA, Wako Pure Chemical Industries, Osaka, Japan) solution (25wt%). The resulting mixture was poured into polystyrene dishes, followed by leaving it at 4 °C for 12 hours for chemical cross-linking of gelatin. Then, hydrogel sheets formed were placed in 100 mM glycine aqueous solution, followed by agitation for 1 hour at room temperature to inactivate the residual unreacted aldehyde groups of GA. The gelatin hydrogel sheet was washed with double-distilled water, freeze-dried, and sterilized with ethylene oxide gas. To remove remaining ethylene oxide gas, the resulting hydrogel sheet was kept at 40 °C for 3 days under vacuum condition.

Application of activated PRP and dressing changes

After debridement of the chronic skin ulcers, activated PRP is applied to them. After the administration of PRP, the wound is dressed with a gelatin hydrogel sheet or a conventional hydrocolloid dressing (DuoActive; Convatec Japan, Tokyo, Japan). The wound is occluded with the above dressing material for 5 days from the beginning of the administration of PRP and treated thereafter by applying ointment such as Azunol ointment (Nippon Shinyaku Co. Ltd., Kyoto, Japan) until 6 weeks after the administration of PRP.

Subsequent therapy

The use of basic fibroblast growth factor (bFGF) will be prohibited from 2 or more days prior to the administration of PRP and during the study period. Negative-pressure wound therapy (NPWT) must also not be performed during the study period. After completion of the study period (6 weeks after PRP administration or the day on which wound closure is judged to be possible), no particular restrictions will be imposed.

Digital photography for healing assessment

Using a digital camera, digital images of the wounds will be taken with a calibrator (CASMATCH; BEAR Medic Corp., Tokyo, Japan) placed on the skin adjacent to the wound. The color and size of the image can be adjusted using the CASMATCH and image editing software (Adobe Photoshop; Adobe Systems Inc., CA, USA) to assess the wound and granulation areas, and wound bed. As with the primary endpoint, the wound evaluation committee members will assess the wound closure.

Primary endpoint

The primary endpoint is "Time until wound closure."

Time until wound closure: The time (number of days) from the administration of PRP to wound closure, spontaneous wound closure, or wound closure by a simple surgical procedure is evaluated. The results of evaluation are confirmed by the wound evaluation committee members consisting of independent physicians other than the physicians involved in the study to ensure the neutrality, objectivity, and reproducibility of the evaluation.

Evaluation criteria for the possibility of wound closure during the investigation are:

(1) Judged to be closed by spontaneous healing

(2) Judged to be closed by a simple surgical procedure (when 2 or more of the 4 conditions below are fulfilled)

- 75% or more of the wound area is covered by granulation tissue.
- Bones, tendons, and underlying tissues are covered by granulation tissue.
- Local infection is controlled clinically (no adverse event due to infection is observed).
- The wound depth is reduced by an average of 50% or more.

Secondary endpoints

- Percentage of wound reduction: Before and after the application of PRP, the wound is photographed once a week at dressing change, and the wound area is measured serially.
- The improvement of blood flow: The skin perfusion pressure (SPP) or ankle brachial index (ABI) is measured at dressing changes before and 1 and 4 weeks after the application of PRP.
- The correlation of "time until wound closure" to the platelet count or growth factor levels in PRP.

Blinding

The wound closure will be independently measured under blinding by central review. Patients will be unblinded, and unblinded investigators will apply PRP and change dressings.

Sample size

This study is a prospective cohort study using historical data as controls and the total number of patients for registration is 30, as a feasible size for a pilot study.

Study schedule

The schedule of study assessments and evaluations is shown in Table 1. The study period will be from the day of PRP application to 6 weeks after PRP application. Data to evaluate the efficacy and safety of this study will be collected at enrollment, baseline, Day 5, and Weeks 1, 2, 3, 4, 5, and 6 after application or the day of wound closure.

Statistical analysis

Statistical analysis will be performed on the following variables:

- Time until wound closure
- Percentage of wound reduction
- The improvement of blood flow
- The correlation of "time until wound closure" to the platelet count or growth factor levels in PRP
- Adverse events related to the application of PRP.

The efficacy is evaluated according to the time from PRP application to wound closure. The survival curves in an existing control group based on historical data and PRP-treated group until wound closure are evaluated by the Kaplan-Meier method, and stratified analysis is performed using the log-rank test. Stratified analysis of the number and percentage of patients who showed wound closure is performed by Fisher's exact test (95% confidence interval).

In addition, the optimal dose of PRP for wound healing is estimated from the platelet count and growth factor concentrations in PRP.

The incidences of adverse events and adverse events that can be causally related to the PRP application will be evaluated by event and severity.

ETHICS

This study is being conducted in compliance with the ICH-GCP and in agreement with the latest revision of the Declaration of Helsinki, Pharmaceutical Affairs Law, and all applicable Japanese laws and regulations, as well as any local laws and regulations and all applicable guidelines.

This protocol and any amendments have Institutional Review Board approval at Kansai Medical University.

Subject consent

Informed consent will be obtained from all potential study participants using the IRB-approved

informed consent form. The clinical investigator informs the potential study subject of all pertinent aspects of the study. The subject must sufficiently understand the contents of the information form before providing written consent. The consent form must be dated and signed by both the investigator and the participant. Subjects are also informed that their medical care will not be affected if they do not choose to participate in this study. The consent form will be retained at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital, and the information form and a copy of the consent form will be handed to the participant. Whenever the investigator obtains information that may affect the participant's willingness to continue participation in the study, the investigator or sub-investigator will immediately inform the participant and record this, and reconfirm the participant's willingness to continue participation in the study.

DISSEMINATION

The findings of this trial will be disseminated through peer-reviewed journals and national and international scientific meetings and will also be disseminated to patients.

DISCUSSION

This study has been designed to address the safety and efficacy of PRP treatment for chronic skin ulcers using a gelatin hydrogel sheet that can sustain the level of growth factors contained in PRP. This study will be the first controlled trial to evaluate the efficacy of PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers. The efficacy of PRP in the treatment of chronic wounds in clinical trials is still controversial.[10-12] Our gelatin hydrogel sheet has been applied in clinical trials mainly as the drug delivery system for bFGF and its efficacy and safety have been reported.[16, 17] bFGF can be used clinically in Japan; however, the application of bFGF is not approved in other countries.[7, 18,19] The clinical usage of growth factors in those countries is limited. PRP can be prepared from

a patient's blood or from donated platelets in all countries. Some bioengineered skin substitutes that provide growth factors secreted by living cells have been reported to be effective for chronic skin ulcers, although they are costly and access is also limited to only a few areas.[20-22] Our gelatin sheet is freeze-dried and can be stored at room temperature for months. Therefore, we can use this combination therapy of PRP and gelatin sheet anywhere when needed and its efficacy might be superior to bioengineered skin substitutes because they have a similar mechanism of action to secrete growth factors in a sustained manner. If successful, this intervention may lead to substantial and important changes in the management of increasingly prevalent chronic skin ulcers, such as diabetes ulcers and venous leg ulcers.

Trial status

Recruitment commenced in November 2014. Recruitment continued until 14 August 2015.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NM, NK, and KK initiated the study design. NM prepared the first draft of the manuscript. NM, NK, TO, TH, KS, and KK provided the expertise in this clinical trial. MK, MY and YK provided contributions from the engineering aspect for gelatin sheets and prepared and supplied them. NM is a grant holder. All authors contributed to the refinement of study protocol and approved the final manuscript.

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Table 1. Schedule of study assessments and evaluations

Clinical assessments, testing, and investigations	At patient registration	Before PRP administration	Day 5 (±2 days)	Week 1 (±7 days)	Week 2 (±3 days)	Week 3 (±3 days)	Week 4 (±7 days)	Week 5 (±3 days)	Last day
Patient background	○								
Wound category	○								
Digital photography of wound		○	○	○	○	○	○	○	○
Blood flow (SPP or ABI)		○		○			○		
Blood tests (peripheral blood (including platelet count), CRP, HbA1c)	○			○					
PRP platelets and GFs		○							
Wound area		○	○	○	○	○	○	○	○
Concomitant medications		○	○	○	○	○	○	○	○
Blood tests		○	○#	○#	○#	○#	○#	○#	○#
Bacteriological tests			○&	○&	○&	○ &	○&	○ &	○&
Adverse events		←							→
Day of wound closure		←							→

○: required
#: Performed on the days of occurrence of adverse events.
&: Performed on the days when infectious signs are suspected.
Last day: after 6 weeks or the day of wound closure

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Abstract

Background

Chronic skin ulcers, such as diabetic ulcers, venous leg ulcers and pressure ulcers are intractable and increasing in prevalence, representing a costly problem in health care. We developed a combination therapy with a gelatin sheet that is capable of providing the sustained release of PRP (platelet-rich plasma). The objective of this study is to investigate the safety and efficacy of autologous PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.

Methods and analysis

Thirty patients with chronic skin ulcers that have not healed with conventional therapy for at least one month are being recruited. The patients will receive PRP after debridement, and the wounds will be covered with a hydrocolloid dressing or gelatin sheet. The efficacy will be evaluated according to the time from the beginning of PRP application to secondary healing or the day on which wound closure is achieved with a relatively simple surgical procedure, such as skin grafting or suturing. All patients will be followed up until six weeks after application to observe adverse events related to the application of PRP and the dressings. This study was designed to address and compare the safety and efficacy of PRP covered with a hydrocolloid dressing versus a gelatin sheet. If successful, this combination therapy may be an alternative to bioengineered skin substitutes containing living cells and lead to substantial progress in the management of chronic skin ulcers.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of Kansai Medical University (KMUNo.0649-1, August 4, 2014: version1.0). The findings of this trial will be disseminated through peer-reviewed journals and national and international scientific meetings as well as to the patients.

Trial registration number: UMIN000015689

Strengths and limitations of this study

- The current protocol will provide evidence to compare the efficacy of PRP covered with a hydrocolloid dressing and that of PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.
- This study is a historical control study.
- Only subgroups are randomized, and the small number of study subjects may affect generalizability.

INTRODUCTION

Chronic skin ulcers caused by diabetes mellitus, venous insufficiency, pressure sores, collagen disease, trauma or radiation are intractable and costly problems in health care.[1-3] In particular, diabetic foot ulcers and venous leg ulcers are frequent and costly complications of the underlying diseases. The prevalence of diabetic foot ulcers ranges from 4% to 10% among diabetes patients, and the lifetime incidence is reported to be as high as 25%. [2,3] It has been reported that venous leg ulcers recur in 72% of cases, and the recurrence rate after skin grafting is 48% within one year.[4] With the development of tissue engineering, bioengineered skin substitutes and genetically derived growth factors have progressed greatly in recent years with respect to the treatment of chronic skin ulcers.[5-7] However, new treatment options for chronic skin ulcers are needed.

Platelet-rich plasma (PRP) is blood plasma enriched with platelets. Autologous PRP is a source of autologous growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). [8,9] PRP has been used clinically in a variety of treatments for bone defects, tendon injuries, cartilage injuries, cosmetic surgeries and chronic ulcers.[10] Regarding the efficacy of PRP in the treatment of chronic wounds, PRP has been described as improving the rate of healing compared with standard care in many reports.[11, 12] On the other hand, no major differences have been found in the healing outcomes of leg ulcers between PRP treatment groups and control groups in other studies.[11, 12]

A drug delivery system (DDS) is a type of technology for enhancing the biological activity of growth factors with a very short in vivo half-life after application. Biodegradable hydrogels of gelatin with an isoelectric point (IEP) of 5.0 have been designed and developed for the sustained release of positively charged growth factors, such as basic fibroblast growth factor (bFGF), PDGF-BB and TGF-β1.[13-15] The efficacy of a sustained release system for epidermal growth

factor (EGF) was also recently reported.[16] PDGF-BB and TGF-β are major growth factors involved in PRP for improving the wound healing process, and enhanced angiogenesis by gelatin microspheres impregnated with PRP has been reported.[13] Additionally, gelatin hydrogel sheets have been used to achieve the sustained release of growth factors and can be applied on wounds directly as a wound dressing.[17] The wound healing effect of PRP covered with this sheet is expected to be enhanced by the sustained release of growth factors from the gelatin sheet as a result of its biodegradation after application.

In this report, we designed an exploratory clinical study to investigate the safety and efficacy of PRP application covered with our gelatin sheet compared with PRP covered with a hydrocolloid dressing in the treatment of chronic skin ulcers. In addition, we estimated the contents of PDGF-BB, TGFβ1 and VEGF in PRP and compared these values and the degree of wound healing associated with the gelatin sheet.

MATERIALS AND ANALYSIS

Primary objective

The objective of this study is to evaluate the safety and efficacy of the administration of PRP in the treatment of chronic skin ulcers that are not expected to heal using conventional treatments. The safety and efficacy of PRP covered with a gelatin sheet versus a hydrocolloid dressing will also be evaluated.

Methods and design

Open-label, non-randomized, controlled clinical trial

Design

This study is a prospective cohort study using historical data as a control. A group treated with PRP application covered with a gelatin sheet and a group treated with PRP application covered

with a hydrocolloid dressing have been set. The patients will be randomized to the gelatin sheet subgroup or the hydrocolloid dressing subgroup, and a randomization-based comparison between these groups is expected to achieve significant improvements in accuracy with a lack of bias. This comparison will provide useful information for designing and conducting future trials.

Setting and participants

This study is being conducted at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital. Patients with chronic skin ulcers are being referred by physicians and identified through a number of wound care clinics in Osaka Prefecture and surrounding prefectures.

Inclusion criteria

1. Patients 20 years of age or older with informed consent
2. The presence of the following chronic skin ulcers:
   - Not healing for at least one month with conventional treatments
   - Not expected to respond to conventional treatments
   - Expected to be closed with autologous skin grafting or suturing after PRP treatment
   - An ulcer area between 3 and 30 cm^2 (that can be applied with autologous PRP)
3. Written informed consent

Exclusion criteria

1. Patients showing unstable hemodynamics due to ischemic heart disease or impaired blood coagulation as in clotting disorders
2. Patients taking anticoagulants or antithrombotics judged by the investigator or sub-investigator to be inappropriate as subjects since the drugs may affect the clotting system

3. Any of the following systemic diseases:
   - Uncontrolled diabetes mellitus (defined as a HbA1c level of ≥10%) according to the latest laboratory data obtained within 28 days before registration)
   - Requiring the continued use of oral corticosteroid therapy (>20 mg/day prednisolone equivalent)
   - A history of malignant tumors with a disease-free interval of three years or less
4. Patients who are or may be pregnant
5. Other patients judged by the investigator or sub-investigator to be inappropriate as subjects of this study

Analysis of subgroups

In this study, patients will be randomized to either the gelatin sheet subgroup or hydrocolloid dressing subgroup at a ratio of 1:1 without stratification. Randomization will be performed by assigning random numbers from random number tables to the treatment conditions.

Interventions

Preparation of PRP

PRP is prepared using the Magellan Autologous Platelet Separator System (Medtronic Inc., Minneapolis, MN) and its basic disposable kit. PRP is prepared by sampling blood using 60 ml syringes of the basic disposable kit filled with an anticoagulant and separating and concentrating platelets to 3-fold or more compared with that observed in the peripheral blood using the Magellan Autologous Platelet Separator System. The obtained PRP is activated as described in our previous study [8,9]. Briefly, a 1:1 (v/v) mixture of 0.5 M $CaCl_2$ (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and autologous thrombin is prepared in advance as an activator. A 10:1 (v/v) mixture of PRP and the activator is incubated for five minutes at room temperature. Platelets of prepared PRP are counted, and the concentrations of

growth factors (TGF-β1, PDGF-BB and VEGF) in activated PRP are also quantified. If platelets cannot be separated and concentrated 3-fold or more compared with that in the peripheral blood, the study will be discontinued.

Preparation of the gelatin sheet

Acidic gelatin with an isoelectric point of 5.0 (Nitta Gelatin, Osaka, Japan) is to be used in this study. Gelatin hydrogel sheets of 12x 3 cm in size are prepared at the Department of Biomaterial, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University[16]. Briefly, 40 g of aqueous gelatin solution (5wt%) preheated at 40°C is mixed with 80 µl of aqueous glutaraldehyde (GA, Wako Pure Chemical Industries, Osaka, Japan) solution (25wt%). The resulting mixture is poured into polystyrene dishes and left at 4°C for 12 hours for chemical cross-linking of gelatin. Then, the formed hydrogel sheets are placed in 100 mM glycine aqueous solution, followed by agitation for one hour at room temperature to inactivate the residual unreacted aldehyde groups of GA. The gelatin hydrogel sheet is washed with double-distilled water, freeze-dried and sterilized with ethylene oxide gas. To remove the remaining ethylene oxide gas, the resulting hydrogel sheet is kept at 40°C for three days under vacuum conditions.

Application of activated PRP and dressing changes

After debridement of the chronic skin ulcers, activated PRP is applied to the lesions. Following the administration of PRP, the wound is dressed with a gelatin hydrogel sheet or conventional hydrocolloid dressing (DuoActive; Convatec Japan, Tokyo, Japan). The wound is occluded with the above dressing material for five days from the beginning of the administration of PRP and treated thereafter by applying ointment, such as Azunol ointment (Nippon Shinyaku Co. Ltd., Kyoto, Japan) or wound dressings, until six weeks after the administration of PRP.

Subsequent therapy

The use of basic fibroblast growth factor (bFGF) will be prohibited from three or more days prior to the administration of PRP and during the study period. Negative-pressure wound therapy (NPWT) must also not be performed during the study period. After completion of the study period (six weeks after PRP administration or the day on which wound closure is judged to be possible), no particular restrictions will be imposed. Concomitant therapies, such as a compression treatment, in cases of venous leg ulcers will be continued and applied before entry, because it is important to evaluate the efficacy of PRP under the same conditions.

Digital photography for the healing assessment

Using a digital camera, digital images of the wounds will be taken with a calibrator (CASMATCH; BEAR Medic Corp., Tokyo, Japan) placed on the skin adjacent to the wound. The color and size of the images can be adjusted using the CASMATCH and image editing software program (Adobe Photoshop; Adobe Systems Inc., CA, USA) to assess the wound and granulation areas as well as the wound bed. As with the primary endpoint, the wound evaluation committee members will assess the extent of wound closure.

Primary endpoint

The primary endpoint is the "time to wound closure."

Time to wound closure: The time (number of days) from the administration of PRP to wound closure, spontaneous wound closure or wound closure with a simple surgical procedure. The results of the evaluation will be confirmed by the wound evaluation committee members consisting of independent physicians separate from the physicians involved in the study to ensure the neutrality, objectivity and reproducibility of the evaluations.

The evaluation criteria for the possibility of wound closure during the investigation are as

follows:

(1) Judged to be closed by spontaneous healing

(2) Judged to be closed using a simple surgical procedure with an ulcer area less than 3 cm^2

(when two or more of the four conditions below are fulfilled)

- 75% or more of the wound area is covered by granulation tissue.
- Bones, tendons and underlying tissues are covered with granulation tissue.
- Local infection is controlled clinically (no adverse events due to infection).
- The wound depth is reduced by an average of 50% or more.

Secondary endpoints

- Percentage of wound reduction: Before and after the application of PRP, the wound is photographed once a week at dressing change, and the wound area is measured serially.
- Degree of improvement in the blood flow: The skin perfusion pressure (SPP) or ankle brachial index (ABI) is measured at dressing changes before and 1 and 4 weeks after the application of PRP.
- Correlations between the "time to wound closure" and the platelet count or growth factor levels in the PRP area.

Blinding

In this study, a data monitoring committee (DMC) was not organized, although the wound closure will be independently measured under blinding by three specialists. The patients will be unblinded, and unblinded investigators will apply PRP and the change dressings.

Sample size

This study is a prospective cohort study using historical data as controls, with a total number of patients for registration of 30, a feasible size for a pilot study.

Study schedule

The schedule of the study assessments and evaluations is shown in Table 1. The study period will be from the day of PRP application to six weeks after PRP application. Data for evaluating the efficacy and safety of this study will be collected at enrollment, baseline, Day 5 and Weeks 1, 2, 3, 4, 5 and 6 after application or the day of wound closure.

Statistical analysis

The statistical analysis will be performed on the following variables:

- Time to wound closure
- Percentage of wound reduction
- Improvement in the blood flow
- Correlations of the "time to wound closure" with the platelet count and/or growth factor levels in the PRP area
- Adverse events related to the application of PRP.

The efficacy will be evaluated according to the time from PRP application to wound closure. The survival curves in an existing control group based on historical data and the PRP-treated group until wound closure will be evaluated according to the Kaplan-Meier method, and a stratified analysis will be performed using the log-rank test. A stratified analysis of the number and percentage of patients who show wound closure will be performed using Fisher's exact test (95% confidence interval).

In addition, the optimal dose of PRP for wound healing will be estimated from the platelet count and growth factor concentrations in the PRP area.

The incidences of adverse events and adverse events that can be causally related to PRP application will be evaluated based on the event and severity. An interim analyses and auditing

will not be planned; however, the investigators will monitor adverse events and other unintended effects of the trial interventions.

ETHICS

This study is being conducted in compliance with the ICH-GCP and in agreement with the latest revision of the Declaration of Helsinki, Pharmaceutical Affairs Law and all applicable Japanese laws and regulations, as well as any local laws and regulations and all applicable guidelines. This protocol and any amendments have received Institutional Review Board approval from Kansai Medical University.

Subject consent

Informed consent will be obtained from all potential study participants using the IRB-approved informed consent form. The clinical investigator informs the potential study subject of all pertinent aspects of the study, and the subject must sufficiently understand the content of the information form before providing written consent. The consent form must be dated and signed by both the investigator and participant. The subjects are also informed that their medical care will not be affected if they do not choose to participate in this study. The consent forms will be retained at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital, and the information form and a copy of the consent form are handed to the participant. Whenever the investigator obtains information that may affect the participant's willingness to continue participation in the study, the investigator or sub-investigator will immediately inform the participant and record this observation and subsequently reconfirm the participant's willingness to continue participation in the study.

DISSEMINATION

The findings of this trial will be disseminated through peer-reviewed journals and national and

international scientific meetings as well as to the patients.

DISCUSSION

This study was designed to address the safety and efficacy of PRP treatment for chronic skin ulcers using a gelatin hydrogel sheet that can sustain the levels of growth factors in the PRP material. This study will be the first controlled trial to evaluate the efficacy of PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers. The efficacy of PRP for the treatment of chronic wounds in clinical trials is still controversial.[10-12] Our gelatin hydrogel sheet has been applied in clinical trials mainly as a drug delivery system for bFGF, and its efficacy and safety have been reported.[17, 18] bFGF can be used clinically in Japan; however, the application of bFGF is not approved in other countries.[7, 19,20] The clinical usage of growth factors in these countries is limited. PRP can be prepared from the patient's blood or donated platelets in all countries. Some bioengineered skin substitutes that provide growth factors secreted by living cells have been reported to be effective for chronic skin ulcers, although they are costly and access is also limited to only a few areas.[21-23] Our gelatin sheet is freeze-dried and can be stored at room temperature for months. Therefore, it is possible to use this combination therapy consisting of PRP and a gelatin sheet anywhere when needed, and its efficacy might be superior to that of bioengineered skin substitutes, as these treatments have a similar mechanism of action in secreting growth factors in a sustained manner. If successful, this intervention may lead to substantial and important changes in the management of increasingly prevalent chronic skin ulcers, such as diabetes ulcers and venous leg ulcers.

Trial status

Recruitment commenced in November 2014. Recruitment will continue until August 14, 2015.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

N.M., N.K. and K.K. initiated the study design. N.M. prepared the first draft of the manuscript. N.M., N.K., T.O., T.H., K.S. and K.K. will treat patients and conduct the clinical trial. N.M. will generate the allocation sequence, enroll participants and assign the participants to the interventions. M.K., M.Y. and Y.K. provided contributions from the engineering aspect for the gelatin sheets and prepared and supplied the sheets. N.M. is a grant holder. All authors contributed to refining the study protocol and approved the final manuscript.

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Table 1. Schedule of the study assessments and evaluations

Clinical assessments, testing, and investigations	At patient registration	Before PRP administration	Day 5 (±2 days)	Week 1 (±7 days)	Week 2 (±3 days)	Week 3 (±3 days)	Week 4 (±7 days)	Week 5 (±3 days)	Last day
Patient background	○								
Wound category	○								
Digital photography of wound		○	○	○	○	○	○	○	○
Blood flow (SPP or ABI)		○		○			○		
Blood tests (peripheral blood (including platelet count), CRP, HbA1c)	○			○					
PRP platelets and GFs		○							
Wound area		○	○	○	○	○	○	○	○
Concomitant medications		○	○	○	○	○	○	○	○
Blood tests		○	○#	○#	○#	○#	○#	○#	○#
Bacteriological tests			○&	○&	○&	○ &	○&	○ &	○&
Adverse events		←							→
Day of wound closure		←							→

○: required
#: Performed on the days of occurrence of adverse events.
&: Performed on the days on which infectious signs are suspected.
Last day: after six weeks or the day of wound closure

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_p1,_title__page
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_p3,_Trial Registration number : UMIN000015689
	2b	All items from the World Health Organization Trial Registration Data Set	In whole paper and the registry of UMIN000015689
Protocol version	3	Date and version identifier	_p3,_Ethics and dissemination
Funding	4	Sources and types of financial, material, and other support	p14,_finding statement_
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	p1_title page, and p14 Author's contribution_
	5b	Name and contact information for the trial sponsor	_p14 Funding statement

	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_p14 Funding statement
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_not applicable
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	_p6,Introduction and p6,Primry objective_
	6b	Explanation for choice of comparators	_p6,Design__
Objectives	7	Specific objectives or hypotheses	_p6,Primry objective___
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	__p6, Methods and design__
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P7, Setting and participants
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	__p7____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	__p8____

	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	p11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	not applicable
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	p9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	p10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	p11 and p19
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	p11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	p13, dissemination
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	p6 and p8
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	p6 and p8
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	p8 and p14

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	p11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	p11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	p10
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	p13
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	p12
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	p12
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	p8
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	p12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	p11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	p12

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	p12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	p12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	p12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	p13
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	p12
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	written in the IRB-approved informed consent form
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	p14
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	written in the IRB-approved informed consent form

Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_p9, written in the IRB-approved informed consent form _
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	____p13____
	31b	Authorship eligibility guidelines and any intended use of professional writers	_____p13_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	IRB-approved informed consent form _
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_not applicable_

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Exploratory clinical trial for combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: Study protocol

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Exploratory clinical trial of combination wound therapy with a gelatin sheet and platelet-rich plasma in patients with chronic skin ulcers: Study protocol

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Abstract

Introduction

Chronic skin ulcers, such as diabetic ulcers, venous leg ulcers and pressure ulcers are intractable and increasing in prevalence, representing a costly problem in health care. We developed a combination therapy with a gelatin sheet that is capable of providing the sustained release of PRP (platelet-rich plasma). The objective of this study is to investigate the safety and efficacy of autologous PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.

Methods and analysis

Thirty patients with chronic skin ulcers that have not healed with conventional therapy for at least one month are being recruited. The patients will receive PRP after debridement, and the wounds will be covered with a hydrocolloid dressing or gelatin sheet. The efficacy will be evaluated according to the time from the beginning of PRP application to secondary healing or the day on which wound closure is achieved with a relatively simple surgical procedure, such as skin grafting or suturing. All patients will be followed up until six weeks after application to observe adverse events related to the application of PRP and the dressings. This study was designed to address and compare the safety and efficacy of PRP covered with a hydrocolloid dressing versus a gelatin sheet. If successful, this combination therapy may be an alternative to bioengineered skin substitutes containing living cells and lead to substantial progress in the management of chronic skin ulcers.

Ethics and dissemination

The study protocol was approved by the Institutional Review Board of Kansai Medical University (KMUNo.0649-1, August 4, 2014: version1.0). The findings of this trial will be disseminated through peer-reviewed journals and national and international scientific meetings as well as to the patients.

Trial registration number: UMIN000015689

Strengths and limitations of this study

- The current protocol will provide evidence to compare the efficacy of PRP covered with a hydrocolloid dressing and that of PRP covered with a gelatin sheet in the treatment of chronic skin ulcers.
- This study is a historical control study.
- Only subgroups are randomized, and the small number of study subjects may affect generalizability.

INTRODUCTION

Chronic skin ulcers caused by diabetes mellitus, venous insufficiency, pressure sores, collagen disease, trauma or radiation are intractable and costly problems in health care.[1-3] In particular, diabetic foot ulcers and venous leg ulcers are frequent and costly complications of the underlying diseases. The prevalence of diabetic foot ulcers ranges from 4% to 10% among diabetes patients, and the lifetime incidence is reported to be as high as 25%. [2,3] It has been reported that venous leg ulcers recur in 72% of cases, and the recurrence rate after skin grafting is 48% within one year.[4] With the development of tissue engineering, bioengineered skin substitutes and genetically derived growth factors have progressed greatly in recent years with respect to the treatment of chronic skin ulcers.[5-7] However, new treatment options for chronic skin ulcers are needed.

Platelet-rich plasma (PRP) is blood plasma enriched with platelets. Autologous PRP is a source of autologous growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β) and vascular endothelial growth factor (VEGF). [8,9] PRP has been used clinically in a variety of treatments for bone defects, tendon injuries, cartilage injuries, cosmetic surgeries and chronic ulcers.[10] Regarding the efficacy of PRP in the treatment of chronic wounds, PRP has been described as improving the rate of healing compared with standard care in many reports.[11, 12] On the other hand, no major differences have been found in the healing outcomes of leg ulcers between PRP treatment groups and control groups in other studies.[11, 12]

A drug delivery system (DDS) is a type of technology for enhancing the biological activity of growth factors with a very short in vivo half-life after application. Biodegradable hydrogels of gelatin with an isoelectric point (IEP) of 5.0 have been designed and developed for the sustained release of positively charged growth factors, such as basic fibroblast growth factor (bFGF), PDGF-BB and TGF-β1.[13-15] The efficacy of a sustained release system for epidermal growth

factor (EGF) was also recently reported.[16] PDGF-BB and TGF-β are major growth factors involved in PRP for improving the wound healing process, and enhanced angiogenesis by gelatin microspheres impregnated with PRP has been reported.[13] Additionally, gelatin hydrogel sheets have been used to achieve the sustained release of growth factors and can be applied on wounds directly as a wound dressing.[17] The wound healing effect of PRP covered with this sheet is expected to be enhanced by the sustained release of growth factors from the gelatin sheet as a result of its biodegradation after application.

In this report, we designed an exploratory clinical study to investigate the safety and efficacy of PRP application covered with our gelatin sheet compared with PRP covered with a hydrocolloid dressing in the treatment of chronic skin ulcers. In addition, we estimated the contents of PDGF-BB, TGFβ1 and VEGF in PRP and compared these values and the degree of wound healing associated with the gelatin sheet.

MATERIALS AND ANALYSIS

Primary objective

The objective of this study is to evaluate the safety and efficacy of the administration of PRP in the treatment of chronic skin ulcers that are not expected to heal using conventional treatments. The safety and efficacy of PRP covered with a gelatin sheet versus a hydrocolloid dressing will also be evaluated.

Methods and design

Open-label, non-randomized, controlled clinical trial

Design

This study is a prospective cohort study using historical data as a control. A group treated with PRP application covered with a gelatin sheet and a group treated with PRP application covered

with a hydrocolloid dressing have been set. The patients will be randomized to the gelatin sheet subgroup or the hydrocolloid dressing subgroup, and a randomization-based comparison between these groups is expected to achieve significant improvements in accuracy with a lack of bias. This comparison will provide useful information for designing and conducting future trials.

Setting and participants

This study is being conducted at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital. Patients with chronic skin ulcers are being referred by physicians and identified through a number of wound care clinics in Osaka Prefecture and surrounding prefectures.

Inclusion criteria

1. Patients 20 years of age or older with informed consent
2. The presence of the following chronic skin ulcers:
    - Not healing for at least one month with conventional treatments
    - Not expected to respond to conventional treatments
    - Expected to be closed with autologous skin grafting or suturing after PRP treatment
    - An ulcer area between 3 and 30 cm^2 (that can be applied with autologous PRP)
3. Written informed consent

Exclusion criteria

1. Patients showing unstable hemodynamics due to ischemic heart disease or impaired blood coagulation as in clotting disorders
2. Patients taking anticoagulants or antithrombotics judged by the investigator or sub-investigator to be inappropriate as subjects since the drugs may affect the clotting system

3. Any of the following systemic diseases:
   - Uncontrolled diabetes mellitus (defined as a HbA1c level of ≥10%) according to the latest laboratory data obtained within 28 days before registration)
   - Requiring the continued use of oral corticosteroid therapy (>20 mg/day prednisolone equivalent)
   - A history of malignant tumors with a disease-free interval of three years or less
4. Patients who are or may be pregnant
5. Other patients judged by the investigator or sub-investigator to be inappropriate as subjects of this study

Analysis of subgroups

In this study, patients will be randomized to either the gelatin sheet subgroup or hydrocolloid dressing subgroup at a ratio of 1:1 without stratification. Randomization will be performed by assigning random numbers from random number tables to the treatment conditions.

Interventions

Preparation of PRP

PRP is prepared using the Magellan Autologous Platelet Separator System (Medtronic Inc., Minneapolis, MN) and its basic disposable kit. PRP is prepared by sampling blood using 60 ml syringes of the basic disposable kit filled with an anticoagulant and separating and concentrating platelets to 3-fold or more compared with that observed in the peripheral blood using the Magellan Autologous Platelet Separator System. The obtained PRP is activated as described in our previous study [8,9]. Briefly, a 1:1 (v/v) mixture of 0.5 M $CaCl_2$ (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) and autologous thrombin is prepared in advance as an activator. A 10:1 (v/v) mixture of PRP and the activator is incubated for five minutes at room temperature. Platelets of prepared PRP are counted, and the concentrations of

growth factors (TGF-β1, PDGF-BB and VEGF) in activated PRP are also quantified. If platelets cannot be separated and concentrated 3-fold or more compared with that in the peripheral blood, the study will be discontinued.

Preparation of the gelatin sheet

Acidic gelatin with an isoelectric point of 5.0 (Nitta Gelatin, Osaka, Japan) is to be used in this study. Gelatin hydrogel sheets of 12x 3 cm in size are prepared at the Department of Biomaterial, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University[16]. Briefly, 40 g of aqueous gelatin solution (5wt%) preheated at 40°C is mixed with 80 µl of aqueous glutaraldehyde (GA, Wako Pure Chemical Industries, Osaka, Japan) solution (25wt%). The resulting mixture is poured into polystyrene dishes and left at 4°C for 12 hours for chemical cross-linking of gelatin. Then, the formed hydrogel sheets are placed in 100 mM glycine aqueous solution, followed by agitation for one hour at room temperature to inactivate the residual unreacted aldehyde groups of GA. The gelatin hydrogel sheet is washed with double-distilled water, freeze-dried and sterilized with ethylene oxide gas. To remove the remaining ethylene oxide gas, the resulting hydrogel sheet is kept at 40°C for three days under vacuum conditions.

Application of activated PRP and dressing changes

After debridement of the chronic skin ulcers, activated PRP is applied to the lesions. Following the administration of PRP, the wound is dressed with a gelatin hydrogel sheet or conventional hydrocolloid dressing (DuoActive; Convatec Japan, Tokyo, Japan). The wound is occluded with the above dressing material for five days from the beginning of the administration of PRP and treated thereafter by applying ointment, such as Azunol ointment (Nippon Shinyaku Co. Ltd., Kyoto, Japan) or wound dressings, until six weeks after the administration of PRP.

Subsequent therapy

The use of basic fibroblast growth factor (bFGF) will be prohibited from three or more days prior to the administration of PRP (wash-out period of bFGF) and during the study period. Negative-pressure wound therapy (NPWT) must also not be performed during the study period. After completion of the study period (six weeks after PRP administration or the day on which wound closure is judged to be possible), no particular restrictions will be imposed. Concomitant therapies, such as a compression treatment, in cases of venous leg ulcers will be continued and applied before entry, because it is important to evaluate the efficacy of PRP under the same conditions.

Digital photography for the healing assessment

Using a digital camera, digital images of the wounds will be taken with a calibrator (CASMATCH; BEAR Medic Corp., Tokyo, Japan) placed on the skin adjacent to the wound. The color and size of the images can be adjusted using the CASMATCH and image editing software program (Adobe Photoshop; Adobe Systems Inc., CA, USA) to assess the wound and granulation areas as well as the wound bed. As with the primary endpoint, the wound evaluation committee members will assess the extent of wound closure.

Primary endpoint

The primary endpoint is the "time to wound closure."

Time to wound closure: The time (number of days) from the administration of PRP to wound closure, spontaneous wound closure or wound closure with a simple surgical procedure. The results of the evaluation will be confirmed by the wound evaluation committee members consisting of independent physicians separate from the physicians involved in the study to ensure the neutrality, objectivity and reproducibility of the evaluations.

The evaluation criteria for the possibility of wound closure during the investigation are as follows:

(1) Judged to be closed by spontaneous healing

(2) Judged to be closed using a simple surgical procedure with an ulcer area less than 3 cm^2 (when two or more of the four conditions below are fulfilled)

- 75% or more of the wound area is covered by granulation tissue.
- Bones, tendons and underlying tissues are covered with granulation tissue.
- Local infection is controlled clinically (no adverse events due to infection).
- The wound depth measured by using a depth gauge is reduced by an average of 50% or more.

Secondary endpoints

- Percentage of wound reduction: Before and after the application of PRP, the wound is photographed once a week at dressing change, and the wound area is measured serially.
- Degree of improvement in the blood flow: The skin perfusion pressure (SPP) or ankle brachial index (ABI) is measured at dressing changes before and 1 and 4 weeks after the application of PRP.
- Correlations between the "time to wound closure" and the platelet count or growth factor levels in the PRP area.

Blinding

In this study, a data monitoring committee (DMC) was not organized, although the wound closure will be independently measured under blinding by three specialists. The patients will be unblinded, and unblinded investigators will apply PRP and the change dressings.

Sample size

This study is a prospective cohort study using historical data as controls, with a total number of patients for registration of 30, a feasible size for a pilot study.

Study schedule

The schedule of the study assessments and evaluations is shown in Table 1. The study period will be from the day of PRP application to six weeks after PRP application. Data for evaluating the efficacy and safety of this study will be collected at enrollment, baseline, Day 5 and Weeks 1, 2, 3, 4, 5 and 6 after application or the day of wound closure.

Statistical analysis

The statistical analysis will be performed on the following variables:

- Time to wound closure
- Percentage of wound reduction
- Improvement in the blood flow
- Correlations of the "time to wound closure" with the platelet count and/or growth factor levels in the PRP area
- Adverse events related to the application of PRP.

The efficacy will be evaluated according to the time from PRP application to wound closure. The survival curves in an existing control group based on historical data and the PRP-treated group until wound closure will be evaluated according to the Kaplan-Meier method, and a stratified analysis will be performed using the log-rank test. A stratified analysis of the number and percentage of patients who show wound closure will be performed using Fisher's exact test (95% confidence interval).

In addition, the optimal dose of PRP for wound healing will be estimated from the platelet count and growth factor concentrations in the PRP area.

The incidences of adverse events and adverse events that can be causally related to PRP application will be evaluated based on the event and severity. An interim analyses and auditing will not be planned; however, the investigators will monitor adverse events and other unintended effects of the trial interventions.

ETHICS

This study is being conducted in compliance with the ICH-GCP and in agreement with the latest revision of the Declaration of Helsinki, Pharmaceutical Affairs Law and all applicable Japanese laws and regulations, as well as any local laws and regulations and all applicable guidelines. This protocol and any amendments have received Institutional Review Board approval from Kansai Medical University.

Subject consent

Informed consent will be obtained from all potential study participants using the IRB-approved informed consent form. The clinical investigator informs the potential study subject of all pertinent aspects of the study, and the subject must sufficiently understand the content of the information form before providing written consent. The consent form must be dated and signed by both the investigator and participant. The subjects are also informed that their medical care will not be affected if they do not choose to participate in this study. The consent forms will be retained at Kansai Medical University Hirakata Hospital and Kansai Medical University Takii Hospital, and the information form and a copy of the consent form are handed to the participant. Whenever the investigator obtains information that may affect the participant's willingness to continue participation in the study, the investigator or sub-investigator will immediately inform the participant and record this observation and subsequently reconfirm the participant's willingness to continue participation in the study.

DISSEMINATION

The findings of this trial will be disseminated through peer-reviewed journals and national and international scientific meetings as well as to the patients.

DISCUSSION

This study was designed to address the safety and efficacy of PRP treatment for chronic skin ulcers using a gelatin hydrogel sheet that can sustain the levels of growth factors in the PRP material. This study will be the first controlled trial to evaluate the efficacy of PRP covered with a hydrocolloid dressing and PRP covered with a gelatin sheet in the treatment of chronic skin ulcers. The efficacy of PRP for the treatment of chronic wounds in clinical trials is still controversial.[10-12] Our gelatin hydrogel sheet has been applied in clinical trials mainly as a drug delivery system for bFGF, and its efficacy and safety have been reported.[17, 18] bFGF can be used clinically in Japan; however, the application of bFGF is not approved in other countries.[7, 19,20] The clinical usage of growth factors in these countries is limited. PRP can be prepared from the patient's blood or donated platelets in all countries. Some bioengineered skin substitutes that provide growth factors secreted by living cells have been reported to be effective for chronic skin ulcers, although they are costly and access is also limited to only a few areas.[21-23] Our gelatin sheet is freeze-dried and can be stored at room temperature for months. Therefore, it is possible to use this combination therapy consisting of PRP and a gelatin sheet anywhere when needed, and its efficacy might be superior to that of bioengineered skin substitutes, as these treatments have a similar mechanism of action in secreting growth factors in a sustained manner. Regarding the limitation of this study, this study included ulcers of various causes that could be potential for bias by different concomitant therapies. Therefore, we sill plan the clinical study limited to one ulcer cause with standardized concomitant therapy in the next step. This is our first trial using the PRP/gelatin hydrogel sheet and we focused mainly on safety in this study and evaluate the efficacy in the next step limited to one ulcer cause.

If successful, this intervention may lead to substantial and important changes in the management of increasingly prevalent chronic skin ulcers, such as diabetes ulcers and venous leg ulcers.

Trial status

Recruitment commenced in November 2014. Recruitment will continue until August 14, 2015.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

N.M., N.K. and K.K. initiated the study design. N.M. prepared the first draft of the manuscript. N.M., N.K., T.O., T.H., K.S. and K.K. will treat patients and conduct the clinical trial. N.M. will generate the allocation sequence, enroll participants and assign the participants to the interventions. M.K., M.Y. and Y.K. provided contributions from the engineering aspect for the gelatin sheets and prepared and supplied the sheets. N.M. is a grant holder. All authors contributed to refining the study protocol and approved the final manuscript.

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Table 1. Schedule of the study assessments and evaluations

Clinical assessments, testing, and investigations	At patient registration	Before PRP administration	Day 5 (±2 days)	Week 1 (±7 days)	Week 2 (±3 days)	Week 3 (±3 days)	Week 4 (±7 days)	Week 5 (±3 days)	Last day
Patient background	○								
Wound category	○								
Digital photography of wound		○	○	○	○	○	○	○	○
Blood flow (SPP or ABI)		○		○			○		
Blood tests (peripheral blood (including platelet count), CRP, HbA1c)	○			○					
PRP platelets and GFs		○							
Wound area		○	○	○	○	○	○	○	○
Concomitant medications		○	○	○	○	○	○	○	○
Blood tests		○	○#	○#	○#	○#	○#	○#	○#
Bacteriological tests			○&	○&	○&	○ &	○&	○ &	○&
Adverse events		←	—	—	—	—	—	—	→
Day of wound closure		←	—	—	—	—	—	—	→

○: required
#: Performed on the days of occurrence of adverse events.
&: Performed on the days on which infectious signs are suspected.
Last day: after six weeks or the day of wound closure