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Case Report

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Treatment of diabetic neuropathy induced non healing foot ulcer using merisis[tm] supercell-platelet rich fibrin matrix: a clinical study

Sanjay Sharma[1], Manoj Bansode[2], Amol Gaikwad[2], Suchit Mali[2], Sarmistha Deb[2], Kaushik D Deb[2*]

[1]*APTA FootSecure, Yostra LabsBangalore, India.*

[2]*BioIntelligence Bangalore, India.*

[*]Corresponding author
Kaushik D Deb, BioIntelligence Bangalore, India.el.

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Abstract

Objective: *Non healing foot ulcers are a major debilitating condition in diabetic patients which leads to limb amputation.In this study we discuss the use ofMERISISTM Supercell plus Platelet Rich Fibrin Matrix (PRFM) kitfor point of care treatments towards limb salvation.*

Method: *We selected diabetic mellitus patients havingDiabetic foot ulcer (DFU) grade 1 or higher. Use of MERISISTM Supercell plus PRFM is a biological matrix of extracellular matrix (ECM) protein plus growth factors derived from peripheral blood to treat the DFU 45 days over.*

Result: *Following treatment the patients showed quick recovery and complete healing of the ulcers.*

Conclusion: *Supercellplus PRFM therapy promotes wound healing in patients suffering from chronic non-healing diabetes foot ulcers. Supercells plus PRFM dressing is a safe and effective treatment modality to promote wound contraction in patients suffering from non-healing diabetic foot ulcers.*

Keywords: Merisis[TM] Supercell plus PRFM, Growth Factor, PRP, Diabetic Foot Ulcer, ChronicNon healing Ulcer, Neuropathy.

Introduction

Diabetes has become the largest global health-care problem of the 21st century. Health surveys predict that the people with diabetes worldwide may double between 2000 and 2030. The International Diabetes Federation forecast that 425 million people globally have diabetes. 73 million in India and 30 million in the United States (US) have diabetes. 12% of global health expenditure, or $727 billion, is directed towards diabetes and its complications, and this number continues to increase at unpredictable rates [5]. There are two types of diabetes mellitus Type 1 and Type 2 diabetes, Type 1 diabetes mellitus was previously known as insulin dependent diabetes mellitus. It is a T-cell mediated autoimmune disease involving destruction of the insulin producing beta cells of islet of Langerhan's of pancreas and Type 2 diabetes mellitus was previously termed as non insulin dependent diabetes mellitus (NIIDM). It is highly complex than type 1 diabetes.

Diabetes causes varieties of acute, chronic, diffuse neuropathy syndromes. Diabetic foot is defined as 'Infection, ulceration or devastation of tissues of the foot associated with neuropathy in the lower extremity of a person with diabetes mellitus [6]. Foot ulcers are one of the major draw backs of diabetes, DFU can lead to infection, gangrene, amputation. On another hand, once DFU has developed, there is an increased risk of ulcer progression that may finally lead to amputation. This medical condition is observed in 15% of total diabetic patients [1].

Diabetic foot complications constitute a major health load amounting to the single largest reason for hospitalization among diabetic patients. It is also gradually recognized that latter stages of difficulty from foot ulcers are associated with serious morbidity and overall reduction in quality of life. Some people may be at a higher risk for diabetic foot ulcers than others if they have poor circulation, history of smoking, high blood sugar, history of foot deformities on immune-compromising medications (steroids) or improper shoes etc.

Diabetic neuropathy is form of nerve damage that is caused by diabetes. Higher blood glucose levels, also called diabetes, and higher levels of fats, such as triglycerides, in the blood from diabetes can damage nerves. Its symptoms depend on the type of diabetic neuropathy. Peripheral neuropathy is one of a type of nerve damage that affects the feet and legs. About 1/3 to 1/2 of people with diabetes have peripheral neuropathy. Symptoms of Peripheral neuropathy include atypical pain, altered sensation, numbness, and hot or burning sensations of the feet and legs of

affected area. Proximal neuropathy is another disabling type of nerve damage of hip, buttock, or thigh. The damage affects one side of body and may rarely spread to the other side. Symptoms slowly improve over duration of months or years. Risk factors of diabetic peripheral neuropathy include duration of disease, poor glycemic control, hypertension, hyperlipidemia, and smoking. Diabetic neuropathy causes disorder due to foot ulceration and amputation, gait disturbance, and fall-related injury.

The aim of DFU treatment is to get a healthy wound closure as expeditiously as possible. Apart from conventional methods to facilitate wound healing various new methods are emerging such as cellular therapies, stem cells and platelet-rich plasma (PRP) therapies [6,7]. Platelets release growth factors from alpha granules which are located in thrombocyte cell membrane include platelet derived growth factor (PDGF), epidermal growth factor (EGF), Fibroblast Growth Factor (FGF), platelet derived angiogenesis factor etc. These factors act locally on wound and accelerate the healing process. Platelet has been used in various studies and has shown very good results in healing of chronic non healing diabetic foot ulcers [1]. The role of growth factors in wound healing is growth factors are stored in the form of α-granules in platelets and when these platelets are activated; they in turn release a multiplicity of growth factors. Later than the formation of platelet coagulum, the activated platelets are scatter among the fibrin strands forming a matrix within the clot, which helps to keep the growth factors within the mesh. They are diffuse out into the surrounding tissue in the end. Growth factors act indigenously to enlist undifferentiated cells to the injury site by chemo attraction and also stimulate mitosis in the undifferentiated cells. Stem cells are pulled to areas of higher concentration of growth factors and cellular movement occurs by forming attachments to the matrix. Growth factors stick to receptors on the stem cell membrane, thereby activating genes controlling cell division. They also attach to cell receptors and control the genetic expression of stem cells via the modulation of signal transduction pathways of secondary proteins, resulting in cellular division and differentiation [10]. Atpresent DFUs were treated with some therapies like hyperbaric oxygen therapy (HBOT), negative pressure wound therapy (NPWT). These biological treatments are improved the time of wound closure. PRP and stem cells, like MSCs could quickly heal the wounds compared to conventional therapies [1].

About MERISISTM Supercells plus PRFM: Supercells are playing a vital role in repair, regeneration and reconstruction of damaged tissue. Platelet rich Fibrin Matrix (PRFM) is a biological matrix of ECM protein plus growth factors derived from peripheral blood. MerisisTM Platelet rich fibrin matrix (PRFM) is an upcoming generation biological product, made up of high concentrated platelets, growth factors and natural fibrin speeding up the healing process. It merges cutting-edge technology with the body's natural ability to heal itself. PRFM is a fibrin matrix in which platelets; cytokines, growth factors and stem cells are trapped and released after a certain time that can serve as a resorbable membrane or gel whichhelp in healing. PRFM copy the natural healing environment in a tissue and boosts tissues repair and regeneration of cells and healing. When PRFM is applied into the damaged area, it activates a mild immune response, which triggers the haling cascade.

In this study we used the MERISISTMSuper cell plus PRFM kit, which is a point of care enclosed sterile system suitable for use in the treatment of DFUs. We hypothesis that Stem cells growth factors plus platelet growth sustained release from the PRFM biological matrix over an period of time will enhance wound healing process in difficult to cure non healing ulcers.

Material and Method

We selected diabetic mellitus patients with type 2 diabetes having DFUs with grade 1 or higher. These DFUs had nothealed for at least 4 weeks after conventional treatments. Four patients were enrolled in this study without a control arm. The grade of wound is recorded according to the standard scoring systems. Results were compared with patients who were anconventional treatments for DFUs.

Patient Preparation

All patients were first physically examined, Doppler ultrasound scan of the body part with DFUs, X-ray of the infected area were recorded. These, patients were classified to wound grades according to classification. The sizes of the DFUs were recorded before commencing treatment.

Preparation of Supercell plus PRFM

According to protocol of PRFM Kit, we collected the 18 ml peripheral blood from patients by using 20 ml syringe. We followed the protocol given on the PRFM kit. 9 ml of blood added into Supercell tube and remaining 09 ml of blood added into PRFM tube. Then centrifuged the both tube at 3400 rpm for 7min to collected the supercell (2-3 ml part from above Gel) from Supercell tube into 1ml insulin syringe and injected the around the wound and for PRFM tube wait 5-10 min after centrifugation to formation of Fibrin gel clot, then this clot is used for dressing of DFU wound.

Patient Preparation and treatment Procedure

Blood collection and PRFM preparation were carried out immediately before application. Before application of PRFM and supercells, we removed senescent or abnormal cells from wound. The freshly prepared approximately 1-2 cm long PRFM was fenestrated using sterile forceps and scissors to introduce uniformly applied on a healthy wound followed by application of a non-absorbable dressing. Adequate rest ensured during the treatment course, to allow drainage of wound exudate, increase moisture and air exchange, and improve intimate application to the wound bed. Supercell and given activator (Biotin and peptide 0.5 ml) mixed and injected to around the ulcer. The secondary dressing of the patient and the dried PRFM was removed from the wound bed after a minimum of 5 days. The procedure was repeated every week. After 1 week, there was depletion in area and the volume of the ulcer. After six sittings of supercell plus PRFM, the ulcer healed completely in 7 weeks [12,13].

After sitting, photographs of the wound taken and healing assessed by measurements of ulcer and volume of ulcer [9]. Wound area calculated by using ellipse formula because ellipse is closer to a wound than a square or rectangle. The formula for area of ellipse is (length x width) and for volume, it is (area x depth) [8].

Inclusive Criteria

The inclusion of patients in this study were based on following

criteria,

1. Wagner 1, 2 and 3, HbA1c of<10, and other co-morbidities being managed well by medication, nutrition, or lifestyle.
2. ABI>0.71
3. Patients with DFU which have failed to respond to conventional therapies for at least 4 weeks.
4. Patients above the age 18.

Exclusion Criteria

1. Patients with unmanaged co-morbidities, leading to non-heapability the wounds, with ABI<0.70.
2. Exclusion criteria were patients not willing to give written informed consent for participation in the study; active infection DFU; uncontrolled diabetes; history of bleeding disorder or thrombocytopenia; Patients on anti-coagulants or anti platelet agents.
3. Pregnant and lactating females.

Diagnostic Tests for Neuropathic DFUs

1. Pressure assessment with the nylon filament Semmes-Weinstein monofilament test
2. Vibration testing with a 128-Hz tuning fork
3. Testing for pinprick sensation
4. Ankle reflex assessment
5. Vibration perception threshold testing
6. Ankle-Brachial Index(ABI)/Toe-brachial index (TBI)

Result

In this study MERISISTM Supercells plus PRFM treatment given to four patients, with age between 30 to 65 years. Figure 1. (a) and Figure 2 (a) shows non healing DFU wound for two patients. Figure 1 (b) shows complete closure of the wound in duration of 8 weeks. Figure 2. (b) shows 25% of wound closure and Fig. 2 (c) shows 75% of wound closure in 6 weeks. Figure 2 (d) shows complete wound closure within 8 weeks.

Table 1: After Two and Four Weeks of treatment.

			After 2 week		After 4 week	
Sr. No.	Patients	M/F	Grade	Percentage of wound area closure	Grade	Percentage of wound area closure
1	P1	M	2	25%	2	55%
2	P2	M	3	30%	3	45%
3	P3	M	3	40%	3	65%
4	P4	M	2	25%	2	60%

Table 2: After Six and Eight weeks of Treatment.

			After 6 week		After 8 week	
Sr. No.	Patients	M/F	Grade	Percentage of wound area closure	Grade	Percentage of wound area closure
1	P1	M	1	90%	0	100%
2	P2	M	2	82%	0	100%
3	P3	M	2	85%	0	100%
4	P4	M	1	75%	0	100%

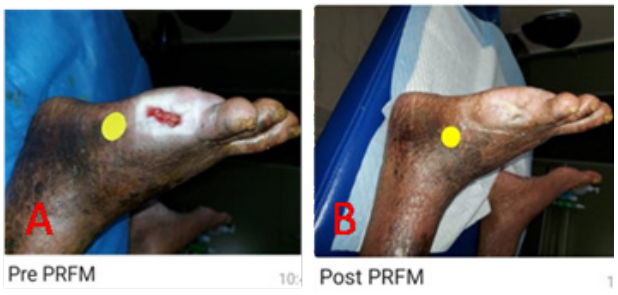

Figure 1: Patient P1; A) DFU before Treatment and B) DFU after Treatment with PRFM.

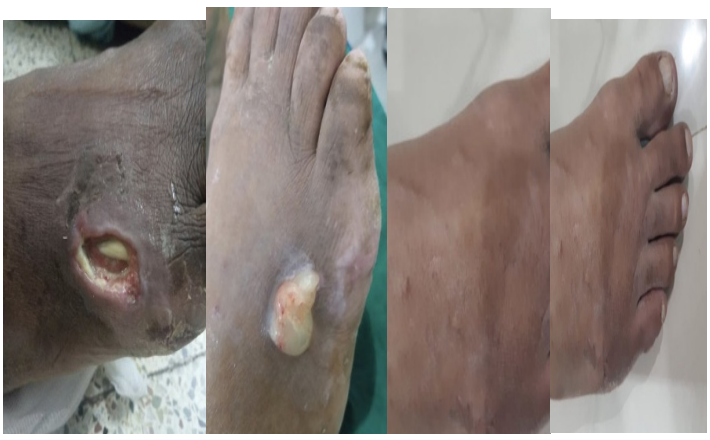

Figure 2: DFUs Patient P4 Picture; A) before treatment, B) PRFM applied on wound, C) Post treatment of Supercells plus PRFM, D) Complete closer of wound.

The results observed after MERISISTM Supercells plus PRFM treatment, given to the four patientsareshown in Tables 1 and 2.

We observed the following progress in wound closure-Patient P1, after MERISISTM Supercells plus PRFM treatment 25% wound closure was observed after 2 weeks, 55% in 4 weeks, after 6 week 90% of wound closure observed.Within 8 weeks wound was completely closed and healed.

In, Patient P2, wound had a grade 3 scorebefore treatment.After MERISISTM Supercells plus PRFM treatmentwithin 2 weeks wound closure started and 30% closed and in subsequent 2 weeks, it closed 45%. After 6 weeks 82%, wound closure was observed andon 7th weekwound was found to be completely closed.

Similarly, Patient P3 with wound grade 3 score,after MERISISTM Supercells plus PRFM treatmentwithin 2 week 40 percent wound closure was observed.After 4 weeks more than half of wound closure was seen i.e. 65%. On 6thweek, 85% wound was closed andwithin 8th week wound was completely closed.

In Patient P4 with grade 2 score wound,after MERISISTM Supercells plus PRFM treatment 25% of wound closure was observed after 2 weeks, 60% wound closure was seen after 4 weeks. After 6th week 75%, wound closure was observed and within 8 weeks wound was completely closed.

Discussion

PRFM is considered as a rich source ofautologous growth factors. PRFM promotes a localized inflammatory response. PRFM enhances wound healing because it contains growth factors that stimulate tissue regeneration and wound closure. The growth factors like PDGF areused alone for enhancing the wound healing, but the application of mixed growth factors gives enhanced results [15]. Aymen Salem and his coworkersconducted study and confirmed that, the use of PRP and PPP increase ulcers healing rate [2]. These results provided a promising method for ulcers treatment. MERISISTMsupercell plus PRFMis enriched with 3X concentrated platelets and also enriched autologous stem cells concentrate. The peripheral blood is a rich source of MNCs, VSELs and other rare stem cell types which are quiescent till activated for repair and regeneration function following an injury or inflammation. When activated with calcium, fibrin will form, and platelets released growth factors gel fibrin formed a barrier to prevent the bacteria contamination into the wound bed [1]. Secondly, the growth factors from platelets stimulate wound healing. This treatment is comparatively less invasive and more effective than antibiotics etc. Diabetic foot ulcers (DFUs) are serious microvascular diabetes-related lesions that are the consequence of several predisposed factors, such as peripheral arterial disease, bone abnormalities, diabetic neuropathy, or infections that, without appropriate management, can lead to limb amputations [7]. PRFM gives better result than PRP because; it acts like drug delivery system. The mean concentration of growth factors in the PRFM concentrates was three times or more than that observed with conventional platelet-rich plasma [14]. In previous study reported by Anirban and coworker (2018) MERISISTM PRFM was shown to have sustained release of tissue regenerative growth factors like PGDF, EGF, FGF2 etc. over 15 days. Similarly, other studies showed that, Growth factors from fibrin matrix released in slowly controlled manner over 1-week time and it was observed that it resulted in better healing of ulcer than PRP [3]. Here we show that MERISISTM supercell plus PRFM treatment for chronic non-healing ulcers is safe, simple, effective and inexpensive therapy without any complication and side effect.

In this study, we treated four non-healing ulcer patients successfully. Total healing was obtained within 8 weeks. Non-healing ulcers often lead to limb amputations. Our treatment protocol could be effectively used for limb salvation. More number of patients are being studied in our clinics.

Conclusion

From our study, we can conclude that our Supercells plus PRFM dressing protocol and therapy promotes wound healing in patients suffering from chronic non-healing diabetes foot ulcers. Supercells plus PRFM dressing is an effective modality to promote wound contraction in patients suffering from diabetic foot ulcers and can be used as an adjunct to conventional mode of treatment.

Competing Interests

The authors declare that they have no competing interests.

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