Biomedical Research and Therapy **2014, 1(2):37-42**
ISSN 2198-4093
www.bmrat.org

DOI 10.7603/s40730-014-0008-3

ORIGINAL RESEARCH

Diabetic foot ulcer treatment by activated platelet rich plasma: a clinical study

Tung Dang-Xuan Tran[1], Phuong Thi-Bich Le[1], Phuc Van Pham[2]

[1]Stem Cell Unit, Van Hanh Hospital, Ho Chi Minh city, Vietnam
[2]Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, Ho Chi Minh city, Vietnam;
*Corresponding author: pvphuc@hcmuns.edu.vn

Received: 25 January 2013 / **Accepted**: 22 February 2014 / **Published online**: 22 March 2014

Abstract— Diabetic foot ulcer is a major complication of diabetes mellitus. It occurred in about 15% of all diabetic patients. To date, the outcome of management of diabetic foot ulcer is poor and low sufficient. Some new therapies were suggested to manage and treat this disease. In almost therapies, management of diabetic foot ulcer relates to debridement of the wound, revascularization, off-loading of the ulcer, antibacterial actions, stimulating granulation, epidermization and angiogenesis. This study aimed to evaluate the effects of activated platelet rich plasma (aPRP) on diabetic foot ulcer healing on volunteer patients. There were 6 patients enrolled in this study. All patients have non-healing foot ulcers. aPRP was isolated from peripheral blood and activated with calcium chloride. Patients were injected with aPRP two times with 14-day interval. All patients were monitored during 12 weeks. The results showed that 100% (6/6) ulcers completely closed after about 7 weeks. This result initially suggests that aPRP injection is efficient method to treat the non-healing foot ulcers. Level of evidence: IV.

Keywords— Platelet rich plasma; Growth factors; Diabetic Ulcer; Wound healing; Chronic Ulcer.

INTRODUCTION

Diabetic foot ulcer is a major complication of diabetes mellitus, and is the major component of the diabetic foot. This medical condition affects to 15% of all patients with diabetes mellitus. Recent study showed that up to 88% of all lower leg amputation related to diabetic foot ulcer (Alvarsson et al., 2012). More importantly the cases of diabetic mellitus patients rapidly increase to 439 million by 2030.

Diabetic neuropathy and peripheral vascular disease are 2 major factors causing diabetic foot ulcers (DFUs) (Wu et al., 2007). The most difficulty of DFUs is non-healing for a long time. The main reason is related to loss of balance between metalloproteinases (MMPs) and MMP inhibitors (Muller et al., 2008). This status is enhanced to become serious when combining with ischemia and vascular disease. The vascular injury and ischemia reduced the oxygen and nutrients to wound. So the wound healing mechanism cannot perform as in the non-diabetic patients. Lack of oxygen and nutrients, epithelial cells at wound can not express essential factors for healing such as VEGF and PDGF; almost of cells at wound changed metabolism and activity (Lobo et al., 2013). These changes in structure and functions of cells and some factors at DFUs delayed the healing process and broke the normal healing process. Hence, when diabetic mellitus patients got DFUs, almost DFUs cannot be healed, and finally patients must be faced with lower leg amputation. In fact, DFU treatment also remains as a challenge.

At the present, DFUs were treated with some physical therapies such as vacuum-assisted closure (Lone et al., 2014; Nather et al., 2010; Ravari et al., 2013), high-voltage pulsed current electrical stimulation (Houghton et al., 2003; Polak et

al., 2014), hyperbaric oxygen therapy (HBOT) (Kranke et al., 2012; Stoekenbroek et al., 2014), negative pressure wound therapy (NPWT) (Zhang et al., 2014)… Some biological therapies also used in DFU treatment. Some growth factors such as EGF (Singla et al., 2012; Tsang et al., 2003; Tuyet et al., 2009), GCSF (Cruciani et al., 2013), NGF (Tiaka et al., 2011), VEGF (Rico et al., 2009)… were evaluated in DFUs. Although these biological therapies improved the time of wound closure, these therapies had not significantly recovered wound healing. In the recent studies, scientists used a pool of growth factors from PRP (Martinez-Zapata et al., 2012; Saad Setta et al., 2011; Villela and Santos, 2010), others used stem cells alone (Blumberg et al., 2012; Kirana et al., 2012; Yang et al., 2013) or in combined with PRP (Cervelli et al., 2009). PRP and stem cells, especially mesenchymal stem cells, could quickly heal the wounds compared to conventional therapies.

This study aimed to apply of autologous PRP in diabetic mellitus patient type 2 DFU treatment. Different to previous publication about PRP usage, in this study, diabetic mellitus patients would be injected with both PRP and PPP to improve the efficacy.

MATERIALS – METHODS

Diabetic mellitus patients

Diabetic mellitus patients type 2 have DFUs with grade I or more. These DFUs cannot heal at least 4 weeks. There were 6 patients enrolled in this study without control. All patients agreed the consent form before enrolling the study. This study approved by Ethical and Medical Committee of Van Hanh Hospital, HCM, VN. The grade of wound is recorded according to the University of Texas (grade and stage) wound classification systems (Oyibo et al., 2001).

Physical examination and treatment

All patients would be done physical examination, Doppler ultrasound scan of the leg with DFUs, X-ray of the foot. Then, patients were classified to grades according to Classification of Texas University. The size of DFUs also recorded before treatment. Only patients satisfied with criteria would be collected 20 mL of peripheral blood to make PRP.

PRP preparation

20 mL of peripheral blood was used to prepare PRP and PPP according to the guideline of New-PRP Pro Kit (Geneworld Ltd., HCM, VN). Briefly, blood was centrifuged at 1.500 rpm in 5 min to obtain plasma. Then, this plasma was centrifuged at 3.500 rpm in 5 min to collect platelets as a pellet at the bottom of the centrifuge tube. Pellet was diluted in 3 mL plasma and was considered as PRP, and remaining plasma was considered as PPP. Both PRP and PPP were activated to release growth factor by Calcium chloride. When calcium chloride was added into PRP, fibrin gel was formed, and this gel was used to dress on the wound, and activated PPP was stored in -20^{0}C for using in the next days.

PRP and PPP application on DFUs

DFUs were dressed by fibrin gel in the first day. After 3 days, from day 4-8, DFUs were injected with activated PPP at the wound bed. During the treatment, diabetic mellitus patients still used antibiotics, NSAID, and vitamin. If after 2 weeks, the wound had not signs of healing, patients continued to be repeated this procedure. DFUs were examined in 12 weeks 2 weeks/time.

RESULTS

The medical conditions of patients enrolled in this study

There were 6 patients with 3 males and 3 females enrolling in this study with average ages 63 with more than 5 years of diabetes mellitus. All patients must use drugs to reduce the glucose, included insulin injection, and get hypertension (4/6) and hypertension combined kidney failure (2/6). By ultrasound and X-ray, all patients received lower leg ischemia from 70-90% (artery). All patients got DFUs more than 5 weeks duration from II-III grade without granulation tissue at treatment of enrolling. The grade of wound is recorded according to the University of Texas (grade and stage) wound classification systems (Oyibo et al., 2001).

Table 1. Medical conditions of patients

Patients	Ages (Years)	Sex	Time of diabetes mellitus (Years)	Ischemia at leg with DFUs	Other diseases
P1	65	M	5	70%	Hypertension
P2	88	M	10	80%	Hypertension, kidney failure
P3	54	F	6	90%	Hypertension
P4	55	M	3	80%	Hypertension, kidney failure
P5	63	F	5	90%	Hypertension
P6	56	M	5	70%	Hypertension

M: male; F: female

Treatment efficacy

Results showed that 100% (6/6) patients completely closed

the DFUs, included 2 cases with whole toes after 12 weeks. The average time for completed healing was 7.1 weeks. The soonest time was 4 weeks. All patients developed granulation tissue after 2 weeks. And wound areas significantly decreased at week 2 after one time of PRP and PPP treatment. This is the safe method to treat DFUs. During 12 weeks, there was not any adverse effect recorded in all patients (**Fig. 1**).

Table 2. DFUs in patients before treatment

Patients	Texas grade*	Size (mm)	Time of DFUs (wks)
P1	IIID	Whole toe	5
P2	IIIC	Foot (50x60)	6
P3	IID	Foot (40x60)	5
P4	IIC	Whole toe	8
P5	IIID	Heel of foot (80x80)	7
P6	IID	Heel of foot (50x60)	6

**The University of Texas wound classification systems*

MMP and MMP inhibitors.

PRP contains at least seven growth factors including epidermal growth factor, platelet-derived growth factor, transforming growth factor-beta, vascular endothelial growth factor (VEGF), fibroblast growth factor, insulin-like growth factor, and keratinocyte growth factor (Pham et al., 2014; Van Pham et al., 2013). Many of these growth factors have important roles in wound healing. PRP stimulates the expression of type I collagen in dermal fibroblasts (Kim et al., 2011), and increases the expression of G_1 cycle regulators, type I collagen to accelerate wound healing (Cho et al., 2012). In fact, after 2 weeks treated with PRP and PPP injection, 100% DFUs developed granulation tissue. And at this time, all wounds significantly closed.

PRP was successfully applied in non-healing DFUs in previous publications. McAleer et al. reported successful use of autologous PRP in chronic lower extremity wound in a 57-year-old man with type 2 diabetes mellitus (McAleer et al., 2006). In the retrospective cohort study of 26.599 patients with DFUs, it showed that 50% of patients healed their wound if treated with PRP (Margolis et al., 2001).

Table 3. DFU healing after 2, 4, 8 and 12 weeks

	After 2 wks		After 4 wks		After 8 wks		After 12 wks	
Patients	Texas grade	Percentage of wound area closure (%)	Texas grade	Percentage of wound area closure (%)	Texas grade	Percentage of wound area closure (%)	Texas grade	Percentage of wound area closure (%)
P1	IIC	30%	IA	75%	0A	90%	0A	100%
P2	IIC	40%	IA	90%	0A	100%	0A	100%
P3	IC	50%	IA	100%	0A	100%	0A	100%
P4	IA	40%	IA	80%	0A	100%	0A	100%
P5	IIC	30%	IIA	50%	IIA	80%	IA	100%
P6	IIC	30%	IIA	60%	0A	100%	0A	100%

**The University of Texas wound classification systems*

DISCUSSION

DFUs are results of healing process failure in diabetic mellitus patients. The lack as well as malfunction of some growth factors broke the natural healing process. PRP and PPP provided almost of growth factor for healing. In fact, all patients went to completely heal the DFUs. PRP is plasma enriched with platelets. When activated with calcium, fibrin will be formed, and platelets released growth factors. In this case, PRP exhibited two important roles for wound healing. Firstly, gel fibrin formed a barrier to prevent the bacteria contamination into the wound bed. Secondly, the growth factors from platelets triggered wound healing and balanced the

Different with our procedure, almost previous published procedure, only PRP was applied on the wound, we used both activated PRP and PPP in wound treatment. Although this study was limited with 6 patients, we also recognized that injection of activated PPP after 3 days of PRP fibrin dressing gave useful benefits to wound healing process. PPP contains very low number of platelets, but it contains a balanced pool of nutrients for cells. DFUs always combined with ischemia that significantly decreases blood flow to DFUs. All epithelial cells and support cells were starved. While PRP provides growth factors for neo-angiogenesis and cell migrations to heal the wound, PPP injection every day will provide nutrients for cells at DFUs. In fact, Saad et

al. compared efficacy of PRP and PPP on wound healing in DFUs and showed that healing in PRP group was significantly faster (P<0.005) than PPP. However, PPP also well stimulated the wound healing in DFUs (Saad Setta et al., 2011).

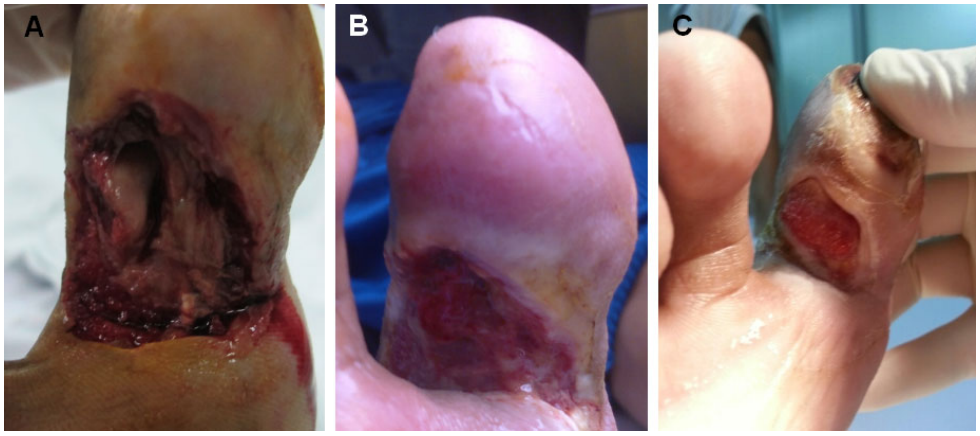

Figure 1. DFU at toe of patient P1. DFU before treating with PRP (A), after treating with PRP 2 wks (B), and 8 wks (C).

Treatment with PRP also showed that was more efficient than others. In a recent study, Lone et al. (2014) used vacuum-assisted closure method to treat DFUs. They showed that 92.85% patients developing granulation tissue by the end of 2 weeks; and 77.78% patients reached 100% granulation at the end of week 5 (Lone et al., 2014).

Conclusion

DFUs seriously effected to life quality of diabetic mellitus patients. Although there are some breakthroughs in the diabetic mellitus foot care to reduce the DFUs, diabetic mellitus patients faced with amputation. In this study, in the limitation of patients, the results showed that application of PRP and PPP efficiently to heal DFUs. The results showed that there was not any adverse effect recorded in all patients during 12 weeks. These results provided a promising method for DFU treatment.

Acknowledgment

This work was funded by grants from GeneWorld Ltd. SHTP, HCM, VN.

Abbreviations

bFGF: basic fibroblast growth factor; DFU: Diabetic foot ulcer; EGF: epidermal growth factor; MMPs: Metalloproteinases; MSCs: Mesenchymal stem cells; PPP: Platelet rich plasma; PRP: Platelet rich plasma; SVF: Stromal vascular fraction; VEGF: Vascular endothelial growth factor

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors read and approved the final manuscript. TDXT, PTBL collected peripheral blood, and applied PRP and PPP on patients. PVP prepared PRP, and PPP using New-PRP Pro kit, prepared the manuscript in cooperation with all other authors.

Open Access

References

Alvarsson, A., Sandgren, B., Wendel, C., Alvarsson, M., and Brismar, K. (2012). A retrospective analysis of amputation rates in diabetic patients: can lower extremity amputations be further prevented? Cardiovascular diabetology *11*, 18.

Blumberg, S.N., Berger, A., Hwang, L., Pastar, I., Warren, S.M., and Chen, W. (2012). The role of stem cells in the treatment of diabetic foot ulcers. Diabetes research and clinical practice *96*, 1-9.

Cervelli, V., Gentile, P., Scioli, M.G., Grimaldi, M., Casciani, C.U., Spagnoli, L.G., and Orlandi, A. (2009). Application of platelet-rich plasma in plastic surgery: clinical and in vitro evaluation. Tissue engineering Part C, Methods *15*, 625-634.

Cho, J.W., Kim, S.A., and Lee, K.S. (2012). Platelet-rich plasma induces increased expression of G1 cell cycle regulators, type I collagen, and matrix metalloproteinase-1 in human skin fibroblasts. International journal of molecular medicine *29*, 32-36.

Cruciani, M., Lipsky, B.A., Mengoli, C., and de Lalla, F. (2013). Granulocyte-colony stimulating factors as adjunctive therapy for diabetic foot infections. The Cochrane database of systematic reviews *8*, CD006810.

Houghton, P.E., Kincaid, C.B., Lovell, M., Campbell, K.E., Keast, D.H., Woodbury, M.G., and Harris, K.A. (2003). Effect of electrical stimulation on chronic leg ulcer size and appearance. Physical therapy *83*, 17-28.

Kim, D.H., Je, Y.J., Kim, C.D., Lee, Y.H., Seo, Y.J., Lee, J.H., and Lee, Y. (2011). Can Platelet-rich Plasma Be Used for Skin Rejuvenation? Evaluation of Effects of Platelet-rich Plasma on Human Dermal Fibroblast. Annals of dermatology *23*, 424-431.

Kirana, S., Stratmann, B., Prante, C., Prohaska, W., Koerperich, H., Lammers, D., Gastens, M.H., Quast, T., Negrean, M., Stirban, O.A., *et al.* (2012). Autologous stem cell therapy in the treatment of limb ischaemia induced chronic tissue ulcers of diabetic foot patients. International journal of clinical practice *66*, 384-393.

Kranke, P., Bennett, M.H., Martyn-St James, M., Schnabel, A., and Debus, S.E. (2012). Hyperbaric oxygen therapy for chronic wounds. The Cochrane database of systematic reviews *4*, CD004123.

Lobo, R., Kiernan, T.J., and Jaff, M.R. (2013). Medical therapy for critical limb ischemia and the diabetic foot: an update. The Journal of cardiovascular surgery *54*, 671-678.

Lone, A.M., Zaroo, M.I., Laway, B.A., Pala, N.A., Bashir, S.A., and Rasool, A. (2014). Vacuum-assisted closure versus conventional dressings in the management of diabetic foot ulcers: a prospective case-control study. Diabetic foot & ankle *5*.

Margolis, D.J., Kantor, J., Santanna, J., Strom, B.L., and Berlin, J.A. (2001). Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes care *24*, 483-488.

Martinez-Zapata, M.J., Marti-Carvajal, A.J., Sola, I., Exposito, J.A., Bolibar, I., Rodriguez, L., and Garcia, J. (2012). Autologous platelet-rich plasma for treating chronic wounds. The Cochrane database of systematic reviews *10*, CD006899.

McAleer, J.P., Sharma, S., Kaplan, E.M., and Persich, G. (2006). Use of autologous platelet concentrate in a nonhealing lower extremity wound. Advances in skin & wound care *19*, 354-363.

Muller, M., Trocme, C., Lardy, B., Morel, F., Halimi, S., and Benhamou, P.Y. (2008). Matrix metalloproteinases and diabetic foot ulcers: the ratio of MMP-1 to TIMP-1 is a predictor of wound healing. Diabetic medicine : a journal of the British Diabetic Association *25*, 419-426.

Nather, A., Chionh, S.B., Han, A.Y., Chan, P.P., and Nambiar, A. (2010). Effectiveness of vacuum-assisted closure (VAC) therapy in the healing of chronic diabetic foot ulcers. Annals of the Academy of Medicine, Singapore *39*, 353-358.

Oyibo, S.O., Jude, E.B., Tarawneh, I., Nguyen, H.C., Harkless, L.B., and Boulton, A.J. (2001). A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. Diabetes care *24*, 84-88.

Pham, P.V., Vu, N.B., Pham, V.M., Truong, N.H., Pham, T.L., Dang, L.T., Nguyen, T.T., Bui, A.N., and Phan, N.K. (2014). Good manufacturing practice-compliant isolation and culture of human umbilical cord blood-derived mesenchymal stem cells. Journal of translational medicine *12*, 56.

Polak, A., Franek, A., and Taradaj, J. (2014). High-Voltage Pulsed Current Electrical Stimulation in Wound Treatment. Advances in wound care *3*, 104-117.

Ravari, H., Modaghegh, M.H., Kazemzadeh, G.H., Johari, H.G., Vatanchi, A.M., Sangaki, A., and Shahrodi, M.V. (2013). Comparision of vacuum-asisted closure and moist wound dressing in the treatment of diabetic foot ulcers. Journal of cutaneous and aesthetic surgery *6*, 17-20.

Rico, T., Green, J., and Kirsner, R.S. (2009). Vascular endothelial growth factor delivery via gene therapy for diabetic wounds: first steps. The Journal of investigative dermatology *129*, 2084.

Saad Setta, H., Elshahat, A., Elsherbiny, K., Massoud, K., and Safe, I. (2011). Platelet-rich plasma versus platelet-poor plasma in the management of chronic diabetic foot ulcers: a comparative study. International wound journal *8*, 307-312.

Singla, S., Singla, S., Kumar, A., and Singla, M. (2012). Role of epidermal growth factor in healing of diabetic foot ulcers. The Indian journal of surgery *74*, 451-455.

Stoekenbroek, R.M., Santema, T.B., Legemate, D.A., Ubbink, D.T., van den Brink, A., and Koelemay, M.J. (2014). Hyperbaric Oxygen for the Treatment of Diabetic Foot Ulcers: A Systematic Review. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery.

Tiaka, E.K., Papanas, N., Manolakis, A.C., and Maltezos, E. (2011). The role of nerve growth factor in the prophylaxis and treatment of diabetic foot ulcers. International journal of burns and trauma *1*, 68-76.

Tsang, M.W., Wong, W.K., Hung, C.S., Lai, K.M., Tang, W., Cheung, E.Y., Kam, G., Leung, L., Chan, C.W., Chu, C.M., *et al.* (2003). Human epidermal growth factor enhances healing of diabetic foot ulcers. Diabetes care *26*, 1856-1861.

Tuyet, H.L., Nguyen Quynh, T.T., Vo Hoang Minh, H., Thi Bich, D.N., Do Dinh, T., Le Tan, D., Van, H.L., Le Huy, T., Doan Huu, H., and Tran Trong, T.N. (2009). The efficacy and safety of epidermal growth factor in treatment of diabetic foot ulcers: the preliminary results. International wound journal *6*, 159-166.

Van Pham, P., Bui, K.H., Ngo, D.Q., Vu, N.B., Truong, N.H., Phan, N.L., Le, D.M., Duong, T.D., Nguyen, T.D., Le, V.T., *et al.* (2013). Activated platelet-rich plasma improves adipose-derived stem cell transplantation efficiency in injured articular cartilage. Stem cell research & therapy *4*, 91.

Villela, D.L., and Santos, V.L. (2010). Evidence on the use of platelet-rich plasma for diabetic ulcer: a systematic review. Growth factors (Chur, Switzerland) *28*, 111-116.

Wu, S.C., Driver, V.R., Wrobel, J.S., and Armstrong, D.G. (2007). Foot ulcers in the diabetic patient, prevention and treatment. Vascular health and risk management *3*, 65-76.

Yang, M., Sheng, L., Zhang, T.R., and Li, Q. (2013). Stem cell therapy

for lower extremity diabetic ulcers: where do we stand? BioMed research international *2013*, 462179.

Zhang, J., Hu, Z.C., Chen, D., Guo, D., Zhu, J.Y., and Tang, B. (2014). Effectiveness and safety of negative pressure wound therapy for diabetic foot ulcers: a meta-analysis. Plastic and reconstructive surgery.

Cite this article as:

Tran, T., Le, P., & Pham, P. (2014). Diabetic foot ulcer treatment by activated platelet rich plasma: a clinical study. *Biomedical Research And Therapy, 1*(2):37-42.