TÜBİTAK

Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2017) 41: 841-847
© TÜBİTAK
doi:10.3906/zoo-1603-58

Effect of dual growth factor administration on oxidative markers during acute stage wound healing in rats

Bahar DİNDAR[1], **Kaan KALTALIOĞLU**[2,*], **Şule COŞKUN CEVHER**[1]
[1]Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey
[2]Vocational School of Espiye, Giresun University, Giresun, Turkey

Received: 25.03.2016 • **Accepted/Published Online:** 12.05.2017 • **Final Version:** 28.09.2017

Abstract: Wound healing is a complex process affected by various conditions, including oxidative stress. The present study explored the time-dependent effects of platelet-derived growth factor (PGDF) and vascular endothelial growth factor (VEGF) administration on oxidative markers during acute stage wound healing. Thirty-six Wistar rats were distributed into three major groups; skin wounds were inflicted in all groups. The wounds were either left untreated (control group), treated topically with blank chitosan gel (blank chitosan gel group), or treated topically with a combination of PDGF and VEGF in chitosan gel (PDGF + VEGF chitosan gel group). Wounds were sampled on postsurgery days 3 and 7; samples were assayed for the oxidant markers nitric oxide (NOx) and thiobarbituric acid-reactive substances (TBARs) and the antioxidant markers glutathione (GSH), ascorbic acid (AA), and superoxide dismutase (SOD) activity. PDGF + VEGF administration increased and decreased NOx levels on days 3 and 7, respectively. PDGF + VEGF administration lowered TBARs levels, compared with blank chitosan gel administration, on day 7. PDGF + VEGF administration increased GSH levels. These results demonstrate that PDGF + VEGF administration changes oxidative status of wound tissue. This study provides valuable insights for the development of therapeutic targets that promote wound healing.

Key words: Wound healing, oxidative stress, PDGF, VEGF

1. Introduction

Wound healing is a physiological process that involves a programmed series of several biological events that include inflammation, proliferation, and remodeling (Lee et al., 2012; Greaves et al., 2013) and is coordinated by growth factors released from various cells (Takehara, 2000; Coskun et al., 2009; Kalay and Cevher, 2012). Platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are two of the most crucially involved growth factors in wound healing as they are essential for proper healing.

PDGF is one of the numerous growth factors that stimulate multiple cellular processes, including cell proliferation, chemotaxis, survival, and differentiation (Heldin, 2012). Various cell types, including platelets, fibroblasts, and macrophages, release PDGF (Barrientos et al., 2008). Recent studies have reported that PDGF plays a vital role in healing (Kaltalioglu and Coskun-Cevher, 2015) in various wound models; it accelerated healing (Breitbart et al., 2003; Steed, 2006), ameliorated inflammation (Pierce et al., 1992), and enhanced cell proliferation (Li et al., 2008). VEGF is another proangiogenic glycoprotein that binds membrane receptors and stimulates vascular endothelial cells by acting as both a chemoattractant and mitogen (Przybylski, 2009; Lauro et al., 2014). Many cell types, including macrophages, platelets, and keratinocytes, can produce VEGF (Duffy et al., 2004). Several scientists have determined that VEGF is involved in angiogenesis and contributes to wound healing (Kirchner et al., 2003; Li et al., 2005; Przybylski, 2009).

Chitosan is a polysaccharide that is obtained from the hard outer skeleton of shellfish; it consists of N-acetylglucosamine and D-glucosamine (Chen et al., 2009). Various studies have suggested that chitosan is biodegradable and biocompatible and contributes to wound healing (Ishihara et al., 2002; Alemdaroglu et al., 2006).

Wound healing proceeds in overlapping stages that need to be carefully and rigorously regulated to ensure proper healing; this is crucial because healing can be affected by various factors, including oxidative stress. Reactive oxygen species (ROS) can damage cells by modifying lipids, proteins, and DNA. As a result, the incurred cellular damage (referred to as oxidative stress) may contribute to the pathogenesis of various diseases (Lobo et al., 2010). As in other metabolic processes,

* Correspondence: kaan.kaltalioglu@gmail.com

oxidative events occur during wound healing too. However, these events are neither entirely beneficial nor entirely detrimental for wound healing. Studies suggest that low ROS [e.g., (O_2^-) and hydrogen peroxide (H_2O_2)] levels serve as second messengers in the healing process (Sundaresan et al., 1995; Guo and Dipietro, 2010). ROS also play a role in eliminating bacterial contamination during wound healing (Sen, 2003; Rodriguez et al., 2008). However, high ROS levels may induce cellular damage, thereby impairing wound healing (Rodriguez et al., 2008; Guo and Dipietro, 2010).

Considering the studies in the literature, we think that dual growth factor administration might be more effective than single factor administration on oxidative events (Coskun et al., 2009; Kalay and Cevher, 2012; Kaltalioglu et al., 2013). Therefore, the purpose of the present study was to simultaneously administer PDGF and VEGF during acute stage wound healing and evaluate resultant time-dependent effects on oxidative events in rats.

2. Materials and methods

2.1. Animals

The Local Animal Ethics Committee (Gazi University) approved the protocol of this study (Ref no: G.Ü.ET-11.010). Thirty-six female Wistar albino rats (healthy, adult, 200–250 g) were utilized. The rats were housed one per cage and given standard rat food and water ad libitum under standard conditions (room temperature, 12-h light/dark cycle). The rats were allocated randomly to one of three experimental groups (Figure).

2.2. Preparation of the chitosan gels

PDGF (P-4056), VEGF (V-3638), and chitosan (C-3646) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Lactic acid (1 g) was added to 50 mL of water. Chitosan (3 g) was added and stirred. After the swelling, the remaining quantity of water (final volume: 100 mL) was added. The prepared gel was stored one night prior to application to trap air bubbles (blank chitosan gel). Then a sufficient quantity of PDGF (417 ng) and of VEGF (417 ng) were combined to prepare a solution; the concentration finally used was 7 + 7 ng/mL (PDGF + VEGF chitosan gel). The gels were stored in a refrigerator at 4–8 °C during the experiment.

2.3. Surgical procedure

The rats were anesthetized with an intramuscular injection of 50 mg/kg of ketamine HCl and 5 mg/kg xylazine HCl (Alfamine and Alfazyne, Alfasan, Woerden, Holland). The dorsum was shaved and cleaned with iodine tincture. Two equal-sized (to be sufficient to analyze all parameters needed), excisional full-thickness skin wounds were carefully created with a surgical blade on both sides of the spine in all animals (wound length = 40 mm, breadth = 2 mm). All wounds were created by the same person and were closed with an interrupted technique at 1-cm intervals with 4/0 silk sutures (Mersilk, Ethicon, Edinburgh, Scotland).

2.4. Treatment

Following the surgery, 0.3 mL of chitosan gel containing PDGF + VEGF was topically applied to each wound once daily, using a needleless syringe. Only rats in the PDGF + VEGF chitosan gel group received this treatment. Treatment was first applied immediately after surgery. Rats in the blank chitosan gel group were treated as per the same regimen; they received the same amount of chitosan gel but without PDGF + VEGF. No treatment was performed in the control group.

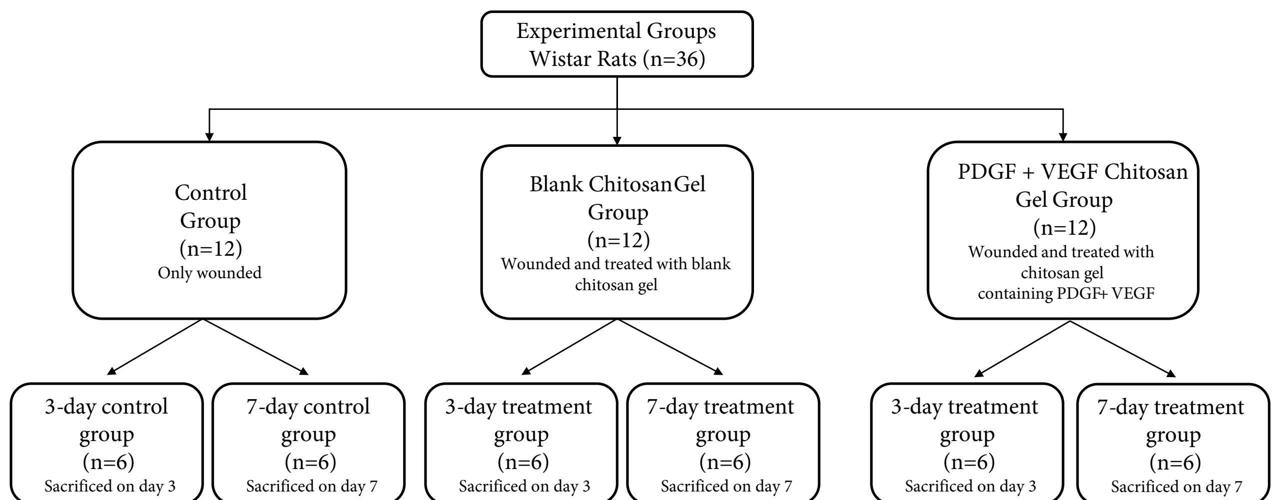

Figure. Experimental groups.

Six rats from each group were sacrificed with intracardiac blood aspiration under anesthesia on days 3 and 7 after wounding; these days were chosen because this is when PDGF (Cheng et al., 2007; Li et al., 2008; Kaltalioglu et al., 2013) and VEGF (Kirchner et al., 2003; Przybylski, 2009) have been shown to be most effective, respectively. Wound samples were collected with a surgical blade and divided into equal six parts. Collected samples were named corresponding to their positions (L1, L2, L3 and R1, R2, R3), directly placed in liquid nitrogen, and stored at −30 °C. Each of the parts was used to analyze different parameters.

2.5. Determination of NOx levels

Reactive nitrogen oxide species (NOx) levels were determined using the Griess reaction. Briefly, samples were homogenized in phosphate buffer (pH 7), centrifuged at 3500 rpm, and treated with 0.3 M sodium hydroxide (NaOH). Following incubation, vanadium trichloride (VCl_3) was added and the mixture was incubated for an additional 30 min at 37 °C. The mixture was then mixed with the Griess reagents, which contain N-(1-naphthyl)ethylenediamide dihydrochloride (NEDD) and sulfanilamide. The absorbance was recorded at 540 nm (UV Mini 1240, Shimadzu, Tokyo, Japan) (Miranda et al., 2001). Sodium nitrite was used as a standard for this assay. Sample absorbance readings were run against the blank.

2.6. Determination of TBARs levels

Briefly, 1 mL of sample that had been homogenized in 0.15 M potassium chloride (KCl) was extracted with 15% trichloroacetic acid. Following centrifugation at 2000 × *g*, the supernatant was added to 1% butylated hydroxytoluene and 0.67% thiobarbituric acid and heated at 100 °C. The absorbance was recorded at 535 nm (UV Mini 1240, Shimadzu, Tokyo, Japan) (Casini et al., 1986). Tetraethoxypropane solution was used as a standard for this assay. Sample absorbance readings were run against the blank.

2.7. Determination of GSH levels

A modified version of the Ellman method was used to determine glutathione (GSH) levels as per the procedure outlined by Aykac et al. (1985). In brief, a sodium chloride/ethylenediaminetetraacetic acid/metaphosphoric acid mixture was mixed with the homogenized sample (homogenized as described in 2.6) for deproteinization. Following centrifugation, 0.5 mL of the supernatant was added to 0.2 mL of dithiobisnitrobenzoate solution and 2 mL of 0.3 M disodium hydrogen phosphate. The GSH level in the tissue sample was determined from its absorbance at 412 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). Sample absorbance readings were run against the blank.

2.8. Determination of AA levels

Ascorbic acid (AA) levels were estimated as previously described (Berger et al., 1989). In brief, homogenized (on ice in 0.35 M perchloric acid) samples were centrifuged for 3 min at 15,000 × *g*. A 400-µL aliquot of either the standard (AA) or sample was mixed with 100 µL of color reagent in a centrifugal analyzer tube. Following incubation, 600 µL of 65% (v/v) H_2SO_4 was added. The absorbance was determined at 520 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). Sample absorbance readings were run against the blank.

2.9. Determination of SOD activity

Superoxide dismutase (SOD) activity was detected using the method previously described by Sun et al. (1988). The homogenized (in ice-cold 0.9% NaCl) sample was centrifuged at 7000 rpm, and an ethanol/chloroform mixture (3:5, v/v) was added to the supernatant. After a second round of centrifugation, the assay reagent [150 µM nitroblue tetrazolium (NBT), 0.6 mM ethylenediaminetetraacetic acid (EDTA), 3 mM xanthine, 400 mM Na_2CO_3, and 1 g/L bovine serum albumin (BSA)] was mixed with the supernatant. Xanthine oxidase was added to this mixture. Following incubation, copper chloride ($CuCl_2$) was added to the mixture. SOD activity was recorded using the absorbance at 560 nm (UV Mini 1240, Shimadzu, Tokyo, Japan). 1 U is defined as the quantity of enzyme causing 50% inhibition of NBT. Sample absorbance readings were run against the blank.

2.10. Statistical analysis

The results are presented as the mean ± standard deviation (SD). Groupwise differences were calculated using one-way ANOVA. Differences with $P < 0.001$ were considered statistically significant.

3. Results

Measurement results are summarized in Tables 1 and 2.

3.1. NOx levels

Blank chitosan gel administration (blank chitosan gel group) significantly increased NOx levels in wound tissue on days 3 and 7 as compared to the control group ($P < 0.001$). Comparing the results of the PDGF + VEGF chitosan gel group with those of the other groups showed that the dual growth factor administration significantly increased NOx levels on day 3 ($P < 0.001$). Moreover, in the PDGF + VEGF chitosan gel group, NOx level was also significantly decreased on day 7 as compared to the blank chitosan gel group ($P < 0.001$).

3.2. TBARs levels

Rats in the PDGF + VEGF chitosan gel group had significantly lower TBARs levels than those in the blank chitosan gel group on day 7 ($P < 0.001$). However, there was no significant ($P > 0.001$) alteration in the PDGF + VEGF chitosan gel group when compared with the other groups on day 3 (Table 1).

Table 1. Oxidant markers on two different intervals in wound tissue. All data are expressed as mean ± standard deviation. * P < 0.001 as compared with the control group. a P < 0.001 as compared with the blank chitosan gel group.

		NOx (µmol/g tissue)	TBARs (nmol/g tissue)
Control group (n = 12)	3-day (n = 6)	477.33 ± 74.66	29.08 ± 5.26
	7-day (n = 6)	396.76 ± 89.75	29.42 ± 7.00
Blank chitosan gel group (n = 12)	3-day (n = 6)	730.66 ± 74.98*	25.35 ± 7.17
	7-day (n = 6)	1093.86 ± 69.50*	42.51 ± 2.84
PDGF + VEGF chitosan gel group (n = 12)	3-day (n = 6)	1471.10 ± 65.96*,a	32.71 ± 8.76
	7-day (n = 6)	411.26 ± 81.42a	27.17 ± 4.41a

Table 2. Antioxidant markers on two different intervals in wound tissue. All data are expressed as mean ± standard deviation. * P < 0.001 as compared with the control group. a P < 0.001 as compared with the blank chitosan gel group.

		GSH (µmol/g tissue)	AA (mg/g tissue)	SOD (U/g tissue)
Control group (n = 12)	3-day (n = 6)	2.23 ± 0.49	0.14 ± 0.02	282.43 ± 12.44
	7-day (n = 6)	3.62 ± 0.73	0.10 ± 0.01	310.65 ± 11.18
Blank chitosan gel group (n = 12)	3-day (n = 6)	2.41 ± 0.71	0.10 ± 0.01	271.71 ± 8.78
	7-day (n = 6)	2.85 ± 0.64	0.23 ± 0.11	309.42 ± 10.49
PDGF + VEGF chitosan gel group (n = 12)	3-day (n = 6)	10.42 ± 2.78*,a	0.15 ± 0.12	280.98 ± 5.63
	7-day (n = 6)	5.14 ± 1.89	0.16 ± 0.08	308.48 ± 12.84

3.3. GSH levels

In the PDGF + VEGF chitosan gel group, GSH levels were also significantly increased on day 3 as compared to the other groups (P < 0.001) (Table 2).

3.4. AA levels

No significant difference in AA level was observed in any of the three groups (P > 0.001) (Table 2).

3.5. SOD activities

No significant difference in SOD activity was observed in any of the three groups (P > 0.001) (Table 2).

4. Discussion

The current study demonstrated that PDGF + VEGF administration affects NOx, TBARs, and GSH levels in wound healing. Additionally, these data suggest that this effect may be time-dependent. Based the data reported here and our previous study, we may infer that dual growth factor administration is more effective than single factor administration on oxidative markers (decreasing TBARs and increasing GSH levels) during wound healing (Kaltalioglu et al., 2013).

Chitosan, used as a vehicle for growth factor administration in our study, showed a statistically significant effect by increasing NOx levels compared to the control group (untreated) (P < 0.001), but it did not show a significant effect on other oxidative markers (TBARs, GSH, AA, and SOD) (P > 0.001). Previous studies have indicated that chitin and its derivatives (such as chitosan) may have different effects (increasing or decreasing) on NO, depending on dosage, source, and preparation methods (Hwang et al., 2000; Yu et al., 2004; Pattani et al., 2009).

NO is a highly reactive biological messenger (Hou et al., 1999; Esplugues, 2002). Various cell types, including macrophages, platelets, endothelial cells, and fibroblasts are capable of producing NO in wounded tissue through the action of three different nitric oxide synthase isotypes: inducible (iNOS), neuronal (nNOS), and endothelial (eNOS) (Frank et al., 2002; Schwentker et al., 2002). Comparing the results of the PDGF + VEGF chitosan gel group with those of the other groups showed that the dual growth factor administration significantly increased NOx levels on day 3 (P < 0.001). VEGF mediates the chemotaxis of macrophages (Shen et al., 1993; Barleon et al., 1996; Sawano et al., 2001) and stimulates vascular endothelial cells by acting as both a chemoattractant and mitogen

(Przybylski, 2009). It is has been reported that VEGF stimulates eNOS and iNOS expression and NO production in endothelial cells (Kroll and Papapetropoulos et al., 1997; Waltenberger, 1998; Park et al., 2002; Koai et al., 2010). In contrast, numerous studies have suggested that PDGF administration suppresses NO production (Scott-Burden et al., 1992; Hishikawa et al., 1996; Kunz et al., 1997; Kaltalioglu et al., 2013). Additionally, in the PDGF + VEGF chitosan gel group, NOx level was also significantly decreased on day 7 as compared to the blank chitosan gel group ($P < 0.001$). This determined difference between days 3 and 7, which is caused by the administration of PDGF + VEGF, may have occurred due to time-dependent changes in cellular status during the healing process; various cell types (e.g., macrophages and endothelial cells) are involved in wound healing and become dominant at different stages in the process.

Lipid peroxidation can be monitored using TBARs levels. There were no significant differences in TBARs levels between the PDGF + VEGF chitosan gel group and the other groups on day 3. However, tissue TBARs levels were significantly lower in the PDGF + VEGF chitosan gel group than in the blank chitosan gel group on day 7 ($P < 0.001$). This decrease on day 7 is similar to that found in our previous study (Kaltalioglu et al., 2013). PDGF + VEGF chitosan gel administration may have an antioxidative effect compared to the blank chitosan gel group on day 7. Additionally, this decrease parallels the simultaneous NOx decrease.

GSH is an important antioxidant tripeptide that protects the cells against oxidative stress (Lushchak, 2012). GSH levels were elevated on day 3 in the PDGF + VEGF group as compared to the other groups ($P < 0.001$). This result is supported by findings from our previous studies (Kaltalioglu et al., 2013). Similarly, Iantomasi et al. (1999) determined that in serum-starved NIH3T3 cells cellular GSH content was increased by PDGF-BB stimulation. The existing literature suggests that both PDGF and VEGF administration increase GSH levels. For instance, Kuzuya et al. (2001) showed that VEGF administration significantly increased GSH levels in bovine aortic endothelial cells. A possible explanation for the increase observed in our study might be that growth factors [like PDGF-BB (Chen et al., 1997)] may trigger signal transduction pathways, thereby causing phospholipase A2 (PLA2) activation and prostaglandin E2 (PGE2) release; GSH plays an essential role in this process (Harvey and Ferrier, 2010; Kaltalioglu et al., 2013). In considering this result, it can be an important indication that dual growth factor administration increases the GSH level in wound tissue during the healing process, especially on day 3.

AA levels and SOD activities were not significantly altered in the PDGF + VEGF chitosan gel group as compared to the other groups ($P > 0.001$). While TBARs levels decrease, AA levels and SOD activity do not change. This suggests that alternative antioxidant defense mechanisms (such as GSH, catalase, and vitamin E) are used to scavenge increased ROS.

In conclusion, PDGF + VEGF administration affects oxidative events in wound tissue by altering NOx, decreasing TBARs, and increasing GSH levels. These results may help elucidate the effect of dual growth factor administration on oxidant markers during acute stage wound healing. The present study also provides valuable insights for the development of therapeutic targets that promote wound healing.

Acknowledgment
The authors would like to thank Assoc Prof Dr Barbaros Balabanlı for his significant contributions to this study.

References

Alemdaroglu C, Degim Z, Celebi N, Zor F, Ozturk S, Erdogan D (2006). An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. Burns 32: 319-327.

Aykac G, Uysal M, Yalcin AS, Kocak-Toker N, Sivas A, Oz H (1985). The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. Toxicology 36: 71-76.

Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marmé D (1996). Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood 87: 3336-3343.

Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M (2008). Growth factors and cytokines in wound healing. Wound Repair Regen 16: 585-601.

Berger J, Shepard D, Morrow F, Taylor A (1989). Relationship between dietary intake and tissue levels of reduced and total vitamin C in the nonscorbutic guinea pig. J Nutr 119: 734-740.

Breitbart AS, Laser J, Parrett B, Porti D, Grant RT, Grande DA, Mason JM (2003). Accelerated diabetic wound healing using cultured dermal fibroblasts retrovirally transduced with the platelet-derived growth factor B gene. Ann Plast Surg 51: 409-414.

Casini AF, Ferrali M, Pompella A, Maellaro E, Comporti M (1986). Lipid peroxidation and cellular damage in extrahepatic tissues of bromobenzene-intoxicated mice. Am J Pathol 123: 520-531.

Chen QR, Miyaura C, Higashi S, Murakami M, Kudo I, Saito S, Hiraide T, Shibasaki Y, Suda T (1997). Activation of cytosolic phospholipase A2 by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin E2 synthesis in mouse osteoblasts cultured with interleukin-1. J Biol Chem 272: 5952-5958.

Chen S, Tsai M, Huang J, Chen R (2009). In vitro antioxidant activities of low-molecular-weight polysaccharides with various functional groups. J Agric Food Chem 57: 2699-2704.

Cheng B, Liu HW, Fu XB, Sun TZ, Sheng ZY (2007). Recombinant human platelet-derived growth factor enhanced dermal wound healing by a pathway involving ERK and c-fos in diabetic rats. J Dermatol Sci 45: 193-201.

Coskun S, Peker EGG, Balabanlı B, Ahıska S, Acarturk F (2009). Effect of transforming growth factor beta 1 (TGF-beta 1) on nitric oxide production and lipid peroxidation in oral mucosal wound healing. Med Chem Res 20: 23-28.

Duffy AM, Bouchier-Hayes DJ, Harmey JH (2004). Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. In: Harmey JH, editor. VEGF and Cancer. Austin, TX, USA: Landes Bioscience, pp. 133-144.

Esplugues JV (2002). NO as a signalling molecule in the nervous system. Br J Pharmacol 135: 1079-1095.

Frank S, Kampfer H, Wetzler C, Pfeilschifter J (2002). Nitric oxide drives skin repair: novel functions of an established mediator. Kidney Int 61: 882-888.

Greaves NS, Ashcroft KJ, Baguneid M, Bayat A (2013). Current understanding of molecular and cellular mechanisms in fibroplasia and angiogenesis during acute wound healing. J Dermatol Sci 72: 206-217.

Guo S, Dipietro LA (2010). Factors affecting wound healing. J Dent Res 89: 219-229.

Harvey R, Ferrier D (2010). Lipid metabolism. In: Harvey R, editor. Lippincott's Illustrated Reviews: Biochemistry. 5th ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins, pp. 173-244.

Heldin CH (2012). Autocrine PDGF stimulation in malignancies. Ups J Med Sci 117: 83-91.

Hishikawa K, Nakaki T, Hirahashi J, Marumo T, Saruta T (1996). Tranilast restores cytokine-induced nitric oxide production against platelet-derived growth factor in vascular smooth muscle cells. J Cardiovasc Pharmacol 28: 200-207.

Hou YC, Janczuk A, Wang PG (1999). Current trends in the development of nitric oxide donors. Curr Pharm Des 5: 417-441.

Hwang SM, Chen CY, Chen SS, Chen JC (2000). Chitinous materials inhibit nitric oxide production by activated RAW 264.7 macrophages. Biochem Biophys Res Commun 271: 229-233.

Iantomasi T, Favilli F, Degl'Innocenti D, Vincenzini MT (1999). Increased glutathione synthesis associated with platelet-derived growth factor stimulation of NIH3T3 fibroblasts. Biochim Biophys Acta 1452: 303-312.

Ishihara M, Nakanishi K, Ono K, Sato M, Kikuchi M, Saito Y, Yura H, Matsui T, Hattori H, Uenoyama M et al. (2002). Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process. Biomaterials 23: 833-840.

Kalay Z, Cevher SC (2012). Oxidant and antioxidant events during epidermal growth factor therapy to cutaneous wound healing in rats. Int Wound J 9: 362-371.

Kaltalioglu K, Coskun-Cevher S (2015). A bioactive molecule in a complex wound healing process: platelet-derived growth factor. Int J Dermatol 54: 972-977.

Kaltalioglu K, Coskun-Cevher S, Tugcu-Demiroz F, Celebi N (2013). PDGF supplementation alters oxidative events in wound healing process: a time course study. Arch Dermatol Res 305: 415-422.

Kirchner LM, Meerbaum SO, Gruber BS, Knoll AK, Bulgrin J, Taylor RAJ, Schmidt SP (2003). Effects of vascular endothelial growth factor on wound closure rates in the genetically diabetic mouse model. Wound Repair Regen 11: 127-131.

Koai E, Rincon Rios T, Edwards J (2010). Vascular endothelial growth factor increases endothelial nitric oxide synthase transcription in huvec cells. WebmedCentral Physiology 1: WMC001111.

Kroll J, Waltenberger J (1998). VEGF-A induces expression of eNOS and iNOS in endothelial cells via VEGF receptor-2 (KDR). Biochem Biophys Res Commun 252: 743-746.

Kunz D, Walker G, Eberhardt W, Messmer UK, Huwiler A, Pfeilschifter J (1997). Platelet-derived growth factor and fibroblast growth factor differentially regulate interleukin 1beta- and cAMP-induced nitric oxide synthase expression in rat renal mesangial cells. J Clin Invest 100: 2800-2809.

Kuzuya M, Ramos MA., Kanda S, Koike T, Asai T, Maeda K, Shitara K, Shibuya M, Iguchi A (2001). VEGF protects against oxidized LDL toxicity to endothelial cells by an intracellular glutathione-dependent mechanism through the KDR receptor. Arterioscler Thromb Vasc Biol 21: 765-770.

Lauro S, Onesti CE, Righini R, Marchetti P (2014). The use of bevacizumab in non-small cell lung cancer: an update. Anticancer Res 34: 1537-1545.

Lee YS, Wysocki A, Warburton D, Tuan TL (2012). Wound healing in development. Birth Defects Res C Embryo Today 96: 213-222.

Li WW, Talcott KE, Zhai AW, Kruger EA, Li VW (2005). The role of therapeutic angiogenesis in tissue repair and regeneration. Adv Skin Wound Care 18: 491-500; quiz 501-502.

Li H, Fu X, Zhang L, Huang Q, Wu Z, Sun T (2008). Research of PDGF-BB gel on the wound healing of diabetic rats and its pharmacodynamics. J Surg Res 145: 41-48.

Lobo V, Patil A, Phatak A, Chandra N (2010). Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev 4: 118-126.

Lushchak VI (2012). Glutathione homeostasis and functions: potential targets for medical interventions. J Amino Acids 2012: ID736837.

Miranda KM, Espey MG, Wink DA (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5: 62-71.

Papapetropoulos A, García-Cardeña G, Madri J, Sessa W (1997). Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. J Clin Invest 100: 3131-3139.

Park JS, Hong GR, Baek SW, Shin DG, Kim YJ, Shim BS (2002). Expression and regulation of endothelial nitric oxide synthase by vascular endothelial growth factor in ECV 304 cells. J Korean Med Sci 17: 161-167.

Pattani A, Patravale VB, Panicker L, Potdar PD (2009). Immunological effects and membrane interactions of chitosan nanoparticles. Mol Pharm 6: 345-352.

Pierce GF, Tarpley JE, Yanagihara D, Mustoe TA, Fox GM, Thomason A (1992). Platelet-derived growth factor (BB homodimer), transforming growth factor-beta 1, and basic fibroblast growth factor in dermal wound healing. Neovessel and matrix formation and cessation of repair. Am J Pathol 140: 1375-1388.

Przybylski M (2009). A review of the current research on the role of bFGF and VEGF in angiogenesis. J Wound Care 18: 516-519.

Rodriguez PG, Felix FN, Woodley DT, Shim EK (2008). The role of oxygen in wound healing: a review of the literature. Dermatol Surg 34: 1159-1169.

Sawano A, Iwai S, Sakurai Y, Ito M, Shitara K, Nakahata T, Shibuya M (2001). Flt-1, vascular endothelial growth factor receptor 1, is a novel cell surface marker for the lineage of monocyte-macrophages in humans. Blood 97: 785-791.

Schwentker A, Vodovotz Y, Weller R, Billiar TR (2002). Nitric oxide and wound repair: role of cytokines? Nitric Oxide 7: 1-10.

Scott-Burden T, Schini VB, Elizondo E, Junquero DC, Vanhoutte PM (1992). Platelet-derived growth factor suppresses and fibroblast growth factor enhances cytokine-induced production of nitric oxide by cultured smooth muscle cells. Effects on cell proliferation. Circ Res 71: 1088-1100.

Sen CK (2003). The general case for redox control of wound repair. Wound Repair Regen 11: 431-438.

Shen H, Clauss M, Ryan J, Schmidt AM, Tijburg P, Borden L, Connolly D, Stern D, Kao J (1993). Characterization of vascular permeability factor/vascular endothelial growth factor receptors on mononuclear phagocytes. Blood 81: 2767-2773.

Steed DL (2006). Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. Plast Reconstr Surg 117: 143S-149S; discussion 150S-151S.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clin Chem 34: 497-500.

Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T (1995). Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. Science 270: 296-299.

Takehara K (2000). Growth regulation of skin fibroblasts. J Dermatol Sci 24 Suppl 1: S70-77.

Yu Z, Zhao L, Ke H (2004). Potential role of nuclear factor-kappaB in the induction of nitric oxide and tumor necrosis factor-alpha by oligochitosan in macrophages. Int Immunopharmacol 4: 193-200.