Digest Journal of Nanomaterials and Biostructures Vol. 3, No.4, December 2008, p. 203 - 208

# DIABETIC WOUND HEALING AND ITS ANGIOGENESIS WITH SPECIAL REFERENCE TO NANOPARTICLES

Ghulam Mohammad[1*], H.P.Pandey[1], Kamlakar Tripathi[b]
[a]*Department of Biochemistry,* [b]*Department of medicine, Banaras Hindu University Varanasi 221 005 INDIA*

Foot ulcers are one of the main complications in diabetes mellitus, with a 15% life time risk in all diabetic patients. The problem and features are infection, ulceration, or gangrene. Neuropathy, poor circulation, and susceptibility to infection are the three major contributors to the development of diabetic foot; which when present, foot deformities or minor trauma can readily lead to ulceration and infection. Not all diabetic foots are preventable, but appropriate preventive measures can dramatically reduce their occurrences. Patients with diabetes display aberrant angiogenesis in various organs, with insufficient activity occurring in impaired wound healing including ulcers. Limited penetration of new blood vessels into the wound would restrict entry of inflammatory cells. VEGF is produced by keratinocytes that, together with macrophages, represent the most important source of this growth factor during normal wound healing. Nanotechnology, not only is widely used in industry, but also has been extensively explored for possible applications in medicine. Angiogenesis induced with the peptide-DNA nanoparticles, gene delivery by using a matrix-based approach in which peptide-DNA nanoparticles were formed with peptides that comprised a minimal-length lysine hexamer domain for initial DNA binding and a pair of cysteine residues for stabilization of the nanoparticle by disulfide crosslinking is helpful in induction of angiogenesis in delayed diabetic wound healing. Side effects of the production, manipulation and use of nanoparticles, and nanosafety has become a priority for application on human beings .

(Received September 3, 2008; accepted September 13, 2008)

*Keywords*: Angiogenesis; Free Radicals; Nanoparticle; Metal oxide; Diabetic wound.

## 1. Impaired wound healing in diabetes

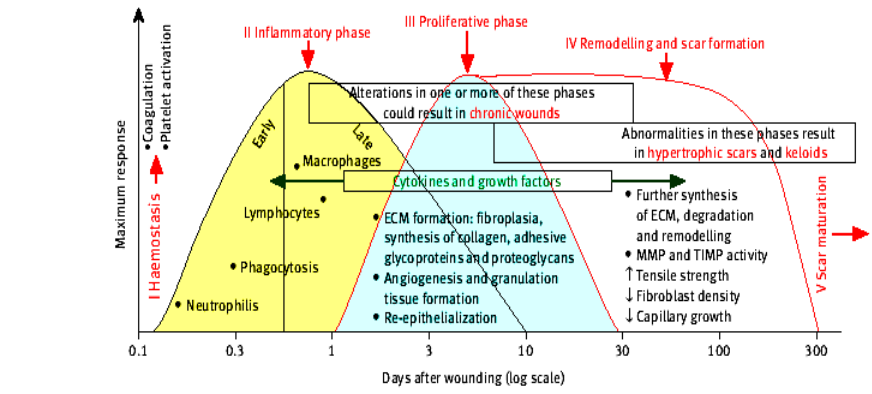
The diabetic foot syndrome represents a major problem in the healthcare of diabetic patients. Understanding the molecular basis of this disease is an important step toward a rational treatment. Due to the systemic character of diabetes, disturbances in several basic cell functions appear to contribute to impaired wound healing. The problem and features of diabetic foot are infection, ulceration, or gangrene. Neuropathy, poor circulation, and susceptibility to infection are the three major contributors to the development of diabetic foot; which when present, foot deformities or minor trauma can readily lead to ulceration and infection [1]. Many essential processes of normal wound healing are regulated in large part by growth factors and proteases, and changes of their expression and activity are relevant for the pathogenesis of the chronic wound. The diabetic foot syndrome is clearly one of the most important complications of diabete. It not only occurs as a typical complication in the late stages of diabetes but also in patients with newly diagnosed diabetes. Despite the postulations of the St. Vincent Declaration that within 5 years the amputation rate has to be reduced by 50%, there are 30,000 amputations reported each year in Germany due to the diabetic foot syndrome [2– 6]. Vascular endothelial growth factor (VEGF) is

* Email: gmkbiochembhu@gmail.com

one of the most potent known angiogenic cytokines and promotes all steps in the cascade process of angiogenesis. In particular, it induces degeneration of the extracellular matrix of existing vessels by proteases, causes migration and proliferation of capillary endothelial cells, and determines tube proliferation of endothelial cells [7]. VEGF action is associated with a variety of physiological and pathological neovascular events, such as embryonic development, tumor growth, and wound repair in particular [8]. VEGF is related to platelet-derived growth factor and has four different isoforms, VEGF121, VEGF165, VEGF189, and VEGF206, which are generated by alternative splicing of mRNA [9]. VEGF is produced by keratinocytes that, together with macrophages, represent the most important source of this growth factor during normal wound healing. Impaired wound healing may be a consequence of normal aging, metabolic derangement such as diabetes, or therapeutic intervention.

## 2. Important of angiogenesis in wound healing

The process by which new blood capillaries grow into a wound space after injury is known as angiogenesis. Wound angiogenesis is an important part of the proliferative phase of healing; in fact the term `granulation tissue' was used by John Hunter in 1787 [10] to describe the appearance of the prominent blood vessels of the initial connective tissue formed in the wound space. Healing of any skin wound other than the most superficial cannot occur without angiogenesis. Not only does any damaged vasculature need to be repaired, but the increased local cell activity necessary for healing requires an increased supply of nutrients from the bloodstream. Moreover, the endothelial cells which form the lining of the blood vessels are important in themselves as organizers and regulators of healing. It is interesting to note that angiogenesis also occurs in many other situations, including solid tumour growth and metastasis; rheumatoid arthritis; psoriasis; scleroderma; placental growth and embryo implantation; and three common causes of blindness - diabetic retinopathy, retrolental fibroplasia and neovascular glaucoma (in fact, diseases of the eye are almost always accompanied by vascularization [11] ) [12], [13]. The process of wound angiogenesis has many features in common with tumour angiogenesis [14].



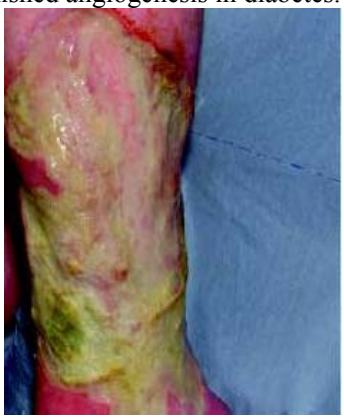

ECM: Extracellular matrix; MMP: Metalloproteinases; TIMP: Tissue inhibitors of metalloproteinases.

Adapted from: Clark R A. In: Goldsmith L A (Editor): *Physiology, biochemistry, and molecular biology of the skin*. 2nd edition, Volume I. New York: Oxford University Press, 1991, p 577.

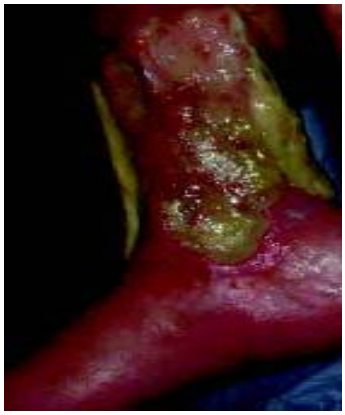
## 3. Angiogenesis and diabetic wound

**Angiogenesis** The formation of new blood vessels from pre-existing capillaries that are then able to penetrate the wound site is an essential component of the wound healing process [15]. Patients with diabetes display aberrant angiogenesis in various organs, with insufficient activity

occurring in impaired wound healing including ulcers [16, 17]. Limited penetration of new blood vessels into the wound would restrict entry of inflammatory cells, as has been reported. In turn, the total amount of factors released by these cells will be decreased. The oxygen supply to the wound will also be poor. Topical administration of high glucose to wounds of non-diabetic rats resulted in inhibition of the normal angiogenic process [18], suggesting a direct role for high glucose levels in diminished angiogenesis in diabetes.



**Diabetic Foot with poor angiogenesis**



**Angiogenesis (Red patches)**

**Process of angiogenesis**

Angiogenesis proceeds concurrently with the formation of new tissue (granulation tissue), which typically begins about 4 days post-wounding [19]. It is stimulated by the chemicals (soluble factors) released by wounded tissue [20]. The resulting processes are tightly regulated by cell-cell interactions, cell-ECM interactions and cell-soluble factor interactions [20]. A blood capillary consists of a hollow tube lined with endothelial cells. The outside of the tube is covered with a layer known as the basement membrane, a major component of which is collagen IV, and which also contains fibronectin and proteoglycans (compounds consisting mainly of polysaccharides but also containing protein) [21]. Angiogenesis begins with degradation of the basement membrane, followed by migration of endothelial cells out of the vessel. These cells then form into a tube which `sprouts' from the old capillary and is extended further into the wound space as the cells behind the leading tip begin to proliferate. The tips of such tubes can branch and eventually join up with other sprouts to form a closed loop through which blood can flow.

**Nanoparticle and angiogenesis**

Nanotechnology, not only is widely used in industry, but also has been extensively explored for possible applications in medicine. Application of nanoparticle in wound healing is the main focus of nanopathology researcher (nanopathology, meaning by that the collection of pathologies due to micro- and nanoparticles). Various nanoparticles is now available to fight with wound healing problem, like use of silver nanoparticle in cleaning the wound infection [22], curcumin nanoparticle as a enhanced wound closing and increase collagen synthesis [23] and our previous article on metal oxide nanoparticle is a cerium oxide (ceria) nanoparticle,metal oxide (ceria) shows scavenger properties against oxidative stress [24]. However, the potential toxicity issues regarding these powerful Nanoparticles are often ignored. Gene delivery system along with nanoparticle is very useful technology for development of recombinant DNA for gene transfer, they are taking advantage of size as we know Nanoparticles are defined as particles with a diameter less than 100 nm, due to the minute size of nanoparticles, the internalization into the body’s tissues appears to be extremely easy, by the use of this technique we can solve the angiogenesis problem in case of diabetic wound. The major problem with impairment of wound healing in diabetes is characterized by decreased angiogenesis this is caused because of low oxygen supply in granulation tissue of wound. The transcription factor hypoxia-inducible factor (HIF) plays this central role in the induction of angiogenesis. One major form is HIF-1 [25, 26],

which consists of a heterodimer of HIF-1α and HIF-1β. Both subunits are constitutively expressed. HIF-1 β is translocated into the nucleus, whereas HIF-1α possesses an oxygen-sensitive degradation domain, spanning from residues 401 to 603 [27]. Gene delivery by using a matrix-based approach in which peptide- DNA nanoparticles were formed with peptides that comprised a minimal-length lysine hexamer domain for initial DNA binding and a pair of cysteine residues for stabilization of the nanoparticle by disulfide crosslinking (28, 29). These nanoparticles can achieve very high transfection efficiency at remarkably low levels of cytotoxicity, probably due to the very low degree of polymerization of the cationic amino acids. Angiogenesis induced with the peptide-DNA nanoparticles encoding HIF-1 induced a 4-fold increase of more mature blood vessels compared with VEGF-A165 protein application, as judged from the amount of dual-stained vascular structures for SMA (as a smooth muscle marker) and CD31 (as an endothelial marker). Literature suggest When the peptide-DNA nanoparticles entrapped in fibrin matrices were applied to fullthickness dermal wounds in the mouse , angiogenesis was increased comparably strongly to that induced by VEGF-A165 protein . In order to adapt this system for gene delivery in wound, a number of changes are currently being made. Most notably, additional bifunctional peptides are being synthesized to bind to integrin receptors expressed on wound fibroblasts but not on macrophages. These peptides will be fused to DNA-binding peptides in an attempt to target the peptide-DNA nanoparticles to fibroblasts for selective uptake and transfection [30].

The nanoparticles are targeted to the neovasculature by linking them with a targeting agent, including, metal nanoshells, which are then irradiated, preferably with a laser, to heat them and ablate the undesired blood vessels. A nanoshell may include a core substrate material having a smaller dielectric permittivity than the preferred metallic material of the outer shell. The nanoparticle may be conjugated or associated with a targeting molecule, where the targeting molecule targets the nanoparticle to regions of neovasculature associated with a disease state. Such targeting molecules can be antibodies, antibody fragments, receptor binding proteins or other proteins or molecules including growth factors. The nanoparticles may also be conjugated with a polymer to reduce opsonization of the nanoparticles. Suitable polymers include polyethylene glycol, the targeting molecules may be conjugated to the nanoparticles by conjugation to the distal end of the polymer.

A nonviral gene carrier, calcium carbonate ($CaCO_3$) nanoparticle, was evaluated for efficient in vitro and in vivo delivery of small interfering RNA (siRNA) targeting vascular endothelial growth factor-C (VEGF-C). The chemically synthesized $CaCO_3$ nanoparticle has a 58 nm diameter and +28.6mV positive surface charge"It is capable of forming a $CaCO_3$ nanoparticle-DNA complex and transferring DNA into targeted cells with high transfection efficiency while effectively protecting the encapsulated DNA from degradation. Furthermore, the $CaCO_3$ nanoparticle-DNA complex has no obvious cytotoxicity, while a liposome-DNA complex exhibited measurable the researchers conclude that $CaCO_3$ nanoparticle is a novel and nonviral system for effective delivery of siRNA for cancer gene therapy [31].

**Drawback of Nanotechnology on Health**

Now that nanotechnologies are a rapidly growing discipline, there is hardly another technology which has over the past few years found such a wide range of applications and been so talked about as nanotechnology. Society is scared by possible side effects of the production, manipulation and use of nanoparticles, and nanosafety has become a priority. Many researchers are investigating the toxicity of nanoparticles towards cells. Inflammation reactions and other cell damage frequently begin with “oxidative” stress, an over-production of reactive oxygen compounds including, for example, so called free radicals or peroxides. These substances can damage cellular proteins and DNA. For this reason the research team investigated different types of metal-containing nanoparticles which are used as catalysts in various chemical reactions and which in catalytic activity are in some cases significantly different, such as titanium oxide, cobalt oxide and manganese oxide particles. The experiments showed that catalytically active nanoparticles such as cobalt oxide and manganese oxide cause significantly more stress to the cells than inert titanium dioxide particles do, the latter having hardly any effect on them. It seems that it is primarily the chemical composition (and therefore the chemical reactivity) of the nanoparticles which makes them dangerous to cells. Water-based solutions of manganese or cobalt salts would

have been significantly less damaging to the cells, since the cell membranes protect them from dissolved heavy metal ions. When the cells are exposed to comparable nanoparticles containing cobalt or manganese, however, they produce up to eight times more reactive oxygen compounds. Nanoparticles therefore seem somehow to "smuggle" the catalytically active metal oxides into the cells, where they then cause oxidative stress. Due to the minute size of nanoparticles, the internalization into the body's tissues appears to be extremely easy. This was shown by experiments in human volunteers with radioactive-labelled carbon nanoparticles that were shown to pass rapidly into the systemic circulation after inhalation. Radioactivity could already be detected in the blood 1 minute after inhalation [33]. Furthermore, animal studies revealed that inhaled nanoparticles were relocated into the liver [33] and the brain [34]. Thus, nanoparticles seem to be able to circumvent the tight blood-brain-barrier and possibly cross the blood-placenta barrier [35, 36]. Moreover, it has been suggested that nanoparticles are involved in thrombus formation in the blood [37, 38]. Today we know that particulate air pollution is associated with enhanced mortality from respiratory and cardiovascular diseases [39].

## 4. Conclusion

The aim of this article was to review the application of nanoparticle with a view toward examining it in the context of angiogenesis in wound healing .we deals about the angiogenesis and its importance in respect with nanoparticle and we are also discussed about the side effect of nanoparticle, this review help in the designing of drug delivery system with minimum toxicity.

## References


[1] Larijani B, Forouzandeh F. Diabetic foot disorders. Iran J Diabetes and Lipid Disord **2**(2), 103 (2003).

[2] Diabetes Care and Research Group in Europe: The Saint Vincent Declaration. Diabet Med **7,** 360 (1990).

[3] Trautner C, Haastert B, Spraul M, Giani G, Berger M: Unchanged incidence of lower-limb amputations in a German city, 1990–1998. Diabetes Care **24,** 855 (2001)

[4] Trautner C, Haastert B, Giani G, Berger M: Amputations and diabetes: a casecontrol study. Diabet Med **19,** 35 (2002)

[5] Trautner C, Haastert B, Giani G, Berger M: Incidence of lower limb amputations and diabetes. Diabetes Care **19**, 1006 (1996)

[6] Standl E: Zur Epidemiologie des diabetischen Fu_syndroms. Diab & Stoffw **9,** 339 (2000)

[7] Ferrara N, Davis-Smyth T: The biology of vascular endothelial growth factor. Endocr Rev **18**, 4 (1997).

[8] Ferrara N: Role of vascular endothelial growth factor in the regulation of angiogenesis. Kidney Int **56**, 794 (1999).

[9] Ferrara N: Molecular and biological properties of vascular endothelial growth factor. J Mol Med **77,** 527 (1999).

[10] J. Hunter, Lectures on the Principles of Surgery, in The Works of John Hunter, J. Palmer (editor).

[11] D.BenEzra, Ocular Circulation and Neovascularization (Documenta opthalmologica proceedings series No. 50), Nijhoff/Junk (1987).

[12] R. Auerbach, W. Auerbach & I. Polakowski. Assays for Angiogenesis : A Review. Pharmacology and Therapeutics, **51**(1), 1 (1991).

[13] A. Desmoulière, M. Redard, I. Darby and G. Gabbiani. Apoptosis Mediates the Decrease in Cellularity During the Transition Between Granulation Tissue and Scar. American Journal of Pathology, **146** (1) 56-66 (1995).

[14] G. F. Whalen & B. R. Zetter, Angiogenesis, in Wound Healing : Biochemical and Physical Aspects, I. K. Cohen (editor), Saunders (1992).

[15] Arnold F, West DC. Angiogenesis in wound healing. Pharmacol Ther **52**: 407 (1991)
[16] Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. Med Res Rev **23**, 117 (2003)
[17] Brem H, Jacobs T, Vileikyte L, Weinberger S, Gibber M, Gill K et al. Wound-healing protocols for diabetic foot and pressure ulcers. Surg Technol Int; **11**, 85 (2003).
[18] Teixeira AS, Andrade SP. Glucose-induced inhibition of angiogenesis in the rat sponge granuloma is prevented by aminoguanidine. Life Sci 1999; **64**: 655–662.
[19] R. A. F. Clark, Overview of Wound Repair, in The Molecular and Cellular Biology of Wound Repair, R. A. F. Clark (editor), Plenum (1996), second edition.
[20] J A. Madri, S. Sankar & A. M. Romanic, Angiogenesis, in The Molecular and Cellular Biology of Wound Repair, R. A. F. Clark (editor), Plenum (1996), second edition.
[21] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts & J. D. Watson, Molecular Biology of the Cell, page 709, Garland (1983), first edition.
[22] Manish Mishra, Hemant Kumar, Kamlakar Tripathi ,Diabetic delayed wound healing and the role of silver nanoparticles Digest Journal of Nanomaterials and Biostructures, **3**(2), 49 (2008).
[23] Vijendra Kumar Mishra,Ghulam Mohammad, Shobha Kant Mishra Down regulation of telomerase activity may enhance by nanoparticle mediated curcumin delivery patient Digest Journal of Nanomaterials and Biostructures, **3**(4), 163-169 (2008).
[24] Ghulam Mohammad, Vijendra K.Mishra, H.P.Pandey. , Antioxidant properties of some nanoparticle may enhance wound Healing in T2DM patient Digest Journal of Nanomaterials and Biostructures, **3**(4), 159-162 (2008).
[25] Huang, L. E. & Bunn, H. F. J. Biol. Chem. **278,** 19575–19578, (2003)
[26] Hewitson, K. S. & Schofield, C. J. Drug Discov. Today **9,** 704–711, (2004)
[27] Huang, L. E., Gu, J., Schau, M. & Bunn, H. F. *Proc. Natl. Acad. Sci. USA* **95,** 7987–7992, (1998).
[28] McKenzie, D. L., Kwok, K. Y. & Rice, K. G. J. Biol. Chem. **275,** 9970–9977,(2000)
[29]. Trentin, D., Hubbell, J. & Hall, H. J. Controlled Release **102,** 263–275,(2005)
[30] Diana Trentin, Heike Hall, Sandra Wechsler, and Jeffrey A. Hubbell Peptide-matrix-mediated gene transfer of an oxygen-insensitive hypoxia-inducible factor-1α variant for local induction of angiogenesis, PNAS **103** (8):2506-2511 (2006)
[31] Jeffrey A. Hubbell, Brian Pullin, Eleonora Simeoni, Yunsuk Jo, Geoffrey Gogniat. Nonviral DNA Vectors in Bone Regeneration European Cells and Materials. **13** (2): 42, (2007)
[32] Nemmar, A., Hoet, P. H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., and Nemery, B., Passage of inhaled particles into the blood circulation in humans. Circulation, **105**, 411-414,( 2002)
[33] Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W., and Cox, C., Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. J Toxicol Environ Health A, **65**, 1531-1543, (2002)
[34] Oberdorster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., and Cox, C., Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol **16**, 437-445,(2004)
[35] Reichrtova, E., Dorociak, F., and Palkovicova, L., Sites of lead and nickel accumulation in the placental tissue. Hum Exp Toxicol, **17**, 176-181, (1998)
[36] Kaiglova, A., Reichrtova, E., Adamcakova, A., and Wsolova, L., Lactate dehydrogenase activity in human placenta following exposure to environmental pollutants. Physiol Res, **50**, 525-528, (2001)
[37] Nemmar, A., Hoylaerts, M. F., Hoet, P. H., Dinsdale, D., Smith, T., Xu, H., Vermylen, J., and Nemery, B., Ultrafine particles affect experimental thrombosis in an in vivo hamster model. Am J Respir Crit Care Med, **166**, 998-1004, (2002)
[38] Gatti, A. M., Montanari, S., Monari, E., Gambarelli, A., Capitani, F., and Parisini, B., Detection of micro- and nano-sized biocompatible particles in the blood. J Mater Sci Mater Med, **15**, 469-472, (2004)
[39] Pope, C. A., 3rd, Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk?, Environ Health Perspect, **108** Suppl 4, 713-723. (2000)