SUMMARY AND CONCLUSIONS

I. Summary

Diabetes is a multisystem disorder and complications of diabetes induce physiological changes in tissues and cells that impair the normal healing process. The pathophysiologic relationship between diabetes and impaired healing is complex. The diabetic wounds get stuck in the inflammatory phase featured by continuing influx of neutrophils that release cytotoxic enzymes.

Wounds that are difficult to heal represent a serious public health problem. The lesions severely affect the quality of life of individuals due to decreased mobility and substantial loss of productivity; they can also cause emotional damage and con-tribute to increase the burden of public expenditures in healthcare.

Physical treatments such as therapeutic ultrasound, Laser and electrotherapy are cited likewise in the literature as important adjuncts in wound management. These therapies seem to be advantageous but they have limitations and do not always achieve satisfactory results.

In recent years nanotechnology has been emerging as a rapidly growing field with numerous applications in science and technology.

This work aimed to evaluate the role of antibacterial nanofibers by incorporating silver nanoparticles into nanofibers using electrospinning as wound dressing on excisional wound healing dynamics in diabetic mice in comparison with physical methods such as therapeutic ultrasound and photostimulation using laser.

In the present study, we prepared different types of nanomaterials such as Ag nanoparticles using chemical reduction process, CA nanofibers and Ag loaded CA nanofibers using electrospinning process. The prepared Ag nanoparticles were characterized using Transmission Electron Microscope (TEM) to determine the morphology and the size of nanoparticles, X-ray Diffraction (XRD) pattern in order to evaluate crystalline state and Particle Size Analyzer (PSA) determine the particle size distribution curve of Ag nanoparticles.

The prepared CA and Ag loaded CA nanofibers were characterized in terms of morphology by scanning and transmission electron microscope, Fourier-transform Infrared spectroscopy (FTIR) to investigate functional groups. The antibacterial activity Ag loaded CA nanofibers with (1, 3 and 5 %) $AgNO_3$ using Disc Diffusion test and Growth kinetics study was determined.

Accordingly Ag nanoparticles, CA and CA loaded with 5% used for wound dressing in excisional wound of diabetic mice in comparison with physical therapy as follow:

Group (I): Diabetic free served as control group (10 mice) injected with citrate buffer.

Group (II): Experimental group (60 mice) injected with Streptozotocin, this group will be divided into the following subgroups:

IIa- Experimental group (10 mice), their open wounds left without treatment.

IIb- Experimental group (10 mice), their open wounds treated with Ag NPs only (50μL every other day).

IIc- Experimental group (10 mice), their open wounds dressed with CA nanofiber only.

IId- Experimental group (10 mice), their open wounds dressed with CA nanofiber contain AgNPs.

IIe- Experimental group (10 mice) treated with insulin combined with laser (650 nm, 150 mW) with a fluence of 5 J/cm^2 5 minutes daily, for 10 days.

IIf- Experimental group (10 mice) treated with insulin combined with pulsed ultrasound (0.5 W/cm^2) 5 minutes daily, for10 days.

- The healing was noticed and assessed during the treatment period and the Healing Factor Was determined.
- Histopatholgical Examination of mice skin during period of healing was done by taking skin from each group in 4th, 10th, 15th days of treatment period.
- Taking after healing period and determine the mechanical properties of healed skin

The results of the present work are:

For Ag nanoparticles they were spherical in shape and there is a variation in particle sizes ranging from 4.32nm to 7.27 nm with an average size 5.79 nm, from the XRD pattern it is clear that silver nanoparticles were essentially crystalline in nature and from particle size analyzer they were in a range of 29-41 nm. The highest fraction of Ag NP present in the solution was of 33.3 nm.

Good quality nanofibers for CA and Ag loaded CA nanofibers with smooth surface were obtained for CA and rough surface for Ag loaded CA nanofibers due to presence of Ag nanoparticles. FTIR showed that there are no major difference between the two samples in the major peak bands. However, it is also seen that the intensities of some peaks increase, this can be attributed to the presence of Ag NPs. The antibacterial activity of CA loaded with Ag Nanoparticles indicated high activity of CA loaded with 5% Ag nanoparticles.

Wound dressing using nanofibers loaded with Ag NPs improve the skin wound healing and the mechanical properties of the treated skin of the diabetic mice. Marked improvement in wound strength and healing due to increase in formation of collagen fibers and activity of the epithelial covering in mice receiving STZ and treated with insulin in combination with dressing with nanofibers loaded with Ag NPs.

Healing factor of different group increases with increasing of the treatment time. Healing factor of the diabetic mice treated with insulin dressed with nanofibers loaded with Ag NPs shows a healing factor higher than that of the normal group.

By comparing results obtained from different treatment modalities, we found that all therapeutic tools facilitate and accelerate diabetic wound healing, and improving scar quality but with different degree, and the best method is to use nanomaterials (nanofibers in conjugation with antimicrobial nanoparticles) which act as scaffold.

Conclusion

1- Nanotechnology is a very promising field that can be used in different application and science like in medicine.

2- Nanomaterials especially metal nanoparticles and nanofibers are wonderful materials that have unique properties than their original materials.

3- Abnormal wound healing that can occur during many pathological states like in diabetic patients is a very dangerous and can lead to mortality in some cases, can be treated using new therapies like using of nanotechnology.

REFERENCES

1. Mishra MH, Kumar K, Tripathi K. Diabetic delayed wound healing and the role of silver nanoparticles. Digest J Nanoma Biostruct 2008; 3: 49-54.

2. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50: 537- 46.

3. Al-Waiban FH, Zhang XY, Andres BL. Low-Level Laser Therapy Enhances Wound Healing in Diabetic Rats: A Comparison of Different Lasers. Photomedicine and Laser Surgery 2007; 25: 72-7.

4. Peter AL. Bacterial resistance to antibiotics: modified target sites. Adv Drug Deliver Rev 2005; 57: 1471-85.

5. Kim JS, Kuk E , Yu KN , Kim J-H , Park SJ , Lee HJ. Antimicrobial effects of silver nanoparticles Nanomedicine: Nanotechnology. Biology and Medicine 2007; 3:95-101.

6. Sweitzer SM, Fann SA, Borg TK, Baynes JW, Yost MJ. What is the future of diabetic wound care? Diabetes Educ 2006; 32: 197-210.

7. Byl NN, McKenzie AL, West JM, Whitney JD, Hunt TK, Sheuenstuhl HA. Low-dose ultrasound effects on wound healing: A controlled study with Yucatan pigs. Arch Phys Med Rehabil 1992;73: 656-64.

8. Abergel P, Lyons RF, Castel JC, Dwyer RM, Uitto J. Biostimulation of wound healing by lasers: experimental approaches in animal models and in fibroblast cultures. J Dermatol Surg Oncol 1997;13:127-33.

9. Conlan M, Rapley JW, Cobb CM. Bio stimulation of wound healing by low-energy laser irradiation: A review. J Clin Periodont. 1996; 23: 492-96.

10. Bisht D, Gupta SC, Misra V, Mital VP, Sharma P. Effect of low intensity laser radiation on healing of open skinwounds in rats. Indian J Med Res 1994; 100: 43-6.

11. Houreldand NN, Abrahamse H. “Low level laser therapy for diabetic wound healing.” The Diabetic Foot 2005; 8: 182-193,.

12. Fahey TJ, Sadaty A, Jones WG, Barber A, Smoller B, Shires GT. Diabetes impairs the late inflammatory response to wound healing. J Surg Res 1991;50: 308-13.

13. Mester E, Koebner N. Laser application in promoting of wound healing. Laser Surg 1980; 8: 83-5.

14. Yaakobi T, Maltz L, Oron U. Promotion of bone repair in the cortical bone of the tibia in rats by low energy laser (He-Ne)irradiation. Calcif Tissue Int 1996; 59: 297-300.

15. Schindl A, Schindl M, Schindl L. Successful treatment of a persistent radiation ulcer by low power laser therapy. J Am Acad Dermatol 1997; 37: 646-8.

16. Lam TS, Abergel RP, Castel JC, Dwyer RM, Lesavoy MA, Uitto J. Laser stimulation of collagen synthesis in human skin fibroblast cultures. Lasers Life Sci 1986; 1:61-77.

17. Lyons RF, Abergel RP, White RA, Dwyer RM, Castel JC, Uitto J. Biostimulation of wound healing in vivo by a helium-neonlaser. Ann PlastSurg 1987; 18: 47-50.

18. Ribeiro MS, Silva DFT, Araujo CEN, Oliveira SF, Pelegrini CMR, Zorn TMT, et al. Effects of low-intensity polarized visible laser radiation on skin burns. A light microscopy study. J Clin Laser Med Surg 2004; 22: 59-66.

19. Gaida K, Koller R, Isler C, Aytekin O, Al-Awami M, Meissl G et al. Low level laser therapy a conservative approach to the burn scar. Burns 2004; 30: 362-7.

20. Hawkins D, Houreld N, Abrahamse H. Low level laser therapy (LLLT) as an effective therapeutic modality for delayed wound healing. Ann. New York Acad Sci 2005; 1056; 486-93.

21. Schindl A, Merwald H, Schindl L, Kaun C, Wojta J, Direct stimulatory effect of low-intensity 670 nm laser irradiation on human endothelial cell proliferation. Br J Dermatol. 2003; 148: 334-6.

22. Peavy GM. "Lasers and laser-tissue interaction," Vet Clin North Am: Small Animal Practice 2002; 32: 517-34.

23. Hawkins D, Abrahamse H. Phototherapy - a treatment modality for wound healing and pain relief. African Journal of Biomedical Research 2007; 10: 99-109.

24. Karu TI, Pyatibrat LV, Afanasyeva NI. Cellular Effects of Low Power Laser Therapy Can be Mediated by Nitric Oxide. Lasers in Surgery and Medicine 2005; 36: 307-14.

25. Schindl A, Schindl M, Pernerstorfer-Sch¨on H, Schindl L. "Low-intensity laser therapy: a review". Journal of Investigative Medicine 2000; 48: 312-26.

26. Loevschall H and Arenholt-Bindslev D. "Effect of low level diode laser irradiation of human oral mucosa fibroblasts invitro". Lasers in Surgery and Medicine 1994; 14: 347- 54.

27. Basford JR. "The clinical status of low energy laser therapy in 1989". Journal of Laser Applications 1990; 2: 57-63.

28. Nemeth AJ. "Lasers and wound healing". Dermatologic Clinics 1993; 11: 783-9.

29. Tavares MR, Mazzer N, Patorello M. Efeito do laser therapeutic on acicatrizaço tendinosa: estudo experimental emratos. Fisioter Bras. 2005; 6: 96-100.

30. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. J PhotochemPhotobiol B. 1995; 27: 219-23.

31. Bibikova A, Belkin V, Oron U. Enhancement of angiogenesis in regenerating gastrocnemius muscle of the toad (Bufoviridis) by low-energy laser irradiation. Anat Embryol (Berl). 1994; 190: 597-602.

32. Bayat M, Vasheghani MM, Razavi N. Effects of Low-level helium-neon laser therapy on the healing of third-degree burn in rats. J Photochem Photobio 2006; 83: 87-93.

33. Maiya GA, Kumar P, Rao L. Effect of low intensity helium-neon (He-Ne) laser irradiation on diabetic wound healing dynamics. J Photomed Laser Surg 2005; 23:187-90.

34. Walker MD, Rumpf S, Baxter GD, Hirst DG, Lowe AS. Effect of low-intensity laser irradiation (660nm) on a radiation- impaired wound -healing model in murine skin. J Laser Surg Med 2000; 26: 41-7.

35. Vladimirov IA , Klebanov GI, Borisenko GG, Osipov AN. Molecular and cellular mechanisms of the low intensity laser radiation effect. J. Biofizika.; 49:339-50.

36. Paul BJ, Lafrayya CW, Dawson AR, Babb E, Bullock F. Use of ultrasound in the treatment of pressure sores in patients with spinal cord injury. Arch Phys Med Rehabil 1960; 41:438-40.

37. Dyson M, Pond JB. The effect of pulsed ultrasound on tissue regeneration. Physiotherapy 1970; 56:136-42.

38. Young SR, Dyson M. Effect of therapeutic ultrasound on the breaking of full-thickness excised skin lesions. Ultrasonics 1990; 28: 170-80.

39. Dyson M, Moodley S, Verjee L, Verling W, Weinman J, Wilson P. Wound healing assessment using 20 MHz ultrasound and photography. Skin Res Technol 2003; 9: 116-21.

40. Nussbaum E, Biemann I, Mustard B. Comparison of ultrasound/ultraviolet-C and laser for treatment of pressure ulcers in patient with spinal cord injury. PhysTher. 1994; 79: 812-23.

41. Riet G, Kessels AG, Knipschild PG. A randomized clinical trial of ultrasound in the treatment of pressure ulcers. Phys Ther. 1996; 76 :1301-11.

42. Lundeberg T, Northstrom F, Brodda-Jansen G, Eriksson SV, Kjartansson J, Samuelson UE. Pulsed ultrasound does not improve healing of venous ulcers. Scand J Rehabil Med 1990; 22:195-97.

43. Cambier DC, Vanderstraeten GG. Failure of therapeutic ultrasound in healing burn injuries. Burns 1997; 23: 248-49.

44. Gan BS, Huys S, Sherebrin MH, Scilley CG. The effects of ultrasound treatment on flexor tendon healing in the chicken limb. J Hand Surg (Br) 1995; 20: 809-14.

45. Lundeberg T, Abrahamsson P, Haker E. A comparative study of continuous ultrasound, placebo ultrasound and rest in epicondylalgia. Scand J Rehabil Med 1998; 20: 99-101.Rehabil. 1992; 73: 656-64.

46. Hess CL, Howard MA &Attinger CE. A review of mechanical adjuncts in wound healing: hydrotherapy, ultrasound, negative pressure therapy, hyperbaric oxygen, and electrostimulation. Ann PlastSurg 2003; 51: 210-8.

47. Ly M, Poole-Warren LA. Acceleration of wound healing using electrical fields: time for a stimulating discussion. Wound Practice and research 2008; 16:138-51

48. Baker KG, Robertson VJ, Duck FA . "A Review of Therapeutic Ultrasound: Biophysical Effects". Physical Therapy 2001; 81: 1351-8.

49. Theron J, Walker JA, Cloete TE. Nanotechnology and water treatment: Applications and emerging opportunities. Critical Reviews In Microbiology 2008; 34: 43-69.

50. Khil MS., Cha DI, Kim HY, Kim IS, Bhattarai N. Electrospun nanofibrous polyurethane membrane as wound dressing. Journal of Biomedical Materials Research Part B: Applied Biomaterials 2003; 67: 675-9.

51. Schneider A, Wang XY, Kaplan DL, Garlick JA, Egles C. Bio functionalized electrospun silk mats as a topical bioactive dressing for accelerated wound healing. Acta Biomaterialia 2009a; 5: 2570-8.

52. Rho KS, Jeong L, Lee G, Seo BM, Park YJ, Hong SD et al . Electrospinning of collagen nanofibers: Effects on the behavior ofnormal human keratinocytes and early-stage wound healing. Biomaterials 2006; 27: 1452-61.

53. Liu SJ, Kau YC, Chou CY, Chen JK, Wu RC, Yeh WL. Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing. Journal of Membrane Science 2010a, Vol.355, No.1-2, pp 53-59.

54. Choi JS, Leong KW, Yoo HS. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF).Biomaterials 2008; 29: 587-96.

55. Liu X, Lin T, Fang J, Yao G, Wang X. Electrospun nanofibre membranes as wound dressing materials. Advances in Science and Technology, Biomedical Applications of Smart Materials, Nanotechnology and Micro/NanoEngineering, 2008; 57: 125-130.

56. Liu X, Lin T, Fang J, Yao G, Zhao H, Dodson M, et al. In vivo wound healing and antibacterial performances of electrospun nanofibre membranes.Journal of Biomedical Materials Research, Part A, 2010b; 94: 499-508.

57. Bolgen N, Vargel I, Korkusuz P, Menceloglu YZ, Piskin E. In vivo performance of antibiotic embedded electrospun PCL membranes for prevention ofabdominal adhesions. Journal of Biomedical Materials Research, Part B: Applied Biomaterials 2007; 81: 530-43.

58. Hong KH . Preparation and properties of electrospun poly (vinyl alcohol)/silver fiber web as wound dressings. Polymer Engineering and Science 2007; 47: 43-9.

59. Chen ZG, Wang PW, Wei B, Mo XM, Cui F Z. Electrospun collagen chitosan nanofiber: A biomimetic extracellular matrix for endothelial cell and smooth muscle cell. Acta Biomaterialia 2010b; 6: 372-82.

60. Ignatova M, Manolova N, Rashkov I. Electrospinning of poly (vinylpyrrolidone)-iodine complex and poly (ethylene oxide)/poly(vinyl pyrrolidone)-iodine complex - a prospective route to antimicrobial wound dressing materials. European Polymer Journal, 2007; 43: 1609-23.

61. Kenawy ER, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders EH et al. Release of tetracycline hydrochloride from electrospun poly (ethylene-co-vinylacetate), poly(lactic acid), and a blend. Journal of Controlled Release, 2002; 81: 57-64

62. Zhou W, He J, Cui S, Gao W. Studies of Electrospun Cellulose Acetate Nanofibrous Membranes .The Open Materials Science Journal 2011; 5: 51-5.

63. Glasser WG. Prospects for future applications of cellulose acetate. Macromol Symp 2004; 208:371–394

64. Yu DG, Chatterton NP, Yang JH, Wang X, Liao YZ. Coaxial electrospinning with triton X-100 solutions as sheath fluids for preparing PAN nanofibers. Macromol Mater Eng 2012a; 297:395–401

65. Es-saheb M, Sherif E-S M, El-Zatahry A, El Rayes MM, Khalil KA. Corrosion Passivation in Aerated 3.5% NaCl solutions of Brass by Nanofiber Coatings of Polyvinyl Chloride and Polystyrene. Int J Electrochem Sci 2012; 7: 10442 –55.

66. Greiner A, Wendorff JH. Electrospinning: A fascinating method for the preparation of ultrathin fibres. Angewandte Chemie-International Edition 2007; 46: 5670-703.

67. Osman AH, Kamel MM, Wahdan MH, Al-gazaly M. Assessment to the Effects of Low Power Diode Laser on Wound Healing in Diabetic Rat. Life Sci J 2013; 10: 1313-21.

68. Nasirian N, Nasirian A. Effect of Low Level Laser Therapy on the Surgical Wound Healing in Hamster. Iranian Journal of Pathology 2012; 7: 236-40.

69. Basavaraj M. Kajagar, Godhi AS, Pandit A, Khatri S. Efficacy of Low Level Laser Therapy on Wound Healing in Patients with Chronic Diabetic Foot Ulcers A Randomised Control Trial. Indian J Surg 2012; 74: 359-63.

70. Kaskos HH, Al-Hasan AH. Effect Of Low Level Laser Therapy On Intra Oral Wound Healing, Al–Rafidain Dent J 2011; 11:105-12.

71. Hussein AJ, Alfars AA, Falih MAJ, Hassan A-NA. Effects of a low level laser on the acceleration of wound healing in rabbits. North Am J Med Sci 2011; 3: 193-7.

72. Alipanah Y, Asnaashari M, Anbari F. The effect of low level laser (GaAlAs) therapy on the post-surgical healing of full thickness wounds in rabbits. Medical Laser Application 2011; 26: 133-8.

73. Mokmeli S, Daemi M, Shirazi ZA, Shirazi FA, Hajizadeh M. Evaluating the Efficiency of Low Level Laser Therapy (LLLT) in Combination With Intravenous Laser Therapy (IVL) on Diabetic Foot Ulcer, Added to Conventional Therapy. Journal of Lasers in Medical Sciences JLMS 2010; 1:8-13.

74. Bayat M, Vasheghani MM, Razavie N, Jalili MR. Effects of low-level laser therapy on mast cell number and degranulation in third-degree burns of rats. JRRD 2008; 45:931-8.

75. Baranauskas GV. Cruz-Höfling MA. The effects of low laser irradiation on angiogenesis in injured rat tibiae. Histol Histopathol 2004; 19: 43-8.

76. Demir H, Balay H, Kirnap M. A comparative study of the effects of electrical stimulation and laser treatment on experimental wound healing in rats. Journal of Rehabilitation Research & Development JRRD 2004; 41: 147-54.

77. Mahran HG. Influence of contact ultrasonic with different power densities on full-thickness wounds healing: An Experimental Study. International Journal of Physiotherapy and Research,Int J Physiother Res 2014; 2: 567-76.

78. Sadraie SH, Kaka GhR, Mofid M, Torkaman G, Monfared MJ. Effects of Low Intensity Pulsed Ultrasound on Healing of Denervated Tibial Fracture in the Rabbit Iran Red Crescent Med J 2011; 13: 34-41.

79. Kavros SJ, Lied DA, Boon AJ, Miller JL, Hobbs JA, Andrews KL. Expedited wound healing with noncontact, low-frequency ultrasound therapy in chronic wounds: A Retrospective Analysis .Advances In Skin & Wound Care 2008; 21: 416-22.

80. Demir H, Menku P, Kirnap M, Calis M, Ikizceli I. Comparison of the Effects of Laser, Ultrasound, and Combined Laser and Ultrasound Treatments in Experimental Tendon Healing. Lasers in Surgery and Medicine 2004; 35: 84-9.

81. Rubin C, Bolander M, Ryaby JP, Hadjiargyrou M. The Use of Low-Intensity Ultrasound to Accelerate the Healing of Fractures. The Journal Of Bone & Joint Surgery JbjsOrg 2001; 83: 259-70.

82. Kataria K, Gupta A, Rath G, Mathura RB, Dhakate SR. In vivo wound healing performance of drug loaded electrospun composite nanofibers transdermal patch. International Journal of Pharmaceutics 2014; 469: 102-10.

83. Lee SJ, Heoa DN, Moona J-H, Ko W-K, Lee JB, Bae MS, et al. Electrospun chitosan nanofibers with controlled levels of silver nanoparticles. Preparation, characterization and antibacterial activity. Carbohydrate Polymers 2014; 111: 53-7.

84. Wu J, Zheng Y, Song W, Luan J, Wen X, Wu Z, et al. In situ synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing. Carbohydrate Polymers 2014; 102: 762-771.

85. Dubský M, Kubinová S, Sirc J, Voska L, Zajíček R, Zajícová A, et al. Nanofibers prepared by needleless electrospinning technology as scaffolds for wound healing. J Mater Sci: Mater Med 2012; 23: 931-41.

86. Leung V, Hartwell R, Yang H, Ghahary A, Ko F. Bioactive Nanofbres for Wound Healing Applications. Journal of Fiber Bioengineering & Informatics 2011; 4: 1-14.

87. Maa Z, Ji H, Tan D, Teng Y, Dongc G, Zhou J ,et al. Silver nanoparticles decorated, flexible SiO_2 nanofibers with long-term antibacterial effect as reusable wound cover. Colloids and Surfaces A: Physicochem. Eng Aspects 2011; 387: 57-64.

88. Miao J, Pangule RC, Paskaleva EE, Hwang EE, Kane RS, Linhardt RJ, et al. Lysostaphin-functionalized cellulose fibers with anti-staphylococcal activity for wound healing applications. Biomaterials 2011; 32: 9557-67.

89. Mathivanan V, Ananth S, Priyanga B, PRABU PG, SELVISABHANAYAKAM. Impact of Silver Nanoparticles on Wound Healing of Freshwater Fish, Anabas testudineus. International Journal of Research in Fisheries and Aquaculture 2012; 2: 17-21.

90. Ghosh S, Upadhay A, Singh AK, Kumar A. Investigation of antimicrobial activity of silver nanoparticle loaded cotton fabrics which may promote wound healing. International Journal of Pharma and Bio Sciences 2010; 1: 1-10.

91. Atiyeh BS, Costagliola M, Hayek SN, Dibo SA. Effect of silver on burn wound infection control and healing: Review of the literature. Burns 2007; 33: 139-48.

92. Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM, et al. Topical Delivery of Silver Nanoparticles Promotes Wound Healing. Chem Med Chem 2007; 2: 129-36.

93. Douglas MacKay, ND, and Alan L. Miller, ND. Nutritional Support for Wound Healing. Alternative Medicine Review. 2003; 8: 359-77.

94. Tortora, G.J., Grabowski, S.R. Principles of Anatomy and Physiology (8th edn). New York: Harper Collins College Publications, 1996.

95. Krasnor, D. Chronic Wound Care: A clinical sourcebook for healthcare professionals (2nd edn). Wayne, Pa: Health Management Publications, 1996 .

96. Flannigan K. Nutritional aspects of wound healing. Advances in wound care 1997; 10: 48-52.

97. Stadelmann WK, Digenis AG, Tobin GR. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg1998; 176: 26-38.

98. Blee TH, Cogbill TH, Lambert PJ. Hemor-rhage associated with vitamin C deficiency in surgical patients. Surgery 2002; 131: 408-12.

99. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: can the dooms day scenario be averted. J Intern Med, 2001; 247: 301-10.

100. Amos A, McCarty D, Zimmet P: The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabetic Med, 1987; 14: S1–S85

101. No author listed. Economic costs of diabetes in the U.S in 2007. Diabetes Care, 2008, 31, 596-615.

102. No author listed. Consensus Development Conference on Diabetic Foot Wound Care: 7-8 April 1999, Boston, Massachusetts. Ameri-can Diabetes Association. Diabetes Care, 1999; 22: 1354-60.

103. No author listed. Epidemiology of lower extremity amputation in centres in Europe, North America and East Asia. The Global Lower Extremity Amputation Study Group. Br. J. Surg., 2000; 87: 328-37 .

104. Falanga, V. Wound healing and its impairment in the diabetic foot. Lancet 2005; 366: 1736-43 .

105. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999; 341: 738-46 .

106. Galkowska H, Olszewski WL, Wojewodzka U Rosinski G, Karnafel W. Neurogenic factors in the impaired healing of diabetic foot ulcers. J Surg Res 2006; 134: 252-8.

107. Davies CE, Hill KE, Wilson MJ et al. Use of 16S ribosomal DNA PCR and denaturing gradient gel electrophoresis for analysis of the microfloras of healing and non-healing chronic venous leg ulcers. J Clin Microbiol 2004; 42: 3549-57.

108. Poole-Warren LA & Ly M. Wound healing. In: Akay M (Ed). Encyclopedia of BME. Hoboken, N.J: John Wiley & Sons Inc., 2006, p.3862-70.

109. Gogia PP. “Physical therapy modalities for wound management,” Ostomy Wound Manag 1996; 42: 46-48, 54: 50-52.

110. Tunér J. The Cochrane analyses - can they be improved? Laser Therapy. 1999; 11: 138-43.

111. Ashford R, Brown N, Lagan K, Howell C, Nolan C, Brady D, et al. Low intensity laser therapy for chronic venous leg ulcers. Nursing Standard 1999; 14: 66-72.

112. Houghton PE. Effects of therapeutic modalities on wound healing: a conservative approach to the management of chronic wounds. Phys Ther Rev 1999; 4 :167-82.

113. Pinfildi CE, Liebano RE, Hochman BS, Ferreira LM. Helium-neon laser in viability of random skin flap in rats. Lasers Surg Med 2005; 37:74-7.

114. Wheeland RG. Lasers for stimulation or inhibition of wound healing. J Dermatol Surg Oncol 1993; 19:747-52.

115. Forney R, Mauro T. Using lasers in diabetic wound healing. Diabetes Tech Ther 1999; 1:189-92.

116. Tunér J, Hode L. Standards for laser therapy studies. J Wound Care 2005; 4: 478-9.

117. Kimura Y, Wilder-Smith P, Matsumoto K. Lasers in endodontics: a review. Int Endod J 2000; 33:173-85.

118. Neiburger EJ. Accelerated healing of gingival incisions by the helium-neon diode laser: a preliminary study. Gen Dent 1997; 45: 166-70.

119. Mester E, Spiry T, Szende B, Tota JG. Effect of laser rays on wound healing. Am J Surg 1971; 122: 532-5.

120. Hopkins JT, McLoda TA, Seegmiller JG, Baxter GD. Low-level laser therapy facilitates superficial wound healing in humans: A Triple-Blind, Sham-Controlled Study. J Athl Train 2004; 39: 223-9 .

121. Qadri T, Miranda L, Tunér J, Gustafsson A. The short-term effects of low-level lasers as adjunct therapy in the treatment of periodontal inflammation. J Clin Periodontol 2005; 32: 714-9.

122. Walsh LJ. The current status of low level laser therapy in dentistry. Part 1. Soft tissue applications. Aust Dent J 1997; 42: 247-54.

123. Damante CA, Greghi SL, Sant'ana AC, Passanezi E. Clinical evaluation of the effects of low-intensity laser (GaAlAs) on wound healing after gingivoplasty in humans. J Appl Oral Sci 2004; 12: 133-6.

124. Posten W, Wrone DA, Dover JS, Arndt KA, Silapunt S, Alam M. Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol Surg 2005; 31: 334-40.

125. Yu HS, Chang KL, Yu CL, Chen JW, Chen GS. Low-energy helium-neon laser irradiation stimulates interleukin-1 alpha and interleukin-8 release from cultured human keratinocytes. J Invest Dermatol 1996; 107: 593-6.

126. Kreisler MB, Haj HA, Noroozi N, Willershausen B. Efficacy of low level laser therapy in reducing postoperative pain after endodontic surgery a randomized double blind clinical study. Int J Oral Maxillofac Surg 2004; 33: 38-41.

127. Tuby H, Maltz L, Oron U. Modulations of VEGF and iNOS in the rat heart by low level laser therapy are associated with cardioprotection and enhanced angiogenesis. Lasers Surg Med 2006; 38: 682-8.

128. Pinheiro AL, Pozza DH, Oliveira MG, Weissmann R, Ramalho LM. Polarized light (400—2000 nm) and non-ablative laser (685 nm): a description of the wound healing process using immunohisto-chemical analysis. Photomed Laser Surg 2005; 23: 485-92.

129. Reddy GK, Stehno-Bittel L, Enwemeka CS. Laser photostimula-tion accelerates wound healing in diabetic rats. Wound Repair Regen 2001; 9: 248-55.

130. Anneroth G, Hall G, Rydén H, Zetterqvist L. The effect of low-energy infra-red laser radiation on wound healing in rats. Br J Oral Maxillofac Surg 1988; 26: 12-7.

131. Bisht D, Mehrotra R, Singh PA, Atri SC, Kumar A. Effect of helium—neon laser on wound healing. Indian J Exp Biol 1999; 37: 187-90.

132. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med 2002; 13: 474-84.

133. Pitaru S, McCulloch CA, Narayanan SA, Cellular origins and differentiation control mechanisms during periodontal development and wound healing. J Periodontal Res 1994; 29: 81–94.

134. Kreisler M, Christoffers AB, Willershausen B, d'Hoedt B. Effect of low-level GaAlAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an in vitro study. J Clin Periodontol 2003; 30: 353-58.

135. Almeida-Lopes L, Rigau J, Zangaro RA, Guidugli-Neto J, Jaeger MM . Comparison of the low level laser therapy effects on cultured human gingival fibroblasts proliferation using different irradiance and same fluence. Lasers Surg Med 2001; 29: 179-84.

136. Pereira AN, Eduardo Cde P, Matson E, Marques MM. Effect of low-power laser irradiation on cell growth and procollagen synthesis of cultured fibroblasts. Lasers Surg Med 2002; 31: 263-267.

137. Azevedo LH, de Paula Eduardo F, Moreira MS, de Paula Eduardo C, Marques MM. Influence of different power densities of LILT on cultured human fibroblast growth: a pilot study. Lasers Med Sci 2006; 21: 86: 9.

138. Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD. Effect of wavelength on low-intensity laser irradiationstimulated cell proliferation in vitro. Lasers Surg Med 2005; 36: 8-12.

139. Kreisler M, Christoffers AB, Al-Haj H, Willershausen B, d'Hoedt B. Low level 809-nm diode laser-induced in vitro stimulation of the proliferation of human gingival fibroblasts. Lasers Surg Med 2002; 30: 365-9.

140. Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K. Determining optimal dose of laser therapy for attachment and proliferation of human oral fibroblasts cultured on titanium implant material. J Biomed Mater Res A 2005;73: 55-62.

141. Pourzarandian A, Watanabe H, Ruwanpura SM, Aoki A, Ishikawa I. Effect of low-level Er:YAG laser irradiation on cultured human gingival fibroblasts. J Periodontol 2005; 76: 187-93.

142. Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. Photochem Photobiol 1994; 59:167-70.

143. Walsh LJ, Murphy GF. The role of adhesion molecules in cutaneous inflammation and neoplasia. J Cutan Pathol 1992;19:161-71.

144. Enwemeka CS. Laser biostimulation of healing wounds: specific effects and mechanisms of action. J Orthop Sports Phys Ther 1988; 9: 333-8.

145. Noble PB, Shields ED, Blecher PDM, Bentley KC. Locomotory characteristics of fibroblasts within a three-dimensional collagen lattice: Modulation by a Helium/Neon soft laser. Lasers Surg Med 1992; 12: 669-74.

146. Pourreau-Schneider N, Ahmed A, Soudry M, Jacquemier J, Kopp F, Franquin JC, Martin PM. Helium-Neon laser treatment transforms fibroblasts into myofibroblasts. Am J Pathol 1990; 137: 171-8.

147. Yu W, Naim JO, Lanzafame RJ. Effects of photostimulation on wound healing in diabetic mice. Lasers Surg Med 1997; 20: 56-63.

148. Lyons RF, Abergel RP, White RA, Dwyer RM, Castel JC, Uitto J. Biostimulation of wound healing in vivo by a helium-neon laser. Ann Plast Surg. 1987; 18: 47-50.

149. Marei MK, Abdel-Meguid SH, Mokhtar SA, Rizk SA. Effect of low-energy laser application in the treatment of denture-induced mucosal lesions. J Prosthet Dent. 1997; 77: 256-64.

150. De Arau´jo CEN, Ribeiro MS, Favaro R, Zezell DM. Zorn TMT. Ultra structural and autoradiographical analysis show a faster skin repair in He–Ne laser-treated wounds. Journal of Photochemistry and Photobiology B: Biology 2007; 86: 87-96.

151. Wheeland RG. Lasers for the stimulation or inhibition of wound healing. J Dermatol Surg Oncol 1993; 19: 747-52.

152. Lyons RF, Abergel RP, White RA, Dwyer RM, Castel JC, Uitto J. Biostimulation of wound healing in vivo by a helium-neon laser. Ann Plast Surg 1987; 18: 47-50.

153. Yu HS, Chang KL, Yu CL, Chen JW, Chen GS. Low-energy helium–neon laser irradiation stimulates interleukin-1 alpha and interleukin-8 release from cultured human keratinocytes. J Invest Dermatol 1996; 107: 593-6.

154. Mester E, Mester AF, Mester A. The biomedical effects of laser application. Lasers Surg Med 1985; 5: 31-9.

155. Schindl A, Schindl M, Schon H, Knobler R, Havelec L, Schindl L. Low-intensity laser irradiation improves skin circulation in patients with diabetic microangiopathy. Diabet Care 1998; 21: 580-4.

156. Schindl M, Kerschan K, Schindl A, Schon H, Heinzl H, Schindl L. Induction of complete wound healing in recalcitrant ulcers by low- intensity laser irradiation depends on ulcer cause and size. Photodermatol Photoimmunol Photomed 1999; 15:18-21.

157. Schindl A, Schindl M, Pernerstorfer-Schon H, Kerschan K, Knobler R, Schindl L. Diabetic neuropathic foot ulcer: successful treatment by low-intensity laser therapy. Dermatology 1999; 198: 314-6.

158. Schindl A, Schindl M, Schindl L, Jurecka W, Honigsmann H, Breier F. Increased dermal angiogenesis after low-intensity laser therapy for a chronic radiation ulcer determined by a video measuring system. J Am Acad Dermatol 1999; 40: 481-4.

159. Bouma MG, Buurman WA, van den Wildenberg FA. Low energy laser irradiation fails to modulate the inflammatory function of human monocytes and endothelial cells. Lasers Surg Med 1996; 19: 207-15.

160. Miller M, Theilade E, Jensen S. Lasers in dentistry: an overview. JADA 1993; 124: 32-5.

161. Neiburger EJ. The effect of low power lasers on intraoral wound healing. NY State Dent J 1995; 61: 40-3.

162. Kuo T, Speyer MT, Ries WR, Reinisch L. Collagen thermal damage and collagen synthesis after cutaneous laser resurfacing. Lasers Surg Med 1998; 23: 66-71.

163. Young S, Bolton P, Dyson M, Harvey W, Diamantopoulos C. Macrophage responsiveness to light therapy. Lasers Surg Med1989; 9: 497-505.

164. Kawalac JS, Pfenningwerth TC, Hetherington VJ, Logan JS, Penfield VK, Flauto JA, et al. A review of lasers in healing diabetic ulcers. The Foot 2004; 14: 68-71.

165. Ohshiro T. Light and Life: A Review of Low Reactive-Laser Therapy, Following 13 Years Experience In Over 12,000 Patients. Laser Therapy 1993; 5: 5-22.

166. Haar G. Therapeutic ultrasound. European Journal of Ultrasound 1999; 9: 3-9.

167. Pye S, Milford C. The performance of ultrasound therapy machines in Lothian region, 1992. Ultrasound Med Biol 1994;20: 347 -59.

168. Maylia E, Nokes LD.The use of ultrasonics in orthopaedics—a review. Tech-nol Health Care 1999; 7: 1-28.

169. Kaufman JJ, Einhorn TA. Ultrasound assessment of bone. J Bone Miner Res 1993; 8:517-25.

170. Moed BR, Kim EC, van Holsbeeck M, Schaffler MB, Subramanian S, Bouf-fard JA, et al. Ultrasound for the early diagnosis of tibial fracture healing after static interlocked nailing without reaming: histologic correlation using a canine model. J Orthop Trauma 1998; 12: 200-5.

171. Dyson M. Role of ultrasound in wound healing. In: Kloth LC, McCulloch JM, Feedar JA, editors. Wound Healing: Alter-natives in Management. Philadelphia, PA: Davis, 1990; 2: 59-65.

172. Qian Z, Sagers RD, Pitt WG. The effect of ultrasonic frequency upon enhanced killing of P. aeruginosa biofilms. Ann Biomed Eng 1997; 25: 69-76.

173. Ennis WJ, Valdes W, Gainer M, Meneses P. Evaluation of clinical effectiveness of MIST ultrasound therapy for the healing of chronic wounds. Adv Skin Wound Care 2006; 19: 437-46.

174. Kavros SJ, Miller JL, Hanna SW. Treatment of ischemic wounds with noncontact, low-frequency ultrasound: the Mayo clinic experience, 2004-2006. Adv Skin Wound Care 2007; 20: 221-6.

175. Herberger K, Franzke N, Blome C, Kirsten N, Augustin M. Efficacy, tolerability and patient benefit of ultrasound-assisted wound treatment versus surgical debridement: a randomized clinical study. Dermatology 2011; 222: 244-9.

176. Huang C, Notten A, Rasters N. Nanoscience and technology publications and patents: a review of social science studies and search strategies. J Technol Transf 2011; 36: 145-72.

177. Gelain F. Novel opportunities and challenges offered by nanobiomaterials in tissue engineering. Int J Nanomed. 2008; 3:415-24.

178. Nel A, Xia T, Mädler L, Li N. Toxic potential of materials at the nanolevel. Science 2006; 311: 622-27. 19.

179. Warheit DB, Webb TR, Colvin VL, Reed KL, Sayes CM. Pulmonary Bioassay Studies with Nanoscale and Fine-Quartz Particles in Rats: Toxicity Is Not Dependent upon Particle Size but on Surface Characteristics. Toxicological Sciences 2007; 95: 270-80.

180. Wang H-S, Fu G-D, Li X-S. Functional Polymeric Nanofibers from Electrospinning. Recent Patents on Nanotechnology 2009; 3: 21-31.

181. Deitzel JM, Kleinmeyer J, Hirvonen JK, Tan NCB. Controlled deposition of electrospun poly (ethylene oxide) fibers. Polymer 2001; 42: 8163-70.

182. Reneker DH, Yarin AL, Zussman E, Xu H. Electrospinning of nanofibers from polymer solutions and melts. Adv Appl 2007; 41: 43-195.

183. Khan AS, Ahmed Z, Edirisinghe MJ, Wong FSL, Rehman IU. Preparation and characterization of a novel bioactive restorative composite based on covalently coupled poly-urethane-nanohydroxyapatite fibres. Acta Biomater. 2008; 4: 1275–87.

184. Lei J, Zhao Z , Li Z , Wang C , Wang W , Wang Z . Ag/AgCl coated polyacrylonitrile nanofiber membranes: synthesis and photocatalytic properties. React Funct Polym. 2011; 71: 1071–6.

185. Xing X, Wang Y, Li B. Nanofibers drawing and nanodevices assembly in poly(trimethylene terephtalate). Optics Express. 2008; 16:10815–22.

186. Niece KL, Hartgerink JD, Donners JJ, Stupp SI. Self-assembly combining two bioactive peptide-amphiphile molecules into nanofibers by electrostatic attraction. J Am Chem Soc. 2003; 125: 7146-7.

187. Huang ZM, Zhang YZ, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electros-pinning and their applications in nanocomposites. Compos SciTechnol. 2003; 63: 2223-53.

188. Martins A, Reis RL. Electrospinning: processing technique for tissue engineering scaffolding. Int Mater Rev. 2008; 53: 257-74.

189. Thompson CJ. , Chase GG, Yarin AL , Reneker DH. Effects of parameters on nanofiber diameter determined from electrospinning model. Polymer 2007; 48: 6913-22.

190. Ahmad Z, Zhang HB , Farook U , Edirisinghe M , Stride E , Colombo P. Deposition of nano-hydroxyapatite particles utilising direct and transitional electro hydrodynamic processes. J Mater Sci Mater Med. 2008; 19: 3093-104.

191. Beachley V, Wen X. Effect of electrospinning parameters on the nanofiber diameter and length. Mater Sci Eng C Mater Biol Appl 2009; 29: 663-8.

192. Ramakrishna S, Fujihara K, Teo WE, Yong T, Ma Z, Ramaseshan R. Electrospun nanofibers: solving global issues. Materials Today 2006; 9: 40-50.

193. Zong X, Kim K, Fang D, Ran S, Hsiao BS, Chu B. Structure and process relationship of electrospun biodegradable nanofibers membranes. Polymer 2002; 43: 4403-12.

194. Kubinova S, Sykova E. Nanotechnologies in regenerative medicine. Minim Invasive Ther Allied Technol. 2010; 19: 144-56.

195. Khang D, Carpenter J, Chun Y, Pareta R, Webster T. Nanotechnology for regenerative medicine. Biomed Micro devices 2010; 12: 575-87.

196. Kumbar SG, Nair LS, Bhattacharyya S, Laurencin CT. Polymeric nanofibers as novel carriers for the delivery of therapeutic molecules. J Nanosci Nanotechnol 2006; 6: 2591-607.

197. Dahlin RL, Kasper FK, Mikos AG. Polymeric nanofibers in tissue engineering. Tissue Eng. Part B. 2011; 17:349-64.

198. Katti DS, Robinson KW, Ko FK, and Laurencin CT. Bioresorbable nanofiber-based systems for wound healing and drug delivery: optimization of fabrication parameters. J Biomed Mater Res B Appl Biomater. 2004; 70: 286-96.

199. Kong H, Jang J. Antibacterial properties of novel poly(methyl methacrylate) nanofiber containing silver nanoparticles. Langmuir 2008; 24:2051-6.

200. Kuffler Damien P. Techniques for Wound Healing with a Focus on Pressure Ulcers Elimination,The Open Circulation and Vascular Journal 2010; 3: 72-84.

201. Wang HS, Fu G-D, Li X-S. Functional Polymeric Nanofibers from Electrospinning, Recent Patents on Nanotechnology 2009; 3:21-31.

202. Son WK, Youk JH, Lee TS, Park WH. Preparation of antimicrobial ultrafine cellulose acetate fibers with silver nanoparticles. Macromol Rapid Commun 2004; 25: 1632-7.

203. Lala NL, Ramaseshan R, Bojun L, Sundarrajan S, Barhate R, Ying-jun L, et al.. Fabrication of nanofibers with antimicrobial functionality used as filters: Protection against bacterial contaminants. Biotechnol Bioeng 2007; 97: 1357-65.

204. Yang DY, Lu B, Zhao Y, Jiang XY. Fabrication of aligned fibrous arrays by magnetic electrospinning. Adv Mater 2007; 19: 3702-6.

205. Jeong SI, Ko EK, Yum J, Jung CH, Lee YM, Shin H. Nanofibrous poly (lactic acid)/hydroxyapatite composite scaffolds for guided tissue regeneration. Macromol Biosci 2008; 8: 328-38.

206. Zhou G, Wang W. Synthesis of silver nanoparticles and their anti-proliferation against human lung cancer cells in vitro. Oriental Journal Of Chemistry. 2012; 28: 651-5.

207. Sheikh FA, Barakat Nasser AM, Kanjwal MA , Chaudhari AA, Jung I-H , Lee JH. Electrospun Antimicrobial Polyurethane Nanofibers Containing Silver Nanoparticles for Biotechnological Applications. Macromolecular Research 2009; 7: 688-96.

208. Sona WK, Youk JH, Park WH. Antimicrobial cellulose acetate nanofibers containing silver nanoparticles Carbohydrate Polymers 2006; 65: 430-4.

209. Soo-Hwan K, Lee H-S, Ryu D-S, Choi S-J, and Lee D-S. Antibacterial activity of silver-nanoparticles against staphylococcus aureus and escherichia coli.korean. J Microbiol Biotechnol, 2011; 39: 77-85.

210. Arora S, Ojha SK Vohora D. Characterisation of Streptozotocin Induced Diabetes Mellitus in Swiss Albino Mice Global Journal of Pharmacology 2009; 3: 81-84.

211. Shalaby TI, NASRA MK, Mohamed MM, Eldine RS. Effect of visible laser therapy on wound healing dynamics in diabetic induced mice. Romanian J Biophys 2013; 23:1-14.

212. Kant V, Gopal A, Pathak NN, Kumar P, Tandan SK, Kumar D. Antioxidant and anti-inflammatory potential of curcumin accelerated the cutaneous wound healing in streptozotocin-induced diabetic rats. International Immunopharmacology International Immunopharmacology 2014; 20: 322-30.

213. Bancroft JD, Stevans A, Turner DR. Theory and practice of histological techniques. 4 th Ed. Churchill Livinigstone, Edinburgh, London, Melbourne, New York 1996.

214. Maiya GA, Kumar P, Nayak BS. Photo-stimulatory effect of low energy helium-neon laser irradiation on excisional diabetic wound healing dynamics in wistar rats. Indian J Dermatol 2009; 54: 323-9.

215. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir.1996; 12: 788-800.

216. Berger TJ, Spadaro JA, Chapin SE, Becker RO. Electrically Generated Silver Ions: Quantitative Effects on Bacterial and Mammalian Cells Antimicrob Agents Chemother 1976; 9: 357-8.

217. Silver S. Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev 2003; 27: 341-53.

218. Nair LS, Laurencin CT. Silver nanoparticles: synthesis and therapeutic applications. Journal of Biomedical Nanotechnology 2007; 3: 301-16.

219. Liau S, Read D, Pugh W, Furr J, Russell A. Interaction of silver nitrate with readily identifiable groups: rel a tionship to the a nti b acterial action of silver ions. Lett Appl Microbiol 1997; 25: 279-83 .

220. Spacciapoli P, Buxton D, Rothstein D, Friden P. Antimicrobial activity of silver nitrate against periodontal pathogens. Periodontal J Res, 2001; 36: 108-13.

221. Ding B, Kimura E, Sato T, Fujita S, Shiratori S. Fabrication of blend biodegradable nanofibrous nonwoven mats via multi-jet electrospinning. Polymer 2004; 45:1895-902.

222. Nagendran A, Mohan DR. Cellulose acetate and polyetherimide blend ultrafiltration membranes: II. Effect of additive. Polym Adv Technol 2008; 19: 24-35.

223. Edgar KJ, Buchanan CM, Debenham JS, Rundquist PA, Seiler BD, Shelton MC, et al. Advances in cellulose ester performance and application. Prog Polym Sci 2001; 26: 1605-88.

224. Lee JH, Lee JH, Nam JD, Choi H, Jung K, Jeon JW, et al. Water uptake and migration effects of electroactive ion-exchange polymer metal composite (IPMC) actuator. Sensor Actuat A 2005; 118: 98-106.

225. Lee JH, Kim DO, Song GS, Lee YK, Jung SB, Nam JD. Direct metallization of gold nanoparticles on a polystyrene bead surface using cationic gold ligands. Macromol Rapid Commun 2007; 28: 634-40.

226. Han TH, Kim DO, Lee YK, Suh SJ, Jung HC, Oh YS, et al. Gold nanostructures formed in ionic clusters of perfluorinated ionomer. Macromol Rapid Commun 2006; 27: 1483-8.

227. Renuga G, Satinderpal K, Chao YF, Casey C, Seeram R. Electrospun nanofibrous polysulfone membranes as pre-filters Particulate removal. J Memb Sci 2007; 289: 210-19.

228. Burger C, Hsiao BS, Chu B. Nanofibrous and their applications. Annual Review of Materials Research 2006; 36: 333-68.

229. Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: A review. Tissue Engineering, 2006; 12: 1197–211.

230. Xie J, Li X, Xia Y. Putting electrospun nanofibers to work for biomedical research. Macromolecular Rapid Communications 2008; 29: 1775-92.

231. Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials 2003; 24: 2077–82.

232. Gallant-Behm CL, Yin HQ, Liu S, Heggers JP, Langford RE, Olson ME, et al. Comparison of in vitro disc diffusion and time kill-kinetic assays for the evaluation of antimicrobial wound dressing efficacy. Wound Repair Regen. 2005; 13:412-21.

233. Watson T. Ultrasound in contemporary physio-therapy practice. Ultrasonics, 2008; 48: 321-70.

234. Bell AL and Cavorsi J. Noncontact ultrasound therapy for adjunctive e treatment of no nhea ling wounds: retrospective analysis. Phys Ther 2008; 88:1517-24.

235. Davis SC, Ovington LG. Electrical stimulation and ultrasound in wound healing. Derm Clinics 1993; 11: 775-81.

236. Ter RG, Kessels AG, and Knipschild PA. Randomized clinical trial of ultrasound in the treatment of pressure ulcers. Phys Ther 1996; 76:1301-11.

237. Dinno MA, Al-Karmi AM, Stoltz DA, Matthews JC, Crum LA. Effect of free radical scavengers on changes in ion conductance during exposure to therapeutic ultrasound. Membr Biochem 1993; 10: 237-47.

238. Goodson WH 3rd, Hunt TK. Wound Healing and the diabetic patient. Surg Gynecol Obstet 1989; 149: 600-8.

239. Dyson M. Effect of laser therapy on wound contraction and cellularity in mice. Lasers Med Sci 1986; 1: 125-30.

240. Oshiro T, Calderhead RG.: Laser Parameters and Power Concept. Low Level Laser Therapy: A Practical Introduction, edited by Oshiro T, editor; Calderhead RG, editor., pp l9–31, John Wiley & Sons, Chichester, London, 1988

241. Kana JS, Hutschenreiter G, Haina D, Waidelich W. Effect of low level laser radiation on healing of open skin wounds in rats. Arch Surg 1981; 116: 293-6.

242. Braverman B, McCarthy RJ, Ivankovich AD, Forde DE, Overfield M, Bapna MS. Effect of Helium Neon and infrared laser irradiation on wound healing in rabbits. Lasers Surg Med 1989; 9: 50-8.

243. Surinchak JS, Alago ML, Bellamy RF, Stuck BE, Belkin M. Effects of low-level energy lasers on the full-thickness skin defects. Lasers Surg Med 1983; 2: 267-74.

244. Labbe RF, Skogerboe KJ, Davis HA, Rettmer RL. Laser photo-bioactivation mechanisms: In vitro studies using ascorbic acid uptake and hydroxyproline formation as biochemical markers of irradiation response. Lasers Surg Med 1990; 10: 201-17.

245. Barnes P, Sell SA, Boland ED, Simpson DG, Bowlin GL. “Nanofiber technology: designing the next generation of tissue engineering scaffolds,” Advanced Drug Delivery Reviews 2007; 59: 1413-33.

246. Chandrasekaran AR, Venugopa J, Sundarrajan S, Ramakrishna S. Fabrication of a nanofibrous scaffold with improved bioactivity for culture of human dermal fibroblasts for skin regeneration. Biomedical Materials 2011; 6: 015001 .

247. Chong EJ, Phan TT, Lim IJ, Zhang YZ, Bay BH, Ramakrishna S, et al. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. Acta Biomaterialia 2007; 3321-30.

الملخص العربي

يعتبر مرض السكرى من الأمراض الخطيرة المنتشرة بكثرة وتكمن خطورتها لما لها من مضاعفات وخاصة إذا تعرض مريض السكرى لجروح فإن التئام هذه الجروح من المشاكل الصعبة التي يواجها المريض ويسبب ذلك حدوث قرح في الجلد يصعب علاجها. ارتفاع تكاليف رعاية جروح مرضى السكري يزيد من الاعباء التي يتكبدها المرضى والمجتمع لذلك فان هناك حاجة لتطوير طرق علاج جديدة، وغير مكلفة وآمنة تزيد من التئام جروح مرضى السكرى وتعتبر تكنولوجيا النانو من أهم التقنيات في القرن ٢١ لما لها من تطبيقات واسعة النطاق في جميع المجالات.

ولقد تمكن العلماء من تطبيق هذه التكنولوجيا في المجال الطبي و ذلك بتصنيع مواد لها أبعاد نانومترية و صفات فريدة تختلف في خواصها عن المواد ذاتها بأبعادها العادية. ومن هذه المواد هناك مواد يطلق عليها الألياف النانومترية التي يمكن استخدامها كضماده للجروح وكذلك يمكن دمج بعض المواد فيها مثل جزيئات الفضة النانومتريه لإستخدامها كمضاد للبكتريا التي يمكن ان تلوث الجروح.

الهدف من الرسالة:

كان الهدف من هذه الرسالة هو تقييم دور الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا المصنع باستخدام طريقة الغزل الكهربائي في تضميد والتئام الجروح في الفئران المصابة بالسكري مقارنة بالطرق الفيزيائيه مثل الموجات الفوق صوتية والليزر.

لتحقيق هذا الهدف تم

١- تحضير مواد نانومترية مثل جزيئات الفضة النانومترية المتكونة عن طريق الإختزال الكيميائي لجزيئات نترات الفضة، والغشاء النانومتري المصنع من اسيتات السليلوزو والمحتوي علي جزيئات الفضة النانومترية عن طريق تحضيرها باستخدام طريقة الغزل الكهربائي لإستخدامهم كضمادة في التئام الجروح ودراسة خواص هذه المواد كالاتى:

- دراسة السطح الخارجي وحجم جزيئات الفضة النانومترية باستخدام الميكروسكوب الإليكترونى النافذ.
- دراسة التركيب اللبلورى لجزيئات الفضة النانومترية باستخدام جهاز حيود الأشعة السينية.
- دراسة السطح الخارجي للغشاء النانومتري و الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا عن باستخدام الميكروسكوب الإليكترونى الماسح.
- دراسة المجموعات الفعالة للغشاء النانومتري و الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا باستخدام جهاز الأشعة تحت الحمراء.
- دراسة تأثير الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في تثبيط نمو البكتريا ونشاطها.
- والفئران المستخدمة وعددها ٧٠ فأر تم حقن ٦٠ فأر منهم بمادة (straptozotocin) بجرعة 180 mg/kg حتي يتم اصابتهم بالسكري و ١٠ المتبقيين لم يتم حقنهم ليكونوا المجموعة الضابطه ثم جرح جميع الفئران وعمل جرح دائري في منطقة الظهر واستخدمت الطرق المختلفة للمساعدة في التئام الجرح و تقسيم الفئران الى مجموعات كالاتي:
  - **المجموعه الاولى** : المجموعه الضابطه. فئران خالية من السكري تركوا بدون علاج لملاحظة التئام الجروح طبيعيا.
  - **المجموعه الثانية** : فئران مصابون بالسكري تم علاجهم بالانسولين فقط.
  - **المجموعه الثالثة** : فئران مصابون بالسكري تم علاجهم بالانسولين وتعريض الجرح لاشعه الليزر المرئي ٦٥٠nm , 150mW حتي التئام الجرح.
  - **المجموعه الرابعة:** : فئران مصابون بالسكري تم علاجهم بالانسولين وتعريض الجرح للموجات الفوق صوتية حتي التئام الجرح.
  - **المجموعه الخامسه:** : فئران مصابون بالسكري تم علاجهم بالانسولين وعلاج الجرح بجرعات يوميه من جزيئات الفضة النانومترية حتي التئام الجرح.
  - **المجموعه السادسه:** : فئران مصابون بالسكري تم علاجهم بالانسولين وتم استخدام الغشاء النانومتري المصنع من اسيتات السليلوز في الغيار للجرح حتي التئام الجرح.

- **المجموعه السابعه:** فئران مصابون بالسكري تم علاجهم بالانسولين وتم وتم استخدام الغشاء النانومتري المصنع من اسيتات السليلوزو المحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في الغيار للجرح حتي التئام الجرح.

- تم ملاحظة التئام الجروح في كل المجموعات خلال فترة العلاج لقياس معامل الالتئام
- تم اخذ عينات من الجلد في اليوم الرابع والعاشر والخامس عشر لفحصها هيستولوجيا بعد التئام الجروح
- تم اخذ الجلد الملتئم وقياس الخاصيه الميكانيكيه له.

نتائج البحث:

وقد أسفرت الدراسة عن النتائج الآتية:

١- التئام الجروح في الفئران المعاجة باستخدام الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا في فترة اقل من باقي المجموعات.

٢- تكون كميات من الكولاجين في طبقات الجلد للفئران المعالجة باستخدام الغشاء النانومتري المصنع من اسيتات السليلوز والمحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا مما يدل علي التئام الجروح.

ومن هذه النتائج نستنتج ان:

- لابد من التطوير في مختلف المجالات وخاصة المجالات الطبية لمحاولة التغلب علي الامراض التي يصعب حلها بالطرق القديمة.
- استخدام تقنيات حديثة مثل استخدام تكنولوجيا النانو قد يساعد في حل العديد من المشاكل في المجالات المختلفة مثل المجال الطبي.

تحضير وتوصيف الغشاء النانومتري المحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا لإستخدامه في التئام الجروح في الفئران المصابة بالسكري مقارنة بالطرق الفيزيائيه للعلاج

رسالة علمية

مقدمة إلى معهد البحوث الطبية– جامعة الإسكندرية
ايفاءا جزئيا لشروط الحصول على درجة

الدكتوراه

فى

الفيزياء الحيوية الطبية

مقدمة من

مايسة السيد سيداحمد مصطفى

بكالوريوس العلوم (كيمياء حيوية خاصة ٢٠٠٧)

جامعة الاسكندرية

ماجستير في الفيزياء الحيوية الطبية
معهد البحوث الطبية
جامعة الإسكندرية
٢٠١١

معهد البحوث الطبية
جامعة الإسكندرية
٢٠١٥

تحضير وتوصيف الغشاء النانومتري المحتوي علي جزيئات الفضة النانومترية المضادة للبكتريا لإستخدامه في التئام الجروح في الفئران المصابة بالسكري مقارنة بالطرق الفيزيائيه للعلاج

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٢٠١١

للحصول على درجة

الدكتوراه

فى

الفيزياء الحيوية الطبية

لجنة المناقشة والحكم على الرسالة	**موافقون**
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