Research Article

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ANTIBACTERIAL WOUND HEALING MESH COATED WITH SYNERGISTIC DRUGS AND CARRIERS TO EVALUATE AGAINST PYOGENIC ORGANISMS

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ABSTRACT

Foot ulcers are the major complication found in the cases of diabetes mellitus. An improvement in the properties of wound healing dressings was achieved through impregnating with antibacterial agents. In the present study, one such method was aimed to reduce the complications of foot ulcer by coating wound dressing mesh materials with two different groups of drugs (synergistic drugs) and a carrier Poly vinyl alcohol (PVA). The *drug coated* (ofloxacin-ornidazole) and *drug-carrier coated* (ofloxacin-ornidazole + PVA) mesh materials were subjected to antibacterial activity against the wound pathogens. The antibacterial activity of the synergistic drugs (ofloxacin-ornidazole) was tested prior coating the mesh. Among the four different concentrates of synergistic drugs, 4X strength exhibited maximum inhibitory zones against all the test organisms. Maximum inhibitory zones of 21mm was observed against *Escherichia coli* and followed by 19mm against *Staphylococcus aureus*. Antibacterial activity of *drug coated* (ofloxacin-ornidazole) and *drug-carrier coated* (ofloxacin-ornidazole + PVA) mesh materials showed potential inhibitory zones against all the test organisms. When compared to *drug coated* materials, *drug-carrier coated* mesh showed more antibacterial activity. This was mainly due to the inhibitory actions of the carrier, PVA in the mixture. Maximum inhibitory zone of 31 mm was recorded against *Escherichia coli* and *Klebsiella pneumoniae* respectively for *drug-carrier coated* materials. Thus the proposed research illustrated the significance of synergistic drug and carrier coatings on wound healing mesh materials. The developed product was thus aimed to prevent from complications like amputation in diabetic foot ulcer patients⁷.

Keywords: Synergistic drugs, Drug carrier, Wound healing, Antibacterial activity, Diabetic foot ulcers

INTRODUCTION

Diabetic foot ulcer is a major complication found in diabetes mellitus¹. The delayed wound healing in diabetic foot disease is due to many factors including peripheral arterial disease, peripheral neuropathy, foot deformity and secondary bacterial infection²⁻³. The 2 most common organisms isolated from the diabetic foot ulcers are Organisms belonging to the *Enterobacteriaceae* family and the species from the *Staphylococcus* genus. Bacterial infection delays in healing of diabetic foot ulcers⁴. A foot ulcer having observable clinical symptoms and/or signs of inflammation such as redness, swelling, heat, and when neuropathy is absent, pain is defined as Bacterial infection in a foot ulcer⁵. Bacterial infection has been regarded as a complication of ulcers in people with diabetes for many years⁶. It is estimated that 50-80% of patients suffering from foot ulcers will become clinically infected. In addition, clinical infection portends a worse prognosis for healing - approximately 20% of patients with an infected foot ulcer will require an amputation⁷.

Foot lesions are one of the major medical, social and economic problem for diabetic people. This infection results in amputation if not treated promptly. Infection with multidrug-resistant organisms increases the cost of management and duration of hospital stay, as well as mortality and morbidity⁸. The most common pathogen isolated from foot ulcers are *Staphylococcus aureus* and are Methicillin-Resistant *S. aureus* (MRSA)⁹⁻¹¹. Methicillin-Resistant *S. aureus* has been increasingly isolated from diabetic foot ulcers and patients with community-acquired MRSA (CA-MRSA) infections often do not exhibit the risk factors compared to patients with hospital-associated MRSA (HA-MRSA) infections. These risk factors include dialysis, recent hospitalization, nursing-home residence and presence of

other co-morbid conditions such as diabetes, chronic pulmonary diseases and chronic renal failure. However, the clinical characteristics and factors that predispose diabetic patients with foot ulcers towards infection with different associated MRSA remain unclear. Virulence factors in *Staphylococcus aureus* are Enterotoxins which results in toxic Shock-Syndrome, pore-Forming Toxins, bi-component leukotoxins and α -Toxin.

Antibiotics such as Gentamycin, Neomycin, and Mupirocin are good antibacterials used commercially. Dressings containing silver and polyherbal preparations come in different formulations and shows good results in healing diabetic foot wounds¹². Sisomycin (0.10%) and acetic acid at concentrations between 0.5% and 5% are effective against *Pseudomonas*, other gram-negative bacilli and beta hemolytic streptococci wound infections. In healing sutured wounds and hypergranulating wounds, Povidone iodine solution dressings are used to suppress or hamper further granulation. Iodine is found to be toxic to human cells as well as bacteria and fungi at high doses¹³⁻¹⁴. Dressings for wounds are made of cotton, wool, natural or synthetic bandages and gauzes¹⁵. The effective strategy for reducing such nosocomial infections is to reduce the dose of microorganisms throughout the healthcare complex using antimicrobial technologies to treat the material surfaces and to maintain the standard of hygiene.

An improvement of the properties of these dressings can be obtained by impregnating them with other materials or compounds to obtain a functional dressing. Gauze and bandage can also be functionalized with topical antimicrobials, which can prevent or reduce bacterial bioburden or reinfection during dressing changes. Commonly used topical antiseptic agents include iodine-releasing agents (e.g. povidone iodine [PVP-I]), chlorine-releasing solutions hydrogen peroxide, chlorhexidine,

silver-releasing agents, and acetic acid. These compounds can be used to either kill or control the growth of micro-organisms in wounds¹⁶⁻¹⁷ and generally are classified as antiseptics or antibiotics and characterized by low specificity to treat wound infection. The problem with straight forward antimicrobial loading into the material leads to antimicrobial resistance. This leads to an important impact on patient outcome by enhancing virulence, delayed from subsequent recovery¹⁸. To avoid the antibiotic resistance character the effects of introducing two different groups of synergistic antimicrobial drugs (a fluoroquinolone drug and a nitroimidazole drug) into the biomedical products gained lot of interest currently. This was well supported from the report of Saginur *et al*¹⁹ (2006); describing that the accepted clinical practice to treat biomedical-associated infections was the use of combination therapy in which two or more antimicrobials are blended at different combinations. So that broader spectrum of activity is achieved at a lower concentration resulting in more effective therapy and decreased resistance.

Based on this concept, a commercial wound dressing mesh materials was impregnated with synergistic drugs (ofloxacin-ornidazole) with and without a carrier (PVA). The antibacterial inhibitory effects of the mesh were investigated against the wound-associated pathogens.

MATERIALS AND METHODS

The present study was carried out in Microbiology Research Laboratory, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, Tamil Nadu, India. The work was completed during the period of December 2017 to March 2018.

Purchase of wound healing mesh material

3M-Tegaderm (Health Vistas, Udaipur, India) wound dressing material was procured from www.healthvistas.com and used in the present study.

Procurement of drugs and carriers

Ofloxacin (Merck), ornidazole (Sigma-aldrich) and poly vinyl alcohol (TCI, USA) was commercially purchased from local chemical suppliers, Coimbatore, India.

Selection of test organisms

Test organisms like *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter sp*, *Staphylococcus aureus*, *Citrobacter sp* and *Staphylococcus epidermidis* was procured from a local diagnostic laboratory, Coimbatore. All the test cultures were cultured and stored at 4°C prior to use. Every time the cultures were freshly prepared in Nutrient broth and used for analysis.

Determining the antibacterial activity of synergistic drugs against the test organisms²⁰

The synergistic antibacterial activity of the drug combinations (Ofloxacin-Ornidazole) was determined against test organisms using a standard well diffusion method. MHA plates were prepared and swabbed evenly over its surface with 12h cultures of each test organisms. In the middle of the plate a 6mm well was cut using a sterile cork-borer. About 50µl of the synergistic drugs were added under sterile conditions. To determine the best concentration, four different concentrations were prepared separately (1X- 10µg, 2X – 20µg, 3X – 30µg and 4X - 40 µg). All the plates were incubated at 37°C for 24h. The inhibitory

zones around the well were measured in millimeter and recorded separately in comparison with the standard antibiotic-sensitivity chart.

Antibacterial coatings of the wound healing mesh material with synergistic drugs²¹

Antibacterial coating of wound healing mesh material using the synergistic drugs was done by a standard two dip-coating technique. The technique started with the preparation of stable slurry with specific amount of synergistic drugs in the molten polyethylene glycol (PEG). PEG (5g) with a predefined molecular weight was mixed with synergistic drugs (500mg) in a glass vial. The mixture was heated at the range of 60 to 70°C in a water bath to obtain homogeneous slurry. The resulting slurry was homogenized in a magnetic stirrer for 5 to 10min. The wound healing mesh disc was cut (10mm) using sterile devices and dip-coated twice with intermittent drying (suspension coating method). The dip-coating procedure was carried out in sterile glass beakers on a shaker (120 rpm) for 30min, with a drying period of about 15 minutes between the two coating procedures, followed by drying at room temperature. All coating steps were carried out under strict aseptic conditions. After coating procedure, the materials were stored at 4°C for upto 15min. In order to increase antimicrobial drug loading and prevent excessive increase in material thickness, the coating process were repeated for replicates of each sample. Subsequently, in order to slow down the release rate of antimicrobial drug from PEG coating and mitigate the friction effect between material surfaces, second coating layer was formed using polyvinyl alcohol (PVA). PVA (drug-carrier) was dissolved in DMSO to acquire a 10 % (w/w) solution. PEG-coated materials were submerged into PVA solution three times for 1min each. The coated materials were left to dry on a clean bench for 1 week at room temperature to remove residual DMSO, followed by determining the antimicrobial activity. The wound healing mesh without PVA was abbreviated as *drug coated (dc)* materials and the mesh coated with PVA was abbreviated as *drug-carrier coated (dcc)* materials.

Evaluating the antibacterial activity of dc and dcc mesh materials

The antibacterial activity of *dc* and *dcc* mesh materials was tested using standard agar diffusion method against five test organisms. MHA plates were prepared by pouring 15 ml of media into sterile Petri dishes. The plates were allowed to solidify for 5 minutes and 0.1% inoculum was swabbed uniformly and allowed to dry for 5 minutes. The *dc* and *dcc* mesh materials with the premeasured size of 10mm in diameter was placed on the surface of medium and the plates were kept for incubation at 37°C for 24 hours. A plain mesh without drugs and carrier was also kept in the plate as control. At the end of incubation, the zone of inhibition formed around each material was measured in millimeter and recorded.

RESULTS AND DISCUSSION

Wound healing using different commercial materials

The traditional dressings, which are generally used during first intervention in wound treatment, prevent wound's contact with outer environment and bleeding²². The best sample of this group is gauze and gauze-cotton composites which have very high absorption capacity. As they cause rapid dehydration whereas they are being removed from the wound surface, they can cause bleeding and damage of newly formed epithelium²³. Therefore, gauze composites with a non-adhesive inner surface are prepared to reduce the pain and trauma which can occur when removing traditional wound dressings from the wound surface. The

commonly used traditional dressing materials in world market are Paraffin gauze dressing containing 0.5% chlorhexidine acetate, Paraffin gauze dressing, Petrolatum gauze dressing, Petrolatum gauze containing 3% bismuth tri-bromophenate, Scarlet Red dressing, Sterile hydrogel dressing, Highly absorbent cotton wool pad, Highly absorbent rayon/cellulose blend sandwiched with a layer of anti-shear high density polyethylene, Absorbent cotton pad. The advantages of using drug coated wound dressing materials include tissue compatibility, occurrence of bacterial resistance, reduced interference with wound healing and prevents contamination, colonisation, critical colonisation and covert infection of microorganisms²⁴.

Antibacterial activity of synergistic drugs against the test organisms

The antibacterial activity of the synergistic drug combinations was determined against five test organisms. Three different concentrations of the drug combinations were tested under in vitro conditions. During the analysis, the higher concentrations (3X strength – 30ug/ml) showed more antibacterial activity against the five test organism. This was evident from the images presented (Fig.1). In Table.1, the inhibitory zones obtained for each drug concentrates against individual test organisms were presented. Among them, *Escherichia coli* and *Klebsiella pneumoniae* exhibited inhibitory zones of 12mm, 16mm, 21mm and 9mm, 12mm, 17mm for 2X, 3X and 4X concentrates respectively. Followed by *Enterobacter sp* and *Staphylococcus aureus* exhibited the inhibitory zones of 10mm, 12mm, 18mm and 9mm, 11mm, 19mm against their respective 2X, 3X and 4X concentrates. *Staphylococcus epidermidis* showed 10mm, 15mm and 18mm of inhibitory zones against the respective concentrates.

Antibacterial activity of dc and dcc mesh materials

The antibacterial coatings of the wound healing mesh materials were evaluated for their potential to retard the biomaterial centered infection causing pathogens. This parameter aids in developing a novel wound healing composite material. The drug coated (dc) and drug-carrier coated (dcc) mesh showed good antibacterial inhibitory zones against all the test organisms tested. Interestingly, drug-carrier coated (dcc) mesh exhibited more inhibitory zones indicating the additional action of the carriers coated in it. PVA acts as the drug carrier in dcc materials revealed

its significance and biological properties like antibacterial potential and constant drug release.

This was evident from the images presented (Fig.2). In Table.2, the inhibitory zones obtained for drug coated (dc) and drug-carrier coated (dcc) mesh against individual test organisms were presented. Among them, *Escherichia coli* and *Klebsiella pneumoniae* exhibited inhibitory zones of 27mm and 28mm for drug coated (dc) and 31mm and 31mm for drug-carrier coated (dcc) mesh respectively. Followed by *Enterobacter sp* and *Citrobacter sp* exhibited the inhibitory zones of 18mm and 25mm against their respective drug coated (dc) and 20mm and 29mm for drug-carrier coated (dcc) mesh samples. *Staphylococcus epidermidis* showed 26mm for drug coated (dc) and 30mm for drug-carrier coated (dcc) mesh samples.

The effective antibacterial activity of synergistic drugs expressed in the present research was mainly due to the following reasons as per the literature survey. The character of synergism mainly depends on the mode of action of a drug. Both fluoroquinolone and nitroimidazole drugs acts on the DNA of bacteria thus targeting the inhibition of DNA synthesis and replication. The mode of action of quinolones involves interactions with DNA gyrase, the originally recognised drug target, and topoisomerase IV, a related type II topoisomerase. DNA gyrase is more sensitive in gram-negative bacteria and topoisomerase IV was more sensitive in gram-positive bacteria. The formation of the ternary complex of quinolone, DNA, and either DNA gyrase or topoisomerase IV occurs through interactions in which quinolone binding appears to induce changes in both DNA and the topoisomerase, which occur separately from the DNA cleavage, a hallmark of quinolone action. Inhibition of DNA synthesis by quinolones requires the targeted topoisomerase to have DNA cleavage capability, and collisions of the replication fork with reversible quinolone-DNA-topoisomerase complexes convert them to an irreversible form²⁵. However, the molecular factors that subsequently generate DNA double-strand breaks from the irreversible complexes and that probably initiate cell death²⁶. The mode of action of nitroimidazole appears to depend on the ferredoxin-mediated reduction of their nitro group, with generation of a reactive metabolite which interact with DNA leading to a subsequent inhibition of nucleic acid and protein synthesis²⁷. The nitro group of the nitro-so-hydroxyl amino moiety is reduced by an electron transport protein in anaerobic bacteria. The reduced drug causes strand breaks in the DNA²⁸.

Table 1. Antibacterial activity of synergistic drugs against the test organisms

S. No.	Test organisms	Zone of inhibition (mm)			
		1X	2X	3X	4X
1	<i>Escherichia coli</i>	0	12	16	21
2	<i>Klebsiella pneumoniae</i>	0	9	12	17
3	<i>Enterobacter sp</i>	0	10	12	18
4	<i>Staphylococcus aureus</i>	0	09	11	19
5	<i>Staphylococcus epidermidis</i>	0	10	15	18

Table 2. Antibacterial activity of dc and dcc mesh materials

S. No.	Test organisms	Zone of inhibition (mm)		
		Control	dc	dcc
1	<i>Escherichia coli</i>	0	27	31
2	<i>Klebsiella pneumoniae</i>	0	28	31
3	<i>Enterobacter sp</i>	0	18	20
4	<i>Citrobacter sp</i>	0	25	29
5	<i>Staphylococcus epidermidis</i>	0	26	30

dc: drug coated
dcc: drug-carrier coated

Fig.1. Antibacterial activity of synergistic drugs against the test organisms

Fig.2. Antibacterial activity of dc and dcc mesh materials

CONCLUSION

The study was aimed to determine the effect of the impregnating drugs and carriers on the product substrate for proving the antimicrobial efficacy, durability and persistence. To reduce the complications of foot ulcer in diabetic patients, wound dressing mesh materials were impregnated with two different groups of drugs (synergistic drugs) and a carrier Poly vinyl alcohol (PVA). The *drug coated* (ofloxacin-ornidazole) and *drug-carrier coated* (ofloxacin-ornidazole + PVA) mesh materials were subjected to antibacterial activity against the wound pathogens. Antibacterial activity of *drug coated* (ofloxacin-ornidazole) and *drug-carrier coated* (ofloxacin-ornidazole + PVA) mesh materials showed potential inhibitory zones against all the test organisms. When compared to *drug coated* materials, *drug-carrier coated* mesh showed more antibacterial activity. This was mainly due to the inhibitory actions of the carrier, PVA in the mixture. Maximum inhibitory zone of 31mm was recorded against *Escherichia coli* and *Klebsiella pneumoniae* respectively for *drug-carrier coated* materials. Thus the proposed research illustrated the significance of synergistic drug and carrier coatings on wound healing mesh materials. The developed product was thus aimed to prevent from complications like amputation in diabetic foot ulcer patients. The finding signifies the method as an effective wound

healing process which is pharmacologically modulated to achieve repair from any types of tissue injury.

REFERENCES

- Turns M. The diabetic foot: an overview of assessment and complications. Br J Nurs 2011; 20 Suppl 15:19-25.
- American Diabetes Association: Standards of medical care in diabetes - 2009. *Diabetes Care* 2009; 32 Suppl 1:13-61.
- Margolis DJ, Kantor J et al., Risk Factors for Delayed Healing of Neuropathic Diabetic Foot Ulcers: A Pooled Analysis. *Arch Dermatol* 2000; 136 Suppl 12:1531-1535.
- Waugh HV, Sherratt JA. Macrophage dynamics in diabetic wound. *Bull Math Biol* 2006; 68 Suppl 1:197-207.
- Lipsky BA, Berendt A. Hyperbaric Oxygen Therapy for Diabetic Foot Wounds: Has hope hurdled hype? *Diabetes Care* 2010; 33 Suppl 5:1143-1145.
- Robson MC, Heggers JP. Delayed wound closure based on bacterial counts. *J Surg Oncol* 1970; 2 Suppl 4:379-83.
- Wu SC, Driver VR et al., Foot ulcers in the diabetic patient, prevention and treatment. *Vascu Health Risk Manag* 2007; 3 Suppl 1:65-76.
- Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V, Grimaldi A. Diabetic foot ulcer

- and multidrug-resistant organisms: risk factors and impact. *Diabet Med* 2004; 21 Suppl 7:710-5.
9. Ellie J C, Goldstein MD, Diane M, Citron BS, Catherine A, Nesbit MS. Diabetic Foot Infections: Bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. *Diabetes Care* 1996; 19 Suppl 6:638-641.
 10. Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med* 2005; 16:567-570.
 11. Tentolouris N, Jude EB, Smirnov I, Knowles EA, Boulton AJ. Methicillin-resistant *Staphylococcus aureus*: an increasing problem in a diabetic foot clinic. *Diabet Med* 1999; 16:767-771.
 12. Rice TT. Metronidazole use in malodorous skin lesions. *Rehabil Nurs* 1992; 17:244-255.
 13. Burnakis TG. Topical metronidazole for decubitus ulcers. *Hosp Pharm* 1989; 24:960-961.
 14. Viswanathan V, Kesavan R, Kavitha KV, Kumpatla S. A pilot study on the effects of a polyherbal formulation cream on diabetic foot ulcers. *Indian J Med Res* 2011; 134:168-173.
 15. Boateng JS, Matthews KH, Stevens HN, Eccleston GM. Wound healing dressings and drug delivery systems: a review. *J Pharm Sci* 2008; 97 Suppl 8:2892-2923.
 16. White RJ, Cooper R, Kingsley A. Wound colonization and infection: the role of topical antimicrobials. *Br J Nurs* 2001; 10 Suppl 9:563-578.
 17. White RJ, Cutting K, Kingsley A. Topical antimicrobials in the control of wound bioburden. *Ostomy Wound Manage* 2006; 52 Suppl 8:26-58.
 18. Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. *Clin Infect Dis* 2003; 36:1433-1437.
 19. Saginur R, Denis MS, Ferris W, Aaron SD, Chan F, Lee C, Ramotar K. Multiple combination bactericidal testing of staphylococcal biofilms from implant-associated infections. *Antimicrob Agents Chemother* 2006; 50 55-61.
 20. Elayarajah B, Rajendran R, Venkatrajah B, GeethuBabu, Devika S. Moist Wound Dressing Fabrications: Carboxymethylation of Antibacterial Cotton Gauze. *J Eng Fiber Fabr* 2013; 8 Suppl 4:78-87.
 21. Boccaccini AR, Notingher I, Maquet V, Jerome R. Bioresorbable and bioactive composite materials based on polylactide foams filled with and coated by Bioglass particles for tissue engineering applications. *J Mat Sci Mat Med* 2003; 14 Suppl 5:443-450.
 22. Sheridan RL, Tompkins RG. Skin substitutes in burns. *Burns* 1999; 25:97-103.
 23. Naimer SA, Chemla. Elastic adhesive dressing treatment of bleeding wounds in trauma victims. *Am J Emerg Med*. 2000; 18:816-819.
 24. Peter Vowden, Kathryn Vowden, Keryln Carville. Antimicrobial dressings made easy. *Wounds Intl* 2011; 2:1.
 25. Laponogov I, Pan XS, Veselkov DA, McAuley KE, Fisher LM. Structural Basis of Gate-DNA Breakage and Resealing by Type II Topoisomerases. *PLoS ONE* 2010; 5 Suppl 6:1-7.
 26. Hooper DC. Mode of action of fluoroquinolones. *Drugs* 1999; 58 Suppl 2:6-10.
 27. Alka mital. Synthetic Nitroimidazoles: Biological Activities and Mutagenicity Relationships. *Sci Pharm* 2009; 77:497-520.
 28. Leiros HKS, Sigrid Kozielski-Stuhrmann, Ulrike Kapp, Laurent Terradot, Gordon A Leonard, Seán M, McSweeney. Structural Basis of 5-Nitroimidazole Antibiotic Resistance- The crystal structure of nima from *Deinococcus radiodurans*. *J Biol Chem* 2004; 279 Suppl 53:55840-55849.

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