DEVELOPING WOUND DRESSING MATERIALS BY FINISHING WITH SYNERGISTIC DRUG COMBINATIONS: DETERMINING ITS ANTIBACTERIAL EFFICACY AGAINST PATHOGENS

M. Roch Sowmia1*, G. Bagyalakshmi2

1Assistant Professor, Department of Fashion Technology & Costume Designing, Shrimati Indira Gandhi College, Trichy
2Associate Professor & Head, Department of Textiles and Clothing, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

ABSTRACT

Colonization and infection of bacteria is the major factor responsible for the delay in wound healing. Antimicrobial dressings can be used on acute or chronic wounds which are critically colonised, or when local and/or systemic infection is already compromising the wound or could compromise wound healing. Combination therapy of drugs can be used for the treatment of drug resistant bacteria. Thus, the present study focuses on developing a significant wound dressing materials with significant antibacterial function. Three fabrics (Cotton-Single Jersey, Cotton-Rib and Cotton-Lycra fabrics) were selected and the best among them was selected by its physical properties. Cotton-Lycra fabric showed significant Air permeability, Bursting strength, Tensile strength and Flexural rigidity values. From the two drug combinations-I (Piperacillin-Tazobactam) and II (Ciprofloxacin-Tinidazole), Piperacillin-Tazobactam combination showed higher antibacterial activity i.e 23mm and 20mm against E. coli and S. aureus. Thus Cotton-Lycra fabric and drug combination Piperacillin-Tazobactam were selected for the development of wound dressing material. EN ISO-20743 was performed to determine the antibacterial activity of the finished fabric. Drug treated fabrics showed inhibitory zones of 27mm against Escherichia coli and 25mm against Staphylococcus aureus. Thus the developed drug treated fabrics can be used as wound dressing materials to promote wound healing and to prevent the bacterial infections.

INTRODUCTION

Wound may be defined as a disruption to the physiological arrangement of the skin cells and a disturbance to its function in connecting and protecting underlying tissues and organs. It may be primary caused by accidental cut, tear, scratch, pressure, extreme temperatures, chemicals, and electrical current, or secondary to surgical intervention or disease (i.e., diabetes, ulcers, or carcinomas) (Boateng et al., 2008). Wound healing is a physiological process, by which the living body repairs tissue damages, restores its anatomical integrity, and regains the functionality of the injured parts. A wound can be closed by primary intention or left to heal by secondary intention, and in both ways
the healing process occurs through a series of overlapping events and is influenced by a number of intrinsic and extrinsic factors (Hutchinson., 1992).

Delay in wound healing in the patients may be due to different factors. One such unknown reason was revealed to be as colonization and infection of bacteria at wound site. This is clinically termed as critical colonization at open wound site (White., 2002). This critical colonization is considered to be as a challenge to majority of medical practitioners. Bandage and wound dressing materials carrying both aerobic and facultative anaerobic bacteria may also be disseminated among the patients and workers. The major penetrating and transmitting organisms are *Escherichia coli*, methicillin resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus epidermis* and anaerobic bacteria like *Bacteriodes fragilis*, *Peptostreptococcus* sp (Elshafei et al., 2011).

In order to reduce the bacterial colonization in wounds and to increase the wound healing process, antimicrobial finishes on the dressing were applied. Antimicrobial dressings can be used on acute or chronic wounds which are critically colonised, or when local and/or systemic infection is already compromising the wound or could compromise wound healing. When choosing an appropriate wound dressing it is vital to assess whether the wound is colonised, critically colonised or infected (Flores and Kingsley., 2007). However, in some cases, high amounts of antibiotics can lead to systemic toxicity. The development of new antibiotics has decreased over the last years, with a small number of antibiotics remaining active in these domains. In addition, antibiotic-resistant microorganisms have considerably increased, due to the overuse and misuse of antibiotics (Das and Horton., 2016). Antibiotic resistant organisms include methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and vancomycin resistant *Enterococcus faecalis* (Fletcher., 2006). This antibiotic crisis is still in progress and affects antibiotic treatments used for both systemic and topical infections (Aumeeruddy et al., 2016).

Inspite of the drug resistance, Saginur et al., (2006) reported 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. The character of synergism mainly depends on the mode of action of a drug. The application of textiles in medicine has a long tradition. An important field of application is wound care and prevention of chronic wounds, in particular pressure sores. Wound dressings and bandages gained great popularity among the textile materials. Woven textiles are mostly used. Despite the fact that traditional textiles fulfilled primary quality approaches like biocompatibility, flexibility, strength, etc. There is an increasing need for specified functions. Along with the technological development of functional textiles, their use in wound healing and prevention of chronic wounds has reached a new quality of interactivity between biological tissues and textiles (Wollina et al., 1998).

Bandages, gauze, medical dressings are some of the knitted medical textiles used in wounds. They cover, protect, prevents infection and promotes wound healing. The medical dressings insulate, attach the drugs to the wound and absorb liquids. when it comes in contact with the skin, wound dressings should possess properties such as good breathability, good hygroscopicity and great sense of comfort. The knitted medical dressings show greater elasticity, extensibility, flexibility and fitness. For simple knitting technology, low viscidity and great flexibility; rib
stitch and weft plain stitch are mostly applied in medical dressings for two dimensional structures. Some three
dimensional structures used in medical dressing are weft multiple composites, weft knitted spacer fabrics and warp
knitted spacer fabrics. They often have absorbent layers for good ability to control heat and moist transfer (Wollina
et al., 1998).

In the present work, the physical properties like Air permeability, Bursting strength, Tensile strength and
Flexural rigidity were determined and the best fabric was selected. Two drug combinations (Ciprofloxacin-Tinidazole
and Piperacillin-Tazobactam) were prepared and its antibacterial activity was evaluated. The best combination was
finished on the fabrics and antibacterial activity was examined by EN ISO-20743 test method.

MATERIALS AND METHODS

Procurement of fabrics

Cotton – Rib, Cotton – Single Jersey and Cotton – Lycra fabrics were the fabrics used in this study and they
were commercially procured from a local shop, Tirupur. The drugs used in the study are Ciprofloxacin, Tinidazole,
Piperacillin and Tazobactam (Sigma - Aldrich).

Determining the physical properties of the fabrics

From the given fabrics one best fabric was selected from the analysis of its physical properties like Air
permeability, Bursting strength, Tensile strength and Flexural rigidity. Air permeability can be measured using an
instrument called Shirley Air Permeability Tester in accordance with Test Method ASTM D-737-96, the air
permeability of textile was examined. Flexural rigidity or stiffness was evaluated according to ASTM D1388-08
(Standard Test Method for Stiffness of Fabric). Bursting strength of the specimens was determined by using a
modified standard ASTM D3787. Tensile strength in the warp and weft directions, was calculated by standard ASTM
D 5035-11 test method

Synthesis of reactive synergistic drugs using beta-cyclodextrin

Two drug combinations (Ciprofloxacin-Tinidazole and Piperacillin-Tazobactam) were prepared and its
antibacterial activity was determined. Drug combination-1 (Piperacillin-Tazobactam) was prepared by dissolving
equal volumes (400mg) of drugs in 2ml deionized water. Similar procedure was carried out for the preparation of
Drug combination-2 (Ciprofloxacin-Tinidazole).

Determining the antibacterial activity of synergistic drugs against the test organisms

The synergistic antibacterial activity of the drug combinations (Ciprofloxacin-Tinidazole and Piperacillin-
Tazobactam) 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 drug combinations were added under
sterile conditions. All the plates were incubated at 37°C for 24h. The inhibitory zones around the well were measured
in millimeter and recorded separately.
Dye-exhaust method to bind reactive antibacterial agents to cotton fabric (Chun and Gamble, 2007)

An exhaust dyeing method was used to bind the reactive drug to the knitted fabric. The dye bath was prepared by adding 0.2ml of Triton-X-100 (Hi media), 2g of sodium sulphate, and 2 ml of the reactive drug combination to 100 ml of de-ionized water. Three, 5 X 5 cm squares of the knitted fabric were submerged in the dyebath heated to 60°C. After 30 mins of incubation, 1.0 g NaOH that had been dissolved in 10 ml of de-ionized water was added. The temperature was then raised to 80°C, and the knitted fabrics heated for another 30 mins. The fabric was then rinsed in de-ionised water and heated for 10 mins at 80°C in de-ionised water, then rinsed and kept in a convection oven at 105°C until dried.

Evaluating the antibacterial activity of the coated materials (EN ISO-20743)

The antibacterial activity of drug combination coated knitted fabrics was tested using standard EN ISO-20743 test method against two 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 drug combinations finished knitted fabrics with the premeasured size of 20mm in diameter was placed on the surface of medium and the plates were kept for incubation at 37°C for 24 hours. Plain knitted fabrics without drugs 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

Air permeability of Cotton-Single Jersey fabric was found to be 96.7cm³/cm²/sec. Cotton-Rib and Cotton-Lycra fabrics showed 93.2cm³/cm²/sec and 101.5cm³/cm²/sec. Bursting strength of Cotton-Single Jersey, Cotton-Rib and Cotton-Lycra fabrics were found to be 123.5, 121.0 and 126.0. Cotton-Single Jersey fabric showed 178.8LWE tensile strength; Cotton-Rib and Cotton-Lycra fabrics showed 172.4LWE and 187.1LWE. Flexural rigidity of the Cotton-Single Jersey, Cotton-Rib and Cotton-Lycra fabrics were found to be 0.24g/cm, 0.23g/cm and 0.24g/cm (Table-1). From the analysis, the Cotton-Lycra fabric showed significant physical properties and Cotton-Lycra was selected for the finishing of drugs and development of wound dressing materials.

Table-1: Physical properties of the Fabrics

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fabric Knit type</th>
<th>Mechanical testing</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Air permeability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cm³/cm²/sec)</td>
</tr>
<tr>
<td>1</td>
<td>Cotton-Single Jersey</td>
<td>96.7</td>
</tr>
<tr>
<td>2</td>
<td>Cotton-Rib</td>
<td>93.2</td>
</tr>
<tr>
<td>3</td>
<td>Cotton-Lycra</td>
<td>101.5</td>
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</table>
Antibacterial activity of the drug combinations by well diffusion method

The antibacterial activity of the Drug combinations- I and II were evaluated by standard well diffusion method (Figure-1). The Drug combination-I (Piperacillin-Tazobactam) showed 23mm and 20mm against *E. coli* and *S. aureus*. Drug combination-II (Ciprofloxacin-Tinidazole) showed 17mm and 15mm against the test pathogens *E. coli* and *S. aureus*. Thus, Drug combination-I (Piperacillin-Tazobactam) is selected for finishing of fabrics.

Figure-1: Antibacterial activity of the Drug combinations by well diffusion method

Qualitative antibacterial activity of the finished fabrics (EN ISO-20743)

The Cotton-Lycra fabrics were finished with Piperacillin-Tazobactam synergistic drugs. The antibacterial activity of the finished fabrics was determined by EN ISO-20743. Drug combination finished fabrics against *Escherichia coli* showed 27mm of inhibitory zones and 25mm against *Staphylococcus aureus* (Figure-2). The significant inhibitory zones is due to the dispersion of drug combination from the fabric and the synergistic activity of the drugs.
Discussion

The present study focuses on developing a significant wound dressing materials with synergistic drug coatings to promote the wound healing and to prevent the bacterial infections. The Cotton-Lycra fabric showed significant physical properties (Air permeability, Bursting strength, Tensile strength and Flexural rigidity) than the other two fabrics (Cotton-Rib and Cotton-Lycra). Drug combination-II showed higher antibacterial activity than drug combination-I. Hence, drug combination-I was selected for finishing on fabrics.

Antibacterial activity of the reactive synergistic drugs (Piperacillin-Tazobactam) obtained during this analysis was found significant. The synergism mainly depends on the mode of action of a drug on the bacterial cell components. If two different drugs has similar mode of action then it was indicated as synergism. This was elaborately defined and discussed for two different groups of drugs called fluoroquinolone and nitroimidazole. Both these drugs act on the DNA of bacteria thus targeting the inhibition of DNA synthesis and replication. Quinolones interacts with DNA gyrase and topoisomerase IV. DNA gyrase is more sensitive in gram-negative bacteria and topoisomerase IV was more sensitive in gram-positive bacteria. Quinolone binding appears to induce changes in both DNA and the topoisomerase which results in formation of the ternary complex of quinolone, DNA, and either DNA gyrase or topoisomerase IV, 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 form (Hooper et al., 1999). Thus the converted irreversible form breaks the generated double-strand DNA leading to cell death (Laponogov et al., 2010). The mode of action of tazobactam inhibits beta lactamase and prevents the destruction of piperacillin. Therefore, in eradication of bacterial infections tazobactam is given with piperacillin to enhance the activity of piperacillin. Mode of action of piperacillin inhibits the synthesis of bacterial cell walls. The developed synergistic drug in the wound healing materials inhibit the colonization of wound pathogens and promotes the wound healing process.
Conflict of Interest

Authors declare no conflict of interest

Conclusion

Three fabrics (Cotton-Single Jersey, Cotton-Rib and Cotton-Lycra fabrics) were selected and the best among them was selected by its physical properties. Cotton-Lycra fabric showed significant Air permeability, Bursting strength, Tensile strength and Flexural rigidity values. From the two drug combinations-I and II (Piperacillin-Tazobactam and Ciprofloxacin-Tinidazole), Piperacillin-Tazobactam combination showed higher antibacterial activity. Thus Cotton-Lycra fabric and drug combination Piperacillin-Tazobactam were selected for the development of wound dressing material. EN ISO-20743 was performed to determine the antibacterial activity of the finished fabric. Drug treated fabrics showed inhibitory zones of 27mm against Escherichia coli and 25mm against Staphylococcus aureus. Thus the developed drug treated fabrics can be used as wound dressing materials to promote wound healing and to prevent the bacterial infections.

REFERENCES


