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Research Article

ANTIBACTERIAL COATINGS OF KNITTED BIOMATERIALS AGAINST BACTERIAL PATHOGENS USING SYNERGISTIC DRUGS

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ABSTRACT

The primary goal of the treatment of wound is rapid closure and a functional and aesthetically satisfactory scar. Wound healing is a dynamic, interactive process involving soluble mediators, blood cells, extracellular matrix, and parenchymal cells. An improvement in the properties of wound healing textile was achieved through impregnating with antibacterial agents. In the present study, one such method was aimed to reduce the complications of wounds by coating wound healing knitted fabric with two different groups of synergistic drugs such as (piperacillin and tazobactam), and a carrier beta cyclodextrin. The *drug coated* (piperacillin-tazobactam) and *drug-carrier coated* (piperacillin-tazobactam+ beta cyclodextrin) knitted fabrics were subjected to antibacterial activity against the wound pathogens. Among the four different concentration of synergistic drugs (piperacillin-tazobactam), 4X strength exhibited maximum inhibitory zones against all the test organisms. Maximum inhibitory zones of 21mm were observed against *Escherichia coli* and 19mm against *Staphylococcus aureus*. Antibacterial activity of *drug-carrier coated* (piperacillin-tazobactam+ beta cyclodextrin) knitted fabric showed maximum inhibitory zone of 31mm against *Escherichia coli* and *Staphylococcus aureus* respectively. When compared to *drug coated* knitted fabrics, *drug-carrier coated* knitted fabrics showed more antibacterial activity. This was mainly due to the inhibitory actions of the carrier; beta cyclodextrin. Thus the proposed research illustrated the significance of synergistic drug and carrier coatings on wound healing knitted fabrics. The developed product was thus aimed to prevent from complications like amputation in wound closure and a functional and aesthetically satisfactory scar.

Keywords: Wound healing, Synergistic drugs, Drug carrier, Antibacterial activity, wound closure

INTRODUCTION

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[1].

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. Cotton, viscose filament, alginate fiber, jute-cell and chitosan are the material used in medical textiles.

Infection with multidrug-resistant organisms increases the cost of management and duration of hospital stay, as well as mortality and morbidity[2]. The most common pathogen isolated from foot ulcers are *Staphylococcus aureus and* are Methicillin-Resistant *S.* aureus (MRSA)[3-5]. Antibiotics such as Gentamycin, Neomycin, and Mupirocin are good antibacterial used commercially. In the process of healing of diabetic foot wounds dressings containing silver and polyherbal preparations shows good results[4]. Sisomicin (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. Povidone iodine solution dressings are used in healing sutured wounds and hypergranulating wounds. Iodine is found to be toxic to human cells as well as bacteria and fungi at high doses[6-7]. Dressings for wounds are made of cotton, wool, natural or synthetic bandages and gauzes[8]. 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.

Antibacterial activity of small ions like silver, zinc, copper and quaternary ammonium compounds is well documented. Silver impregnated textiles are used as wound dressings for infected wounds or wounds at high risk of infection. Whereas linkages between biocidal moieties and cellulose are covalently formed

on reactive hydroxyl groups, polyamides, polypropylene and polyester lack such reactive sites. Quaternary ammonium salts have a positively charged nitrogen ion that can interact with the negatively charged groups of anionic dyes. These intermolecular interactions inside fibers serve as binding forces to enhance the durability of the biocidal agents once attached. Dye molecules can be used as bridges to bind functional antimicrobial groups to chemically stable synthetic polymers. Quantitative antimicrobial evaluations of treated fabrics reveal that there are significant reductions in bacterial load on surface contact[9].

Ofloxacin, penicillin and other antibiotics have been applied to polyester grafts. A collagen coating was used for binding chloramphenicol and rifampicin. A fibrin sealant was employed to bind gentamycin. More recently, ciprofloxacin and ofloxacin were used unmodified as dyes for polyester fibers. Pad heating was employed as well. The preliminary data were encouraging enough to conduct in vitro testing in rabbits. The best results were obtained with pad heating of a mixture of both antibiotics[10]. The use of small molecules to prepare textiles is of hygienic interest, i.e. impregnation of towels, bed covers, underwear, etc [11-12]. In addition, antimicrobial activity can also reduce odor, which is of interest for wound dressing in the treatment of chronic wounds as well as for clothes[13].

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 wound[14-16] 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 recovery[17]. 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*[18].,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, commercial knitted fabrics were impregnated with synergistic drugs (piperacillin-tazobactam) with and without carriers (beta cyclodextrin). The antibacterial inhibitory effects of the drug+carrier coated knitted fabrics were investigated against the wound-associated pathogens.

MATERIALS AND METHODS

Textile material

The fabric from a commercial producer used for various purposes in the healthcare centre was used as the test fabric. 100% knitted fabric was selected and sterilized prior experimentation. The fabric was cut into squares (swatches), approximately 5 cm x 5 cm, before being treated. After treatment, the swatches were wrinkle removed and sterilized in prior to the antibacterial assay.

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.

Synergistic antimicrobial compound

The synergistic drugs (piperacillin-tazobactam) were used in this study were purchased commercially from the medical pharmaceutical concern. The drugs were checked for their purity based on their specific wavelength using UV-VIS spectrophotometer.

Cross-linker or carrier

Biodegradable cross- linker or drug-carrier was used to treat or coat the knitted fabric based on their mode of application. For non-implantable knitted fabrics, carrier beta-cyclodextrin was used based on their biodegradable properties.

WOUND HEALING TEXTILE STUDIES

Methods of treating synergistic drugs

In the present research a method was described to treat the synergistic drugs with hospital based knitted fabrics. The method was as described by Chun and Gamble, (2007)[19] for reactive dye method.

Reactive dye method (Chun and Gamble, 2007)[19]

Synthesis of reactive synergistic drugs using beta-cyclodextrin

Synthesis of reactive drug-A (piperacillin) was accomplished by suspending 400 mg piperacillin (Ranbaxy) in 2ml de-ionized water in an ice bath at 5^0 C. To this suspension, 2ml of beta-cyclodextrin (Hi media) was added. The suspension was maintained at 5^0 C during drop-wise addition of 1.0 N NaOH to dissolve completely.

Synthesis of reactive drug-B (tazobactam) was accomplished by suspending 200 mg tazobactam (Sigma chemical Co) in 2ml de-ionized water in an ice bath at 5^0 C. To this suspension, 2ml of beta-cyclodextrin was added. The suspension was maintained at 5^0 C during drop-wise addition of 1.0 N NaOH to dissolve completely.

Dye-exhaust method to bind reactive antibacterial agents to cotton fabric

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 (A and B) to 100 ml of de-ionized water. Three, 5 X 5 cm squares of the knitted fabric were submerged in the dyebath heated to 60^0 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 80^0 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 80^0 C in de-ionised water, then rinsed and kept in a convection oven at 105^0 C until dried.

Determining the antibacterial activity of synergistic drugs against the test organisms

The synergistic antibacterial activity of the drug combinations (piperacillin-tazobactam+beta cyclodextrin) 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.

Evaluating the antibacterial activity of *dc* and *dcc* cotton materials

The antibacterial activity of *dc* and *dcc* knitted fabrics 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* (piperacillin-tazobactam+beta cyclodextrin) knitted fabrics with the premeasured size of 10mm in diameter was placed on the surface of medium and the plates were kept for incubation at 37ºC for 24 hours. Plain knitted fabrics 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.

Table 1: Application pattern of reactive drugs on knitted fabric materials

Synergistic drugs	Cross-linker	Fabric used
Piperacillin-tazobactam	Beta-cyclodextrin	Knitted fabric

Table 2: Antibacterial activity of synergistic drugs against the test organisms

S. No	Test organisms	Zone of inhibition (mm)			
		1X	2X	3X	4X
1	*Escherichia coli*	0	12	16	21
2	*Klebsiella pneumoniae*	0	9	12	17
3	*Enterobacter sp*	0	10	12	18
4	*Staphylococcus aureus*	0	9	11	19
5	*Staphylococcus epidermidis*	0	9	14	18

Table 3: Antibacterial activity of *dc* and *dcc* knitted fabrics materials

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

dc: drug coated, dcc: drug-carrier coated

Figure 1: Anti-bacterial activity of synergistic drugs (piperacillin-tazobactam)

RESULTS AND DISCUSSION

Synthesis of reactive synergistic drug using beta-cyclodextrin

The antimicrobial synergistic drugs used for the research were reactively synthesized with specific cross-linker beta-cyclodextrin separately. The combinations of synergistic drugs with cross-linker for binding with knitted fabrics were mentioned in Table 1.

Antibacterial activity of synergistic drugs against the test organisms

The antibacterial activity of three different concentrations of 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 (Figure 1). In Table 2, 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. About 9mm, 14mm and 18mm of inhibitory zones were observed against *Staphylococcus epidermidis* the respectively.

Antibacterial activity of *dc* and *dcc* knitted fabric materials

The antibacterial coatings of the knitted fabrics 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*) knitted fabrics showed good antibacterial inhibitory zones against all the test organisms tested. Interestingly, drug-carrier coated (dcc) (piperacillin-tazobactam+beta cyclodextrin knitted fabric exhibited more inhibitory zones indicating the additional action of the carriers coated in it. Beta cyclodextrin 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. In Table 3, the inhibitory zones obtained for *drug coated* (*dc*) and *drug-carrier coated* (*dcc*) (piperacillin-tazobactam+beta cyclodextrin knitted fabrics 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 and 31mm for *drug-carrier coated* (*dcc*) (piperacillin-tazobactam+beta cyclodextrin) knitted fabrics respectively. Inhibitory zones of 18mm and 25mm against their respective *drug coated* (*dc*) and 20mm and 29mm for *drug-carrier coated* (*dcc*) (piperacillin-tazobactam+beta cyclodextrin) knitted fabrics samples were observed on *Enterobacter sp* and *Citrobacter* sp. *Staphylococcus epidermidis* showed 26mm for *drug coated* (*dc*) and 30mm for *drug-carrier coated* (*dcc*) knitted fabric samples. These results confirmed that *drug-carrier coated* (*dcc*) (piperacillin-tazobactam+beta cyclodextrin) knitted fabrics samples show good antibacterial inhibitory zones against wound pathogens.

The 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. 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[20]. Thus the converted irreversible form break the generated double-strand DNA leading to cell death[21]. In present study 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 beta cyclodextrin contains cyclic oligosaccharides which forms non-covalent complexes with of drugs and alter their physicochemical properties. The potential sites for chemical modification is the primary and secondary hydroxyl groups of the native (α, β, γ-) cyclodextrins. By incorporating these agents into drug delivery systems, as a physical mixtures, these agents covalently bound conjugates or cross-linking agents, resulting in constant drug release. Therefore these drug and carrier combination shows good antibacterial activity against diabetic wound pathogens.

CONCLUSION

The study was aimed to determine the effect of synergistic activity of the impregnating drugs and carriers on the product substrate for proving the antimicrobial efficacy, durability and persistence. To reduce the complications of wound infections in patients, wound healing cotton materials were impregnated with two different groups of drugs (synergistic drugs) and a carrier (DL-Lactic acid & beta cyclodextrin. The *drug coated* (piperacillin-tazobactam) and *drug-carrier coated* (piperacillin-tazobactam+beta cyclodextrin) knitted fabric materials were subjected to antibacterial activity against the wound pathogens. Potential inhibitory zones against all the test organisms were observed on *drug coated* (piperacillin-tazobactam) and *drug-carrier coated* (piperacillin-tazobactam+beta cyclodextrin) knitted fabric materials showed. When compared to *drug coated* materials, *drug-carrier coated* (piperacillin-tazobactam+beta cyclodextrin) knitted fabric materials showed more antibacterial activity. This was mainly due to the inhibitory actions of the carrier beta cyclodextrin, in the mixture. Maximum inhibitory zone of 31mm was recorded against *Escherichia coli* and *staphylococcus aureus* respectively for *drug-carrier* (piperacillin-tazobactam+beta cyclodextrin) *coated* materials. Thus the proposed research illustrated the significance of synergistic drug and carrier coatings on wound healing knitted fabric materials. The developed product was thus aimed to prevent from complications like amputation in wound infection patients. This method signifies an effective wound healing process to achieve repair from any types of tissue injury.

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