Study of antimicrobial property of knitted spacer fabric treated with 1-Tetradecanaminium, N,N-dimethyl-N-[3-(trimethoxysilyl)propyl], chloride

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ABSTRACT – REZUMAT

Study of antimicrobial property of knitted spacer fabric treated with 1-Tetradecanaminium, N,N-dimethyl-N-[3-(trimethoxysilyl)propyl], chloride

The current study helps to understand the efficacy of antimicrobial treatment done on a weft-knitted spacer fabric. The antimicrobial agent used in the study is from the class of quaternary ammonium salts. The fabric is evaluated for antimicrobial efficacy. It is also cross-compared with the drug and some advanced wound dressings available in the market for its antimicrobial and anti-biofilm activity.

Knitted spacer fabric treated with 1-Tetradecanaminium, N,N-dimethyl-N-[3-(trimethoxysilyl)propyl], chloride (QAS) shows good antimicrobial properties and is shown to exhibit a broad range of antimicrobial effects against gram-positive bacteria, gram-negative bacteria and also fungus and yeast. The rapidity of killing and durability of microbial activity make it a very suitable material for infection control in applications like wound care materials. The spacer fabric can behave as foam in the management of exudates in wounds.

The efficacy of this material compared with other dressing materials yields substantial evidence of its activity as a wound dressing material when it comes to management of microbial contaminations including biofilm prevention and disruption. Such material can find use in the management of chronic wounds and is a subject matter of further studies with clinical evidence.

Keywords: *spacer fabric, knitted, antimicrobial, QAS, biofilm*

Studiul proprietăților antimicrobiene ale distanţierelor tricotate tratate cu clorură de 1-Tetradecanaminiu, N,N-dimetil N-[3-(trimetoxisilil)propil]

Studiul actual ajută la înțelegerea eficacității tratamentului antimicrobian efectuat pe un distanţier tricotat. Agentul antimicrobian utilizat în studiu este din clasa sărurilor cuaternare de amoniu. Materialul textil este evaluat pentru eficacitatea antimicrobiană. De asemenea, este comparat cu medicamentul și cu unele pansamente avansate disponibile pe piață pentru activitatea sa antimicrobiană și anti-biofilm.

Distanţierul tricotat tratat cu clorură de 1-Tetradecanaminiu, N,N-dimetil-N-[3-(trimetoxisilil)propil]- (QAS) prezintă proprietăți antimicrobiene și dovedeşte că are o gamă largă de efecte antimicrobiene împotriva bacteriilor gram pozitive, bacteriilor gram negative și, de asemenea, împotriva fungilor şi levurilor. Rapiditatea uciderii și durabilitatea activității microbiene îl fac un material foarte potrivit pentru controlul infecțiilor în aplicații, precum materialele de îngrijire a rănilor. Materialul distanțier se poate comporta ca spumă în managementul exsudatelor din răni.

Eficacitatea acestui material, în comparație cu alte materiale de pansament, oferă dovezi substanțiale ale activității sale ca pansament în cazul gestionării contaminărilor microbiene, inclusiv prevenirea și perturbarea biofilmului. Un astfel de material poate fi utilizat în gestionarea plăgii cronice și este subiectul unor studii ulterioare cu dovezi clinice.

Cuvinte-cheie*: material distanţier, tricot, antimicrobian, QAS, biofilm*

INTRODUCTION

The antimicrobial property of textiles is a subject matter of great importance in modern days. It finds a wide application in various areas from usages like apparel, hygiene clothes, and protective barrier textiles to areas as broad as medical textiles. There are various underlying technologies of the primary mode of action of these technologies. The rise of antimicrobial technology in textiles has helped conventional use textiles to expand its usage in the field of applications like, medical, pharmaceutical, protective, engineering, agricultural, and food industries. The choices of antimicrobial agents, and treatment techniques on textiles are guided mainly by the efficacy of testing and the durability of the treatment after repetitive laundry washes. Various methods are employed for the development of antimicrobial fabrics from impregnation to coatings and surface grafting to in-situ polymerisation [1–3]. Surface modifications with unconventional methodologies like plasma, gamma radiations and electron beam bombardment are also widely studied [2].

Microorganisms like Bacteria are essentially made up of semi-permeable cell walls. If the cell wall is disturbed externally for any reason, the bacterial cell cannot survive. This property is used as the basic mechanism of bactericidal activity of textiles. There can be other ways by which the cell content can be interfered with and made ineffective for the bacteria to replicate. In such a case the bacteria remains alive

but does not replicate. This phenomenon of antibacterial activity is termed a bacteriostatic property of textiles. Thus the word bactericidal is used when the bacteria is killed, whereas the term bacteriostatic is used when the bacteria is not allowed to replicate and grow in numbers. Both these activities are called antibacterial activity of textiles. In general, almost all bactericidal agents act as bacteriostatic agents at lower concentrations [1].

The literature cites that the modes of action of the antimicrobial agents' preliminary are as below [1]:

- Protein coagulation of the microbes.
- Disruption of the cell wall of microbes causing the contents of the cell to be exposed/damaged.
- Removal of free sulphydryl group essential for the functioning of enzymes.
- A compound resembling the essential substrate of the enzyme diverts or misleads the enzyme essential for the metabolism of the cell and causes cell death.

The most common category of the chemicals/compounds that are employed to make any textiles antibacterial is either of one of the below as depicted in table 1.

The most conventional practices used to bind the antimicrobial agent on textiles are [1]:

– fibre reaction and formation of metastable bonds;
– interaction with thermosetting agent;
– formation of co-ordination compound (Binders/Linkers);
– ion exchange methods;
– high energy radiations – In-situ grafting/polymerisation.

When one looks at the antimicrobials that are commonly used for textiles, except the metal ions or natural materials like chitosan, not many are preferred in wound dressing applications because the compound is expected to touch breached skin. The biggest concern with these compounds is that the compound may leach into the wound along with the wound dressings during its usage. It may cause cell toxicity and interfere with the normal wound-healing mechanism of the body. Such interaction in the wound is not desirable. Certain interactions can hinder normal wound healing conditions of the wound, even if they are very effective in fighting the microbial burden on the wound.

Silver seems to be the most commonly used compound when it comes to wound dressing applications of dressings. Some of the most prominent wound dressing materials that are based on silver-based antimicrobial technology are [1]:

– Acticoat – A nanocrystalline Silver based resin;
– AlphaSan – Silver Sodium hydrogen zirconium phosphate, for silver ions release;
– Actisorb Silver 220 – Silver ion-based dressing;
– Aquacel Agr – Sodium carboxymethyl cellulose with 1–2% Silver in ionic form;
– Novaron – Zirconium Silver phosphate, for silver ion release.

The main difference between microbes and bacteria is that microbes represent microscopic organisms. The most common of the seven groups of microbes are bacteria, archaea, protozoa, algae, fungi, viruses, and multicellular animal parasites. On the other hand, bacteria are a form of single-celled microbes.

All type of antimicrobial agents listed in table 1, needs to be leached from the dressing to go into the wound and encounter the microbes in the wound and on the dressing. Hence toxicity of the product becomes a crucial matter. Ideally, If they can be immobilised on the dressing and the sphere of influence of

Table 1

COMMON TYPE OF ANTIMICROBIAL AGENTS USED IN TEXTILES [2,3]

Type	Action	Merits	Demerit	Applications
Ag and other metals like Gold, copper etc.	Producing reactive oxygen species, demolition of protein, lipid and DNA	Effective and durable	Chance of depletion	Cotton, Wool, Silk, Polyester, Nylon and regenerated Cellulose
QACS	Formation of the complex with microbes, denaturing protein, and disturbing DNA to reduce propagation	Effective and durable	Often hazardous	Cotton, Polyester, Nylon and Wool
Polybiguanide	Damaging lipids, leakage of cytoplasmic sources	Effective and durable	Large amount required	Cotton, Polyester and Nylon
Triclosan	Prohibiting lipid biosynthesis, cell membrane integrity depletion	Effective and durable	Breaks into toxic dioxin	Polyester, Nylon, Polypropylene, Cellulose acetate and Acrylic
N-halamines	Binding with microbes, preventing enzymatic and metabolic processes	Effective and durable	Needs regeneration, and can cause odour	Cotton, Polyester, Nylon and Wool
Chitosan	Blocking protein synthesis, obstructing transportation of solutes toward cells	Eco-Friendly	Poor durability, opposing effect on handle	Cotton, Polyester, and Wool

the antimicrobial action can be extended, then the fullest potential of the compound can be exploited, without compromising the cell toxicity matter.

Quaternary ammonium salts (QAS) have a promising future as antimicrobial agents for textiles as they can be immobilised on the surface of textiles and have been studied to cause no bacterial resistance built up due to the unique nature of its kill which is attributed to physical rupture of the cell wall of microbes.

As per the literature, the antimicrobial compound (QAS) is bonded by silanol (a hydrolyzed silane) and it is covalently bonded to receptive surfaces (chemisorption). This bonding is then made even more durable by the silanol functionality, which homopolymerises (bonds to its neighbouring molecule). After the molecule has homopolymerised, it becomes an integral and permanent part of the product even on materials with which it cannot react covalently [4]. Figure 3 shows the matrix of cross-polymerized chemicals and its mechanism of action. Thus after treatment, cationic sites are created on the base substrate that acts as an active layer of swords [5, 6].

As this agent is positively charged (cations) and microbes are negatively charged, nearby the microbes are drawn into the active surface of the antimicrobial agent and killed. The active component responsible for the microbial kill is the edge that blows the microbes, the long molecular chain acts like a sword that pierces the cell membrane of all microbes that come in contact with it [5, 7].

This stabbing and an “electrocution” of the anionic biochemical in the membrane of pathogens resulting from the positive charge means that the antimicrobial will be fully effective as long as the surface of the treated substrate remains intact. Since it is not consumed and does not dissipate, the antimicrobial's active portion is not depleted and continues to control microbial growth. The mechanism of action is physical control, unlike chemical controls as seen in the case of leaching biocides and drugs [6]. Figure 3 shows the schematic diagram of the mechanism of microbial kill.

The current study helps to understand the efficacy of antimicrobial treatment done on a weft-knitted spacer fabric. The antimicrobial agent that is been used in this study is a quaternary ammonium salt. The fabric is evaluated for its antimicrobial efficacy and is cross-compared with the drugs and some advanced wound dressings available in the market for its antimicrobial and anti-biofilm activity.

MATERIALS AND METHODS

Materials

The spacer fabric used in this study was made by the technique of weft-knitted spacer fabric production. It is constructed with the use of Polyethylene terephthalate [PET] and polyurethane yarns in the composition of 90% Polyethylene terephthalate and 10% Polyurethane. The fabric is produced on a 24 gauge interlock knitting machine by spacer knitting technology. The structure has three distinct surfaces. The face is knitted by the cylinder needles with Polyethylene terephthalate yarn of 150/108 D and the back is knitted by the dial needles with Polyethylene terephthalate yarn of 150/108 D. The middle layer is made up of 40 D Monofilament Polyethylene terephthalate yarns that connect the front layer (cylinder loops) with the back layer (dial loops). 40 D polyurethane yarn is used as the elastomeric yarn to impart stretch to the structure. The Cylinder to dial height on the machine decides the space between the face and back layers of the fabric and hence it is optimally set to have 1.8 mm height of the fabric. The material is three-dimensional (3D) in its construction, unlike conventional gauze dressings. Figure 1 shows the image of the spacer fabric along with a cross-sectional view of the material.

It must be noted that Polyethylene terephthalate is a known biocompatible material. Further, it is a very stable material against biofluids of the wound and it does not deteriorate in long-term usage. Hence the material can be a good choice when used in wound care requirements. Table 2 enlists the details of the fabric construction.

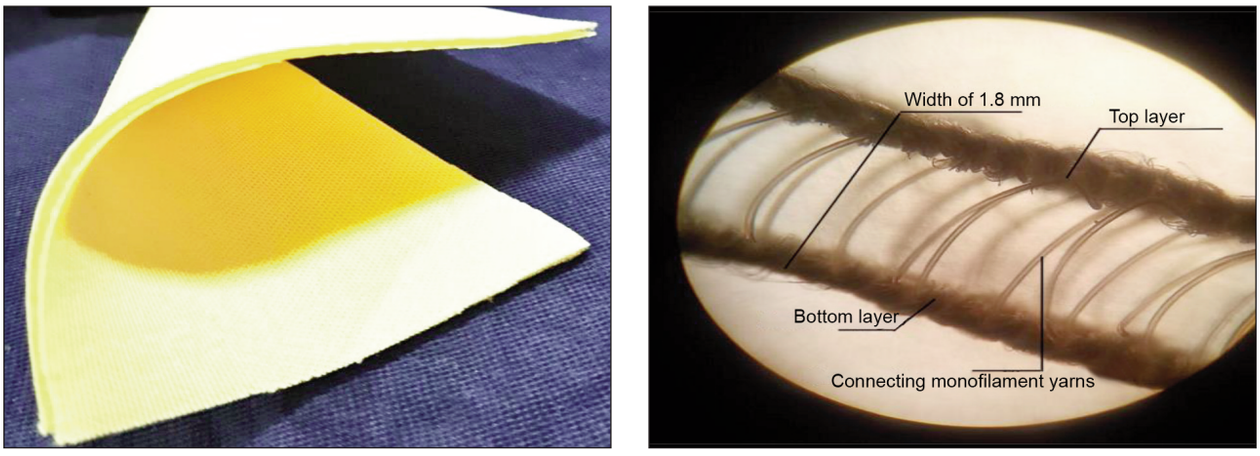


Fig. 1. Image of spacer fabric with a cross-sectional view of the material

Table 2

CONSTRUCTION DETAILS OF SPACER FABRIC	
Property	**Value**
Fabric content	90% PET/ 10% Polyurethane
Yarn at the face of the fabric	150/108 D Polyester filament yarns
Separator yarn at the centre	40 D, monofilament yarn
Yarn at the back of the fabric	150/108 Polyester filament yarns
Polyurethane yarn	40 D
GSM of fabric	300
Fabric thickness	1.8 mm

Methods

The fabric was impregnated [1] in a padding bath containing 15 gpl solution of Quaternary Ammonium Salt (QAS) called 1-Tetradecanaminium, N,N-dimethyl-N-[3-(trimethoxysilyl)propyl]-,chloride (DMTAC as abbreviated in this article). The chemical was procured from Sigma Aldrich-India. Various stabilizers and linking primers were used along with pH-balancing chemicals. The most common linkers used for such compounds are amino acids.

The fabric was squeezed under the stenter mangles and then it was dried and cured in the stenter frame at 180°C for around 35 seconds of residence time. After treatment, the fabric was washed at 40°C with a wetting agent and dried at 140°C. The fabric after treatment was an active antimicrobial fabric. Figure 2 shows the schematic representation of the process used for the treatment of knitted spacer fabric. The active component of QAS is supposed to be cross-linked on the surface of the spacer fabric imparting it the antimicrobial property. The mechanism of kill is documented in literature as the physical rupture of the pathogen's cell wall by long aliphatic chains of the cross-linked chemical [5].

The textile material thus formed is evaluated for various physical properties as listed in the table 3.

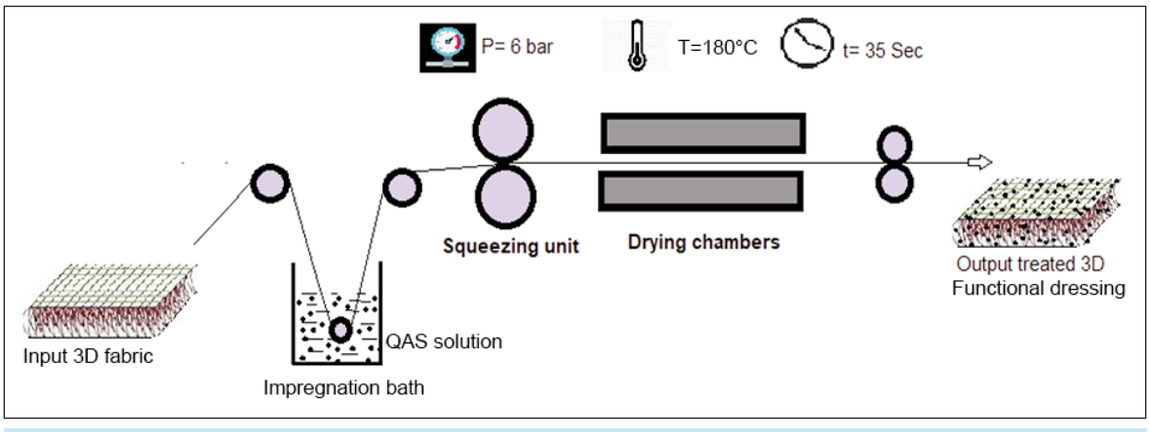


Fig. 2. Schematic representation of the manufacturing process

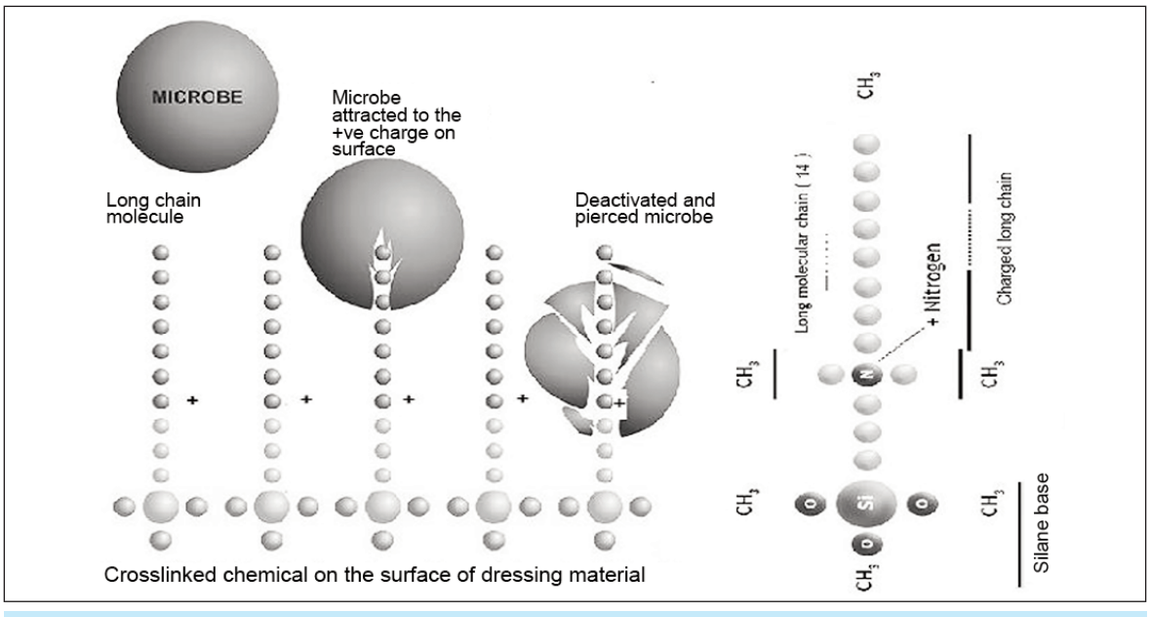


Fig. 3. Schematic representation of the mechanism of microbial Kill (Source: Aegis Microbe Shield System)

Table 3

PHYSICAL PROPERTIES OF THE ANTIMICROBIAL SPACER FABRIC			
Property	**Instrument used**	**Method/Standard used**	**Value**
Weight	GSM cutter- Weigh Scale	GSM	298
Air permeability	Air permeability tester	ASTM D 737	110 $cm^3/cm^2/s$
Elasticity	Extension relaxation method	British Pharmacopeia (BP)	43%
Stiffness (Bending length)	Stiffness tester	BS 3356	4.3 cm
Overall moisture management capacity	Moisture management tester	AATCC 195	0.6033
Water vapour permeability	Water vapour permeability tester	ASTM E 96-95 Option B	2203 $Gm/Met^2/24$ h
Water vapour resistance	Sweating hot plate method	ISO 11092	4.7423 M^2Pa/W
Water holding capacity	Gravimetric	On weight method (OWF)	360%
Synthetic blood holding capacity	Gravimetric	On weight method(OWF)	538%
Bursting strength	Bursting strength tester	ASTM D 3746	183 PSI
Tearing strength	Elmendorf tester	ASTM D 1424	14.1 lbf

Figure 4 shows the chemical reaction depicting the Cross-linking and binding of DMTAC on the Surface of PET spacer fabric. It can be seen that the DMTAC molecule not only gets bonded on the surface of PET fibre but also gets cross-bound with each other to form a 3D matrix through – Si-O-Si-bonds.

Methodology of evaluation

FESM micrographs

FESM (Field emission scanning microscopy, ZEISS, Germany EVO Model) was conducted at various magnification levels of the spacer fabric to obtain micrographs of the structure. The images were obtained for the structure before and after cross-linking of QAS on the surface of the structure.

Quantitative evaluation of antimicrobial activity (ASTM E2315)

The treated spacer fabric was evaluated for quantitative values of antimicrobial activity by ASTM E2315 [4] for three gram-positive bacteria, three gram-negative bacteria and yeast. Various contact periods were used to understand the broad spectrum effect spread over a long period

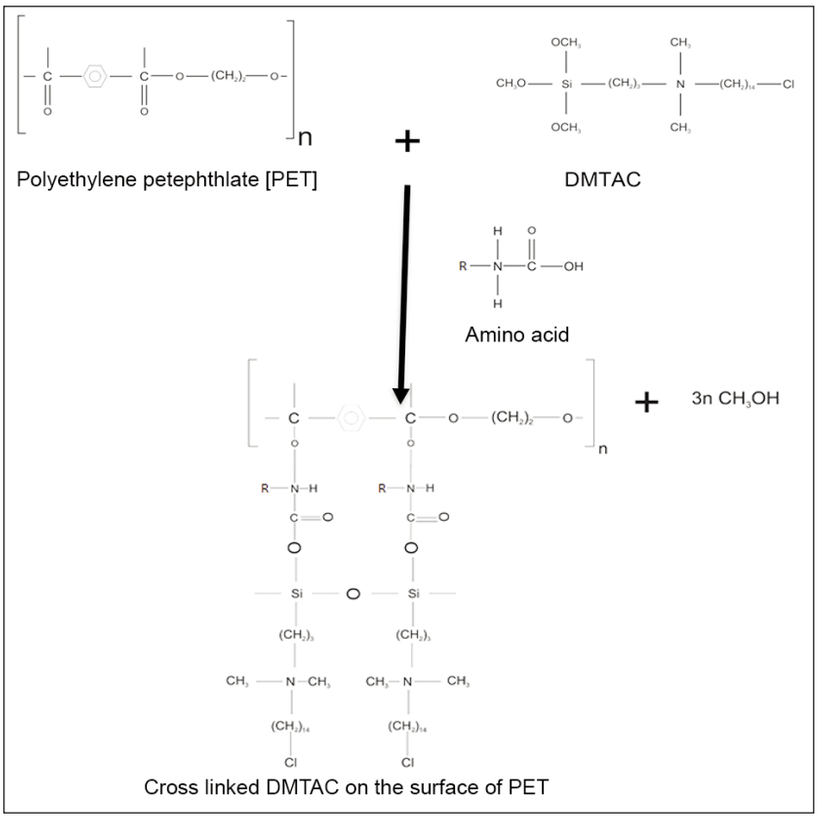


Fig. 4. Chemical reaction depicting the cross-linking and binding of DMTAC on the Surface of PET spacer fabric

The rapidity of microbial kill by ASTM D 6329-98

This test was performed to analyse the rapidity of killing microorganisms. As per standard protocol followed, the test was conducted for Staphylococcus Aureus ATCC 12600 and *Escherichia Coli* NCIM 2065. The test was also conducted to test the rapidity of killing the most drug-resistant strain called methicillin-resistant staphylococcus aureus (MRSA) ATCC 43300. Table 4 summarises the results of the test.

Antimicrobial effectiveness testing USP 51

The effectiveness of antimicrobial testing was evaluated using the test method, which is defined in US Pharmacopeia Standards chapter 51 most commonly known as USP 51. Though the method is widely used for liquid preservative contents, it also is useful to assess the effectiveness of the bactericidal activity of surfaces. Especially so where

challenge organisms are cultured on the surface of the component and then the washing of surface growth is taken to know the viable count of extract and compared with the zero time readings to study the preservative action of the subject matter [8, 9].

Evaluation of efficacy with comparator technologies

The efficacy of treated antimicrobial spacer fabric concerning various parameters like broad range microbial kill, rapidity of kill and long durability of kill of microbes makes this material suitable for applications like antimicrobial wound care dressing. Such properties are essential in wound dressing to tackle the infections that may hamper the normal wound healing cycle [10–12]. Hence the microbicide efficacy of this treated spacer fabric was evaluated with the two most common dressing technologies available in the advanced wound care market. One material was a polyester spacer fabric with silver-based antimicrobial and the other was cotton-based gauge dressing with QAS named Polydiallyldimethylammonium chloride (pDADMAC).

Table 4

COMPARATOR TECHNOLOGIES OF ANTIMICROBIAL WOUND DRESSINGS USED IN THE STUDY

Test item code	Technology	Material form
Reference 1	Elemental Silver-based antimicrobial	Polyester knitted spacer fabric
Reference 2	QAS - pDADMAC	Cotton mesh
Test Item	QAS - TMDAC	Polyester knitted spacer fabric

In this various studies like the Disc Diffusion test, Broth test rate of kill and biofilm prevention and disruption assay were performed.

RESULTS AND DISCUSSIONS

FESM Micrographs

FESM (Field emission scanning microscopy) was conducted at various magnification levels of the spacer fabric structure to obtain micrographs of the structure. The images were obtained for the structure before and after cross-linking of DMTAC on the surface of the structure. It can be seen from figure 5 that there is a layer of cross-bonded chemicals on the surfaces of the yarns. Micrographs A and C also shows clearly the presence of the monofilament yarn between the groups of multifilament yarns. This coated layer of DMTAC is now immobilised on the surface of the spacer fabric that is present on all layers i.e. on both the faces and also the middle interconnecting layer of fabric.

Quantitative evaluation of antimicrobial activity (ASTM E2315)

ASTM E 2315 method of quantitative assessment of antimicrobial activity for 3-gram positive strains, 3-gram negative strains and yeast was done [7]. The

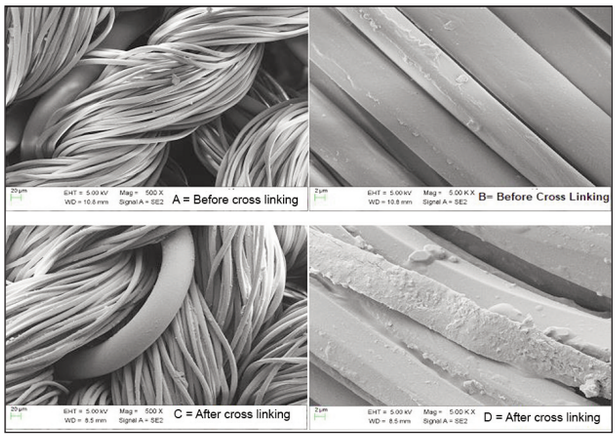


Fig. 5. FESM Images of the material before and after QAS cross-linking

positive control used was sterile nonwoven polyester viscose wipes saturated with Neolone P.E-Phenoxyethanol and the negative control was untreated sterile nonwoven polyester viscose wipes.

In this method, a control tube using nutrients was prepared without inoculation of microorganisms. The bacterial culture is added in 10 ml sterile nutrient broth and vortex and let to stand still for 15–20 min. This was then diluted with a suitable medium to 1×10^6 organisms (CFU/ml).

The treated knitted spacer fabric is cut to size such that it is capable of absorbing 1.0 +/– 0.1 ml standard inoculum. The positive and negative controls are also made ready. The test item, positive control and negative control are placed inside sterile screw cap bottles. The test inoculum (1 ml) was added to the surfaces of the items using a Micropipette. The contact time of one hour, 1 hour, 4 hours, 24 hours and 48 hours was used in the study. After the study time, the specimen was transferred into a 250 ml culture bottle and 100 +/– 1 ml of 0.05 % neutralizing solution was added and shaken vigorously for 1 minute. Serial dilutions (10^o, 10^1, 10^2 and 10^3) were done with sterile water. The dilution is plated on suitable nutrient agar plates for bacteria and fungus. The Incubation of 36 hrs at 37°C for bacteria and 5–7 days at 25°C for fungus was done. After incubation, the number of colonies was counted and noted for different contact times. The evaluation was done by reporting the number of microbes recovered on the 0^{th} contact time and the required contact time.

The percentage reduction of microbes was counted as per the below formula:

$$R = 100\ (B - A)/B \qquad (1)$$

Where R is percentage reduction, A – the number of microbes recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period and B – the number of microbes recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at “0” contact time).

Table 5

QUANTITATIVE ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF TREATED SPACER FABRIC					
S.no	**Test organisms**	**% of reduction**			
		1 hour	**4 hour**	**24 hour**	**48 hour**
1	*Staphylococcus aureus* ATCC 6538	99.99	99.99	99.9999	99.9999
2	*Listeria monocytogenes* ATCC 19115	99.99	99.99	99.9999	99.9999
3	*Enterococcus faecalis* ATCC 29212	99.99	99.99	99.9999	99.9999
4	*Escherichia coli* ATCC 25922	99.99	99.99	99.9999	99.9999
5	*Pseudomonas aeruginosa* ATCC 15442	99.99	99.99	99.9999	99.9999
6	*Klebsiella pneumoniae* ATCC 4352	99.99	99.99	99.9999	99.9999
7	*Candida albicans* ATCC 10231	90	90	99.9999	99.9999

Time-based kill results for different strains show that the kill rate is at least 4 log reduction within 1 hour except for *Candida albicans* yeast which as per literature needs long-duration contact for effective kill.

Table 5 shows that knitted spacer fabric has excellent broad-spectrum antimicrobial activity.

As seen in the literature survey it can be evidenced that the antimicrobial action of the QAS is attributed to its surface action after it is immobilized on the surface of the textile. Irrespective of the class of pathogen, the mechanism of killing is effective in the physical lyses of the pathogen [5, 10]. The study shows a relatively lower percentage of kills in short intervals of time for a yeast called Candida albicans, as it needs more incubation times for the yeast, which is a known medical fact.

The test results show that the treated spacer fabric is showing good antimicrobial activity for a broad range of microbes for an extended period of 48 hours as studied by ASTME 2315.

The rapidity of microbial kill by ASTM D 6329-98

This test was performed to analyse the rapidity of killing microorganisms. As per standard protocol (ASTMD 6329-98) followed, the test was conducted for *Staphylococcus Aureus* ATCC 12600 and *Escherichia Coli* NCIM 2065. The test was also conducted to test the rapidity of killing the most drug-resistant strain called methicillin-resistant *Staphylococcus Aureus* (MRSA) ATCC 43300. Table 6 summarises the results of the test.

From the test, it is clear that the antimicrobial spacer fabric exhibits the property of instantaneous kill when it comes in contact with microbes. The kill property is also seen in a very drug-resistant strain like MRSA. This strain is called a superbug because it has become resistant to many known and commonly used antibiotics. This is essentially because the mechanism of kill as documented for the QAS is the physical rupture of the cell wall of pathogens. Irrespective of the resistance built up for the drugs, it still is vulnerable to the physical rapture.

Antimicrobial effectiveness testing USP 51

In this test method, the challenge microorganisms used were *Candida albicans* ATCC10231, *Aspergillus brasiliensis* ATCC16404, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC9027 and *Staphylococcus aureus* ATCC6538. Table 6 shows the % kill rate of microbes over some time up to 28 days.

The test results show that the antimicrobial effect of the treated spacer fabric is effective for up to 28 days. The efficacy does not drop for a long period. This is in line with the literature findings that claim that the antimicrobial once bonded to the surface of the textile is not depleted as it is not consumed in the process of microbial kill [5]. Hence the spacer fabric may be used for applications that need extended protection against microbes.

Evaluation of efficacy with comparator technologies

Disc Diffusion Test

Ciprofloxacin, a known antibiotic was used as positive control for evaluation. Discs marked as positive Control (C), Test Item (TI), Reference 1 (Ref1) and Reference 2 (Ref2) were cut and placed on 90 mm

Table 6

RAPIDITY OF KILL ASTM D 6329-98 OF TREATED SPACER FABRIC									
Test organism	**Inoculum strength**	**Test organism's kill rate at specific time intervals (%)**							
		30 sec	**1 min**	**10 min**	**30 min**	**1 hrs**	**4 hrs**	**8 hrs**	**12 hrs**
Staphylococcus Aureus ATCC 12600	$1.06x0^6$ CFU/0.5 ml	-	99.47	99.97	99.99	99.99	99.99	99.99	99.99
Escherchia Coli NCIM 2065	$1.06x0^6$ CFU/0.5 ml	-	99.48	99.98	99.99	99.99	99.99	99.99	99.99
MRSA ATCC 43300	$1.02x0^6$ CFU/0.5 ml	99.25	99.52	99.98	99.98	99.99	99.99	99.99	99.99

Table 7

USP 51 ANTIMICROBIAL EFFECTIVENESS OF TREATED SPACER FABRIC				
Test organism	**Inoculum concentration (CFU's/ml) results**	**% Kill rate seen over some time**		
	Start Concentration	**Day 7**	**Day 14**	**Day 28**
P.aeruginosa	$2.00x10^7$	100.0	100.0	100.0
E. coli	$3.00x10^7$	100.0	100.0	100.0
S. aureus	$11.00x10^6$	100.0	100.0	100.0
Candida albicans	$2.00x10^6$	99.99	99.99	99.99
Aspergillus Niger	$1.00x10^6$	100.0	100.0	100.0

agar plates spread with 100 µl of ~10^8 CFU/ml of each bacterial strain and incubated at 37±1°C for 24 hours and zone of inhibition (ZOI) i.e radius in mm around the discs were measured using a transparent ruler [11, 12].

The test organisms used were *Escherichia coli* (E. coli) ATCC 25922, *Pseudomonas aeruginosa* (P. aeruginosa) ATCC 9027, *Staphylococcus aureus* (S. aureus) ATCC 25923, *Candida albicans* (C. albicans) ATCC90028. The same strains were used in all the comparator studies.

The positive control in the case of fungus was Flucanazole which is a known fungicidal drug. The findings of the ZOI in comparison to positive controls as listed in table 8.

From table 8, it is clear that there is no significant zone of inhibition seen around the treated fabric as against known antibiotics and fungicidal compounds. This may be attributed to the fact that the cross-bonding of DMTAC on the surface of the fabric gives it a three-dimensional cross-linked matrix structure. This is called immobilisation of the DMTAC on the surface of the spacer fabric. Due to this immobilisation, the active ingredient does not leach from the surface of the fabric, unlike the drugs. A small zone seen around the material may be attributed to quorum sensing, which is the cell-to-cell communication in bacterial flora, which hints at the vulnerable bacteria near the hazard zone to stay away from the hazard and maintain distance [13–15].

After the above assay, the content then was transferred to the nutrient broth and continued incubation at 37±1°C for 24 hours and plated on nutrient agar plates to measure the residual number of microbes in the discs. Mean Log_{10} CFU/ml of broth was plotted for Control (C), Test Item (TI), Ref 1 and Ref 2 discs by using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple

Table 8

ZOI COMPARATORS AGAINST KNOWN ANTIBIOTIC AND FUNGICIDAL COMPOUND			
Test organisms	**Name of the test item**	**ZOI (mm)**	
		Mean	**SD**
E. coli ATCC 25922	treated spacer fabric	0.00	0.00
	Ciprofloxacin – 1 µg/Disc	11.00	1.00
P. aeruginosa ATCC 9027	Treated spacer fabric	1.2	0.40
	Ciprofloxacin	9.50	0.50
S. aureus ATCC 25923	Treated spacer fabric	0.00	0.00
	Ciprofloxacin	11.55	0.95
C. albicans ATCC90028	Treated spacer fabric	0.9	0.65
	Flucanazole – 25 µg/Disc	3.60	0.40

Comparison Test, P<0.05 were chosen as the criterion for statistical significance. Figure 6 shows the bacterial load reduction with a 6 mm disc with known drugs (Ciprofloxacin) whereas figures 7 to 9 show the comparator with Ref1 and Ref2 when tested with different challenge microorganisms for a 12 mm disc.

From the study, it can be seen that the treated spacer fabric exhibits very efficient antimicrobial inhibition properties when compared with known drugs for

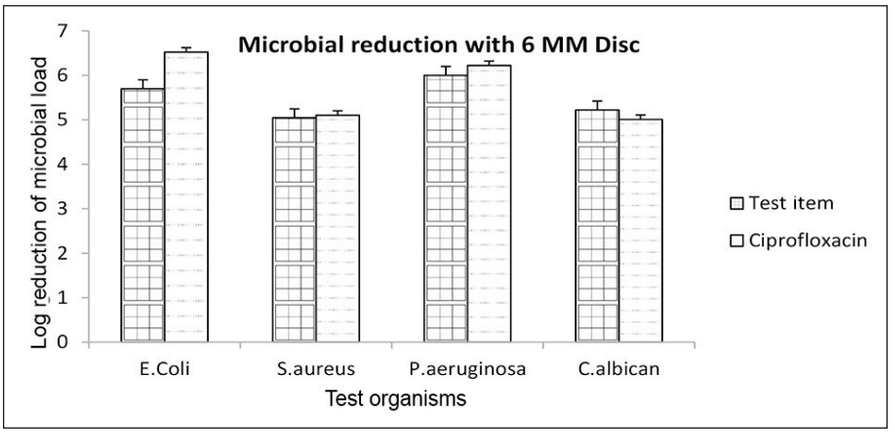


Fig. 6. Microbial load reduction on the treated spacer fabric compared to Ciprofloxacin

different strains of microbes that are gram-positive, gram-negative and yeast.

In comparison with dressing material with elemental silver and the compound pDADMAC, the treated spacer fabric has a very efficient activity for different strains of microbes that are gram-positive, gram-negative and yeast. It is because of this property of microbial load reduction the treated spacer fabric can be used in areas like as wound dressing material.

Broth test – Time rate of Kill

In this method, 15 millimetres (mm) discs of Test Item (TI), Ref 1 and Ref 2 were cut and placed into 24 well plates containing ~10^7 CFU/ml in a nutrient broth of *P. aeruginosa* and *S. aureus* respectively and incubated at 37±1°C. Control (C) tubes were incubated with only ~10^7 CFU/ml in a nutrient broth of *P. aeruginosa* and *S. aureus* respectively. After incubation, the broth was sampled at 1, 5, 15, 30 and 60 minutes plated on agar plates and incubated for 24 hours at 37±1°C. The mean Log_{10}CFU/ml of broth was plotted for C, TI, Ref1 and Ref 2 discs by using one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. $P<0.05$ was chosen as the criterion for statistical significance. Figures 8 and 9 show the effect of time on the kill properties of the materials.

The time rate of kill study shows that the treated antimicrobial spacer fabric exhibits similar properties in terms of its kill when compared with reference and reference 2. Hence it can be considered at par with infection-reducing properties of the established advanced antimicrobial wound dressing.

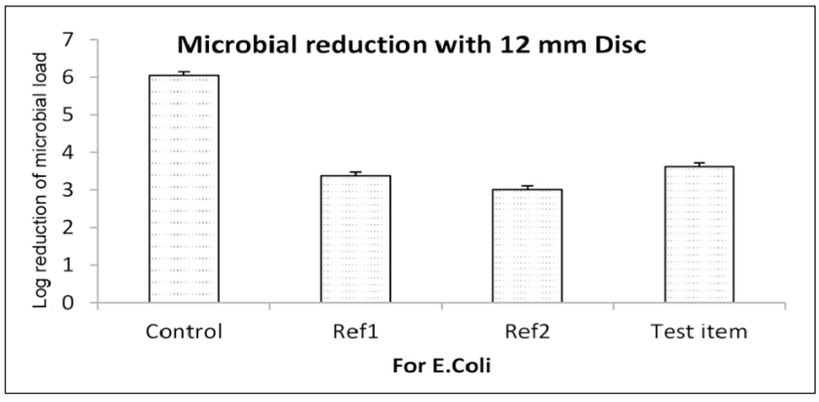


Fig. 7. Microbial load reduction comparisons with comparators for *E. coli* organism

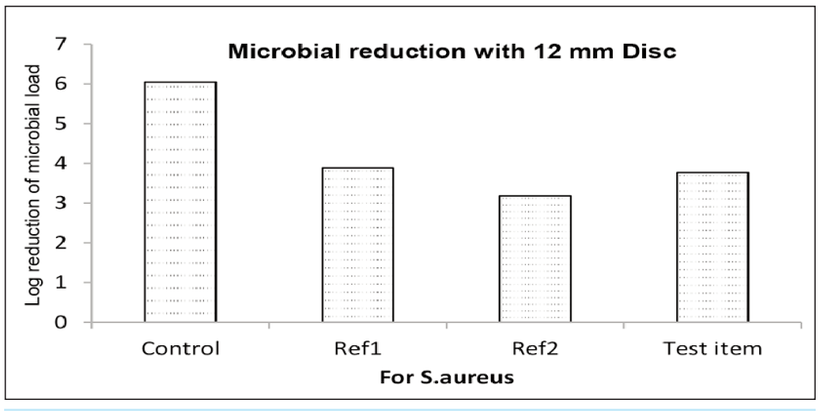
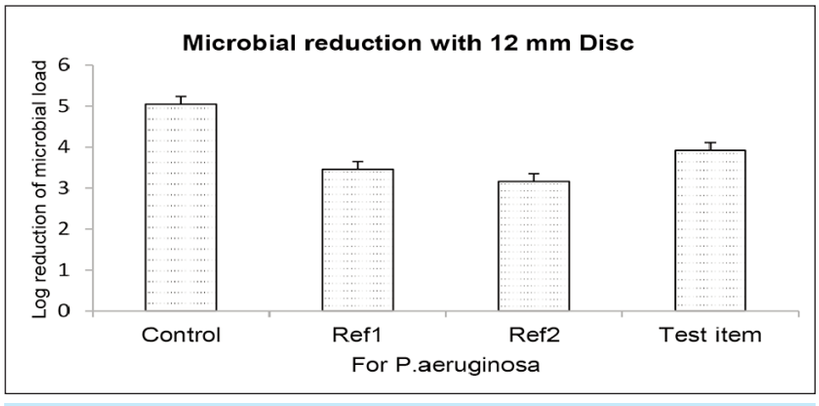


Fig. 8. Microbial load reduction comparisons with comparators for *S. aureus* organism



9. Microbial load reduction comparison with comparators for *P. aeruginosa* organism

Biofilm prevention and disruption assay

Biofilms are described as complex microbial communities. The microorganisms synthesize and secrete a protective matrix that attaches the biofilm firmly to a living or non-living surface [16]. Biofilms are dynamic heterogeneous communities that are continuously changing [15].

They may consist of a single bacterial or fungal species, or more commonly, may be poly microbial, i.e. contain multiple diverse species of microbes [16, 17].

In short, a biofilm can be described as bacteria embedded in a thick, slimy barrier of sugars and proteins that is very difficult to penetrate. The biofilm barrier acts as a protective layer against the microorganisms from external threats. Almost all chronic wounds have biofilm formed in them.

For this study, two assays were made one was a prevention assay and the other was a disruption assay. In this method, 15 millimetres (mm) discs (to cover the entire surface area of each well) of Test Item (TI),

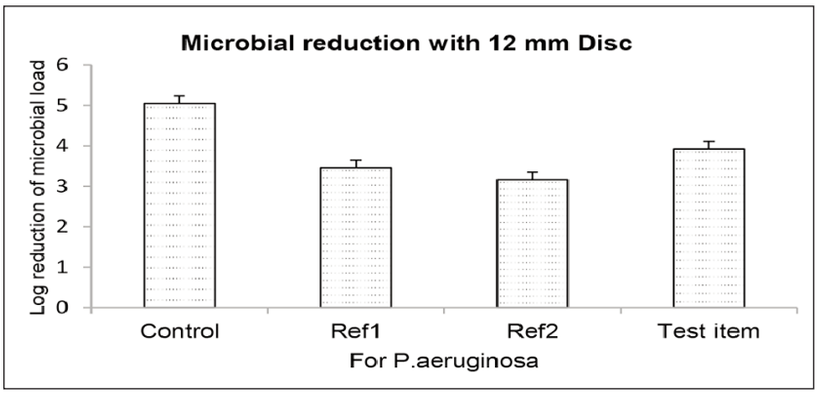


Fig. 10. Microbial load reduction comparison with comparators for *C. albican* organism

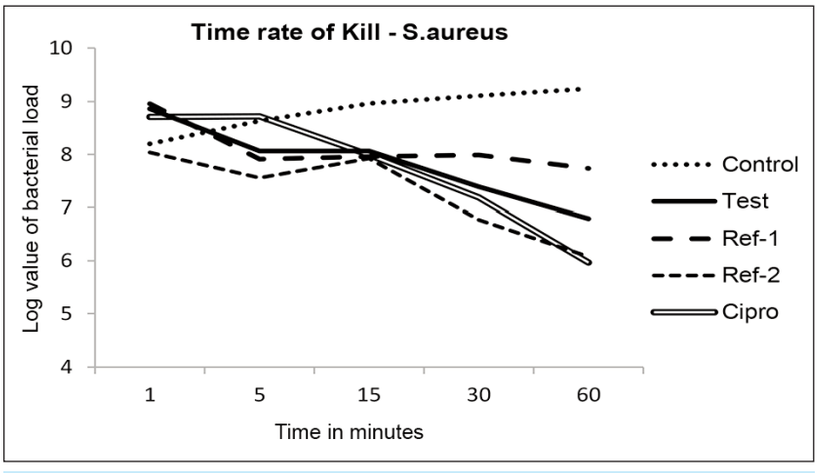


Fig. 11. The time rate of kill of treated spacer fabric with comparators for *S. aureus*

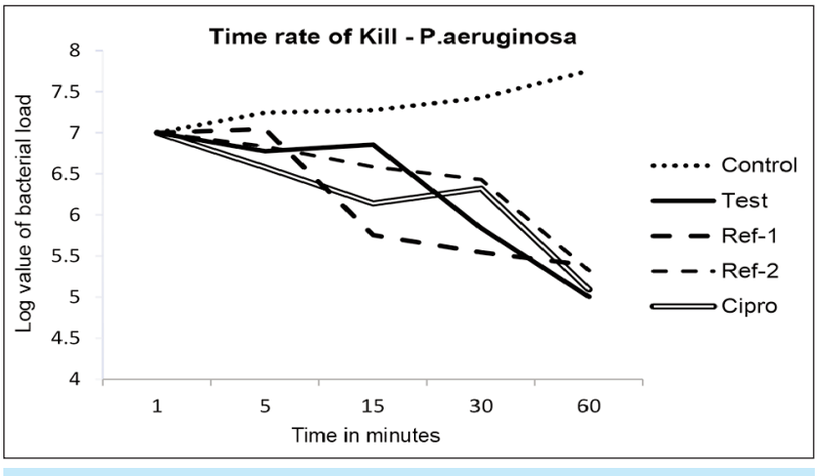


Fig. 12. Time rate of kill of treated spacer fabric with comparators for *P. aeruginosa*

Ref 1 and Ref 2 were cut and placed into 24 well plates containing ~10^7 CFU/ml in Tryptic Soy Broth (TSB) of P. aeruginosa and S. aureus respectively and incubated at 37±2°C for the Prevention assay. Similarly, 15 millimetre (mm) discs of Test Item (TI), Ref 1 and Ref 2 were cut and placed into 24 well plates containing overnight grown culture of P. aeruginosa and S. aureus respectively in TSB and further incubated for 24 hours at 37±2°C for the Disruption assay. Culture control (C) contained only ~10^7 CFU/ml in Tryptic Soy Broth (TSB) in both the Prevention and Disruption assay.

After incubation, the contents were removed and washed four times with Phosphate buffer saline (pH 7.2). A 500 µl of 0.1% crystal violet stain was added to each well and incubated for 20 minutes at room temperature, washed and dried. Subsequently, 500 µl of ethanol supplemented with 2% acetic acid was added to each well for 30 min. The Optical Density (OD) of stained adherent Biofilm was obtained by using a Tecan plate reader at 570 nm wavelength Mean optical density (OD) of C, TI, B and T discs by using one-way analysis of variance (ANOVA) followed by followed by Dunnett's Multiple Comparison Test. $P<0.05$ was chosen as the criterion for statistical significance. Figures 13 and 14 show the findings for the prevention assay and figures 15 and 16 show the findings for the disruption assay.

Higher optical density (OD) signifies a high amount of biofilm formation which is an extracellular matrix secreted when the planktonic bacteria proliferate and attach to surfaces. Known drug ciprofloxacin is a proven biofilm prevention as well as biofilm targeting entity. Hence the OD is seen to be the least among all the comparators. The silver-based dressing also shows good activity for *S. aureus*. This may be due to the free leaching of silver ions from the dressing.

In comparison, the developed spacer fabric shows comparable biofilm prevention properties in comparison to elemental silver and pDADMAC. This means when used in wound management, they would not go to the stage where biofilms are formed. It helps in the prevention of biofilm formation.

In case of biofilm disruption, especially for *P. aeruginosa* strain the treated spacer fabric is capable of disrupting the formed biofilm. Thus the material can be seen as a promising solution for the application of new wound dressings.

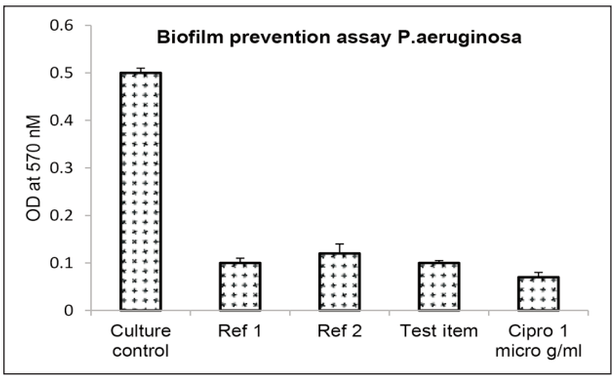


Fig. 13. Comparison of Biofilm Prevention assay for *P. aeruginosa*

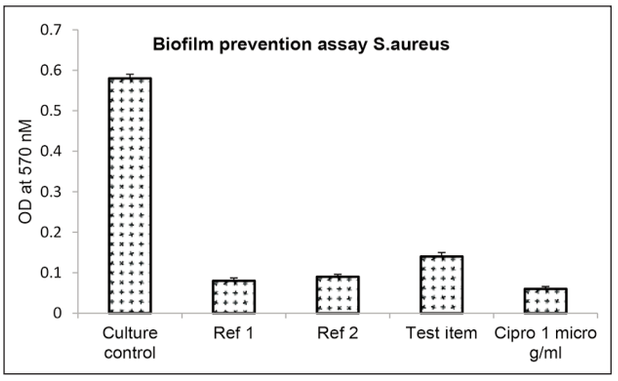


Fig. 14. Comparison of Biofilm Prevention assay for *S. aureus*

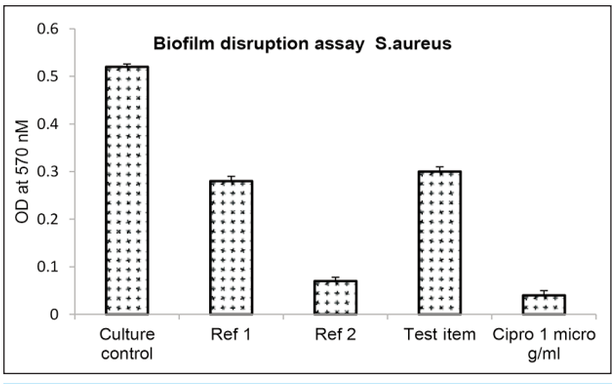


Fig. 15. Comparison of Biofilm Disruption assay for *S. aureus*

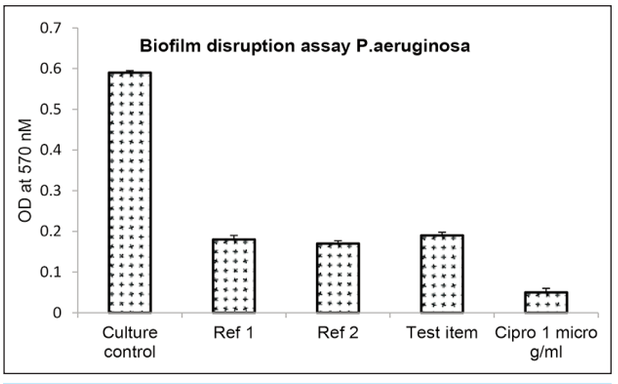


Fig. 16. Comparison of Biofilm Disruption assay for *P. aeruginosa*

CONCLUSION

Knitted spacer fabric treated with QAS has shown good antimicrobial properties in this study. It is seen that the treated fabric with DMTAC exhibits a broad range of antimicrobial efficacy against gram-positive bacteria, gram-negative bacteria and also fungus and yeast. The rapidity of kill was seen to be very effective even against resistant strains like MRSA. It was also seen that the fabric had good biofilm prevention and biofilm disruption actions. This is a very important property when the fabric is used as a wound dressing. Chronic and infected wounds are hard to heal because of the biofilms present in them. The antimicrobial and anti-biofilm properties exhibited by this dressing make it a suitable material for infection control in wound dressings. This spacer material can also behave as foam in the management of exudates in wounds. The efficacy of this material compared with other dressing materials yields substantial evidence of its superior activity as a wound dressing material when it comes to management of microbial contaminations including biofilm prevention and disruption. Such material can find use in the management of chronic wounds and is a subject matter of further studies with clinical evidence.

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