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Antimicrobial properties of Theruptor 3D-hydrocellular wound dressing: An *in vitro* study

Richie Gupta [a], KVNN Santosh Murthy [b], K.R. Bhagavan [c], Ashok Kumar Moharana [d], Michael Rodrigues [d], Deepak TS [d,*]

[a] Department of Plastic Surgery, Fortis Hospital-Shalimar Bagh, Gurgaon, Haryana, India
[b] Advanced Wound Healing Clinics, Navya Multi-Speciality Hospital, Nalgonda, Telangana, India
[c] Department of Surgery, K.S. Hegde Medical Academy, Mangaluru, India
[d] Healthium Medtech Limited, Bangalore, Karnataka, India

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ABSTRACT

Background: Colonized wound infections become a major public health concern. Infections may lead to delayed healing process or severe complications. Thus, the incorporation of antimicrobial agents such as silver, iodine, etc., into the dressing material provides protection against microbes. However, these agents have limited functional usage. Recently, Dimethyl tetradecyl [3-(trimethoxysilyl)propyl] ammonium chloride (DTAC) based 3D-hydrocellular wound dressings have emerged. Therefore, we evaluated the short- and long-term antibacterial efficacy of Theruptor 3D-hydrocellular wound dressing.

Methods: The antimicrobial activity of the dressing was evaluated using modified method of AATCC100. Inoculums of 27 differential microbes including gram-positive bacteria, gram-negative bacteria, and fungus were obtained. The fabric samples were inoculated with a different microbial sample in a sterile petri plate. The samples were incubated for short (1 min, 30 min, 1h, and 4h) and long (7, 14, and 28 days) term durations.

Results: Initially, the growth of test microorganisms ranged between 7.9×10^6 to 2.38×10^7 CFU/ml at 0 min. After respective time intervals, the growth of all the tested microbes was significantly reduced in a time-dependent manner ($p < 0.05$). The dressing material achieved zero CFU/ml at 4 h. Moreover, it showed a significant reduction of >5 log at 1 h till 28 days.

Conclusion: Based on the "physical kill mechanism", Theruptor 3D-hydrocellular wound dressing not only provides protection against a broad spectrum of pathogens but for a wide-ranging time period i.e., 1 min to 28 days that ensures effective and significant wound healing. Thus, it may consider as a promising advancement in the wound care settings.

1. Introduction

Skin is the outermost bodily tissue that protects against pathogens and external damages. It also plays a vital role in regulating water and temperature, and immunological surveillance [1,2]. However, the leading cause of wound infections is the patient's own skin microflora. Microorganisms gain entry through the damaged skin and get colonized in the wound [3]. As a result, these pathogens not only hinder the wound healing process but increase the risk of diseases. Thus, the microbial load can be considered as a significant factor in delayed healing [4]. In mathematical terms, microbial load in the wound is inversely proportional to the tendency of wound healing [5].

With the advent of deadly microbes such as *Corynebacterium* spp., *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus,* etc., and drug-resistant organism like Methicillin-resistant staphylococcus aureus (MRSA), colonized wound infections become a major public health concern [6]. Wound infection occurs as a result of complex interactions of these pathogenic organisms within the wound. Such infections not only delay the healing process but may lead to severe complications resulting in prolonged hospital stays and increased cost of wound care. In severe cases, highly infected wound may lead to amputation of body parts. In fact, the wound infection may turn from minor injury representing initial infection symptoms such as pain, swelling, and redness to severely infected wound with biofilm formation

* Corresponding author. Healthium Medtech Limited, RMZ NorthStar, Cowrks, 12th Floor, Adjacent to RMZ Galleria Mall, Yelahanka, Bangalore, 560064, India.
E-mail address: deepak.ts@healthiummedtech.com (D. TS).

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causing nausea, chills, or fever, if left unattended [5]. Wound infections can occur in either hospital or community settings through various forms of contact such as touch of a contaminated caregiver, surgical instruments, germs in the air, patient's own flora, etc. Thus, preventing wound infection in the early stages or whenever possible, is vitally important aspect of wound management.

The emerging risk of wound infections has motivated new approaches in modern wound care settings to improve healing procedures. For example, antibiotics and antimicrobial agents such as silver, iodine, polyhexamethylene biguanide (PHMB), etc., have been incorporated into dressing material for the prevention of wounds against pathogen invasion and contamination [7]. These therapeutic agents are the powerful tool to reduce the growth of microbial cells at the wound site by obstructing the metabolic pathways and functioning of the microbes. Several experimental and clinical studies in the literature have reported the microbicidal properties of silver, iodine, or PHMB [8–10]. Surprisingly, most of these studies have determined only the short-term efficacy of these antimicrobial wound dressings. Despite the widespread usage of these dressings, the antimicrobial agents have functional or usage limitations in terms of the half-life of elution. For instance, elution of silver in the wound causes argyria and organ system dysfunctions, and iodine dressings cannot be used in thyroid patients or patients with iodine allergy [10]. Moreover, the active ingredient of medicated dressing would leach out from the dressing to act on the wound site to protect against the pathogens. This leads to the depletion of the active component over a period of time. Therefore, there is a need to develop a new technology that is non-toxic, non-leachable, kills microbes effectively, does not cause drug resistance in microbes, and fastens the healing process. With the advancements in technology, the attention has been shifted to Dimethyl tetradecyl [3-(trimethoxysilyl)propyl] ammonium chloride (DTAC) technology-based wound dressings. DTAC is a cationic surfactant used at the concentration of 1% w/w [11]. Based on DTAC technology, 3D-hydrocellular wound dressing is formulated as a 3-dimensional knitted fabric which is marketed under various brand names of Trushield and Theruptor [Healthium Medtech Limited, India]. The characteristics of Theruptor 3D-hydrocellular wound dressing are shown in Fig. 1. The 3-dimensional knitted fabric comprises of DTAC-bounded polyethylene terephthalate (90% w/w) and polyurethane material (10% w/w), forming a hydrocellular structure. DTAC-based dressing acts as a physical barrier against contaminants, helps in gaseous exchange, maintains the moist environment, and manages the exudates effectively. They are primarily used for exuding, minor, chronic and surgical wounds, and first and second-degree burns [12]. The exceptional properties of DTAC technology are the "physical kill mechanism" for microbial protection and the non-leachability of DTAC into the skin or out of the dressing. The cross-linking property ensures that the active component remains bounded to the surface of the dressing. In addition, the DTAC dressings as antimicrobial, cover a broad spectrum of microbes and eradicate the infection load at the wound site. Only few studies in the literature have focussed on the leaching properties of these antimicrobial agents.

Based on this background, the present study aims to evaluate both short (0, 1, 4, 30 min, and 4 h) as well as long (7, 14, and 28 days) term antimicrobial efficacy of Theruptor 3D-hydrocellular DTAC-based wound dressing on a broad spectrum of microbes including gram positive-bacteria, gram-negative bacteria, and fungi. Moreover, the non-leaching property of Theruptor 3D-hydrocellular wound dressing was also assessed.

2. Materials and methods

2.1. Microbial samples and reagents used

A broad spectrum of test microorganisms including *Corynebacterium Diphtheriae*, *Propionibacterium acnes, Citrobacter freundii*, *Bacteroides fragilis*, *Morganella morganii*, *Streptococcus, Fusobacterium nucleatum, Prevotella melaninogenica, Veillonella parvula, Acinetobacter baumannii, Aspergillus niger, Candida albicans, Burkholderia cepacian, Enterobacter cloacae, Serratia marcescens, Providencia rustigianii, Klebsiella pneumonia, Methicillin resistant staphylococcus aureus, Proteus vulgaris, Staphylococcus aureus, Pseudomonas aerugionosa,* and *Aspergillus brasiliensis* were purchased from the American Type Culture Collection (ATCC), Virginia, USA. *Stenotrophomonas maltophila, Proteus mirabilis, Escherichia coli, Micrococcus* sp., and *Staphylococcus epidermidis* were purchased from the National Collection of Industrial Microorganisms (NCIM), Pune, India. Theruptor 3D-hydrocellular wound dressing made up of polyethylene terephthalate (90% w/w) and polyurethane material (10% w/w), was obtained from Healthium Medtech Ltd., Bengaluru, India. Soybean casein digest agar and Sabouraud dextrose agar were procured from Microxpress, Tulip Diagnostics (P) Ltd, India. Letheen broth and phosphate buffer saline were purchased from Himedia Pvt. Ltd., India.

2.2. Regulatory compliance of microbiological testing

The tests in the study were conducted in compliance with Quality Management System as per the International Organization for Standardization (ISO) ISO/IEC 17025:2017.

2.3. Plate count method

The AATCC 100 method is a very robust approach commonly used for the quantitative evaluation of antimicrobial fabric performance worldwide. US FDA recommends this test for porous substrates such as wound dressings. The AATCC 100 method evaluates both bacteriostatic

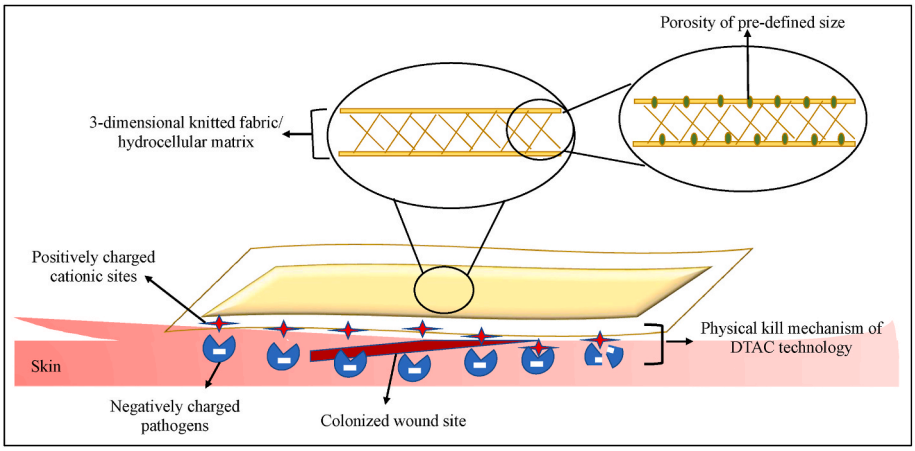

Fig. 1. Characteristics of Theruptor 3D-hydrocellular wound dressing.

(growth inhibition i.e., the microbes remain in the stationary phase) or bactericidal (killing of bacteria) properties of textile material over a 24-h period of contact. It mainly consists of six key stages: preparation of samples, sterilization, inoculation, incubation, washing/shaking out, and counting after serial dilutions. The microorganisms are incubated in favorable and optimum conditions to promote their growth [13]. In the present study, the antimicrobial activity of the dressing was evaluated using modified method of AATCC 100. Modification method was employed in terms of duration of contact which otherwise is standard i.e., 24 h.

For the same, the test fabric sample of size 5 cm × 5 cm, was cut from Theruptor 3D-hydrocellular wound dressing and was placed in 90 mm sterile petri plates, as described earlier [14]. Briefly, the test swatches were inoculated with 1 ± 0.1 ml appropriate dilution of microbial culture. The swatches were immediately transferred (contact time = 0 minutes (min)) into 100 ± 1.0 ml of sterile Leethen broth. The sample was shaken vigorously for 1 min. After that, inoculums were immediately covered with a 4 × 4 cm sterile thin film to prevent evaporation of the suspension. Further, 10-fold serial dilutions were prepared in serial diluents (up to 10^{-6}). The samples were incubated for differential experimental periods. Different sets of organisms (Table 1) were tested to evaluate the antimicrobial efficacy of fabric material for short (1 min, 30 min, 1 h, and 4 h) and long (7, 14 and 28 days) term durations. After completion of the incubation period, the microbial colonies were counted to calculate the arithmetic mean of the duplicate plates.

2.4. Log reduction and percentage reduction

The average of the common logarithm of the number of viable microbial colonies recovered from the initial (contact time = 0 min; N_0) and respective time periods (1 min, 30 min, 1 h, 4 h, and 7 days; N_a) of tested sample was calculated. The antimicrobial activity was calculated by:

$$R = N_0 - N_a$$

Further, the percentage reduction was calculated by:

$$\text{Percentage reduction } (\%) = [(B\text{-}A)/B] \times 100$$

A = colonies obtained at initial contact time (0 min)
B = colonies obtained at different contact times (1 min, 30 min, 1 h, 4 h, and 7 days)

2.5. Leachability testing by UV–visible spectrophotometry

The UV–visible spectra of the antimicrobial compound i.e., dodecyl trimethyl ammonium chloride (DTAC), was recorded using a UV–visible spectrophotometer. Methanol was taken as the reference solution.

2.6. Statistical analysis

Data were analyzed using GraphPad Prism (v.8). The data for the antimicrobial efficacy of fabric material was compared using analysis of variance (ANOVA) and repeated measures ANOVA. A p-value < 0.05 was considered as statistically significant.

3. Results

3.1. Short term evaluation of wound dressing material against microbes

Initially, the growth of test microorganisms ranged between 7.9 × 10^6 to 2.38 × 10^7 CFU/ml at 0 min. After respective contact time intervals i.e., 1 min, 30 min, 1 h, and 4 h, the growth of all the tested microbes was found to be significantly reduced in a time-dependent manner ($p < 0.05$). After 4 h of contact, the dressing material achieved zero CFU/ml for all the tested microbes providing short-term antimicrobial benefits (Table 2).

Further, the average logarithm value of viable microbes was calculated. At 0 min, the log values ranged between 6.9 and 7.38, which significantly reduced with time. There was no microbial growth after 4 h of contact time (Table 3). Based on these, log reduction for all microbial samples was calculated at different periods of incubation. The Theruptor 3D-hydrocellular wound dressing showed >1 log reduction at 1 min, 5 log reduction at 30 min and >5 log reduction at 1 h and 4 h for all tested microorganisms. Lastly, the percentage reduction was calculated and

Table 1
List of tested microorganisms, their classification and laboratory culture conditions.

Microbial strain	ATCC/NCIM No.	Classification	Culturing media	Incubation Temperature (°C)	Incubation Time (h)
Corynebacterium Diphtheriae	13812	Gram-positive bacteria	SCDA	30–35	24–48
Propionibacterium acnes	6919	Gram-positive bacteria	SCDA	30–35	24–48
Citrobacter freundii	43864	Gram-negative bacteria	SCDA	30–35	24–48
Bacteroides fragilis	25285	Gram-negative bacteria	SCDA	30–35	24–48
Morganella morganii	25829	Gram-negative bacteria	SCDA	30–35	24–48
Streptococcus	12392	Gram-positive bacteria	SCDA	30–35	24–48
Fusobacterium nucleatum	25586	Gram-negative bacteria	SCDA	30–35	24–48
Prevotella melaninogenica	25845	Gram-negative bacteria	SCDA	30–35	24–48
Veillonella parvula	10790	Gram-negative bacteria	SCDA	30–35	24–48
Acinetobacter baumannii	19606	Gram-negative bacteria	SCDA	30–35	24–48
Burkholderia cepacian	25416	Gram-negative bacteria	SCDA	30–35	24–48
Enterobacter cloacae	11439	Gram-negative bacteria	SCDA	30–35	24–48
Serratia marcescens	14756	Gram-negative bacteria	SCDA	30–35 °C	24–48
Stenotrophomonas maltophila	5530	Gram-negative bacteria	SCDA	30–35	24–48
Proteus mirabilis	NCMI 2241	Gram-negative bacteria	SCDA	30–35	24–48
Providencia rustigianii	12013	Gram-negative bacteria	SCDA	30–35	24–48
Micrococcus sp	NCMI 5655	Gram-positive bacteria	SCDA	30–35	24–48
Staphylococcus epidermidis	NCMI 5270	Gram-positive bacteria	SCDA	30–35	24–48
Klebsiella pneumonia	4352	Gram-negative bacteria	SCDA	30–35	24–48
Methicillin resistant staphylococcus aureus	BAA-44	Gram-positive bacteria	SCDA	30–35	24–48
Proteus vulgaris	13315	Gram-negative bacteria	SCDA	30–35	24–48
Staphylococcus aureus	6538	Gram-positive bacteria	SCDA	30–35	24–48
Escherichia coli	8739	Gram-negative bacteria	SCDA	30–35	24–48
Pseudomonas aeruginosa	9027	Gram-negative bacteria	SCDA	30–35	24–48
Candida albicans	10231	Yeast	SDA	20–25	48–72
Aspergillus brasiliensis	16404	Fungus	SDA	20–25	48–72
Aspergillus niger	16404	Fungus	SDA	20–25	48–72

SCDA= Soybean casein digest agar; SDA= Sabouraud dextrose agar; h = hours.

Table 2
Short-term antimicrobial efficacy of Theruptor 3D-hydrocellular dressing.

Microbial strain	0 min	1 min	30 min	1 h	4 h	p-value
Corynebacterium Diphtheriae	1.89×10^7	1.87×10^6	69	9	NG	**<0.0001**
Propionibacterium acnes	1.69×10^7	1.65×10^6	145	18	NG	**<0.0001**
Citrobacter freundii	1.75×10^7	1.69×10^6	85	11	NG	**<0.0001**
Bacteroides fragilis	1.82×10^7	1.79×10^6	96	13	NG	**<0.0001**
Morganella morganii	1.81×10^7	1.75×10^6	59	9	NG	**<0.0001**
Streptococcus	1.88×10^7	1.68×10^6	78	8	NG	**<0.0001**
Fusobacterium nucleatum	1.71×10^7	1.42×10^6	86	6	NG	**<0.0001**
Prevotella melaninogenica	1.61×10^7	1.54×10^6	57	7	NG	**<0.0001**
Veillonella parvula	1.59×10^7	1.26×10^6	75	9	NG	**<0.0001**
Acinetobacter baumannii	1.83×10^7	1.28×10^6	98	14	NG	**<0.0001**
Burkholderia cepacian	1.87×10^7	1.35×10^6	76	10	NG	**<0.0001**
Enterobacter cloacae	1.92×10^7	1.75×10^6	82	12	NG	**<0.0001**
Serratia marcescens	1.85×10^7	1.58×10^6	46	6	NG	**<0.0001**
Stenotrophomonas maltophila	1.53×10^7	1.38×10^6	53	7	NG	**<0.0001**
Proteus mirabilis	1.82×10^7	1.31×10^6	58	8	NG	**<0.0001**
Providencia *rustigianii*	1.53×10^7	1.51×10^6	79	9	NG	**<0.0001**
Micrococcus sp	1.93×10^7	1.26×10^6	82	11	NG	**<0.0001**
Staphylococcus epidermidis	1.95×10^7	1.49×10^6	89	13	NG	**<0.0001**
Klebsiella pneumonia	2.38×10^7	1.35×10^6	93	15	NG	**<0.0001**
MRSA	2.29×10^7	1.85×10^6	132	17	NG	**<0.0001**
Proteus vulgaris	1.76×10^7	9.4×10^5	112	19	NG	**<0.0001**
Aspergillus brasiliensis	7.9×10^6	8.2×10^5	75	12	NG	**<0.0001**

MRSA = Methicillin resistant staphylococcus aureus, NG = No growth, min = minutes, h = hours.

the data showed >90% of antimicrobial efficiency of Theruptor 3D-hydrocellular dressing at 1 min, and >99% at 30 min. At 1 h, 100% reduction was observed for *Corynebacterium Diphtheriae, Morganella morganii, Streptococcus, Fusobacterium nucleatum, Prevotella melaninogenica, Serratia marcescens, Stenotrophomonas maltophila,* and *Proteus vulgaris* and 99.99% for other microbes (Table 3).

3.2. Long term evaluation of wound dressing material against microbes

After evaluating the short-term anti-microbial benefits of wound dressing, the fabric was subjected to long term effect of microbes i.e., 7 days. No growth of any of the microbes was observed on the fabric material after 7 days (Table 3). Further, a set of microbes such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger,* and *Candida albicans* were inoculated and tested for 14 and 28 days. The initial (0 h) logarithm of viable microbes ranged between 5.5 and 6.81. The Theruptor 3D-hydrocellular wound dressing showed a significant reduction of >5 log after 7, 14, and 28 days for the tested microorganisms (Fig. 2 and Table 4).

3.3. Anti-microbial compound has non-leaching property

The anti-microbial (DTAC) compound was diluted with methanol in the ratio of 1: 10 and subjected to UV–Visible spectrophotometry. The spectrophotometric analysis revealed the absorbance of 205.9 nm for the anti-microbial compounds and 340.7 nm for methanol with zero ppm of leachability at different incubation periods and leachate temperatures.

4. Discussion

This is the first *in vitro* study in the literature that predominates the antimicrobial efficacy of DTAC technology-based Theruptor wound dressings. The findings of the present study affirm the effectiveness of Theruptor wound dressing against a broad spectrum of microbes for shorter and broader time ranges i.e., 1 min to 28 days.

Concisely, wound healing is a dynamic physiological phenomenon that restores and repairs an injury of the skin. It involves a cascade of biological events *viz.* hemostasis, inflammation, migration, proliferation, and remodeling [15,16]. All these phases occur in a synchronized manner. These phases can be affected by microbial invasion and colonization which causes prolonged inflammatory stages of wound. The cascade of these events results in delayed healing. Thus, to accelerate the healing process by protecting the wound against pathogens, antimicrobial dressings are considered as a powerful tool in hospital settings [17,18].

Overwhelming evidence in the literature showed good therapeutic candidates in the arena of wound dressings. For instance, the incorporation of antibiotics or antimicrobial agents such as silver, iodine, PHMB, chlorhexidine, etc., into dressing material prevents the wounds against pathogens and improves the healing process [7]. These agents possess good antimicrobial activity. But they cause cytotoxicity and antimicrobial resistance in certain conditions [19–22]. Thus, a new technology i.e., DTAC-based wound dressing has been materialized that uses a "physical kill mechanism" for microbial protection (Theruptor 3D-hydrocellular wound dressing; Healthium Medtech Limited, India). In the present study, we evaluated short (0, 1, 4, 30 min, and 4 h) and long (7, 14, and 28 days) term antimicrobial efficacy and non-leaching properties of Theruptor 3D-hydrocellular wound dressing against a broad spectrum of microbes.

The infected wounds are generally polymicrobial. Thus, the therapeutic agent should be effective against a broad spectrum of microbes [23]. Therefore, in the present study, a total of 27 different microbial strains were inoculated on DTAC-based dressing material. A significant decline in the bacterial count was observed with a 99.99% of reduction after 30 min and beyond. At 4 h of contact time, no colonies of any of the tested microbes were observed. Notably, 100% of the reduction was achieved against 8 bacteria *viz. Stenotrophomonas maltophila, Proteus mirabilis, Morganella morganii, Streptococcus, Fusobacterium nucleatum, Prevotella melaninogenica, Corynebacterium diphtheriae,* and *Serratia marcescens.* In concordance to our data, Erdem and Sanli (2008) evaluated the antibacterial activity of DTAC-impregnated fabric against *Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli* strains. The authors observed that the unwashed fabrics were susceptible to *Staphylococcus aureus* as compared to treated fabric, suggesting DTAC efficacy against microorganisms. Further, the authors also assessed their antibacterial activity after 5, 10, and 20 washings and found a significant decrease in the efficacy of DTAC after each washing [24].

Since studies determining the antimicrobial efficacy of dressing during a prolonged period are limited therefore, we checked the microbicidal properties of DTAC-based dressing for a longer duration i.e., 7, 14, and 28 days. The test results of microbial elimination during prolonged duration also delivered remarkable results. No microbial growth of any tested organisms was observed at any time period of experimental study (7, 14, and 28 days). Moreover, >5 log reductions in microbial colonies were estimated after 4 h of contact time. Lastly, the

Table 3
Log reduction and percentage reduction of microbial growth in short- and long-term.

Microbial strain	0 min	1 min		30 min		1 h		4 h	7 d
	(Initial log value)	LR	PR (%)	LR	PR (%)	LR	PR (%)	LR and PR (%)	
Corynebacterium Diphtheriae	7.28	1.01	90.11	5.44	99.99	6.33	100	NG	NG
Propionibacterium acnes	7.23	1.01	90.24	5.07	99.99	5.97	99.99	NG	NG
Citrobacter freundii	7.24	1.01	90.34	5.31	99.99	6.2	99.99	NG	NG
Bacteroides fragilis	7.26	1.01	90.16	5.28	99.99	6.15	99.99	NG	NG
Morganella morganii	7.26	1.02	90.33	5.49	99.99	6.31	100	NG	NG
Streptococcus	7.27	1.04	91.06	5.38	99.99	6.37	100	NG	NG
Fusobacterium nucleatum	7.23	1.08	91.70	5.3	99.99	6.45	100	NG	NG
Prevotella melaninogenica	7.21	1.02	90.43	5.45	99.99	6.36	100	NG	NG
Veillonella parvula	7.2	1.1	92.08	5.32	99.99	6.25	99.99	NG	NG
Acinetobacter baumannii	7.26	1.15	93.01	5.27	99.99	6.11	99.99	NG	NG
Burkholderia cepacian	7.27	1.14	92.78	5.39	99.99	6.27	99.99	NG	NG
Enterobacter cloacae	7.28	1.04	90.89	5.37	99.99	6.2	99.99	NG	NG
Serratia marcescens	7.27	1.07	91.46	5.61	99.99	6.49	100	NG	NG
Stenotrophomonas maltophila	7.18	1.04	90.98	5.46	99.99	6.33	100	NG	NG
Proteus mirabilis	7.26	1.14	92.80	5.5	99.99	6.36	100	NG	NG
Providencia *rustigianii*	7.18	1	90.13	5.28	99.99	6.23	99.99	NG	NG
Micrococcus sp	7.29	1.19	93.47	5.37	99.99	6.25	99.99	NG	NG
Staphylococcus epidermidis	7.29	1.12	92.36	5.34	99.99	6.18	99.99	NG	NG
klebsiella pneumonia	7.38	1.25	94.33	5.41	99.99	6.2	99.99	NG	NG
MRSA	7.36	1.09	91.92	5.24	99.99	6.13	99.99	NG	NG
Proteus vulgaris	7.25	1.28	94.66	5.2	99.99	5.97	99.99	NG	NG
Aspergillus brasiliensis	6.9	0.99	89.62	5.02	99.99	5.82	99.99	NG	NG
Staphylococcus aureus	6.81	-	99.47	-	99.99	-	99.99	5.63	99.99
Escherichia coli	6.74	-	99.48	-	99.99	-	99.99	6.74	99.99

MRSA = Methicillin resistant staphylococcus aureus, LR = Log reduction, PR = Percentage reduction, NG = No growth, min = minutes, h = hours, d = days, % = percentage.

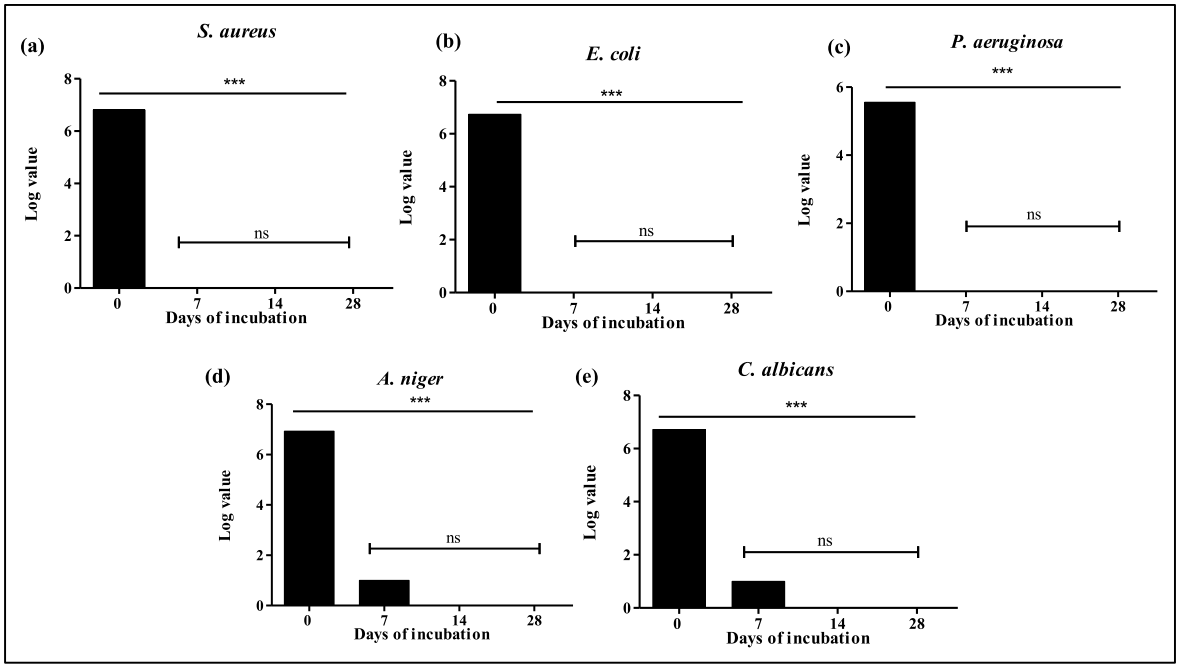

Fig. 2. Long term antimicrobial efficacy of Theruptor 3D hydrocellular wound dressing. Bar-grouped plots representing colony formation by different microbes in log values. (a) *Staphylococcus aureus*, (b) *Escherichia coli*, (c) *Pseudomonas aeruginosa*, (d) *Aspergillus niger*, and (e) *Candida albicans*. Significant reduction in microbial growth was observed from 0 to 7 days and beyond. ns: non-significant; ***$p < 0.0001$.

spectrophotometric analysis revealed zero ppm of leachability at different incubation periods and leachate temperatures. In contrast to our study, Barbour et al. (2016) compared the toxicity of commercially available chlorhexidine digluconate and chlorhexidine hexametaphosphate (CHX-HMP) wound care materials. The authors found that the toxic effects of chlorhexidine digluconate were more on human placental cells as compared to CHX-HMP [25]. In another contrasting study, Nesporova et al. (2020) evaluated the antimicrobial efficacy, silver penetration, DNA damage, and cytotoxicity of silver dressings *in vitro* and on porcine skin *in vivo*. The authors revealed a significant elevation in the expression of DNA damage marker i.e., γ-H2AX with silver dressing, corresponding with the amount of silver in the skin. Further, a significant correlation between the amount of silver released in culture media and *in vitro* cytotoxicity was observed. Silver dressings also caused oxidative stress, reduction in viability, and elevation in the production of pro-inflammatory IL-6. i.e, corroborating the damaging

Table 4
Log values and log reduction of microbial growth after 7, 14, and 28 days.

Microbial strain	0 h	7 days			14 days			28 days		
	LV	LV	LR	PR (%)	LV	LR	PR (%)	LV	LR	PR (%)
Staphylococcus aureus	6.81	1	5.63	99.99	0	6.81	99.99	0	6.81	99.99
Escherichia coli	6.74	0	6.74	99.99	0	6.74	99.99	0	6.74	99.99
Pseudomonas aeruginosa	5.56	0	5.56	99.99	0	5.56	99.99	0	5.56	99.99
Aspergillus niger	6.93	1	5.79	99.99	0	6.93	99.99	0	6.93	99.99
Candida albicans	6.72	1	5.72	99.99	0	6.72	99.99	0	6.72	99.99

LV = Log values, LR = Log reduction, PR = Percentage reduction, h = hours, % = percentage.

effects of silver in wound care [26]. In a prospective clinical study, Brouillard et al. (2018) studied the systemic absorption of silver and evaluated the toxicity of silver-impregnated dressings in 40 patients with chronic inflammatory wounds. The authors reported raised level of silver after 1 month of treatment in 20 patients and found wound area, anaemia, and malnutrition as predictive factors for systemic silver absorption. Thus, the long-term application of silver dressings, especially in elder patients who suffer from malnutrition and anaemia are highly discouraged [27]. Similarly, other studies also reported the cytotoxic effects of several commercially-used antibacterial agents impregnated in wound dressings [20,21].

Evidence in the literature suggesting the antimicrobial efficacy of dressings during a prolonged period are limited. To the best of our knowledge, this is the first study that evaluated both the short- and long-term efficacy of DTAC-based wound dressing against 27 different pathogens including gram positive-bacteria, gram-negative bacteria, and fungi, unlike several other drug-impregnated dressings with short-term anti-microbial activities only. Fruitful attempts have been made to utilize and enrich the available technology in dressing for both short- and long-term microbicidal benefits. Aside these strengths, this study has a major limitation in real-time i.e., it provides *in vitro* evidence only. However, clinical studies and trials are underway that are aimed at assessing the antimicrobial efficacy of Theruptor 3D-hydrocellular wound dressing (CTRI/2021/12/038800, CTRI/2021/12/038884, CTRI/2022/03/041044, and CTRI/2022/03/040898) [28]. Further studies are required to understand the non-leaching nature of DTAC in clinical scenario.

5. Conclusion

Conclusively, DTAC-based dressing can revolutionize the field of modern wound care. The DTAC technology uses a physical kill mechanism to eliminate the pathogens from the wound without leaching out in the skin or out of the dressing. These dressings not only displayed antimicrobial efficacy against a broad spectrum of microbes but also found effective in a broader time range i.e., 1 min to 28 days. Such properties are vital in managing all stages of wound care by ensuring long-term protection against possible causes of infections. In addition, the use of 3D-hydrocellular dressings will reduce the frequent changes of wound dressing hence, it will help in cutting down the cost of wound management. Based on these features, DTAC-based 3D-hydrocellular wound dressing can be considered a promising technology for preventing pathogen proliferation and enhancing wound healing conditions.

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Ethical approval

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Consent

Not applicable.

Author statement

Study concept or design: Richie Gupta; KVNN Santosh Murthy; KR Bhagavan; Ashok Kumar Moharana; Michael Rodrigues; Deepak TS.

Data collection: Richie Gupta; KVNN Santosh Murthy; KR Bhagavan; Ashok Kumar Moharana; Michael Rodrigues; Deepak TS.

Data analysis or interpretation: Richie Gupta; KVNN Santosh Murthy; KR Bhagavan; Ashok Kumar Moharana; Michael Rodrigues; Deepak TS.

Writing the paper: Richie Gupta; KVNN Santosh Murthy; KR Bhagavan; Ashok Kumar Moharana; Michael Rodrigues; Deepak TS.

Registration of research studies

Not applicable.

Guarantor

Deepak TS.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ashok Kumar Moharana, Michael Rodrigues, and Deepak TS are full time employees of Healthium Medtech Limited, who are the manufacturers of Theruptor 3D-hydrocellular wound dressing. Any of the other authors have not received any financial aid from Healthium Medtech Limited.

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References

[1] Chua AWC, Tan BK, Foo CL, Tan KC, Chong SJ, Khoo YC. Skin tissue engineering advances in severe burns: review and therapeutic applications. Burns & Trauma 2016;4:3–17.
[2] Paul W, Sharma CP. Advances in wound healing materials: science and skin engineering. Smithers Rapra Tech; 2015.
[3] Liesenfeld B, Moore D, Mikhaylova A, Vella J, Carr R, Schultz G, et al. Antimicrobial wound dressings- mechanism and function. In: Symposium on advanced wound care; 2009.
[4] Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001;14(2):244–69.
[5] Davies CE, Hill KE, Newcombe RG, Stephens P, Wilson MJ, Harding KG, et al. A prospective study of the microbiology of chronic venous leg ulcers to re-evaluate the clinical predictive value of tissue biopsies and swabs. Wound Repair Regen 2007;15:17–22.
[6] van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: an update. Infect Dis Clin 2020;34(4):709–22.

[7] Sood A, Granick MS, Tomaselli NL. Wound dressings and comparative effectiveness data. Adv Wound Care 2014;3(8):511–29.
[8] To E, Dyck R, Gerber S, Kadavil S, Woo KY. The effectiveness of topical polyhexamethylene biguanide (PHMB) agents for the treatment of chronic wounds: a systematic review. Surg Technol Int 2016;29:45–51.
[9] Daeschlein G. Antimicrobial and antiseptic strategies in wound management. Int Wound J 2013;10:9–14.
[10] Dissemond J, Böttrich JG, Braunwarth H, Hilt J, Wilken P, Münter KC. Evidence for silver in wound care - meta-analysis of clinical studies from 2000-2015. J Dtsch Dermatol Ges 2017;15(5):524–35.
[11] Venkatrajah BV, Malathy VB, Elayarajah RR, Rammohan R. Synthesis of carboxymethyl chitosan and coating on wound dressing Gauze for wound healing. Pakistan J Biol Sci 2013;16:1438–48.
[12] https://www.healthiummedtech.com/products/.
[13] AATCC 100: 20U4 Test method for Anti-bacterial finishes on Textile materials: Assessment developed by American association of textile chemists and colorists.
[14] Shankar M, Ali S, Gupta M, Moharana AK, TS D. Antimicrobial efficacy of Trushield™ Gloves: an in vitro experimentation-based review. Indian J Surg 2022. https://doi.org/10.1007/s12262-022-03410-7.
[15] Ninan N, Thomas S, Grohens Y. Wound healing in urology. Adv Drug Deliv Rev 2015;82–83:93–105.
[16] Bowden LG, Byrne HM, Maini PK, Moulton DE. A morphoelastic model for dermal wound closure. Biomech Model Mechanobiol 2016;15(3):663–81.
[17] Rizzello L, Pompa PP. Nanosilver-based antibacterial drugs and devices: mechanisms, methodological drawbacks, and guidelines. Chem Soc Rev 2014;43 (5):1501–18.
[18] Vowden K, Vowden K, Carville K. Antimicrobials made easy. Wounds Int 2011;2 (1):1–6.
[19] Aramwit P, Muangman P, Namviriyachote N, Srichana T. In vitro evaluation of the antimicrobial effectiveness and moisture binding properties of wound dressings. Int J Mol Sci 2010;11:2864–74.
[20] Cheong SH, Yang YI, Choi MY, Kim MH, Cho KR, Lim SH, et al. Lung injury induced by the pulmonary instillation of povidone–iodine in rats. J Anesth 2012;26:70–9.
[21] Horner C, Mawer D, Wilcox M. Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter? J Antimicrob Chemother 2012; 67:2547–59.
[22] Medicines and Healthcare Products Regulatory Agency. Medical device alert. Ref. MDA/2012/075. All medical devices and medicinal products containing chlorhexidine-risk of anaphylactic reaction due to chlorhexidine allergy. London, UK: MHRA; 2012.
[23] Sunil KP, Raja BP, Jagadish RG, Uttam A. Povidone iodine—revisited. Indian J Dent Adv 2011;3(3):617–20.
[24] Erdem AK, Sanli NO. The evaluation of antibacterial activity of fabrics impregnated with dimethyltetradecyl (3-(trimethoxysilyl) propyl) ammonium chloride. IUFS J Biol 2008;67(2):115–22.
[25] Barbour ME, Maddocks SE, Grady HJ, Roper JA, Bass MD, Collins AM, et al. Chlorhexidine hexametaphosphate as a wound care material coating: antimicrobial efficacy, toxicity and effect on healing. Nanomedicine 2016;11(16):2049–57.
[26] Nešporová K, Pavlík V, Šafránková B, Vágnerová H, Odráška P, Žídek O, et al. Effects of wound dressings containing silver on skin and immune cells. Sci Rep 2020;10(1):15216.
[27] Brouillard C, Bursztejn AC, Latarche C, Cuny JF, Truchetet F, Goullé JP, et al. Silver absorption and toxicity evaluation of silver wound dressings in 40 patients with chronic wounds. J Eur Acad Dermatol Venereol 2018;32(12):2295–9.
[28] http://ctri.nic.in/Clinicaltrials/advsearch.php. [Accessed 20 June 2022].