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Preclinical Evaluation of CuraVue ® : A Novel Dual-Action Antimicrobial Silicone Adhesive Securement Dressing

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Additional Declarations: Competing interest reported. B.A., S.A.H., R.M.A., R.Z., F.F.A., A.M.A., F.A.., W.A.S and M.E.S. are members of the research team at Incura Inc. Incura Inc. is the organization that developed and holds the intellectual property rights for CuraVue® (the antimicrobial dressing evaluated in this manuscript). These authors have direct employment relationships with Incura Inc., which is developing and commercializing the technology described in this study. The study was designed to include objective preclinical evaluation methods, with comparison against an established commercially available product (Mepitel®) using standardized antimicrobial assessment protocols. The in vivo study was conducted in the UResearch Animal Facility, which is AAALAC-accredited, under the supervision of certified vets. The authors affirm that all data presented in this manuscript are reported accurately and objectively, and that the study design, methodology, and conclusions were not influenced by any desire to obtain favorable results for CuraVue®. Also, to ensure data integrity validation of the antimicrobial results was done in an external GLP-compliant and ISO-accredited contract testing organization (Mass Laboratuvar ve Danışmanlık Hizmetleri A.Ş.,).

Abstract

Background

Catheter-related bloodstream infections (CRBSIs) are among the most important challenges facing health care workers, leading to morbidity and mortality all over the world. CuraVue® is an antimicrobial adhesive securement dressing loaded with Chlorhexidine and Silver that is designed to offer sustained broad-spectrum antimicrobial activity over 7 days, thus proving it to be an efficient dressing at vascular access and device insertion sites, allowing reduction of the incidence of CRBSIs.

Results

Seven antimicrobial studies were carried out to assess CuraVue® antimicrobial activity, namely Disc Diffusion Testing (CLSI M02), Quantitative Contact-kill Test (ISO 22196), Quantitative Time-kill Test over 7 Days (Modified ISO 22196), Minimum Inhibitory Concentration (MIC) Detection, Static Coupon Biofilm Assay (ASTM E3435), assessment of Chlorhexidine and Silver Spatial Uniformity Distribution, and In Vivo Evaluation of Antimicrobial Efficacy in a Rat Skin Abrasion Colonization Model. Disc diffusion testing showed measurable zones of inhibition against all tested strains. The ISO 22196 contact-kill assay showed ≥ 99.99% reduction against all tested organisms, with exceptional activity against *P. aeruginosa* (R = 6.00). The 7-day time-kill assay gave mean R values ≥ 4.0 for *S. aureus*, *E. coli*, and *P. aeruginosa*, with no significant decline over time (linear regression, $p > 0.05$). MIC testing showed bacterial inhibition. The biofilm assay demonstrated strong antibiofilm activity (> 99% inhibition against the three tested strains). Spatial uniformity testing confirmed consistent antimicrobial activity across all regions of the dressing. An ISO-certified testing laboratory validated CuraVue® antimicrobial activity, showing > 5.22 log and > 5.12 log reductions against E. coli and S. aureus, respectively, confirming its strong, sustained antimicrobial efficacy. In the rat skin abrasion colonization model, CuraVue® significantly reduced bacterial burden compared to the negative control.

Conclusions

CuraVue® demonstrated broad-spectrum and sustained antimicrobial activity throughout the 7 day wear period supported by antibiofilm formation activity and localized microbial suppression on skin surface as proven in an *in vivo* animal model. These results indicate that CuraVue® should be considered as an effective antimicrobial protective dressing for vascular access and device insertion sites.

Background

Healthcare-associated infections (HAIs) are among the most common adverse events occurring in the context of health service delivery, with catheter-related bloodstream infections (CRBSIs) contributing substantially to preventable morbidity and mortality worldwide. According to the World Health Organization (WHO), on average, 1 in 10 patients is affected by HAIs, with low- and middle-income countries reporting higher frequency [1]. In low- and middle-income countries, surgical site infections

occur at an average rate of 11.8% (1.2%–23.6%), while rates are lower in high-income countries, though surgical site infections still lead to longer hospital stays, antimicrobial resistance, higher healthcare costs, and psychological stress globally [2, 3].

The microbial etiology of CRBSIs and central line-associated bloodstream infections (CLABSIs) is diverse and has evolved over the years. Gram-negative bacilli (Enterobacteriaceae), Gram-positive cocci (*Staphylococcus aureus*), and *Candida* species were consistently reported as leading causative pathogens in both adult and pediatric populations. Specifically, Candida spp. were reported to dominate adult intensive care units (ICUs), and Enterobacteriaceae were reported in adult, oncology, pediatric wards, and PICUs [4–7]. It is critical to note, however, that surveillance data indicate that pathogen distribution varies across care locations due to varying national clinical practices. In this context, preventing CRBSIs at the device–skin interface is integral to reducing colonization and improving patient outcomes.

A prospective surveillance study on peripheral venous catheter-related bloodstream infection (PVCR-BSI) rates was conducted over more than 6 years in 246 intensive care units that are members of the International Nosocomial Infection Control Consortium in 14 countries in the Middle East, showing that there were 470 PVCR-BSI cases (2.32 per 1,000 PVC-days). Patients with PVCR-BSI had higher mortality (29.36% vs. 10.38%) and longer hospital stays (16.84 vs. 5.94 days) than those without infection. The microorganism profile showed that *Staphylococcus aureus* accounted for 14%, *Escherichia coli* (7%), and *Pseudomonas aeruginosa* (5%). The study also showed that PVCR-BSI rates found in Middle Eastern ICUs were much higher than rates published from the USA, Australia, and Italy [8].

P. aeruginosa is one of the most prevalent pathogens causing a range of acute and chronic nosocomial infections. *P. aeruginosa* has intrinsic resistance to several antibiotics [9]. Gram-negative infections are increasingly frequently caused by *P. aeruginosa*, particularly in people with weakened immune systems [10]. It is a common cause of nosocomial infections and the most commonly isolated pathogen from patients hospitalized for more than a week [11]. *Pseudomonas* infections are dangerous and complex. As an opportunistic pathogen, *P. aeruginosa* has a complex regulatory system that can resist harsh external and internal environments, which causes substantial morbidity, debilitating diseases, shortened lifespan, and high mortality in humans. The virulence factors of *P. aeruginosa* include lipopolysaccharides, adherence factors, elastase, secretion systems, and outer membrane vesicles [12].

Perioperative personnel must focus on implementing infection prevention measures to improve clinical outcomes and reduce health care costs. Dressing selection plays a pivotal role in protecting the insertion site, maintaining a barrier to microbial entry, and facilitating early detection of local complications. Although transparent film dressings permit close inspection of insertion sites, their ability to prevent microbial colonization is largely passive. To elaborate, such dressings rely mainly on creating a physical barrier rather than active antimicrobial activity [13]. Recent research developments have achieved the incorporation of chlorhexidine (CHG), silver, or both into adhesive securement dressings to enhance protection against CRBSIs [14]. However, there remains a need to rigorously develop and evaluate new

protective adhesive dressings with a broader antimicrobial spectrum and optimized performance [14, 15].

The present study aims to evaluate the antimicrobial performance and preclinical protective efficacy of CuraVue®. CuraVue® is a sterile, single-use antimicrobial adhesive securement soft silicone-based dressing containing chlorhexidine and silver as dual active antimicrobial agents. CuraVue® was developed by engineering the silicone adhesive layer to incorporate chlorhexidine and silver salts within a single matrix while preserving adhesive performance and optical transparency. Particular emphasis was placed on incorporating stable silver salts, with minimal color change observed during and after the manufacturing process. The stability of the resulting formulation was further supported by an accelerated aging study equivalent to three years of storage. This dual-agent design is intended to provide localized broad-spectrum antimicrobial activity at the dressing–tissue interface for up to seven days.

The primary objective of developing CuraVue® is to strengthen prevention efforts against CRBSI. To emphasize, CuraVue®'s dual-antimicrobial optimized formulation is designed to overcome drug-resistant strains of bacteria that are known to cause severe bloodstream infections with adverse complications and treatment challenges, such as MRSA and Pseudomonas species. CuraVue® formulation is designed to provide broad-spectrum antimicrobial activity while maintaining gentle skin adhesion. When combined with chlorhexidine, which disrupts cytoplasmic membranes and precipitates cellular contents, silver may offer complementary or synergistic activity across Gram-positive and Gram-negative bacteria as well as fungi, while potentially lowering the risk of resistance and enhancing efficacy at the skin–catheter interface [16]. The device is intended to secure medical devices and protect percutaneous insertion sites while providing localized antimicrobial protection and allowing continuous visual monitoring of the underlying skin [17].

There are extremely few in vivo models that specifically simulate early extraluminal contamination at catheter insertion sites. Thus, this paper presents both in vitro and in vivo methods to determine if CuraVue® has broad-spectrum antimicrobial activity and sustained antimicrobial activity for up to 7 days. The hypothesis was that CuraVue® will demonstrate consistent in vitro broad-spectrum antimicrobial activity and significantly lower microbial burden of challenging bacterial strains for up to 7 days in vitro and in vivo, thus supporting its potential to reduce catheter-related bloodstream infection risk in clinical practice.

Material and Methods

This study aims to assess CuraVue® antimicrobial activity against a broad spectrum of organisms and to detect the uniformity and sustainability of its action over the time of its use that can be extended to 7 days thus proving its potential to be an efficient dressing that can reduce the incidence of Catheter-related bloodstream infections (CRBSIs). Six in vitro and one in vivo antimicrobial studies were done, along with the validation of the antimicrobial effect in an external lab.

Material

Silicone Dressings

CuraVue® is a sterile, single-use antimicrobial adhesive securement dressing composed of a transparent polyurethane film coated with a soft silicone adhesive layer containing chlorhexidine and silver as dual active antimicrobial agents. The dressing is designed with a U-shaped geometry to accommodate catheter placement while maintaining continuous skin contact and allowing visual inspection of the insertion site. A commercially available transparent silicone contact layer dressing, Mepitel®, was used as the negative control. Mepitel® does not contain any incorporated antimicrobial agents and therefore serves as an antimicrobial-deficient comparator.

Bacterial Strains: Five bacterial strains commonly associated with CRBSI were used for the antimicrobial testing of CuraVue®. For the minimum inhibitory concentration (MIC) determinations, disc diffusion assays and contact kill assay. The following strains were used: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC *37833*), *Escherichia coli* (ATCC 25922), methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC 43300) isolate, and a *Candida albicans* (ATCC 10231) isolate. These organisms were selected to represent both Gram-positive and Gram-negative bacteria, and yeast that are associated with CRBSIs.

For the Assessment of the Spatial Uniformity Distribution, antibiofilm activity, and the time-kill assay, the following strains were used: *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC *37833*), and *Escherichia coli* (ATCC 25922)

For the *in vivo* evaluation of CuraVue® in a rat skin abrasion colonization model, *S. aureus* (ATCC 29213) was used as the challenge organism, as it is considered a leading cause of catheter-related and device-associated infections [8, 11].

For the ISO 22196 assay, the strains used were *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538P). For the ASTM E2149 assay, *Escherichia coli* (ATCC 25922) was used.

Rats used in the *in vivo* experiment

Sprague-Dawley male rats (275–300 g, pathogen-free, immunocompetent) were used in this experiment.

Methods

Assessment of the Antimicrobial Activity

Disc Diffusion:

As recommended by CLSI M02, 2025 [18], strains were prepared in sterile saline at 0.5 McFarland (1.0–2.0 × 10^8 CFU/mL) and then spread on Mueller-Hinton agar plates (MHA). A 2 × 2 cm^2 from each sample was added to each plate and gently pressed to make sure that each piece was in full contact with the agar. The plates were incubated at 37°C for 24 h. Then, the zone of inhibition was measured as the distance from the film edge to the outer edge of the clear zone (mm) in at least two perpendicular directions, or the total diameter (film + zone) along two axes, with the subtraction of the film size.

In Vitro Quantitative Contact-Kill Test:

Antimicrobial susceptibility testing was done according to ISO 22196:2011 guidelines [19]. Specimens were prepared by cutting them to 4 × 4 cm and adding them to sterile plates. Isolates were cultured overnight and diluted in nutrient broth to reach a load of 10^2–10^5 CFU/cm^2.

The inoculum was covered with a sterile plastic film smaller than the test film, and the initial bacterial count (T_0) was determined by serial dilution and plating on TSA agar plates. All plates were incubated at 37°C for 24 hours. To preserve humidity, wet filter papers were placed adjacent to each sample, and the plates were placed in sealed bags [20]

After incubation, saline was added (10 mL per specimen), then shaken; serial dilution was performed to count the colonies and calculate the R value ($R = \log_{10}(B) - \log_{10}(C)$), where B = mean CFU recovered from untreated control after 24 h and C = mean CFU recovered from treated sample after 24 h; an R value ≥ 4 indicates very strong antimicrobial activity [19].

In Vitro Quantitative Time-Kill Test over 7 Days:

Specimens were prepared by cutting them to 4 × 4 cm pieces and adding them to sterile plates. Isolates were cultured overnight and diluted in nutrient broth to achieve a target load (often on the order of 10^2–10^5 colony-forming units per cm^2). The concentration was detected by serial dilution and plating on TSA agar plates.

The inoculum was covered with a sterile plastic film smaller than the test film, and the initial bacterial count (T_0) was determined by serial dilution and plating on TSA agar plates. All plates were incubated at 37°C for 7 days. To preserve humidity, wet filter papers were placed adjacent to each sample, and the plates were placed in sealed bags.

Incubation was performed for different periods (12 hours, 24 hours, 4 days, and 7 days). After incubation, saline (10 mL per specimen) was added, and the specimen was then shaken. Serial dilution was performed to enumerate colonies and calculate R values.

R value = $\log_{10}(A0) - \log_{10}(C)$, where A0 = mean CFU recovered from test samples immediately after inoculation; C = mean CFU recovered from the treated sample after each incubation time. An R value ≥ 4 indicates very strong antimicrobial activity [20].

Minimum Inhibitory Concentration (MIC) Detection

Following the CLSI protocol in M07, 2024 [21], film extracts were prepared by placing a cut sample (2 × 2 cm) into a sterile tube with 4 mL of Mueller-Hinton broth (MHB), then incubated at 37°C for 24 h with gentle shaking (≈ 100–150 rpm) to allow antimicrobial components to diffuse from the film; then serial dilutions were prepared in a 96-well microtiter plate. Inoculum preparation by serial dilutions was performed to obtain approximately 1×10^7 CFU/mL. When 10 µL of this diluted inoculum is added to each well (final volume ~ 200–210 µL), the final inoculum should be ~ 5×10^5 – 1×10^6 CFU/mL. The plates were incubated at 37°C for 24 hours. The MIC was detected by adding 10 µL of freshly prepared AlamarBlue (resazurin) dye and incubating for an additional 75 minutes (2 hours for *P. aeruginosa and E. coli)*. The minimum inhibitory concentrations (MICs) were determined according to the reference broth microdilution procedure recommended by the CLSI and the Manual of Clinical Microbiology. The MIC is defined as the lowest concentration of antimicrobial agent that produces no visible growth after 20-h incubation in Mueller-Hinton broth at 37°C [21, 22].

The resazurin-based assay is considered a better method than optical density measurements (OD600) because it is less susceptible to changes due to the optical density of the added drug. Viable bacterial cells reduce blue-colored, non-fluorescent resazurin to pink-colored, highly fluorescent resorufin. The change of color from blue to pink indicates bacterial growth. The minimum concentration of the extract that maintains the blue color (no color change) is considered the MIC [23]

Static Coupon Biofilm Assay: Viable Count (CFU)

Antibiofilm activity was detected according to the ASTM E3435 assay: Standard Practice for Testing Antimicrobial or Disinfectant Efficacy Against Biofilms Grown on a Medical Device or Surface by the Biofilm Surface Test Protocol (BSTP) [24].

To evaluate the antimicrobial efficacy, the dressings were first prepared by cutting them into uniform discs of approximately 12 mm in diameter. One coupon was placed into each well of a 24-well plate, including CuraVue®, non-antimicrobial controls, and a planktonic growth control consisting of wells containing bacterial inoculum without a coupon. The plate is then incubated to confirm bacterial viability via OD_{600} measurement after 24 hours.

An overnight bacterial culture was then grown and serially diluted to approximately 10^6 CFU/mL in fresh medium to prepare the inoculum. Then, 2 mL of the inoculated broth was added to each well containing a dressing coupon. The plates were incubated statically for 24 hours at 35–37°C to encourage surface attachment and biofilm formation. Following incubation, planktonic cells were removed by carefully aspirating the media, and each coupon was gently rinsed three times using a well-to-well transfer method: the coupon was transferred to a new well containing fresh PBS, gently agitated, and then moved to a second rinse well to avoid re-exposure to detached planktonic cells. To recover biofilm cells, each coupon was transferred into 10 mL of PBS, vortexed for 30 seconds, sonicated in a water-bath sonicator

at approximately 40 kHz for 5–10 minutes, and vortexed again for 30 seconds to dislodge surface-attached biofilm cells.

The resulting suspension was serially diluted 10-fold, plated onto tryptic soy agar (TSA), and incubated for 24 hours. Colony-forming units (CFU) were counted, and biofilm density was calculated as CFU per coupon. Finally, the log reduction was determined using the formula: Log Reduction = $\log_{10}$(Control CFU) – $\log_{10}$(Antimicrobial CFU) [25].

Assessment of Antimicrobial Activity Uniformity

Antimicrobial activity measured through the disc diffusion assay is used as a functional indicator of CHG and silver. This method evaluates the biological effect of the antimicrobial agents rather than their absolute concentration, which provides a clinically relevant assessment of performance. By assessing antimicrobial activity across multiple predefined regions of the dressing, this experiment enables evaluation of both radial and circumferential uniformity, adapted to the U-shaped geometry of CuraVue®. The experimental design includes two complementary antimicrobial assessment approaches to provide a comprehensive evaluation of spatial uniformity. For whole dressing testing, intact CuraVue® dressings were applied directly onto inoculated Mueller-Hinton agar plates to evaluate uniformity of antimicrobial activity across the entire dressing surface (Fig. 1-A1). For region-specific assessment, the dressing was subdivided into three predefined spatial zones based on distance from the geometric center of the device (Fig. 1A2&B1).

The antimicrobial activity of the CuraVue® dressing was assessed using a modified disc diffusion method following CLSI M02 guidelines [18]. Bacterial suspensions of *S. aureus, E. coli*, *and P. aeruginosa* were adjusted to a 0.5 McFarland standard and uniformly inoculated onto Mueller-Hinton agar plates. Two sample approaches were used: intact dressings applied in their native U-shaped geometry, and segmented samples from three predefined spatial regions (central insertion-site coverage, intermediate adhesive matrix, and peripheral edge). Following aerobic incubation at 35–37°C for 18–24 hours, antimicrobial activity was quantified by measuring zones of inhibition (ZOI) in two perpendicular directions.

Validation of the Antimicrobial Activity in an ISO-Certified Lab

The validation of the product's antimicrobial activity was performed by Mass Laboratuvar ve Danışmanlık Hizmetleri A.Ş., a GLP-compliant and ISO-accredited contract testing organization, employing standardized methodologies (ISO 22196 and ASTM E2149) under controlled experimental conditions.

ISO 22196: For each test strain (*Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538P), 0.4 mL of standardized bacterial suspension (2.5–10 × 10^5 CFU/mL) was inoculated onto each specimen. For the initial count determination (U_0), 10 mL of Soya Casein Digest Lecithin Polysorbate Broth (SCDLP Broth) was added to the three untreated specimens immediately after inoculation. Serial

dilutions were prepared, and 1 mL aliquots were plated onto Plate Count Agar (PCA), followed by incubation at 35 ± 2°C for 40–48 hours to enumerate viable bacteria (CFU/cm²).

R value was calculated as: $R = \log_{10}(B) - \log_{10}(C)$, where B = mean CFU recovered from the untreated control after 24 h and C = mean CFU recovered from the treated sample after 24 h; an R value ≥ 4 indicates very strong antimicrobial activity [19].

ASTM E2149: Test specimens were weighed (1 g ± 0.1 g) and inoculated with 50 mL of bacterial suspension (*Escherichia coli* ATCC 25922) at a concentration of $1.5 \times 10^5 – 3 \times 10^5$ CFU/mL. Both test and inoculum-only flasks were shaken for 60 minutes at 35°C. Serial dilutions were prepared in triplicate and plated onto PCA to determine bacterial counts (CFU/mL) at both initial and contact time points. For sustained activity assessment, an extraction method was employed in accordance with ISO 10993-12 using 0.9% saline as the extraction solution. One sample was analyzed on day 0, while the remaining seven samples were incubated at 37°C and analyzed sequentially over 7 days [26].

Preclinical Evaluation of Antimicrobial Efficacy in a Rat Skin Abrasion Colonization Model

A controlled, randomized *in vivo* study using male Sprague-Dawley rats evaluated the antimicrobial efficacy of CuraVue®, compared to a non-antimicrobial dressing (negative control (Mepitel®)).

Following anesthesia, a standardized 2 × 2 cm superficial scratch lesion was created on the dorsum to simulate a catheter insertion site, sterilized with ethanol, and inoculated with 50 µL of *Staphylococcus aureus* ATCC 25923 at 10^7 CFU/mL. Dressings (2.5 × 2.5 cm) were applied with gentle pressure, and swab samples were collected from the lesion area at consecutive time points up to 7 days (0, 1 h, 3 h, 24 h, 48 h, 72 hours, 5 days, and 7 days). Swab samples were cultured on tryptic soy agar, and bacterial growth was semi-quantitatively graded from no colonies (complete inhibition) to heavy growth across all four quadrants. Scores were assigned to each plate to interpret the results. The sample size was chosen to be 6 animals per group, which is documented in the literature as sufficient to detect moderate to large effect sizes at an alpha level of 0.05 with approximately 80% statistical power [27].

Table 1
Interpretation of Semi-Quantitative Grade Scores for Bacterial Growth

Shape of colonies	Growth	Score
No colonies	Complete inhibition	0
Few colonies only in the 1st quadrant:	Scant growth	1
Heavy growth in the 1st quadrant + colonies in the 2nd quadrant	Mild growth	2
Heavy growth in the 1st quadrant + colonies in the 2nd and 3rd quadrants	Moderate growth	3
Heavy growth in the 1st quadrant + colonies in the 2nd ,3rd and 4th quadrants	Heavy growth	4

Statistical Analysis

All statistical analyses were performed using SPSS and R. Statistical evaluation, including descriptive statistics (mean ZOI, SD, %CV) and equivalence testing using the Two One-Sided Tests (TOST) with a ± 2 mm margin, was conducted to assess spatial uniformity across the dressing [28, 29]. Linear regression was performed in the time-kill assay to detect if CuraVue®'s antimicrobial activity changed significantly over the 7-day test period. In the animal model experiment, the antimicrobial efficacy of CuraVue® (Test Group, G2) was evaluated compared to the Negative Control (Mepitel®)(G1). A Kruskal-Wallis test was performed at each time point (1 hour, 3 hours, 24 hours, 48 hours, 72 hours, 5 days, and 7 days). This non-parametric test was selected because the growth score data (0–4 scale) are ordinal and did not meet the normality assumptions required for one-way ANOVA [27]. All experiments were performed in triplicates.

Results

Disc Diffusion

CuraVue®’s ability to inhibit microbial growth was primarily assessed using the Disk Diffusion assay against the Mepitel® dressing. CuraVue® showed measurable zones of inhibition (ZOI) around the dressing against all five tested strains, with no ZOIs recorded with Mepitel®. The mean inhibition zones were 2.5mm for *S. aureus*, 2.5 for *E.coli*, 1.0 mm for *P.aeruginosa*, 1.5 mm for *C. albicans*, and 1.5 mm for methicillin-resistant *Staphylococcus aureus* (*MRSA)* (Table 2).

Table 2
Zones of Inhibition (ZOI) against S. aureus, E.coli, P.aeruginosa, C. albicans, and methicillin-resistant Staphylococcus aureus (MRSA).

Sample	**Average ZOI (mm)** ***S. aureus***	**Average ZOI (mm)** ***E.coli***	**Average ZOI (mm)** ***P. aeruginosa***	**Average ZOI (mm)** ***C. albicans***	**Average ZOI (mm)** **MRSA**
CuraVue®	2.5	2.5	1	1.5	1.5
Mepitel® (Negative control)	No measurable ZOI	No measurable ZOI	No measurable ZOI	No measurable ZOI	No measurable ZOI

In vitro Quantitative Contact-kill Test

The standardized method, ISO 22196, is widely used to evaluate the antimicrobial properties of treated plastics and non-porous surfaces under controlled temperature, humidity, and inoculum concentrations [19]. The quantitative contact kill assay measured the reduction in recoverable viable microorganisms following 24-hour contact. CuraVue® showed strong antimicrobial activity against all five tested microorganisms, including *S. aureus*, *P. aeruginosa*, *E. coli*, *C. albicans* and MRSA. All tested organisms showed R values greater than 4, indicating strong antimicrobial effectiveness according to ISO 22196 interpretation criteria. CuraVue® showed the greatest antimicrobial reductions against *P. aeruginosa* (R value = 6), indicating a broad spectrum of antimicrobial activity against different bacterial strains (Table 3). Mepitel® showed extensive microbial growth across all tested strains, confirming the absence of intrinsic antimicrobial activity.

Table 3
Results of the antimicrobial testing against one fungal and four bacterial strains.

Sample	CFU/ml	R value
S. aureus		
CuraVue®	1600	4.80
Negative control (Mepitel®)	10^8	
E.coli		
CuraVue®	8660	4.06
Negative control (Mepitel®)	10^8	
P. aeruginosa		
CuraVue®	100	6.00
Negative control (Mepitel®)	10^8	
Candida albicans		
CuraVue®	11000	4.49
Negative control (Mepitel®)	3.4*10^8	
MRSA		
CuraVue®	10000	4.60
Negative control (Mepitel®)	4*10^8	

In Vitro Quantitative Time-Kill Test over 7 days

The time-kill assay extends the assessment of CuraVue®'s persistent antimicrobial activity over 7 days by measuring the log10 reduction in viable counts relative to the initial inoculum recovered immediately after inoculation. CuraVue® showed sustained antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* over the 7-day test period (Table 4). Mean R values were ≥ 4.0 for all three organisms (*E. coli*: 4.36 ± 0.28; *P. aeruginosa*: 4.20 ± 0.39; *S. aureus*: 4.18 ± 0.35), indicating ≥ 99.99% reduction in viable bacterial counts.

Linear regression analysis was performed to assess whether CuraVue® 's antimicrobial activity changed significantly over the 7-day test period. No statistically significant time-dependent changes were observed for *S. aureus* (slope = -0.0222, p = 0.9185), *E. coli* (slope = 0.0954, p = 0.5528), or *P. aeruginosa* (slope = 0.2398, p = 0.2025), indicating that CuraVue® 's antimicrobial activity remained consistent and sustained throughout the 7-day wear period.

Table 4
Summary of the time-kill results

Organism	Mean R ± SD	R ≥ 4.0	Time Effect
E. coli	4.36 ± 0.28	YES	No significant change
P. aeruginosa	4.20 ± 0.39	YES	No significant change
S. aureus	4.18 ± 0.35	YES	No significant change

Minimum Inhibitory Concentration (MIC) detection

To measure the lowest CuraVue® 's extract concentration required to inhibit detectable microbial growth, the Minimum Inhibitory Concentration assay was performed. The MIC was detected and evaluated by the change in the dye color. CuraVue® achieved 100% MIC against *S. aureus*, *E. coli*, MRSA, and *C. albicans and* MIC of 85% against *P. aeruginosa*. The complete inhibition of all tested organisms confirms that CuraVue® is a highly effective antimicrobial dressing with broad-spectrum activity suitable for clinical use. (Table 5; Supplementary Figs. 1–5).

Table 5
MIC values of CuraVue®

Sample	Av. MIC (%) *S. aureus*	Av. MIC (%) *E.coli*	Av. MIC (%) *P. aeruginosa*	Av. MIC (%) *C. albicans*	Av. MIC (%) *MRSA*
CuraVue®	100	100	85	100	100

Static Coupon Biofilm Assay of CuraVue®: Viable Count (CFU)

The static coupon assay assessed the recovery of viable surface-associated bacteria after 24 hours of static incubation with the dressings CuraVue® against Mepitel® under the conditions supporting biofilm formation. Compared to Mepitel®, CuraVue® showed strong antibiofilm activity against all tested microorganisms by reducing the viable counts by 5.27 log for *S. aureus*, 4.11 log for *E. coli*, and 2.49 log for *P. aeruginosa*. These reductions corresponded to greater than 99.99% for *S. aureus* and *E. coli*, and approximately 99.68% for *P. aeruginosa* (Table 6).

Table 6
Mean results, R value, and percent reduction in the static coupon inhibition assay

Sample	Mean CFU/mL	R value	% Reduction
S. aureus			
CuraVue®	163.33	5.27	> 99.99%
Negative control (Mepitel®)	30,666,666.67	--	--
E.coli			
CuraVue®	1,666.67	4.11	> 99.99%
Negative control (Mepitel®)	21,666,666.67	--	--
P. aeruginosa			
CuraVue®	12,933,333.33	2.49	99.68%
Negative control (Mepitel®)	4,000,000,000	--	--

Assessment of Antimicrobial Activity Uniformity

The spatial assessment examined CuraVue®'s antimicrobial activity across the dressing's central, intermediate and peripheral regions. CuraVue® produced measurable zones of inhibition across all evaluated regions against three bacterial strains *S. aureus, E.coli and P. aeruginosa.* Mean regional ZOI against *S. aureus* was 3.33mm, 2.33mm for *E. coli*, and 1.33 to 2mm for *P. aeruginosa*. Mepitel® produced no measurable ZOI in any region under evaluation (Tables 7–10). The results of the two one-sided tests (TOST) for *S. aureus* show that CHG is uniformly distributed across the dressing. CuraVue® showed complete spatial uniformity (all regions statistically equivalent). CuraVue® spatial uniformity is confirmed for all three organisms (*S. aureus*, E. coli, *P. aeruginosa*).

Table 7
Mean ZOI against S. aureus, E.coli and P. aeruginosa

Organism	Sample	Whole (mm)	Region A (mm)	Region B (mm)	Region C (mm)	Overall Mean (mm)
S. aureus	CuraVue®	3.67	3.33	3.33	3.33	3.42
	Mepitel®	0	0	0	0	0
E. coli	CuraVue®	3.00	2.33	2.00	2.33	2.42
	Mepitel®	0	0	0	0	0
P. aeruginosa	CuraVue®	1.33	1.33	2.00	1.67	1.58
	Mepitel®	0	0	0	0	0

Table 8
TOST summary table for S. aureus

Comparison	Mean Diff (mm)	90% CI	Within ± 2 mm	Equivalence Conclusion
Within CuraVue®				
Region A vs Region B	0.00	(-0.94, 0.94)	Yes	**Equivalent**
Region A vs Region C	0.00	(-0.94, 0.94)	Yes	**Equivalent**
Region B vs Region C	0.00	(-0.94, 0.94)	Yes	**Equivalent**
CuraVue® vs Mepitel®				
Whole vs Whole	3.67	(2.94, 4.40)	No	**Not Equivalent** (CuraVue® superior)
Region A vs Region A	3.33	(2.64, 4.02)	No	**Not Equivalent** (CuraVue® superior)
Region B vs Region B	3.33	(2.64, 4.02)	No	**Not Equivalent** (CuraVue® superior)
Region C vs Region C	3.33	(2.64, 4.02)	No	**Not Equivalent** (CuraVue® superior)

Table 9
TOST results for E. coli

Comparison	Mean Diff (mm)	90% CI	Within ± 2 mm	Equivalence Conclusion
Within CuraVue®				
Region A vs Region B	0.33	(-0.78, 1.44)	Yes	**Equivalent**
Region A vs Region C	0.00	(-0.78, 0.78)	Yes	**Equivalent**
Region B vs Region C	0.33	(-0.78, 1.44)	Yes	**Equivalent**
CuraVue® vs Mepitel®				
Whole vs Whole	3.00	(2.28, 3.72)	No	**Not Equivalent** (CuraVue® superior)
Region A vs Region A	2.33	(1.68, 2.98)	No	**Not Equivalent** (CuraVue® superior)
Region B vs Region B	2.00	(1.42, 2.58)	No	**Not Equivalent** (CuraVue® superior)
Region C vs Region C	2.33	(1.68, 2.98)	No	**Not Equivalent** (CuraVue® superior)

Table 10
TOST test results for P. aeruginosa

Comparison	Mean Diff (mm)	90% CI	Within ± 2 mm	Equivalence Conclusion
Within CuraVue®				
Region A vs Region B	0.67	(-0.22, 1.56)	Yes	**Equivalent**
Region A vs Region C	0.34	(-0.44, 1.12)	Yes	**Equivalent**
Region B vs Region C	0.33	(-0.22, 0.88)	Yes	**Equivalent**
CuraVue® vs Mepitel®				
Whole vs Whole	1.33	(0.72, 1.94)	Yes	**Equivalent**
Region A vs Region A	1.33	(0.72, 1.94)	Yes	**Equivalent**
Region B vs Region B	2.00	(1.42, 2.58)	No (upper bound exceeds)	**Not Equivalent** (CuraVue® superior)
Region C vs Region C	1.67	(1.06, 2.28)	No (upper bound exceeds)	**Not Equivalent** (CuraVue® superior)

Validation of the Antimicrobial Activity

The validation of CuraVue® 's antimicrobial activity was assessed using ISO 22196 and ASTM E2149 methodologies, including testing after conditioning periods extending to seven days [19, 26] under controlled experimental conditions. For the ISO 22196 assay, recoverable counts of *E. coli* ATCC 8739 and *S. aureus* ATCC 6538P were below the reported detection limit of 10 CFU/cm² after 24 hours of contact with CuraVue®, whereas growth was recovered from the untreated controls (Table 11). In the ASTM E2149 assessment, recoverable *E. coli* ATCC 25922 counts were below the reported detection limit of 30 CFU/mL at every evaluated conditioning time from day zero through day seven, following the specified 60-minute contact period. Tables 11 and 12 show the high R values (> 4) in both experiments .

Table 11
Summary of Antimicrobial Activity Results (ISO 22196) performed in the ISO certified lab.

Contact Time	Untreated Sample (U_0) – Initial	Untreated Sample (U□) - After 24 h	Treated Sample (A□) - After 24 h	Log Reduction (R)	Reduction (%)
E. coli ATCC 8739					
24 hours	1.6 × 10³ CFU/cm² (3.21 log)	1.8 × 10⁵ CFU/cm² (6.22 log)	< 10 CFU/cm² (< 1.00 log)	> 5.22 log	> 99.999%
S. aureus ATCC 6538P					
24 hours	1.2 × 10³ CFU/cm² (3.06 log)	1.3 × 10⁶ CFU/cm² (6.12 log)	< 10 CFU/cm² (< 1.00 log)	> 5.12 log	> 99.999%

Table 12
Summary of Antimicrobial Activity Results (ASTM E2149) performed in an ISO certified lab

Day	Inoculum After Contact Time (B) (CFU/mL)	Treated Sample After Contact Time (A) (CFU/mL)	Log Reduction (B - A)	Reduction (%)
Day 0	1.5 × 10⁵ (5.19 log)	< 30 (< 1.48 log)	> 3.71 log	100%
Day 1	1.1 × 10⁵ (5.03 log)	< 30 (< 1.48 log)	> 3.55 log	100%
Day 2	1.2 × 10⁵ (5.07 log)	< 30 (< 1.48 log)	> 3.59 log	100%
Day 3	1.8 × 10⁵ (5.26 log)	< 30 (< 1.48 log)	> 3.78 log	100%
Day 4	1.1 × 10⁵ (5.06 log)	< 30 (< 1.48 log)	> 3.58 log	100%
Day 5	1.1 × 10⁵ (5.03 log)	< 30 (< 1.48 log)	> 3.55 log	100%
Day 6	1.6 × 10⁵ (5.21 log)	< 30 (< 1.48 log)	> 3.73 log	100%
Day 7	1.9 × 10⁵ (5.28 log)	< 30 (< 1.48 log)	> 3.80 log	100%

Preclinical Evaluation of Antimicrobial Efficacy in a Rat Skin Abrasion Colonization Model

The assessment of CuraVue® as an antimicrobial dressing was further done in a rat model by infecting their skin with *Staphylococcus aureus* ATCC 25923 and scoring the bacterial load infecting each rat at each assigned time. The negative control (Mepitel®) showed consistently higher bacterial growth scores

(overall mean = 2.90 ± 0.79) compared to CuraVue® (overall mean = 1.71 ± 0.55). Kruskal-Wallis tests showed statistically significant differences between groups at 6 of 7 time points (1 h, 3 h, 24 h, 48 h, 72 h, and 7 days; all $p < 0.05$). At 5 days, a borderline trend was observed ($p = 0.0647$) (Table 13).

Table 13
The mean results obtained from each group (mean score) — Rat Skin Abrasion Colonization Model

Group	1h	3h	24h	48h	72h	5 days	7 days
G1 (Mepitel®)	3	2.83333	2.66667	2.83333	3	2.83333	3.16667
G2 (CuraVue®)	1.666667	1.5	1.333333	1.5	1.333333	1.666667	1.333333

Table 14
Overall Mean by Group of the results — Rat Skin Abrasion Colonization Model

Group	Overall Mean	Overall SD
CuraVue®	1.71	0.55
Negative Control (Mepitel®)	2.90	0.790

Kruskal-Wallis Test Results:

Table 15
Kruskal-Wallis Test Results — Rat Skin Abrasion Colonization Model

Time Point	χ^2	df	p-value	Significance	Interpretation
1 hour	7.857	1	0.0051	**	Significant
3 hours	7.663	1	0.0056	**	Significant
24 hours	4.031	1	0.0447	*	Significant
48 hours	4.632	1	0.0314	*	Significant
72 hours	7.620	1	0.0058	**	Significant
5 days	3.413	1	0.0647	Ns	Borderline (trend)
7 days	4.407	1	0.0358	*	Significant

Discussion

Central venous catheters (CVCs) are indispensable in modern clinical practice due to their ability to provide reliable vascular access during critical and perioperative care. The frequent use of CVCs often creates a route for skin microorganisms to enter the catheter and colonize its surface [30]. This is further

complicated by catheter related blood stream infection (CRBSI) that is considered the most lethal and costly complication of central venous catheterization [31]. Despite strict implementation of standard evidence-based techniques, significant rates of CRBSIs persist in many settings, particularly in inpatient wards and outpatient hemodialysis facilities [32, 33]. Thus, this necessitates the development and availability of biocompatible antimicrobial dressings [34, 35]. The incorporation of antimicrobial substances into adhesive layers contacting the skin entails antimicrobial activity at the skin covered by the dressing.

The present study introduces CuraVue®, an antimicrobial adhesive securement silicone dressing containing chlorhexidine and silver as antimicrobial agents. It is developed for microbial infection control at the insertion site pertaining to its dual antimicrobial incorporation within its silicone adhesive layer. A comprehensive set of in vitro and in vivo assessments was performed to evaluate the dressing's antimicrobial performance, with emphasis on its activity over seven-day wear time, as well as the uniformity of antimicrobial activity across the dressing regions, and localized microbial control at skin-contacting surface. Skin microflora was found to be suppressed in healthy volunteers' skin when covered by adhesive layers with a chlorhexidine antimicrobial agent [36]. The incorporation of silver in the dressing gives synergistic effect with chlorhexidine, leading to the enhancement of the antimicrobial efficacy at the skin–catheter interface with a broad spectrum effect by targeting pathogens through multiple complementary mechanisms [37].

The antimicrobial functionality of CuraVue® against Gram-positive, Gram-negative microorganisms and yeast is supported by the presence of measurable inhibition zones (ZOI). The ZOIs against all tested strains support CuraVue® antimicrobial activity.

CuraVue® whole dressing was assessed using the CLSI disc diffusion test against Mepitel® [18]. For diffusion-based assays involving polymeric or adhesive dressings, ZOIs are relatively small due to the localized gradual release of the antimicrobial agents around the dressing area [38]. However, CuraVue® produced mean inhibition zones of 2.5mm, 2.5mm, 1.0mm, 1.5mm and 1.5mm (Table 2) when tested against multiple bacterial strains *S. aureus, E.coli*, *P.aeruginosa, C. albicans*, and methicillin-resistant *Staphylococcus aureus* (*MRSA)*, respectively. These persistent ZOIs are better translated when compared to Mepitel® that showed complete absence of ZOIs, supporting the whole dressing antimicrobial activity and controlled skin infection underneath.

The functional significance of CuraVue®'s antimicrobial activity is well demonstrated through its ability to control microorganisms during contact with the dressing. The quantitative contact-kill assay detects the antimicrobial effect of treated plastic surfaces, thus extending the diffusion and extract-based observations [19]. CuraVue® demonstrated strong reductions exceeding 4-log as compared to Mepitel® against all five tested strains, including *Staphylococcus aureus*, MRSA, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* indicating significant antimicrobial performance according to ISO 22196 interpretation criteria (Table 3). [20]. This strong activity was further confirmed over seven days through the Time Kill assay as CuraVue® maintained the antimicrobial activity against *S. aureus*, *E. coli*,

and *P. aeruginosa* over the clinically relevant 7-day wear period. CuraVue® mean R values for all three tested organisms reflect more than 99.99% reduction in bacterial viability which remained stable over time (linear regression, $p > 0.05$) (Table 4, supp. Tables 1–4, Figs. 3 and 4), indicating sustained antimicrobial protection.

The 6.00-log reduction against *P. aeruginosa* in the contact kill assay and a 2.49-log reduction in the static coupon assay further demonstrates activity against this organism under the direct-contact conditions evaluated. *P. aeruginosa* is considered a very challenging pathogen due to its intrinsic resistance to many antimicrobial agents, its ability to form biofilms on catheter surfaces and medical devices, and its association with severe healthcare-associated infections, including CRBSIs [39].

Additionally, testing CuraVue®'s minimum inhibitory concentration extract demonstrated noticeable antimicrobial activity against all tested pathogens (Table 5, supp Figs. 1–5) [21].

The evidence of CuraVue®'s antimicrobial functionality was further assessed in the dressing's ability to reduce viable surface-related bacteria as compared to Mepitel® in conditions promoting bacterial attachment and biofilm formation. Biofilm presents a challenge for localized microbial control as it is usually formed by highly resistant bacteria to antibiotic treatments or medical devices placed in the body [40, 41]. The greater reductions against *S. aureus* and *E. coli*, alongside the smaller but substantial 2.49-log reduction against *P. aeruginosa* (Table 6), indicate that the magnitude of this effect varied among the tested organisms. The difficulty of eradicating *P. aeruginosa* biofilms is mentioned in many studies, including a human plasma-based biofilm model study where silver- and octenidine-containing wound dressings failed to give bactericidal or bacteriostatic activity, while even effective agents such as cadexomer-iodine showed incomplete eradication due to incomplete biofilm lysis [42]. Since the dressing was present during bacterial attachment and growth, the results present the ability of the dressing to limit viable microbial accumulation during development, rather than the eradication of a pre-existing mature biofilm.

Since CuraVue® is designed to contact the skin across several regions at the insertion site, spatial assessment of its activity is essential. Dividing the whole dressing into central, intermediate, and peripheral regions demonstrated functional antimicrobial distribution across the sampled adhesive areas of the dressing. The spatial uniformity of antimicrobial activity across the CuraVue® dressing was assessed using the Two One-Sided Tests (TOST) approach with a predefined equivalence margin of ± 2 mm. TOST is a statistically important method that tests for equivalence instead of failing to detect a difference [28, 29]. The 90% confidence intervals for the comparisons done between all the dressing predefined spatial zones as mentioned in Tables 8–10 fell within the ± 2 mm equivalence margin for all the three tested microorganisms. The equivalence margin was selected based on the studies done on the disk diffusion assay where a regional difference in mean ZOI of ≤ 2 mm was considered a reflection of methodological variability in the assay rather than a significant variation in antimicrobial activity [42, 43].

The results shown in Tables 8–10 prove that CuraVue® provides spatially uniform antimicrobial activity across its entire U-shaped dressing surface, supporting its intended function in reducing microbial colonization surrounding catheter insertion sites and contributing to reliable infection prevention across the whole dressing [4]. This emphasizes that the observed functionality was distributed across the sampled sections; hence, connecting to the dressing's intended use of providing microbial growth control across the area of skin contact.

Performing quantitative antimicrobial assays under additional test conditions and via external certified labs further strengthens the claimed antimicrobial activity. Thus, the antimicrobial evaluation done via Mass Laboratuvar ve Danışmanlık Hizmetleri A.Ş., provided further evidence using different bacterial strains and different methodology when it came to the time-kill assay (ASTM E2149 instead of our method, which is a modified form of the ISO 22196 assay). Recoverable counts were below the reported detection limits in both experiments, including the *E. coli* assessment following incubation for up to seven days. (Tables 11 and 12). These findings support the overall evidence of strong broad spectrum antimicrobial activity, noting that the differences in controls, strains, and exposure conditions require each result to be interpreted within its own method.

The in-vivo skin colonization model was done to confirm the in-vitro antimicrobial activity on an animal model in which skin colonization with *S. aureus* was done; then the bacterial inhibition was assessed over a period of seven days to verify the efficacy of CuraVue® against skin colonization that can lead to blood stream infection. CuraVue® showed sustained antimicrobial activity with significantly lower bacterial growth scores compared to the negative control at most time points throughout the 7-day study period (Tables 13 and 14, Fig. 5, Supp. Figures 6–8), confirming sustained antimicrobial protection throughout the 7-day period. These findings support the clinical utility of CuraVue® for the prevention of catheter-related infections [45]. The findings give a preclinical link between the performed lab assessments and the targeted site of action [46].

Taken together, these comparative assessments confirm the broad spectrum antimicrobial functionality of CuraVue® dressing and its capacity to sustain microbial control under challenging conditions for up to 7 days, which contribute to its significance in limiting microbial colonization at catheter insertion sites.

Conclusion

In this study, CuraVue®, a transparent silicone adhesive dressing loaded with chlorhexidine and silver, was investigated for its functional antimicrobial activity against a wide spectrum of bacteria as well as Candida yeast. Several methods were utilized to assess and confirm its spectrum and strength of antimicrobial efficacy. CuraVue® demonstrated antimicrobial activity against *S. aureus*, *MRSA*, *E. coli*, *P. aeruginosa*, and *Candida albicans*, with utmost antimicrobial activity against challenging strains of *P. aeruginosa*. Direct contact assessments confirmed substantial reductions in microbial recovery in the contact kill assay which remained consistent up to the seventh day in the time kill assay. The significant

reduction in viable surface-associated bacteria under conditions supporting biofilm formation further demonstrated CuraVue®'s antimicrobial activity. Consistent zones of inhibition observed during uniformity assessment of the entire dressing and its segmented areas confirm CuraVue®'s uniform antimicrobial activity throughout the dressing. The suppression of localized bacterial growth over seven days in the rat skin colonization model confirmed the antimicrobial activity on a biological skin surface for up to 7 days. This assessment package presents a preclinical foundation for CuraVue®'s established functionality in providing sustained and localized microbial suppression, thus reducing incidences of CVCs and CRBSI colonization at catheter insertion sites.

Abbreviations

WHO
World Health Organization
HAIs
Health care-associated infections
CVC
Central venous catheter
CRBSI
Catheter-related bloodstream infection
CFU
Colony-forming unit
CVCs
Central venous catheters
NNIS
National Nosocomial Infections Surveillance System
CDC
Centers for Disease Control and Prevention
ICUs
Intensive Care Units
PICUs
Pediatric Intensive Care Units
PVCR-BSIs
peripheral venous catheter-related bloodstream infection
CHG
chlorhexidine
hFB
Human fibroblast
FBS
fetal bovine serum
PBS

phosphate-buffered saline
EDTA
ethylenediaminetetraacetic acid
DMSO
dimethyl sulfoxide
ATCC
American Type Culture Collection
ASTM
American Society for Testing and Materials
ISO
international organization for standardization
S. aureus
Staphylococcus aureus
E.coli
Escherichia coli
P. aeruginosa
Pseudomonas aeruginosa
CLSI
Clinical & Laboratory Standards Institute
ZOI
Zone of Inhibition
MRSA
Methicillin-resistant *Staphylococcus aureus*
MIC
Minimum Inhibitory Concentrations
MHA
Mueller–Hinton Agar
MHB
Mueller–Hinton Broth
OD
Optical Density
CFU
Colony Forming Unit
TOST
Two One-Sided Tests

Declarations

Ethics approval:

To assess the antimicrobial efficacy of the dressing in a rat model, female Sprague-Dawley rats (125–150 g) were obtained from UResearch. All medical procedures and care were performed according to the National Research Council's Guide for the Care and Use of Laboratory Animals. The study was approved by the Institutional Animal Care and Use Committee (IACUC) of Cairo University (URAF-F-14-25). A clinical trial number is not applicable for this study.

Consent for publication:

Not applicable. This manuscript does not contain any individual person's data in any form. All animal experiments were conducted in accordance with institutional guidelines and approved by the relevant ethics committee.

Competing Interests

B.A., S.A.H., R.M.A., R.Z., F.F.A., A.M.A., F.A.., W.A.S and M.E.S. are members of the research team at Incura Inc. Incura Inc. is the organization that developed and holds the intellectual property rights for CuraVue® (the antimicrobial dressing evaluated in this manuscript). These authors have direct employment relationships with Incura Inc., which is developing and commercializing the technology described in this study. The study was designed to include objective preclinical evaluation methods, with comparison against an established commercially available product (Mepitel®) using standardized antimicrobial assessment protocols. The in vivo study was conducted in the UResearch Animal Facility, which is AAALAC-accredited, under the supervision of certified vets. The authors affirm that all data presented in this manuscript are reported accurately and objectively, and that the study design, methodology, and conclusions were not influenced by any desire to obtain favorable results for CuraVue®. Also, to ensure data integrity validation of the antimicrobial results was done in an external GLP-compliant and ISO-accredited contract testing organization (Mass Laboratuvar ve Danışmanlık Hizmetleri A.Ş.,).

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Author Contribution

B.A. performed data curation and data analysis and wrote the manuscript. F.F.A. carried out validation and quality control of the product. R.M.A., A.M.A., and A.F. contributed to the research and development of the product. A.M.A. and A.F. also managed the project. R.Z. and S.A.H. wrote, reviewed and edited the manuscript. R.Z. prepared the graphical abstract. M.E.S. conceptualized the study and supervised the work. W.A.S. supervised the work. All authors read and approved the final manuscript.

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Availability of data and materials:

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

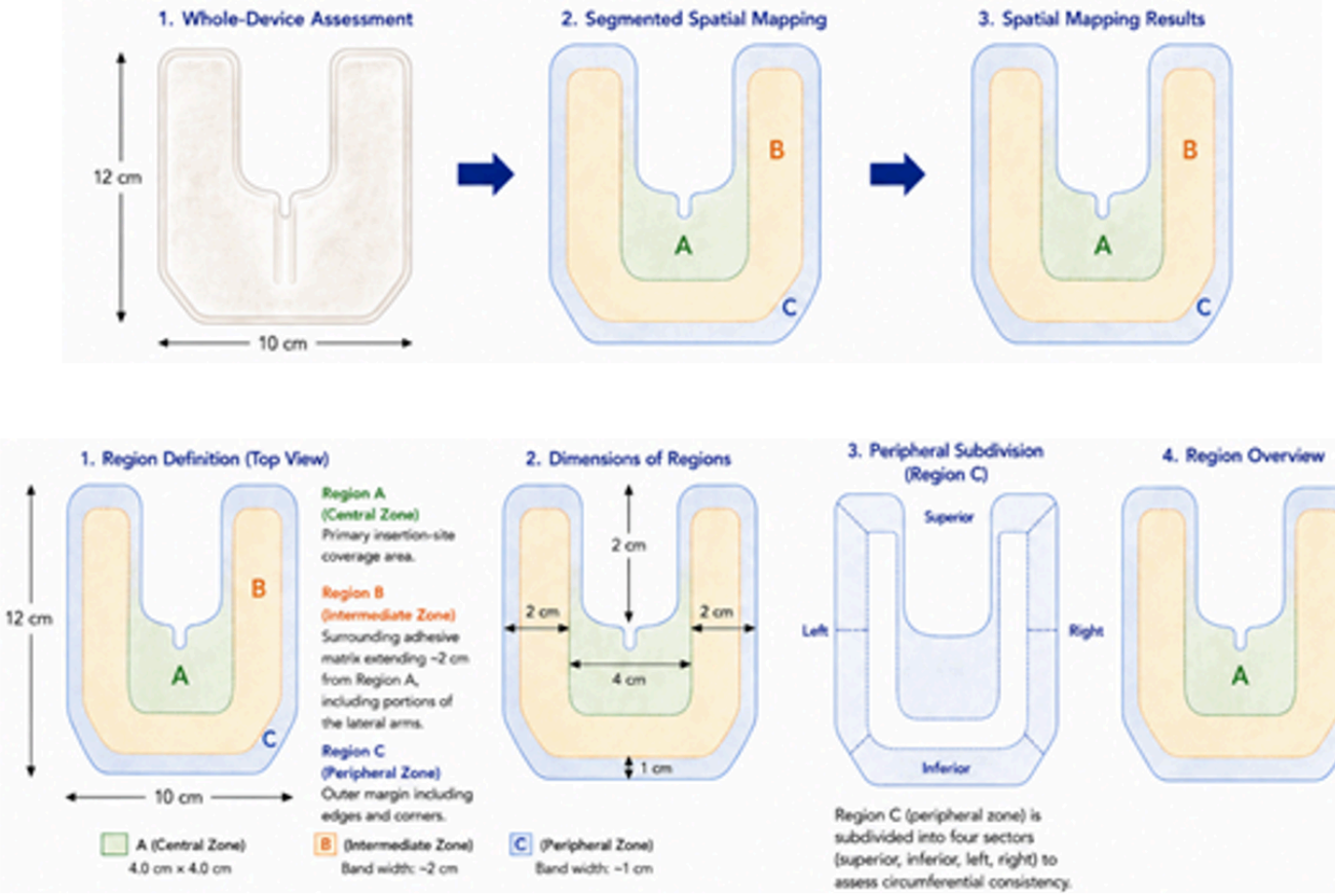


Figure 1

Experimental Design and Spatial Segmentation for Assessment of Antimicrobial Spatial Uniformity Across CuraVue®.The experimental design and spatial segmentation strategy used to assess antimicrobial spatial uniformity across the CuraVue® U-shaped antimicrobial dressing. Section A illustrates the overall experimental workflow, including whole-device assessment (A1) and segmented spatial mapping (A2). Section B illustrates the predefined spatial regions used for analysis, including the central region (Region A), intermediate region (Region B), and peripheral region (Region C), together with peripheral subdivision of Region C (B3). Antimicrobial activity was evaluated using the CLSI M02 disc diffusion methodology with Mepitel® as the negative control.

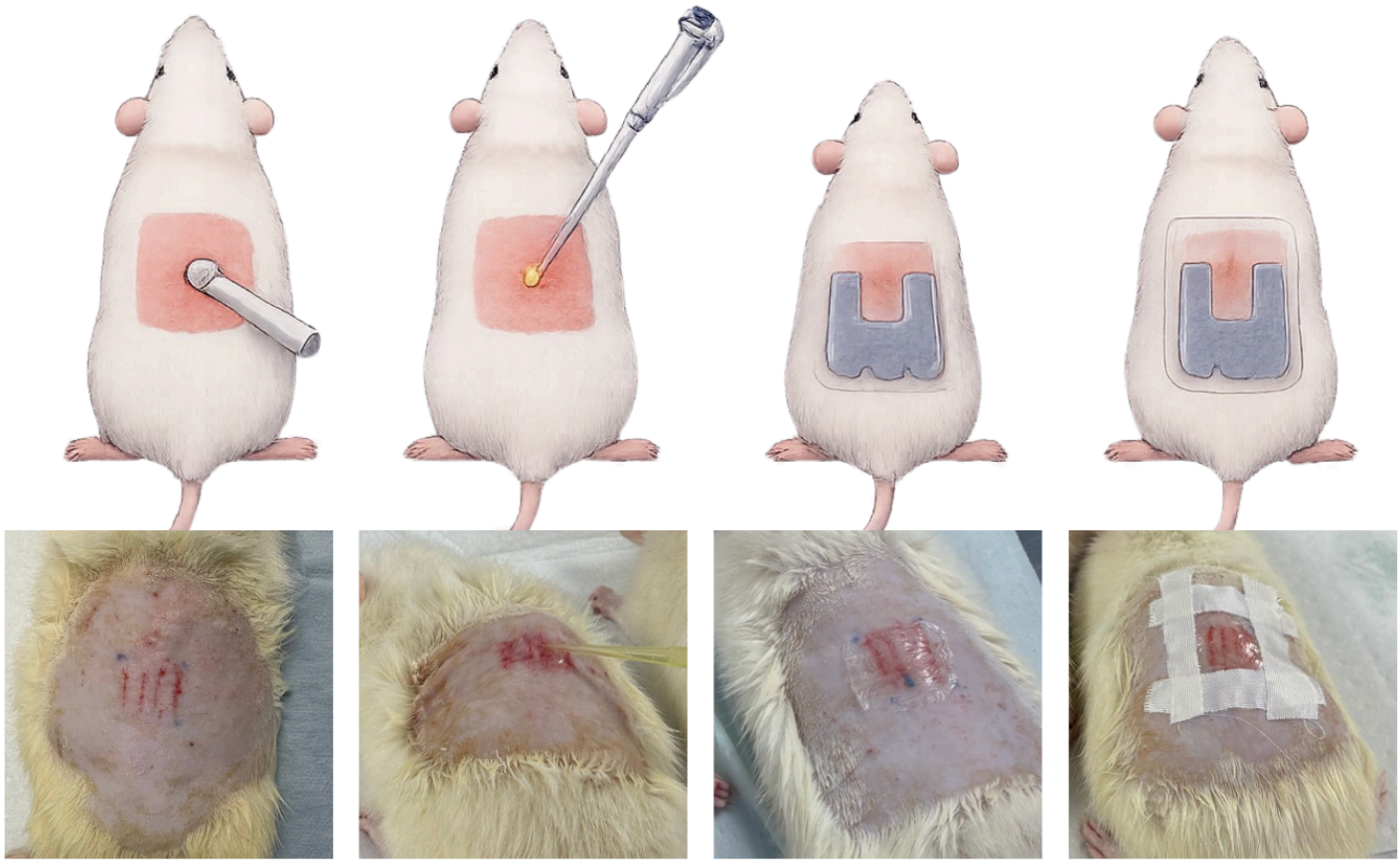


Figure 2

Schematic Illustration of the Rat Skin Abrasion Colonization Model. The diagram illustrates the procedural steps of the study. The representative photographs show the detailed steps of the experimental procedure.

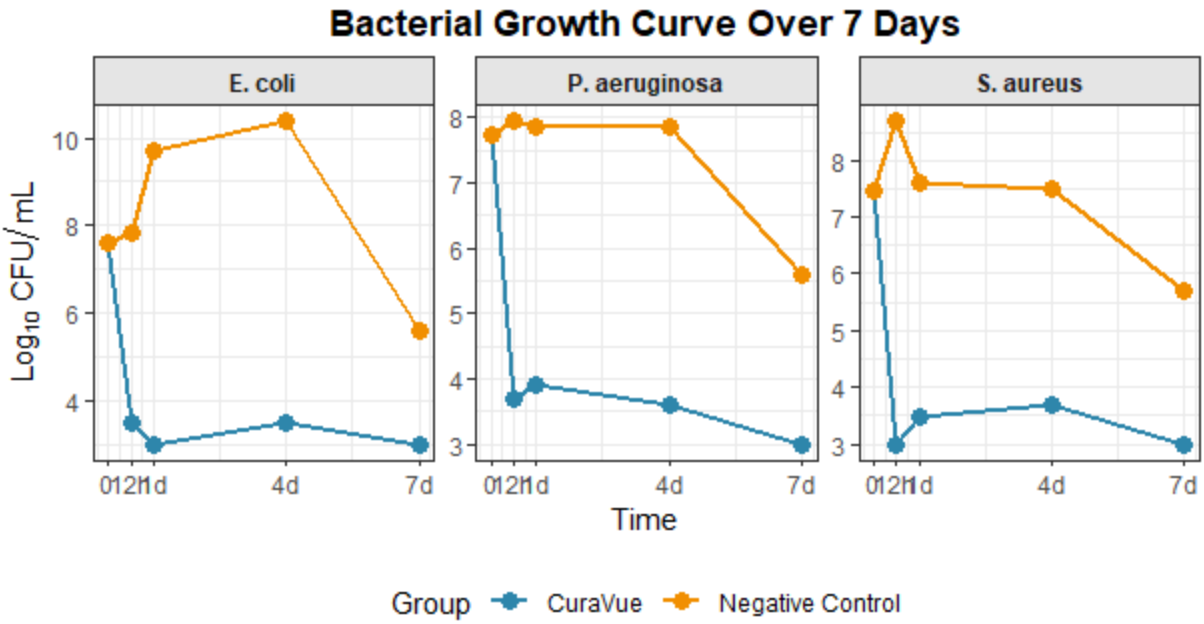


Figure 3

Bacterial growth curve over a 7-day interval in a time-kill assay

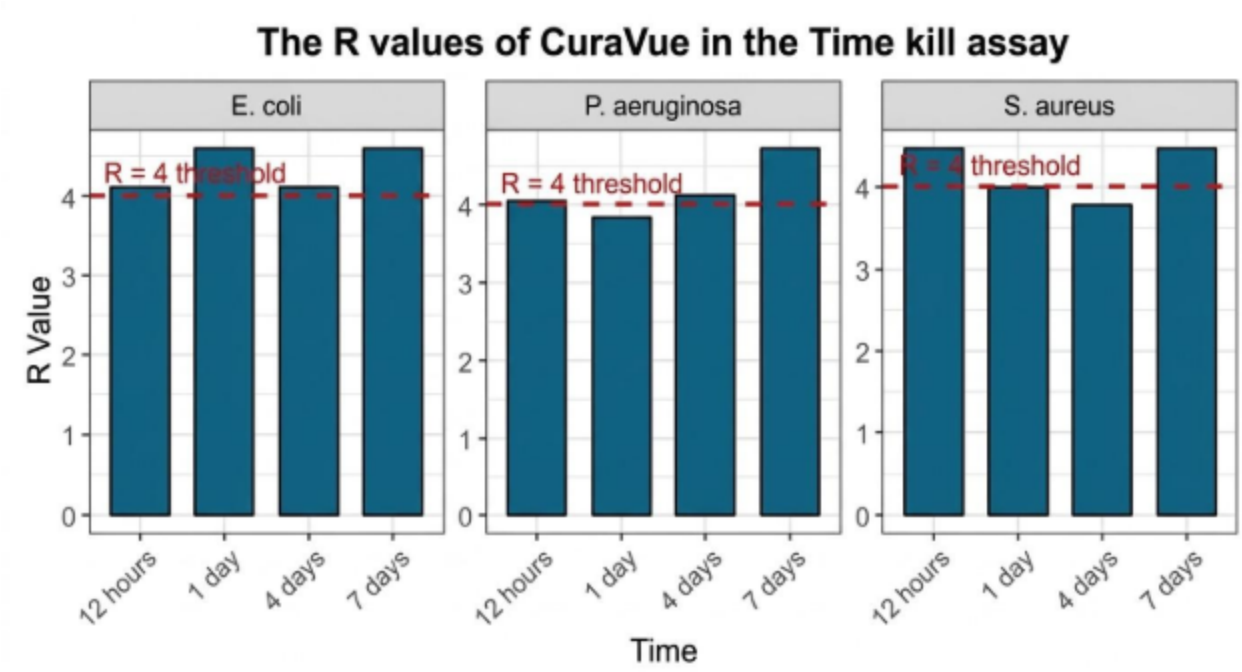


Figure 4

R values of the time-kill assay

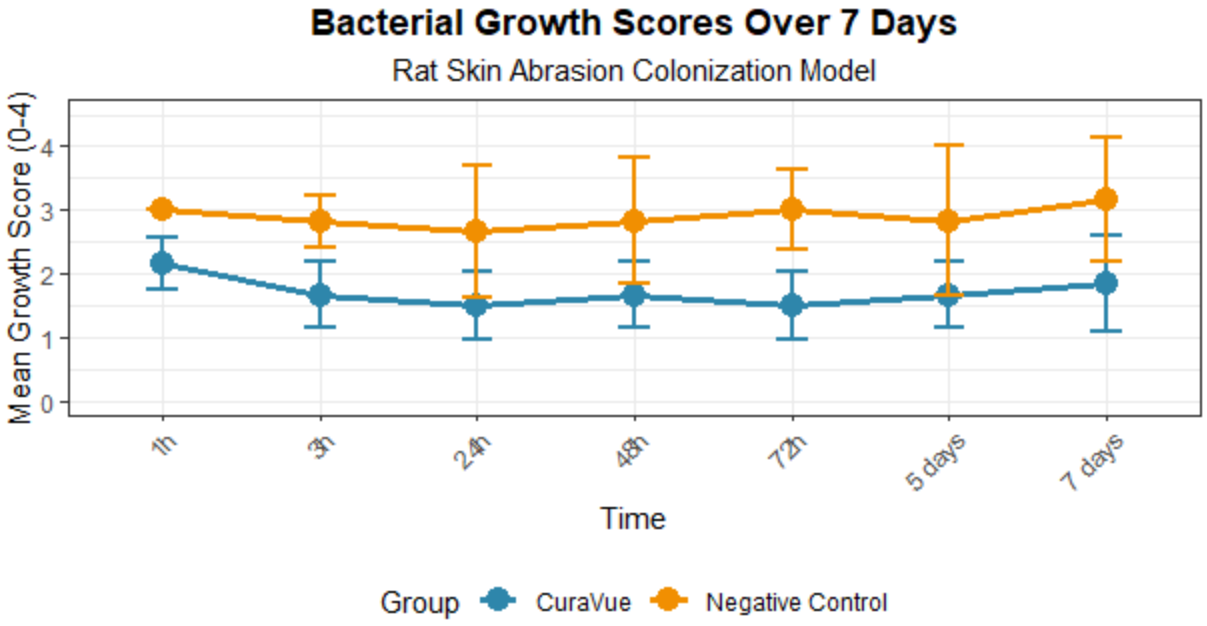


Figure 5

*Bacterial growth scores over 7 days. Data are presented as mean growth scores (0-4 scale; 0 = no growth, 4 = heavy growth) with error bars representing the standard error of the mean (SEM) for each group (n = 6 replicates per group). Negative Control (G1) showed consistently high growth scores throughout the experimental period. CuraVue® (G2) demonstrated significant antimicrobial activity, with low growth scores maintained from 1 hour through 7 days. Statistical analysis was performed using the Kruskal-Wallis test with Bonferroni-corrected post-hoc comparisons (*p < 0.05, **p < 0.01, ***p < 0.001 compared to Negative Control; p < 0.05 between treatment groups) [27].*

Supplementary Files

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- GraphicalAbstract.docx
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