

Effect on Microbial Growth of a New Skin Protectant Formulation

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Objective: Evaluate the effect of a new investigational skin protectant formulation on the growth of various microorganisms *in vitro*.

Approach: An *in vitro* laboratory assay with various species of gram-positive bacteria, gram-negative bacteria, and yeast grown on agar plates was used to verify that a new investigational product used for the management of incontinence-associated dermatitis (IAD) does not support microbial growth.

Results: The investigational product did not support the growth of all organisms tested for 48h in these assays. The results demonstrate the barrier properties of this investigational formulation against bacteria and yeast that are relevant to incontinent patients.

Innovation: IAD accompanied by skin damage is difficult to manage with currently available products. A new skin protectant that can be applied as a liquid and polymerizes into a breathable film *in situ* even in the presence of exudate (as shown previously) has been developed and tested to ensure that it does not support microbial growth.

Conclusion: This work verifies that this new product does not support microbial growth *in vitro* using organisms relevant for the intended application.

Keywords: skin protectant, incontinence-associated dermatitis, microbial growth, *in vitro*



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INTRODUCTION

INCONTINENCE-ASSOCIATED dermatitis (IAD) is a skin condition caused by the effects of urine and stool on the skin. Exposure to moisture and irritants can alter the skin pH and normal flora, and increase its susceptibility to damage from friction.^{1,2} Secondary infections often occur in IAD patients, given the susceptibility of moist and warm denuded skin; in fact, IAD is associated with an increased risk of localized cutaneous

infection.³ The most common organisms are *Candida albicans*, from the gastrointestinal tract, and *Staphylococcus* species, from the perineal skin.⁴ The treatment of IAD focuses on three main goals: (1) removing irritants from the skin and protecting it from further exposure to urine or stool, (2) eradicating cutaneous infection such as candidiasis, and (3) alleviating, containing, or diverting incontinence-related urine or stool.³ Products used to manage IAD should

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not promote the growth of microorganisms, and ideally form a barrier to prevent them from penetrating the weakened skin.

A new skin protectant was developed to manage moderate to severe IAD. It consists of a customized acrylic polymer, combined with 2-octyl cyanoacrylate. Cyanoacrylates are generally recognized as having antimicrobial properties.⁵ The formulation combines a cyanoacrylate with a polymer in a novel chemistry, and we wanted to verify that the property of not supporting microbial growth was preserved. The purpose of this study was therefore to evaluate the effect of the new skin protectant on the growth of various microorganisms in an *in vitro* assay.

CLINICAL PROBLEM ADDRESSED

The skin denudement observed in severe cases of IAD is particularly challenging to manage because it often presents as multiple small open areas of irregular shape over a contoured body surface. These open areas are exudative and standard barrier film products, ointments, and even many pastes do not adhere to the wet weeping surface. In cases of frequent or continuous incontinence, the damaged skin is constantly exposed to moisture and irritants such as urine and/or feces. The skin is at a high risk for further breakdown and maceration.⁶ The investigational product described herein is applied as a liquid to effectively cover the desired area and conform to the topography. It polymerizes *in situ* into a film within ~30 s even in the presence of exudate as previously shown in an animal model.⁷ This film is breathable and prevents irritants from reaching the skin surface. It remains intact even under conditions of continuous or repeated exposure. It provides an easy-to-clean surface after incontinence episodes and does not need to be reapplied each time. The results presented here demonstrate that in an *in vitro* assay, it does not support the growth of microorganisms relevant to incontinence patients.

MATERIALS AND METHODS

Description of new formulation

The investigational product is a solution based on a patented acrylate chemistry, which forms a durable, transparent elastomeric barrier upon application to skin. A customized acrylic polymer, combined with 2-octyl cyanoacrylate, creates the film structure. The film formers are delivered from a well-tolerated solvent. Prior testing done includes cytotoxicity, irritation, sensitization, genotoxicity, and systemic toxicity based on the criteria of expected use (>30 days in contact with a breached skin barrier) and guidance covering the biological evaluation of medical devices outlined in EN ISO 10993-1:2009. The results (not shown) supported a conclusion that the product is safe for its intended use. Additional testing in three animal models has also been performed.⁷

Microbial growth inhibition assay

In this study, the formulation was tested by forming a film covering a partial area of the agar in Petri dishes and seeding the agar with various microorganisms (10 bacterial species and 3 yeasts). Figure 1 illustrates the deposition of the film on the agar plates, and Table 1 lists the various organisms tested. The organisms were purchased as individual single-use KWIK-STIK™ devices from Microbiologics, Inc. (St. Cloud, MN), except for *Staphylococcus aureus* USA100 (clinical isolate from a previous study) and *Pseudomonas aeruginosa* PA-01 (gift from Dr. Gregory Schultz, University of Florida).

Sterile tryptic soy agar (TSA) and Sabouraud's dextrose agar (SDA) (Difco; Becton, Dickinson and Company, Sparks, MD) plates were poured in 90 × 15 mm polystyrene Petri plates and allowed to solidify. Plates were inverted and stored at room temperature for 24 h.

Pure culture stocks of all microbes were streaked onto TSA for bacteria and SDA for yeasts and grown overnight at 35°C. Broth cultures were prepared by

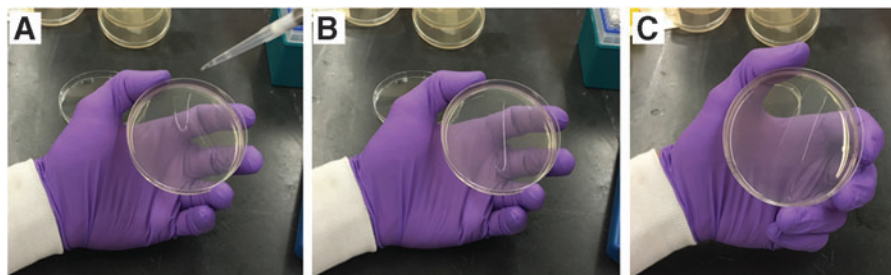


Figure 1. Application of investigational skin protectant to agar plates. (A) A pipette is used to deposit 100 μL of the investigational product in one spot at the edge of the agar plate. (B) The plate is tilted vertically to have the drop spread linearly across the plate. (C) The plate is tilted horizontally to have the solution spread to form a broader line.

Table 1. Microorganisms tested for growth inhibition by the investigational product

Microorganism	Comments
Gram-positive bacteria	
<i>Staphylococcus aureus</i> USA100 (clinical isolate from previous study)	MRSA, clinical isolate
<i>S. aureus</i> ATCC 33592	MRSA
<i>S. aureus</i> BAA-44	Multidrug resistant
<i>S. epidermidis</i> ATCC 35984	Biofilm former
<i>Enterococcus faecalis</i> ATCC 51299	VRE
<i>E. faecium</i> ATCC 700221	VRE
Gram-negative bacteria	
<i>Pseudomonas aeruginosa</i> BAA-2108	Multidrug resistant
<i>P. aeruginosa</i> PA-01 (from Dr. Gregory Schultz, University of Florida)	Biofilm former
<i>Acinetobacter baumannii</i> BAA-1605	Multidrug resistant
<i>Escherichia coli</i> BAA-2469	Multidrug resistant, NDM-1
<i>Klebsiella pneumoniae</i> BAA-2146	Multidrug resistant, NDM-1
<i>Enterobacter cloacae</i> ATCC 23355	Drug resistant
<i>Serratia marcescens</i> ATCC 14756	Reference strain
Yeast	
<i>Candida albicans</i> ATCC 10231	Reference strain
<i>C. tropicalis</i> ATCC 9968	Reference strain
<i>C. glabrata</i> ATCC 15545	Reference strain

NDM-1, New Delhi metallo-beta-lactamase multidrug-resistant strain; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*.

inoculating 5 mL of TSB with an isolated colony from the stock plates and incubated overnight at 35°C. The broth cultures were centrifuged to a pellet and resuspended in phosphate-buffered water (PBW). Suspensions of each organism were prepared using McFarland turbidity standards to a density of $\sim 10^8$ colony forming unit (cfu)/mL in PBW. Suspensions were diluted 1:1000 in PBW to a final concentration of $\sim 10^5$ cfu/mL.

The investigational product (100 μ L of solution) was pipetted and transferred to the top edge agar surface of the Petri plate while holding it at a slight angle. After the solution ran to the opposite edge, the plate was tilted to the right, which allowed the film to spread, creating an ~ 2 cm width band down the center of the plate (Fig. 1). Plates were allowed to dry in a biological safety cabinet with the lids slightly ajar for 15 min (normal dry time on skin is

about 30 s; additional time was allowed in this experiment to ensure full polymerization in this different environment). Two milliliters of the diluted bacteria (10^5 cfu/mL suspension) was pipetted onto the film. The plate was rotated until the entire surface had been contacted by the bacterial suspension. The plates were allowed to stand for 60 s, after which the remaining suspension was decanted from the plate. The plates were left in a biological safety cabinet with the lids slightly ajar for 10 min to allow any pooled inoculum to dissipate. The plates were then incubated inverted for 48 h at 35°C. Plates were observed at 24 and 48 h for growth. Every organism was run in duplicate.

Results were recorded and photos of each plate were taken. A successful result was indicated by a clear zone with no microbial growth where the investigational product was applied.

RESULTS

Every plate displayed a clear zone (no microbial growth) where the investigational product was applied, and a full microbial lawn on the rest of the plate, confirming viability and growth of the organism. Figure 2 shows control plates and Figure 3 shows representative samples for each of the various microorganisms.

DISCUSSION

Our results show that the new investigational skin protectant did not support the growth of several gram-positive organisms, gram-negative organisms, and yeasts in a laboratory assay over a period of 48 h.

As mentioned previously, various other formulations containing cyanoacrylates have been shown to provide a barrier to bacterial penetration. Bhende *et al.*⁸ used a laboratory assay with agar plates containing a pH indicator to indicate actively growing bacteria and showed that the cyanoacrylate tissue adhesive formulation tested (Dermabond[®], 2-octylcyanoacrylate) was an effective microbial

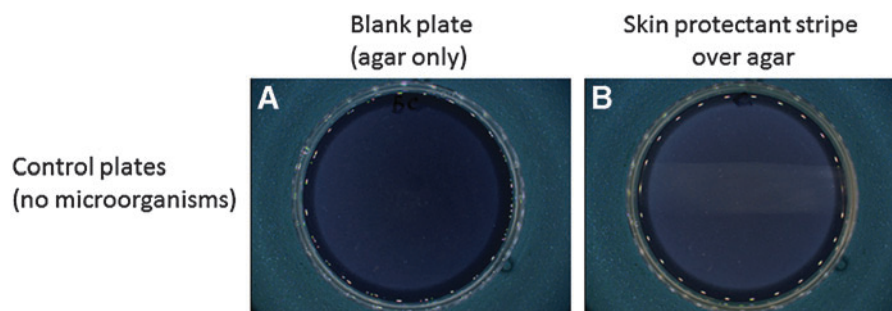


Figure 2. (A) Control plate with agar only. (B) Plate with a strip of skin protectant (a low level of haziness is inherent to the product).

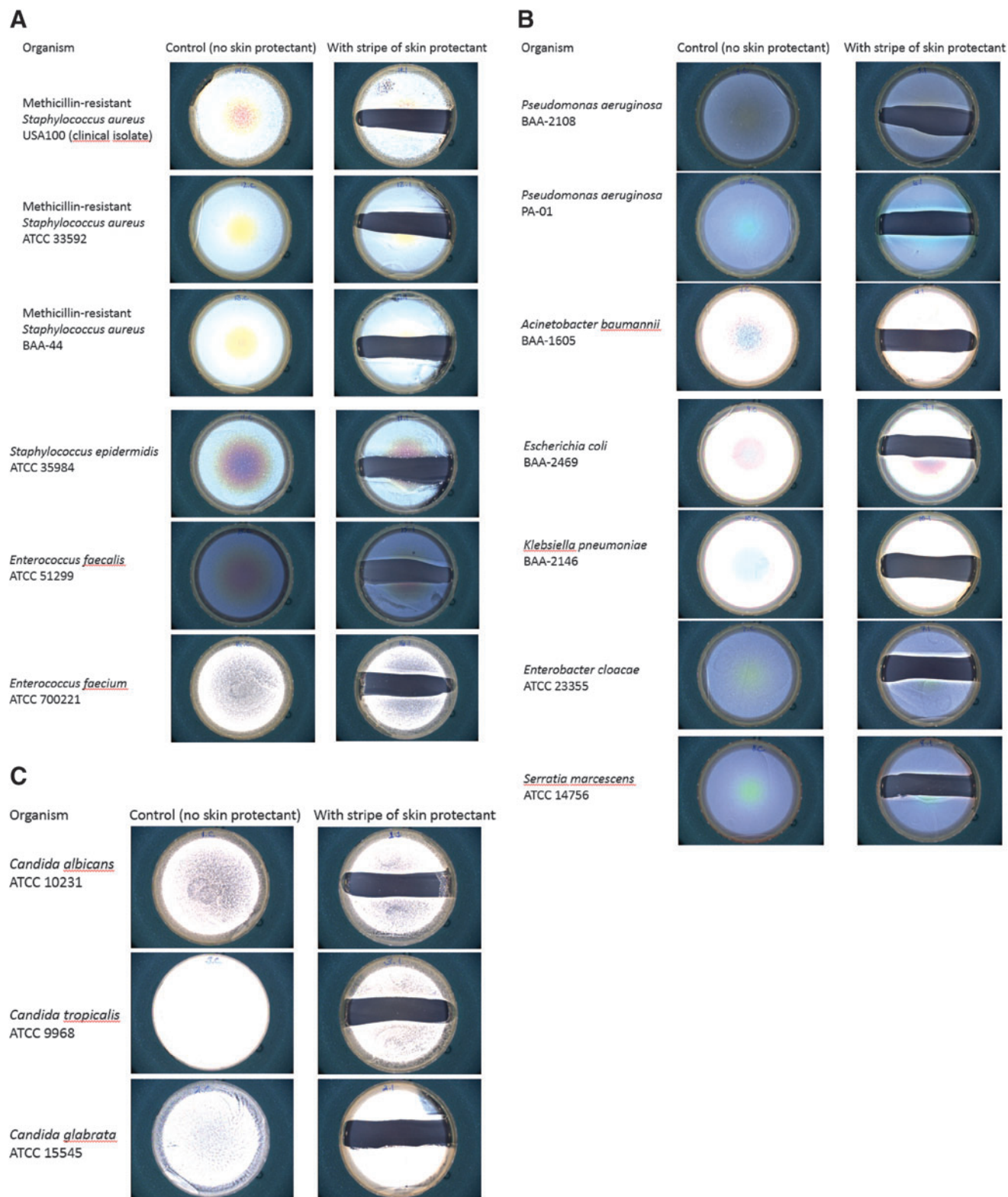


Figure 3. Agar plates after a 48-h incubation. *Left:* Plate seeded with microorganism. *Right:* Plate covered with a strip of investigational formulation and then seeded with microorganism. **(A)** Gram-positive organisms. **(B)** Gram-negative organisms. **(C)** Yeasts.

barrier. As they pointed out, this type of test is much more sensitive than an animal infection model, since penetration of one organism (1 cfu) would theoretically lead to a positive test, whereas logarithmically higher numbers of organisms would be required to establish an infection in an *in vivo* model due to the animals' inherent infection resistance/immune response. Narang *et al.*⁹ obtained similar results while performing their experiment in two different ways (also using Dermabond), either polymerizing the cyanoacrylate film directly on the agar as we did or using a preformed film in case the polymerization affected the agar (which it did not). Another agar plate method intended for a different application was used by Simonova *et al.*¹⁰ to test 2 cyanoacrylate adhesives (Dermabond, 2-octylcyanoacrylate; and Histoacryl[®], butylcyanoacrylate) for their ability to prevent the migration of microbes inoculated around catheters inserted in the agar at a 30° angle. Their results showed no visible growth under the cyanoacrylate and no penetration along the catheter tunnel. Romero *et al.*¹¹ have shown zones of inhibition *in vitro* for different cyanoacrylate chemistries (Superbonder[®], ethyl-cyanoacrylate; and Histoacryl, butylcyanoacrylate) for gram-positive bacteria, but not gram negatives. They hypothesize that the antibacterial effects of cyanoacrylate are greater in gram-positive bacteria than in gram-negative bacteria, possibly because the latter is protected by an outer carbohydrate capsule. Similar zone of inhibition results were also described by Friedman *et al.*¹² using Neucrylate[™] (1-hexyl-2-cyanoacrylate). On the contrary, Olson *et al.*¹³ showed *in vitro* adhesion of *S. epidermidis* to VetBond[™] (n-butyl-2-cyanoacrylate), followed by biofilm formation. Their method involved the circulation of medium containing bacteria with a peristaltic pump, which is different than the inoculation on Petri plates. This is, to our knowledge, the only publication describing actual bacterial growth on a cyanoacrylate, and our own results with a different strain of this biofilm former organism are contrary to those reported in that article. Matsumoto *et al.*¹⁴ have described the absence of bacteriostatic and bactericidal effects *in vitro* for individual monomers of n-butyl, isobutyl, and methyl-2 cyanoacrylate. Finally, Jang *et al.*¹⁵ have shown no *in vitro* antibacterial activity of octylcyanoacrylate against clinical strains of *P. aeruginosa* isolated from otitis media patients, which is consistent with other results discussed above regarding gram-negative bacteria. We believe that it is difficult to strictly compare *in vitro* results from different studies because the bacterial strains used were different, the inoculates were different, the volumes and chemical compositions of cyanoacry-

lates used were different, and mostly because the cyanoacrylate we describe here is a novel formulation combining other ingredients. In addition to these *in vitro* methods, pig models of partial-thickness wounds were used in which a cyanoacrylate-based formulation was applied to wounds either before (barrier study) or after (antimicrobial study) a bacterial inoculum challenge. The formulation protected wounds from external pathogens (compared to a first aid dressing and air exposed) and also created an environment that was not conducive to the growth of *S. aureus* and *P. aeruginosa* and was able to reduce the number of organisms recovered from the wounds compared to the other treatments.¹⁶ Another study has been done to look at the effect of using a cyanoacrylate product (InteguSeal[®], n-butyl cyanoacrylate) as a preoperative sealant in trauma surgery patients and found that this intervention reduced microbial contamination on sutures during surgery, without, however, changing overall wound contamination.¹⁷ Finally, Dohmen¹⁸ reviewed the literature on the impact of microbial skin sealants to prevent surgical site infections (SSIs) and concluded that a cyanoacrylate skin sealant can prohibit endogenous bacteria migration (by immobilizing any bacteria having survived the skin antiseptics) and actively reduce bacterial growth, making it an attractive option to reduce SSIs.

The ability of cyanoacrylate to immobilize bacteria could prove to be a helpful strategy to help prevent infections on wounded skin. Recent research has shown that many wound dressings dramatically reduce the antimicrobial activity of local wound antiseptics and wound irrigating agents (*in vitro* data).¹⁹ If the antiseptic properties of wound cleaning agents are also compromised during their clinical use because of possible negative interaction or interference with other wound care products, then an additional component of treatment, including bacterial immobilization, could potentially be beneficial to successfully fight infection.

This study has limitations. Agar is different from skin and may not reflect the clinical situation. The mechanism of action has not been addressed; however, one could speculate that since adherence of bacteria or fungi to target tissue is an important first step in infection, a cyanoacrylate product bound to skin could likely prevent infection by inhibiting the adhesion of microorganisms to the tissue and preventing deeper penetration in the tissue. Joost *et al.*²⁰ have shown that the extracellular adherence protein of *S. aureus* is more highly expressed in deep chronic wounds than in superficial wounds, supporting the hypothesis that adherence could contribute to the development of chronic infection. Microbial attachment is also recognized as the first

step in the establishment of biofilms, and chronic wounds have been shown to harbor biofilms.^{21,22}

The present *in vitro* results confirm that the new formulation does not support the growth of the microorganisms tested on its surface nor does it allow them to penetrate through it to reach the nutrient layer below. When combined with the other information gathered thus far on this investigational product, that is, biocompatibility testing (results not shown), animal testing,⁷ and clinical testing (manuscript accepted for publication), this novel skin protectant shows promise for the management of IAD.

INNOVATION

The innovative property of the investigational skin protectant presented here is that it can form a durable, long-lasting polymeric film *in situ* even in the presence of exudate.⁷ This film is breathable and capable of preventing irritants from reaching the skin surface. This study demonstrates that the film also does not support the growth of microorganisms on it and prevents them from penetrating through it to reach the nutrient layer below in an *in vitro* microbial culture assay.

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This work was done in the laboratories of 3M.

KEY FINDINGS

- The investigational skin protectant forms a film that does not support the growth of microorganisms.
- The investigational skin protectant forms a film barrier to microorganisms (bacteria and yeast) associated with incontinence.

AUTHOR DISCLOSURE AND GHOSTWRITING

J.S. and S.F.B. are employees of 3M. Both authors contributed to writing this article and no ghostwriters were used to write this article.

ABOUT THE AUTHORS

Joseph J. Stoffel, BS, in Microbiology from the University of Wisconsin–La Crosse. He currently resides in Minnesota and works for 3M. His work at 3M has included research and development in the area of advanced wound care, biofilm prevention, food safety, diagnostics, and inhalation drug delivery using *in vitro* and *in vivo* assays, clinical research, and analytical chemistry. **Stéphanie F. Bernatchez, PhD**, in Interdisciplinary Sciences from the University of Geneva, Switzerland. She currently resides in Minnesota and works for 3M. Her work at 3M has included research and development in the area of advanced wound care using *in vitro* and *in vivo* assays, as well as clinical research work.

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Abbreviations and Acronyms

- IAD = incontinence-associated dermatitis
 MRSA = methicillin-resistant *Staphylococcus aureus*
 PBW = phosphate-buffered water
 SDA = Sabouraud's dextrose agar
 SSIs = surgical site infections
 TSA = tryptic soy agar
 VRE = vancomycin-resistant *Enterococcus*