Clinical Utility of PNA-FISH for Burn Wound Diagnostics: A Noninvasive, Culture-Independent Technique for Rapid Identification of Pathogenic Organisms in Burn Wounds

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Burn injury results in an immediate compromised skin state, which puts the affected patient at an immediate risk for infection, including sepsis. For burn patients that develop infections, it is critical to rapidly identify the etiology so that an appropriate treatment can be administered. Current clinical standards rely heavily on culture-based methods for local and systemic infection testing, which can often take days to complete. While more advanced methods (ie, MALDI or NAAT) have improved turnaround times, they may still suffer from either the need for pure culture or sensitivity and specificity issues. Peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) offers a way to reduce this time from days to hours and provide species-specific identification. While PNA-FISH has had great utility in research, its use in clinical microbiology diagnostics has been minimal (including burn wound diagnostics). This work describes a nonculture-based identification technique using commercial available U.S. FDA-approved PNA-FISH probes for the identification of common clinical pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, present in burn wound infections. Additionally, calcofluor white was included for identification of *Candida albicans*. All three pathogens were identified from a tri-species infected deep-partial thickness rat burn wound model. These species were clearly identifiable in swab and tissue samples that were collected, with minimal autofluorescence from any species. Although autofluorescence of the tissue was present, it did not interfere or was otherwise minimized through sample preparation and analysis. The methodology developed was done so with patient care and diagnostic laboratories in mind that it might be easily transferred to the clinical setting.

Following burn injury, the primary barrier of the host, the skin, is immediately compromised, placing the patient at high risk for infection.[1–3] Some of the most common pathogens to infect burn patients include *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as the fungi, *Candida albicans*.[1,3–6] According to the U.S. Center for Disease Control and Prevention, the aforementioned pathogens are classified at a serious threat level to the public.[7] Without rapid diagnosis and treatment of an infected burn wound, the patient could face sepsis, which is one of the leading causes of death for burn victims with severe burns more than 40% total body surface area (50–84% mortality rate).[1,3] Regardless if the patient is septic, current methods for identification of a pathogen(s) in a wound or biological fluid may take days.[8–10] During this time, a burn patient may experience increased sequelae from wound infection, including becoming septic and/or succumb to septicemia, as mortality rates may increase by almost 8% per hour following diagnosis of sepsis.[11] Diagnostic techniques for assessing wounds, such as matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry and nucleic acid amplification tests (NAAT), have improved turn-around times, but still have limitations. MALDI-TOF provides faster results, but is still a culture-based technique that requires a pure culture.[12,13] NAAT offer a culture-independent method, but can suffer from sensitivity and specificity issues due to recognition of dead/live organisms, low starting volumes, easy contamination, and inhibitors of reactions.[14,15] As a means to abate sepsis (and other sequelae of infection), an apt diagnosis of etiologies present in the burn wound would allow for rapid diagnosis with greater specificity in treatment selection while reducing delays in treatment initiation. Ideally this would help reduce treatment times, hospital stays, medical costs, and, most importantly, mortality rates of burn patients.[16]

Peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) has been extensively utilized within research, but rarely applied in the clinical setting.[17] PNA-FISH provides a method of rapid identification of different microorganisms (including bacteria and fungi) at the genus and species level based

on ribosomal RNA targets.[10,17] This species-specific technique has been applied in the past to identify or characterize microorganisms in multiple scenarios, including multi-species biofilms, blood cultures (sepsis),[18,19] chronic wounds,[20] and respiratory tract infections.[21] A resistance profiling study demonstrated that PNA-FISH is capable of identifying antibiotic resistant-specific strains of microorganisms.[22] Furthermore, it is one of the only techniques to provide the location of species-specific organisms within tissue specimens.[23] Minimal utilization of PNA-FISH within the clinical community (including burn diagnostics) is largely due to inadequate standardization and/or commercialization of diagnosis methods.[17]

To help reduce the risk of sepsis and improve burn wound care management, we describe a rapid culture-independent technique for the isolation and identification of three clinical pathogens in infected burn wounds from an in vivo rat burn infection model utilizing PNA-FISH probes and calcofluor white (CW). Currently, the OpGen® AdvanDx PNA-FISH® bacterial kits applied in this study are approved by the U.S. Food and Drug Administration (FDA) for diagnostic use in blood culture analysis, but not at tissue level. CW is a common diagnostic microbiology staining technique, readily available in the clinical microbiology laboratory. Techniques described herein were developed to provide a simple, high-resolution, noninvasive, and rapid method for detecting and identifying pathogens commonly isolated from infected burn wounds.

METHODS

Bacterial and Fungal Strains

Bacterial strains *Pseudomonas aeruginosa* (12-4-4(59))[24] and *Staphylococcus aureus* (TCH 1516), and fungal strain *Candida albicans* (MYA 2876) were utilized in this study. For planktonic cultures, strains were cultured overnight in Luria-Bertani broth at 35°C with shaking at 300 rpm. For the in vivo infection study, cells were cultured at 37°C overnight in Tryptic Soy Broth and then subcultured to mid-log phase before inoculation onto the burn wound.

Ethics Statement and Animal Housing

Male Sprague-Dawley rats (*Rattus norvegicus*) age 3 to 6 months and weighing approximately 380 to 420 g were allowed to acclimate to the research facilities for a minimum of 1 week and provided access to food and water ad libitum. Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study (IACUC number A17-031). The facility where this research was conducted is fully accredited by AAALAC International.

Planktonic Cell Preparation and Detection

Overnight planktonic cell cultures (approximately 18 h growth) were prepared with OpGen® AdvanDx QuickFISH® *S. aureus*/CNS PNA FISH® (Cat. No. KT005) and GNR Traffic Light® PNA FISH® kits (contains *P. aeruginosa* probe; Cat. No. KT011) based on manufacturer's instructions. These

kits are reported by the manufacturer to have a sensitivity of 10^5 colony forming units per mL, based on blood culture analysis. Briefly, all microorganisms were diluted 1:10 into a single suspension with 1× phosphate buffered saline (PBS). Glass slides were initially flamed and allowed to cool before adding 10 μl of suspension, along with a single drop of AdvanDx GN Fixative Solution. Slides were fixed at 55°C for 20 min. After fixation, a single drop of each probe was added and smeared across the sample with a sterile pipette tip to ensure complete coverage. Hybridization was achieved at 55°C for 30 minutes and then slides were washed in AdvanDx Wash Solution at 55°C for an additional 30 minutes. Slides were removed from wash, air-dried, and then coverslip (24 mm × 50 mm) placed after addition of 10 μL of CW (ie, Fluorescent Bright 28, Sigma–Aldrich Cat. No. F3543, 1 mg/ml 1:1 Methanol:Water) and AdvanDx Mounting Medium.

In vivo Tri-species Burn Wound Infection and Sample Collection

In vivo samples were collected from infected burn wounds of male Sprague-Dawley (*n* = 3) rats using a previously described model of deep-partial thickness burn infection that was modified to investigate a tri-species infection.[25] In brief, a 10% total body surface area burn (based on Meeh's formula)[26] was induced on the dorsum of anesthetized rats, followed by immediate surface inoculation of 10^5 CFU/wound of each microorganism (*S. aureus*, *P. aeruginosa*, and *C. albicans*; grown to mid-log state) suspended in 1× PBS. On day 3 post-injury, the rats were euthanized and the wound was excised and subjected to biopsy punches, tissue sectioning (histology), and complete swabbing of the entire remaining wound bed as part of sample collection approach.

TRI-SPECIES BURN WOUND SWAB SAMPLE COLLECTION AND PATHOGEN DETECTION

A Puritan® Opti-Swab™ Liquid Amies Collection & Transport System equipped with a large HydraFlock® swab (Puritan Diagnostics, Puritan Medical Products Co. LLC, Ref LA-106) was utilized to collect organisms from the wound bed. Following the collection of tissue sections and biopsy punches, the swab was wetted with the provided Amies-medium in the transport tube, expressed on the tube wall to remove excess liquid within tube, and then swabbed across the entire remaining wound bed, including biopsied sites, using the Z-swab method.[27] Care was taken not to collect any large tissue debris, which can negatively affect assay specificity and sensitivity.

Upon completion of swabbing, the swab was returned to the transport tube, sealed, and pulsed briefly, followed by continuous vortexing for 5 seconds at 2500 rpm. Approximately 200 to 500 μl of vortexed sample was added to an EZ Single Cytofunnel® containing White Filter cards (Thermo Scientific, Ref. No. A7871003) containing a blank glass slide. Samples were cytospun at 2000 rpm for 8 minutes at room temperature using the high acceleration setting on a Thermo Scientific Shandon Cytospin® 4. Speeds and times were based on the instrument manual's recommendations for bacteria.

Slides were then carefully removed from funnels and samples were fixed with one drop of GN Fixative Solution at 55°C

for 20 minutes. Before hybridization, samples were emulsified with the fixative to help with both fixation and imaging of the sample. Following fixation, samples were stained, washed, and coverslip placed with CW and mounting media, as previously discussed for the planktonic cells (above).

HISTOLOGICAL PROCESSING AND APPLICATION OF PNA-FISH PROBES AND CW STAIN

Histological cross-sections of the wound bed were obtained and immediately fixed in 10% buffered formalin in PBS for a minimum of 48 hours before being processed and embedded in paraffin wax. Tissue sections of 4 μm were found to be ideal for PNA-FISH probes. Tissue sections were deparaffinized in three changes of xylene for 5 minutes each change, rinsed with distilled water, and fixed with one drop of GN Fixative Solution for 20 minutes at 55°C. After fixation, one drop of GNR Traffic Light and one drop of *S. aureus* PNA-FISH probes were placed on each tissue section. A coverslip was placed on each sample to diffuse probe across the sample surface, while minimizing air bubbles formation. Sections were hybridized for 45 minutes at 55°C and then the coverslip removed while slides incubated in 1× Wash Solution (AdvanDx) for 1 to 2 minutes. Samples were then washed without agitation for an additional 45 minutes at 55°C in 1× Wash Solution. Following the final wash, coverslips were placed over the tissue samples with CW and mounting media as described for the planktonic cell samples (above).

IMAGING OF PNA-FISH STAINED SAMPLES

All samples were imaged using a Leica DM6 fluorescent microscope and CCD camera (Leica DFC365 FX). The fluorescent microscope was equipped with standard Leica filter sets with the following names and excitation/emission wavelength ranges: DAPI (ex 325–375, em 435–485), GFP (ex 450–490, em 500–550), and Texas Red (ex 540–580, em 592–668). The objectives used in this study included a 10× HC PL APO, 0.40 NA; 40× ACS APO Oil, 1.15 NA; and a 63× ACS APO Oil, 1.3 NA. All images were acquired with the same imaging settings for each channel including exposure time and fluorescence light intensity. Images were adjusted for optimal brightness and contrast using Leica software LASX and ImageJ (NIH). Images for individual figures were adjusted with the same settings. Figures were prepared with Microsoft PowerPoint 2013 and GraphPad Prism 7.

RESULTS

Specificity and Sensitivity of PNA-FISH Probes and CW Stain on a Tri-Species Suspension of Clinically Relevant Pathogens

Before analysis of in vivo samples, the selected PNA-FISH probes and CW stain were validated on a planktonic tri-species suspension to ensure specificity and sensitivity of the method. PNA-FISH probes demonstrated specificity toward their respected analytes; *P. aeruginosa* and *S. aureus* as seen in the red and green channels, respectively (Figure 1A). *Candida*

albicans cells were successfully identified with the CW stain, which provided clear visualization of the budding yeasts in the blue channel. Ill-defined ovals larger than *S. aureus* were also seen in the green channel (see arrowhead, Figure 1A); however, an overlay of the channels revealed these to be the *C. albicans* yeast. Unstained slides of monospecies cultures showed minor autofluorescence from *S. aureus* and *C. albicans*, but not *P. aeruginosa*, in the green channel only (Figure 1B). Autofluorescence was negligible in the red and blue channels for all pathogens (data not shown).

Identification of Pathogens from Swab Samples of Infected Burn Wound

After establishing a detection method for planktonic cultures of the pathogens, multiplexing of the PNA-FISH probes and CW stain was tested on swabbed specimens collected from a rat burn wound that was infected with all three pathogens 3 days before collection. As shown in Figure 2, all three pathogens were successfully collected and identified from the swab specimens. In the red channel, *P. aeruginosa* rods are clearly visualized without any artifacts. *Staphylococcus aureus* was readily distinguishable in the green channel from autofluorescing tissue debris. Though tissue debris was also seen in the blue channel, *C. albicans* was readily visualized in both budding and hyphal forms. Turnaround time was 2.5 to 3 hours from receipt of specimens.

Observation of Pathogenic Organisms in Histological Sections

Histological sections of the burn wound were stained to assess the feasibility for off-label use. Unstained overlays of the blue channel with the red (Figure 3A) and green (Figure 3C) channels revealed notable autofluorescence of the tissue in the red and green channels. Within the unstained tissue there were also notable voids in the red and green channels that were comparable in shape and size to *C. albicans* seen in the blue channel of stained tissue. Following staining, the tissue components become significantly more visible in the blue channel (Figure 3 and Supplementary Figure S1), as compared with the unstained sections. Fluorescence in the red and green channels was increased slightly following staining, most notably in the actual burn tissue (see Supplementary Figure S1 for individual channels of stained and unstained histological sections).

All three pathogens were visualized in several areas of the examined wound tissue. *Pseudomonas aeruginosa* (red) and *C. albicans* (blue) were seen congregated together below the surface of the skin (Figure 3B). An extensive layer of *C. albicans* was also seen across the wound surface, in proximity to several clusters of *S. aureus* (green) on top (Figure 3D). In these regions, minimal dermal tissue was noted along the surface, either due to lack of staining or presence. *Candida albicans* was detected as clusters of hyphae, along with potential chlamydospores.

DISCUSSION

When clinicians are presented with a burn patient, one of their biggest challenges is to prevent and control infection if it

A

B
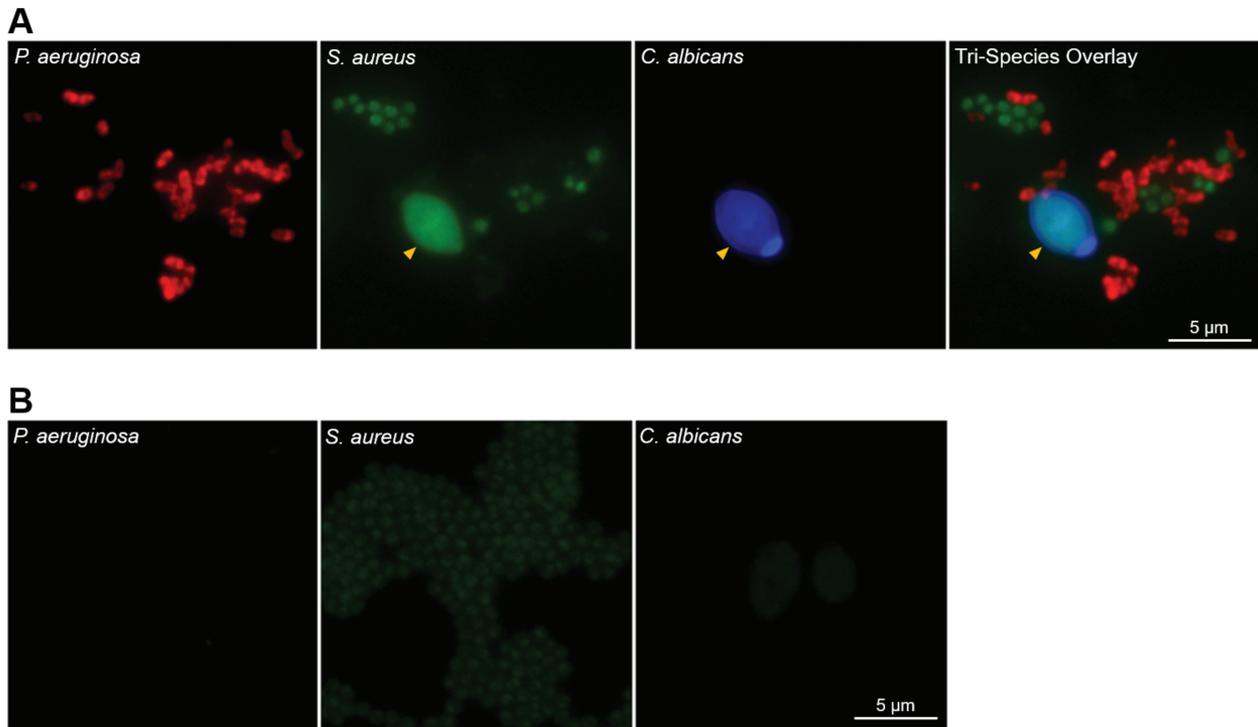
Figure 1. Validation of multiplexed PNA-FISH and CW on planktonic cell cultures. A. Tri-species planktonic cell cultures were stained to both validate the procedure and serve as controls for in vivo studies. The PNA-FISH probes showed specificity toward their respective bacteria. CW was specific to *Candida albicans* (blue); however, it also increased the fluorescence of the fungi in the green channel (arrowhead). B. Unstained monospecies cultures demonstrated faint autofluorescence of both Staphylococcus *aureus* and *C. albicans* in the green channel; however, no autofluorescence was seen from *Pseudomonas aeruginosa* in any channel. All images were taken with 63× objective.
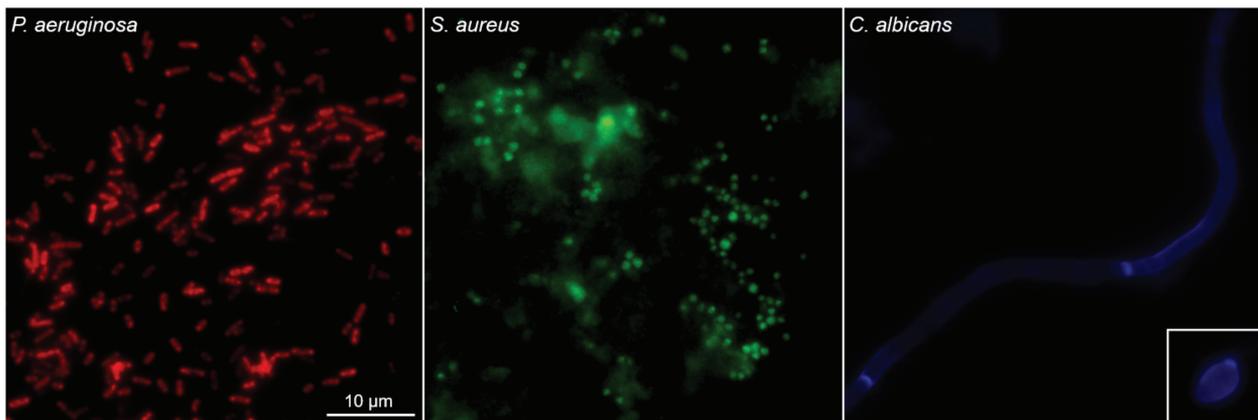
Figure 2. Pathogenic organisms identified from swabs of partial-thickness burn wounds. Each panel represents an individual channel and location for visualization of the microorganisms. *Pseudomonas aeruginosa* was clearly seen in the red channel as bright clusters of rods with minimal staining of the tissue debris. In the green channel, there were several clusters of *Staphylococcus aureus* that could be distinguished from the autofluorescent nature of the tissue debris. Tissue debris autofluorescence was also seen in the blue channel (not shown in panel), but *Candida albicans* was still clearly identifiable in both hyphae and bud form (inset, same scale). All images were taken with 63× objective. Single scale bar applies to all panels.

occurs and, more specifically, sepsis. Therefore, early detection of infectious agents is critically important for the care of burn patients, especially for individuals suffering from large body surface area burns. Here, we adapted PNA-FISH kits that are FDA-approved for blood culture analysis as a rapid method for culture-independent, wound swab and tissue analysis. This approach expands the utility of the PNA-FISH technique as a diagnostic tool for prevention and treatment toward burn wound infections. The ability to rapidly detect pathogens

present in a burn wound using a noninvasive swab or histological specimen could allow clinicians to provide a more rapid diagnosis with a more individualized treatment plan.

The PNA-FISH probes, as well as the CW stain, were shown to be effective and specific at labeling all three pathogens before ex vivo testing. Unstained control slides revealed autofluorescence in only the green channel for *S. aureus* and *C. albicans*, which has been demonstrated previously by other groups.[28,29] However, this natural fluorescence was not
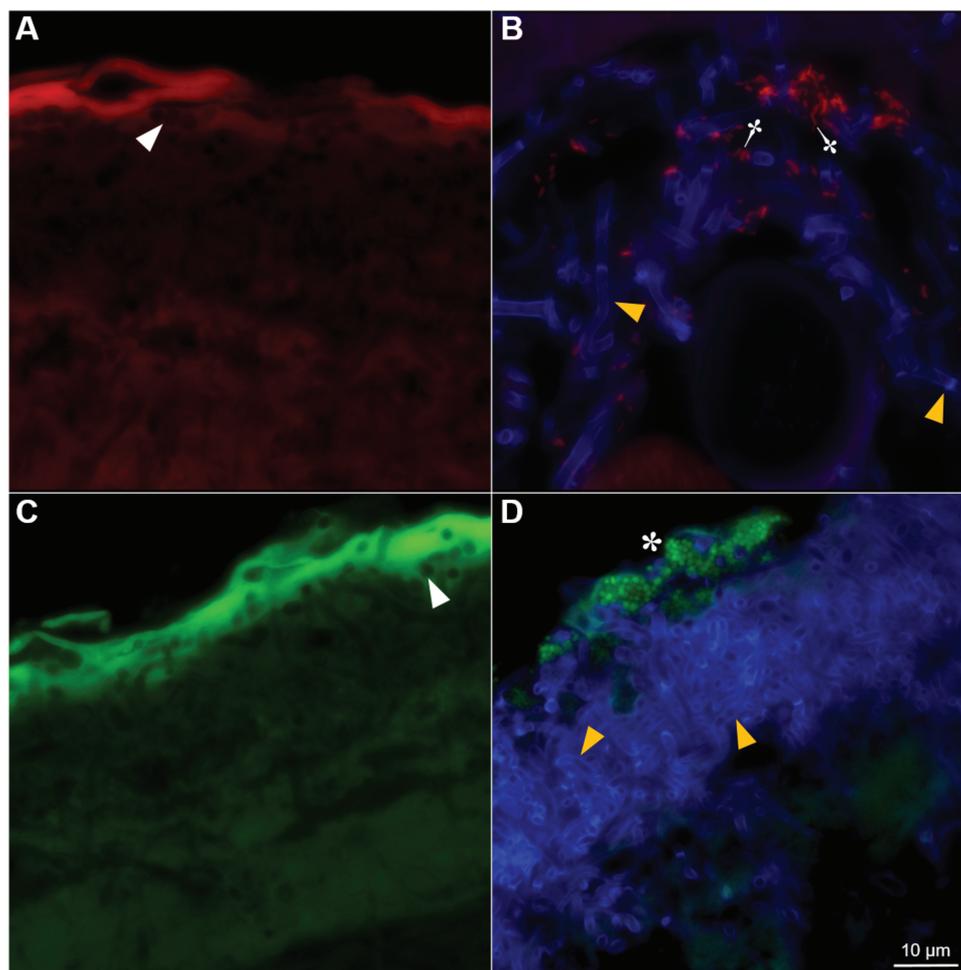
Figure 3. PNA-FISH and CW treatment of histological sections reveals pathogenic niches throughout the burn wound. Overlays of the blue channel with the red (A, B) and green (C, D) channels for unstained (A, C) and stained (B, D) tissue. Unstained sections showed autofluorescence in the red and green channels, as wells as voids that resembled *Candida albicans* (white arrowheads), which could be seen throughout the stained sections (yellow arrowheads). Red *Pseudomonas aeruginosa* rods (B, dagger) and green *Staphylococcus aureus* cocci (D, asterisk) were also seen intermingled with *C. albicans*. All images were taken with 63× objective.

enough for identification in complex scenarios (ie, multispecies) due to limited brightness and ill-defined morphologies. This optimization step served to not only validate the performance of the probes/stain, but also to provide a control slide for sample analysis. In the clinical setting, this control slide would serve as an important reference for routine diagnostic testing of burn samples.

Combining a swab sample with the multiplex probe/staining technique used in this study made it possible to successfully differentiate three pathogens within an infected burn wound. Based on morphology and the PNA-FISH probes, *P. aeruginosa* and *S. aureus* cells were easily identified in the red and green channels, respectively. Adding the CW stain improved the overall sensitivity of the assay for pathogens via detection of *C. albicans*. It should be noted that while this technique provides a simple and quick method for identification of pathogenic organisms, it is important to minimize tissue debris during the preparation of the wound sample in order to provide the best results for down-stream screening. Initial attempts on lysates from homogenized biopsy punches resulted in large amounts of tissue debris that clogged the

cytospin funnels. Not only did this result in more background fluorescence, but it also minimized sample deposition onto the slide, rendering it unfit for pathogen identification. Brief centrifugation could remove the debris; however, this could result in pelleting and removal of the larger fungal elements along with the debris. By contrast, the swab technique provides a less invasive method of sample acquisition that minimizes tissue debris while offering improved total wound coverage (compared with a biased single biopsied area). Autofluorescence from debris (ie, tissue) can be further minimized by properly emulsifying and spreading the sample across the slide (postcytospin) to provide optimal staining and imaging of the sample.

In addition to swab samples, tissue sections from the burn wound were also collected and treated with PNA-FISH probes and CW stain to determine if the multiplex technique could be applied to intact burn tissue. All three pathogens were discovered along the surface of the wound and in some cases penetrating deeper into the tissue. *Pseudomonas aeruginosa* and *C. albicans* were readily identified due to the increased fluorescent intensities of the probe and stain, respectively, over

the background intensity. *Pseudomonas aeruginosa* was found scattered throughout the wound, penetrating into the tissue. *Candida albicans* was mostly found in the hyphal form (as seen with the swab isolates), which were seen as thick layers near the surface or as single hyphae penetrating deeper into the tissue. *Staphylococcus aureus* was the most difficult to identify within the intact tissue sections due to its size and weaker fluorescent intensity of the probe; however, it could be found in several clusters throughout the tissue. While PNA-FISH staining techniques for tissue have been utilized by many groups in the research field, it is not used extensively in the clinical community.[25,30–32] Limitations to this type of sampling include burn wound integrity, as well as slower turnaround times (about 1–2 days), which are both mitigated by the swab technique. However, if it is possible to collect an intact sample, it may provide further insights into not only the types of pathogens present, but also the depth (ie, tissue penetration) and degree of the infection. For the clinical community, this information could alter treatments based on identification and locations of individual pathogens.

Although the assay presented here demonstrated success in identifying several pathogens, there are some limitations which could be improved upon in the future. As mentioned above, the green channel presented a few challenges with autofluorescence of some pathogens and tissue. This could be overcome with another fluorescent probe that has limited auto-fluorescence in its respective channel. PNA-FISH blood cultures kits that test for *C. albicans* are available from OpGen®; however, identification was also based on a green probe and these fungal kits have yet to meet U.S. FDA approval. From a clinical and treatment standpoint, it is important to identify fungal presence initially with CW so that further testing for species differentiation can be done. Although not able to differentiate fungal species, CW provides a quick initial screen for presence of fungal organisms and does not interfere with PNA-FISH identification of the bacteria, as confirmed by others.[33] For future research and potential clinical use, kits could be assembled to provide color panels for bacterial and fungal identification within burn wound (or other) tissue, as is currently available for blood culture samples through companies like OpGen®. These kits could be potentially designed with probe combinations that are more reflective of pathogens faced in different clinical wound settings.

PNA-FISH is not a new technique for the scientific research community, but bridging the gap to actual clinical use has not been very successful. The use of similar techniques using clinical samples have been previously reported, but usually limited to research and typically in a fashion not amenable to clinics due to cost or logistics (ie, confocal microscopy). The rapid diagnostic methods described herein could be easily transferred to the clinic for pathogenic detection at the species' level and assessment of burn wounds with the hope of abating sepsis through more accurate treatment strategies.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Burn Care & Research* online.

REFERENCES

1. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev 2006;19:403–34.
2. Kennedy P, Brammah S, Wills E. Burns, biofilm and a new appraisal of burn wound sepsis. Burns 2010;36:49–56.
3. Nunez Lopez O, Cambiaso-Daniel J, Branski LK, Norbury WB, Herndon DN. Predicting and managing sepsis in burn patients: current perspectives. Ther Clin Risk Manag 2017;13:1107–17.
4. Tredget EE, Shankowsky HA, Rennie R, Burrell RE, Logsetty S. Pseudomonas infections in the thermally injured patient. Burns 2004;30:3–26.
5. Erol O, Simavlı S, Derbent AU, Ayrım A, Kafalı H. The impact of copper-containing and levonorgestrel-releasing intrauterine contraceptives on cervicovaginal cytology and microbiological flora: a prospective study. Eur J Contracept Reprod Health Care 2014;19:187–93.
6. Santucci SG, Gobara S, Santos CR, Fontana C, Levin AS. Infections in a burn intensive care unit: experience of seven years. J Hosp Infect 2003;53:6–13.
7. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013. CDC, Editor. Atlanta; 2013.
8. Dowd SE, Sun Y, Secor PR, et al. Survey of bacterial diversity in chronic wounds using Pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol, 2008;8:43.
9. Vila J, Gómez MD, Salavert M, Bosch J. Methods of rapid diagnosis in clinical microbiology: clinical needs. Enferm Infecc Microbiol Clin 2017;35:41–6.
10. Harris DM, Hata DJ. Rapid identification of bacteria and Candida using PNA-FISH from blood and peritoneal fluid cultures: a retrospective clinical study. Ann Clin Microbiol Antimicrob 2013;12:2.
11. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589–96.
12. Stevenson LG, Drake SK, Murray PR. Rapid identification of bacteria in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2010;48:444–7.
13. La Scola B, Raoult D. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One 2009;4:e8041.
14. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis 2004;4:751–60.
15. Vaneechoutte M, Van Eldere J. The possibilities and limitations of nucleic acid amplification technology in diagnostic microbiology. J Med Microbiol 1997;46:188–94.
16. Bauer KA, Perez KK, Forrest GN, Goff DA. Review of rapid diagnostic tests used by antimicrobial stewardship programs. Clin Infect Dis 2014;59 Suppl 3:S134–45.
17. Frickmann H, Zautner AE, Moter A, et al. Fluorescence in situ hybridization (FISH) in the microbiological diagnostic routine laboratory: a review. Crit Rev Microbiol 2017;43:263–93.
18. Søgaard M, Stender H, Schønheyder HC. Direct identification of major blood culture pathogens, including Pseudomonas aeruginosa and Escherichia coli, by a panel of fluorescence in situ hybridization assays using peptide nucleic acid probes. J Clin Microbiol 2005;43:1947–9.
19. Peleg AY, Tilahun Y, Fiandaca MJ, et al. Utility of peptide nucleic acid fluorescence in situ hybridization for rapid detection of Acinetobacter spp. and Pseudomonas aeruginosa. J Clin Microbiol 2009;47:830–2.
20. Rhoads DD, Wolcott RD, Sun Y, Dowd SE. Comparison of culture and molecular identification of bacteria in chronic wounds. Int J Mol Sci 2012;13:2535–50.
21. Rudkjøbing VB, Thomsen TR, Alhede M, et al. The microorganisms in chronically infected end-stage and non-end-stage cystic fibrosis patients. FEMS Immunol Med Microbiol 2012;65:236–44.
22. Werner G, Bartel M, Wellinghausen N, et al. Detection of mutations conferring resistance to linezolid in Enterococcus spp. by fluorescence in situ hybridization. J Clin Microbiol 2007;45:3421–3.
23. Petrich A, Rojas P, Schulze J, et al. Fluorescence in situ hybridization for the identification of Treponema pallidum in tissue sections. Int J Med Microbiol 2015;305:709–18.
24. Karna SL, Chen T, Chen P, et al. Genome sequence of a virulent Pseudomonas aeruginosa strain, 12-4-4(59), isolated from the blood culture of a burn patient. Genome Announc 2016;4:e00079–16.

25. Brandenburg KS, Weaver AJ Jr, Qian L, et al. Development of Pseudomonas aeruginosa biofilms in partial-thickness burn wounds using a Sprague-Dawley rat model. J Burn Care Res 2019;40:44–57.

26. Gilpin DA. Calculation of a new Meeh constant and experimental determination of burn size. Burns 1996;22:607–11.

27. Dow G, Browne A, Sibbald RG. Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy Wound Manage 1999;45:23–7, 29.

28. Graus MS, Neumann AK, Timlin JA. Hyperspectral fluorescence microscopy detects autofluorescent factors that can be exploited as a diagnostic method for Candida species differentiation. J Biomed Opt 2017;22:16002.

29. de Jong NW, van der Horst T, van Strijp JA, Nijland R. Fluorescent reporters for markerless genomic integration in Staphylococcus aureus. Sci Rep 2017;7:43889.

30. Lefmann M, Schweickert B, Buchholz P, et al. Evaluation of peptide nucleic acid-fluorescence in situ hybridization for identification of clinically relevant mycobacteria in clinical specimens and tissue sections. J Clin Microbiol 2006;44:3760–3767.

31. Kim DH, Hong YK, Egholm M, Strauss WM. Non-disruptive PNA-FISH protocol for formalin-fixed and paraffin-embedded tissue sections. Biotechniques 2001;31:472, 475–2, 476.

32. Cerqueira L, Fernandes RM, Ferreira RM, et al. Validation of a fluorescence in situ hybridization method using peptide nucleic acid probes for detection of Helicobacter pylori clarithromycin resistance in gastric biopsy specimens. J Clin Microbiol 2013;51:1887–1893.

33. Rasconi S, Jobard M, Jouve L, Sime-Ngando T. Use of calcofluor white for detection, identification, and quantification of phytoplanktonic fungal parasites. Appl Environ Microbiol 2009;75:2545–53.