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Diagnostics for Wound Infections

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Abstract

Significance: Infections can significantly delay the healing process in chronic wounds placing an enormous economic burden on health care resources. Identification of infection biomarkers and imaging modalities to observe and quantify them has seen progress over the years.

Recent Advances: Traditionally, clinicians determine the presence of infection through visual observation of wounds and confirm their diagnosis through wound culture. Many laboratory markers including white blood cell count, erythrocyte sedimentation rate, C-reactive protein, procalcitonin, presepsin, and bacterial protease activity have been quantified to assist diagnosis of infection. Moreover, imaging modalities like plain radiography, computed tomography, magnetic resonance imaging, ultrasound imaging, spatial frequency domain imaging, thermography, autofluorescence imaging, and biosensors, have emerged for real-time wound infection diagnosis and showed their unique advantages in deeper wound infection diagnosis.

Critical Issues: While traditional diagnostic approaches provide valuable information, they are time-consuming and depend on clinicians' experience. There is a need for non-invasive wound infection diagnostics that are highly specific, rapid, accurate and do not require extensive training.

Future Directions: While innovative diagnostics utilizing various imaging instrumentation are being developed, new biomarkers have been investigated as potential indicators for wound infection. Products may be developed to either qualitatively or quantitatively measure these biomarkers. This review summarizes and compares all available diagnostics for wound infection including those currently used in clinics and still under development. This review could serve as a valuable resource for clinicians treating wound infections as well as patients and wound care providers who would like to be informed of the recent developments.

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1.0 SCOPE AND SIGNIFICANCE

Through decades of research and development, tremendous improvements have been made in the efficiency and accuracy of wound infection diagnostics. In this review, we provide a comprehensive view on different aspects of wound infection diagnostics, ranges from approaches that are actively used in the clinic to innovative studies that are undergoing laboratory development, from clinical biological assay to imaging modalities as well as sensor-based instrumentation. The ultimate goal of the review is to provide both wound care providers and patients a better evaluation of available wound infection diagnostics.

2.0 TRANSLATIONAL RELEVANCE

Many new technologies have been created in recent years to diagnose infected wounds [1, 2]. In addition to physiological assays, traditional imaging modalities such as radiography and magnetic resonance imaging, as well as new hybrid imaging techniques including single photon emission computed tomography/computed tomography and positron emission tomography/magnetic resonance imaging, are being utilized as wound infection diagnostic tools [3]. Innovative imaging approaches such as ultrasonography, thermography, and multispectral imaging have gained prominence to fulfill the need for fast, cost-effective and accurate diagnosis of wound infection [4], though they have been developed for research use only.

3.0 CLINICAL RELEVANCE

Wound infections encumber not only the patients but also the healthcare systems worldwide that provide care for the patients [5]. While existing tools and techniques have provided reasonable success in wound infection management, emerging diagnostic instruments and technologies hold tremendous potential in the clinical management of wounds. There is great potential in using these techniques to provide additional information about wound infection diagnosis and direct further treatment plans. Insight into these diagnostics can improve the accuracy of clinical validation in light of the wide diversity of wound types, anatomic locations and tissue involved.

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4.0 BACKGROUND

Chronic wounds affect approximately 20 million individuals worldwide and annual cost for their treatment and management is estimated to be over $31 billion [5-7]. Many factors contribute to delay in the wound healing process including chronic metabolic disease (diabetes), neurological defect, vascular insufficiency, nutritional deficiency, aging and infection [8, 9]. Among these, infection is a major factor that delays the wound healing rate and requires immediate treatment. A wound is characterized as a chronic wound when it does not progress through the normal stages of healing within three months or relapses.

There are three stages in the wound infection continuum namely contamination, colonization and infection [10]. Wound contamination is the presence of non-replicating microbes in an open wound. The presence of small numbers of microbes do not affect normal inflammatory responses and wound healing processes [11]. However, microbial replication causes wound colonization. The increased number and persistent presence of microbes can prolong the inflammatory phase of wound healing and lead to further tissue damage [12]. When the microbes migrate deep into the wound bed and reproduce rapidly, they can trigger either a local or systemic immune reaction with the characteristics of infection [13]. Furthermore, some microbes can produce a complex protective glycocalyx–also called biofilm–which makes the infected wounds hard to be detected and treated [14]. The presence of biofilm is a hallmark of chronic wounds and most chronic wounds possess biofilms [15]. Certain microbial phenotypes enhance inflammation causing further tissue damage and protect from antimicrobial therapy as well as the immune system [16, 17]. Within less than 24 hours of infection [18], the invading microbes can skew early innate immune responses to allow the establishment of the biofilm phenotype [19]. With further progressing, the wound infection may jump from topical wounds to systemic complications such as cellulitis, osteomyelitis, and septicemia.

To diagnose wound infection, most practitioners rely on clinical characteristics 98% of the time, followed by patient-reported symptoms (88%) and wound cultures (70%) [20]. Clinical signs of a superficial infection include purulent drainage, abnormal granulation tissue, abnormal foul odor, increasing temperature, additional breakdown, edema, induration and erythema [21]. Due to the varieties of wound infections and considering the

history of wound infection diagnosis, wound cultures have become the gold standard in infection diagnosis. Traditionally, techniques including swab culture (Levine technique), needle aspiration, and tissue biopsy are used to identify pathogens in the wound bed [22]. Among them, swab culture is the most frequently applied approach since it is simple, cheap, and convenient. However, this invasive approach can be time-consuming . Therefore, fast and non-invasive diagnostics that can detect a wide range of microbes regardless of wound types and locations are greatly needed.

5.0 DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

5.1 Visual Observations

Before using any diagnostic tool, most clinicians suspect wound infections by visually inspecting the wounds for signs and symptoms of infection. Typical signs of an acute infection include pain, erythema, heat and purulent exudate. However, this may be modified in a chronic wound setting by underlying comorbidities. In addition to these signs, an infected chronic wound may show signs of delayed healing, discolored and friable granulation tissue, serous exudate, epithelial bridging/pocketing in granulation tissue, foul odor and wound breakdown [12, 23, 24]. Acute wound infection has apparent signs and symptoms since it is generally caused by single organism while chronic wound infection has less obvious signs due to multiple pathogens [25]. Anyhow, due to the wound infection continuum, it is vital that clinicians must be able to distinguish colonization, in which multiplication of organisms interferes with wound healing without invading, from bacterial infection [10]. Unfortunately, the visual inspection does not provide any definite determination. Signs like changes in exudate, granulation tissue, discoloration of the wound bed and wound bridging can be associated with colonization rather than infection. A comprehensive list (Table 1) summarizing signs and symptoms at different stages of the wound infection continuum can be a useful tool for clinicians to assess patients' wounds [25]. As demands on healthcare systems increase, expanding the role of point-of-care medical personnel (i.e. nurses) in assessing the state of wounds is essential. The Bate-Jensen wound assessment tool [26], a holistic approach to record wound parameters such as size, depth, necrotic tissue type, necrotic tissue content, edge characterization, and undermining presence, can be used for wound diagnosis and captures much of the

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information listed above. This can help to streamline the diagnosis and move towards implementing therapy in a timelier manner.

Since a delayed wound healing process is an important indicator for wound infection, wound mapping tools such as wound tracing, scaled photographs, and planimetry can be used to indirectly detect wound infection by monitoring wound size changes [27]. If there is no noticeable improvement of the wound within three weeks, the wound could be infected [28]. It should be noted that the impediment to wound healing can be associated with many factors other than an infection. Therefore, further investigation is inevitably needed to confirm the diagnosis.

5.2 Odor Sensing and Pain Assessment

Odor sensing and pain assessment are also widely used by the clinicians for wound infection diagnosis. Wound odor (or malodor) is often led by necrosis or extremely poor vascularization of tissues, promoting bacteria as well as fungal colonization or infection. Hence it is clinically used as an indication for wound infection [29]. Deteriorating, highly exuding and fungating wounds usually possess malodor due to the fermentation of amino acids in anaerobes to malodorous organic amines [30].

Wound pain has been considered as the most frequent sign of heralding the onset of infection [31]. The pain mainly comes from the cellular injury and host immunological reactions caused by the growth, multiplication, and invasion of the microorganisms [32]. Wound pain assessment tools can help to assess the nature and severity of pain. Thorough and frequent pain assessment is critical for early detection of infection as well as comprehension of the patient's distress and discomfort [33]. Though neither provides a definite prediction of wound infection, both odor sensing and pain assessment cannot identify microbial species and the extent of colonization which are important parameters for the instruction of further treatment. This necessitates further clinical/laboratory assessments to acquire quantitative information within the infected wounds.

5.3 Clinical and Laboratory Assessments

5.3.1 Wound Culture

Wound cultures are performed when an infection is suspected through visual observation. The culture is used to confirm the initial diagnosis, identify species of resident microorganisms, and determine effective antibiotics to treat the wound [34]. Current

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literature provide three techniques on laboratory methods for diagnosing wound infections including deep-tissue biopsy, needle aspiration, and swab culture [35].

A deep-tissue biopsy is a qualitative and quantitative culture of wound tissue, which is accomplished by using an aseptic technique in obtaining a tissue sample via punch biopsy, needle biopsy, or a scalpel [36]. Through microscopic examinations, biopsy results are generally reported as the number of organisms per gram of tissue. Compared with other tests, deep-tissue biopsy result is considered more conclusive and accurate for the detection of microorganisms invading wound tissue which makes the technique the gold standard for identifying wound infection [37]. However, the biopsy procedure itself is not only a time consuming, costly, invasive, painful, and require special equipment as well as special training, but also has the risk for postsurgical trauma, wound disruption, and bacteremia is fairly high [35]. Most importantly, the feasibility of biopsy procedure varies from the anatomic location of the wound, patient's comorbidities, and/or provider's willingness. Therefore, it is usually not the primary choice for wound cultures.

Needle aspiration of wound fluid is a common technique used in puncture wounds or post-surgical wounds with suspected abscess. This method can obtain microbes below the surface of the wound via inserting a fine-gauge needle into the tissue to aspirate fluid and to quantify the concentrations of microbes [38]. Although needle aspiration is less invasive compared to tissue biopsy, it is still painful and the results are not always reproducible [37].

Swab culture is the most commonly used technique in the clinic due to its practical, non-invasive, reproducible, and inexpensive features. It has been reported that swab culture has sufficient correlation with tissue biopsy to identify causative organisms in an infected wound [39-41]. Among existing approaches, the Levine technique is considered one of the best methods to obtain a swab culture [42]. Usually, for quantitative swab cultures, the wound fluid stained swab is placed in 1mL of diluent and vortexed to release microorganisms. After incubation under aerobic condition, the type and the number of bacteria is measured and reported as the number of organisms per swab [21]. The major concern associated with swab culture is that only the surface colonizing bacteria will be reflected instead of the pathogenic strain invading deeper tissues. Moreover, swab

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cultures can be unreliable in the context of biofilm infection [43]. A comparison of these three wound culture techniques has been listed in Table 2.

Many different types of micro-organisms may exist in chronic wounds. Even though different clinical approaches may be able to identify the type of micro-organisms in wounds, these methods are unable to determine a causative organism or combination of organisms in complex chronic wounds. Such complexities need to be appreciated and considered in the development of wound infection diagnostics.

5.3.2 Laboratory Markers

In addition to the wound culture techniques, laboratory markers can also be measured to aid the diagnosis of wound infection. These markers include C-reactive protein (CRP), procalcitonin (PCT), presepsin, microbial DNA, and bacterial protease activity (BPA).

In response to inflammation and infection, CRP, a peptide produced in the liver, is stimulated by cytokines primarily Interleukin-6 and employed in complement binding and phagocytosis by macrophages [44]. By using light scattering from an aggregation of CRP-specific antibody, the concentration of CRP can be measured within 15 to 30 minutes [45]. Several studies have investigated the relationship between CRP production and wound infection. For example, a recent study showed that a higher grade of diabetic foot infection possessed a significantly higher level of CRP [46]. Moreover, elevated serum CRP levels were found to correlate well with incisional surgical site infection [47]. Although elevated CRP is frequently associated with acute infection, it has been suggested that CRP measurement be considered as supportive data instead of being used for direct diagnosis of infection [45, 48].

PCT is a peptide hormone secreted by non-neuroendocrine parenchymal cells, can be measured by time-resolved amplified cryptate emission, and serum level of PCT can increase 500~8000 times in patients with severe sepsis compared with healthy individuals [49]. A series of studies have reported that patients with bacterial infections have elevated PCT levels [50]. It has also been shown that PCT values were significantly higher among patients who developed post-surgical infections compared with those who did not [2]. However, due to the relatively low levels of PCT in infected individuals (ng/mL), a high-sensitivity PCT test may be needed to detect infection at an early phase [51].

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Presepsin, a soluble CD14 subtype, has been considered as a new, emerging, early indicator for diagnosis and prognosis of wound infections. It is secreted by monocytes and functions to stimulate the phagocytosis of monocytes within innate immunity responses [52]. Presepsin has been investigated as a potential biomarker for the detection of bacterial infection using presepsin ELISA kits. Subsequently, a highly sensitive, and fully automated PATHFAST presepsin measurement system was developed for faster processing [53]. Based on the results of several clinical studies, presepsin levels in patients with systemic bacterial infections were significantly higher than those without infections [54-56]. However, there are several limitations associated with this approach, such as the influence of pathophysiological conditions on its concentration [57], its elevation caused by the burn instead of infection [58], and by chronic kidney syndromes [59].

As an adjunct to wound cultures, culture-independent investigation of the microbial DNA applying pyrosequencing [60], PCR-denaturing gradient gel electrophoresis [61], and quantitative real-time PCR [62] have identified a greater range of bacteria than traditional culture techniques. However, these identification techniques are costly and they require special training as well as instruments that make them less translational into clinical application. Recently, DxWound, a PCR based microbial DNA analysis system, has been developed to provide accurate and sensitive detection of an array of microbes including aerobic bacteria, anaerobic bacteria, and fungi [63]. Moreover, the procedure is easy to handle with results available within one day. This comprehensive testing although not rapid, has great potential in diagnosing wound infection.

Bacterial protease is a large and diverse group of proteases produced exclusively by all microorganisms and functions mainly in degrading host tissue proteins to sustain the bacteria [64]. Bacterial proteases are often secreted into the infected wound environment and therefore can be utilized as diagnostic markers for wound infection. Moreover, the measurement of BPA can help to understand the pathological behavior of the microorganisms within the wound and provide valuable information on wound infection [65]. The most common and basic method to assess BPA is the incorporation of substrates of bacterial proteases, such as collagen, gelatin, and casein, into microbiological agar and a zone of clearance in the agar will appear where extracellular bacterial protease presents [66]. However, this technique is time-consuming and lacks specificity. A point-of-care swab-

based test, WOUNDCHEK™ Bacterial Status, has been developed by WOUNDCHEK Laboratories to help clinicians detect BPA within 15 minutes [67]. The product was recently approved by the US Food and Drug Administration for clinical use in the diagnosis and prognosis of non-healing wounds. While this device is designed to detect one enzyme produced by an organism that might not be associated with a wound infection, this device has not been approved for infection diagnosis in the United States. It must be noted that some lab markers like CRP and PCT are non-specific as they can be elevated by conditions unrelated to infection. A table that summarizes and compares all the available laboratory markers for wound infection diagnosis is listed for better comprehension (Table 3).

5.4 Imaging Modalities and Instrumentations

5.4.1 Traditional Imaging Modalities

In the clinic, to confirm the diagnosis from visual observation/blood test and to examine the existence of systemic/deep tissue infection, several current imaging modalities are frequently applied to aid in the diagnosis of wound infections. These imaging modalities include plain radiography (X-ray), computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging, positron emission tomography (PET), a single-photon emission computerized tomography (SPECT) and combined imaging modalities, such as PET/CT and SPECT/CT. These imaging modalities are not used as primary diagnostic tools for wound infection due to the fact that they require specific instruments, imaging facility, are costly, and cannot quickly identify infection status in complex chronic wounds.

The initial examination of patients with soft-tissue infections typically begins with plain radiography. This approach is widely used due to its simple operation, low cost, and wide availability [68]. However, it should be noted that plain radiography is non-specific and cannot distinguish between infection and other conditions, such as trauma, venous insufficiency, systemic causes of subcutaneous edema, and deep venous thrombosis [69].

Computed tomography (CT) plays an important role in the diagnosis of soft-tissue infection and intra-abdominal abscesses due to its wide availability, fast scanning speed, high spatial resolution, and multi-planar reformatting capabilities [70].

Magnetic resonance imaging (MRI) has been used for the diagnosis of soft-tissue infection due to its high spatial and contrast resolution. MRI provides anatomic and

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pathophysiologic information about the extent of infection within both soft tissue and the underlying bone [71]. However, it is difficult to distinguish foreign bodies from adjacent structures, such as scar tissue, calcifications, and tendons in superficial wounds [3].

Ultrasound imaging stands out among other imaging modalities because it is portable, cost-effective, readily available and does not provide exposure to radiation [72]. As air can interfere with ultrasound imaging, when utilized for intra-abdominal infection diagnosis, loops of intestines with intra-luminal air can obscure its detection capabilities [72]. Operator skill is also a major factor in distinguishing an abscess from other causes of skin swelling [73].

Positron Emission Tomography (PET) relies on developing a three-dimensional image from the accumulation of specific radioisotopes and resulting emission of gamma photons via positron annihilations to construct a three-dimensional image of the area of isotope accumulation. One of the drawbacks of this approach is that the equipment and radioisotope tracers used to perform these scans are expensive.

5.4.2 Innovative Imaging Modalities

Several innovative imaging approaches are still being developed in laboratories. These imaging approaches include spatial frequency domain imaging (SFDI), thermography, and fluorescence imaging. These fast and non-invasive imaging technologies have a great potential to revolutionize wound infection diagnosis.

Spatial frequency domain imaging (SFDI) is a new noncontact imaging approach that can quantify volume fraction of tissue chromophores, such as oxy-hemoglobin, deoxy-hemoglobin, and water [74]. It can study the optical properties of wound tissue by separating and quantifying absorbed and scattered incoherent monochromatic light [75]. The technique has been applied to identify infection in burn wound through quantifying changes in absorption and reduced scattering that correlate with bacterial infection [76]. Although SFDI technology is a new entrant in the field of cutaneous wound research, it is promising for diagnosis of burn wound infection and has great potential in assessing infections in diabetic wounds as well as pressure ulcers [77, 78].

Thermography is a technique wherein an infrared camera is applied to measure infrared radiation emitted from the wound tissue [79]. As a marker of inflammation, the elevated temperature has been shown in multiple studies to diagnose infection around

wound site [80]. The recent emergence of smartphone-based thermography has opened doors for the development of simple, portable and inexpensive techniques to detect and monitor wound healing [81, 82]. Although the technique has limited accuracy and specificity [83], it can offer complementary information on wound infection and has the potential to be utilized remotely from the clinic.

Luminescence imaging, an emerging imaging modality that captures visible photons emitted by Cherenkov radiation [84], has recently been used to detect infection in skin wounds [85]. A portable luminescence imaging device for detecting both inflammatory responses and infection in superficial wounds has been developed and tested using a full-thickness cutaneous wound model in pigs [85]. Since reactive oxygen species (ROS) levels can increase by an order of magnitude in an infected wound [86], this imager can display 2D ROS activity distribution in real-time through visualizing ROS-associated luminescence. Moreover, by analyzing ROS intensity and distribution within infected wounds on a pig model, this approach has been developed to distinguish infected wounds from uninfected ones. With unique properties like being simple, non-invasive, real-time, and portable the imager has shown great potential in being utilized in clinics as a wound infection diagnostic tool.

Autofluorescence imaging is becoming an innovative approach for the diagnosis of wound infection due to the light-absorbing properties of endogenously produced bacterial porphyrins. Bacterial porphyrins are not only important for the metabolism of molecular oxygen and diatomic gases but also involved in gene regulation [87]. Many clinically relevant bacterial species, such as *S. aureus*, MRSA, *Escherichia coli*, *Enterococcus spp.*, *Proteus spp.*, *Klebsiella pneumonia*, and *Enterobacter spp.*, have been detected using this approach without application of a contrast agent [88, 89]. A handheld portable autofluorescence imaging device has been developed and tested to detect bacterial infection around diabetic foot ulcers in real time [90, 91]. Moreover, a product, MolecuLight i:X™, has been commercialized based on the prototype. However, the device cannot detect infection and all causative microorganisms since not all of them possess porphyrins. Finally, the wound must be free of blood and blood clots because the presence of blood can block fluorescence signal [92]. The device may be used to detect microbial 'hot spots' in wound

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therefore helping to assist with wound debridement and other treatment decisions at the point-of-care [93, 94].

5.5 Detection of Biofilms

Biofilms on wounds may be suspected when wounds do not progress for multiple weeks [95]. They show no evident signs until they are large and produce a viscous exopolymeric substance [95]. Diagnosis of biofilms on wounds is difficult since there are no point-of-care tools that can detect biofilms. While standard clinical microbiology culture method can be used to identify infectious microbes especially on superficial wounds, it cannot be used to identify the main pathogen in the biofilm located in deep tissue [96]. Microscopic techniques, especially bright-field, scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) are some of the most reliable diagnostic methods for biofilms on wounds [97-100]. By visualization of debridement samples from diabetic foot wounds, a study has shown that bright-field microcopy, CLSM and environmental SEM were able to visualize the microcolonies associated with the biofilm phenotype and biofilm structure [99]. Molecular techniques such as 16S rRNA polymerase chain reaction, Bacterial tag-encoded FLX amplicon pyrosequencing, full ribosomal amplification, cloning and Sanger sequencing, partial ribosomal amplification and pyrosequencing , and peptide nucleic acid fluorescence *in situ* hybridization, have been explored to detect microbes in biofilm (please see review [96]). More recently, investigations have probed the presence of mono or multi microbes in biofilms using combined SEM and molecular techniques like peptide nucleic acid fluorescent *in situ* hybridization with CLSM [101]. With increasing evidence on the important role of biofilm in wound healing, there is tremendous need for reliable biofilm detection techniques that can serve at the point-of-care and serve as an adjunct tool for wound care specialists. Coincidentally, a recent study has shown that the creation of a fast diagnosis tool for biofilm might be possible. This study show that the presence of biofilms in wounds can be identified by using a wound blotting technique with pre-wet nitrocellulose membrane and then stained with a polysaccharide staining dye, ruthenium red or alcian blue [102].

5.6 Microwave-Microfluidic Device

A small and inexpensive microwave-microfluidic biosensor was recently developed for rapid, contactless and non-invasive quantification of *E. coli* within medium solutions to

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increase the efficacy of clinical wound infection assessment [103]. By measuring the variation of resonant amplitude and frequency responses of the microwave system, different concentrations of bacteria can be detected in solutions with different pH values. The minimum prepared optical transparency of bacteria was tested at an OD_{600} value of 0.003 which indicates the high sensitivity of this device. Moreover, the growth of bacteria can be monitored over time and that reveals the potential to use the device for rapid and real-time monitoring of bacterial infection in the wound. A table that summarizes and compares all the available imaging modalities and instrumentations for wound infection diagnosis is listed for better comprehension (Table 4).

6.0 SUMMARY

There is an exponentially growing population with wounds and associated co-morbidities that increase the risk of wound infections. Consequently, there is an urgent need to develop rapid, inexpensive, non-invasive, accurate, simple and specific techniques to assist wound care providers in the diagnosis and monitoring of wound infections. Current techniques used in clinical practice including visual observation for clinical signs and symptoms, clinical/laboratory assessments, and imaging modalities/instrumentations are speculative, expensive or sometimes off-target. The recent development of wound infection diagnostic products, such as PCR kits DxWound and handheld portable bacteria imager MolecuLight i:X™, may be considered as small but significant steps before we reach our ultimate goal of developing inexpensive, non-invasive, accurate, simple and specific wound infection diagnostic devices.

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7.0 TAKE-HOME MESSAGES

- Wound infection is one of the main factors for delayed wound healing.
- The wound infection continuum has three stages including contamination, colonization, and infection.
- Current wound infection diagnostics like the visual observation of signs and symptoms of wound infection cannot identify these stages.
- Wound culture is costly, time-consuming, and lacks accuracy.
- Multiple laboratory markers have been used for wound infection diagnosis, such as white blood cell count, CRP, PCT, presepsin, Microbial DNA, and BPA.
- Other than traditional imaging modalities like MRI, CT, ultrasound, PET, SPECT/CT, new imaging modalities including SFDI, thermography, luminescence imaging, and autofluorescence imaging has been used for wound infection diagnosis. Summary and comparison of all the imaging modalities have been listed.
- Microscopic and molecular techniqueshave been used for the detection of biofilms on wounds.
- Several new testing kits and devices have been developed and investigated for wound infection diagnosis as well.

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Paul Renick has a potential research conflict of interest due to employment as a full-time staff scientist at Smith & Nephew, plc. and his graduate studies are funded through an employee tuition reimbursement program. A management plan has been created to preserve objectivity in research in accordance with UTA policy.

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Abbreviations and Acronyms

BPA: Bacterial protease activity

CLSM: Confocal laser scanning microscopy

CRP: C-reactive protein

CT: Computed tomography

ESEM: Environmental scanning electron microscopy

MRI: Magnetic resonance imaging

PET: Positron emission topography

PCT: Procalcitonin

ROS: Reactive oxygen species

SEM: Scanning electron microscopy

SFDI: Spatial frequency domain imaging

SPECT: Single photon emission computed tomography

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Table 1. Signs and symptoms within wound infection continuum

Contamination	All open wounds may contain microorganisms. They will not multiply or persist until suitable nutritive and physical conditions are available for each microbial species, or they successfully evade host's defenses. Consequently, their presence is only transient and wound healing is not delayed.
Colonization	Microbial species successfully grow and divide, but do not cause damage to the host or initiate wound infection.
Local infection	Covert (subtle) signs of local infection: Hypergranulation (excessive 'vascular tissue'); Bleeding, friable granulation; Epithelial bridging and pocketing in granulation tissue; Wound breakdown and enlargement; Delayed wound healing beyond expectations; New or increasing pain; Increasing malodor.
	Overt (classic) signs of local infection: Erythema; Local warmth; Swelling; Purulent discharge; Delayed wound healing beyond expectations; New or increasing pain; Increasing malodor.
Spreading infection	Extending in duration +/- erythema; Lymphangitis; Crepitus; Wound breakdown/dehiscence with or without satellite lesions; Malaise/lethargy or nonspecific general deterioration; Loss of appetite; Inflammation, swelling of lymph glands.
Systemic infection	Severe sepsis; Septic shock; Organ failure; Death.

International Wound Infection Institute (IWII) Wound infection in clinical practice. Wounds International 2016

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Table 2. Comparisons among wound culture techniques

	Descriptions	Advantages	Disadvantages
Deep-tissue biopsy	Obtain tissue sample via punch/needle biopsy or a scalpel; Quantitative results acquired by microscopic examinations.	Conclusive and accurate result for detecting invading microorganisms; Gold standard for wound infection diagnosis.	Time-consuming, costly, invasive, painful, require special equipment and special training; High risk for postsurgical trauma, wound disruption, and bacteremia.
Needle aspiration	Obtain microbes below the surface of the wound via inserting a fine-gauge needle into tissue to aspirate fluid.	Feasible for small open wound and detecting subcutaneous microorganisms; Less invasive.	Time-consuming, painful; May underestimate bacterial isolates.
Swab culture	Press sterile culture swab against the wound base to extract wound fluid; Using eluent for incubation and quantification.	Practical, noninvasive, reproducible, and inexpensive; Has sufficient correlation with tissue biopsy outcome.	Time-consuming; Can't detect pathogenic strain invading deeper tissues; Weak in detection of biofilm infection.

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Table 3. Laboratory markers for wound infection diagnosis

	Description	Pros and cons	Sensitivity	Specificity
C-reactive protein (CRP)	In response to inflammation and infection, CRP is stimulated by cytokines primarily IL-6. By using light scattering from aggregation of CRP-specific antibody, concentration of CRP can be measured.	✓ Simple; Fast; Non-invasive. x Short half-life; Nonspecific; Cannot determine microbial species.	High	Low
Procalcitonin (PCT)	PCT is a peptide hormone secreted by non-neuroendocrine parenchymal cells and can be measured by time-resolved amplified cryptate emission. Patients with bacterial infections have elevated PCT levels.	✓ Fast; Non-invasive. x Costly; Nonspecific; Require high-sensitivity facilities; Cannot determine microbial species.	High	Low
Presepsin	Presepsin is a soluble CD14 fragment released into blood when monocytes are activated during an infection. It can be quantified by either an assay kit or PATHFAST presepsin	✓ Fast; Non-invasive; High sensitivity; Prognostic. x Altered by other pathophysiological conditions; Nonspecific; Cannot determine	High	Low

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	measurement system.	microbial species.		
Microbial DNA	Culture-independent investigation of the microbial DNA can identify a greater range of bacteria species than traditional culture techniques. It can also be detected by DxWound, a PCR based microbial DNA analysis system.	✓ Accurate; Sensitive; Non-invasive; Can determine microbial species. x Costly; Require special training and instruments.	High	High
Bacterial protease activity (BPA)	Bacterial proteases are often secreted into the infected wound environment to degrade host tissue proteins for bacterial sustenance.	✓ Fast; Non-invasive; Prognostic. x Nonspecific; Cannot determine microbial species.	Medium	Low

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Table 4. A summary of imaging modalities and instrumentations for wound infection diagnosis

	Application in Wound Infection Diagnosis	Pros and cons	Sensitivity	Specificity
Plain radiography	Initial examination of soft-tissue infections.	✓ Simple operation, low cost, wide availability; Radioactive; Reveals inflammatory changes; x Can distinguish swelling due to infection from fractures; Nonspecific findings; x. Misled by other conditions.	Low	Low
Computed tomography	Diagnosis of soft-tissue infection and intra-abdominal abscesses; Evaluation of deeper structures and the extent of surrounding inflammations; Identification of small infected collections.	✓ Wide availability; Fast scanning speed; High spatial resolution; multiplanar reformatting capabilities; high penetration depth. x. Radioactive, sometimes requires contrast	High	High

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		agent.		
Magnetic resonance imaging	Diagnosis of soft-tissue infection; Can provide anatomic and pathophysiologic information about the extent of infection within both soft tissue and the underlying bone.	✓ High spatial/contrast resolution; Non-radioactive x. Costly; Low availability; Requires special training/facility; Hard to distinguish foreign bodies from adjacent structures within superficial wounds.	High	High
Ultrasound imaging	Diagnosis of skin and soft tissue infections. Can evaluate suspected radiolucent foreign bodies.	✓ Fast; Accurate; Cost-effective; Portable; Available in many clinics; No ionizing radiation. x. Interference from air; Low penetration depth; Relies on operator's skill.	Medium	Medium
Positron emission tomography (PET)	Diagnose and predict remission of antibiotic treatment for diabetic foot infections by developing 3D imags	✓ High resolution 3D imaging; High penetration depth. x Costly; Short tracer half-life; Requires	High	High

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	from ccumulation of radioisotopes.	special training/facility; Possible misdiagnosis resulting from sterile inflammation.		
Spatial frequency domain imaging (SFDI)	Identify burn wound infection by quantifying volume fraction of tissue chromophores.	✓ Noncontact; Distinguishes infected and non-infected burn wounds. x. Limited scanning area and wound types.	High	High
Thermography	Use an infrared camera to measure infrared radiation emitted from the wound tissue. Smart phone based thermography has been developed for diabetic foot ulcer detection and wound healing prediction.	✓ Simple; Portable; Cost-effective; Real-time imaging; Non-invasive; Remote diagnosis. x. Limited accuracy and specificity.	Low	Medium
Luminescence imaging	Portable imager distinguishes infected wounds from uninfected ones in a	✓ Simple; Portable; Cost-effective; Real-time imaging; Non-invasive;	Medium	Medium

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	pig model based on intensity and distribution of visible photons emitted y Chernow radiation.	Remote diagnosis. x. Limited specificity; Requires more research		
Autofluorescence imaging	A handheld portable device diagnoses bacterial infection in diabetic foot ulcers in real time by detecting autofluorescence due to the light absorbing properties of endogenously produced bacterial porphyrins.	✓ Simple; Portable; Cost-effective; Real-time imaging; Non-invasive; Sensitive; Remote diagnosis. x. Low specificity; Can not determine microbial species.	High	Low
Microwave-microfluidic biosensor	A microwave-microfluidic biosensor for quantification of E. coli within medium solutions to increase the efficacy of clinical wound infection assessment; The growth of bacteria can be monitored over time.	✓ Small; Cost-effective; Rapid; Contactless; Real-time measurement; Non-invasive; Sensitive. x. Limited detectable bacterial species	High	N/A