Survey of fungi and yeast in polymicrobial infections in chronic wounds

• **Objective:** To assess the incidence, abundance and species diversity of fungi in chronic wounds, as well as to describe the associations of major fungi populations.
• **Method:** Comprehensive molecular diagnostic reports were evaluated from a total of 915 chronic wounds in a retrospective study.
• **Results:** Of the 915 clinical specimens, 208 (23%) were positive for fungal species. These samples were further compared in a compiled dataset, and sub-classified among the four major chronic wound types (decubitus ulcer, diabetic foot ulcer, non-healing surgical wound, and venous leg ulcer). The most abundant fungi were yeasts in the genus *Candida*; however, *Curvularia*, *Malessezia*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, *Engodontium* and *Trichtophyton* were also found to be prevalent components of these polymicrobial infections. A notable bacterial/fungal negative correlation was found to be apparent between *Staphylococcus* and *Candida*. There were also significant relationships between both bacterial and fungal genera and patient metadata including gender, diabetes status and cardiovascular comorbidities.
• **Conclusion:** This microbial survey shows that fungi are more important wound pathogens and opportunistic pathogens than previously reported, exemplifying the impact of these under-reported pathogens. With the application of modern cost-effective and comprehensive molecular diagnostics, clinicians can now identify and address this significant component of chronic wound bioburden with targeted therapies, thereby improving healing trajectories.

• **Conflict of interest:** SED and RW are owners of Pathogenius Diagnostics which is a clinical diagnostic laboratory, SED and RW are owners of Research and Testing Laboratory which develops molecular methods for clinical diagnostics. JK and CEJ are owners of SEMT which licenses lipogels for wound care.

fungi; yeast; biofilms; chronic wounds

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Chronic infections, which include chronic wounds, comprise 60–80% of infectious diseases occurring in humans.[1] The bioburden associated with biofilm phenotype infection (clinical or subclinical) contributes to the chronicity and delayed healing of practically all chronic wounds.[2–4] Regardless of whether we attribute this barrier to healing to the net bioburden linked to biofilm phenotypes, we have observed a notable improvement in healing and prognosis of patients when biofilm-based wound care principles are employed. Recently, we have also demonstrated that the use of comprehensive molecular diagnostics combined with biofilm-based wound care protocols and targeted therapies based on these molecular tools and clinical biofilm principles can significantly improve healing rates, prognosis and outcomes.[5] The bioburden of chronic wounds is a symbiotic, multi-species opportunistic pathogenic biofilm infection that apparently confers reduced sensitivity to systemic antibiotic therapy and adaptive strategies that are not otherwise attributable to the individual organisms within the microbial census.[1,6–23]

Accurately diagnosing the polymicrobial infections of chronic wound biofilm/bioburden can be difficult and, in turn, present significant challenges in determining optimal, individual treatment regimens. Studies of bacterial diversity using bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) (i.e. molecular diagnostics) on venous leg ulcers,[24] diabetic foot ulcers,[25] surgical site infections[26] and pressure ulcers,[27] have demonstrated that many of the bacteria that are dominant and ubiquitous in chronic wounds are simply not identified using routine or traditional culture-based diagnostic techniques. This is largely due to the fact that the majority of component organisms that populate wounds are fastidious (hard to grow), slow-growing (require days or weeks to propagate), require very specialised growth media or growth conditions (anaerobic, microaerobic, complex media and supplementation etc.), or are simply unable to be grown using traditional culture techniques. Another factor hindering identification is that communal associa-

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tions among species can be lost when biofilms are disrupted.[28–36]

Fungal infections have been reported in patients with chronic rhinosinusitis, infections of shunt devices, osteomyelitis, phaeohypomycosis, keratitis, dermatitis, diabetic foot ulcers and onoychomycosis and are recognised pathogenic agents in burn, surgical and traumatic injuries, as well as in patients that are immunocompromised as a result of radiation, transplant and malignancy.[37–55] The diversity of fungal species found in wounds has become more important as the use of topical antibiotics has become more prevalent for treating non-systemic bacterial infections.[42,43] However, until recently the best way to identify the species diversity of pathogenic fungi was through traditional culture methods, which is limited by the ability of definitive conditions and media to grow specific fungi similar to the discussion above for bacteria, or through fluorescent *in situ* hybridisation, or quantitative polymerase chain reaction (qPCR) techniques;[56] each of these require the researcher or clinician to have prior knowledge or predict which species may be present and anticipate specific pathogens *a priori*. Thus, the current understanding of fungal diversity and occurrence in wounds is profoundly limited and in need of further elucidation through investigation.

Previously, our group has employed an universal fungal diagnostic approach to identify specific fungi present in three chronic wounds; however, this limited sample population did not have sufficient power to elucidate fungal abundance or diversity.[37]

The purpose of the research presented here is to retrospectively evaluate clinical wound diagnostic samples using modern comprehensive molecular diagnostic approaches (Pathogenius Diagnostics, Lubbock, TX) in order to illustrate the prevalence and contribution of fungi within the polymicrobial environment of chronic wounds. These diagnostic methods were used to quantify the incidence and relative abundance of fungal diversity in wound biofilms within this group, as well as to describe any trend or tendency of cohabitation of fungal and bacterial species.

Materials and method

Sample collection

During a 4-month period, following presentation at the Southwest Regional Wound Care Centre for treatment of chronic wounds, all patients described herein (n=609) were enrolled into the study in compliance with Western Institutional Review Board approved protocol 56-RW-004 WIRB Protocol #20062347.

A total of 915 diagnostic samples, obtained by sharp debridement as per standard care, were identified for analysis. The samples were immediately transported to the in-house CLIA/CAP certified diagnostic laboratory with concurrent samples subsequently provided to the Research and Testing Laboratory (Lubbock, TX) and Pathogenius Diagnostics (Lubbock, TX). Diagnostics were performed using level I and level II wound pathogen diagnostic assays (Pathogenius Diagnostics, Lubbock, TX). The level I diagnostic test is a finite panel of the most commonly occurring bacteria and genetic antibiotic resistance factors in chronic wounds. Level II is a comprehensive diagnostic test (i.e most bacteria and fungi known can be detected and identified without *a priori* assumptions). Reports for level II results were utilised based on bacterial and fungal identifications with the capability of >95% sequence identity.

Wounds were classified into four major categories:
- Pressure ulcers (n=23)
- Diabetic foot ulcers (DFU) (n=83)
- Non-healing surgical wounds (n=11)
- Venous leg ulcers (VLU) (n=41).

Wounds that were not assignable to one of the four major wound types were classified as a general chronic wound (n=50).

Quantitative PCR validation of diagnostic results

Using qPCR with SYBR GreenqPCR Master Mix (SABiosciences, Fredrick, MD) and clinically validated *Candida albicans* and *C. orthopsilosis/parapsilosis* species-specific primers (Pathogenius Laboratories, Lubbock, TX), 100 of the 915 wound samples were selected to validate the species identities generated by the BLASTn process. Wounds were selected if they were diagnosed with *C. albicans*, *C. parapsilosis*, *C. orthospilosis*, or *C. glabrata*. Additionally, 17 samples indicated by diagnostics to contain only bacteria were examined as negative controls. These same negative control samples were also used as positive controls created by addition of an equivalent of ~10^4 colony forming units (CFUs) of *C. albicans* DNA. Samples were considered to have species-positive results when peaks were observed before 35 cycles of the qPCR.

Evaluation of bacteria to fungi ratios in chronic wounds

Relative ratios of fungus to bacteria were determined with qPCR using bacteria and fungus universal quantitative primers (Research and Testing Laboratories, Lubbock, TX) on 174 of the fungus-positive wounds. Serial dilutions of known concentrations of *C. albicans* and *Pseudomonas aeruginosa* DNA were used to generate reference samples in order to determine relative molecular enumeration of both bacteria and fungi. This was then applied to extrapolate the relative normalised percentages of both fungi and bacteria determined by the level II assay, thereby providing a normalised polymicrobial popula-

Table 1. Survey of fungi in 208 positive wounds (continued opposite)

	No. of samples	Avg. %	SD	Min. %	Max. %
Candida albicans	97	46.09	44.92	0.02	100.00
Candida parapsilosis	94	40.66	42.24	0.01	100.00
Curvularia lunata	73	28.60	34.93	0.01	100.00
Candida tropicalis	37	34.14	43.27	0.02	99.79
Trichophyton mentagrophytes	27	32.68	41.39	0.01	99.89
Candida glabrata	16	28.29	40.76	0.01	99.49
Rhizopus oryzae	3	58.59	51.76	0.56	99.99
Candida orthopsilosis	59	4.22	14.35	0.03	97.33
Fusarium moniliforme	13	27.24	34.89	0.03	90.14
Physcia adscendens	6	38.12	44.82	0.07	98.21
Malassezia restricta	43	13.40	27.59	0.01	100.00
Phoma rabiei	28	11.77	18.80	0.04	91.18
Fusarium equiseti	15	4.34	7.37	0.23	28.53
Cladosporium herbarum	44	9.32	20.45	0.01	93.75
Fusarium sporotrichioides	17	2.75	7.54	0.04	30.08
Kodamaea ohmeri	1	99.92	0.00	99.92	99.92
Phoma betae	2	36.19	45.93	3.71	68.66
Aspergillus terreus	5	20.46	43.77	0.27	98.76
Ulocladium botrytis	48	8.73	17.73	0.04	100.00
Aureobasidium pullulans	41	19.85	27.91	0.02	89.74
Candida athensensis	5	14.83	13.18	0.95	33.20
*Myrothecium spp**	21	8.32	14.92	0.03	49.60
Aspergillus niger	5	17.94	34.96	0.03	80.07
Candida silvicultrix	2	16.59	9.21	10.07	23.10
*Pyrenophora spp**	18	1.05	3.14	0.01	13.57
Bipolaris eleusines	16	1.47	3.22	0.03	13.04
Malassezia sympodialis	15	15.27	28.98	0.15	89.83
Phyllosticta pyrolae	7	12.72	21.08	0.03	52.14
Engyodontium album	25	22.56	32.71	0.03	100.00
Saccharomyces martiniae	2	36.43	49.22	1.63	71.24
Humicola fuscoatra	3	27.15	45.13	0.52	79.26
Phoma medicaginis	21	3.97	4.39	0.07	17.11

tion profile. As a simplified example, if qPCR universal assays for bacteria and fungi in a wound indicated that the bacterial and fungal components were in equal proportions (i.e. bacteria = 10^4 cells/mg and fungi = 10^4 cells/mg; a 1:1 ratio). Then again as a simplified example, a relative proportion of *C. albicans* determined to be 50% of the total fungal component and *P. aeruginosa* determined to be 50% of the bacterial component could be normalised, based on these 1:1 ratios to estimate that each of these species were 25% of the total normalised microbial population. Further, we can determine that there were 5×10^3 *C. albicans* and 5×10^3 *P. aeruginosa*.

Statistics

Correlation analysis and ANOVA analyses were performed with XLStat 2010 (Addinsoft USA, New York, NY) using taxonomic designations at the genera level using bacterial and fungal wound diversity normalised data (n=174). For ANOVA, metadata was used to determine if there were any significant relationships between bacterial and fungal genera and patient metadata, while correlation analyses were used to determine if there were notable relationships in the normalised populations of fungi and bacteria among chronic wounds.

Results

A total of 208 wounds out of 915 total wounds (23% of specimens) tested positive for fungi within the 4-month study period. Within the 208 samples a total of 48 different species of fungi, in 34 genera, were identified as occurring in at least five wounds. An additional six genera for which no species could be determined (likely novel or previously uncharacterised species) were identified in more than five chronic wounds each. The most abundant fungi were yeasts in the genus *Candida* (Table 1). All of the 40 most abundant fungi genera (Table 1) have previously been reported as human pathogens with the exception of *Pyrenophora* and *Myrothecium*.

Wounds were examined by type (Table 2) and the most dominant (highest average percentage per wound type) and the most ubiquitous (occurring in the most samples) species of fungus were determined. *C. albicans* and *C. parapsilosis* were observed at high levels in all wound classifications. Although each wound type had a different cohort of fungal species, *Candida* species comprised at least two of the top four species in each classification. *Malessezia restricta* and *Curvularia lunata* were also commonly found in four of the five wound types. In DFUs, non-healing surgical wounds and VLUs, *Candida* consisted of three of the four most dominant and most ubiquitous genera. *Candida tropicalis* was the third member of the genus in DFUs (dominant) and VLUs (ubiquitous and dominant), whereas *Candida*

dublinensis was the third most ubiquitous member of the genus in DFUs (ubiquitous).

Based on normalised fungal and bacterial populations, there was a significant negative correlation between the occurrence of *Staphylococcus* and *Candida* ($p<0.001$; Pearson −0.316). *Candida* also had significant ($p<0.05$) negative correlations to other fungi such as *Alternaria*, *Cochliobolus*, and *Engyodontium*. There were significant ($p=0.004$) positive correlations (Pearson 0.219) between several bacterial genera such as *Finegoldia* and *Pseudomonas*; however, there were no other significant correlations between bacteria and fungi except for *Clostridium* and *Cladosporium* ($p=0.024$; Pearson 0.260). Two fungi (*Aureobasidium* and *Catenulostroma*) had the highest positive correlation ($p<0.0001$; Pearson 0.801). Microbial population correlations are graphically illustrated in Fig 1 for the most predominant genera.

Based on ANOVA using least square means or the normalised percentages of fungi and bacteria and Tukey post hoc analyses, there were several notable significant ($p<0.05$) variations in the normalised microbial populations in relation to patient metadata. *Corynebacterium* were significantly higher in female subjects (6.8 versus 1.9%), *Pelomonas* were higher in patients with diabetes (3.7 versus 0.4), *Kluyveromyces* were higher in surgical site infections compared with all other ulcers (14.0 versus <1.0), *Pichia* was higher in subjects with immobility (5.2 versus 0.2), *Veillonella* were higher in pressure ulcers than other ulcers (3.2 versus <0.1) and were also significantly higher in relation to immobility, and finally *Cochliolobus* and *Engyodontium* were higher in subjects with cardiovascular complications. Metadata summaries are provided in Table 3.

Candida species diagnoses were validated with qPCR. None of the fungus negative samples were reactive with any of the *Candida* spp. validation assays, neither of the species-specific assays reacted with *C. glabrata*, and only *C. albicans* samples were positive with *C. albicans* assay. Samples containing either *C. parapsilosis* or *C. orthopsilosis* were all found to be positive using *C. orthopsilosis/parapsilosis* assay. Similarly, none of the samples that were not diagnosed with *C. orthopsilosis* or *parapsilosis* showed were found to be positive with the *C. orthopsilosis/parapsilosis* qPCR assays. These results indicate a 100% specificity and sensitivity for the qPCR assays in relation to the sequencing based diagnostic within this dataset.

Quantification of relative abundance of bacteria versus fungus revealed that 57/174 normalised fungal positive wounds microbiomes had fungal ratios, accounting for less than half of the microbial component, whereas 60 wounds had fungal percentages between 50% and 75%. In 33 wounds, the fungal contribution to the wound microbiome was between

Table 1. Survey of fungi in 208 positive wounds (continued)

	No. of samples	Avg. %	SD	Min. %	Max. %
Candida oregonensis	4	27.33	46.19	0.35	96.18
Exserohilum rostratum	14	2.76	3.45	0.02	12.40
Candida dubliniensis	48	0.96	4.37	0.02	30.36
Candida viswanathii	10	2.15	6.04	0.04	19.33
Candida sojae	4	5.76	10.33	0.03	21.21
Penicillium janthinellum	16	12.93	23.68	0.19	84.62
Keissleriella cladophila	21	1.50	3.02	0.03	12.41
Bipolaris sorokiniana	11	7.02	11.06	0.11	34.83

The primary identification based on percent sequence identity as described in the materials and methods is indicated. Generic names followed by spp. indicate samples where resolution between more than one species in the genus was not possible. The total number of samples in which each taxon was recovered is provided, along with the average non-normalised percentage (Avg %) of the fungal population among samples testing positive for each taxon, the standard deviation (SD) and the range of percentages among the respective positive samples (Min % and Max %).
* Genera not previously associated with wounds or human pathology

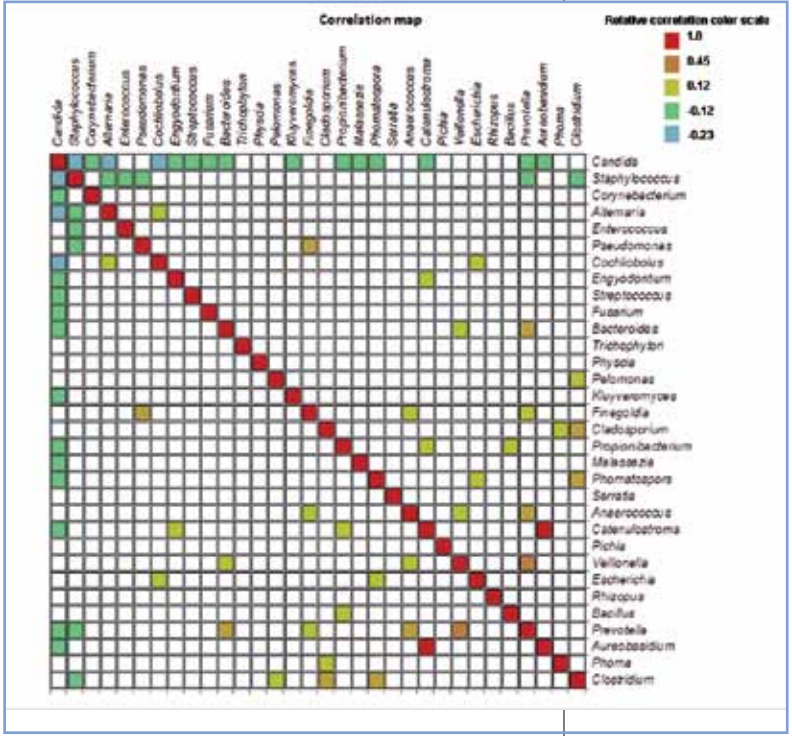

Fig 1. Correlation matrix for normalised bacterial and fungal microbiome of wounds. The significant correlations are indicated by coloured cells. The relative correlation is indicated by the figure legend with blue and green indicating negative correlations and yellow through to red indicating positive correlations. A legend showing the average correlation values is provided. White cells indicate no significant relationship

Table 2. Most ubiquitous and dominant fungal species per wound type

Wound type	n	Most ubiquitous	Dominant
Chronic wound	50	*Curvularia lunata* *Ulocladium botryti* *Candida albicans* *Candida parapsilosis*[4] *Malessezia restricita*[4]	*Curvularia lunata* *Candida albicans* *Candida parapsilosis* *Aureobasidium pullulans*
Decubitus ulcer	23	*Candida albicans* *Candida parapsilosis* *Curvularia lunata*[3] *Aureobasidium pullulans*[3] *Malessezia restricita*[4] *Cladiosporium herbarum*[4]	*Candida albicans* *Candida parapsilosis* *Malassezia sympodialis*
Diabetic foot ulcer	83	*Candida parapsilosis* *Candida albicans* *Candida orthopsilosis* *Candida dubliniensis*	*Candida parapsilosis* *Candida albicans* *Candida tropicalis* *Trichophyton mentagrophytes*
Venous leg ulcer	41	*Candida albicans* *Curvularia lunata* *Candida parapsilosis* *Candida tropicalis*	*Candida tropicalis* *Candida albicans* *Curvularia lunata* *Candida parapsilosis*
Non-healing surgical wound	11	*Candida albicans*[1] *Candida glabrata*[1] *Curvularia lunata*[2] *Ulocladium botrytis*[2] *Malessezia restricta*[2] *Engodontium album*[2]	*Candida albicans* *Candida glabrata* *Ulocladium botrytis* *Candida glabrata*

Top five fungal species in each wound type is listed, based on the percentage of wounds/type in which a given fungus was observed (most ubiquitous) as well as the number of wounds (two or greater) in which a given fungus was the dominant species (>50%). Species were only listed if they occurred in more than two wounds. Species marked with superscript numbers had equal ranking. Pressure ulcer only had three species which were dominant in more than two wounds.

75% and 90% and, surprisingly, 24 fungus positive wounds had relative abundances of fungus >90%. Thus, 117 of the 174 population normalised chronic wounds had fungal percentages accounting for >50% of the normalised microbial bioburden.

Discussion

These results demonstrate that fungal pathogens are significant to the microbial census in a significant number of chronic wounds. Specifically, the high relative abundance of fungus within the polymicrobial ecology of wound infections suggests that, when fungus is present, it is can be a major contributor to the bioburden or biofilm of wounds. Furthermore, the diversity of fungal genera and species represented in and among such wounds is higher than previously suspected or reported. The overall incidence of fungi within this population of 915 chronic wounds (22.7% of wounds over 4 months) and the abundance within each specific wound illustrates how under-reported and underappreciated the contribution of yeast and fungi has been in chronic wounds, to date.

The overall prevalence of fungi in chronic wounds may seem extreme but, with the evolution of diagnostic methodologies now available (Pathogenius Diagnostics, Lubbock, TX), it is to be expected that new opportunistic pathogens will be identified. Chronic wounds are known to have fungal infections.[38,57–61] However, based on historical prevalence data from 'pre-molecular diagnostics' when culture-based evaluation was utilised, the percentage of wounds found to be positive for fungi/yeast was less than 2%, suggesting that culture may not be adequate for routine diagnoses.

Only two genera of fungi identified in this study have not previously been reported in association with human infections (*Pyrenophora* and *Myrothecium*). Both of these fungal genera are known as plant pathogens.[62–64] Of course, other taxa, such as *Rhizopus*, were originally observed initially as plant pathogens[65,66] and later described from human infections including cutaneous infections.[58,67–69] Given the close association of humans and plants via agriculture, indoor decorations and gardening, it is plausible that routine diagnosis utilising compre-

Table 3. Summary of metadata for the subjects with fungal positive diagnostic results

Variable	Categories	%
Gender	Female	50.575
	Male	49.425
Race	Asian	0.575
	Black	9.195
	Hispanic	52.299
	White	36.207
	Unknown	1.724
Wound type	Chronic wound	25.287
	Decubitus ulcer	9.770
	Diabetic foot ulcer	40.805
	Surgical site infection	5.747
	Venous leg ulcer	18.391
Diabetes	Without	44.828
	With	55.172
Cardiovascular disease	Without	71.839
	With	28.161
Immobility	Without	95.402
	With	4.598

Variables include the gender, race, type of wound, and comorbidities such as diabetes, known cardiovascular diseases, and immobility (quadriplegia, paraplegia). The percentage of each category for each variable is also provided. No relationship was noted between fungi positive and fungi negative results among the metadata

hensive molecular methods may identify fungi previously recognised only as plant-associated genera in other human chronic infections and therefore as potential opportunistic human pathogens. Thus, rather than view the occurrence of *Pyrenophora* and *Myrothecium* in human wounds as aberrant, it may be more appropriate to view it as an expected result empowered by improved diagnostic methods. The high occurrence levels of *Curvularia*, *Aureobasidium*, and *Malessezia* in general chronic wounds and PUs, *Cladosporium* in PUs, *Ulocladium* in general chronic wounds, *Curvularia* in VLUs, and *Trichophyton* in DFUs are not unexpected as all have been implicated as opportunistic human pathogens in skin diseases such as phaeohypomycosis, keratitis, dermatitis and onychomycosis.[39,47,51,70–75]

The overall abundance of *Candida* species illustrates the importance of this genus in human wound pathology. A total of 40 *Candida* species were found, including five primary opportunistic pathogens associated with candidiosis (*C. albicans*, *dubliniensis*, *glabrata*, *parapsilosis* and *tropicalis*). The observation of *C. glabrata* should be highlighted as this specific yeast is becoming increasingly recognised as an important clinical opportunistic pathogen. Recently, this species has been described as the second most common cause of candidiasis and is resistant to azole antifungals.[76] Interestingly, *C. orthopsilosis* was also observed at moderately high levels. This taxon was a recently identified species[77] closely related to *C. parapsilosis* and it is plausible that *C. orthopsilosis* is a common, but yet unrecognised, human pathogen.

Clinical observations

Regarding the care of chronic wounds with a fungal component, it is apparent that when the treatment is designed to singularly address the bacterial contribution, ignoring the fungal component will result in inadequate treatment of at least 23% of wounds (based on the data yielded by this study). Thus, wound healing trajectories may not be optimal, may fail to progress in a positive healing trajectory or worsen as the bacterial component is deselected in favour of the fungal.

Direct clinical observations from the Southwest Regional Wound Care Centre have continually demonstrated that if the fungal populations are also targeted when diagnosed through molecular methods, there is a definitive and significant improvement in the healing trajectory.[5] Therefore, to design an appropriately targeted treatment, it is critical that the correct fungal genera or species are identified and the relative contribution is quantified. Thus, the quantitative molecular techniques described herein, which are capable of identifying an expansive range of fungal species, have already been shown to be important tools in the diagnosis and subsequent treatment of chronic wound infections.[5] The case studies below are simple examples of the importance of fungal diagnostics and targeted therapies.

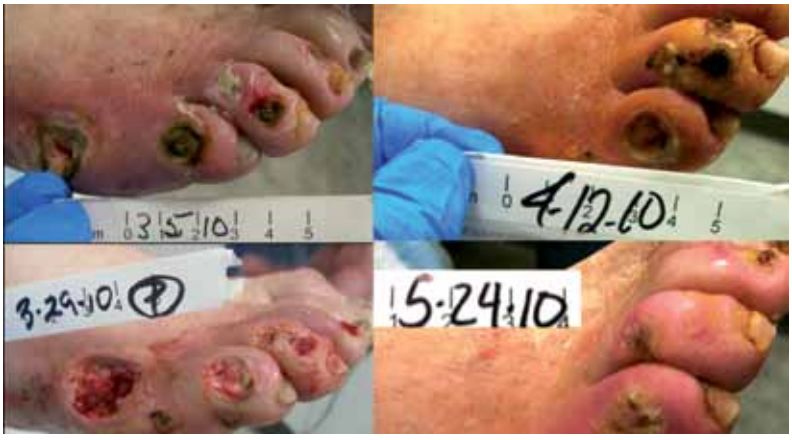

Fig 2. Case study 1. *Candida parapsilosis* infection in a diabetic foot with vascular complications that was resolved with effective biofilm-based wound care combined with accurate and comprehensive diagnosis of a fungal component in the polymicrobial infection

• **Case 1** A 62-year-old white female with insulin-dependent type 2 diabetes along with critical limb ischemia, presented with a severe DFU involving the toes and the right fifth metatarsal head on 22 February 2010 (Fig 2). There was no significant improvement during the first month of treatment, which included weekly debridements, Iodosorb gel and advanced dressings. On 18 March 2010, after requisitioning a level II test (Pathogenius Diagnostics, Lubbock, TX), the patient was shown to have a wound fungal component consisting of *C. parapsilosis* (99%) and an anaerobic bacterial component which included *Finegoldia magna* and *Anaerococcus vaginalis*. The *C. parapsilosis* was treated with terbinafine 1%, along with metronidazole and clindamycin for anaerobes in a proprietary Sanguitec Lipogel (Southeastern Medical Technologies, LLC). A re-evaluation on 19 April 2010 demonstrated that the *Candida* component of the wound bioburden was still present. The patient's specific topical gel was revised to ketoconazole as the antifungal agent with continued clindamycin and metronidazole to continue to address the anaerobic bacteria. Subsequently, the wounds on her right foot and toes were completely resolved, despite the fact that the patient could not be revascularised.

• **Case 2** A 62-year-old Hispanic female in general good health sustained a dog bite to her right lower leg on 6 April 2010 (Fig 3). The wound deteriorated despite 10 days of Keflex 500mg p.o.t.i.d., followed by 5 days of Bactrim DS one p.o.b.i.d. along with local wound care. The patient presented on 3 May

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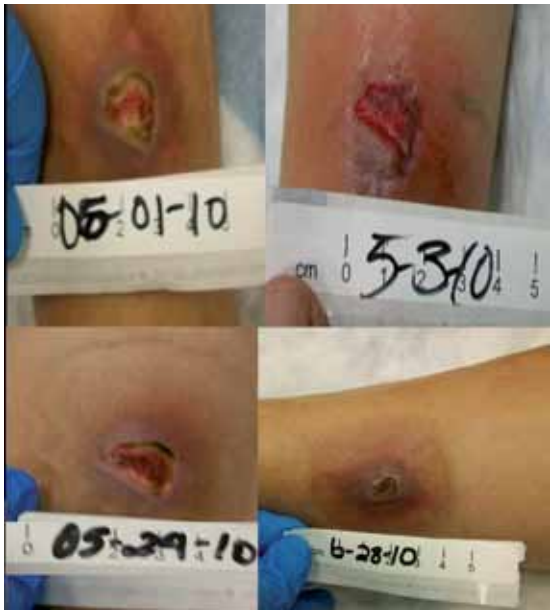

Fig 3. Case study 2: *Fusarium moniliforme* containing polymicrobial infection in a dog bite wound that failed to heal until fungal diagnosis and infection specific-treatments were implemented

2010 with marked tenderness, erythema and swelling. A clinical specimen obtained that day showed very little total bacteria (Pathogenius Diagnostics) but the fungus genera *Fusarium* was diagnosed. It took approximately 7 days to identify the fungus and obtain a personalised topical therapy from a specialty pharmacy (Southeastern Medical Compounding, LLC). During this time, the wound deteriorated with increased exudate and tenderness. The patient was started on terbinafine in a Sanguitec Lipogel base on 17 May 2010. The pain diminished over the first week and the patient demonstrated complete re-epithelialisation by 28 June 2010.

• **Case 3** An 85-year-old white female with no other major medical problems presented with pyoderma gangrenosum in 2007 (Fig 4). The wound, on the patient's right lateral ankle, had waxed and waned for several years. There was an exacerbation on 10 March 2010 with significant erythema, tenderness and exudation. A culture on that date revealed *C. albicans* comprising 100% of the fungus present and only a small amount of *Staphylococcus epidermidis* were present on bacterial analysis. The patient was started on ketoconazole plus vancomycin in a Sanguitec Lipogel base.

At the following visit, the patient reported decreased pain and erythema as well as reduced exudate and oedema were noted clinically. The patient's wound was then re-evaluated diagnostically, which showed *C. albicans* remained present but had declined in concentration on the relative diagnostic scale. Ketoconazole was continued and the patient continued to show steady improvement until nearly complete resolution.

Conclusion

The fungal composition of biofilm infections is an important factor to consider when diagnosing chronic wound infections. Although *Candida* species were the most ubiquitous in this study, several other genera of fungi play an important role in multispecies biofilm opportunistic infections. Due to this diversity and limitations in previous or traditional identification techniques, comprehensive, quantitative molecular diagnostic methods are the most efficient and effective ways to appropriately identify and characterise the complex bacterial and fungal components of such polymicrobial infections. As more wounds are examined using these diagnostic techniques, organisms not previously associated with clinical infections will be reported and previously unknown and uncharacterised fungi not previously associated with human infection will be identified. Fungal species not currently recognised as contributing pathogens may ultimately be elucidated as important contributors in the composition of biofilms and polymicrobial wound infections as well as their recalcitrance to healing. The concepts of pathogenesis must be revised from the overly simplified one-organism paradigm to fully embrace and account for the synergistic pathogenic effects of polymicrobial infections and biofilm infections. ■

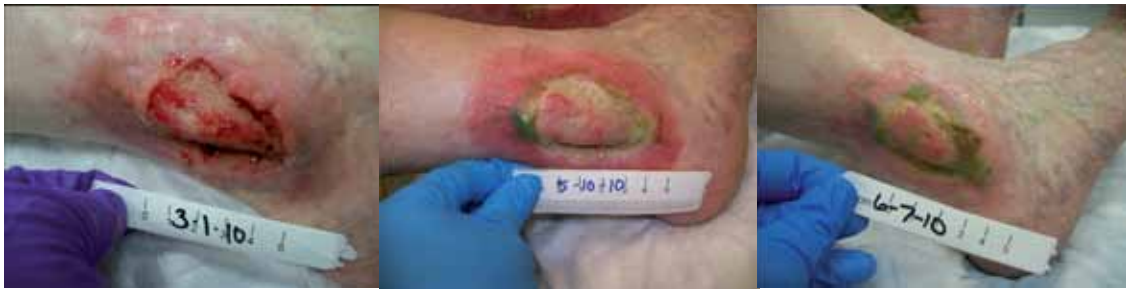

Fig 4. Case study 3: *Candida albicans* pyoderma gangrenosum infection that was continuing to deteriorate. On correct diagnosis of a fungal component, the infection was controlled and the wound healing progressed rapidly

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