Aim: Increasingly biofilms have been linked to delayed healing in chronic wounds. The slow growth of mature biofilms and host components (protein, red blood cells and serum) inhibit antimicrobial activity. In this study two wound dressings were evaluated in an in vitro model against mature biofilms grown in both simple and clinically relevant growth medium.

Method: Antibiofilm efficacy of silver hydrofibre dressing* antibiofilm (SHFAB) and Cadexomer iodine (CI) dressings was assessed in an in vitro biofilm model using wound simulation media (WSM) containing damaged tissue protein, red blood cells and serum, and a simple medium (n=4 for each dressing on each media type). Slow growing mature biofilms (72 hours old) of Staphylococcus aureus and Pseudomonas aeruginosa were challenged with the dressings for 24 and 72 hours. Antibiofilm effect was then assessed by replicate plating.

Results: CI demonstrated good antibiofilm activity (both organisms) in the simple media, and against S. aureus in the WSM. SHFAB had no/ limited effect in all conditions.

Conclusion: Good antibiofilm effect was observed with the CI dressing compared to the SHFAB across all conditions. Although wound proteins provide a higher challenge to topical antimicrobials, activity was more prominently impeded in the silver dressing.

Previous clinical evidence indicates that CI has a significant effect on bioburden in chronic wounds, accompanied by higher healing rates compared to standard care, and reduced treatment costs and surgical revision requirements. Such evidence combined with known antibiofilm efficacy in vitro suggests a role for CI in successful treatment against biofilms in chronic wounds.

CI = IODOFLEX◊, SHFAB = AQUACEL™Ag+Extra,
◊Trademark of Smith & Nephew, ™All Trademarks acknowledged
* Hydrofiber™
Aim: To try to establish a template for the early diagnosis and correct management for necrotizing soft tissue infections.

Method: We discuss a number of 10 cases of complicated soft tissue infections admitted to our Plastic Surgery Unit between 2012 – 2014, taking into account the initial diagnosis, risk factors, treatment, complications and evolution.

Results / Discussion: The complicated skin and soft tissue infections usually present with erythema, tumefaction and pain affecting a patient with cronical diseases. In our series, the most frequent responsible patogen was *Staphylococcus aureus* and the worst prognosis was associated with *Streptococcus* infections, cirrosis and alcoholism. At least two operative procedures were required with local complications such as non-healing or dehiscence wounds, chronic pain or persistent edema. The LRINEC Score was concordant with the necessity of surgical management.

Conclusion: Cellulitis and necrotizing fasciitis are two overlapping entities also known as necrotizing soft tissue infections. For the surgeon this is a shrewed adversary, the clinical presentation being quite unspecific. Regardless it is essential to make an early diagnosis as the operative treatment can prove life-saving.
Aim: To analyze operative tactic and following wound management in patients with Fournier gangrene and abdominal wall necrotizing fasciitis in order to optimize both infection management and further closure of the soft tissue defects. To assess the role of newer options for wound management in these patients.

Method: Retrospective cohort study of the practice in our clinic/case series, including 11 consecutive male patients for the period February 2014 – February 2015.

Results / Discussion: Design of incisions varies widely between surgeons and depends on the extent of the infectious process. In general scrotum management is done via single midline incision (8 patients) or separate lateral incisions (2 patients). Abdominal wall is controlled also via one or two incisions. Marked tendency toward single curvilinear incision or small number of separate curved incisions was noted, that eases further closure by operative and non-operative measures.

Conclusion: Properly chosen access is of crucial importance for achieving complete debridement. It has to allow easy exploration of neighboring areas in case of infection progression and also have to be comfortable for discharge management and dressing. Contemporary dressings and negative pressure therapy (NPT) provide good options for shortening the duration of treatment, providing better comfort for the patient and achieving easier wound closure, reducing the number of required reoperations.
Aim: *Nocardia Farcinica* is a rare species of the *Nocardia* genus that causes localized and disseminated infections in immunocompromised patients. The literature is scarce, however, there is an increasing number of cases reported since first recognized as a human pathogen in 1975. These cases are helping guide the diagnosis and treatment. There have been reports of a 39% death rate associated with *N. Farcinica* despite aggressive antibiotic therapy, therefore, early diagnosis and appropriate treatment is paramount. Cutaneous manifestations have proven to be uncommon, but when present, the bacteria is difficult to eradicate particularly in open wounds.

Method: We present a case of a 66-year-old female with a history significant for rheumatoid arthritis, on chronic Plaquenil, who had a fibrinoid lesion excised from her right foot. Her post-operative course was complicated with dehiscence and development of a non-healing wound. Superficial cultures originally grew out *Staphylococcus Aureus* but the wound continued to drain despite antibiotic therapy. Further investigation revealed *N. Farcinica*. She was treated for six months with trimethoprim-sulfamethoxazole and healed.

Results / Discussion: This patient had a non-healing wound with cultures positive for *S. Aureus* initially resistant to multiple antibiotics. Her wound cultures were sent to a specialized medical research center where *N. Farcinica* was discovered as the bacteria contributing to her non-healing wound.

Conclusion: Nocardiosis is an uncommon gram-positive bacterial infection caused by strains of the aerobic genus *Nocardia*. It is poorly studied and sparsely documented. Our patient responded aptly to six months of oral trimethoprim-sulfamethoxazole deeming it an appropriate antibiotic therapy.
Aim: To investigate the sudden unexplained increase in mortality around 2nd post burn week in our burn center.

Method: A detailed study in 2008 on 100 burn patients was done. In this study a routine biopsy was send from burn wounds on the 7th, 14th and 21st post burn day for fungal culture. On the basis of a high incidence (12%) of fungal wound infection in this preliminary study, another study was done in 2010 and 2011. In this study of 24 months duration, wound samples for 90 patients in whom there was clinical suspicion of fungal infection were sent.

Results / Discussion: 32 of these patients were diagnosed with fungal wound invasion (FWI) thus establishing an incidence of 44% from suspected cases. Surprisingly C. Albicans was not grown in any patient whereas C. Nonalbicans grew in 90% samples, of which 33% grew C. Tropicalis and 40% grew C. Krusei. The increasing incidence of C. Nonalbicans infection especially C. Tropicalis and C. Krusei is alarming because unlike C. Sibicans which is found as a commensal, C. Ttropicalis and C. Krusei when isolated is always associated with deep fungal invasion with higher mortality and indicates nosocomial infection.

Conclusion: The outbreak of this new microbial infection in burn wound is a major challenge to any burn unit which requires a clear understanding of how and why they occur if they are to be prevented and controlled. Empirical broad spectrum antifungal treatment has to be started on clinical suspicion and same has to be escalated or de-escalated according to final culture report.
Aim: To assess the effects of the antimicrobial honey * on surgical site infection rates in women undergoing caesarean section (CS).

Method: All women presenting for CS were offered the antimicrobial honey* as a dressing to the wound as a single application when the wound was dressed at the end of the procedure. This was a retrospective cohort comparison service evaluation. All women were followed up and examined for surgical site infection for 30 days after CS. Infection rates in the antimicrobial honey* cohort were compared with infection control data of the Caesarean cohort in the previous time period.

Results / Discussion: A single application of the antimicrobial honey* dressing reduced SSI by 60% from a rate of 5.42%, n=590 to 2.15%, n=186, (p=0.042). The annual extrapolated potential saving to the NHS across the UK of using the antimicrobial honey* as a single-application achieving this level of wound infection reduction is £5,105,337.

Conclusion: The antimicrobial honey* offers a simple, clinically and cost-effective intervention to significantly reduce SSI in women undergoing Caesarean section and could potentially save considerable surgical infective morbidity in other patients undergoing surgery.

* Surgihoney (SH)
AQUACEL SURGICAL DRESSING REDUCES THE RATE OF SSI IN POST SURGICAL WOUND: OUR CLINICAL EXPERIENCE

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¹Aou Policlinico Universitario Di Messina; Plastic Surgery

Thursday, May 14, 2015

E-poster session: Infection

Aim: Surgical wound infections are a frequent complication of surgery with a high clinical and economic impact. Modern technology applied to the wound care has allowed us to better manage these complications due to the contribution of antibacterial sutures and silver containing Hydrofiber surgical wound dressing.

Method: From March 2013 to November 2014 two groups (the first one treated with a new silver containing Hydrofiber surgical wound dressing and the second one with traditional dressings) of surgical wounds (belonging to 4 CDC classes) were observed and the results were evaluated by clinical and microbiological assessment for a total of 28 patients undergoing surgery for abdominoplasty post-bariatric, incisional hernias complicated, syndrome of Fournier and revision scars. The first one treated with a new silver containing Hydrofiber surgical wound dressing and the second one with traditional dressings.

Results / Discussion: The data analysis of the two groups showed an advantage in terms of both infection prevention and comfort of the patients treated with silver containing Hydrofiber surgical dressing compared to the conventional dressing group. In the silver Hydrofiber group there was a decrease in bacterial load and a reduced incidence of dehiscence of the surgical wound especially in the highest CDC classes with improved patient comfort even under compression bandages compared to the conventional dressings group. All patients underwent to postoperative antibiotic treatment.

Conclusion: The 7 days “in situ” permanence of the silver containing Hydrofiber surgical dressing leads to better cost effectiveness when compared with conventional dressings.
Aim: The aim of this poster is to demonstrate how an audit following the implementation of a “Mothers’ Pathway” on a busy maternity unit demonstrated a significantly reduced number of readmissions for surgical site infection following caesarean section. The maternity unit had a higher than average SSI rate for mothers following caesarean section.

Method: The number of mothers being re-admitted to hospital following caesarean section with surgical site infection was audited following the implementation of the “Mothers’ Pathway” and compared to the same months for the previous year.

Pre-Admission /Induction = Antibacterial wash prescribed for mothers booked for a caesarean section or induced.

Theatre = Dedicated theatre personnel. Skin preparation changed to meet standards in general theatres, meeting national guidelines.

Post-Op Care = Changed post operative dressing. Dressing remains in situ for 5 days. All skin closures are removed on day 5 along with the dressing.

Other = Appropriate use of oral antibiotics.

Tissue Viability support provided by specialist team.

Results / Discussion: We compared the same 6 month in 2012 and 2014, before and after changes.

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<tr>
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<th>2012</th>
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<tr>
<td>Readmission for Surgical Site Infection (SSI)</td>
<td>20</td>
<td>14</td>
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<tr>
<td>Cases requiring surgical intervention</td>
<td>10</td>
<td>3</td>
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<td>Cases requiring anti-biotic therapy</td>
<td>8</td>
<td>11</td>
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Conclusion: As a result of implementing the new “mothers’ pathway” there was a reduction in the number of severe SSI which required surgical intervention. This improves the experience for new mothers and impacts on their health related quality of life.
Aim: To evaluate the efficiency of povidon-iodine (PVP-I) formulation in treating porcine biofilm-containing skin wounds and to verify applicability of the porcine biofilm model.

Method: Ten skin excisional wounds were cut on a minipig back. Subsequently, multispecies biofilm was implanted into the wounds. After three days post inoculation (p.i.), five wounds on one side of the back were treated with a PVP-I soaked gauze. The untreated wounds were covered with gauze wet with saline. Biopsies and swabs of the wounds were analyzed 7, 10, 14, 17 and 24 days p.i. to determine colony forming units of the implanted bacterial species. We have previously found genes that were significantly altered between biofilm-containing and control uninfected porcine wounds. Expression of the genes was analysed by qRT-PCR in the biopsies from the treated and the untreated wounds.

Results / Discussion: Due to the PVP-I treatment a decrease in bacterial counts was apparent in the swabs from the wound surfaces, yet there was no alleviation in bacterial burden in the biopsies. Also, the treated and untreated infected wounds did not differ (p > 0.05) in expression of the inflammation-related genes (IL-8, CCL-20, SAL-1, FOLR-2, CDO-1), antiapoptotic transcript (BCL2A1), oxidative-stress gene (SOD-2), matrix modifying gene (MMP-1) and components of extracellular matrix (COL1A1, LUM, ASPN).

Conclusion: The results suggest that the infected wound model was sensitive enough for antimicrobial testing. PVP-I acted on bacteria, although only superficially, and thus more powerful treatment than PVP-I is needed for biofilm eradication that would also result in faster healing.
Aim: To investigate the biofilm forming potential (BFP) and biofilm architecture of *Trichophyton rubrum*.

Method: The biofilm forming potential (BFP) of *T. rubrum* was performed using a standard microtitre plate method. To investigate the architecture and characteristics of *T. rubrum* biofilms a poloxamer biofilm model was employed. In this model *T. rubrum* was inoculated into 1ml tryptic soy broth (TSB) and incubated. After 24 hours samples were centrifuged. The pellet formed was then stained with 200µl of calcofluor white stain and live/dead viability stains. 100µl of the suspension was then added to 900µl poloxamer (biofilm inducer state) or agar (quasi biofilm state). 100µl samples of the suspension were added to glass microscope slides and incubated at 35°C (±3°C). Daily samples were viewed with light microscopy and confocal laser scanning microscopy (CLSM). In addition total viable cell counts were performed to establish the growth rate of the fungus over time.

Results / Discussion: *T. rubrum* was shown to be an avid biofilm former (microtitre plate method). Furthermore *T. rubrum* demonstrated its ability to form mature biofilms within 72 h (poloxamer biofilm model). Under CLSM the *T. rubrum* biofilm was found to contain extensive biomass and extracellular polymeric substances (EPS). In addition there was evidence of a coordinated network of hyphae which architecturally was dissimilar to growth of *T. rubrum* in agar.

Conclusion: Understanding the structure and growth characteristics of biofilms formed by *T. rubrum* may help to contribute to the development of new antibiofilm agents that are appropriate for skin and wounds colonised with *T. rubrum*. 
A NOVEL IN-VITRO MODEL TO INVESTIGATE THE FORMATION, REGROWTH AND DESTRUCTION OF BIOFILMS ON WOUND DRESSINGS

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Thursday, May 14, 2015
E-poster session: Infection

Aim: To develop a novel in-vitro model that can be used to investigate the formation, regrowth and destruction of biofilms on and within wound dressings.

Method: Three 1cm² samples of different wound dressings (fibre, foams etc.) were inoculated with 200µl of 1 x 10⁶ cfu/ml of Staphylococcus aureus and/or Pseudomonas aeruginosa and/or Candida albicans. Each dressing was then incubated at 35°C (±3°C) for 1hr to allow for irreversible adhesion and biofilm growth. After 1hr, each dressing was flushed with sterile water for 30 sec and placed within a 2ml syringe so that the dressing samples filled up to 0.5ml of the syringe. A needle and tubing was connected to the syringe and tryptic soy broth (TSB) only was slowly dripped (a continuous flow model – to simulate exudate flow) through each biofilm ‘infected’ wound dressing at a rate of approximately 16ml/hr. Over the seven day test period the numbers of microbes disseminating from each wound dressing was taken (specific growth rates and doubling times were calculated). In addition confocal laser scanning microscopy (CLSM) images were taken.

Results / Discussion: It was found that all wound dressings supported the growth of biofilm. A difference between wound dressing types and biofilm development was evident. Furthermore the dissemination of microorganisms from all the dressings evaluated increased significantly over time indicating growth of the biofilms in all wound dressings.

Conclusion: Dissemination of microbes, still within the biofilm state, from wound dressings increased over time. Dissemination of microbes from wound dressing biofilms into the wound bed will increase the wounds microbial bioburden and increase the risk of infection.
Introduction: Pseudomonas aeruginosa is one of the most problematic wound pathogens. The blue-green pigment pyocyanin is one of its many virulence factors, with toxic effects on the wound tissue. When P. aeruginosa is present in a wound, the wound dressing may be visibly stained by pyocyanin, and the question has been raised as to whether this green coloration indicates that the dressing material promotes bacterial growth.

Aim: The aim of this study was to investigate, in vitro, any correlation between P. aeruginosa growth in the presence of a dressing and pyocyanin staining of the dressing material.

Method: The growth and pyocyanin production by P. aeruginosa (PAO1) cultures were studied in the presence of commercially available wound dressings of different materials and brands. Bacterial numbers were quantified by viable plate counts, and pyocyanin was measured by absorbance spectrophotometry.

Results / Discussion: No difference in bacterial growth was observed in the presence of any dressing. The amount of pyocyanin increased over time in all cultures, however the cultures with dressings always resulted in lower pyocyanin concentrations than the control. The lowest levels were found in cultures surrounding the polyurethane foam dressings in which pyocyanin was found to accumulate.

Conclusion: There is no evidence that any of the examined materials promote growth of P. aeruginosa. Instead one may speculate that in the clinical setting, the more pyocyanin that is found in the dressing, the less of this harmful virulence factor will be left in the wound tissue. Also, the more visible the pyocyanin, the easier infection could be detected.
Aim: Patients with Diabetes Mellitus have an increased risk of infection due to immunopathy and it carries a significant morbidity if it involves the hands. This study is to investigate the clinical characteristics and surgical outcomes of upper limb infections in patients with Diabetes Mellitus.

Method: One year retrospective review of patient with diabetes admitted to our orthopaedic ward for upper limb infections requiring intravenous antibiotics and/or surgical intervention.

Results / Discussion: Twenty one patients fits the inclusion criteria of whom majority are woman (71%) with average age of 50 years old. More than a quarter (29%) out of them were newly diagnosed diabetes. Patient with established diabetes had average duration of 5.5 years after initial diagnosis. The average Glycosilated Hemoglobin (HbA1C) level was 11%. On average, patient had onset of symptoms of 10 days before presenting to the hospital. Upon presentation, only one patient (5%) had fever with majority (67%) of site affected involving the hands. For patients requiring operative intervention, the average waiting time was 2.2 days and average hospital stay was 8.8 days. Three patients required radical intervention i.e amputation or disarticulation due to uncontrolled infection and delayed presentation. Culture studies did not yield any causative organism in 31% of patients.

Conclusion: Serious morbidity of the upper limbs can be caused by unawareness and delayed presentation of infections involving upper limbs in diabetic patients. This study further highlight the importance of good glycemic control and patient education regarding the condition.
Aim: Extracellular matrix (ECM) molecules play an integral role in the process of skin wound healing. In a non-healing chronic wound the surrounding tissue environment changes, due to impaired ECM synthesis, the presence of bacterial biofilms and an increasingly alkaline pH. The aim of this study was to determine any differences in the release of ECM molecules from fibroblasts when cultured in different pH and bacterial conditioned media.

Method: *S. aureus* and *P. aeruginosa* were cultured in DMEM media overnight before diluting to equivalent of $10^8$ CFU/mL. Bacteria were removed and used at planktonic CM. *S. aureus* and *P. aeruginosa* biofilms were grown before then being cultured in DMEM media for 24 hours. Media was then collected and filtered and used as biofilm CM. Normal skin fibroblasts (NF) and chronic wound fibroblasts (CF) were cultured for 24 hours in pH-adjusted DMEM media (6, 7.5 and 9) or in pH-adjusted planktonic and biofilm conditioned media (CM). Cell media was collected after 24 hours and analysed for the presence of ECM molecules.

Results / Discussion: Synthesis of collagen and fibronectin was decreased in the presence of bacteria which were further reduced in alkaline conditions. However, glycosaminoglycan synthesis was significantly increased in pH9 and bacterial conditions.

Conclusion: An alkaline pH environment, similar to that found in chronic wounds, had negative effects on the synthesis of ECM molecules from CWF cells. Shifting the pH of a chronic wound towards a more acidic environment and eliminating any bacteria present, should improve wound healing.
Aim: To assess whether a bacteriological analysis of a wound swab may be supportive to the clinical assessment of an infected chronic wound.

Method: Patients attending an outpatient wound clinic and having a chronic wound for more than 3 weeks were clinically assessed for the presence or absence of signs and symptoms of chronic wound infection as presented in the international consensus document of the World Union of Wound Healing Societies (WUWHS). Health care professionals at the wound clinic were specifically trained in using these criteria.

In addition, a standardized wound swab according to the Levine technique was taken by 3 professionals. Chronic wounds clinically assessed as infected and non-infected were compared with the qualitative and quantitative microbiological findings of the swabs taken.

Results: The clinical assessments of the chronic wounds were not significantly related to the qualitative or quantitative bacteriological results of the swabs taken. More in depth analysis of the clinical assessments of patients suffering from diabetes mellitus or peripheral arterial disease showed the same results.

Conclusion: A swab may be representative for the type and amount of planktonic bacteria present in a chronic wound, but maybe does not reflect the type and number of bacteria present in a biofilm sufficiently. When a swab appears to be not supportive to the clinical assessment of a chronic wound infection, the suggested clinical criteria by the WUWHS to diagnose chronic wound infection can be questioned.
[EP193] BACTERIAL PROTEASE ACTIVITY IN CHRONIC WOUND FLUID, A POTENTIAL INDICATOR OF PATHOGENICITY EVEN IN THE ABSENCE OF OVERT SIGNS OF INFECTION

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¹Woundchek Laboratories
²Serena Group

Thursday, May 14, 2015

E-poster session: Infection

**Aim:** Pathogenic bacteria produce proteases, virulence factors, in the process of invading their host. Bacterial protease activity (BPA), therefore, may be a useful method of detecting the presence of pathogenic bacteria in wounds.

**Method:** 366 patients with chronic wounds from 6 wound care centres in the United States underwent assessment for the signs of critical colonisation/infection using validated assessment criteria. The wounds were swabbed to assess bacterial protease activity levels and quantitative bacterial load.

**Results / Discussion:** Approximately 70% of wounds had more than $10^5$ colony forming units per ml. However, only 18% of wounds exhibited signs of critical colonisation/infection. In contrast, nearly half of all wounds swabbed had elevated bacterial protease activity. Of these BPA positive wounds more than half had no signs of critical colonisation/infection. We suspect that these wounds had pathogenic bacteria but had not yet progressed to overt infection.

**Conclusion:** Increased bioburden in wounds impairs healing and can lead to systemic infection. Some chronic wounds fail to exhibit the classic signs of infection. As a result, clinical examination can under-diagnose infections in chronic wounds. Moreover, current culture techniques have limited reliability on their own, frequently leading to the over-diagnosis of infection. Testing wound fluid for bacterial protease activity may be a useful method for detecting the presence of pathogenic bacteria, at a clinically significant stage in the infection continuum, even before the signs of infection are apparent.