

## **Oxygen Dependent Killing of Bacteria in Wounds of Diabetic Patients**

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### **Abstract**

*In the present study diabetic patients of both sexes at an aged ranging between 47 to 58 years were treated for wound infection on their feet due to diabetes mellitus. The patients were treated for five days twice a day in an oxygen rich foot bath. The oxygen foot bath had good effect on bacteria localized in the wound on feet since 66% of the patient totally recovered from bacterial infections on their feet. Furthermore, witnesses from the included subjects and their physicians clearly point at a patient amelioration.*

### **Introduction**

Diabetic foot ulcers occur as a result of various factors, such as mechanical changes in conformation of the bony architecture of the foot, peripheral neuropathy, and atherosclerotic peripheral arterial disease, all of which occur with higher frequency and intensity in the diabetic subjects compared to the normal population.

Diabetes causes nonenzymatic glycation predisposes ligaments to stiffness. Neuropathy in diabetic subjects causes loss of protective sensation and loss of coordination of muscle groups in the foot and leg, both of which increase mechanical stresses during ambulation.

Diabetic foot lesions are responsible for more hospitalizations than any other complication of diabetes. Diabetes is the leading cause of non-traumatic lower extremity amputations in the World, with approximately 5% of diabetics developing foot ulcers each year and 1% requiring amputation.

Physical examination of the extremity having a diabetic ulcer can be divided into examination of the ulcer and the general condition of the extremity, assessment of the possibility of vascular insufficiency, and assessment for the possibility of peripheral neuropathy (Hsiao-Chuan L, et al. 2014).

The staging of diabetic foot wounds is based on the depth of soft tissue and osseous involvement. A complete blood cell count should be done, along with assessment of serum glucose, glycohemoglobin, and creatinine levels (Guideline, 2014, International Expert Committee 2009, Vehik K, Beam CA, Mahon JL, et al. 2011).

A vascular surgeon and/or podiatric surgeon should evaluate all patients with diabetic foot ulcers so as to determine the need for debridement, revisional surgery on bony architecture, vascular reconstruction, or soft tissue coverage.

In the present study we show an alternative way of treating foot wounds and the method can be used to avoid deep soft tissue wounds by preventive use of oxygen rich foot bath.

## **Material and Methods**

### **Patients**

This study was limited to infected wounds of 15 diabetic patient presented to a diabetic clinic in Khartoum, Sudan between January and February 2015. The age of the patients range from 47 to 58 years and both sexes were represented. Determination of infection was made clinically by looking for signs of inflammation. None of the patients has taken any antibiotic for the last 30 days prior to the study. No other history of illness besides diabetes mellitus was known when the study started.

### **Treatment of Subjects**

The patients in this pilot study were treated by foot bath with 2% of BioCool® twice a day for 5 days. When the results obtained from this first group of subjects indicated that we had achieved the objective of the study, the study was terminated. The BioCool® can be used at a concentration between 0.05 to 4.00 % deepening on which microorganism killing is aimed at. The temperature of the foot bath should be 37<sup>0</sup> C for optimal effect. This has previously been tested and 2% have been found to be optimal for killing of all types of microorganisms both pro and eukaryotic ones (Sandström G., Eriksson J-O., and Saeed A., 2014).

### **Material**

Substance: The test substance (BioCool®) was obtained from the company BioCool AB, Skellefteå, Sweden. Hereafter described as test substance. The test substance has been described on the open market as BioCool®. The mechanism by which the microorganism is killed is a chemical reaction resulting in singlet oxygen. Singlet oxygen is known from other biological systems to have killing effect on microorganisms (Kniel et al., 2003) . The substance has previously been tested and found to kill *Escherichia coli* (Nandi et al., 2010). The Swedish governmental owned company FOA-test performed the test. In the present study test substance was tested at concentration of 2%.

### **Specimen Collection**

Two cultures were taken from every wound, before and after treating with the substance. The wound and area surrounding it was cleansed with sterile physiologic glucose solution by means of a sterile compress passed over the wound surface to reduce the amount of contaminating bacteria. The swab was held in contact with the wound for at least 5 s before any debridement was done. At the end of debridement, a deep tissue was taken. Deep tissue was defined as the last non-viable tissue removed from the deepest area of the wound.

All swabs were placed in a tube and delivered to the microbiology laboratory was usually made within 30 min. Cultures were performed by standard procedures. Bacterial isolates were identified to the species level.

### **Results**

In table 1 it is described the outcome of treatment of patients with the substance. Out of the group of subjects treated with the substance 66 % showed no growth of bacteria at all although all of them had since and symptoms of deep infection wounds to start with. The other 24 % of subjects showed a clear decrease in bacterial growth after treatment although not a complete lack of bacteria in the wounds. The normal flora of the subjects was not affected dramatically since the normal flora was fully recovered when treatment was stopped. Moreover no difference between sexes could be found and no effect failure due to age was noted.

Patient 7-15 had the following bacteria *Staphylococcus aureus*, Beta haemolytic streptococcus, *Pseudomonas aeruginosa* and *Klebsiella* spp. These patients were excluded from the study since for one way or another they had to stop treatment for the study. Such reasons could be risk of gangrene, antibiotic treatment or amputation of legs.

**Table 1**

No. of Patient	Sex	Age	Before treating with the substance	After treating with the substance
1.	F	58	Staphylococcus aureus Pseudomonas aeruginosa	No growth
2.	M	53	Staphylococcus aureus Beta Haemolytic streptococcus	Beta Haemolytic Streptococcus
3.	F	51	Coagulase-negative Staphylococcus aureus and enterococci	No growth
4.	M	50	Staphylococcus aureus Pseudomonas aeruginosa	No growth
5.	F	47	Klebsiella spp.	No growth
6.	M	44	Staphylococcus aureus Proteus spp.	Staphylococcus

### **Discussion**

Hyperbaric oxygen therapy (HBOT) is a medical treatment in which the patient breathes 100% oxygen inside a pressurized treatment chamber. The aim is to increase the oxygen level in the tissue. It is used in cases where partial or total tissue hypoxia occurs such as acute limb ischemia, diabetic foot lesion, and arterial ulcers.

Limb-threatening diabetic foot infections are usually polymicrobial. Commonly encountered pathogens include methicillin-resistant staphylococcus aureus,  $\beta$ -hemolytic streptococci, enterobacteriaceae, pseudomonas aeruginosa, and enterococci. Anaerobes, such as bacteroides, peptococcus, and peptostreptococcus, are rarely the sole pathogens but are seen in mixed infections with aerobes. Antibiotics selected to treat severe or limb-threatening infections should include coverage of gram-positive and gram-negative organisms and provide both aerobic and anaerobic coverage. Patients with such wounds should be hospitalized and treated with intravenous antibiotics (The DCCT Research Group. 1993, Boulton AJ, Malik RA, Arezzo JC, Sosenko JM., 2004, Armstrong DG, Lavery LA, Kimbriel HR, Nixon BP, Boulton AJ., 2003., Armstrong DG, Lavery LA, Wu S, Boulton AJ., 2005)

Mild to moderate infections with localized cellulitis can be treated on an outpatient basis with oral antibiotics such as cephalexin, amoxicillin with clavulanate potassium, moxifloxacin, or clindamycin. The antibiotics should be started after initial cultures are taken and changed as necessary.

The substance (BioCool®) is here shown to be an alternative treatment of infected feet. The effect of the substance has been disclosed to be optimal at 2% but from 0.05 to 4% the substance at 37°C can be used as previously shown. If the oxygen bath will be used on a daily basis a concentration of 0.05 % is recommended (Sandström G., Eriksson J-O., and Saeed A., 2014). Not only affected nails and warts are showing dramatic improvement but also skin and feet in general are refined (Sandström G., Eriksson J-O., and Saeed A., 2014) Moreover, witnesses from the included subjects and their physicians clearly point at a patient amelioration.

### **Acknowledgement**

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