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An electrochemical sensor for fast detection of wound infection based on myeloperoxidase activity

Martin Hajnsek[a,1], Doris Schiffer[b,f,*,2,6], Daniel Harrich[a,1], Daniel Koller[c,3], Vanessa Verient[b,2], Job v.d. Palen[d,4], Andrea Heinzle[b,2], Barbara Binder[e,5], Eva Sigl[b,2], Frank Sinner[a,1], Georg M. Guebitz[f,6]

[a] *Joanneum Research Forschungsgesellschaft mbH HEALTH, Neue Stiftingtalstrasse 2, 8010 Graz, Austria*
[b] *Austrian Centre of Industrial Biotechnology, Petersgasse 14, 8010 Graz, Austria*
[c] *Department of Molecular Bioscience, Humboldtstr. 50, 8010 Graz, Austria*
[d] *Medical School Twente, Medisch Spectrum Twente and Department of Research Methodology, Measurement, and Data Analysis, University of Twente, 7513 ER Enschede, The Netherlands*
[e] *Department of Dermatology, Medical University of Graz, 8010 Graz, Austria*
[f] *Institute for Environmental Biotechnology, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Straße 20, 3430 Tulln an der Donau, Austria*

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ABSTRACT

Infection can lead to severe complications during wound healing. We have developed an electrochemical sensor for fast and simple detection of wound infection based on the quantification of myeloperoxidase activity as a marker for infection.

Applicability of the enzyme was confirmed with a correlation study with silver standard wound diagnostics. Significant higher enzyme activities comparing non infected and infected wound fluids were determined ($P = 0.01$). To eliminate supplemental substrate addition, the chlorination activity of the enzyme – the formation of hypochlorous acid (HOCl) from chloride and hydrogen peroxide – was investigated in different wound fluids and correlated with the peroxidation activity measurements. Significant activity differences were likewise obtained ($P = 0.01$). Based on this we constructed an electrochemical hydrogen peroxide sensor system for the quantification of chlorination activity in wound fluids. Furthermore, immobilized glucose oxidase was integrated into the system to provide hydrogen peroxide required by myeloperoxidase.

Infected wound fluids were indeed identified by using the sensor system quantifying the consumption of hydrogen peroxide consumed by myeloperoxidase. Thereby, immobilized glucose oxidase was shown to produce enough hydrogen peroxide for the myeloperoxidase reaction from glucose present in wound fluids. There is a strong need for a simple but effective sensor system to determine infections in wounds. This sensor measuring hydrogen peroxide consumption could effectively identify infected wound fluids based on the myeloperoxidase activity.

* Corresponding author at: Austrian Centre of Industrial Biotechnology, Institute for Environmental Biotechnology, Konrad-Lorenz-Straße 20 3430 Tulln an der Donau, Austria Tel.: +43 2272/66280 562; fax: +43 2272 66280 503.

E-mail addresses: martin.hajnsek@joanneum.at (M. Hajnsek), doris.schiffer@acib.at (D. Schiffer), daniel.harrich@gmx.at (D. Harrich), daniel.koller@uni-graz.at (D. Koller), vanessa-verient@gmx.at (V. Verient), J.vanderPalen@mst.nl (J.v.d. Palen), andrea.heinzle@acib.at (A. Heinzle), barbara.binder@klinikum-graz.at (B. Binder), eva.sigl@gmx.net (E. Sigl), frank.sinner@joanneum.at (F. Sinner), guebitz@boku.ac.at (G.M. Guebitz).

[1] Tel.: +43 316 876 4123; fax: +43 316 8769 4123.
[2] Tel.:+43 316 873 9319; fax: +43 316 873 8815.
[3] Tel.:+43 316 873 9319; fax: +43 316 873 8815.
[4] Tel.: +53 489 3574.
[5] Tel.: +43 316 385 13055.
[6] Tel.: +43 2272 66280 501; fax: +43 2272 66280 503.

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1. Introduction

Infection is a common problem in chronic wounds resulting in prolonged hospital stays, non-healing wounds and increasing mortality of the patients. For example about 1% to 2% of individuals are estimated to suffer from leg ulceration during their lifetime requiring a substantial portion of the health care budget and the number is likely to increase as the population ages [1–4]. The correct identification of infection is a complex issue as not all clinical signs such as redness (*rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*) and impairment of function (*functiolaesa*) can be consistently observed. The concept of critical colonization is also controversial and not universally accepted [4–6]. Diverse guidelines have been developed to prevent upcoming infections [7,8]. Clinical examination by itself is unreliable for the diagnosis of wound infection. Quantitative biopsies of ulcers showed that 28% of participants had bacterial counts greater than 10^5 but were lacking any clinical signs of infection [9]. Despite ongoing research with a focus on markers for wound healing, most of the available sensors still rely on bacterial biochemistry, odor, temperature or pH changes which are either time-consuming or unspecific [10–14]. Wound fluid *per se* has an enormous potential for fast detection of infection and for monitoring wound healing [15–18]. As the recruitment of neutrophil granulocytes is one of the earliest events in wound repair, the analysis of neutrophil-derived enzymes has a new perspective in wound status monitoring [19,20]. We present a new sensor supported strategy for fast diagnosis of wound infection based on the detection of neutrophil derived myeloperoxidase (MPO). MPO is a heme containing protein that uses hydrogen peroxide (H_2O_2) to oxidize not only chloride ions yielding antimicrobial HOCl, but also phenolic compounds like guaiacol [21–23]. Since the recruitment of neutrophils increases with bacterial invasion, the enzyme MPO-activity indicates the infection status at a very early stage [24,25]. We used amperometric detection of H_2O_2 to determine the MPO chlorination activity in wound fluids. MPO requires H_2O_2 which was supplied by *in situ* generation by immobilized glucose oxidase to avoid instable H_2O_2 containing system reagents. Devices for fast diagnosis of infections are applicable in medical centers focussed on dermatological illnesses as well as in home care and retirement homes. They address not only long term ulcer wounds observed frequently but also wounds after amputations or decubitus wounds as well as post operative wounds. A fast and easy applicable device should facilitate the recognition of an infection in a wound and therefore allow adequate wound treatment to avoid sepsis.

2. Methods

If not stated otherwise all chemicals were purchased from Sigma Aldrich, MO, USA.

2.1. Sample collection and preparation

Wound fluids from post-operative wounds, ulcer and decubitus wounds were collected by swabbing the wound bed with a nylon swab (Microrhelogics, Brescia, Italy) followed by dilution in 0.9% sodium chloride (NaCl) for further analysis. Uninfected wound fluids from blisters served as negative controls. Permission to collect wound fluid was obtained from the Ethics Committee of the Medical University of Graz, Austria..

2.2. Microbiological analyses

Before sample collection, wounds were cleaned with 0.9% NaCl (Sigma Aldrich, MO, USA) to remove superficial bacteria. Swabs were taken of the most contaminated and/or deep site of the wound bed and/or wound edges. The samples were analyzed with MALDI TOF techniques, addressing the analyses of the occurring species, as well as gram staining dependent microscopy evaluations were performed to determine the bacterial load [26]. After gram staining, the slides were screened (magnification 1000×) and the bacterial count was reported. The semi quantitative reporting system was subdivided from +(<1) to +++(>100) counts per ocular field [27,28]. The microbiological test results were categorized as “infected”, “critical”, or “good healing”, by looking for the presence of potential pathogenic microorganisms (ppmos) relative to the general microbiological flora. The following definitions were used:

The results were evaluated by a microbiologist. Additionally an experienced medical doctor also examined the wounds following standard diagnostic procedures. *E.g.* a good healing wound was characterized due to size reduction of the wound bed as well as a good granulation and epithelization.

2.3. Myeloperoxidase activity

For the detection of MPO-peroxidation activity 10 μL of wound fluid or MPO solution was mixed with 290 μL substrate solution containing 99 mM guaiacol and 0.017% (w/w) H_2O_2 in 50 mM potassium phosphate buffer pH 7.0 [25]. Enzyme standards containing 1.0 to 5.0 U/mL MPO (Human Myeloperoxidase, Planta, Vienna, Austria) were used. The formation of tetra-guaiacol was measured every 1 s for 100 s at 470 nm in 96 well plates using a Tecan infinite M200 platereader (Tecan, Maennedorf, Switzerland).

Detection of MPO-chlorination activity was based on trapping of HOCl formed from chloride and H_2O_2 by using taurin to produce stable taurinechloramine (MPO activity assay, Northwest, Vancouver, Canada) [29]. Taurinechloramine then reacted with 5-thio-2-nitrobenzoic acid forming colorless end products, resulting in a reduction of measurable absorbance with increased production of taurinechloramine, measured at 412 nm in 96 well plates.

2.3.1. MPO activity in the presence of glucose oxidase

Glucose Oxidase (GOD, from *Aspergillus niger*) was used to generate H_2O_2 which is required by MPO to produce HOCl. A 300 mM β-D-glucose solution (Glucose, Roth, Karlsruhe, Germany) was prepared in 100 mM potassium phosphate buffer at pH 7.0, and stirred for at least 3 h to gain oxygen saturation. GOD was diluted in 100 mM potassium phosphate buffer to concentrations of 0 U/mL, 10 U/mL, 20 U/mL and 30 U/mL. MPO (Sigma Aldrich, MO, USA) was diluted to concentrations of 2 U/mL. For the measurements, 100 μL of the GOD containing buffers were incubated with 100 μL of MPO buffers (2 U/mL; containing 61.5 mM guaiacol and 300 mM β-D-glucose) and peroxidation activity was measured in triplets in 96 well plates. The reaction was monitored over 45 min at 470 nm.

2.4. Glucose measurement

Glucose levels of 10 wound fluids, classified as infected, critical and good healing by a microbiologist and additionally by an experienced medical doctor, were determined according to the protocol provided by ABCAM (Cambridge, UK; Glucose detection kit, ab102517). In this assay, glucose is specifically oxidized to a colored product which was quantified spectrophotometrically at $\lambda = 450$ nm. The kit can detect glucose concentrations in the range of 20 μM–10 mM. The measurements were performed in 96 well plates using a Tecan infinite M200 platereader (Tecan, Maennedorf, Switzerland).

2.5. Sensor

A screen-printed amperometric H_2O_2 sensor was used to assess MPO activity in wound fluid samples. The sensor was used in a three electrode setting comprising a silver/silver chloride (Ag/AgCl)