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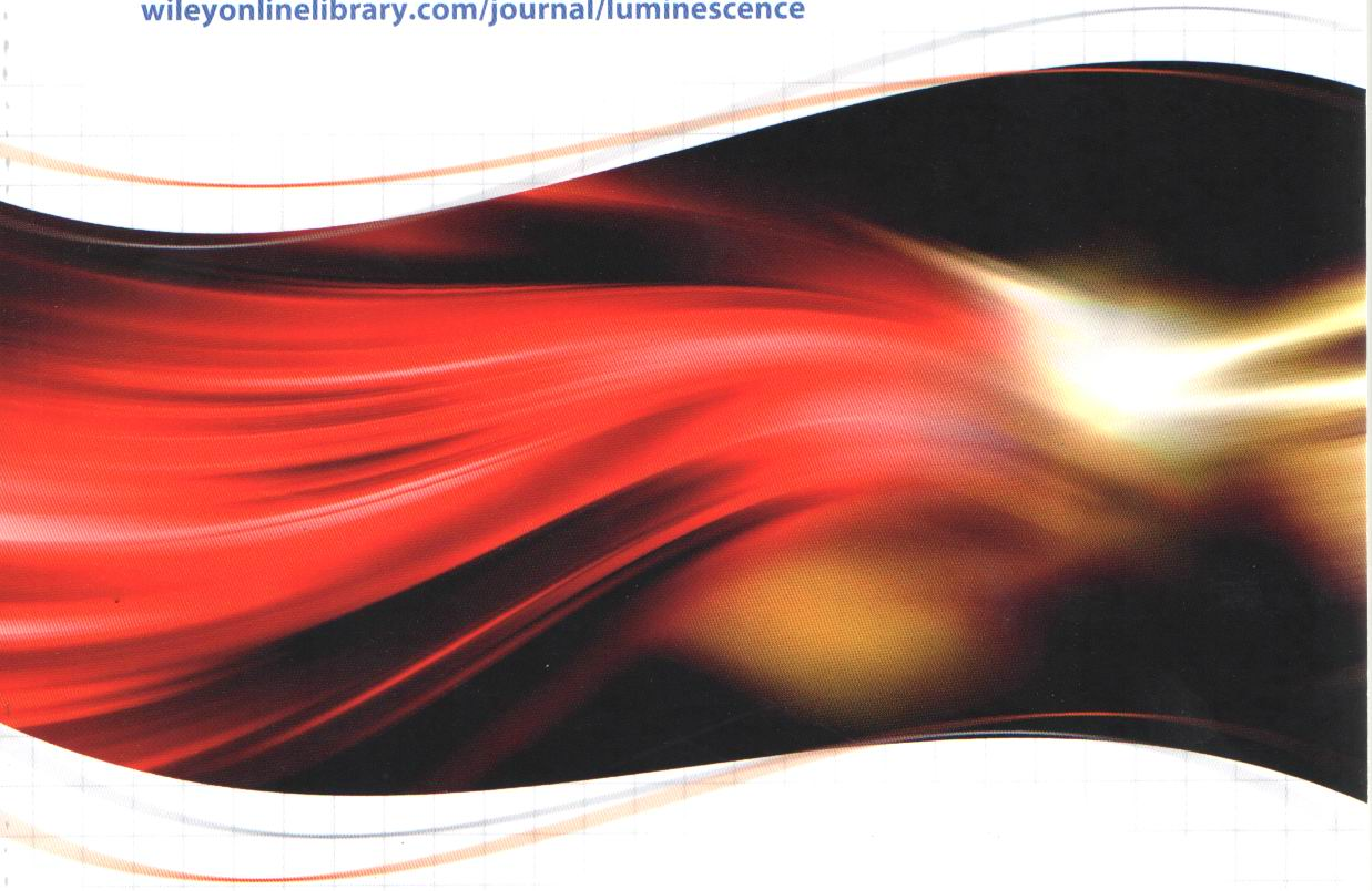

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O0038

Protective effect of deferoxamine, as a potential anti-inflammatory chemical, on ROS-mediated cell and DNA damage in chemiluminescence systems

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Inhibition of iron-mediated generation of reactive oxygen species (ROS) by a synthetic chemical might help treat many (non)infectious diseases in humans and animals. Deferoxamine (DFO) would be one of the chemicals of choice for that purpose; as a naturally occurring sideramine, the DFO is specifically used to treat patients with iron-mediated disorders and inflammatory diseases; medical application of DFO is undoubtedly increasing. In the first study, ROS scavenging effect of DFO against superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) was examined by chemiluminescence (CL) assays; effect of DFO on the kinetics of some CL systems was also studied. In the second study, the protective effect of DFO on DNA and neutrophils was investigated using agarose gel electrophoresis and CL systems, respectively; using cellular and acellular CL assays the effects of DFO was assessed on: CL from added OCl^-, flow cytometry assays of necrosis, apoptosis, CL-based bactericidal capacity of neutrophils and phagocytosis and killing of *Escherichia (E.) coli* and *Staphylococcus (S.) aureus* by neutrophils. Briefly, isolated bovine blood neutrophils were exposed with 0 and 0.1 mM of DFO for 1 and 18 h depending on the assay. Further, blood neutrophils were exposed with the DFO for 3 h and phagocytosis and killing activities against *S. aureus* and *E. coli* were examined. Our study strongly confirms the scavenging effects of DFO on $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ in the luminol and orthophenanthroline CL systems. Maximal quenching capacity of DFO was observed in Fenton's reaction where participation of catalyst, iron ions, to the CL system is central. DFO also strongly protected DNA from ROS-mediated damages. Mechanistically, the observed quenching effect of DFO on ROS clearly revealed the static part of quenching properties of DFO for ROS generation systems. Though the effect of DFO on extracellular ROS greatly diminished, the DFO-treated neutrophils showed a remarkably increased phagocytosis-depended luminal CL. No neutrophils necrosis and apoptosis was induced by DFO. Phagocytosis rates and killing of *E. coli* and *S. aureus* by DFO-treated neutrophils were significantly higher than those of non-treated ones. Our results show the extracellularily anti-oxidant and pro-phagocytic properties of pharmacologically relevant level of DFO for bovine neutrophils. Application of bovine neutrophils as a cellular model for CL system revealed that DFO behaved differently in cellular and acellular CL. The scope of the enhancing effects of the *in vitro* DFO on neutrophil functions should be considered in mammals as a pharmacologically relevant chemical for clinical implications.

O0039

Bioluminescent Magnetotactic Bacteria as powerful Bioanalytical tool for Lab-On-A-Chip analysis

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The increasing need for rapid, robust and cost-effective toxicity screening systems led to the development of miniaturized analytical devices exploiting the potentiality of genetically engineered living cells for biosensing.

The incorporation of living cells within a miniaturized system offers the advantage of small reagent and sample volume requirements combined with the possibility to move the cells in different areas of the chip designed to carry out specific functions. However several critical issues still need to be addressed such as limit of detection, sensitivity and reproducibility [1,2].

In an effort to obtain bioreporters with enhanced analytical performance suitable to chip integration, we produced smart whole-cell biosensors using genetically engineered bioluminescent magnetotactic bacteria (BL-MTB). MTB, which have the ability to produce magnetosome chain, (i.e. magnetite nanoparticles enveloped in a phospholipid membrane) to orient according to an external magnetic field [3], have been genetically engineered to express bioluminescent reporter proteins and used as a whole cell biosensors based on reported gene technology.

As first proof of concept *Magnetospirillum gryphiswaldense* strain was genetically engineered to constitutively express the red-emitting click beetle luciferase (CBR, λmax = 610 nm) and used as general toxicity sensor.

A simple microfluidic chip has been fabricated by using multilayered polydimethylsiloxane (PDMS) constituted by three parallel diamond shape incubation chambers connected via microchannels to separate detection areas. BL-MTB were incubated with different cell-toxic compounds (e.g., bile acids, DMSO) and then transferred and magnetically trapped in the detection area which is placed in contact with a CCD sensor [4].

BL images were acquired upon addition of the D-luciferin substrate and light emission inhibition as a result of cell toxicity was evaluated with ImageJ software to obtain quantitative measurements.

Despite the analytical performance of BL-MTB is not yet competitive with BL microbial biosensors, our preliminary results represent the first step toward the obtainment of a new class of biosensors based on magnetic bioluminescent bacteria that can be integrated into microfluidic platforms and controlled by external magnetic fields [5].

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