Development of Enzyme Responsive Biosensors Based on Nanoporous Silicon Rugate Filters for the Detection of Bacterial Enzymes

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In recent years, porous silicon (pSi) has emerged as a promising material for (bio)medical applications, including drug delivery, tissue engineering and biosensing, due to its biocompatibility and its tunable nanoporous structure [1,2]. In particular, pSi electrodes as well as photonic structures offer attractive features in dye-free biosensing, e.g. for enzymes [3,4]. These may possess the potential for future (bio)medical applications, e.g., to detect bacterial infections via high local concentrations of hydrolytic enzymes secreted by bacteria in biofilms.

Here we report on the development of an enzyme sensor with visual read-out based on pSi Rugate filters. The Rugate filter was fabricated by electrochemical etching of highly doped silicon in hydrofluoric acid (HF) solution, followed by functionalization with an enzymatically degradable polymer [3,5]. The deposition and degradation of poly(lactic acid) (PLA) by the enzyme proteinase K was analyzed by reflectometric interference spectroscopy (RIFS) and afforded a red shift of the characteristic fringe pattern by 60 nm after filling the pores with PLA. A corresponding blue shift of 59 nm was observed after degradation of the PLA by proteinase K. Moreover, a clearly discernible color change of the Rugate filter from green to blue was discernible by bare eye after PLA degradation. The scope of the concept was expanded by using poly(ε-caprolactone) (PCL) instead of PLA, which is susceptible to enzymatic attack of lipase. In the future Rugates filters with patterned functionalities consisting of arrays of different degradable polymers may afford the multiplexed detection of several microbial enzymes and thereby allow for the fast identification and discrimination of the corresponding bacteria.

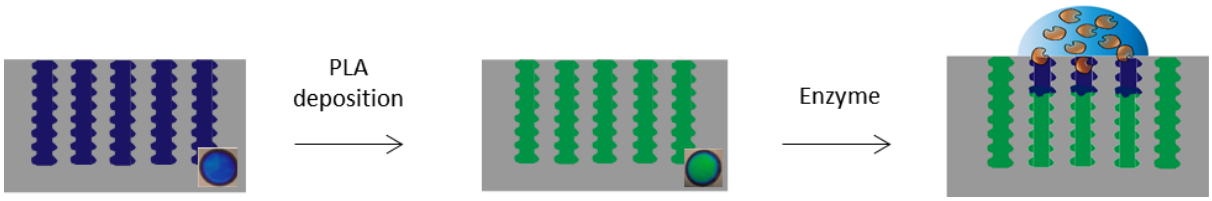

Figure 1. Color change of the Rugate filter indicates the deposition of PLA in the pores and the subsequent enzymatic degradation, which serves as a means to detect the enzyme.

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Acknowledgment: The authors thank Dr. Sergey I. Druzhinin and Dr. Daniel Wesner for many useful discussions as well as financial support by the German Academic Exchange Service (DAAD, Ph.D. stipend to QA). Part of this work was performed at the Micro- and Nanoanalytics Facility (MNaF) of the University of Siegen.