Sensing and Bio-Sensing Research 3 (2015) 65–73

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Fiber-optic protease sensor based on the degradation of thin gelatin films

Bastien Schyrr [a,b,*], Stéphanie Boder-Pasche [a], Réal Ischer [a], Rita Smajda [a], Guy Voirin [a]

[a] CSEM Centre Suisse d'Electronique et de Microtechnique SA, Jaquet-Droz 1, CH-2002 Neuchâtel, Switzerland
[b] AMI Adolphe Merkle Institute, Université de Fribourg, Chemin des Verdiers 4, 1700 Fribourg, Switzerland

ARTICLE INFO

Keywords:
Sensor
Optical fiber
Matrix metalloproteinases
Evanescent wave
Gelatin

ABSTRACT

Despite increasing interest *in situ* monitoring of proteolytic activity in chronic wound is not possible and information can only be obtained by sampling wound exudate. In this context, we developed an evanescent wave (EW) fiber-optic sensor to quantify protease activity directly in the wound bed. Detection is based on the degradation of thin gelatin films deposited on the fiber core by dip-coating, which serve as a substrate for proteases. After staining with a chlorophyllin copper sodium salt biocompatible dye, EW absorption occurs proportionally to the dye concentration, which is detected by the variation in light transmission intensity. The sensor response varies proportionally to enzymatic activity, showing sensitivity against MMP-2 and MMP-9 down to 2 μg/mL and 10 μg/mL, respectively. In addition, it is sensitive to film thickness and crosslink density, thus allowing tuning of the sensitivity and lifetime. Designed to be totally biocompatible and low cost, this miniature sensor has potential for use as a point-of-care disposable device in a clinical environment to assist physicians with quantitative information about the wound healing process.

1. Introduction

Proteases play a crucial role in the control of physiological processes including wound healing [1], tissue remodeling [2,3] cell migration [4], and immune response [5]. Disruption of their activity is implicated in many pathologies and diseases [6–9]. Therefore, quantification of proteolytic activity in biological samples and tissues is of utmost interest for medical diagnoses and drug development [10]. In this perspective, a large variety of detection schemes have been developed, principally based on fluorescence [11–13]. Despite their high sensitivity, these assays often require time-consuming sample preparation and long incubation times. This reduces efficiency and can affect enzyme activity. In addition, they necessitate sophisticated laboratory equipment. In a different approach, researchers have focused on the development of single-use sensors to be available as point-of-care devices. Promising systems have thus been reported based on the erosion of thin protease substrate films [14–19]. By depositing the film on an appropriate transducer (electrochemical, piezo-electric, optical), the degradation process can be converted into a quantifiable signal in real-time. For example, poly(ester amide) films were used for the detection of chymotrypsin using a combination of quartz crystal microbalance (QCM) and electrochemical impedance spectroscopy (EIS) [14], by surface plasmon resonance (SPR) [17], or scanning photoinduced impedance microscopy (SPIM) [15]. However these systems require extraction of biological samples, and often involve complex fabrication processes or measurement setups which preclude their clinical use.

In order to provide rapid assessment of protease activity directly in biological samples (i.e. *in situ*), we developed a portable system based on optical fibers as transducers to monitor the enzymatic degradation of thin polymer films. Detection relies on evanescent wave (EW) absorption to probe the degradation of a stained substrate film replacing a portion of the fiber cladding. This approach offers several advantages. The existence of the EW only close to the fiber surface allows detection even in highly absorbing media and in very small volumes. Detection is performed by measuring variation in transmitted light intensity, which can be achieved using simple optical and optoelectronic components. As opposed to EIS-based systems which are limited to materials with good insulating properties [20], this versatile approach is suitable to any kind of natural or synthetic substrates (provided a refractive index lower for the film than for the core). Due to their small size and flexibility, fiber-optic sensors are furthermore adapted

* Corresponding author at: Chemin du Vieux Collège 14, 1008 Prilly, Switzerland. Tel.: +41 79 464 00 77.
E-mail address: bastien.schyrr@unifr.ch (B. Schyrr).

http://dx.doi.org/10.1016/j.sbsr.2014.12.004
2214-1804/

for the realization of miniaturized systems capable of remote sensing. In addition, they are immune to external electromagnetic interferences and safer due to the absence of circulating current like in electrode-based systems, which encourages their use in clinical applications [21,22]. Fiber-optic sensors have been successfully implemented for the detection of analytes such as pH [23,24], oxygen [25,26], glucose [27,28], and bacteria [29]. However, this is to our knowledge the first report on their use to quantify protease activity.

This study describes the development of an EW fiber-optic sensor for the detection of matrix metalloproteinase 2 (MMP-2) and 9 (MMP-9) activities. In the context of pressure and venous leg ulcers, an elevated and persistent activity of these proteases has been associated with a loss of extracellular matrix (ECM) homeostasis and a chronic inflammatory state, which result in the disruption of the wound healing process [8,30–32]. The protease-sensitive film consists in glutaraldehyde (GTA)-crosslinked cold water fish gelatin deposited by dip-coating on the core of a poly(methyl methacrylate) (PMMA) optical fiber. Gelatin was chosen due to its recognized biocompatibility, non-immunogenicity [33], low price, excellent film forming properties [34,35], and degradation by most MMPs involved in the ECM remodeling process, principally MMP-2 and MMP-9 [36]. As such, it has been widely used to quantify the overall proteolytic activity [37,38,19,39]. The gelatin film was stained with chlorophyllin copper sodium salt to visualize its degradation by the enzyme using EW absorption of transmitted light. The system was integrated as a portable autonomous device, providing a new point-of-care diagnostic tool to identify large protease imbalances in the wound bed, which will complete clinical symptom evaluation and support treatment decisions.

2. Experimental

2.1. Chemicals

Gelatin from cold water fish skin, glutaraldehyde 50% photographic grade (GTA), 3-chloro-4-methyl phenol 99% (CMP), chlorophyllin copper sodium salt (E141), hexamethylenediamine (HMD), sodium tetraborate decahydrate, sodium bicarbonate, sodium borohydride, *N*-Hydroxysuccinimide (NHS), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide chlorohydrate (EDC), zinc sulfate heptahydrate, isopropanol and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich (Buchs, Switzerland). Methanol, ethanol, acetone and nitric acid 65% were purchased from Merck (Zug, Switzerland). Hellmanex III was purchased from Hellma GmbH & Co. KG (Müllheim, Germany). Lyophilized human serum was obtained from Roche Diagnostics (Rotkreuz, Switzerland). Native human MMP-2 was purchased from Sino Biological Inc. (Beijing, China) and recombinant human MMP-9 (catalytic domain) from Randox diagnostics (Antrim, United Kingdom). All chemicals were used without further purification, and all solutions were prepared from deionized water.

2.2. Preparation of PMMA optical fibers and slides

The cladding of 200 μm diameter PMMA optical fibers (SuperEska, Industrial Fiber Optics Inc., Tempe, USA) was removed over 4 cm following a reported procedure [16]. A lint-free lens tissue (Peca Products Inc., Beloit, USA) was saturated with acetone and slid along the fiber for approximately 10 s. Water was then added to the tissue to result approximately in a 1:1 v/v water/acetone mixture, and the tissue was rubbed along the treated region. The procedure was repeated 5 times. The uncladded portion was then cleaned by sonication in a solution of 2% (v/v) Hellmanex III in deionized water for 30 min at 40 °C, and rinsed profusely with deionized water. An identical cleaning procedure was used for PMMA slides. Surface modification of PMMA was then performed by soaking in a solution of 10% (w/w) HMD in 100 mM borate buffer pH 11.5 at room temperature overnight [40]. The surfaces were washed several times with deionized water and dried by nitrogen blowing. Derivatization of the aminated surface was achieved by reaction in a solution of 0.5% (v/v) GTA in deionized water for 20 min, yielding aldehyde-functionalized PMMA surfaces. The surfaces were washed twice with deionized water and stored in deionized water until use. Immediately before dip-coating, they were dried by nitrogen blowing. Glass slides were employed as substrates for profilometry and free-aldehyde assays. In this case, the surfaces were cleaned by sonication in isopropanol for 10 min and rinsed with deionized water. The substrates were made hydrophilic by immersion in a solution of HNO_3 30% for 5 min to improve surface wetting by the gelatin/GTA mixture and the adherence of the resulting film. The glass slides were then rinsed in deionized water and dried by nitrogen blowing immediately before dip-coating.

2.3. Preparation of gelatin layer

Cold water fish gelatin (1 g) was dissolved in 10 mM PBS pH 7.4 (10 mL) by heating at 60 °C for 1 h with stirring. The solution was cooled down to room temperature, and CMP (10 mg) dissolved in ethanol (100 μL) was added as an antiseptic. In a typical formulation, GTA (18 μL, 1.0% (w/w) with respect to gelatin content) was then added drop wise and the solution was stirred in a closed vial for 3 h. These formulations are referred to as 1.0XL. Formulations with 0.6% (w/w) (0.6XL), 0.8% (w/w) (0.8XL) and 1.2% (w/w) (1.2XL) GTA were prepared in a similar way using 10.8, 14.4 and 21.6 μL GTA, respectively. Deposition onto GTA-derivatized surfaces was performed at room temperature using a NIMA dip-coater (DC Mono 160, KSV Nima, Espoo, Finland) at withdrawal speeds in the range between 50 and 400 mm/min, typically at 200 mm/min. After deposition, the films were dried at room conditions for 4 days (temperature 21 °C ± 2 °C, relative humidity (RH) 40–50%), followed by 7 days at 90% RH inside a closed desiccator using deionized water saturated with zinc sulfate heptahydrate [41]. Gelatin staining was done using carbodiimide chemistry [42]. Chlorophyllin copper sodium salt (72.42 mg) was dissolved in methanol (1 mL), and diluted with PBS (49 mL) to a final concentration of 2 mM. EDC (47.9 mg, 5 mM) and NHS (11.51 mg, 2 mM) were then added. The solution was stirred for 5 min and poured onto the gelatin-coated substrates. Reaction was carried out at room temperature for 3 h with mild shaking. The films were then soaked in PBS for 24 h and treated with an aqueous solution of sodium borohydride (1 mg/mL), using 4 baths of 15 min each [43]. The coated surfaces were washed in deionized water overnight and immersed in a 0.1% (w/v) solution of CMP prepared by dissolving CMP (50 mg) in ethanol (500 μL) and diluting it with deionized water (49.5 mL). The films were stored at room conditions until use (40–50% RH). The sensing fiber preparation process is summarized in Fig. 1.

2.4. Viscosity measurements

The kinematic viscosity of the gelatin solution was measured using a Ubbelohde viscometer (size II, range 20–100 cSt, $K = 0.996$, Fisher Scientific, Loughborough, United Kingdom). The gelatin solutions (10% (w/w) in 10 mM PBS pH 7.4) were mixed with GTA (1.0–1.5% (w/w) based on gelatin weight) and transferred to the Ubbelohde tube. The flow-through time at room temperature was recorded and used to determine the kinematic viscosity of the solution using the tube capillary constant K.