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Supplementary Material

Matrix Metalloproteinase Biosensor Based on a Porous Silicon Reflector

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Western blotting method

Protein was precipitated from human wound fluid samples using standard trichloroacetic acid methods as previously described.[1] Briefly, wound fluid was precipitated by addition of 4 volumes of trichloroacetic acid (TCA) in acetone. The samples were kept on ice for 1 hr before centrifuging at 15000 rpm for 20 min. The pellet was washed with 1 mL of cold acetone, centrifuged and the pellet dried and resuspended in SDS-PAGE loading buffer with β-merchaptoethanol. After heating at 95 °C for 5 min, the samples were centrifuged to remove any undissolved material. Western blotting was performed as previously described.[2] Briefly, sample proteins were run on a 10 % sodium dodecyl sulfate-proacrylamide gel electrophoresis gels at

100 V for 1.5 hr. Recombinant human MMP-1 (R&D Systems) was used as a control (100 ng) and 40 μL of wound fluid samples were loaded onto the gel. Protein transfer was performed onto nitrocellulose by wet transfer (Bio-Rad Laboratories) using standard Towbins buffer with 10 % methanol at 100 V for 1 hr. The membrane was blocked for 1 hr using 5 % skim-milk-blocking buffer and then incubated at 4 °C overnight with primary antibody (anti-MMP-1, Santa Cruz sc-58377 (1:1000) added in phosphate buffered saline solution containing 5 % skim milk powder and 0.1 % Tween20). The membrane was then washed in 5 % skim-milk-blocking buffer and then a secondary horseradish peroxidase-conjugated antibody (goat anti-mouse-HRP, Dako P0447, 1:1000) was added for 1 hr at room temperature. Washes were performed before detection of horseradish peroxidase by Super Signal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology, Rockford, IL) and captured using GeneSnap analysis program (SynGene, Frederick, MD).

The presence of MMP-1 in wound fluid was confirmed using Western Blotting analysis (Figure S1). Recombinant MMP-1 was loaded at 100 ng as a positive control. The band detected in the wound fluid samples was weaker than 100 ng MMP-1 control indicating that the level of MMP-1 in these samples was very low. The results confirm that the optical biosensor platform designed in this study is sensitive and selective enough to detect low concentration of MMP in the wound fluid.

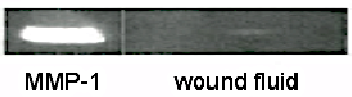

Fig. S1. The Western Blotting analysis for wound fluid sample and MMP-1 as a positive control.

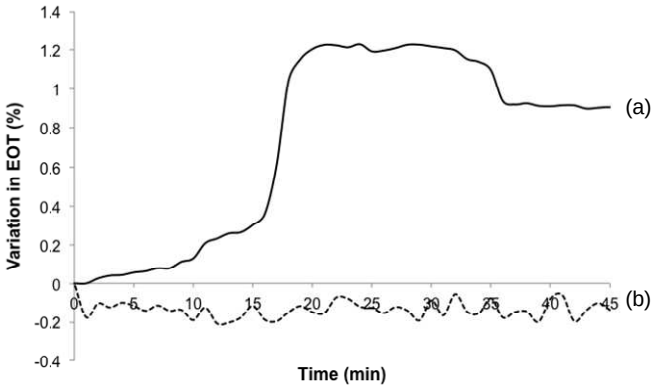

Fig. S2. MMP-1 biosensing experiments on the pSi surface with MMP inhibitor. In (a) (same as Fig. 6(a)) at minute 10-25, MMP-1 solution (25 μg/mL) was injected. In (b), the sample was exposed to buffer without MMP-1 at minute 10-25.

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[2] Z. Kopecki, R. M. Arkell, X. L. Strudwick, M. Hirose, R. J. Ludwig, J. S. Kern, L. Brucker-Tuderman, D. Zilikens, D. F. Murrell, A. J. Cowin, *Jornal of Pathology* 2011, *225*.