

Minimally Invasive Enzyme Microprobes: An Alternative Approach for Continuous Glucose Monitoring

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Continuous glucose monitoring has been shown to improve glycemia in diabetes.^{1,2} To minimize the pain, inconvenience, and discomfort associated with long-term implanted sensors, minimally invasive technologies are being developed, including microneedles that remove small volumes of subcutaneous interstitial fluid (ISF).³ We describe here an alternative approach for continuous monitoring of glucose in ISF that involves using solid microprobes that access the interstitium in a minimally invasive and less painful manner than using a lancet to draw capillary blood. The glucose sensor is a small (~1 cm²) wearable patch covered with arrays of solid microprobes, which penetrate the stratum corneum and monitor glucose in ISF within the epidermis. Unlike microneedles, solid microprobes are not susceptible to clogging and their minimally invasive nature may be less traumatic than existing subcutaneous sensors, reducing the risk of sensor drift and protein adsorption that degrades performance.^{4,5}

The microprobes were made by photolithography using backside illumination⁶ adapted to create 500–750 μm long structures with tip diameters of 10 μm. Sensitivity to glucose was achieved by assembling glucose oxidase (GOx; EC 1.1.3.4) on the gold-coated microprobes using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide coupling followed by a Nafion membrane incorporating tetrathiafulvalene (TTF) as an electron transfer mediator. *In vitro* studies with these devices have demonstrated that they perform well in the clinically relevant range (2–20 mM; **Figure 1**), with a linear response to increasing glucose and measured currents in the microampere domain.

Data are presented for glucose measurement, but the platform may be employed with other enzymes such as lactate oxidase or beta-hydroxybutyrate dehydrogenase. It is also possible to partition the microprobe array and immobilize different enzymes across multiple subarrays for continuous multianalyte sensing. Partitioning may also allow multiple sensors to be used in a “voting” system for the same analyte, thus improving sensor reliability.

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Abbreviations: (GOx) glucose oxidase, (ISF) interstitial fluid, (TTF) tetrathiafulvalene

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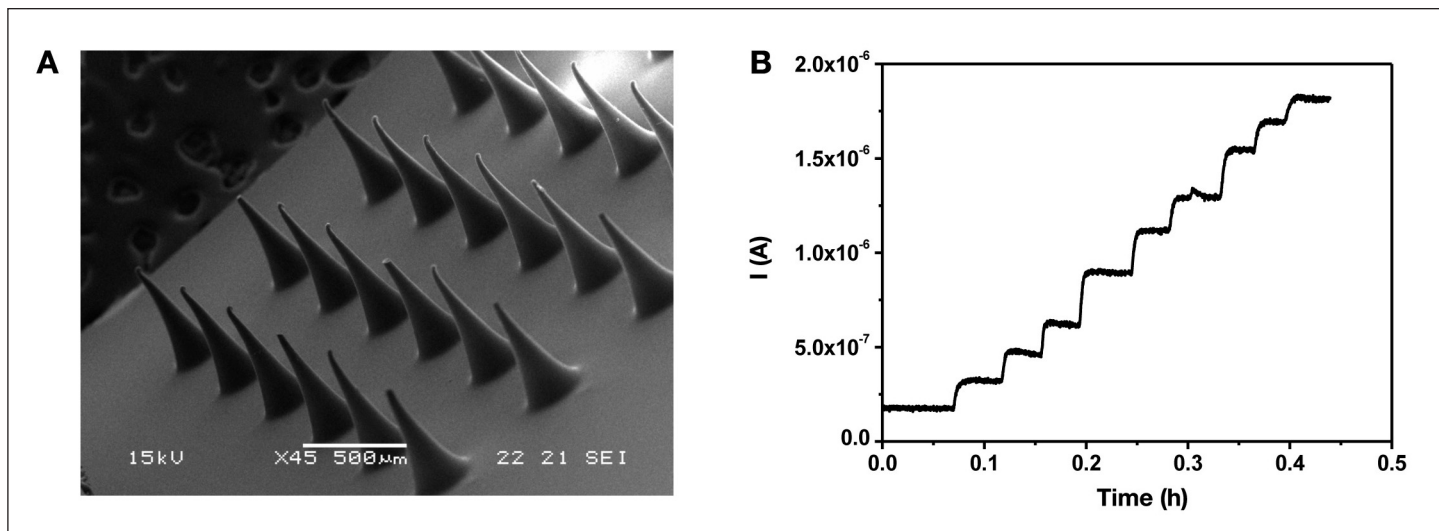


Figure 1. (A) Scanning electron microscope image showing 600 μm high microprobe array. (B) Calibration of TTF/GOx-modified gold microprobes performed in 0.1 M phosphate-buffered saline pH 7.4 background solution; each step represents an increase of 2 mM in glucose concentration.

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