Miniature Enzyme-Based Electrodes for Detection of Hydrogen Peroxide Release from Alcohol-Injured Hepatocytes

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Supporting Information

ABSTRACT: Alcohol insult to the liver sets off a complex sequence of inflammatory and fibrogenic responses. There is increasing evidence that hepatocytes play a key role in triggering these responses by producing inflammatory signals such as cytokines and reactive oxygen species (ROS). In the present study, we employed a cell culture/biosensor platform consisting of electrode arrays integrated with microfluidics to monitor extracellular H₂O₂, one of the major ROS types, produced by primary rat hepatocytes during alcohol injury.

The biosensor consisted of hydrogel microstructures with entrapped horseradish peroxidase (HRP) immobilized on an array of miniature gold electrodes. These arrays of sensing electrodes were integrated into microfluidic devices and modified with collagen (I) to promote hepatocyte adhesion. Once seeded into the microfluidic devices, hepatocytes were exposed to 100 mM ethanol and the signal at the working electrode was monitored by cyclic voltammetry (CV) over the course of 4 h. The CV experiments revealed that hepatocytes secreted up to 1.16 μM H₂O₂ after 3 h of stimulation. Importantly, when hepatocytes were incubated with antioxidants or alcohol dehydrogenase inhibitor prior to alcohol exposure, the H₂O₂ signal was decreased by ~5-fold. These experiments further confirmed that the biosensor was indeed monitoring oxidative stress generated by the hepatocytes and also pointed to one future use of this technology for screening hepatoprotective effects of antioxidants.

Toxic effects of alcohol on the liver have long been studied. Excessive consumption of alcohol, resulting in alcohol hepatitis, associated with hepatosteatosis, is an early stage of alcoholic liver disease and may contribute to the progression of fibrosis, leading to cirrhosis. As a result of many clinical and experimental studies, it has been found that the production of reactive oxygen species (ROS) due to alcohol intake causes oxidative stress that leads to a sequence of inflammatory and fibrogenic processes. ROS are reduced or activated derivatives of oxygen [superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH⁺)], chemically reactive, and extremely toxic toward most biological components. This leads to chemical modifications (oxidation, peroxidation, nitration, etc.) of lipids, proteins, and DNA that affect the integrity of the cell and its whole metabolism. Much work has been focused to study the generation of ROS from Kupffer cells or liver resident macrophages; however, more recently attention has turned to the role of hepatocytes as initiators and triggers of fibrogenic and inflammatory signals produced during liver injury. Hepatocytes are parenchymal cells of the liver constituting 70–80% of the cell mass in this organ. There are reports describing contributions of hepatocytes to ROS production in the liver during alcohol injury; however, quantification of oxidative stress generated by hepatocytes has not been undertaken to the best of our knowledge.

Hydrogen peroxide (H₂O₂) is the most chemically stable ROS type that is produced both as the byproduct of alcohol metabolism and due to the degradation of more reactive ROS types (e.g., superoxide anion). It is freely miscible with water, can penetrate through cell membranes readily, and is cytotoxic at high concentrations. Despite a growing interest in the detection of H₂O₂ there are few experimental tools available for real-time monitoring of H₂O₂ release from cells. There are fluorescence-based methods for detecting H₂O₂. For example, AM-etidium (10-acetyl-3,7-dihydroxyphenoxazine) is another fluorescent probe that fluoresces upon interaction with H₂O₂. Another fluorescent probe that fluoresces upon interaction with H₂O₂ and may be used to monitor extracellular peroxide. Both intra- and extracellular fluorescent probes for detecting H₂O₂ suffer from several challenges that include photobleaching, auto-oxidation, and autofluorescence, which make quantitative measurements difficult.

Electrochemical measurements may be used for sensitive detection of electroactive species and often provide a viable alternative to fluorescence-based detection. Several reports have described electrochemical biosensors for in vitro and in vivo detection of H₂O₂ and other ROS produced by cells. Amatore et al. studied the release of ROS and reactive nitrogen species from macrophages that were cultured in a detection
chamber containing a three-electrode system and were stimulated by the microinjection of a calcium ionophore. Cheah et al. developed a microfluidic device for heart tissue perfusion with real-time electrochemical monitoring of ROS release. Previously, our laboratory has reported on the development of enzyme-carrying miniature electrodes and have employed these electrodes for the detection of H₂O₂ production from macrophages.

In the present work, we wanted to extend the use of miniature enzyme-based biosensors to measure H₂O₂ generated by primary hepatocytes during alcohol injury. The sensor was fabricated by depositing horseradish peroxidase (HRP)-containing poly(ethylene glycol) diacrylate (PEG-DA) hydrogel on top of miniature gold electrodes (see Scheme 1). The primary hepatocytes were seeded onto the biochip surface, attaching and spreading around the gel-covered electrodes. The cells and electrodes were then integrated into a microfluidic device and injured in situ by alcohol exposure. Production of H₂O₂ by injured hepatocytes was monitored over the course of 4 h using cyclic voltammetry (CV) and was found to reach ≈1 μM. Pretreatment of hepatocytes with antioxidants prior to alcohol insult was shown to significantly diminish the levels of extracellular H₂O₂. To the best of our knowledge, this study is one of the first to attempt quantification of extracellular oxidative stress generated by injured hepatocytes. A microsystem with electrochemical biosensors integrated at the site of cells inside the microfluidic channels may become an important tool for toxicology studies and screening effectiveness of liver protective therapeutics.

## MATERIALS AND METHODS

### Chemicals and Reagents

PEG-DA (MW 575), PEG-DA (MW 258), 2-hydroxy-2-methyl-propiophenone (photoinitiator), 99.9% toluene, H₂O₂, HRP, and glutaraldehyde (Glu) were purchased from Sigma, U.S.A. Chromium (CR-4S) and gold etchants (Au-5) were purchased from Cyantek Corporation (Fremont, CA). Positive photoresist (S1813) and its developer solution (MF-319) were bought from Shipley (Marlborough, MA). (3-Acryloxypropyl)trichlorosilane was from Gelest, Inc. (Morrisville, PA). 5- and 6-Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-DCFDA), N-acetyl cysteine (NAC), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 4-methylpyrazole were purchased from Sigma, U.S.A. Phosphate-buffered saline (PBS 0.1 M, pH 7.4) solution was purchased from Fisher Scientific. Poly(dimethylsiloxane) (PDMS) and silicone elastomer curing agent were purchased from Dow Corning (Midland, MI).

A 10 mg mL⁻¹ solution of HRP was prepared in PBS solution of pH 7.4. 2% (v/v) solution of Glu was prepared in deionized water. The prepolymer hydrogel enzyme solution was prepared by adding 20 μL of HRP solution, 2 μL of Glu, and 2% (v/v) solution of the photoinitiator in 25 μL of PEG-DA (MW 575 and 258). Two different PEG-DAs were used to improve attachment of the hydrogels to the substrate. We found that attachment of hydrogel constructs made out of a 1:1 mixture of PEG 575 and 258 was better than PEG 575 alone. While attachment properties of PEG 258 alone were excellent, the nonfouling properties were compromised. The mixture of two PEG types allowed us to improve adhesion of gel constructs while retaining nonfouling properties. The prepolymer hydrogel enzyme solution was stirred overnight to react off all the aldehyde groups of Glu to the primary amines of the enzyme molecules.

Different concentrations of H₂O₂ and 100 mM ethanol were prepared in 0.1 M PBS solution of pH 7.4.

### Isolation of Primary Hepatocytes

Primary hepatocytes were isolated from adult female Lewis rats weighing 125–200 g (Charles River Laboratories, Boston, MA), using a standard two-step collagenase perfusion procedure. Typically, 100–200 million hepatocytes were obtained with viability ≈90% as determined by trypan blue exclusion. Primary hepatocytes were maintained in Dulbecco’s modified Eagle’s medium (DMEM).
supplemented with epidermal growth factor, glucagon, hydrocortisone sodium succinate, recombinant human insulin, 200 units mL$^{-1}$ penicillin, 200 mg mL$^{-1}$ streptomycin, and 10% fetal bovine serum.

**Micropatterning of Au Electrodes on Glass.** The micropatterned Au arrays were prepared using a previously described standard photolithography and metal-etching method. The glass slides with dimension of 7.5 cm x 2.5 cm sputtered with Cr (15 nm) and Au (100 nm) were purchased from Lance Goddard Associates (Santa Clara). A protective layer of a positive photoresist was spin-coated (2000 rpm for 30 s) on top of the Au-coated slide and soft-baked at 115 °C for 1 min. The electrode array pattern made in AutoCAD was formed on the slides with a mask aligner, and the final patterned slides were obtained using the wet etching method. In this way, an array of eight electrodes of circular shape (300 μM diameter) was obtained for fabricating the microfluidic platform.

**Formation of HRP-Carrying Hydrogel Microstructures on Au Electrode Arrays.** The micropatterned glass slides, containing photoresist on top of Au surface, were treated with oxygen plasma for 10 min and incubated in 0.05% solution of (3-acryloxypropyl)triethoxysilane in toluene for 1 h under nitrogen atmosphere to obtain a self-assembled monolayer of silane on the glass regions. The photoresist layer protected the Au region of the slide from the silane modification. These slides were then sonicated in acetone to remove the photoresist from Au regions. The prepolymer hydrogel solution (PEG–HRP–Glu containing 2% photoinitiator) was coated onto the Au electrodes of the patterned slides. These slides were exposed to UV radiation (60 mJ/cm²) using an Omnicare 1000 light source for 3.5 s through an aligned photomask on top of the electrodes. As a result liquid prepolymer was cross-linked into hydrogel microstructures formed on top of Au electrodes. Enzyme-carrying hydrogel microstructures were made larger (600 μm) than Au electrodes (300 μm) so that acryl group of the silane could cross-link to the PEG-DA providing more stability to the enzyme–hydrogel onto the glass surface (see Figure 1). The UV-exposed PEG-DA precursor gets polymerized and subsequently cross-links to the silane layer on the glass substrate, while the unexposed regions were later washed from the surface using deionized water. To seed primary hepatocytes around the PEG–HRP electrodes, the micropatterned glass slides were incubated in collagen (0.2 mg mL$^{-1}$) solution for about 30 min and were kept at 37 °C in an incubator. The collagen gets adsorbed onto silane-modified glass regions and not on the gel-coated Au electrode regions of the surface due to the nonfouling properties of PEG. Subsequently, primary rat hepatocytes were seeded for about 24 h at 37 °C onto these surfaces and then enclosed inside microfluidic channels to commence on-chip detection experiments. Scheme 1 (steps 1–6) describes the stepwise fabrication of enzyme–hydrogel electrodes and subsequent cell attachment.

![Figure 1. (A) PDMS covered sensing chip containing eight micropatterned Au electrodes (four in each working channel). (B) An image of a PEG–HRP polymerized Au electrode. (C) Microfluidic device for on-chip electrochemical experiments containing flow-through Ag/AgCl as reference (connected to the outlet), Pt wire as counter (in the inlet), and patterned Au as working electrodes.](image-url)
Integration of Sensing Surfaces with Microfluidic Channels for Electrochemical Detection of H$_2$O$_2$. PDM5-based microfluidic channels were designed and fabricated according to procedures described by us previously. The microfluidic device consisted of two channels of dimension $10 \text{ mm} \times 3 \text{ mm} \times 0.1 \text{ mm}$ and a volume of $3 \mu\text{L}$ with the inlets and outlets punched with a blunt 16-gauge needle (Figure 1A). Along with the two fluidic channels, a network of auxiliary channels was fabricated on the same PDMS surface and was used for suctioning the device onto glass substrates carrying cells and electrodes.

All the electrochemical measurements were done inside the microfluidic device connected with a precision syringe pump (Harvard Apparatus, Boston, MA) for generating controlled flow. A CHI instrument (842B, CH Instruments, Austin, TX) was employed for the voltammetric experiments. The electrochemical cell consisted of a flow-through Ag/AgCl (3 M KCl) reference electrode inserted at the outlet, a platinum wire counter electrode placed in the inlet of the microfluidic device, and Au working electrodes positioned inside the fluidic channels (Figure 1C). The solutions were made in 0.1 M PBS for all the electrochemical measurements. The contact pads of the sensing Au arrays were connected to a home-built multiplexing setup capable of collecting CV spectra at prescribed time intervals from individual members of the electrode array.

Cyclic voltammetry was used to characterize responses of PEG−HRP electrodes to H$_2$O$_2$. In order to create a calibration curve, known concentrations of H$_2$O$_2$ prepared in PBS were infused into the microfluidic device containing enzyme-based electrodes. The cyclic voltammograms (CVs) were taken from 0.7 to $-0.7 \text{ V}$ at the scan rate of 50 mV/s. The calibration curve was constructed by plotting the absolute value of reduction current at $-0.4 \text{ V}$ versus H$_2$O$_2$ concentration.

To analyze H$_2$O$_2$ production from alcohol-injured cells, primary hepatocytes were seeded onto the surface of the biochip. The surface was pretreated with 0.2 mg mL$^{-1}$ of collagen (I) in order to promote hepatocyte attachment. Subsequently, hepatocytes were suspended in cell culture medium at a concentration of $10^6$ cells mL$^{-1}$ and incubated at $37 \degree \text{C}$ over the surface of the biochip for 1 h. The unattached cells were then washed away using PBS, and the remaining cells were allowed to recover for at least 12 h prior to assembling the PDMS channels on top of the biochip. The injury experiments were commenced by injecting 100 mM ethanol into the fluidic channel. CV measurements were made every 15 min during the 4 h alcohol stimulation experiment.

Fluorescence-Based Detection of Peroxide. Hepatocytes were seeded into the wells of a 96-well plate precoated with 0.2 mg/mL collagen (I). After 24 h of incubation with cells, certain wells were incubated with NAC (0.6128 μM) or Trolox C (0.1997 μM) for 24 h, or 4-MP (2 mM) for 1 h, or left unprotected by antioxidants. After oxidant incubation, the cells were washed and then incubated with CM-DCCFDA (10 μM) for 45 min. After washing away the residual CM-DCCFDA not taken up by the cells, ethanol (100 mM) was added to the protected and unprotected cells and their fluorescence was measured every 10 min for 3 h using plate reader (Tecan Safire 2) employing excitation/emission of 485 nm/530 nm. Plates were stored in a tissue culture incubator at $37 \degree \text{C}$ when not in use.

RESULTS AND DISCUSSION

Fabrication and Electrochemical Characterization of Enzyme-Based Biosensors. PEG is a biocompatible polymer known for its excellent nonfouling properties. This biomaterial has been used widely to minimize unwanted protein adsorption and cell attachment in tissue engineering and biosensor development. In addition to possessing excellent nonfouling properties, PEG-based hydrogels also provide a hydrated environment that helps retain high levels of activity in entrapped enzymes. As shown in Figure 1B, the PEG−HRP sensor was fabricated by polymerizing enzyme−hydrogel on top of micropatterned Au electrodes. The glass slides were modified with acryl silane to promote attachment of hydrogel micropatterns (Scheme 1). HRP is an oxidoreductase enzyme that catalyzes the reduction of H$_2$O$_2$ according to eq 1 below.

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O $$

The CV measurements were carried out to characterize the enzyme−hydrogel electrodes. Given that H$_2$O$_2$ is an electroactive compound we wanted to assess the oxidation/reduction of this compound at the electrodes containing or lacking HRP. Figure 2A shows CVs of the hydrogel electrodes in PBS solution in absence (Figure 2A, curve i) and presence of HRP (Figure 2A, curve ii) in the PEG hydrogel. The incorporation of redox enzyme in the hydrogel matrix results in increased current response in the positive and negative potential range (Figure 2A, curve ii) as compared to the response for only hydrogel electrode (Figure 2A, curve i). This is attributed to the incorporation of electrochemically active enzyme into PEG gel. Subsequently, an increase in reduction current was obtained upon the addition of H$_2$O$_2$ into the microfluidic channel containing PEG−HRP microelectrodes indicating enhanced reduction of H$_2$O$_2$ (diffused into the hydrogel) by HRP (Figure S1, Supporting Information). Conversely, the microelectrodes made with pure PEG did not show much change in current when challenged with H$_2$O$_2$ (Figure S2, Supporting Information). This validates that the HRP augments the reduction of H$_2$O$_2$. About a 60-fold increase in the reduction current was found with the PEG−HRP as compared to the PEG-only electrode.

Calibration of the prepared enzyme sensor was performed by infusing known concentrations of H$_2$O$_2$ into the microfluidic device containing PEG−HRP microelectrodes and monitoring the reduction current using CV in the range of 0.7 to $-0.7 \text{ V}$. The injected H$_2$O$_2$ diffuses into the porous hydrogel matrix and reacts biochemically with the HRP present inside the hydrogel (Figure 2B). The reduction current was found to increase with increasing H$_2$O$_2$ concentration. Figure 2C illustrates the calibration plot (reduction current versus H$_2$O$_2$ concentration at $-0.4 \text{ V}$) for detection of exogenous H$_2$O$_2$. The low operating potential ($-0.4 \text{ V}$) obviates interference due to other species such as ascorbic acid, uric acid, etc. The lowest concentration of H$_2$O$_2$ that could be detected by each sensing electrode of area 0.070714 mm$^2$ was found to be 0.2 μM. The sensitivity of the sensor was calculated to be 27.5 nA μM$^{-1}$ mm$^{-2}$. The stability of the enzyme−hydrogel electrode was tested at a regular interval of 1 week by infusing a 50 μM concentration of H$_2$O$_2$ in the microfluidics channel containing the PEG−HRP sensor. The biochip was stored in PBS at 4 °C when not in use. The value of reduction current was monitored each time and plotted against number of weeks. The reduction current did not change.
over the course of 4 weeks (Figure S3, Supporting Information). This verifies stable entrapment of enzyme molecules inside the gel constructs. The stability of enzyme electrodes was also tested at 37 °C under cell culture conditions. We did not observe changes in sensor response over 2 days under cell culture conditions. This stability was sufficient to conduct ethanol injury experiments. The stability of the enzyme at varied temperatures is attributed to the biocompatible environment provided by the hydrated gel matrix. There are several reports in the literature confirming stability of HRP up to ∼50 °C when encapsulated in gel matrixes.31,32

**Electrochemical Detection of Hepatic H₂O₂ Released During Alcohol Injury.** To conduct alcohol injury experiments the hepatocytes were seeded onto collagen (I)-coated surfaces containing enzyme electrodes. The cells captured inside the biochip were kept in a tissue culture incubator at 37 °C for 24 h. As seen in Figure 3, parts A and B, which shows images of hepatocytes taken immediately after seeding and 12 h post seeding, cells formed a monolayer with bright cell borders, prominent nuclei, and cuboidal morphology indicative of the hepatic phenotype. It is of note that hepatocytes did not attach on top of the electrodes due to the nonfouling properties of PEG. We found that, in cases when nonfouling properties were compromised and hepatocytes attached to the gel/electrode region, the sensor’s response was compromised (results not shown). An estimated 50 000 cells were cultured inside each channel during the peroxide detection experiment.

The cells were stimulated by infusing 100 mM ethanol into the microfluidic channel. This level of ethanol is pathophysiological and is similar to the levels observed during acute alcohol injury.17,33 The resultant release of H₂O₂ was detected with CV by sequentially addressing H₂O₂ sensing electrodes located inside the microfluidic channel. Measurements were made every 15 min for up to 4 h using a home-built multiplexer coupled with automated data collection. The flow inside the fluidic channel was stopped prior to conducting electrochemical measurements. An increase in reduction current of H₂O₂ was observed after ∼60 min of incubation with 100 mM ethanol, pointing to the production of H₂O₂ from hepatocytes injured with alcohol. The reduction current was found to saturate after 3 h. Figure 3C shows representative response of hepatocytes to 100 mM ethanol. When testing less than 100 mM concentration of ethanol we did not observe a change in the reduction current over time. The H₂O₂ produced from hepatocytes was determined to be ranging from 0.29 μM

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**Figure 2.** (A) CV plots of (i) PEG and (ii) PEG–HRP polymerized onto miniature Au electrodes in the potential range of 0.7 to −0.7 V at the scan rate of 50 mV/s. The better current obtained with HRP-incorporated PEG is attributed to the redox property of the enzyme. (B) CV plots of the PEG–HRP electrode as a function of H₂O₂ injected into the microfluidic device: (i) 1, (ii) 5, (iii) 10, (iv) 20, (v) 50, and (vi) 100 μM. The reductive current increases with increasing concentration of H₂O₂. This indicates enhanced enzymatic reaction between HRP and H₂O₂. (C) Calibration plot obtained for the PEG–HRP electrode showing the absolute value of the reduction current as a function of different H₂O₂ concentrations (current values at −0.4 V).

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![cv plots](image-url)
after 1 h to 1.16 μM after 3 h (Figure 3D). Thus, the rate of 
H₂O₂ release from ~12,000 stimulated hepatocytes around 
each PEG−HRP sensing electrode was found to be 6.5 nM
min⁻¹ from 1 to 3 h. It is worth noting that the response time 
for detection of extracellular peroxide by hepatocytes is likely a 
function of the biosensor sensitivity. Our laboratory has 
previously demonstrated that sensitivity may be improved by 
conjugating enzymes with Au nanoparticles.34 It is therefore 
conceivable that more sensitive biosensors will enable earlier 
detection of H₂O₂ from hepatocytes and may enable cellular 
responses to lower alcohol concentrations.

A number of control experiments were carried out to ensure 
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A number of control experiments were carried out to ensure 
that the signal observed in Figure 3C was indeed due to 
extracellular H₂O₂. Exposure of enzyme electrodes to 100 mM
ethanol did not cause a change in reduction current. Similarly, 
cultivation of hepatocytes around enzyme electrodes in the 
absence of alcohol did not result in an electrochemical signal 
(results of both experiments not shown). This suggests that 
the generation of the electrochemical signal required the presence 
of hepatocytes and injury with alcohol.

It may be noted that most of the electrochemical biosensors 
reported in literature are used to monitor ROS production 
from immune cells7,18,30,35 but not from hepatocytes. The 
prepared electrochemical enzyme−hydrogel-based sensor is 
able to detect H₂O₂ in a wide range of concentrations and 
shows sensitive detection of H₂O₂ secreted from alcohol-
injured hepatocytes in PBS without any external redox indicator.

**Figure 3.** Sensor surface (A) just after hepatocyte seeding onto the collagen-modified silane region around the PEG−HRP electrode and (B) after incubation of the sensor chip at 37 °C in an incubator for 24 h. (C) CV measurements taken from hepatocytes exposed to 100 mM ethanol. Increase in reduction current with time indicates enhanced cellular production of H₂O₂. (D) Plot showing enhancement in reduction current (at −0.4 V) due to cellular production of H₂O₂.

**Effects of Antioxidants on Detection of H₂O₂.** Development and screening efficacy of antioxidants is an important part of liver toxicology and liver therapeutics. To demonstrate potential utility of our biosensor for liver toxicology studies we assessed the effects of commercial antioxidants such as NAC36 and Trolox37 on oxidative stress production in alcohol-injured hepatocytes. These antioxidants are known to protect the cells against oxidative stress possibly by the scavenging activity.37 The purpose of these investigations was to monitor the protective effect of these antioxidants on hepatocytes toward production of ROS. These experiments also served as additional controls for verifying that the electrochemical signals measured in the vicinity of injured hepatocytes were due to oxidative stress.

The hepatocytes cultured around enzyme electrodes were 
incubated with either 0.6128 μM NAC or 0.1997 μM Trolox in DMEM for 24 h prior to alcohol injury. The extra antioxidant 
molecules were washed out by infusing 0.1 M PBS into the 
microfluidic channels. Cells were then exposed to 100 mM 
ethanol, and electrochemical measurements were performed as 
described in the previous section of this paper. Figure 4 
compiles the reduction current values from experiments where 
hepatocytes were protected with antioxidants prior to injury 
versus a control scenario where hepatocytes were injured in the 
absence of antioxidant treatment. This set of data clearly 
demonstrates that antioxidant treatment caused a dramatic 
decrease in the reduction current in the electrochemical signal 
measured at the sensing electrode. Importantly, peroxide signal
Fluorescence-based detection of H$_2$O$_2$ was used to corroborate CM-DCFDA, a cell-permeable intracellular dye. In the results of electrochemical sensing. These experiments utilized antioxidants that prevent the release of H$_2$O$_2$ and thus can be used as an intracellular probe to monitor oxidative stress in hepatocytes. Alcohol dehydrogenase is accountable for breaking down the ethanol into acetaldehyde, and activity of this enzyme is connected to generation of peroxide and that the levels of this ROS could be decreased by antioxidants prior to alcohol insult and were observed to generate significantly lower levels of oxidative stress as result. The functionality of this microsystem may be enhanced in the future by integrating additional biosensors for inflammatory molecules produced by liver cells during injury.

**ASSOCIATED CONTENT**

Supporting Information
Performance of hydrogel (PEG-DA) electrodes containing and lacking enzyme (HRP); stability of HRP inside the PEG-DA hydrogel matrix. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

The authors thank Professors Natalie Torok and Jian Wu for helpful suggestions. The authors also thank Ms. Dipali Patel for primary hepatocyte isolation. The financial support for these studies was provided in part by NIH (AA017939).

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