Microelectrode Sensors for *in Vivo* Detection of Radiopharmaceuticals

Maria Theresa B. Lee,† Carl J. Seliskar,†
William R. Heineman,*,† and Anthony J. McGoron‡

Department of Chemistry, University of Cincinnati
P.O. Box 210172, Cincinnati Ohio 45221-0172
Department of Radiology, Division of Nuclear Medicine
College of Medicine, University of Cincinnati
Cincinnati, Ohio 45267-0577
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Radiopharmaceuticals are used in nuclear medicine to image specific organs for the purpose of diagnosis or to provide selective delivery of radiation for therapy of cancerous tissues and traumatic lesions.[1] Nuclear medicine procedures are used extensively for noninvasive detection and assessment of abnormalities in the heart.[2] One strategy for developing heart imaging radiopharmaceuticals explores ^{99m}Tc coordination compounds with an overall charge of 1+ and a certain degree of hydrophobicity.[3] In general, the chemical composition of the injected radiopharmaceutical is known. However, the specific chemical form that is actually responsible for the image may be altered due to *in vivo* reactions.

Recent developments in the area of *in vivo* sensing have been achieved with microelectrodes. The use of carbon fibers for detection of neurotransmitters in the brains of test animals has been well documented.[4] Since many radiopharmaceuticals are electroactive, electrochemical sensors offer a possible avenue for continuous, direct, *in vivo* monitoring of a radiopharmaceutical after injection into a test animal.

We have developed a microelectrode carbon-fiber sensor with a coating of Nafion gel to monitor a specific chemical form of an agent after injection and as it localizes in and images an organ. The sensor has been shown to measure $[Re(DMPE)_3]^+$ where DMPE is 1,2-bis(dimethylphosphino)ethane,[5] a nonradioactive analog of the prototype cationic lipophilic ^{99m}Tc imaging agent $[^{99m}Tc(DMPE)_3]^+$ in the heart of a live rat. This Re complex was also detected in isolated organs such as the lungs, muscle, kidney, and liver. Furthermore, as far as we know, this is the first time that a chemical sensor has been developed for *in vivo* measurement of a radiopharmaceutical analog.

The sensor consists of a carbon-fiber microelectrode with a thin polymer coating (Figure 1). $Re(DMPE)_3^+$ is detected by the following oxidation when the potential of the sensor is scanned from −200 to 400 mV

$$Re(DMPE)_3^+ \rightarrow Re(DMPE)_3^{2+} + 1e^-$$

This same electrode reaction occurs at both bare carbon fibers and at polymer coated electrodes. A key feature of the sensor

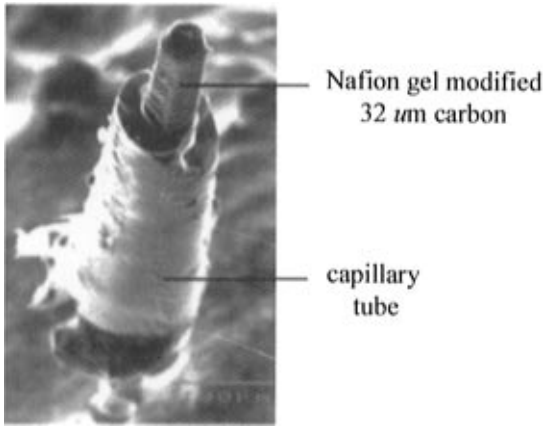

Figure 1. Scanning electron micrograph of a Nafion gel modified carbon fiber microelectrode tip.

is its small size, which stems from using a 32 μm carbon fiber electrode. The polymer film, which provides the necessary selectivity and detection limit to monitor $[Re(DMPE)_3]^+$ *in vivo*, is a gel consisting of a mixture of Nafion and tri-*n*-butyl phosphate (7.5:2.5 by weight of 5% Nafion [Aldrich]; tri-*n*-butyl phosphate [Fisher]). Nafion has been shown to concentrate hydrophobic cationic coordination compounds through interaction with its tetrafluoroethylene backbone and fixed-charge sulfonate ion exchange sites.[6] The tri-*n*-butyl phosphate functions as a gelling agent which also enhances the hydrophobicity of the film thereby improving the ability of Nafion to preconcentrate hydrophobic compounds.[7] Nafion gel modified microelectrodes have been shown to detect $[Re(DMPE)_3]^+$ in the presence of potential interferents in biological tissue such as ascorbic acid and uric acid which are also oxidizable.[8]

Male rats (250−300 g) were anesthetized with pentobarbital-sodium and given heparin intraperitoneally. The test rats (n = 3) were given 1.4 mL/kg of 1 mM $[Re(DMPE)_3]CF_3SO_3$ in saline and 5% ethanol by tail vein injection, and control rats (n = 2) were given the vehicle (minus the analyte). Thirty min after analyte injection the organs were removed, placed in sealed vials, and stored on ice until use. For the electrochemical study, each isolated organ (except blood) was placed in a dish with a few drops of saline. A Pt auxiliary electrode and a bare Ag/AgCl wire reference electrode were used. Square-wave voltammetry was the technique used for electrochemical detection.

Voltammograms recorded with sensors inserted 1−2 mm into the left ventricle wall of hearts removed from a control rat and a test rat are shown in Figure 2(parts a and b, respectively). No distinguishable voltammetric wave was observed for the control heart (Figure 2a), indicating acceptable selective exclusion of interfering electroactive species such as ascorbate and urate. A very pronounced peak at +40 mV was observed (Figure 2b) for the heart perfused with $[Re(DMPE)_3]^+$. This peak is consistent with electrochemical oxidation of $[Re^I(DMPE)_3]^+$ that has partitioned from the heart tissues into the Nafion gel film of the sensor. The actual location of the tip is not known but is probably in the extracellular fluid or at least in the area of disrupted vasculature and cellular tissue. After the microsensor was removed from the heart, it was recalibrated *in vitro*. Comparison of the voltammograms obtained from the excised heart with that obtained in a 1×10^{-5} M $[Re(DMPE)_3]^+$ solution (Figure 2c) shows similarities in both the peak current and potential. The amount of $[Re(DMPE)_3]^+$ that diffused to the

* Author to whom correspondence should be addressed.
† Department of Chemistry.
‡ Department of Radiology.
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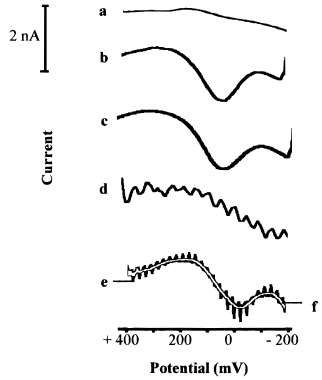

Figure 2. Square wave voltammograms at Nafion gel modified microelectrode sensor. (a) Sensor inserted in control heart isolated from rat without injection of $[Re(DMPE)_3]^+$. (b) Sensor in heart isolated from rat 30 min after injection of 1×10^{-3} M $[Re(DMPE)_3]^+$ into test rat. The voltammogram was taken 2 min after the sensor was inserted into the heart. (c) Sensor used in **b** removed from rat, soaked in 0.15 M NaCl for at least 30 min to remove all $[Re(DMPE)_3]^+$ and then soaked in 1×10^{-5} M $[Re(DMPE)_3]^+$ solution for 2 min. (d) Sensor inserted in the heart of a live anesthetized rat before injection of the 1×10^{-3} M $[Re(DMPE)_3]^+$ solution. (e) Sensor inserted in the heart of a live anesthetized rat 45 min after injection of the 1×10^{-3} M $Re(DMPE)_3^+$ solution. The scan was taken about 5 min after the Nafion modified sensor was inserted in the heart. The same condition as in **d** was observed during the scan. (f) Voltammogram in **e** after seven-point smoothing. Square-wave voltammetry parameters: scan limits, −200 to 400 mV; square-wave amplitude, 25 mV; frequency, 15 Hz; step potential, 4 mV; samples per point, 256.

carbon surface of the gel-modified microelectrode following insertion into the organ appears to correspond to a tissue concentration of ca. 1×10^{-5} M $[Re(DMPE)_3]^+$ (1% of the injected dose) which is consistent with published values for uptake of $[Re(DMPE)_3]^+$ and $[Tc(DMPE)_3]^+$ in the rat heart determined by radioactivity.[9] Although agreement between the sensor and biodistribution values is good, this must be considered an approximation until more is known about the effect of heart tissue on the rate of diffusion of $[Re(DMPE)_3]^+$ to the Nafion gel surface.

The ability of the sensor to function in other excised organs and blood from rats injected with $[Re(DMPE)_3]^+$ was evaluated. In all of the organs tested, except the brain, voltammetric waves indicating the presence of $[Re(DMPE)_3]^+$ in the tissue surrounding the sensor tip were obtained. Figure 3 shows the measured peak currents for $[Re(DMPE)_3]^+$ reported by sensors inserted for 30 min in various excised organs and blood. The sensor peak current is directly proportional to the concentration of $[Re(DMPE)_3]^+$ in the tissue. The relative magnitudes of these currents correlate well with the reported biodistribution of $[^{186}Re(DMPE)_3]^+$ in rat.[9a] It is significant that no signal was obtained for the brain, which is consistent with the view that the charged $[Re(DMPE)_3]^+$ complex should not cross the brain-blood barrier.[3b]

The use of the sensor for detecting $[Re(DMPE)_3]^+$ in a live anesthetized rat after injection was also explored. An anesthetized rat was placed on a mechanical respirator through an endotracheal tube, and the chest was opened by a midsternal thoracotomy. The heart was supported in a pericardial cradle. Before analyte injection, the microelectrode sensor was slowly inserted into the beating heart with a micromanipulator such that the sensor tip was 1−2 mm into the wall of the left ventricle. A square wave voltammogram obtained prior to injection of $[Re(DMPE)_3]^+$ yielded the voltammogram shown in Figure 2d.

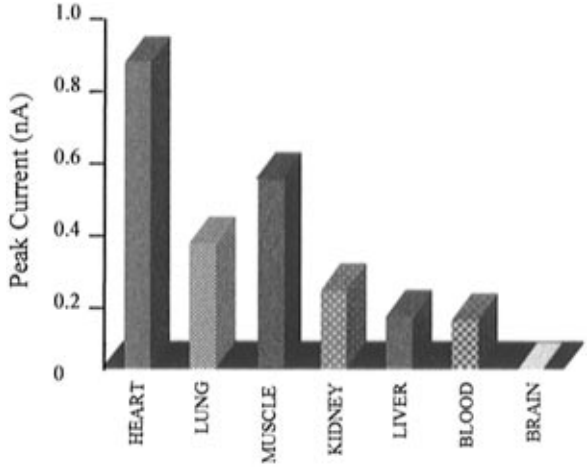

Figure 3. A bar graph of peak currents from square wave voltammograms obtained at sensors inserted into different organs isolated from a test rat. The current is the maximum signal obtained 30 min after inserting a sensor. See Figure 2 for square wave scan parameters.

The appearance of the unusual recurring spikes/pulses coincides with the respiration rate of the rat induced by the mechanical respirator. A 25-point smoothing of the signal (using a moving average) produced a curve that resembles Figure 2a, namely, the voltammogram for the control excised heart. Similar to the conclusion from the voltammogram of Figure 2a, no observable wave was obtained from a sensor inserted into the live control heart, which confirms the absence of signal due to interferents.

A voltammetric scan recorded 45 min after $[Re(DMPE)_3]^+$ was injected into the anesthetized rat shows a peak at −40 mV (Figure 2e) due to oxidation of $[Re(DMPE)_3]^+$ at the sensor. Recurring pulse/spikes similar to those found in the control heart, but at a higher frequency than in Figure 2d, correspond to the heart rate of the rat and not the respiration rate since the respirator was turned off while the voltammogram was recorded. A seven-point smoothing of the signal revealed a more obvious peak for $[Re(DMPE)_3]^+$ as shown in Figure 2f. Following the procedure used to obtain voltammogram **c** (Figure 2) gave a voltammogram for $[Re(DMPE)_3]^+$ with the same peak potential as in voltammograms **e** and **f**. The peaks in Figure 2 (parts e and f) are shifted about 80 mV negative with respect to those shown in Figure 2 (parts b and c). We attribute this shift in peak potential to a difference in the half-cell potential of the silver/silver chloride quasi-reference electrodes used in the two experiments, rather than to a change in the rhenium complex itself such as substitution of a DMPE ligand with two Cl^-, which results in a much greater shift in the potential.[10] The reference electrode size was minimized by using a silver wire coated with silver chloride, which was immersed, unprotected, directly into the saline solution bathing the organs.

The data presented demonstrate that a Nafion gel modified microelectrode sensor can detect $[Re(DMPE)_3]^+$ not only in the excised organs but also *in vivo* in the beating heart of an animal following intravenous injection. Thus, sensors of this type can provide a new analytical tool for studying electroactive radiopharmaceuticals. The possibility now exists to monitor *in vivo* an imaging agent in real time as it distributes into different organs. Simultaneous monitoring in different organs could be accomplished by using an array of microsensors.

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