

Single Cell Viability Measured by Scanning Electrochemical Microscopy and Live/Dead™ Staining*

K.M. Jeerage, T.L. Oreskovic, N. Goldstein, and D.S. Lauria

Materials Reliability Division, National Institute of Standards and Technology, 325 Broadway, Boulder, CO 80305

Metabolic activity is an unambiguous indicator of cell viability and health. A widely utilized probe molecule for *cell cultures* is resazurin, which is reduced to resorufin by living cells. Scanning electrochemical microscopy (SECM) can probe the metabolic activity of *single cells* via O₂ reduction [1]; respiration causes a decrease in the O₂ concentration above living cells. However, based on its standard reduction potential, oxidized ferrocenemethanol (FcCH₂OH) may act as an alternate electron acceptor. By locally oxidizing FcCH₂OH at a microelectrode, we probe metabolic activity using feedback measurements, which have higher resolution than passive measurements of O₂ diffusion profiles. If FcCH₂OH is regenerated by the cell, the current will increase when the microelectrode is positioned above a cell. This is known as positive feedback and has been reported for HeLa cells [2]. However if FcCH₂OH diffusion is blocked by the cell, the current will decrease when the microelectrode is positioned above a cell. This is known as negative feedback and has been reported for COS-7 cells [3].

Living cells have been probed via SECM using a variety of redox mediators; the information obtained depends critically on the interaction of the probe molecule and the living cell. Cell-impermeable mediators such as Fe(CN)₆^{3-/4-} or Ru(NH₃)₆^{3+/2+} are always blocked by the membrane, leading to negative feedback or topography [4, 5]. On the other hand, cell-permeable mediators, most notably quinones, can participate in cellular redox processes. Here mediator concentration becomes critical, as intracellular redox processes are not detectable when the concentration of mediator in solution far exceeds the concentration of redox centers. Therefore, while intracellular redox activity is obtained at a low (40 μM) concentration of menadione or 1,2-naphthoquinone [4], at high (1000 μM) concentration, many redox mediators detect only topography [5], despite intracellular regeneration. Both menadione and 1,2-naphthoquinone are reduced by the microelectrode, meaning that the cell must re-oxidize them to obtain positive feedback.

We examined adherent HeLa cells in cell culture medium containing 500 μM FcCH₂OH. The three electrode system comprised a Pt microelectrode of about 5 μm radius, a Pt wire counter electrode, and a Ag/AgCl reference electrode. All experiments utilized the reversible, one-electron oxidation of FcCH₂OH, which is diffusion-limited at E = 0.5 V vs. Ag/AgCl. When the potential on a Pt microelectrode is stepped to this potential, it reaches a steady, diffusion-limited current (I_{lim}). First the microelectrode was brought into contact with the culture medium and positioned near a group of cells. Approach curves were acquired by moving the microelectrode towards the polystyrene substrate at 1.0 μm/s; because the microelectrode was positioned away from the cells, the normalized current (I_{tip}/I_{lim}) vs. distance curve matched simulated curves for approach to an insulating surface [6]. Approach was halted when I_{tip}/I_{lim} ~ 0.8, corresponding to an imaging height of 10 μm. Images were obtained at 40.8 μm/s or 61.2 μm/s.

*Contribution of the U.S. Department of Commerce; not subject to copyright in the U.S.

As the microelectrode oxidizes FcCH_2OH , the cell must re-reduce it for positive feedback to be observed. In a series of images, we detected both positive and negative feedback (FIG. 1). Cells in two groups (a,b) regenerated FcCH_2OH , whereas several other cells (c,d) registered only slightly in the electrochemical image. Three individual cells (e,f,g) acted primarily as barriers to FcCH_2OH diffusion. Obtaining positive feedback at high ($500\ \mu\text{M}$) concentration suggests a large number of reduced electron carriers are able to react with FcCH_2OH . This is consistent with participation in the metabolic reactions of the electron transport chain, analogous to the resazurin/resorufin redox couple. We suspected that cells exhibiting negative feedback were dead, such that there were no reduced electron carriers available. We used a live/dead™ cell viability assay to test this hypothesis. This assay utilizes calcein acetoxymethyl, which penetrates the cell membrane and is hydrolyzed by esterases, giving rise to green fluorescence throughout the cytoplasm. Dead cells with compromised membranes allow ethidium homodimer-1 to penetrate the cell membrane and bind nucleic acids, producing red fluorescence. All cells probed electrochemically (except c) fluoresced green (FIG. 2). Electrochemical measurements therefore distinguish live, metabolically active cells from live, metabolically inactive cells.

However there is debate about the reliability of this assay [7], as cells with uncompromised membranes may retain esterase activity after death. Cells e, f, and g could thus be apoptotic. An alternate explanation for the observed negative feedback is that topographic effects exceed intracellular redox processes in these cells. We are exploring this possibility through modeling. We have observed similar results for another immortal cell line (SW-13 cells) and are expanding this work to cells used in tissue engineering [8].

References

- [1] T. Kaya et al., *Biosens. Bioelectron.* 18 (2003) 1379.
- [2] A.J. Bard et al., *Biosens. Bioelectron.* 22 (2006) 461.
- [3] X. Zhao et al., *Can. J. Chem.* 85 (2007) 175.
- [4] B. Liu et al., *J. Electroanal. Chem.* 500 (2001) 590.
- [5] J.M. Liebetrau et al., *Anal. Chem.* 75 (2003) 563.
- [6] J.L. Amphlett et al., *J. Phys. Chem. B* 102 (1998) 9946.
- [7] B. Rauch et al., *Am. J. Vet. Res.* 67 (2006) 1280.
- [8] Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

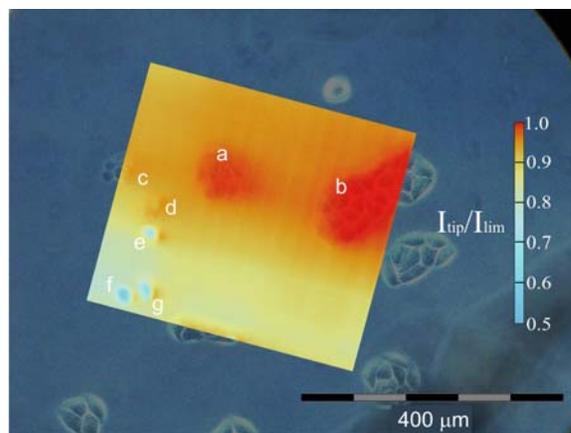


FIG. 1. SECM image overlain onto optical image.

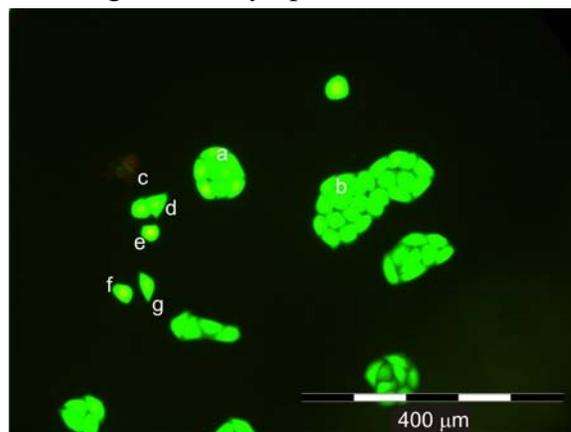


FIG. 2. Live/dead image of region shown in FIG. 1.