Nanoprobes for Imaging Single Cells under Low-Dose Radiation

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Reactive oxygen species (ROS) are produced by a variety of cellular mechanisms in response to low-dose radiation. Radiolysis of water, a major component of the intracellular environment, produces a variety of highly reactive radicals (superoxide and peroxide). The formation of these species occurs almost immediately, and it has been assumed that their role in the response to radiation is due solely to oxidative damage within the cell. However, it is becoming clear that intracellular oxidation / reduction reactions can be perturbed by this initial change in ROS and remain altered over extended periods of time, leading to acute and chronic changes in metabolism, cell signaling and gene expression. Techniques to measure quantitatively the early events associated with irradiation of cells are crucial to understanding the molecular mechanism of ROS generation, but are currently lacking. Given the potential interaction between primary responding cells and those altered by secreted factors or indirect effects, the ability to probe ROS in single cells would be especially effective. To address this deficiency, we develop a nanoscale sensor that will measure intracellular ROS formation upon exposure to low doses of ionizing radiation, in real time and in a quantitative manner. We begin by developing a probe for hydrogen peroxide (H_2O_2) as a prototypic ROS. A highly sensitive fluorescence assay using Amplex red is employed in this study for the detection of H_2O_2: in the presence of horse-radish peroxidase (HRP), H_2O_2 reacts stoichiometrically with the non-fluorescent Amplex Red to generate the red-fluorescent oxidation product resorufin (Fig 1).

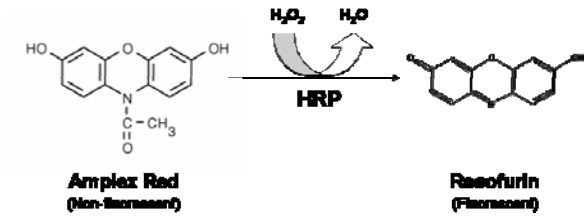

Figure 1. Amplex red assay for H_2O_2 detection

In this report, HRP-doped nanosensors are prepared by immobilizing HRP in silica nanoparticles during base-catalyzed hydrolysis of tetraethylorthosilicate (TEOS). (Fig. 2)

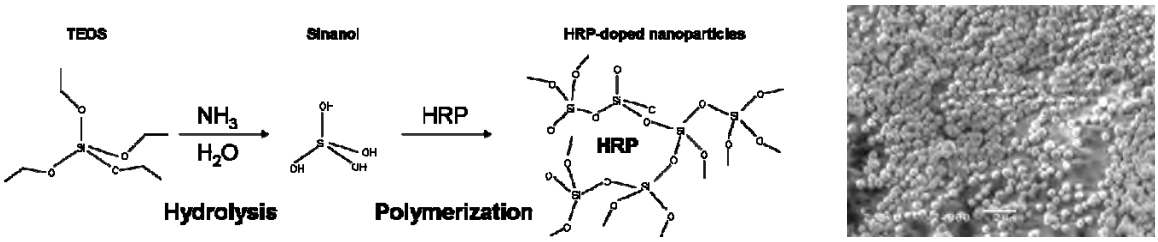

Figure 2. Synthesis of HRP-doped silica nanoparticles from base-catalyzed hydrolysis of TEOS. The picture on the right shows the SEM micrograph of the nanoparticles (The scale bar in the image is 2μm).

Activity and sensitivity to H_2O_2 of Encapsulated HRP were investigated. HRP-doped nanoparticles were suspended in sodium phosphate buffer (pH 7.4) and aliquots of 100μM Amplex red working solution and H_2O_2 standard solution were added. The resulting mixture was incubated at 37 °C for 30 min. followed by fluorescence measurements (excitation at 550 nm, emission at 585 nm) The fluorescence intensities obtained using the encapsulated HRP correlated closely with those of the enzyme in aqueous solution, indicating that the encapsulation into the nanoparticles preserved the enzyme activity. A limit-of-detection (LOD) of 25 nM for H_2O_2 was determined using the calibration curve shown in Fig. 3. This LOD is equivalent to the LOD obtained using a solution phase Amplex red assay, indicating that the diffusion of Amplex red and H_2O_2 and their interaction with the nanoparticle does not impose a restriction in comparison to the solution phase assay. Reusability of the HRP-encapsulated nanoparticles for multiple cycles was also demonstrated. These results show the great potential of using such a simple, reliable, and highly sensitive bionanosensor for studying the effect of low-dose radiation on cells by measuring ROS in localized environment within a cell.

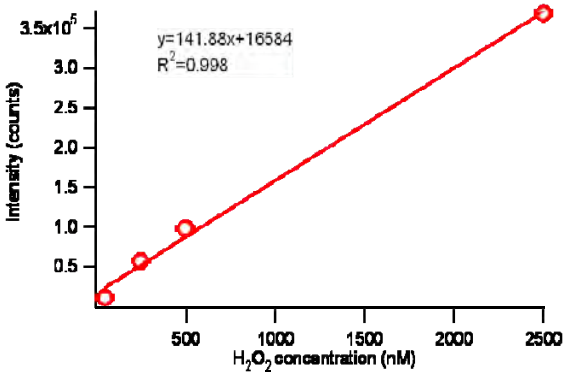

Figure 3. Calibration curve for H_2O_2 detection using HRP-doped silica nanoparticles