

# In vitro Validation of ROS Nanosensors in Renal Cancer Cells

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This research aims to synthesize and validate gold-based nanosensors that will be able to measure the concentration of hydrogen peroxide ( $H_2O_2$ ) and thus differentiate renal cancer cells from normal cells. Non-fluorescent carboxy- $H_2DCFDA$ , which becomes green fluorescent when it is oxidized, was conjugated to gold nanoparticles (AuNPs) to detect  $H_2O_2$ . Therefore, it was hypothesized that: (1) carboxy- $H_2DCFDA$  conjugated gold nanoparticles (carboxy-AuNPs) will be more sensitive in detecting  $H_2O_2$  than carboxy- $H_2DCFDA$  and (2) compared to normal cells, renal cancer cells treated with carboxy-AuNPs will show higher values of fluorescence intensity. The saturated concentration of carboxy- $H_2DCFDA$  in 1 mL of AuNPs was obtained to be  $10\mu L/mL$  through the experiment performed with a spectrophotometer. The carboxy-AuNPs was tested its sensitivity in detecting 100mM of  $H_2O_2$  with a fluorescence plate reader and it was 11.6 times more sensitive than carboxy- $H_2DCFDA$ . The kidney cell line (293T) and the renal cancer cell lines (786-O and A498) were harvested and plated for the fluorescence intensity test. When applied to the cells, carboxy-AuNPs showed lower values of fluorescence intensity compared to carboxy- $H_2DCFDA$ . Interestingly, however, carboxy- $H_2DCFDA$  could differentiate normal cells from renal cancer cells based on their fluorescence intensity values. Therefore, this research suggests that carboxy- $H_2DCFDA$  can be applied to cells to detect the presence of  $H_2O_2$  and possibly differentiate the cancer cells from normal cells based on their fluorescence intensity values. It also suggests that the sensitivity of carboxy- $H_2DCFDA$  in cells cannot be enhanced by the gold nanoparticles.