



**CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY**

Name(s) Shamik Mascharak	Project Number S0419
Project Title Synthesis of Fluorescent Silica Nanoparticles Conjugated with RGD Peptide for Detection of Invasive Human Breast Cancer	
<p style="text-align: center;">Abstract</p> <p>Objectives/Goals The objectives of this project were to synthesize fluorescent silica nanoparticles (FSNPs) with RGD peptide covalently linked on the surface amino groups and to employ these FSNPs as fluorometric probes to detect invasive human breast cancer cells via strong interaction between RGD peptide and integrin proteins on the cell surfaces.</p> <p>Methods/Materials 3-aminopropyltriethoxysilane (APTS), fluorescein-5-isothiocyanate (FITC), tetraethylorthosilicate (TEOS), cyclohexane, n-hexanol, triton X-100, 3-(Trihydroxysilyl)propylmethylphosphonate (THPMP), DAPI, Perkin-Elmer Fluorescent Spectrometer, Zeiss HAL 100 Fluorescent Microscope, JEOL 1200 EX TEM. Methods: First, a batch of 69 mg of APTS and 5.25 mg FITC was dissolved in 1 mL of absolute ethanol under dry N₂ atmosphere to isolate the fluorescent silane reagent. Next, 50 microL of the FITC-APTS conjugate, 15 microL of THPMP (to prevent aggregation), 100 microL TEOS and 100 microL ammonium hydroxide were reacted in a water/oil microemulsion. After 24 hours, the microemulsion system was destabilized with ethanol, and the FSNPs were collected by centrifugation. The FSNPs were then washed and centrifuged (2000 RPM, 10 min), and checked for quality via TEM and fluorescence microscopy (515 nm). For tumor targeting, the FSNPs were conjugated to RGD peptide with SPDP as the coupling reagent. The fluorescence properties of the peptide-nanoparticle conjugates were checked via fluorescent spectroscopy and TEM imaging. Both RGD-FSNP conjugates and FSNPs were then added to MCF7 (non-invasive breast cancer cells), MDA-MB 435 (transformed human breast cancer cells), and MDA-MB 231 (metastasized breast cancer cells) grown in 8-well trays (2 days, 5% CO₂ atmosphere). After 2 hrs, the cells were thoroughly washed, fixed, stained with DAPI, and subjected to fluorescence microscopy.</p> <p>Results The FSNPs synthesized in this project showed a narrow range of dispersivity (70 nm). They are stable in aqueous buffer over weeks and strongly fluoresce at 515 nm. The FSNP-RGD peptide conjugates selectively got attached to the high concentration of integrins expressed on the surface of the metastasized cancer cells. In the case of the non-invasive cells, the expression of integrin was low and hence such cells showed very few FSNPs on the cell surface.</p> <p>Conclusions/Discussion The results confirm that FSNP-RGD conjugates are excellent imaging tools for cancer detection.</p>	
Summary Statement My project is aimed at synthesizing a non-toxic, fluorometric nanoparticle probe to detect invasive human breast cancer cells.	
Help Received Michael Rose (a UCSC grad student) helped in the synthesis and cell experiments; Walter Bray of UCSC provided the cells; Dr. Yang of UCSC helped in obtaining the TEM images.	