NDnano
Notre Dame Nanoscience and Technology

NDnano Summer Undergraduate Research
2023 Project Summary

1. Student name & home university: Mónica Leal Palma, Universidad de las Américas Puebla (UDLAP).

2. ND faculty name & department: Dr. Meenal Datta and Dr. Ryan Roeder, Aerospace and Mechanical Engineering (AME).

3. Summer project title: Nanoparticle-mediated reprogramming of cells to fight cancer.

4. Briefly describe new skills you acquired during your summer research:

- Cell culture
- RNA extraction and qPCR
- Immunofluorescence staining
- Fluorescence microscopy
- Animal training, mice work

5. Briefly share a practical application/end use of your research:

The main objective of this project is to study the ability of fluorescein isothiocyanate labeled silica (FITC-SiO_2) nanoparticles (NPs) of targeting and reprogramming cancer-associated fibroblasts (CAFs) into a tumor-restraining state, in order to help reduce tumor growth.

6. 50- to 75-word abstract of your project:

Fibroblasts are important for maintaining the structure and function of tissues, but when cancer signals are present, they can adopt an activated phenotype marked by the expression of different biomarkers. These cancer-associated fibroblasts (CAFs) are an abundant component of the tumor microenvironment (TME) in many solid tumors, and are known to promote tumor progression, metastasis, immunosuppression, and treatment resistance. The objective of this study is to investigate fluorescein isothiocyanate labeled silica (FITC-SiO_2) nanoparticles (NPs) as a platform for engineering fibroblast-specific antibodies to enable targeting and reprogramming of CAFs into a tumor-restraining state.

7. References for papers, posters, or presentations of your research:

1. Han, et al. Biomarker Research, 2020, 8:64.
2. Nallathamby, et al. Journal of Materials Chemistry B, 2016,4 (32), 5418-5428.
3. Leal Palma, Mónica. (2023, July 21). Nanoparticle-mediated reprogramming of cells to fight cancer. [Research Presentation]. NDnano Research Reviews, Notre Dame, IN, United States.
4. Leal Palma, Mónica. (2023, July 28). Nanoparticle-mediated reprogramming of cells to fight cancer. [Poster Presentation]. University of Notre Dame Summer Research Symposium, Notre Dame, IN, United States.

Project summary that describes problem, project goal and your activities / results:

Fibroblasts are important for maintaining the structure and function of tissues, but when cancer signals are present, they can adopt an activated phenotype marked by the expression of biomarkers such as fibroblast activation protein alpha (FAPα) and alpha smooth muscle actin (αSMA). These cancer-associated fibroblasts (CAFs) are an abundant component of the tumor microenvironment (TME) in many solid tumors, and are known to promote tumor progression, metastasis, immunosuppression, and treatment resistance. However, a subset of CAFs (e.g., CAV1^{high} CAFs) are also known to promote anti-tumor activity[1]. Nanoparticles (NPs) have been used to target cells due to enhanced targeting specificity, high payload, protection of delivered biomolecules and imaging capabilities.

The objective of this study is to investigate fluorescein isothiocyanate (FITC) labeled silica (FITC-SiO_2) NPs as a platform for engineering fibroblast-specific antibodies to enable targeting and reprogramming of CAFs into a tumor-restraining state (Fig. 1).

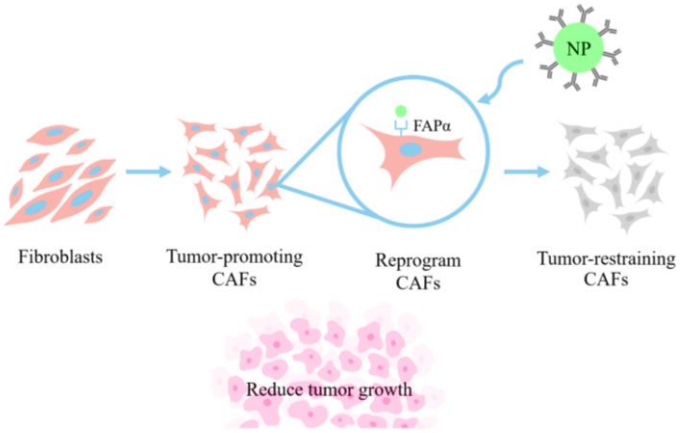

Fig 1. Schematic representation of the project summary.

FITC-SiO_2 NPs, were synthesized using a modified Stöber method. FITC-SiO_2 NPs exhibited unchanged hydrodynamic size of ~124.8 nm and fluorescence intensity for a week in water, indicating aqueous stability and sufficient fluorescence for longitudinal imaging. Proof of concept antibody conjugation was demonstrated by functionalizing FITC-SiO_2 NPs with COOH groups and subsequently conjugated with immunoglobulin G (IgG) antibody using EDC/NHS chemistry (Fig. 2-3)[2]. Antibody bioconjugation was confirmed by visible agglomeration when mixing IgG conjugated FITC-SiO_2 NPs with protein A biobeads (Fig. 3C). In contrast, NPs without IgG and mixed with protein A biobeads exhibited the fluorescent of NPs without any agglomeration.

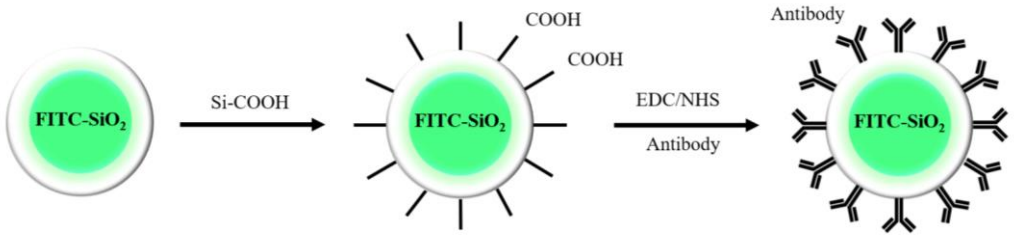

Fig 2. Diagram showing the synthesis of FITC-SiO_2 NPs, functionalization with COOH groups and bio-conjugation with antibodies.

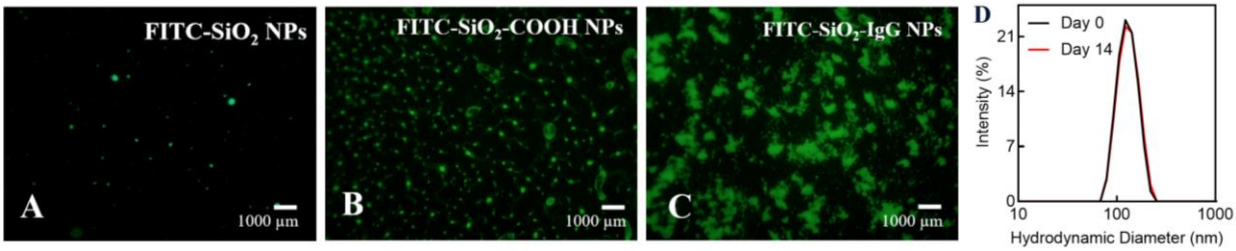

Fig 3. Nanoparticle imaging and characterization. **A**, fluorescence image of FITC-SiO_2 NPs. **B**, fluorescence image of FITC-SiO_2 NPs after functionalization with COOH groups. **C**, fluorescence image of FITC-SiO_2-COOH NPs conjugated with IgG antibodies and mixed with protein A biobeads. **D**, plot of the FITC-SiO_2-COOH NPs hydrodynamic size measurement by Dynamic Light Scattering (DLS).

FITC-SiO_2 NPs conjugated with FAP antibody are being investigated for their ability to target and reprogram TGF-β-transformed CAFs in vitro. NIH3T3 murine fibroblasts were cultured and treated with human recombinant TGF-β. The activation of the murine fibroblasts following TGF-β treatment was evaluated by qPCR and immunofluorescence staining. A preliminary pilot study to investigate the biodistribution of NPs and evaluate their performance *in vivo* was performed. Mice were injected via intramammary with 100 mg/kg dose (mice #884 and #886) and 50 mg/kg dose (mice #885 and # 887). NPs exhibited nontoxicity after 24h, at both doses exhibited strong and stable imaging signal for 24 h after injection (Fig. 4).

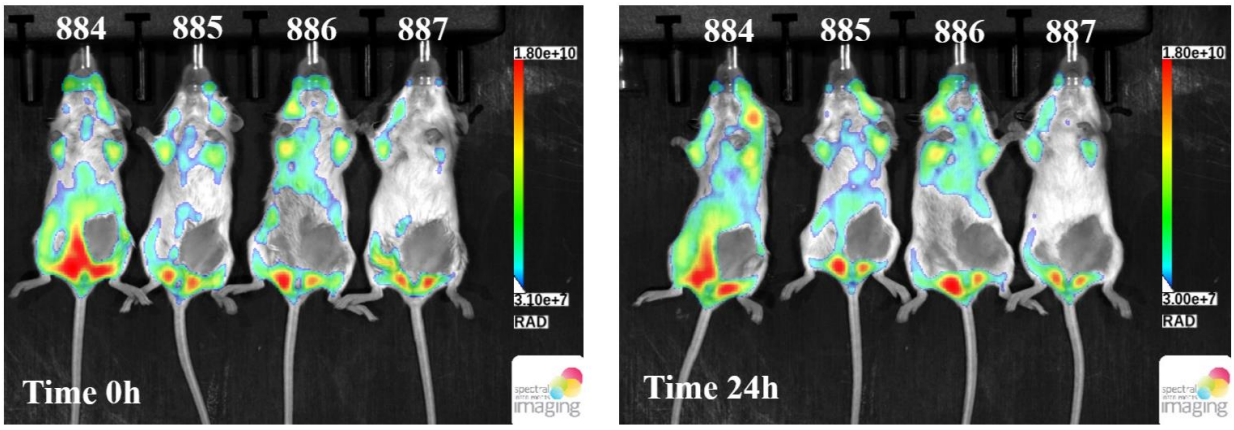

Fig 4. Live imaging of the nanoparticle biodistribution pilot study in a health mouse model at time 0 and after 24 h.

The future work would be focused on the co-culture TGF-β-transformed CAFs with varying concentrations of SiO_2-FAPα NPs and evaluate the target and reprogram ability by immunostaining and qPCR. In addition to investigate the in vivo NP biodistribution, targeting and reprograming in a tumor bearing mouse model using live imaging, confocal imaging, and histology.