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14. ABSTRACT Each person's cancer is as unique as his or her fingerprint, which explains unpredictable responses to therapies and poses new biotechnology challenges for tumor characterization on the molecular level. For these reasons, it is of pivotal importance to develop novel molecular profiling methodologies for diagnosis, prognosis and individually tailored therapeutics of patients based on the biology of their tumors. We proposed to develop oligonucleotide tagged quantum dots and antibodies for multiplexed imaging of prostate cancer specimens.					
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Annual Report: PC061345-Molecular Profiling of Prostate Cancer Specimens Using Multicolor Quantum Dots

PI: X. Gao, Department of Bioengineering, University of Washington

Introduction:

There is increasingly compelling evidence that cancer varies both genetically and phenotypically between patients who have identical histologic and tissue types and stages of cancer. Each person's cancer appears to be as unique as his or her fingerprints. This uniqueness helps explain the variable and unpredictable responses of tumors in individual patients to therapies. The prognosis and choice of therapy for prostate cancer is currently based mainly on three parameters obtained at the time of diagnosis - clinical stage, serum prostate specific antigen, and the Gleason grade of the cancer. The grade which is based on microscopic tumor architecture, has a value between 2 (well differentiated and indolent) and 10 (poorly differentiated and rapidly progressive).¹ Studies have demonstrated a direct correlation between grade and clinical measurements of disease outcome, including time to tumor recurrence and probability of dying of tumor. However, the Gleason system has limitations. It is (1) subject to interobserver variability; (2) does not stratify patients into a large number of categories (>85% of tumors are grade 6 or 7); and (3) does not provide molecular information. Molecular biomarkers that can be localized in needle biopsy tissue using immunostains have been developed to better predict the biology of prostate cancers. Knowing the molecular profile of a prostate cancer raises the prospect of therapy targeted to specific molecules. We proposed a multivariate molecular pathology approach for prostate cancer diagnostics, prognostics as well as prediction of the outcome of therapies using oligonucleotide tagged semiconductor quantum dots and specific antibodies as shown in **Figure 1**.

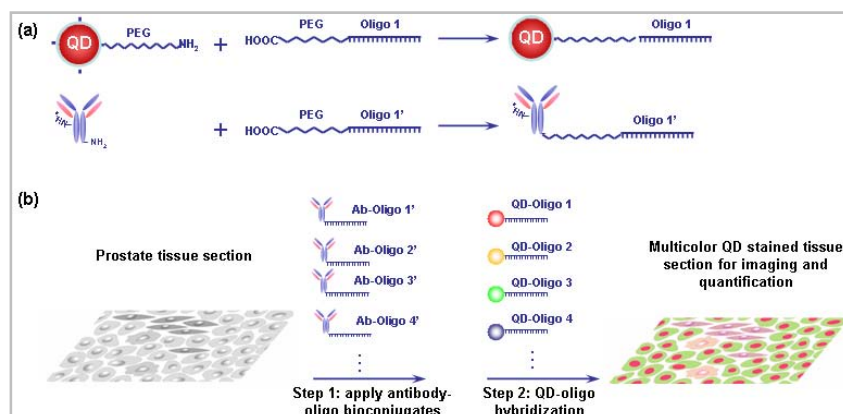


Figure 1. Schematic illustration of FINISH technology. (a) to prepare FINISH probes, QDs and antibodies are first tagged with complimentary pairs of oligonucleotides via covalent bond. (b) a two-step assay of first applying all the antibody probes, then multicolor QDs tagged with complimentary oligonucleotides. If the target is present, hybridization will occur and vice versa. Thus, the antibody binding assay is transformed into multiplexed and robust DNA hybridization

Body:

In our proposed research, there are two specific Aims. Aim 1 focuses on probe preparation; Aim 2 focuses on application of the technology on cells and tissues; and Aim 3 focuses on the comparison of this new technology with the conventional low-throughput technologies. In the past year, we started with QD preparation, QDs with emission wavelength ranging from 480-650 nm with high quantum yield have been synthesized based on literature procedures.²⁻⁵ The QDs were

characterized by optical absorption, fluorescence emission, transmission electron microscopy, and dynamic light scattering, to ensure sufficient quantum yield and monodispersity. The resulting QDs were not water-soluble. We have converted them into hydrophilic nanoparticles by using amphiphilic copolymers. We have identified a series alkyl modified polymaleic anhydride polymers that are well-suited for nanoparticle solubilization. Our result show that polymers with molecular weight ranging from 15,000 to 25,000 works the best due to their high solubility in hydrophilic solvents and strong binding affinity to nanoparticle surface.

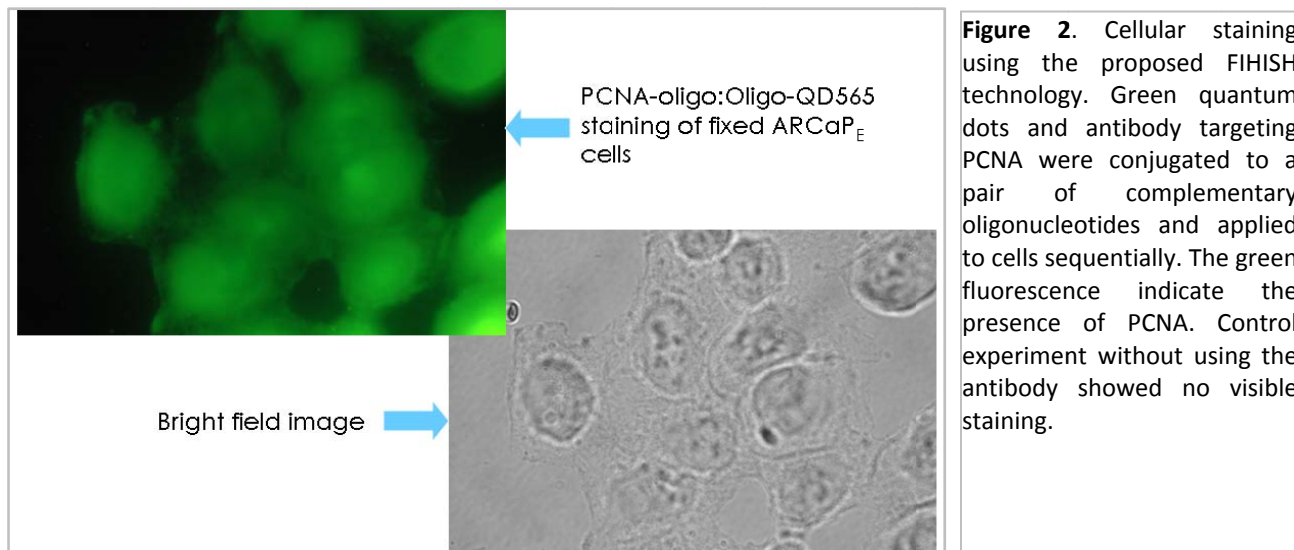
In parallel to the nanoparticle synthesis, we have also designed and synthesized multiple oligonucleotide sequence pairs (16 base long) that have approximately the same melting temperatures (T_m) and no or minimum similarity to endogenous DNA in mammalian cells using commercially available software and database (IDT oligo design & NCBI BLAST). In addition, to minimize non-specific binding and potential steric hindrance from nanoparticles, each oligonucleotide strand was inserted with a polyethyleneglycol (PEG) spacer. The carefully selected sequences are listed in Table 1.

Table 1. Oligonucleotide sequences selected

No.	func. group	spacer	5'	oligonucleotide	3'
1	NH2	PEG54		CGTCGCACCAAGAAAT	
1'	NH2	PEG54		ATTCTTGGTGCGACG	
2	NH2	PEG54		TAGACTTGCCATACGT	
2'	NH2	PEG54		ACGTATGGCAAGTCTA	
3	NH2	PEG54		AATTCTTGAGACCAGG	
3'	NH2	PEG54		CCTGGTCTCAAGAATT	
4	NH2	PEG54		TGAAGACCTGGCCAAT	
4'	NH2	PEG54		ATTGGCCAGGTCTTCA	
5	NH2	PEG54		TTGATGTGGGTGGGAA	
5'	NH2	PEG54		TTCCACCCACATCAA	
6	NH2	PEG54		ATCTGCCCAAACCTCCA	
6'	NH2	PEG54		TGGAGTTTGGGCAGAT	
7	NH2	PEG54		TTCCAAGCGTCATCT	
7'	NH2	PEG54		AGATGACGCTTGGGAA	
8	NH2	PEG54		TCTTTGGGACGCTGAA	
8'	NH2	PEG54		TTCAGCGTCCCAAAGA	
9	NH2	PEG54		GTGTCTCGTGGCTACC	
9'	NH2	PEG54		GGTAGCCACGAGACAC	
10	NH2	PEG54		TCACCGAGCGATTTCT	
10'	NH2	PEG54		AGAAATCGCTCGGTGA	

We then proceeded with the bioconjugation. The complimentary oligonucleotides are linked to carboxy-QDs and antibodies using carbodiimide coupling reagent EDAC. The resulting

bioconjugates were thoroughly purified to remove any free oligonucleotide, which could potentially compete with specific binding. For technology testing, we stained ARCaP cells (one of the best model cell lines in prostate cancer research) using the bioconjugates. As shown in **Figure 2**, the fluorescence staining are specific and very bright, which serves as a functional test of the newly designed fluorescent probes.



Key Research Accomplishments

- Synthesis of multicolor quantum dot nanoparticles
- Water-solubilization of quantum dots and surface functionalization with carboxylic acids
- Design and synthesis of oligonucleotides with similar T_m and minimum similarity to endogenous DNA in mammalian cells
- Bioconjugation of oligonucleotides to antibodies and quantum dots using carbodiimide chemistry
- Purification of the bioconjugates using size-exclusion chromatography
- Cellular staining of PCNA antigen in ARCaP cells

Reportable Outcomes

- Training of Ph.D. student Pavel Zrazhevskiy. This project will become a major section of his Ph.D. thesis when he graduates

- Book chapter entitled “Molecular profiling of cancer cells and tissues using multicolor quantum dots” by P. Zrazhevskiy and X. Gao accepted for publication in *Encyclopedia of Inorganic Chemistry* edited by CM Lukehart and RA Scott.

Conclusion

In conclusion, we have successfully prepared the quantum dots, oligonucleotides and their conjugates, and met the milestones for year 1. In the second half of the project period, we will continue our current effort to further develop this technology for multicolor staining of cells and tissue section.

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Appendices N/A