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Quantifying Serum miRNA using DNA-Gold Nanoparticles: A Modern Approach to Diagnosing Pancreatic Cancer

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ABSTRACT

Pancreatic Cancer (PC) is the third leading cause of cancer-related mortality in America with nearly 50,000 deaths annually. Furthermore, PC's low 5-year survival rate, 11% overall, is highly dependent on the stage during which it is diagnosed and treated. When confined to the pancreas, the 5-year survival rate has been shown to reach as high as 42%, a nearly four-fold increase. **Due to significant mortality differences dependent on PC stage, there is an utmost clinical need to diagnose PC as early as possible to maximize the chance of favorable patient outcomes.** Most PC diagnostic methods currently involve imaging techniques including x-rays, CT, MRI, US, PET, ERCP. Issues with these techniques include levels of discomfort for the patient and higher costs and commitment to diagnose PC. For this reason, noninvasive diagnostic tests, such as CA 19-9 and CEA antigen blood tests are widely used to better inform patient outcomes. Current blood tests struggle to show appropriate specificity or sensitivity to reliably diagnose PC, including the limited ability to discriminate between benign and malignant tumors. Lacking validity may lead to patients over or under-investing in PC treatment. **In comparison to blood antigens, microRNA (miRNA) is a biomarker that can be much more capable of diagnosing PC.** The miRNAs consist of strands of around 20 base pairs in length that are transcribed in the body for gene regulation, controlling the expression of over half of our total genes. Aberrant expression of miRNAs are associated with PC. Current diagnostic tests that utilize miRNA biomarkers (e.g., microarray, qPCR) lack the sensitivity and limit of detection (LOD) required to assay normal versus aberrant miRNA levels since they are designed to detect longer nucleotide chains. Current methods are also cumbersome to use as they require extreme precaution and resources to collect data and avoid contamination. **We propose new nanoparticle-based DNA-AuNP technology that will run a real time *in situ* assay of a patient's serum sample to more reliably inform PC diagnosis early on.**

HYPOTHESIS AND OBJECTIVES

Hypothesis

DNA-Au Nanoparticles (DNA-AuNP) allow for sensitive and reliable quantification of miRNA in situ through a serum sample. This can be used to help better diagnose PC.

Objectives

1) Synthesize and characterize (hydrodynamic diameter, UV spectra, etc.) DNA-AuNPs with attached probes.

2) Run kinetic DNA-AuNP assays on samples spiked with varied levels of miRNA-21 and miR-221 to test quantification.

3) Run kinetic DNA-AuNP assays on human serum training samples to observe PC diagnostic potential.

EXPERIMENTAL DESIGN AND ASSAY MECHANISM

Synthesis and mechanism of DNA-AuNP probes for quantification of miRNAs

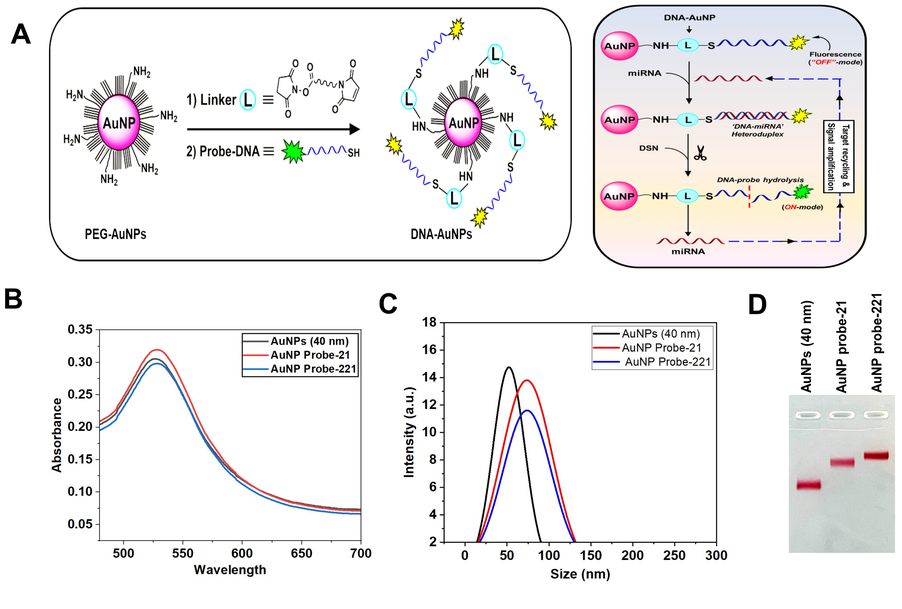
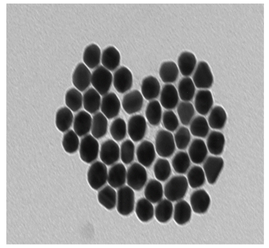


Figure 1:. **(A)** Representative scheme for the synthesis and mechanism of DNA-AuNPs (1) When DNA probes hybridize with complementary miRNAs, the resulting heteroduplex is recognized by the DSN enzyme, allowing it to hydrolyze the probe strand and cleave it from the heteroduplex. 2) Once separated from the AuNP, FAM is then able to fluoresce and be detected. 3) miRNAs in the heteroduplex are not targeted by DSN, allowing them to be reused and amplify the signal. **(B)** UV-vis spectra of 40 nm AuNPs and DNA-AuNPs. (Red: AuNP probe-21 and blue: AuNP probe-221). **(C)** DLS spectra of AuNPs, indicating ~15 nm increment in hydrodynamic diameter (ø) of DNA-AuNPs observed upon surface modification. **(D)** Gel electrophoretic analysis of DNA-AuNPs. A reduced electrophoretic mobility of DNA-AuNPs other than Linker-AuNPs revealed successful loadings of DNA-probes on PEG-coated AuNPs.

RESULTS

Characterization of DNA-AuNP probe & Summary of AuNPs characterization

A



B

Summary of the AuNPs characterization

AuNPs type[a]	Size (DLS, nm)	PDI[b] (DLS)	ζ-potential (NTA, mV)
40 nm AuNPs	44.8 ± 19	0.15	− 37.2 ± 2.9
AuNP Probe-21	77.0 ± 20	0.19	− 35.1 ± 3.1
AuNP Probe-221	61.2 ± 16	0.18	− 34.3 ± 3.6

C

D

Summary of the calculation of No. oligos per AuNP (N_{Oligo})

miRNA-specific DNA-AuNPs[a]	Mole fraction (χ) of PEG-NH_2[b]	[FAM][c] nM	[AuNP][d] nM	DNA/AuNP[e]
miR-21	0.15	224 ± 10	3.1	71 ± 8
miR-221	0.15	329 ± 8	2.3	137 ± 5

Figure. 2. Spectroscopic characterization 40 nm AuNPs and DNA-AuNPs. (A) Representative TEM image of DNA-AuNPs (miR-21 specific), showing stable, uniform size and monodisperse particle core after surface functionalization (Scale bar 100 nm). **(B)** Summary of AuNPs characterization (DLS: Dynamic light scattering, PDI: Polydispersity index, NTA: Nanoparticle tracking analysis). **(C)** Evidence-based selection of a 9-miRNA panel. Differentially expressed miRNAs in pre-cancerous IPMNs (KC mouse model) & in PC was determined from comparison of ours (Batra et al. Oncotarget, 2015. 6(37): p. 40295-309, 3 datasets, 22 upregulated miRNAs), & 2 others meta-analyses (Chhatriya et al. Pancreas, 2012. 41(5): p. 685-90, 8 datasets, 17 upregulated miRNAs; and Zhang et al. Sci Rep, 2019. 9(1): p. 123, 10 datasets, top 48 miRNAs), from literature review. **(D)** Summarized table showing number of Oligos per AuNP (NOligo) determined by measuring the concentration of the probe (fluorescence assay) per concentration of AuNPs (UV Visible spectroscopy).

Real-time kinetic fluorescence assay (quantification of miR-21 and miR-221)

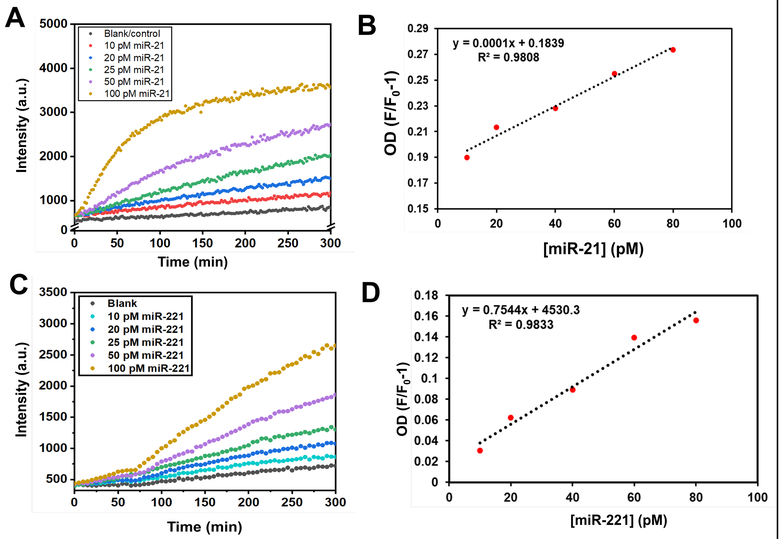
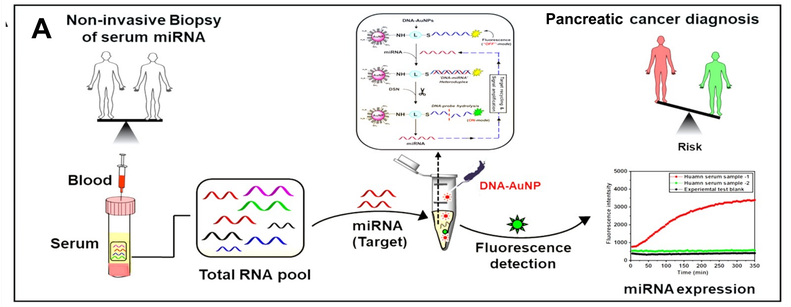


Figure 3. Fluorescence spectra and standard calibration curves. Kinetic real-time monitoring of FI using variable concentrations of synthetic miRNA targets. **(A)** Graph of FI versus time for miR-21 standards, **(B)** Standard calibration curve for miR-21. **(C)** Graph of FI versus time for miR-221 standards. **(D)** Standard calibration curve for miR-221. F:Fluorescence intensity of the sample and F_0 Fluorescence intensity of the blank.

DNA-AuNPs probe-based quantification of human PC serum miRNA



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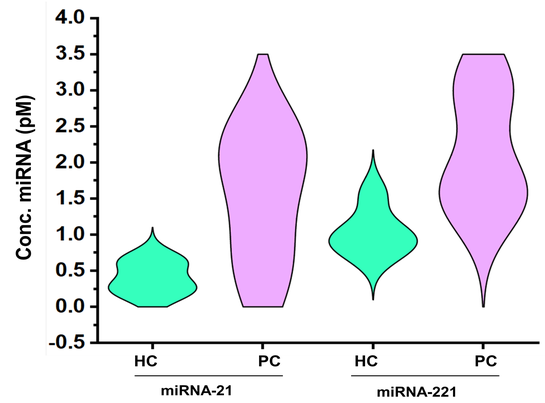

Figure 5. DNA-AuNP-based biopsies of serum miRNAs and assessment of their diagnostic performances in PC diagnosis. (A) A pictorial diagram displaying the scheme for Non-invasive biopsies of serum miRNAs. **(B)** Violin plot representing the quantitative expression of serum miRNA-21 and miR-221 samples: [HC (n= 6) and PCa (n=6)]. Data are shown as mean ± SD of triplicates.

❖ ***Tested miRNAs efficiently differentiated PC patients from the healthy controls, confirming their diagnostic potential in PC diagnosis.***

CONCLUSIONS

1) **Successfully synthesized and stored 40nm Gold Nanoprobes for miRNA detection.**
2) **Characterized our DNA-AuNPs by measuring hydrodynamic diameter, UV spectra, and electrophoretic mobility indicating that DNA-AuNP nanoprobes are stable, uniform sized.**
3) **DNA-AuNP kinetic assays were successfully run with a training set of PC and control serum samples.**
4) **Gold Nanoprobes offer non-invasive and PCR-free detection of miRNA biomarkers that may be a future diagnostic tool for PC.**

FUTURE DIRECTIONS

1) **Run DNA-AuNP kinetic assay on larger training and blank sets (n > 100).**
2) **Utilize multiple fluorophores to facilitate multiplexing and quantify multiple miRNAs at once.**
3) **Apply DNA-AuNP based miRNA assays to help better diagnose other cancers.**
4) **Use different probes to test relationship between different miRNAs' upregulation and PC.**

REFERENCES

1) **American cancer Society – Cancer Facts and Figures-2022**
2) **B.L. Jackson, A. Grabowaska, H. Ratan BMC Cancer 2014, 14, 930-940**
3) **B.A. Walter, V.A. Valera, P. Pinto, M. J. Mario J. of Cancer 2013, 350-357**
4) **F. Degliangeli, P. Kshirsagar, V. Brunnetti, P. Pompa, R. Fiammengo JACS 2014 136, 2264**
5) **P. Kshirsagar, SK Batra et al. Volume 43, July 2022, 102566**