Sequence-specific microRNA detection by induced electroosmosis flow inside a borosilicate capillary

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Significance

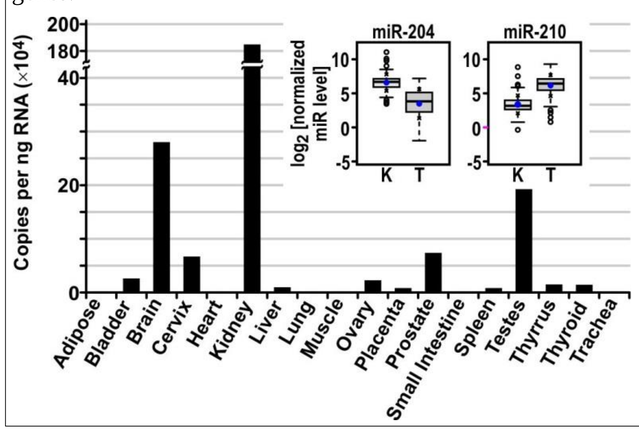
Recently, there have been significant efforts in the identification and isolation of cancer-related biomarkers in body fluids such as circulating tumor cells, extracellular vesicles, cell-free DNA and microRNAs (miRNAs). Cancer detection based on circulating biomarkers is advantageous over the traditional tissue biopsy because it is minimally invasive and can serve as better representatives of the primary and metastatic sites and metastatic sites.

To this day, a few hundred miRNAs have been identified in human cells, and several have been shown to have either pro-oncogenic or tumor suppressing activities. MiRNAs are short, noncoding, ~22-nucleotide-long RNAs that regulate gene expression primarily at the post-transcriptional level. The primary function of miRNAs is to inhibit translation of target genes, but they can also process mRNAs for cellular decay and degradation. MiRNAs can be passively leaked from apoptotic cells or actively released by exosomes secreted from cells in to the blood stream. Traces of circulating miRNAs have been identified in blood serum, plasma, urine and sweat. Thus, they have a remarkable potential to be utilized as non-invasive diagnostic, prognostic, and predictive cancer biomarkers.

The overall goal of this project is to demonstrate the feasibility of a new nanopore sensing concept for sensitive and cost-effective detection of cancer-related miRNA biomarkers that does not rely on polymerase chain reaction (PCR) amplification and does not require any special reagents other than a complementary sequence capture probe conjugated to polystyrene beads.

Target miRNAs

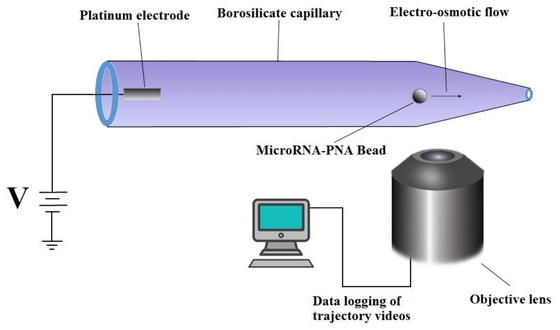
We have focused on the analysis of two miRNAs relevant for the growth of clear cell renal cell carcinoma (ccRCC). CcRCC is a malignant kidney cancer distinguishable by the early loss of the von Hippel-Lindau tumor suppressor protein (VHL), leading to the accumulation of the hypoxia inducible transcription factor (HIF) and induction of HIF-responsive genes.



Here we are targeting two miRNAs highly relevant for growth of ccRCC: (a) miRNA-210, which is a target of HIF and has pro-oncogenic activities and (b) miRNA-204, which is induced by VHL tumor suppressor and has tumor suppressing activities. As a model system we used human 786-O RCC cell line where VHL gene is inactivated and which expresses high levels of miRNA-210 and low levels of miRNA-204, and an isogenic cell line with reconstituted VHL, where levels of miRNA-210 are decreased and levels of miRNA-204 are induced as a result of VHL activity.

Nanopore-based sensor

A sequence-specific nucleic acid detection scheme has been developed by adapting a nanopore-based sensing technique and an assay of complementary probe peptide nucleic acid (PNA) conjugated to polystyrene beads.



1μm diameter borosilicate micropipette was fabricated by laser-assisted puller-Sutter P2000.

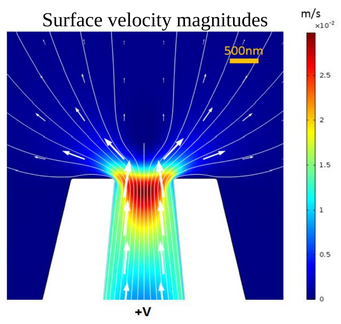
Beads motion trajectory was recorded and monitored by the high-resolution camera under the microscope.

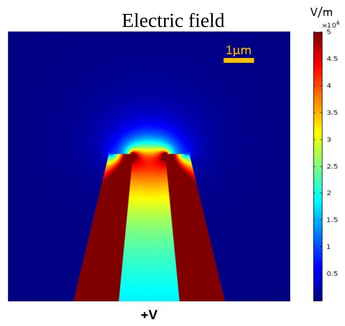
Signature ionic current were measured as beads blocked or passed through the pore.

Novelty of Detection Principle

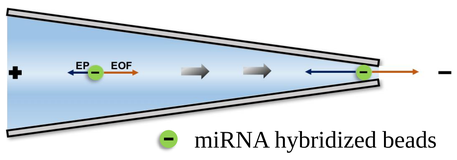
Electroosmosis flow inside the glass capillary is directed towards the tip of the pore and outwards under the applied bias. As illustrated in the following COMSOL simulation results, the size of the arrows and thus, the magnitude of fluid velocity is larger near the capillary surface compare to the bulk of the solution.

The conical shape of the pore leads to a non-uniform distribution of electrical field. The near tip zone of the capillary has relatively higher magnitude of electric field compared to the lower region farther from the pore.



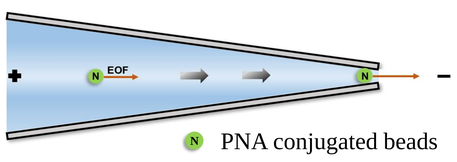


MiRNA detection is based on the unique electrical signals obtained by induced electroosmosis flow as a driving force to translocate the cargo beads toward the sensing zone and block the pore.



miRNA hybridized beads

MiRNA hybridized beads possess negative surface charge. At the tip of the pore, the strong non-uniform electric field results in opposing electrophoretic force (EP) which balance or exceed the electroosmotic force (EOF).



PNA conjugated beads

Neutral PNA-beads or non-complementary control beads experience mainly the elelctroosmostic force at the tip of the pore. As a result of low surface charge or loosely non-specifically bound RNA molecules.

Results and Discussion

1μm diameter pore blockades by 2.36μm beads

Target experiment: ***Serration shaped ionic current drops***

Control experiments: ***Right angle shaped ionic current drops***

miRNA204 detection results:

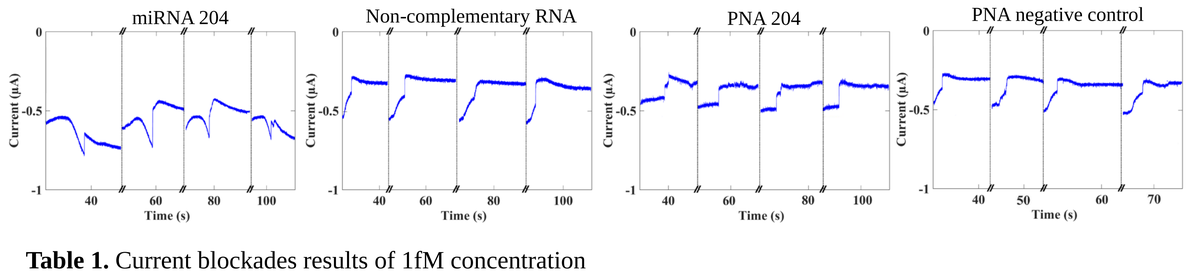
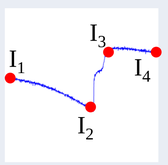
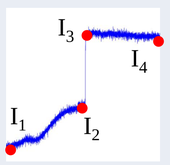
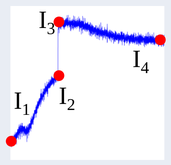
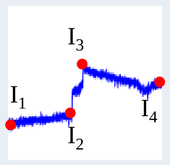


Table 1. Current blockades results of 1fM concentration

Current	miRNA 204	Non-complementary RNA	PNA 204	PNA negative control
blockade shape				
I_1 (μA)	-0.5103	-0.5654	-0.4334	-0.4289
I_2 (μA)	-0.6885	-0.4675	-0.3252	-0.4166
I_3 (μA)	-0.4057	-0.3181	-0.2401	-0.3471
I_4 (μA)	-0.5194	-0.3155	-0.2685	-0.3448

miRNA210 detection results:

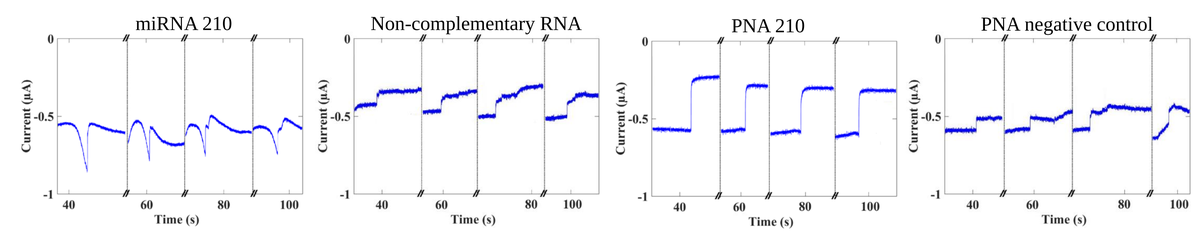
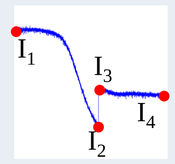
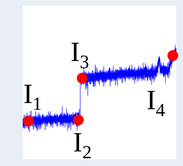
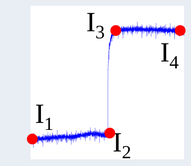
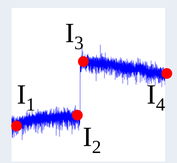


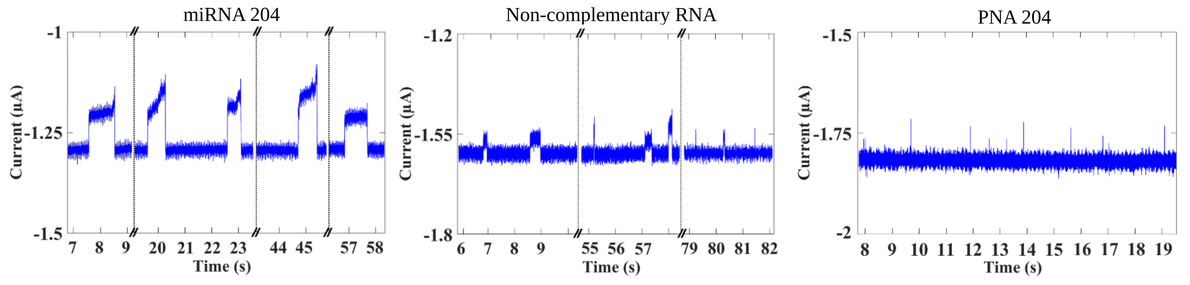
Table 2. Current blockades results of 10fM concentration

Current	miRNA 210	Non-complementary RNA	PNA 210	PNA negative control
blockade shape				
I_1 (μA)	-0.4385	-0.4318	-0.625	-0.5677
I_2 (μA)	-0.7957	-0.4266	-0.606	-0.5628
I_3 (μA)	-0.6598	-0.3625	-0.3519	-0.4881
I_4 (μA)	-0.6836	-0.3029	-0.3487	-0.5071

Passage of 0.97μm beads through the 1μm diameter pore

Different velocity of beads carrying target miRNA and noncomplenteray RNA due to opposing electrophoretic force:

$$v_{target} < v_{non-complemetary\ RNA} < v_{PNA}$$



- Target experiment: ***dwelling time = 1260ms***
- Non-complementary RNA experiment: ***dwelling time = 260ms***
- PNA 204 experiment: ***dwelling time = 1ms***

Conclusion

- In the case of glass nanopore sensing, electroosmotically driven system showed better detection sensitivity compare to the electrophoretically driven systems.
- The unique current blockades shapes and the long pulses dwelling times reprent the miRNA detection by our system.
- The concentration detection limit of our sensor are 1fM for miRNA204 and 10fM for miRNA210.

References

1. “VHL-Regulated MiR-204 Suppresses Tumor Growth through Inhibition of LC3B-Mediated Autophagy in Renal Clear Cell Carcinoma,” O. Mikhaylova, Y. Stratton et al, Cancer Cell, 21 (4), 532–546 (2012).

2. “Sequence-specific nucleic acid detection from binary pore conductance measurement,” L. Esfandiari, H.G. Monbouquette et al. J. Am. Chem. Soc., 134(38), 15880–6 (2012).