Detection of miRNA using NanoGap as *in vitro* diagnostic test for endometriosis

Introduction

Endometriosis is a condition of the female reproductive system where tissue similar to the uterine lining is also present in places other than the womb [1]. It is a very painful condition, especially during menstruation. When women seek medical help for this condition, laproscopic surgery is the current golden standard to diagnose endometriosis. Because this is such an invasive procedure, not many fertile women are willing to undergo it. Therefore there is an unmet need for a noninvasive test that can reliably diagnose endometriosis in women.

Biomarkers

A panel of circulating mi-RNAs that are dysregulated in endometriosis are chosen for specificity, sensitivity, and if dysregulation of the mi-RNAs in other diseases. The panel of mi-RNAs can be seen in Table 1.

Table 1: Panel of miRNAs for use in detection of endometriosis [2][3][4]

Type	Expression in endometriosis
miR-143	Upregulated
miR-199a	Upregulated
miR-200a	Downregulated
miR-122	Upregulated

Samples

Population: Woman between 15 - 49 [5] who are infertile and/or show symptoms of endometriosis; painful periods, pain with intercourse, pain with bowel movements or urination, excessive bleeding, infertility. [6]

Sample collection: Peripheral blood samples are collected into collection tubes (5-10 mL)

Time of sampling: In the afternoon. During the proliferative phase of the menstruation cycle.

Working principle

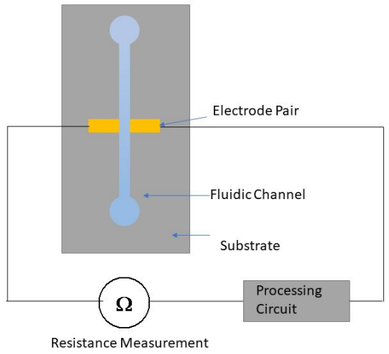
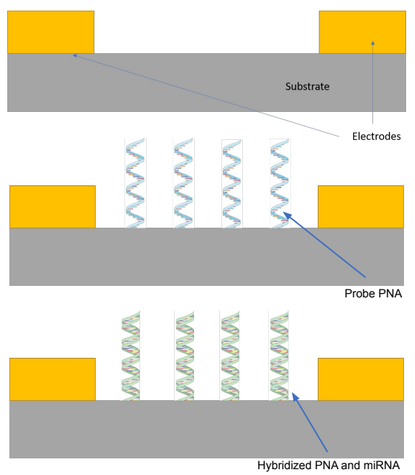


Figure 1: Single channel viewed from above



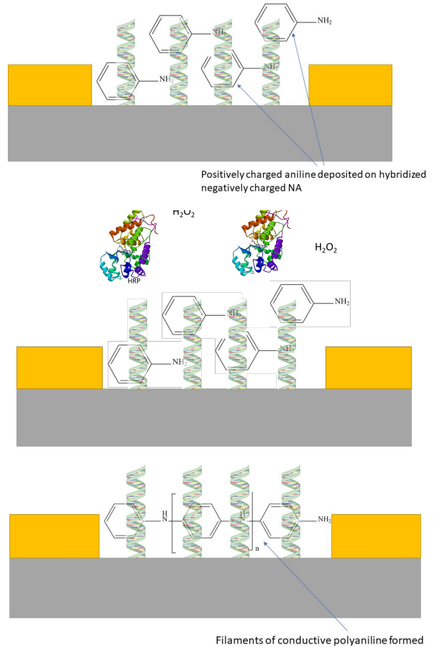


Figure 2: Cross section of the device. Working principle explained

Discussion

- Main problem with miRNA: small size of the miRNA and sensitivity [5].
- Nanogap sensors: very specific compared to other detection methods, especially in the case of miRNA.
- Reported specificity of two miRNA samples: 20:1 between perfectly matched and mismatched miRNA [6].
- Nanogap sensors: provide a linear response for concentrations between 10fM to 20pM [6], well within our measuring parameters.
- LOD = 5 fM [6](lower than most other forms of miRNA detection).
- Nanogap sensors: able to provide S/N ratios above 3 at the low and up to 10 at the high concentrations [5][6].

This, combined with the multiple different miRNAs we measure, will reduce uncertainty and improve measurement quality to be able to compete with existing technologies and other microfluidic devices.

[1] Endometriosis - NHS [Internet]. [cited 2020 Nov 3]. Available from: https://www.nhs.uk/conditions/endometriosis/ [2] Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril. 2020 Jun 1;113(6):1232–41. [3] Moga MA, Bălan A, Dimienescu OG, Burtea V, Dragomir RM, Anastasiu CV. Circulating miRNAs as Biomarkers for Endometriosis and Endometriosis-Related Ovarian Cancer—An Overview. J Clin Med [Internet]. 2019 May 23 [cited 2020 Nov 3];8(5):735. Available from: www.mdpi.com/journal/jcm [4] Maged AM, Deeb WS, El Amir A, Zaki SS, El Sawah H, Al Mohamady M, et al. Diagnostic accuracy of serum miR-122 and miR-199a in women with endometriosis. Int J Gynecol Obstet [Internet]. 2018 Apr 1 [cited 2020 Nov 3];141(1):14–9. Available from: http://doi.wiley.com/10.1002/ijgo.12392 [5] [1] "Facts about endometriosis « Endometriosis.org." [Online]. Available: http://endometriosis.org/resources/articles/facts-about-endometriosis/. [Accessed: 04-Nov-2020]. [6] [1] "Endometriosis - Symptoms and causes - Mayo Clinic." [Online]. Available: https://www.mayoclinic.org/diseases-conditions/endometriosis/symptoms-causes/syc-20354656. [Accessed: 04-Nov-2020]. [5] Ezat Hamidi-Asl, Ilaria Palchetti, Ehteram Hasheminejad, Marco Mascini, A review on the electrochemical biosensors for determination of microRNAs, Talanta, Volume 115, 2013, Pages 74-83, ISSN 0039-9140, https://doi.org/10.1016/j.talanta.2013.03.061. [6] Yi Fan, Xiantong Chen, Alastair D. Trigg, Chih-hang Tung, Jinming Kong, and Zhiqiang Gao, Detection of MicroRNAs Using Target-Guided Formation of Conducting Polymer Nanowires in Nanogaps, Journal of the American Chemical Society 2007 *129* (17), 5437-5443 DOI: 10.1021/ja067477g

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