Project Feasibility Analysis
--- Novel Non-invasive Diagnosis of Endometriosis

Reporter: Hanwen Gu

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Content:

1. Introduction

1.1. Project introduction

1.1.1. Project name

Detection and Diagnosis of Endometriosis

1.1.2. Field of study

Synthetic Biology, Biology, Biomedical Engineering

1.1.3. Duration of study

Jan 2020 - November 2020

1.1.4. Organization

Biology, BME, CHE department of the University of Rochester

1.1.5. Project leader

Emily Schiller

1.2. Overview of research background

Endometriosis is a disease characterized by the presence of tissue, similar to the type that lines the inside of the uterus, which grows outside of the uterus and on other organs in the abdomen (Dunselman et al., 2014).

Endometriosis affects more than 10% of women globally, which is more than 200 million women in total, and this does not even account for cases within the LGBTQIA+ community (Dunselman et al., 2014). Symptoms include chronic fatigue, excessive menstrual bleeding, painful bowel movements, burning urination, inflammation, scarring, infertility, and damage to nearby organs and tissues. Patients with endometriosis also tend to have more sensitive vaginal canals, which lead to pain during intercourse, and even when inserting tampons or menstrual cups (Bese T et al., 2003). Despite experiencing these symptoms throughout most of their lives,

both endometriosis patients and healthcare professionals often fail to recognize that they are signs of this chronic disease. (Dr. Ashley Gubbels, July 2020, Personal Interview).

As we performed research and interviews with stakeholders, we found that an early, reliable, accessible, and non-invasive diagnostic method would allow professionals to diagnose endometriosis in patients within a narrower window of time after the onset of symptoms. Thus, avoiding an extensive number of unnecessary invasive diagnostic and therapeutic procedures and years of unresolved, unbearable pain. This early detection could help patients take advantage of an earlier reduction of disease burden, improvement of fertility, and prevention of progression of the disease (Signorile, P. G., & Baldi, A. 2018).). Therefore, we conclude that the development of a non-invasive, accessible, and reliable test for endometriosis is both responsible and good for society.

2. Project plan

2.1. **Research Object:** Endometriosis

2.1.1. Introduction

Endometriosis is a disease characterized by the presence of tissue, similar to the type that lines the inside of the uterus, growing outside of the uterus and on other organs in the abdomen (Dunselmanet al., 2014). Endometriosis most commonly involves the ovaries, fallopian tubes, and tissue lining the pelvis. Rarely, endometrial tissue may spread beyond the pelvic organs. Even with endometriosis prevalence and severity, there are currently more than 11% of patients with undiagnosed endometriosis around the globe [Louis, G. M. B., Hediger, et al., 2011]. There are no current diagnostics available except for exploratory surgery, such as laparoscopy, or auxiliary imaging methods like ultrasound (Manero et al., 2009). The current diagnostic methods are either invasive, expensive, require the involvement of professionals, or do not have sufficient specificity to allow for a definitive diagnosis of endometriosis and need multiple evaluations for proper confirmation (Kinkel. et al., 2006).

2.1.2. Causes (Mayo Clinic Staff, 2019)

2.1.2.1. **Problems with the menstrual period flow.** Retrograde menstrual flow is the most likely cause of endometriosis. Some of the tissue shed during menstrual periods flows through the fallopian tube into other areas of the body, such as the pelvis.

2.1.2.2. **Genetic factors.** Because endometriosis runs in families, the disease may have a genetic component.

2.1.2.3. **Immune system problems.** A faulty immune system may fail to find and destroy endometrial tissue growing outside of the uterus. Immune system disorders are more common in women with endometriosis.

2.1.2.4. **Hormones**. The hormone estrogen appears to promote endometriosis. Research is underway to determine whether endometriosis may be connected to hormonal disorders.

2.1.2.5. **Surgery**. During surgery to the abdominal area, such as a Cesarean (C-section) or hysterectomy, endometrial tissue could be picked up and unintentionally moved.

2.1.3. **Symptoms (Mayo Clinic)**

2.1.3.1. Pelvic pain ≥ six months

2.1.3.2. Unbearable pain during intercourse

2.1.3.3. Painful bowel movements

2.1.3.4. Painful urination

2.1.3.5. Chronic fatigue

2.1.3.6. Low back pain

2.1.3.7. Diaphragmatic/lung pain

2.2. Research content

The current gold-standard diagnostic method, laparoscopy, is invasive and often occurs over ten years after onset due to a lack of both patients' and medical professionals' awareness (Dr Gubbels, Personal Interview, June). That delay in diagnosis could lead to a more

severe progression of endometriosis and thus result in increased damage to patients' organs (Ballard, K.,et al, 2006).

2.3. Research significance

This research aims to help patients get the proper diagnosis easily and in time to avoid unnecessary suffering and years of pain.

2.4. Goal

Our project goals are to reduce the cost and risk of an endometriosis diagnosis.

(1) A non-invasive, accurate, affordable diagnostic method

The current gold-standard diagnostic method utilizes laparoscopy, which involves a small incision in the abdomen with a camera's aid. We plan to accomplish the goal to reduce the cost and risk of an endometriosis diagnosis by creating a non-invasive, affordable, and reliable method of diagnosis which utilizes menstrual effluent.Color changes would indicate the risk of having endometriosis.

(2) An improvement to the menstrual cup and a redesigned portable cup sterilizer

Patients with endometriosis tend to have a more sensitive vaginal canal and a heavier menstrual blood flow (Shreya, 2016). Using a menstrual cup or tampon could result in pain, and those who choose to use pads instead might require an increased number of pads due to more massive bleeding (Shreya, 2016). This may pose environmental and health problems, as increased pad or tampon usage results in increased waste and a higher risk of infection as menstrual cups are reused (Mitchell, M. A., Bisch, S., et al., 2015). Endometriosis patients require a better cup that can fit comfortably and hold more blood than typical menstrual cups (Dr Gubbels, Personal Interview, June). A portable sterilizer is beneficial to quickly disinfect the cup, as the traditional method of boiling to sterilize typically takes more than 20 minutes. Additionally, in the diagnostic method specified above, the cup itself also serves as the blood collection tool for this non-invasive endometriosis diagnostic, thus decreasing waste by reusing the cup.

2.5. Organization

Institution of higher education and research

2.6. Features and innovations

Menstrual effluent is the ideal sample for the detection of endometriosis as it allows for non-invasive collection methods and has been shown to contain reliable biomarkers for this condition due to it containing shed endometrial cells (Nayyar et al., 2020, Warren et al., 2018). As opposed to peripheral blood samples, which can be collected at any stage in the menstrual cycle, using menstrual effluent ensures that specimen collection only occurs during menstruation, which minimizes the risk of hormone-related fluctuations in biomarker levels (Warren et al., 2018). Therefore, the use of menstrual effluent will allow us to create a fast and reliable diagnostic panel for endometriosis while also employing a novel early and noninvasive detection technique.

In order to collect the menstrual effluent samples, we designed a special menstrual cup for endometriosis patients. During both the literature review and from our discussions with professionals, we learned that patients with endometriosis have heavier flows (Shreya, 2016), meaning on average they use more than 2,400 feminine hygiene products per year (Shreya, 2016) and that they have more sensitive vaginal canals, meaning they feel more pain during tampon or menstrual cup use (Diva Cup, Interview with Sophie Zivku). Therefore, to tackle these problems, we created a customized 3D-printed menstrual cup that is easy to use, reduces leakage during the pullout process, offers better comfortability, and is more sustainable for the environment compared to other feminine hygiene products (i.e., pads and tampons).

As our team approached stakeholders, including gynecologists, menstrual cup manufacturers, and patients, we saw the need to clean menstrual cups when there is limited access to water and when no water boiler is available. Using the cup without proper cleaning would have a strong odor, with the potential of infections. Further, using a UV sterilization method could reduce the amount of water usage during cleaning. Therefore, our team decided to advance with the idea of using a UV sterilization method to improve sustainability and provide an alternative to the existing cleaning method.

2.7. Research status and development

Lateral Flow Assay

A lateral flow assay is a type of sandwich immunoassay that has high target specificity, a rapid run time, and a colorimetric output that can also be quantified with the use of a sensor. Unlike other types of immunoassays, a lateral flow assay can be run with minimal equipment (an initial step of centrifugation to isolate blood plasma) and can be performed rapidly in a clinical or laboratory setting. In this project, there were also opportunities to improve the sensitivity of the design to meet the minimum detection thresholds for our biomarkers. As such, this type of design would meet all of our goals for a point of care diagnostic.

Gold Nanoparticles

Gold nanoparticles have quickly become a standard tool for diagnostic assays due to their ability to stably conjugate with detection probes, as well as their unique optical properties. These molecules shift color from red to purple upon aggregation, allowing for both naked-eye detection and quantification using imaging software. We determined that labeling our antibodies with GNPs would be the perfect way to address our sensitivity concerns by increasing our assay's sensitivity and allowing for biomarker detection at concentrations as low as one ng/mL. This threshold could be further lowered through the use of a gold enhancement solution, which relies on chemical methods to increase the intensity of the colorimetric signal of the GNPs. Additionally, this method would not require expensive, complicated laboratory equipment, thus helping to improve the accessibility of our diagnostic method.

Menstrual Effluent

Menstrual effluent (ME) was chosen as our testing sample because of recent evidence demonstrating the diagnostic potential of this source. Most importantly, this sample collection would be painless and non-invasive, presenting a significant improvement over current methods, which require surgical excision of endometrial tissue for diagnosis.

Biomarker Selection

IGFBP-1

Recent studies have shown that the stromal fibroblast cells of endometriosis patients have a significantly reduced decidualization capacity that can be measured in menstrual effluent (Nayyar et al., 2020, Warren et al., 2018). This is important because cellular decidualization is critical for proper tissue growth and which may be implicated in the aberrant tissue growth seen in endometriosis (Warren et al., 2018). Consequently, there is a significant decrease of insulin growth factor-binding protein-1 (IGFBP-1) production in menstrual effluent with reduced decidualization capacity after cAMP stimulation in endometriosis patients (Warren, 2018), as well as an increase in levels of prolactin (PRL) in serum (Braz, 2006; Esmeilzadeh, 2015; Mirabi, 2019). While both IGFBP-1 and PRL are implicated as measures of decidualization capacity, the team decided against the use of PRL as the biomarker due to the absence of numerical values or threshold levels in current literature. In contrast, IGFBP-1 has reliably been measured in menstrual effluent with exact threshold values for a diagnosis of endometriosis that provides high specificity and sensitivity for our design.

IL-1B, IL-6, IL-8, and TNF-a

Various interleukins and cytokines frequently came up in our searches for biomarkers of endometriosis. Research frequently found elevated levels of IL-1β, IL-6, IL-8, and TNF-α in the serum of endometriosis patients, as implicated in the elevated immune response that has been hypothesized in the pathophysiology of endometriosis and its associated infertility (Malvezzi et al., 2019). While these molecules are implicated in a variety of disorders, we decided to include these biomarkers in our panel due to their potential as therapeutic targets. In fact, there are some approved immunotherapy options available for IL-1β, IL-6, and TNF-α, although they have not yet been studied for the treatment of endometriosis.

CA125

Although CA125 was not indicative of endometriosis on its own, it could enhance our design's specificity and sensitivity in conjunction with other biomarkers selected. Thus, CA125 was included in the final design of our novel diagnostic method.

BioBrick Selection

Bacterial Production of Antibodies

We will be using the engineered *E. coli* strain SHuffle as the chassis for our plasmid. This strain has been altered to have an oxidative cytoplasm, which allows for the efficient production of full-length immunoglobulin proteins (Lobstein et al., 2012). By designing these plasmids, we aim to make antibody production an easy and cost-effective method for the mass production of our diagnostic tools and for future iGEM teams.

3. Research and development

3.1. Team experience

The three advisors, Dr Stein, Dr Chen, and Dr Soufan, have decades of experience in biology research, and other advisors also have strong connections with the industry, which will be useful in the research and development of our product.

All members of the team have experience in biology or engineering research laboratories.

One of the team members is an endometriosis patient herself. She provides the team with first-hand knowledge of the disease.

3.2. Necessary Conditions

3.2.1. Necessary requirements for implementation

All proper equipment for testing the diagnostic panel including centrifuge, shaker, homogenizer, microscope, solder, -80 fridge, T-75 flask, are present in the laboratory.

3.2.2. Advantages

The team has a strong science and engineering background and good connections with the industry and leading researchers in the field.

3.2.3. Limitations

Due to COVID-19, our team has extremely limited access to the laboratory to perform experiments supporting our models.

Our team also has limited marketing and business development resources due to large science and engineering team setup.

3.2.4. Solution

- Use clinical data to model the result of the diagnostic panel.
- External help from the Simon Business School

3.3. Members of the research group

Members:

Emily Laskey, Wetlab Manager - Major: Biochemistry and Psychology

Emily Schiller, Team Leader - Major: Cell and Developmental Biology

Ethan Chen, Wiki Designer - Major: Biomedical Engineering

Gabe Isaacson, Fundraising Manager - Major: Microbiology

Heather Shi, Outreach Manager - Major: Microbiology;

Helen Shammas, Hardware Manager - Major: Biomedical Engineering

Isabel Lopez, Graphic Designer - Major: Biochemistry

James Tang, Software Manager - Major: Cell and Developmental Biology; Economics

Linh Hoang, Modeling Manager - Major: Biomedical Engineering

Meghan Martin, Public Relations Manager - Major: American Sign Language and Biochemistry

Nello Gu, Policy & Practice Manager - Major: Biomedical Engineering

Zivile Vebraite, Collaboration Manager - Major: Cell and Developmental Biology

Advisors :

Anne Meyer, Associate Professor, Department of Biology

Nancy Chen, Assistant Professor, Department of Biology

Omar Soufan, Mechanical Engineering BS, Biomedical Engineering

Lynn Sidor, Ph.D. Student, Department of Biology

Ram Gona, Ph.D. Candidate, Department of Material Science

Roger White, Ph.D. Student, Department of Biology

Consultant:

Peter K. Gregersen, MD, Professor, Feinstein Institutes for Medical Research
Dr. Gregersen is the head of Robert S. Boas Center for Genomics and Human Genetics and Professor of Molecular Medicine and Medicine at Hofstra North Shore-LIJ School of Medicine. His research focuses on the genetics and biology of endometriosis.

Christine Metz, Ph.D., Professor, Hofstra-North Shore-LIJ School of Medicine.
Dr. Metz's research focuses on inflammation, a complex biological response to infection and injury, in both pregnant and non-pregnant populations. Throughout her career, she has studied numerous conditions that affect women, including rheumatoid arthritis, endometriosis, cardiovascular disease, preeclampsia, and preterm labor. Her work identifying ways to reduce and prevent dysfunctional inflammation has been funded by the American Heart Association, the National Institutes of Health, and the NY State Department of Public Health. Dr. Metz has published over 110 peer-reviewed research papers and book chapters and has been an inventor or co-inventor on five patents.

Dr Gubbels, M.D.
Dr. Gubbels is a fellowship-trained minimally invasive gynecologic surgeon. She specializes in chronic pelvic pain conditions such as endometriosis, interstitial cystitis, pelvic muscle pain, and neuropathic pain conditions.

Sophie Zivku
Sophie is the Corporate Communications Manager at Diva International Inc, one of the largest corporations that manufactures menstrual cups and is also devoted to women rights.

Dr Idhaliz Flores
Dr Flores is a professor of Basic Sciences, and OB-GYN at the Ponce Health Sciences University (PHSU) in Ponce, Puerto Rico. Since 2001, she has directed the Endometriosis Research Program (ERP) of the Ponce Research Institute. Her research interests include

discovery of molecular biomarkers, inflammatory, and genetic/epigenetic factors of endometriosis and infertility.

4. Research funds

4.1. Sources of funds

University of Rochester Funding
Fundraising activities including 5K virtual Marathon

4.2. Cost Analysis

A theoretical Cost analysis including the direct and indirect material cost for the ideal experimental setting, potential labor cost during production are proposed. A comparison of the direct material cost is then compared to the price of the traditional diagnosis methods.

TABLE I: Direct Material Cost

Name	Amount	Amount needed	Total Price	Price per case
1x PBS	20L	30ml	$432	$6.48
DMEM	6 x 500 mL	10ml	$150	$0.50
FBS	100 mL	10ml	$100	$10
Penicillin/streptomycin	20 mL	20ml	$12.19	$12.19
Glutamine	5g	3g	$41.40	$24.84
Normocin (1:500)	500 mg	20 mg	$153	$6.12
Sodium phosphate buffer	1 gal	50ml	$57	$0.75
Bovine albumin	10g	3g	$81	$24.30
Antibody - total 18 antibodies	100 unit	1 unit	$7,200	$72
Gold Nanoparticle	10	0.2	$695	$13.9
Blocking buffer	500 ml	50 ml	$260	$26
Target Molecule	50kUnits	1kU	$571	11.42
HiFlow 180 nitrocellulose membrane - 25 mm x 10 cm per 5 test strips (5 mm wide)	120 unit	1 unit	$436	$21.80

application pad, conjugate pad, absorbent pad	400 unit	1 unit * 3 pads	$99	13.365
2 mM Hydroxylamine in H2O	100g	0.01g	$59.60	$0.01
1% HAuCl4 in H2O	10g	0.001g	$443	$0.04
Wire	30 ft	0.4	$15	$0.20
Battery	24	2	$10	$0.83
Outer Case	1	1	$2	$2
Silicon	1	1	$3	$3
Switch	1	1	$0.23	$0.23
UVC bulb	13	2	$13.00	$2.00
Silicon	1	1	$4	$4
			Total:	$245

TABLE II: Fixed Cost

Heparin Test Tube	**1000**	**1**	**$638**	**$0.64**
CO2 / 37 degree Celsius incubator	**1**	**1**	**$400**	**$400**
T-75 flasks	**100**	**3**	**$313**	**$9.39**
96-well plates	**25**	**1**	**$134**	**$5.36**
Shaker	**1**	**1**	**$1,000**	**$1,000**
-20 Freezer	**1**	**1**	**$800**	**$800**
			Total:	**$220.66**

TABLE III: Labor Cost

Direct	($/hr)	(people)	(hrs/unit)		($/unit)
Helper	10	2	0.3		6
Technician	14	1	0.2		2.8
Operator	20	3	0.5		30
Engineer	40	1	0.2		8
				Total:	**$46.8**

Cost and Features Comparison (Source: www.healthgrades.com)

TABLE IV: Diagnosis method comparison in the United States

Name	Cost ($)	Non-invasive	Definitive Diagnosis

Pelvic Exam	350/examination	√	×
Ultrasound	225/examination	√	×
MRI	700/examination	√	×
Laparoscopy	9,360/ case	×	√

TABLE V: Treatment method comparison in the United States (Gao, X., Outley, J., et al., 2006) https://www.tandfonline.com/doi/abs/10.3111/13696998.2010.549532

Name	Cost ($)	Non-invasive	No Recurrence	Side Effect
Hormonal contraceptives	800/yr	×	x	√
Gn-RH (Gonadotropin Releasing Hormone)	4400/6 months	×	x	√
Laparoscopy	9,360/case	×	x	√
Hysterectomy	8413/case	×	x	√

Table V above shows only the direct material costs for diagnosis and treatment. It is difficult to find reliable data on indirect costs including hospitalization, loss in work production, and continued post-surgical treatment cost in recent years in the United States due to lack of research in endometriosis around the world. Longer delays in obtaining proper diagnoses for patients result in higher costs of treatment (Dr Ahsely Gubbels, June 2020, Personal Interview). Due to the progression of the disease and its symptoms, at later stages of the disease individuals may need larger doses or combinations of medications to experience relief and may experience more frequent hospitalizations (Winkel, C. A. 2000). It also should be noted that none of the

treatments available right now can guarantee non-recurrence or absence of side effects (Rizk, B.,2014.). However, early diagnosis typically means earlier treatment and more treatment options, which could significantly improve quality of life, mental health, and work productivity for patients (Nnoaham, et al., 2011).

Disclaimer:

Due to limited lab access during the COVID-19 pandemic, the estimation of our method is theoretical based on our designed protocol. More lab work is required to validate the accuracy of our result as well as the actual cost of production, research, and development. The described type of diagnostic test is used for pre-screening purposes only. Confirmation of any medical condition requires consultation with a physician. All analyses above are based on national data in the United States, and all costs are considered out-of-pocket as opposed to insurance coverage in order to understand the direct cost.

5. Expected Situation Analysis

5.1. Analysis of economic and social benefits

Endometriosis has estimated annual costs of the United States $12,419 per patient, comprising one-third of the direct health care costs with two-thirds attributed to the loss of productivity (Simoens S, et al, 2012). Decreased quality of life is the most important predictor of direct health care and total costs (Simoens S, et al, 2012). It has been estimated that there is a mean delay of 6.7 years between symptom onset and surgical diagnosis of endometriosis, and each affected patient loses, on average, 10.8 hours of work weekly, mainly owing to reduced effectiveness while working (Bese T et al., 2003).

5.2. Marketing and Application prospect

The global endometriosis market is forecasted to reach the US $2.42 Billion by 2026, according to a new report by Reports and Data in 2020.

The market is projected to witness considerable growth during the forecast period. A significant factor in this regard is the increasing occurrence rate of the condition.

In context to Surgery Type, the laparoscopy segment yielded the highest revenue of USD $0.87 Billion in 2018, with the fastest growth rate of 3.2% during the forecast period. The effectiveness of this surgery in accurately diagnosing endometriosis and even eliminating it in mild to moderate conditions with just a small incision resulted in increased demand and revenue generated by the procedure.

Key participants in the development of endometriosis diagnostics include Johnson & Johnson Services Inc., Bayer AG, Neurocrine Biosciences Inc., Meditrina Pharmaceuticals Inc., AbbVie Inc., Astellas Pharma Inc., AstraZeneca Plc, Evotec AG, Debiopharm Group and Teva Pharmaceutical Industries Ltd.

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