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The Development of Multi-modal, pH-sensitive Quantum Dots for the Detection of Breast Cancer

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Abstract: In the current decade, a significant amount of women have been diagnosed with breast cancer. There is a one in eight chance that women will develop breast cancer. It consists of a high fatality rate and tragic death due to the existing barriers set by current methods that are accessible as a treatment for breast cancer. For example, most treatment plans include a combination of surgery, radiation, hormone therapy, chemotherapy, and targeted therapies. However, due to the collateral damage from multiple follow-up surgeries and subsequent infections, some patients are even averse to starting or continuing treatment. Chemotherapy can result in fatigue, pain in the fingers and feet, increased risk of infection, and more. Hormone therapy can result in similar symptoms but also include nausea, muscle and joint pain, and headaches. Especially breast biopsy, the removal of a sample of breast tissue for diagnosis, is very painful for patients. It takes multiple days to get a diagnosis. It also results in soreness, swelling, or bruising at the biopsy site. This article will attempt to address the current limitations of breast cancer diagnosis by suggesting an innovative method on multimodal pH and progesterone-sensitive quantum dots to detect breast cancer faster and cheaper. Just a sample of blood is needed from the patient, and the quantum dot sensor will detect the cancerous cells through the emission of wavelengths of about 475 and 1300 nm with a cyan color and infrared radiation. These wavelengths can be translated to quantitative graphs with a spectrophotometer. With its optical sensor, the quantum dot will significantly reduce the price of getting a diagnosis, and it will be able to diagnose the patient almost immediately. Further, it does not require a trained professional to diagnose, which is a significant improvement over the current techniques.

Keywords: pH-lemon sensor; Breast cancer; Quantum dots; Progesterone; Multimodal

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1. Introduction

1.1. Breast cancer

Breast cancer is a disease where breast cells grow unmanageably to form tumors. Progressive states of severity

indicate four stages of breast cancer; pathologists determine stages and look at cell morphology, making visual inspection (**Figure 1**) [1]. It is known that 23% of breast cancers are not diagnosed accurately (false-negative) [2]. Therefore, this research will develop an innovative method for multi-modal identification and validation. Furthermore, abundant and complex steps of redundant examination and guided biopsy are required to clarify the existence of cancer cells. Hence, this study will use a primarily accurate method to simplify detecting cancer cells. This study will be making prominent use of pH sensors, thus identifying the pH of the cells; the extracellular pH of cancer cells is between 6.7 and 7.1, while the normal cell is around 7.4 [3–4].

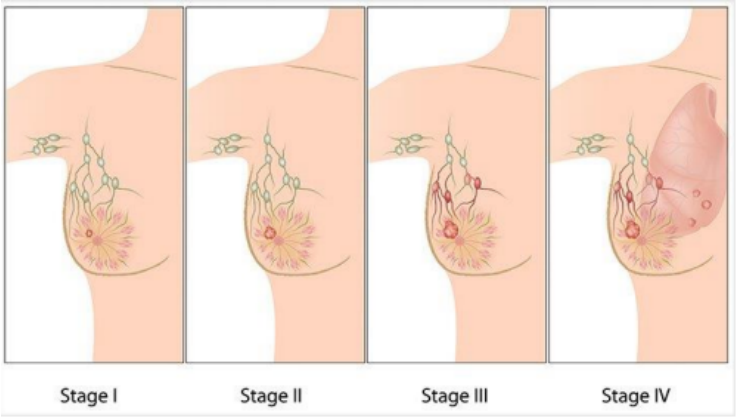

Figure 1. Examination diagram of breast cancer, ordered by stage of its severity

Malignant tumors are cancerous and aggressive because they spread (metastasize) to other parts of the body as they invade and damage surrounding tissues. However, benign tumors are noncancerous and do not spread, so they do not cause systemic health issues compared to malignant tumors (**Figure 2**) [5].

Despite the numerous treatments for breast cancer, this study wanted to propose a new detection method that utilizes quantum dots, pH sensors, and progesterone hormones. This innovative method aims to allow for early detection of breast cancer stage, which can enhance survival and treatment.

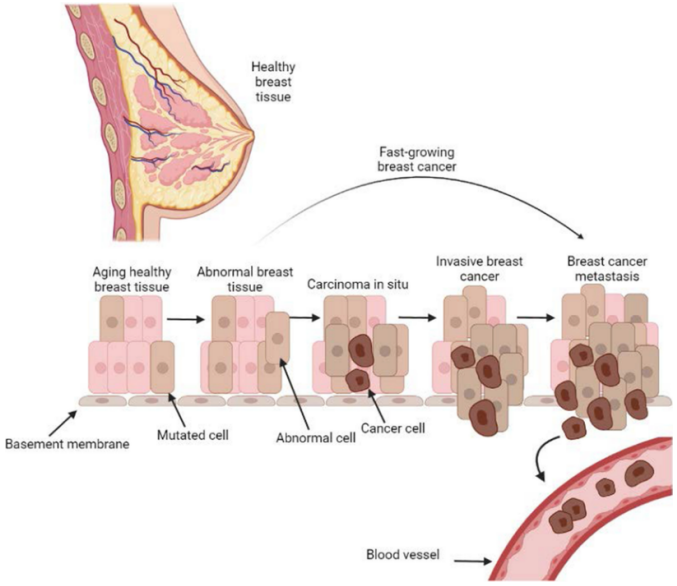

Figure 2. Diagram of the progression of breast cancer and how the cancer cells travel to other parts of the body by categorizing benign and malignant tumors

1.2. Quantum dots

A multi-modal sensor serves to have two different signals from the same probe which are anatomical and metabolic. Here, the usage of quantum dots in the process will be anatomical. Quantum dots are nanocrystalline semiconductors that exhibit three-dimensional quantum confinement. They are band gap tunable, meaning their optical and electric properties can be engineered to fit certain properties. The specific wavelengths and properties are dependent on size and shape (**Figure 3**) [6]. The size directly influences the energy levels that quantum dots' electrons can occupy. These electrons can move to a higher energy level when energy is absorbed. When returning to their original level, they emit energy in the form of light. The energy levels between the levels depend on the size of the quantum dot. Smaller quantum dots emit light with shorter wavelengths and vice versa. Quantum dots are applied to different fields, such as photovoltaics, light-emitting diodes, photoconductors and photodetectors, biomedicine and environment, and catalysis [7]. The original research was primarily done in group IV and III-V elements, such as Cadmium and Lead. However, Cadmium has been proven to be toxic due to the release of free Cd2+ ions and the generation of reactive oxygen species (ROS) [8]. The research investigating the cellular uptake amounts of four types of CdSe/ZnS quantum dots by *Phanerochaete chrysosporium* indicated that these four types of quantum dots were highly accumulated in the mycelia. Light irradiation causes the photooxidation of quantum dots in living cells, which causes electron transfer from quantum dots to O2. This generates ROS, which forms hydroxyl radicals when the unpaired holes on the quantum dots react with water. Serious harm such as metabolic dysfunction, DNA nicking and breaking, and even cell death, can be caused by these hydroxyl radicals [9]. Lead quantum dots can cause oxidative stress, direct damage to the cell membrane, morphological alterations, genotoxicity, and various types of cell death, such as apoptosis and necrosis [10]. Today, there are nontoxic alternatives such as indium, carbon, phosphorous, or graphene. This study will be using silicon for the material, which is also safe. Neither acute nor chronic (14 days) toxicity was observed by cell morphology, viability, ATP production, ROS production, and DNA damage at doses of 50–200 μg mL-1 [11]. This study has chosen silicon out of other nontoxic materials because it can emit wavelengths of about 1300 nm, enough to be detected through the breast tissue [12].

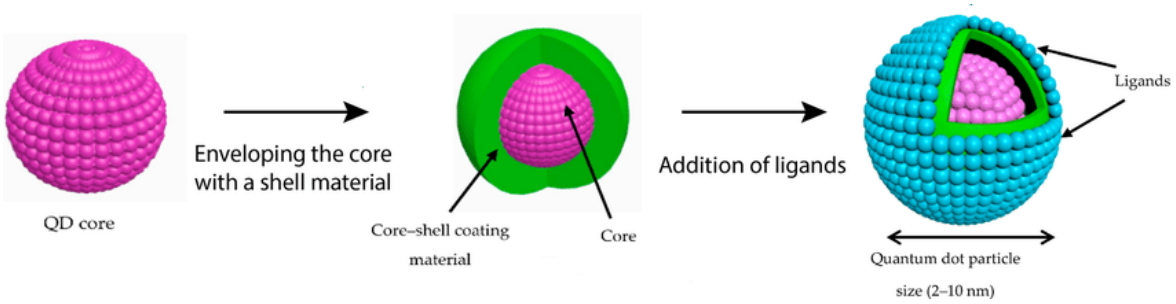

Figure 3. Schematic of the new quantum dot sensor through chemical functionalization with shell material and ligands

1.3. pH sensor

Focusing on the metabolic, the multi-modal sensor will serve to determine the function of the cells using a pH sensor. The pH sensor indicates the acidity and alkalinity of a substance. This study will measure the pH of the cancer cells to determine the acidity and provide validity of cancer cells. This study has chosen to use a visual pH sensor called "pH-Lemon", which was developed by researchers at the University of California, San Diego. Specifically, it was pioneered by Martin Griesbeck and colleagues. This pH sensor is based on the fusion of two fluorescent proteins, mTurquoise2 and Enhanced Yellow Fluorescent Protein (EYFP) for acidic compartments

(**Figure 4**) [3, 13]. Based on the fusion of mTurquoise2 and EYFP, the pH spectrum investigated can detect the pH range from 2 to 10. Some publications report different ranges of numbers, but this study will use this range as it is the most relevant for the proposal made. To briefly explain the conventional pH range that applies to most pH-related theories: if the value x is the pH of something specified, the substance is acidic when x is lower than 7, if x equals 7 the substance is neutral, and if it is greater than 7, it is alkaline [13–14]. Connecting it to the color spectrum, it has a color range from cyan to yellow. Additionally, this study will be taking advantage of a fluorescence detection system to validate the results and enhance the degree of accuracy. Here, the fluorescence ratio will be converted into pH levels and this study will calculate the average pH level from both optical inspection–color spectrum of pH-lemon sensor and statistical inspection which is a fluorescence detection system.

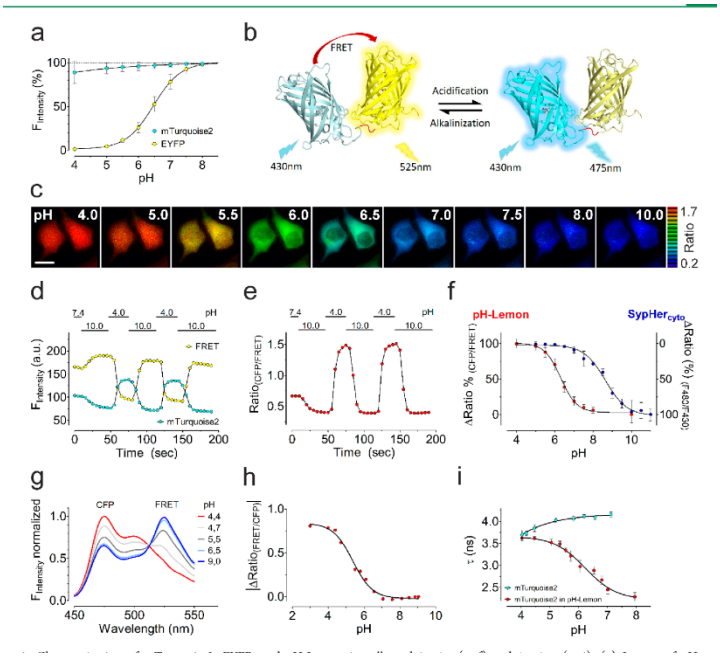

Figure 4. Characterization of mTurquoise2, EYFP, and pH-Lemon in cells, in situ (a−f), and in vitro (g−i). (a) Impact of pH on the fluorescence intensities of mTurquoise2 and EYFP. i) Representative fluorescence lifetimes of mTurquoise2 alone (cyan circles) or mTurquoise2 as FRET donor within pH-Lemon (red circles) at different pH. Data represents an average ± SD of 3−58 cells per pH

1.4. Progesterone

Progesterone is an endogenous steroid hormone that plays a crucial role in the human reproductive system because it produces signaling events that stimulate crucial chemical messengers (**Figure 5**) [15]. Since this hormone is necessary for pregnancy, it is also named the “pregnancy hormone” because it supports women to get pregnant and maintain pregnancy. Prolonged exposure to increased progesterone levels induces early menarche, late menopause, and shorter menstrual cycles. As a result, there is an increased risk of breast cancer. However, early full-term pregnancies play a role in protecting against progesterone receptor (PR-positive) breast cancers [16]. Therefore, an increased risk of cancer risk is associated with a shorter menstrual cycle as a result of higher progesterone levels during the luteal phase, which occurs after ovulation in the menstrual cycle to prepare the body for potential pregnancy [17].

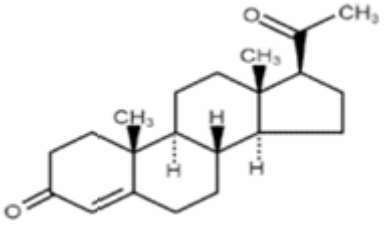

Figure 5. Chemical structure of progesterone

2. Method and approach

This study will measure the pH utilizing the pH-sensitive sensor and quantum dot to simplify the redundant biopsy process. Firstly, this study will purchase the quantum dot from the following companies: Nanosys Inc. (Shoei Electronic Materials Inc.), NnCrystal US Corporation (NN-Labs), and Quantum Materials Corporation with the sensor attached to the blood sample. The sensor sample will be measured in the cuvette spectrophotometer to determine the wavelength of the color and the color of the pH sensor. The fluorescence will indicate if the sample contains cancer cells. The first aim is to create a polyethyleneimine-modified quantum dot (**Figure 6**).

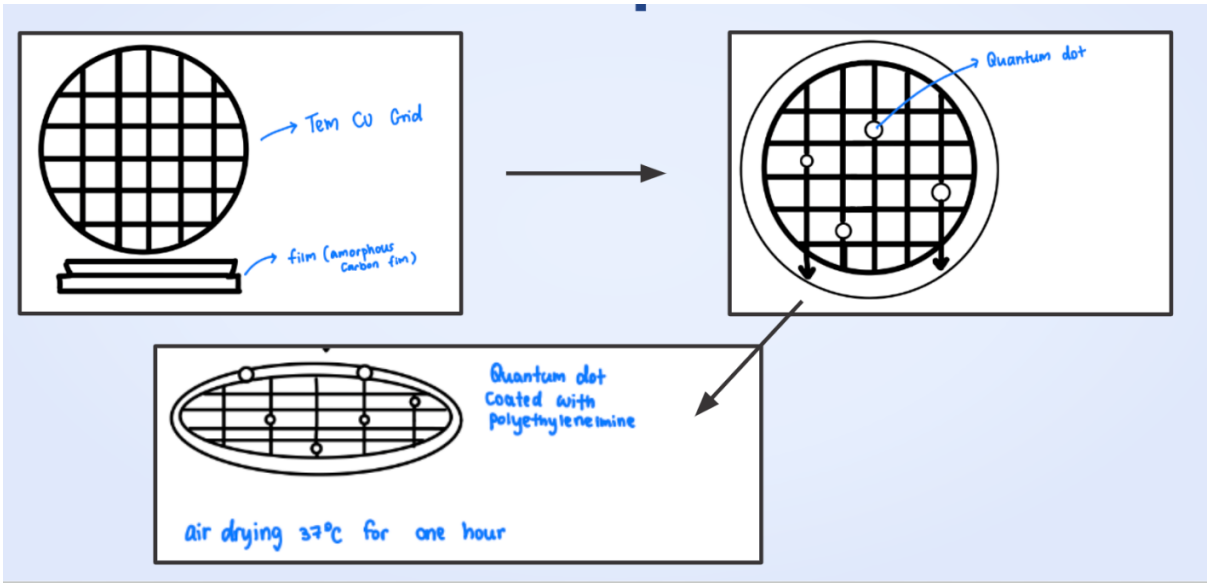

Figure 6. Diagram of the first step of the first aim, where this study coats the surface of the quantum dot with polyethyleneimine

The first step is to coat the surface of our quantum dot with polyethyleneimine (PEI). PEI is a fundamental and positively charged polymer with repeating units composed of the amine group and two carbon aliphatic CH_2CH_2 spacers [18–19]. The amine groups will be able to bind with the bromine-tethered progesterone, which will then bind to the progesterone receptors on the breast cancer cells. The surface passivation of PEI with rich amino groups supports stabilizing the surface energy traps on the quantum dot [20–22]. This study will then drop the quantum dot solution onto a copper grid, followed by air drying at 37°C for one hour and the copper mesh

will be coated with an amorphous carbon film. More specific steps are entailed in Yang's research paper, which states: "The effect of quantum dot size and poly(ethylenimine) coating on the efficiency of gene delivery into human mesenchymal stem cells" (**Figure 7**) [23].

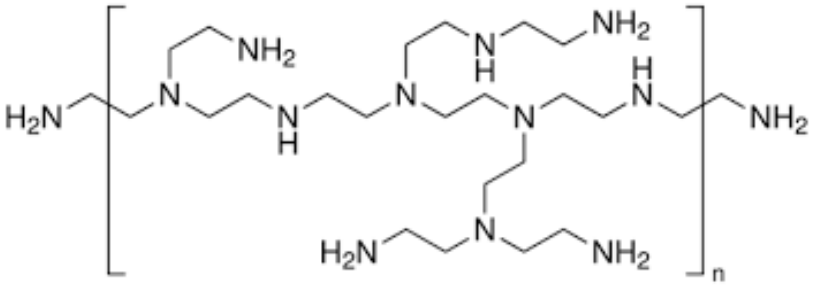

Figure 7. The chemical structure of a polyethyleneimine

After coating the quantum dot with PEI, this study will purchase the bromine-modified progesterone and bind it with a quantum dot. From 21-hydroxyprogesterone, the neutral series of conjugates with zero and six-carbon spacers (1, 2) will be synthesized, as shown in **Figure 8**. The synthesis of 1 will begin with the bromination of the 21-hydroxyl group utilizing carbon tetrabromide and triphenylphosphine. To bind the finished bromine-tethered progesterone with the quantum dot, this study will use Do3A, t-Bu ester K2CO3, and 40% of NBu4OHCH3CN at 90 degrees. By using PEI (2.71 g, 6.9 mmol), quantum dots, K2CO3 (2.86 g, 20.7 mmol), and a catalytic amount of 40% Tetrabutylammonium Hydroxide in anhydrous acetonitrile, the mixture will be refluxed for 16 hours. The reaction will occur when the mixture is monitored by TLC and concentrated in a vacuum. The crude residue will be purified by flash column chromatography with dichloromethane: methanol (15:1), an eluent to afford a quantum dot bond with bromine-tethered progesterone. (**Figure 9**) [24].

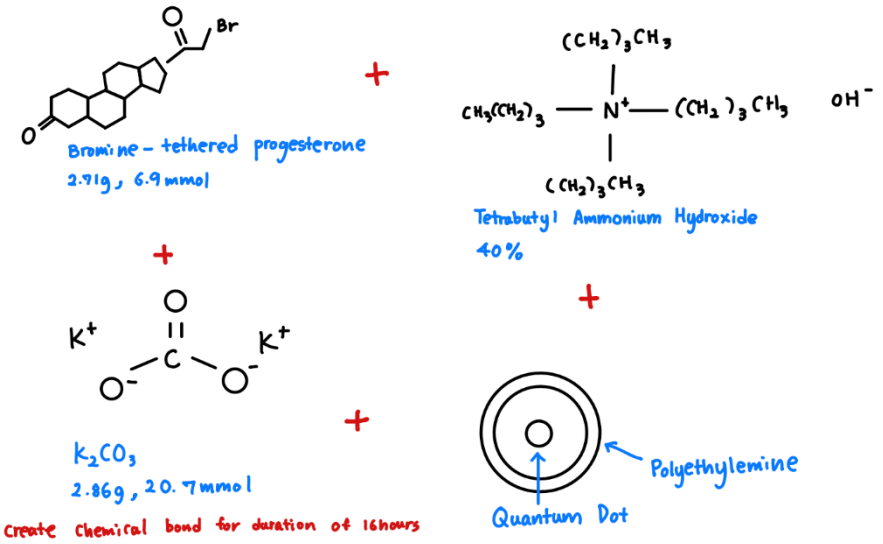

Figure 8. Step two of the first aim where this study functionalizes the coated quantum dot with the bromine-tethered progesterone by creating a chemical bond for a duration of 16 hours

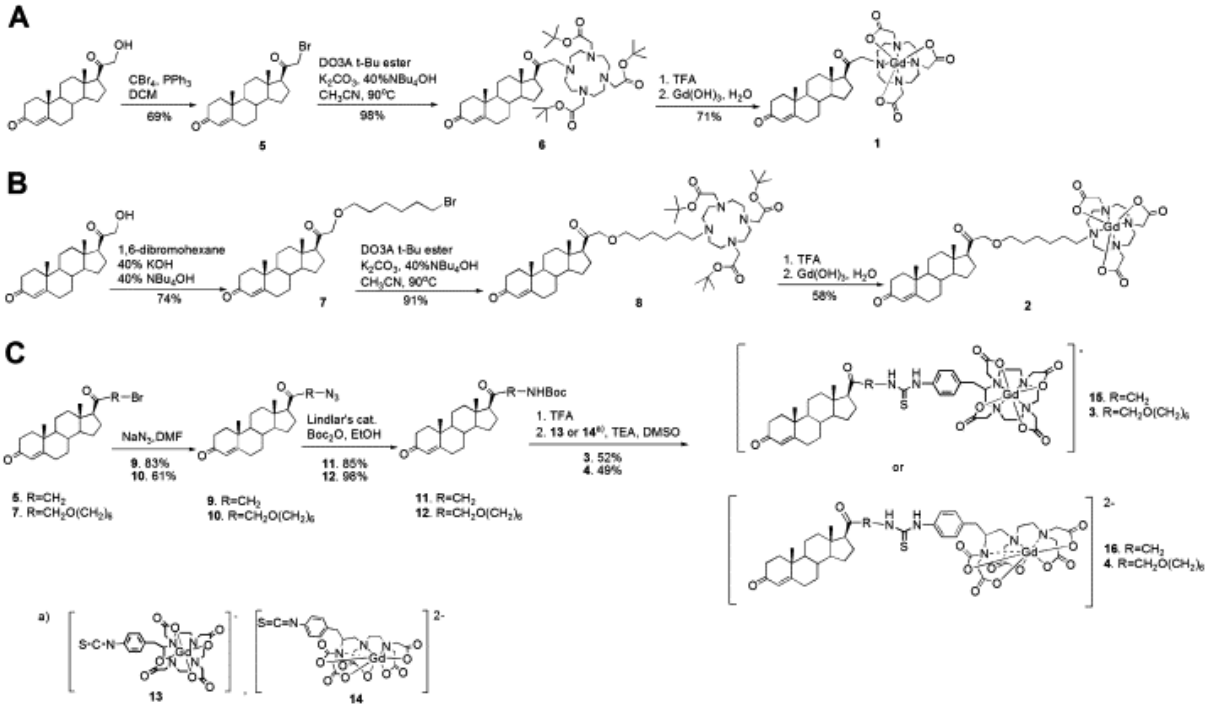

Figure 9. Diagram of the production of bromine-tethered progesterone. Instead of utilizing the DO3A-tris t-butyl ester in step 6, this study will bind the progesterone with the functionalized quantum dot

The study then collected the product after the successful functionalization of quantum dots. Now, this study combines quantum dots, polyethyleneimine, and progesterone, and modifies it with a pH-lemon sensor molecule. This study will have a multimodal sensor to ensure accurate and responsive detection. Firstly, this study will prepare the pH lemon sensor by engineering the pH-lemon genetic construct, which consists of two proteins—pH-stable mTurquoise2 fused to the pH-sensitive EYFP.

The study will then check whether the solution from part A (quantum dot-polyethyleneimine-progesterone) is well spread in a suitable buffer. Afterward, this study will use EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) to activate carboxyl groups on the quantum dot and polyethyleneimine surface, which forms an NHS ester that can react with the amine groups on the pH-Lemon sensor [25]. Then, This study will combine the activated quantum dot solution, polyethyleneimine, and progesterone conjugate with the purified pH-Lemon sensor for pH detection. The reaction will undergo a while, and the changes will be monitored through the fluorescence properties of the pH-Lemon sensor [3]. Afterward, this study will set up the fluorescence detection system using a fluorescence microscopy setup capable of detecting both quantum dot fluorescence and the pH-Lemon's signal. This study will then observe the pH color change from cyan to yellow by exciting at the appropriate wavelength for pH-Lemon, which has a constant range for each fluorescence protein.

The pH-lemon sensor can attach to the cancer cells and glow in the indicated color in the pH-lemon spectrum. The wavelength also depends on the type of pH sensor used. Looking at the wavelength of each protein, mTurquoise2 has an excitation range of 420–450 nm and an emission range of 460–490 nm (**Figure 10**) [26–27]. Contrastingly, EYFP has an excitation range of 490–520 nm and an emission range of 520–550 nm (**Figure 11**) [28]. Energy transfer theory will be applied to the emission of wavelengths. The study has selected

mTurquise2 as the protein that emits, and EYFP is the absorber; mTurqouise2 will emit in wavelengths of 460–520 nm, and EYFP will absorb at a higher nm. As mentioned above, it will emit a wavelength of 530–560 nm when excited. Thus, the excited protein, EYFP, will produce a yellow color. Following emission ranges allow pH-lemon sensors to measure pH changes meticulously through visible shifts in the fluorescence intensity and wavelength of two proteins–mTurquoise2 and EYFP–especially in the acidic to neutral range, approximately from 4 to 7.5. The study will convert the fluorescence ratio gained through the calibration curve to determine pH values accurately. This is vital for the research since it is suitable for measuring extracellular pH in abundant conditions [29].

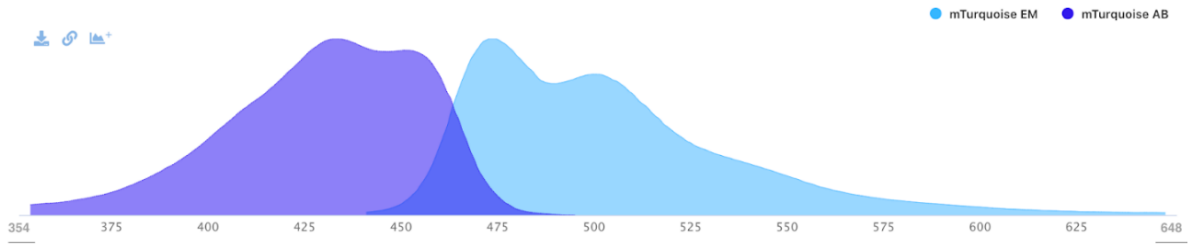

Figure 10. Wavelength graph of mTurquoise2

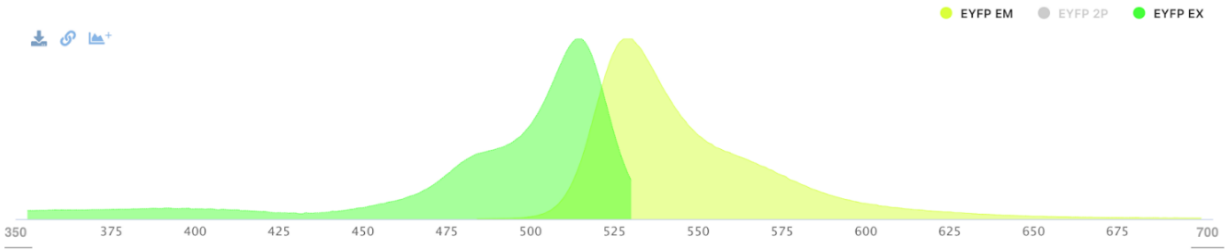

Figure 11. Wavelength graph of EYFP (Enhanced Yellow Fluorescent Protein)

3. Characterization

First, the study will sample blood from a potential breast cancer patient. The location will be in the upper outer quadrant, where about 52% of breast cancers are found. If no cancer cells are found, the study will sample blood from the other quadrants [30–31]. Then, insert the quantum dot sensor and observe changes in the color of the sample. In a cuvette spectrophotometer, input the blood and the quantum dot sensor solution and translate the wavelengths into a graph of two peaks — one for the pH sensor, in between wavelengths of 460 to 560 nm, and one for the quantum dot, of about 1300 nm. If two peaks reaching a maximum at about 475 nm and 1300 nm are detected, it would indicate the presence of a breast cancer cell and its location.

4. Expected results

After the experiment, here are the expected outcomes. The experiment has two parts, which are labeled as A and B in total. Firstly, in the former part, when the quantum dot attaches itself to the cell, the progesterone will bind

to the progesterone receptor on the surface of cancer cells and emit a wavelength of about 1300 nm, which will be detectable by the spectrophotometer. Moving on to the latter part, when a ph-lemon sensor attaches itself to the cancerous cells that were profound from quantum dots, it will detect the lower pH by lighting up with cyan color and producing excitation wavelength in the range of 420–450 nm with mTurquoise2 as a critical indicator. Nonetheless, when it is a normal cell, it will detect higher pH, appearing as a yellow color and producing excitation wavelengths in the range of 490–520 nm EYFP as a critical indicator [26–28].

5. Discussion and conclusion

The two possible pitfalls of the detection method are: 1) If the pH-lemon sensor produces overlapping wavelength peaks when comparing the emitted light for the extracellular pH of a cancer cell and a normal cell, it means that it is hard to differentiate wavelengths from a cancer cell versus a normal cell. Since the cancer cell's extracellular pH is from 6.7 to 7.1 while the normal cell's extracellular pH is about 7.4, perhaps the sensor cannot accurately emit easily differentiated wavelengths for each. 2) The fluorescence is not robust enough for researchers to detect easily. To overcome these possible pitfalls, the solution is to 1) find a more sensitive pH sensor with a narrow pH range, which will provide a more accurate result, and 2) choose an alternative quantum dot of Cd/Pb Core-Shell proven to have wavelengths of 1500–1700 nm, or find a nontoxic quantum dot with a higher wavelength [32].

For possible future research, the researchers will first develop and test the current design for the quantum dot sensor. If it succeeds, the researchers will develop, test, and modify the current quantum dot sensor to eliminate cancer cells. This will vastly improve the future of both diagnosing and treating breast cancer. Furthermore, if the technology works, it will make the breast cancer detection method a cost-effective, fast way to assess whether the patient has breast cancer and, if so, locate its cells and organs. Instead of misdiagnosing 20% of the total breast cancer patients who need treatment, this will hopefully diagnose all the patients. With its low cost, perhaps more of the younger population will visit for screening. Since the technology does not require a trained professional, it can be applied to save patients in areas where trained professionals are unavailable. Improving the problem of biopsy and other diagnosis methods in the past, the innovative quantum dot sensor diagnosis will detect cancer cells within hours.

In conclusion, this study proposed a pH-sensitive quantum dot sensor for detecting progesterone receptors for earlier detection and diagnosis of breast cancer patients. The proposal includes two approaches to enhance the effectiveness of the detection method. The cost-effectiveness of this quantum dot sensor will significantly encourage patients with breast cancer to be diagnosed, particularly among the younger population, to visit the hospital for a quick breast cancer screening, which can lead to higher survival rates. Through improved detection, this quantum dot sensor's successful validation and effectiveness could enhance the lives of many breast cancer patients.

Disclosure statement

The authors declare no conflict of interest.

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