ウィスパリング ・ギャラリー・モードによるリガンド―受容体相互作用の新規高感度検出法

High performance novel sensing of receptor-ligand interactions by whispering gallery mode resonators

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Malaria is one of the most important diseases in the world causing high levels of morbidity and mortality, killing almost 1 million people every year. Development of rapid and sensitive diagnosis techniques for malaria is still an important need for robust and effective treatment regimens. Hemozoin is a bio-crystalline metabolite of *Plasmodium* parasites. During blood circulation of *Plasmodium* parasites, hemozoin is continuously produced, released and captured by immune cells, activating immune-mediated signaling cascades. Because hemozoin crystals are the unique signature of malaria parasites, detection of hemozoin in circulating blood or serum with high sensitive platforms, even at the single nanocrystal/molecule resolution, is the ideal platform for the early diagnosis of malaria disease. Furthermore, the highly sensitive platforms which can detect hemozin accurately would be beneficial to study hemozoin's interaction with immune cells.

Whispering-gallery-mode (WGM) resonators have recently been emerged as new platforms for sensing DNA, proteins and pathogens such as viruses. With an international collaboration, we have recently explored the use of WGM resonators both in air and liquid as label-free detection and measurement of hemozoin crystals with single crystal resolution. We have further explored the detection of changes during hemozoin and various molecule interactions.

研究目的

It is extremely important to develop highly sensitive technologies to detect very small molecules, both in air and water, for various applications such as early pathogen detection which can prevent outbreaks. Malaria is one of the important diseases in the world causing high levels of morbidity and mortality. Development of rapid and sensitive techniques for malaria diagnosis is still an important need for robust and effective treatment regimens.

Whispering-gallery-mode (WGM) resonators have emerged as a new and very versatile nano/micro-photonics sensing platform that relies on the ultrahigh-quality (Q) (enabling longer interaction time) and the microscale mode volume (enabling high optical intensity via the confinement of light field) of optical resonances. As such, WGM resonators enable high-fidelity optical measurements of biological phenomena with superior sensitivity (1, 2). WGM resonators have been shown to be feasible platform for the detection of viruses and nanoparticles at single-particle resolution (3, 4), as well as nucleic acid interactions.

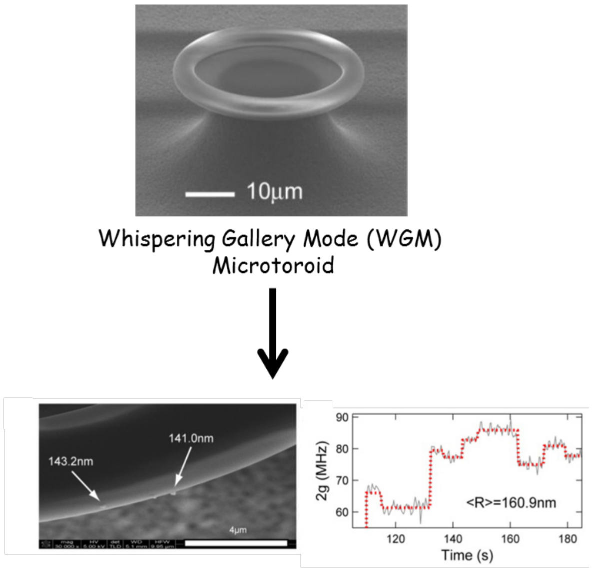
Hemozoin is a nano-sized crystalline by-product of *Plasmodium* parasites. During blood circulation of *Plasmodium* parasites, hemozoin is continuously produced, released and captured by immune cells. Because hemozoin crystals are the unique signature of malaria parasites, detection of hemozoin in circulating blood or serum with high sensitive platforms, even at the single nano-crystal/molecule resolution, will enable the early diagnosis of malaria disease (5). Hence, we have recently used WGM resonators for the first time in biological fluids to detect hemozoin crystals, a by-product of *Plasmodium* parasites, for the early detection of malaria disease (5).

Hemozoin is a biologically active molecule that interacts with various immune cells and immune-mediated signaling cascades. In this research project, we have investigated the possibility of using high-Q WGM resonators (which can detect hemozin accurately) to study hemozoin’s interaction with immune system molecules.

研究経過

1) The mode splitting in active and passive WGM optical resonators as a novel self-referencing technique for detecting perturbations in close proximity to the resonator has been introduced recently by co-investigators Ozdemir and Lang. Using the mode splitting technique, detection and single-shot size measurement of individual nano-scale objects, including polystyrene, potassium chloride and gold particles as well as Influenza A virions, with the minimum detectable size of 20 nm has been achieved (4). This technique has been applicable in aquatic environment that hemozoin crystals could be detected easily with WGM microtoroid resonators even in biological fluids (5).

To perform WGM sensing for the detection of hemozoin crystals (Figure), microtoroids were fabricated from silica-on-silicon wafer with diameters D=~50 μm and hemozoin crystals deposited on a microtoroid resonator. The mode splitting experiment was performed in an aquatic environment. The results of this study highlighted that highly sensitive early-phase detection of malaria infection from serum is possible with WGM resonators.



Single hemozoin particle detection

2) It has recently been shown that hemozoin crystals play a role in host immune system modulation by malaria parasites and interact with the host immune system. PI Coban and the others have tried to elucidate these interactions; however, there have been technical limitations. For instance, we have used Raman microscopy to investigate hemozoin’s effect on a single cell level (6). The Raman spectroscopy as a label-free method detected the biochemical changes occurring in macrophages during the first few hours of hemozoin uptake. There is a high possibility that innate arm of the immune system which is evolved to recognize immunologically active components derived from microbes are activated in these macrophages after the hemozoin uptake. One question that remained to be addressed is: How can the receptor-mediated recognition of hemozoin cooperate at the level of ligand recognition? A previous study by our group showed by using circular dichroism (CD) spectrum analysis that there are some interactions between TLR9 protein and hemozoin crystals (7). This implies light-polarization dependent response of these crystals. This is interesting, because we have shown in our studies with WGM resonators that the response of the WGM microresonator is very much dependent on the orientation of the hemozoin in the sensing volume and the polarization of the probing light field. Therefore, this implies that sophisticated methods such as mode splitting in active and passive WGM optical resonators can be further utilized as a novel self-referencing technique for detecting perturbations due to the presence of hemozoin crystals in close proximity to the resonator to probe such interactions at single crystal level. Currently, we are designing new fluidic platform to employ our

already-established WGM platform for studying such interactions.

考察

WGM resonators are very sensitive microscale photonic systems that can enable high-performance sensing platform to detect biological materials and their interactions. Ultra-high-Q and small mode volumes provide a superior sensitivity surpassing the performance of traditional interferometric or Surface Plasmon Resonance (SPR) techniques. Interestingly, plasmonic components can be easily incorporated to WGM resonators to make use of the best of two platforms: Highly localized field of plasmons (nanoscale mode volume in plasmonic particles systems versus microscale mode volume in WGM resonators) and ultra-high-Q (over 10 million in WGM versus a few thousands in plasmons). Now, these WGM resonators are used for wide range of applications including detection of biohazardous microbes in air or label-free sensing of single molecules not only in air but in biological fluids such as hemozoin detection in aqueous environment. We have also shown the performance enhancement in WGM resonators using intrinsic gain mechanisms such as Raman gain in the material from which WGM resonators are fabricated to compensate optical losses to increase sensing resolution as well as to turn WGM resonators into microlasers to significantly enhance detection and simplify the detection system for nanoparticle detection (8). We hope that this innovative tool will help understanding how microbes are recognized and further designing molecules for the development of new drugs and/or vaccines at least against malaria. We are now developing our systems to detect hemozoin interactions in real time by monitoring the response of WGM miroresonators and microlasers.

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