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研究課題名（和文）Establishment of a novel　platform of Raman microscope for diagnosis of hydrogel-generated　cancer stem cells.

研究課題名（英文）Establishment of a novel platform of Raman microscope for diagnosis of hydrogel-generated cancer stem cells.

研究代表者

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研究成果の概要（和文）：ヒドロゲル基板および高度なデータ前処理パイプラインと統合されたラマンイメージングシステムが、がん幹細胞の存在を検出するための効果的なプラットフォームとして機能できることが観察されました。高度な前処理ラマン データ パイプラインを使用することで、ステムネス マーカーを発現する可能性が高いハイドロゲル上で培養されたがん細胞のラマン スペクトルを、標準的な培養条件から得られたラマンスペクトルと比較することが可能になります。

研究成果の学術的意義や社会的意義
Cancer stem cells drive cancer resistance, metastasis, and drug resistance, making it urgent to develop platforms for detecting their presence in tumors and studying their properties. My research aims to create such a platform, enhancing early detection and understanding of cancer stem cells.

研究成果の概要（英文）：It was observed that a Raman imaging system integrated with a hydrogel substrate and an advanced data preprocessing pipeline can serve as an effective platform for detecting the presence of cancer stem cells. By using an advanced preprocessing Raman data pipeline, it becomes possible to compare the Raman spectra of cancer cells cultured on hydrogels, which are likely to express stemness markers, with those obtained from standard culture conditions. This approach enhances the sensitivity and specificity of cancer stem cell detection and provides insights into the metabolic alterations that underpin their stemness properties. Consequently, this platform holds great potential for improving cancer diagnosis and treatment by enabling the early detection and characterization of cancer stem cells, ultimately contributing to more targeted and effective therapeutic strategies.

研究分野：biophotonics

キーワード：Raman spectroscopy　cancer stem cell　machine learning　material science

１．研究開始当初の背景

Cancer is a complex and heterogeneous disease, characterized by uncontrolled cell growth and the ability to invade other tissues. A critical aspect of cancer biology that has gained significant attention in recent years is the concept of cancer stem cells (CSCs). CSCs are a small subpopulation of cells within a tumor that possess the ability to self-renew and differentiate into various cell types found in the tumor. This subpopulation is thought to play a crucial role in cancer progression, metastasis, and resistance to conventional therapies. CSCs are believed to be the driving force behind tumor growth and recurrence. Unlike the bulk of tumor cells, which may be sensitive to chemotherapy and radiation, CSCs exhibit several unique properties that enable them to survive and proliferate under adverse conditions. Despite their critical role in cancer biology, the rarity of CSCs within the bulk tumor and the difficulty in maintaining their stemness state pose significant challenges to their study. Very little is known about their properties or how to effectively detect them in clinical applications. Current techniques often struggle to isolate and characterize CSCs due to their scarcity and the dynamic nature of their phenotype.

２．研究の目的

Due to the rarity of cancer stem cells (CSCs), it is essential to develop a platform that can maintain their stemness and allow us to explore their properties in detail. To achieve this, we propose to bridge material science, biophotonics, and machine learning. This interdisciplinary approach aims to create advanced platforms that not only sustain the stemness of CSCs but also facilitate comprehensive studies of their unique characteristics and behaviors. By integrating these cutting-edge fields, we seek to develop innovative methodologies that will enable us to better understand CSCs, ultimately leading to the discovery of biomarkers specific to CSCs and a deeper understanding of their plasticity. Our research has demonstrated that hydrogel samples can mimic the extracellular matrix of CSCs and reprogram cancer cells into CSCs. Additionally, Raman imaging has shown great promise as an imaging modality to detect biochemical variations in cells. By coupling hydrogel samples, high-speed Raman microscopy, and advanced analytical methodologies, we believe we can detect and better understand the properties of CSCs. This integrated approach will provide new insights into CSC biology and potentially lead to the development of novel therapeutic strategies to target these elusive cells effectively.

３．研究の方法

Hydrogel platform

We develop hydrogel samples that mimic the extracellular matrix of cancer stem cells (CSCs). These hydrogels are used to maintain the stemness of CSCs and to reprogram cancer cells into CSCs. By optimizing the composition and properties of these hydrogels, we enhance their

effectiveness in preserving CSC characteristics. Different cancer cell lines are utilized to test the elevation of stemness in these cells when cultured on the hydrogel platforms.

High-speed Raman for biochemical state visualization.

We utilize high-speed Raman microscopy to detect biochemical variations in cells. Raman imaging is employed to monitor the changes in CSCs maintained on hydrogel-based platforms. The Raman spectra obtained from these imaging sessions are analyzed to identify specific biomarkers and metabolic states associated with CSCs. This technique provides detailed insights into the biochemical environment of CSCs, facilitating a better understanding of their properties, as well as allow to visualize the distribution of several biomolecules in the spatial dimension in a label free manner without disturbing the metabolic state of cells.

Data analysis.

Machine learning algorithms specifically designed to work with hydrogel samples were employed. The developed local Gaussian weighted background preprocessing algorithm effectively minimizing hydrogel substrate interference in cell signal detection was used. Machine learning algorithms, such as random forest and ridge logistic classifiers, are used to extract and quantify meaningful information from the data. This analytical approach aids in the discovery of novel biomarkers specific to CSCs and enhances our understanding of their plasticity.

４．研究成果

It was shown that optimized hydrogels substrate could reprogrammed diverse cancer cell lines including myoblast cell model, breast cancer cell line or again brain cancer cell line. The high -speed measurement of Raman imaging of live cell image on hydrogel has been successfully realized which is the first time to see in Japan. These measurements have been compared with controlled measurement done on quartz bottom dishes. In addition to this complex measurement, the algorithm developed to compare Raman data acquired on different substrate have shown promising possibilities to compare the biochemical states of cells cultured on these two types of substrates which is the first time to observe. Indeed, the minimization of diverse experimental factor that degrade the possibility to recover biological information has been grandly minimize, so that the comparison of cells among different date of measurement and different devices is robustly comparable. Hence, the use of machine learning algorithms such as random forest algorithm and ridge logistic classifier learned similar features in the recognition of cancer stem cells. In contrast the different algorithm proposed in the literature to standardize Raman data could not achieve a robust result among different experiments, and machine learning algorithm were were sensitive to diverse set of spectral to differentiate cancer stem cell metabolic state. The proposed standardizing Raman data method conjugated with machine learning algorithm shown that lipid alteration and repartition in the spatial dimension is a marker of cancer stem cell that can be used to detect them in large biological samples. In addition, some alteration of the cytochrome -c activity have been detectable by the classifier algorithms.

In parallel, some co-culture system mixing cancer cell and macrophage have shown the elevation

of cancer stem cell markers.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　2件／うち国際学会　1件）

１．発表者名 Jean-Emmanuel Clement, Zannatul Ferdous,Masami Tsuda, Shinya Tanaka, Jian Ping Gong, Katsumasa Fujita, Tamiki Komatsuzaki
２．発表標題 Gaussian Weighted Background Correction for Raman images : cancer stem cell detection
３．学会等名 19th europen conference on the spectroscopy of biological molecule（国際学会）
４．発表年 2022年

１．発表者名 Jean-Emmanuel Clement, Zannatul Ferdous,Masami Tsuda, Shinya Tanaka, Jian Ping Gong, Katsumasa Fujita, Tamiki Komatsuzaki
２．発表標題 Establishment of a novel platform of Raman microscope for diagnosis of hydrogel-generated cancer stem cells.
３．学会等名 The 45th Annual Meeting of the molecular biology Society of Japan (BSJ) 2022（招待講演）
４．発表年 2022年

１．発表者名 Zannatul Ferdous, Jean-Emmanuel ,Masami Tsuda, Shinya Tanaka, Jian Ping Gong, Katsumasa Fujita, Tamiki Komatsuzaki
２．発表標題 Morphological analysis of hydrogel induced cancer stem cell
３．学会等名 The 60th Annual Meeting of the Biophysical Society of Japan (BSJ) 2022（招待講演）
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関