様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 ２６ 日現在

機関番号：１７３０１
研究種目：若手研究
研究期間：2019～2022
課題番号：１９Ｋ１５３８８

研究課題名（和文）Multifunctional Au/Silk Nanoparticles Prepared by Facile Co-Precipitation Method and Evaluation for Delivery, Imaging and Targeting in Combination Cancer Therapy

研究課題名（英文）Multifunctional Au/Silk Nanoparticles Prepared by Facile Co-Precipitation Method and Evaluation for Delivery, Imaging and Targeting in Combination Cancer Therapy

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交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：本研究は、がん治療における薬物送達、画像化、ターゲティングに使用する初の金/シルクタンパク質ナノ粒子を簡便な共沈法で作製することを目指す。得られたナノ粒子は、サイズ分布が狭く、陰イオン型と陽イオン型の抗がん薬を効果的に負荷することができる。これらのナノ粒子は、薬物の取り込みと放出性に優れ、生体環境で安定し、細胞レベルでは無毒性である。また、特定の波長で照射すると、薬物放出量が増加する。このシステムは、レーザー照射と抗がん薬の組み合わせによって細胞アポトーシスを誘導し、がん治療の改善に貢献することが期待される。

研究成果の学術的意義や社会的意義
本研究の科学的な意義は、効果的な薬物送達と制御された放出を実現する多機能薬物送達システムの達成にある。得られたAu/シルクタンパク質ナノ粒子は、がん細胞への検出、送達、治療反応のモニタリングなど、すべてを提供するオールインワンのシステムとなる。
調査によると、Au/シルクナノ粒子が薬物送達システムにおいて初めて作製され、ターゲティングされることになる。Auナノ粒子は、共役によってLSPRを調整することができる独特なプラズモニック特性を持ち、薬物の放出応答や有効性に影響を与えるフォトサーマル効果を表現することができる。

研究成果の概要（英文）：The research aims to establish Au/silk nanoparticles for cancer treatment using a facile co-precipitation method. These nanoparticles showed narrow size distribution, successfully loaded both anionic and cationic anticancer drugs, and exhibited moderate drug uptake, good stability, and minimal toxicity. Irradiation with a wavelength similar to the absorption band of Au significantly increased drug release, up to double the amount with longer irradiation duration. Increasing the irradiation time or Au content in Au/silk nanoparticles resulted in nearly 90% drug release at pH 7. Combining hyperthermia and anticancer drugs induced cell apoptosis, as confirmed in vitro experiments with DOX and MitC on 4T1 breast cancer cells. Laser irradiation using Au/silk nanoparticles decreased cell viability, which was further enhanced with longer irradiation or higher Au content.

研究分野：Nanomaterials

キーワード：Silk protein　Gold　Hybrid　Nanomaterials　Drug delivery　Cancer therapy　Photothermal　Biocompatibility

１．研究開始当初の背景

In recent decades, extensive research and development efforts have been dedicated to the field of drug delivery, with a strong focus on utilizing nanotechnology to create versatile drug carriers. This innovative approach has shown great potential in combination cancer therapy, as it enables precise tumor targeting, efficient delivery of therapeutic substances, imaging capabilities, and monitoring of drug response. While numerous models have been explored and tested, the selection of appropriate materials and the optimization of carrier preparation remain ongoing challenges to ensure both safety and effectiveness. In response to these requirements, silk protein has emerged as an ideal candidate, offering a biodegradable and biocompatible matrix for incorporating noble metallic nanoparticles as modifiers and drug molecules as therapeutic agents. This system, with its adjustable structures and compositions, represents an advanced form of drug carriers that holds significant promise for enhancing cancer therapy.

２．研究の目的

This study aims to achieve three main objectives. Firstly, to establish a facile synthesis protocol for the formation of hybrid nanoparticles. Secondly, to explore the correlation between the characteristics of nanoparticles and their performance in drug delivery and anticancer activities. Finally, to evaluate the potential of the Au/silk system in applications related to drug delivery and cancer therapy.

３．研究の方法

The research project was carried out through three main steps: (1) Preparation of Au NPs and silk NPs individually; (2) Incorporation of Au into silk matrix through several methods and the most effective one was chosen to fabricate a series of Au/silk NPs with various compositions; (3) Characterization and evaluation of Au/silk properties and performance in drug delivery.

４．研究成果

(1) Results for 2019-2020 period

Silk nanoparticles were obtained with a uniform morphology and adjustable sizes ranging from 10 to 100 nm. The fabrication process involved careful monitoring of three types of silk protein solutions to achieve various morphologies and structures using the reprecipitation method. The surface charges of the synthesized silk nanoparticles could be modified under different preparation conditions, allowing successful loading of both anionic and cationic anticancer drugs. These silk nanoparticles exhibited moderate drug uptake and release, good stability in biological environments, and low cellular toxicity.

Au nanoparticles, synthesized with a size of 13 nm, underwent post-treatment to remove excessive ions before being incorporated into the silk matrix via a co-precipitation method. The structure of the resulting Au/silk nanoparticles was

confirmed through TEM measurements. The surface modification of Au was found to initially affect the fabrication yield of Au/silk nanoparticles. The Au/silk nanoparticles, slightly larger in size, showed negligible cytotoxicity and exhibited tunable plasmonic properties by varying the contents of Au. Additionally, Au-based metallic nanoparticles were successfully fabricated, demonstrating promising plasmonic properties.

The obtained Au/silk hybrid nanoparticles inherited the properties of silk nanoparticles and displayed dual pH- and photothermal-responsive drug release behavior. These behaviors were observed under simulated cellular pH conditions of 4.5, 6, and 7.4. The pH-responsive drug release was associated with the functions of silk side groups, while the correlation between photothermal-responsive drug release behavior and the content and structure of Au is currently being investigated.

(2) Results for 2021-2022 period

The fabrication of Au/silk nanoparticles was further optimized to control their size and compositions. Similar to silk nanoparticles, the surface charges of these nanoparticles could be modified. All the obtained nanoparticles exhibited moderate drug loading, good stability in biological environments, and minimal cellular toxicity. Upon irradiation at a wavelength similar to the absorption band of Au, the drug release from the nanoparticles dramatically increased. Furthermore, increasing the irradiation time or Au content in Au/silk nanoparticles led to nearly 90% drug release at pH 7, which was slower when using silk nanoparticles alone. This enhanced drug release was primarily driven by the increased temperature, which facilitated the dissociation of the absorbed drug, while the thermal hydrolysis of silk protein played a supporting role. The photothermal effect of Au nanoparticles induced low- or high-grade hyperthermia (43 and 45 °C, respectively) and promoted cell apoptosis when combined with anticancer drugs. These findings were confirmed through in vitro experiments using two types of anticancer drugs, DOX and MitC, on the 4T1 cell line.

Overall, the investigation of these nanocarriers with various types of drugs loaded revealed pH-responsive drug release associated with silk side groups and initial evidence of photothermal-responsive drug release related to the swelling and biodegradation of silk protein. The potential synergistic effect between the pharmaceutical activities of anticancer agents and hyperthermia was demonstrated. Varying the contents of Au in Au/silk nanoparticles had negligible effects on drug loading efficiency. However, increasing the irradiation time or Au content in the nanoparticles enabled drug release of nearly 90% at pH 7, which was relatively slower when using silk nanoparticles alone. The enhanced drug release was primarily attributed to the temperature increase, facilitating the dissociation of the absorbed drug. The photothermal effect of Au nanoparticles induced hyperthermia and promoted cell apoptosis when combined with anticancer drugs, as confirmed through in vitro experiments using DOX and MitC on the 4T1 cell line.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acsanm.1c02363	査読の有無 有
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〔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