ACS APPLIED BIO MATERIALS

www.acsabm.org Article

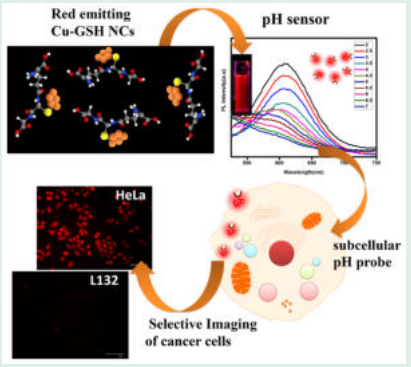
Label Free, Nontoxic Cu-GSH NCs as a Nanoplatform for Cancer Cell Imaging and Subcellular pH Monitoring Modulated by a Specific Inhibitor: Bafilomycin A1

Neeli Chandran,[‖] Prajit Janardhanan,[‖] Manikanta Bayal, Unnikrishnan Unniyampurath, Rajendra Pilankatta,* and Swapna S. Nair*

Cite This: *ACS Appl. Bio Mater.* 2020, 3, 1245−1257 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Metal nanoparticles-based sensors invoked much research attention in the biomedical field, especially in applications involving live cell imaging and monitoring. Here, a simple cost-effective method is adopted to synthesize glutathione coated copper nanoclusters (Cu-GSH NCs) with strong bright red fluorescence (625 nm). The clusters were found to be containing five Cu(0) atoms complexed with one molecule of glutathione (GSH) as evidenced by MALDI-TOF MS analysis. The synthesized Cu-GSH NCs system responds linearly to the pH in the acidic and alkaline ranges with a high degree of in vitro pH reversibility, projecting its potential as a real time pH sensor. Higher intensity emission observed in acidic conditions can be exploited for its employability as cellular organelle markers. The imaging and sensing potential of Cu-GSH NCs in the live human adenocarcinoma cell line, the HeLa cells, was tested. The treatment of HeLa cells for 48 h imparted deep red fluorescence, owing to the lower level of intracellular pH in cancer cells. In contrast, the imaging using normal cell lines (L-132, lung epithelial cell line) showed significantly lower fluorescence intensity as compared to that of HeLa cells. The subcellular pH-dependent fluorescence emission of Cu-GSH NCs was further assessed by treating HeLa cells with proton pump (V-ATPase) inhibitor Bafilomycin A1, which increases the vesicular pH. Interestingly, the fluorescent intensity of HeLa cells decreases with increasing concentration of Bafilomycin A1 in the presence of Cu-GSH NCs, as evidenced by the fluorescence microscopic images and quantitative fluorescent output. Accordingly, the developed Cu-GSH NCs system can be employed as an efficient pH-based bioimaging probe for the detection of cancer cells with an implied potential for the label free subcellular organelle tracking and marking. Importantly, the Cu-GSH NCs can be used for live cell pH imaging owing to their high degree of reversibility in sensing of pH variation.



KEYWORDS: *Cu GSH nanoclusters, pH sensor, bioimaging, HeLa cells, organelle marker, cancer cell imaging*

1. INTRODUCTION

Subcellular or single-cell pH has an essential role in the homeostasis of many cellular events. It plays vital roles in pathological and physiological processes, which include enzyme activity, protein degradation, and function of different cellular organelles.[1] Inside the cell, lysosomes possess the lowest intracellular pH (4.7) and the highest is found in mitochondria (pH 8.0).[2,3] Atypical variations in the pH values may lead to functional disorders of organelles resulting in unhealthy populations of cells. A discrepancy in the intracellular pH has been observed in the case of diseases such as Alzheimer's, stroke, and cancer.[3,4] Abnormality in intracellular pH and uncontrolled growth are the two major indicatives of cancerous cells.[5] Hence, effective detection of intracellular pH of living cells helps in the early detection and diagnosis of cancer. Different types of cellular pH measuring techniques and devices are available with the advent of nanoengineered sensors for both in vitro and in vivo applications. Protein capped nanoparticle systems are developed by researchers as cellular pH sensors[6] which can be used for bioimaging. However, most of the chemical dye-based pH sensors are found to be less stable against photobleaching, especially in the presence of high intensity laser-based imaging and are cytotoxic in nature. Hence, label free nanoplatforms based on quantum dots (QDs) and nanoparticles (NPs) emerge as ideal substitutes in these contexts.

Nanomaterial-based biosensors are gaining popularity in the biomedical field.[7−9] Metal and non-metal nanoparticles are being widely used as a tool for the precise detection of specific

Received: December 9, 2019
Accepted: January 20, 2020
Published: January 20, 2020

https://dx.doi.org/10.1021/acsabm.9b01036
ACS Appl. Bio Mater. 2020, 3, 1245−1257