Developing A Chlorophyll-Based Near-Infrared Fluorescent Probe for Cancer Cell Imaging

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Introduction: Near-Infrared (NIR) fluorescence-based imaging is a noteworthy and safer strategy for cancer cells/tissues imaging compared to radiological imaging. NIR fluorescence offers deep tissue penetration and have minimal obstruction by autofluorescence and photon scattering [1]. There are several NIR dyes including indocyanine green (ICG) [2] and IR-1061 [3] that allow high-resolution tissue imaging. However, these dyes possess some low-quality characteristics which limit their use, namely photo instability, toxicity, poor water solubility, and short half-lives [4]. Therefore, more efficient and effective alternatives are urgently required to provide the desired clinical outcomes. Chlorophyll (Chl) is a natural dietary NIR fluorescence emitting substance which has the potential to serve as a lead NIR imaging candidate for cancer administration [5].

Objective: Developing a biocompatible NIR imaging dye from natural resources that can facilitate cancer cells/tissues imaging for improved detection. Chlorophyll is a natural NIR fluorescent alternative that is widely present in plants and green vegetables. Hence, we aim to extract Chl from dietary herbs and vegetables for cancer imaging.

Methods: We selected 12 different leaves such as basil, bay leaf, collard, dill, kale, lettuce, mint, oregano, rosemary, sage, spinach, and thyme to assess the fluorescence distribution of chlorophyll. Each leaf was imaged in triplicates using the IVIS *In Vivo* Imaging System to detect Chl with excitation and emission wavelengths at 600/710 nm. Based on fluorescent intensity levels, next, we selected the 6 most fluorescent leaves (Bay leaf, collard, lettuce, mint, oregano, and spinach) and extracted the Chl dye using ethanol. The extracts were again visualized with IVIS system for Chl detection. Further, a Dynamic Light Scattering (DLS) system was used to measure size distribution, surface charge and the concentration of these Chl extracts. In order to determine the Chl internalization in cancer cells, AsPC-1 (pancreatic) and SK-HEP-1 (liver), two cell lines were treated with bay leaf (highest fluorescence) extracted Chl at different concentrations (10, 20, 30, 40 and 50 µg) for an hour and imaged under red channel using an EVOS Imaging System.

Results: Whole leaf IVIS imaging revealed that spinach had the maximum Chl fluorescence of ~ $1.92x10^{12}$ and the lowest was in thyme ~ $8.60x10^{10}$. On the other hand, extracted Chl was most detectable in bay leaf extract (~ $4.98x10^{10}$). Additionally, physicochemical characterization of extracted Chl from these leaves suggested the particle size range of ~ 50 to 230 nm (Bay leaf 62.7 nm), zeta potential of ~ -20 to -25 (Bay leaf -24.76) and the obtained concentrations were, $1.11x10^{12}$ for bay leaf (Highest) and $5.01x10^{9}$ for lettuce (Lowest). Moreover, cellular internalization data obtained using a fluorescent microscopy indicated a dose dependent increase in the bay leaf extracted Chl fluorescence in both cell lines. However, the fluorescence level was more prominent in the SK-HEP-1 cells compared to AsPC-1.

Discussion: NIR fluorescent dyes play a significant role when it comes to the early stage cancer detection. Chlorophyll has NIR fluorescent excitation ~ 604 nm and emission ~ 700 nm which offers the capability of high-resolution imaging with deep tissue penetration, making it ideal for cancer imaging. Extraction process of Chl from dietary leaves is highly scalable and reproducible, herein, we have screened different leaves for Chl fluorescence, and it was evident that bay leaf exhibited the highest yield and Chl fluorescence.

Conclusions: Taken together, our data suggested that Chl extracted from dietary resources, is a potent biocompatible alternative for NIR fluorescence which can be applied to cancer cells and tissues/tumors for enhanced detection resolution.

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