# Enhancement of Protoporphyrin IX Accumulation by Alginate-Incorporated and Folic Acid-Conjugated Chitosan Nano-particles for Colorectal Cancer Photodynamic Detection

Yang, S.-J., Tsai, K.-C. ,Lin, F.-H., Shieh, M.-J.*

Institute of Biomedical Engineering, National Taiwan University, Taipei 100, Taiwan

## Abstract

***Introduction:*** The incidence of colorectal cancer is increasing worldwide and its prognosis remains poor. In general, survival is inversely related to extent of tumor spread at time of detection. Relative rates of survival of 50 % at three years and 40 % at five years have remained unchanged since the 1960s. Prognosis is excellent with detection at an early stage. Unfortunately, small colorectal neoplasia and early cancer are frequently overlooked during endoscopy. Therefore, a powerful and highly-sensitive tool for the detection of precancerous lesions would be of great value.

***Background:*** Photo-diagnosis is one of the most promising and non-invasive methods for detecting malignant or premalignant tissue. Currently, detection of abnormal tissue usually involves the use of an exogenous chromophore, such as protoporphyrin IX (PpIX), excited by optima light to generate fluorescence in cancer lesions. The 5-aminolevulinic acid used in the study, a precursor in heme group synthesis, is totally degraded intracellularly and converted to PpIX. Because the decomposition rate of PpIX in cancer cells differs from that in normal cells, the photosensitive fluorophore, PpIX, can be used to detect cancer lesions.

***Purpose:*** We aimed to synthesize an alginate-incorporated and folic acid-conjugated chitosan nano-particle as a suitable vehicle for carrying 5-aminolaevulinic acid (5-ALA) to enhance the detection of colorectal cancer *in vivo* after a short-term uptake period.

Table 1. The average particle size, zeta-potential and loading efficiency of chitosn-based nano-particles.

| Sample | Z-average size (nm) | PDI [a] | Zeta-potential (mV) | Loading efficiency of 5-ALA (%) |
|---|---|---|---|---|
| CN | 73.3 ± 3.56 | 0.252 | 24.4 ± 3.27 | — |
| CNA | 76.2 ± 1.28 | 0.226 | 24.8 ± 1.30 | 44.5 ± 5.68 |
| fCN | 87.1 ± 3.95 | 0.381 | 22.7 ± 0.52 | — |
| fCNA | 84.3 ± 1.57 | 0.339 | 23.1 ± 1.19 | 34.8 ± 4.76 |
| fCAN | 114.9 ± 2.05 | 0.401 | 22.6 ± 1.08 | — |
| fCANA | 116.1 ± 3.08 | 0.377 | 21.8 ± 1.10 | 26.7 ± 1.83 |

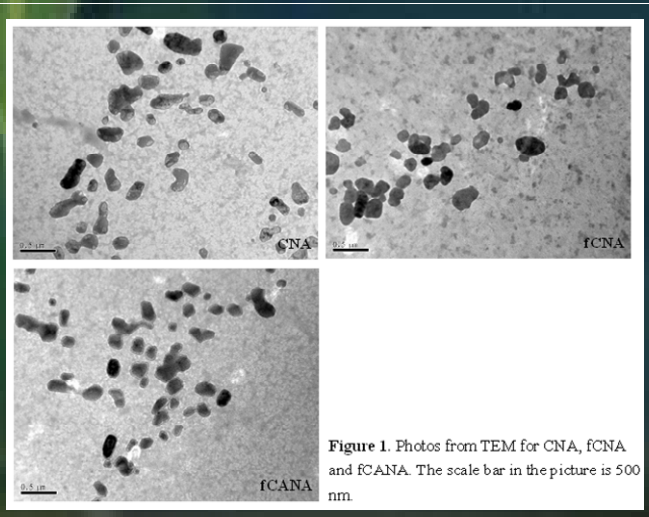


**Figure 1**. Photos from TEM for CNA, fCNA and fCANA. The scale bar in the picture is 500 nm.

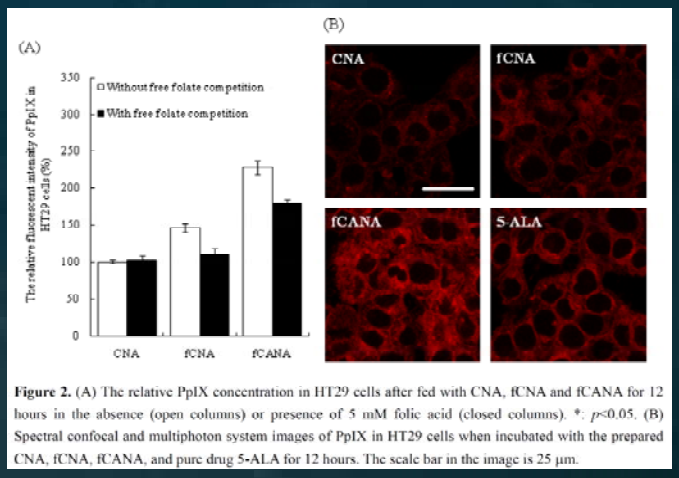


**Figure 2.** (A) The relative PpIX concentration in HT29 cells after fed with CNA, fCNA and fCANA for 12 hours in the absence (open columns) or presence of 5 mM folic acid (closed columns). *: $p<0.05$. (B) Spectral confocal and multiphoton system images of PpIX in HT29 cells when incubated with the prepared CNA, fCNA, fCANA, and pure drug 5-ALA for 12 hours. The scale bar in the image is 25 μm.

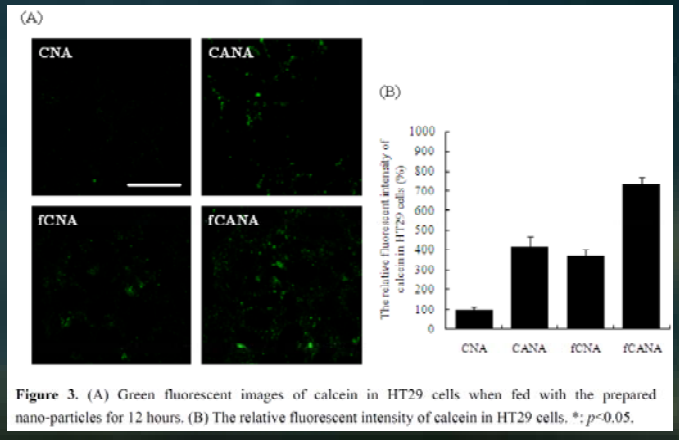


**Figure 3.** (A) Green fluorescent images of calcein in HT29 cells when fed with the prepared nano-particles for 12 hours. (B) The relative fluorescent intensity of calcein in HT29 cells. *: $p<0.05$.

***Conclusion:*** The use of alginate and folic acid-chitosan conjugate appears to be an ideal vector for colorectal-specific delivery of 5-ALA for fluorescent endoscopic detection.