

# The development of multifunctional nanocomplexes for drug delivery in oral cancer therapy

Cheng FY<sup>1</sup>, Tsai CH<sup>1</sup>, Shieh DB<sup>1,2</sup>, Huang JS<sup>1,2\*</sup>

<sup>1</sup> Institute of Oral Medicine, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan  
<sup>2</sup> Department of Stomatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan  
huangjs@mail.ncku.edu.tw

## ABSTRACT

Oral cancer has been denoted as the sixth most common cancer in the world. Chemotherapy is one of the major therapeutics for advanced oral cancer cases. Advances in drug delivery system draw a niche for cancer treatment due to improved local therapeutic concentration, side effect control and pharmacokinetic profile. A novel drug delivery system made of superparamagnetic iron oxide nanoparticles (SPIONs) covalently linked with alginate was developed for oral cancer therapy. The results suggest that Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles could effectively release doxorubicin in a spatial-temporal controlled manner and even achieved higher release rate in cancer intracellular compartments. These data suggest that Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate could be a potential candidate of advanced drug delivery systems for advanced cancer therapy.

**Keywords:** superparamagnetic iron oxide nanoparticles, alginate, carbodiimide reaction, drug delivery system, oral cancer

## 1 INTRODUCTION

Oral cancer is the sixth most common cancer in the world and one of the leading causes of cancer death with an average 5-year survival rate of only around 50%. Unfortunately, this figure does not improve during the past decades. Most of the advanced cases rely on chemotherapy to prolong their life expectancy. Thus, it is important to develop more effective chemotherapeutic approach for clinical management of such malignancy.

Doxorubicin is one of the most effective anti-cancer drugs. It is prescribed often in combination with other anti-cancer drugs for the treatment of various types of cancer. Doxorubicin is a subgroup of anthracycline, which is a branch of antibiotics produced by *Streptomyces Peucetius* and a variety of *Cassius*. Anthracycline, doxorubicin, daunorubicin, adriamycin and epirubicin are anti-neoplastic antibiotics with a wide range of clinical applications. Doxorubicin acts against a number of tumors, such as sarcomas and carcinomas. this drug has severe side effects,

such as cardiac and renal toxicity. Hence, the use of doxorubicin for treatment of cancer patients was limited in certain aspects. Much efforts has been taken to solve these issues and to reduce the adverse effects. Among which is to confine the drugs to tumor locale through nanoparticle delivery systems.

Recently, drug delivery systems were intensively investigated to carry bioactive agents in time- and spatial-controlled manners for the improvement of overall therapeutic efficacy. The aim of this study is to develop and evaluate a novel drug delivery nano-system made of superparamagnetic iron oxide nanoparticles (SPIONs) core covalently linked with alginate to achieve augmented targeting and therapeutic efficacy in oral cancers.

## 2 MATERIALS AND METHODS

### 2.1 Preparation and characterization of Fe<sub>3</sub>O<sub>4</sub> nanoparticles, Fe<sub>3</sub>O<sub>4</sub>/alginate nanoparticles and Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles.

Alginate-conjugated SPION (SPION-alginate) is prepared by mixing magnetite (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles functionalized with the -NH<sub>3</sub><sup>+</sup> group and alginate, followed by carbodiimide reaction. The hydrodynamic diameter, zeta potential and the surface modification of alginate to Fe<sub>3</sub>O<sub>4</sub> nanoparticles of SPION-alginate are characterized by dynamic light scattering (DLS) and fourier transform infrared spectroscopy (FT-IR), respectively.

### 2.2 In vitro release profile of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles

Doxorubicin (DOX), an anticancer drug, is encapsulated into the drug delivery system by suspending DOX mixed in SPION-alginate solution followed by dropping the mixture into 10 mM CaCl<sub>2</sub> aqueous solution (DOX-loaded SPION@Ca-alginate). The encapsulation efficiency and loading capacity are determined by fluorescence spectrometer with excitation wavelength of 485 nm and emission wavelength of 590 nm; the drug releasing profiles

of DOX-loaded SPION@Ca-alginate at four different media (ddH<sub>2</sub>O, cytoplasm mimicking buffer and PBS at pH 7.4 or 5.5) are monitored every 24 hours over 7 days period..

### 2.3 In vitro cytotoxicity evaluation of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles

OEC-M1 cells were seeded at 5×10<sup>3</sup> cells/well in a 96-well plate. After 24 hours, serial dilutions of free DOX and DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles were added to the cells with final concentrations ranging from 0.1 μM to 2.0 μM (DOX concentration), and were incubated for additional 24 hours. Then, MTT tests were applied to verify the vital cell counts in various DOX concentrations.

## 3 RESULTS

The hydrodynamic diameter and zeta potential of SPION-alginate is 80.3±20.6 nm and -39.00±0.34 mV, respectively. The FT-IR spectra of SPION-alginate reveal successful conjugation of alginate to Fe<sub>3</sub>O<sub>4</sub> nanoparticles. The encapsulation efficiency and loading capacity reached 61% and 1218.21 μg/mg Fe, respectively when the ratio of DOX to Fe is 2:1 (weight to weight). In vitro drug releasing profiles of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles showed an initial releasing phase occurred within 24 hours then reached saturation at 48 hour. The cumulative releasing profile of DOX is environment-pH dependent and the highest releasing was found to be at pH 5.5 (44.88 ± 1.76 % at the 7<sup>th</sup> day). [Figure 1]

The cumulative DOX concentration released from the nanoparticles present in the four different buffer systems increased in the following order: deionized water < PBS (pH 7.4) < PBS (pH 5.5) < cytoplasm mimicking buffer. The releasing profiles of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles were independent of calcium concentration in the solution (Figure 1. CaCl<sub>2</sub>: A. 10.0mM. B. 5.0mM. C. 2.0mM.) that may entrap doxorubicin in the alginate matrix. [Figure 2]

The in vitro cytotoxicity assay showed that DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate has a strong association between Ca<sup>2+</sup>-crosslinking density and their in vitro cytotoxicity. The efficacy was enhanced by lowering Ca<sup>2+</sup>-crosslinking density. At low DOX concentration of 0.5 μM, nanoencapsulation delivered superior cancer cell cytotoxicity that lower crosslinking by CaCl<sub>2</sub> (2.5mM) gave better cytotoxicity response than higher crosslinking and free DOX. This may be caused by the delayed DOX release to prolong effective exposure time to the cancer cells. [Figure 2] However, at high DOX concentration, despite the same trend of calcium concentration dependent cancer cell cytotoxic response was observed, free DOX of the same concentration delivered better anti-cancer efficacy.

This may attribute to the fact that higher DOX concentration could be achieved in their free form when compared to prolonged lower concentration exposure. These results suggested that a critical concentration exist for optimized DOX loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles to achieve the best in vitro and in vivo treatment outcome and should be further explored. Besides, in vitro toxicity study could not reflect the hemodynamics and pharmacokinetics of the delivery system in real disease model. Further in vivo validation should be performed to reach a solid conclusion.

**Figure 1. In vitro release profile of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles.**

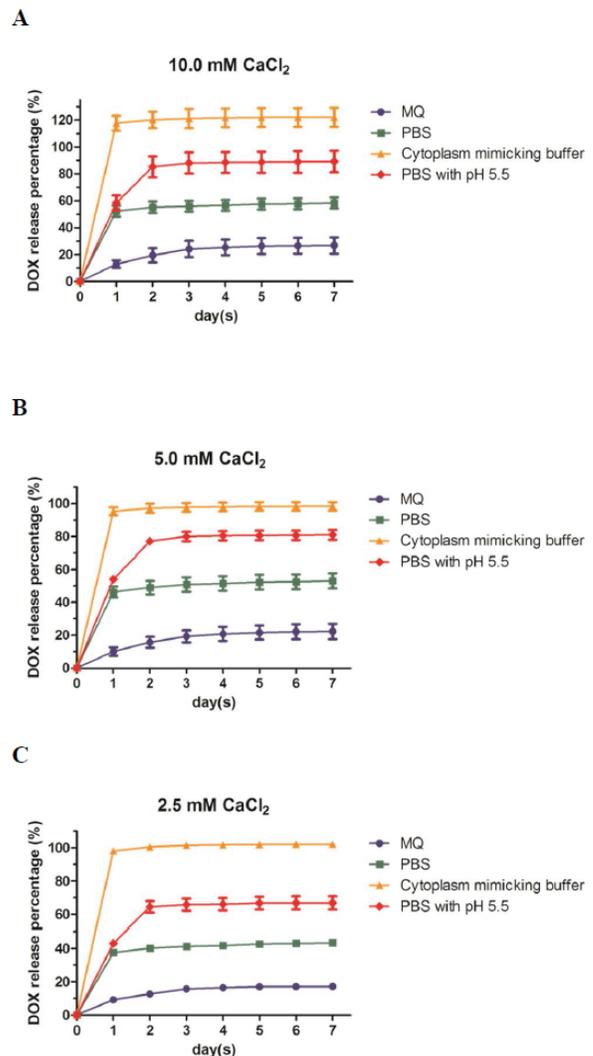


Figure 1: Ten microgram of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles were added in deionized water, PBS, cytoplasm mimicking buffer, and PBS with pH value at 5.5 (total volume: 500 μL). The alginate on nanoparticles was crosslinked by (A) 10.0 mM CaCl<sub>2</sub> (B) 5.0 mM CaCl<sub>2</sub> and

(C) 2.5 mM CaCl<sub>2</sub>. The buffers were removed for quantification of released DOX and replenished every day with new buffers. The released DOX were determined by fluorescent spectrometer (excitation wavelength: 485 nm; emission: wavelength: 590 nm).

**Figure 2. In vitro cytotoxicity evaluation of DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles**

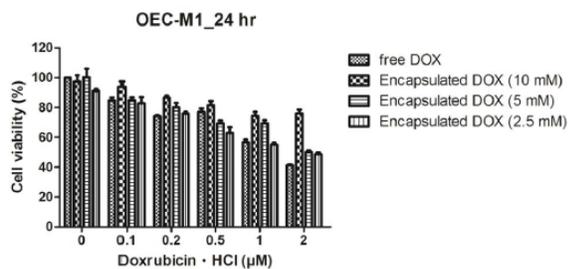


Figure 2: DOX loaded Fe<sub>3</sub>O<sub>4</sub>@alginate nanoparticles were crosslinked with different calcium concentrations (2.5, 5 and 10mM). These nanoparticles at 20 ppm equivalent iron concentration were analyzed for cytotoxicity to OEC-M1 oral cancer cell line after 24hrs of exposure. The free DOX of the same loading concentration was served as the control.

## 4 DISCUSSION

In this study, we succeeded in the preparation of Fe<sub>3</sub>O<sub>4</sub> nanoparticles with further biocompatible modification of alginate on the surface to derive the core shell nano drug carrier by modifying a synthesis procedure previously described by Shieh et al.'s. The Fe<sub>3</sub>O<sub>4</sub>@alginate nanoparticles were characterized by DLS for their size distribution and FT-IR for their surface chemistry as well as TEM for ultrastructure features.

In selected cases of localized recurrent oral cancer patients, re-operation or re-irradiation were considered. However, for most patients, palliative chemotherapy is the standard treatment of choice. Current systemic anti-neoplasm therapy usually induced severe side effects and did not significantly improve overall survival.

In this study, we presented a novel cancer chemotherapy with nano drug delivery system consists of an Fe<sub>3</sub>O<sub>4</sub> nanoparticle core and alginate shell cross-linked by calcium ions. Such system could preferentially release anti-cancer drug such as DOX to the cancer cells in a controlled slow release mode while boost up local concentration of chemotherapeutic agents through enhanced permeation and retention effect (EPR). Our results suggest that Fe<sub>3</sub>O<sub>4</sub>@Ca-

alginate nanoparticles could release anti-cancer drugs preferentially in cytoplasm of the cancer cells (in cytoplasmic mimic buffer) and performed a slow release for over one week period in tissue fluid mimic environment (neutral pH PBS). Besides, the nanocarrier present acidic environment triggered release (pH 5.5 PBS) thus would preferentially deliver therapeutic agents in the cancer microenvironment that is known to be acidic (pH 4~5.8) due to Warber's effect. (Figure 1) Such property would significantly reduce the potential off-target side effects and the required frequent dose of chemotherapeutic agents thus encourage better patient compliance.

Besides, iron oxide nanoparticles are generally recognized to be biocompatible and has been applied in clinical settings such as MRI contrast agent (Resovist for example) due to their superparamagnetism. Such T2 weighted MR imaging contrast further allowed atrgeting validation of chemotherapeutics through MRI. Such property encouraged further design of integrated diagnosis and therapy in one platform.

Due to the high surface to volume ratio, the alginate moiety on nanoparticles are capable of carrying a significant payload containing even a cocktail of multiple drugs to simultaneously inhibit cancer signaling pathways in synchronized manner. Future integration and optimization of the cocktail delivery is warrented. Moreover, our in vitro release study suggest efficient intracellular release than outside cells.

Nanoparticles have also been reported to direct the therapeutics to the target cell nuclei to exert improved molecular level control of anti-cancer effects. Nanoparticles are also known for better tissue penetration due to their extremely small form factor and the unique size of the Fe<sub>3</sub>O<sub>4</sub>@Alginate designed in this study further distinguished normal versus cancer permeation by EPR effect for improved tumor tissue accumulation for an extended period. The in vitro cytotoxicity data indicates that DOX-loaded Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate nanoparticles exhibit excellent therapeutic efficacy. These data suggest Fe<sub>3</sub>O<sub>4</sub>@Ca-alginate may be a candidate for future anti-cancer drug delivery systems for advanced malignant disease management.

## 5 CONCLUSION

Our results suggest that the  $\text{Fe}_3\text{O}_4@Ca$ -alginate can serve as a nanocarrier for cancer chemotherapy, and we expect that this system will improve therapeutic efficacy and minimize side effects for future anticancer therapy.

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