Multifunctional Calcium Phosphate Nanocomposite for Synchronous Sensing and Controlled Drug Delivery

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Statement of Purpose: Pharmaceutical nanosystems capable of performing multiple tasks such as controlled drug delivery, bioimaging and sensing can enhance efficacy of many therapeutic and diagnostic protocols.[1,2] The **objective** of this work is to understand the chemistry of calcium phosphate (CaP) interaction with drug molecule and fluorescent organic moiety for sensing and controlled drug release. Our **hypothesis** is that, a multifunctional pH sensitive fluorescent CaP nanosystem will help in controlled drug delivery and simultaneously sense the release of the drug. The **rationale** is once we delineate the stimuli responsive release behavior of the drug molecule and the sensing ability of the system, we will gain insight to develop CaP based sensing and controlled intracellular drug delivery system. Herein, we report a novel CaP with alendronate (AD) encapsulated in CaP and modifying the surface with rhodamine B (RDB) dye to engineer a multifunctional nanosystem that is capable of sensing the delivery of the therapeutic by a change in the fluorescence.

Methods: The synthesis of CaP/AD nanocomposite was carried out by mixing 1.0M $Ca(NO_3)_2$ .4 H_2O (Sigma-Aldrich Inc. (St. Louis, MO)) solution with 0.67M $(NH_4)_2HPO_4$ (Alfa Aesar (MA)) solution having 10 mM alendronate trihydrate (Sigma-Aldrich Inc. (St. Louis, MO)) at pH 9.5. Rhodamine B (RDB) was loaded on the surface of CaP/AD nanocomposite by treating with 4 x 10^{-3} M RDB solution adjusted to pH 9.0. The concentration of alendronate and rhodamine B were determined by using a Spectronic UV-visible spectrophotometer (GENESYS 5, ThermoFisher, Madison, WI). The synthesized nanocomposites were characterized by TEM (Philips CM 200) to determine the dimensions. FTIR spectra were recorded on a Thermo FTS-1000 FTIR spectrometer. The hydrodynamic diameter was measured by dynamic light scattering using a NICOMP particle size analyzer (NICOMP^{TM} 380, Santa Barbara, CA, USA).

Results: The CaP/AD nanocomposite synthesized by precipitation method was successful in loading 32.64 mg/g of AD. The CaP/AD nanocrystals displayed average dimension of 20 × 44 nm, and RDB modified CaP/AD displayed average dimension of 21 x 44 nm. Their mean hydrodynamic diameter was estimated as 47.4 ±5nm and 55.1 ±8 nm, respectively. The AD doping in CaP was confirmed by ATR-FTIR technique. Peaks of AD in the range of 1200-900 cm^{-1} were present in the spectrum of CaP/AD with a small shift. *In* vitro release study of AD from CaP/AD nanocomposite was performed at two different pH of 5.0 and 7.4 (Figure 1(a)). The amount of AD released from the CaP/AD nanocomposite system reached about 81% in 48 h in pH 5.0. When the pH of the release medium was increased to 7.4, only 19% of AD was released. This finding indicates pH responsive drug-release pattern. The RDB modified CaP/AD was found to possess fluorescence property when excited at a wavelength of 530 nm. In order to better understand the solution phase behavior, RDB-CaP/AD nanoparticles were dispersed in PBS solutions of pH 5.0 and 9.5. The emission spectrum of the RDB-CaP/AD nanoparticles in solution of pH 5.0 was ~1.4 times higher compared to RDB-CaP/AD in pH 9.5. In solution of pH 9.5 most of the dye is attached to the surface of CaP/AD due to the low solubility of CaP, which results in quenching of the fluorescence of RDB and hence lower fluorescence was observed. The higher fluorescence of RDB-CaP/AD when exposed to an acidic media (pH 5.0) is due to the release of RDB from CaP/AD surface. The pH dependent fluorescence emission of RDB-CaP/AD was evaluated by measuring the fluorescence intensity of RDB-CaP/AD in PBS of pH 5.0 and 7.4 (Figure 1(b)). Higher fluorescence emission was observed for RDB-CaP/AD in PBS of pH 5.0 as compared in PBS of pH 7.4 due to higher release of RDB as a consequence of faster dissolution of CaP/AD in acidic environment.

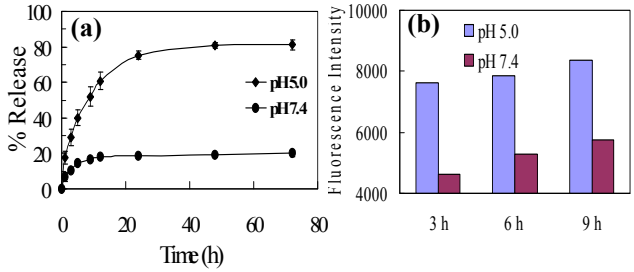

Figure 1. (a) Release profiles of AD from CaP/AD and (b) Fluorescence of RDB released from RDB-CaP/AD at pH 5.0 and 7.4.

Conclusions: A multifunctional pH sensitive CaP based nanocarrier was synthesized. The average dimension of CaP/AD and RDB-CaP/AD nanocomposites determined by TEM were found to be 20 × 44 nm and 21 x 44 nm, respectively. CaP/AD nanocomposite exhibited pH triggered response, i.e., drug was released in acidic conditions (pH 5.0), but the release was retarded in physiological pH (7.4). Higher fluorescence was found for RDB-CaP/AD in PBS of pH 5.0, compared to RDB-CaP/AD in pH 7.4. The results indicate that this novel nanomedical system which combines controlled drug delivery as well as sensing at the same time has an excellent potential for simultaneous diagnosis and therapy. Financial support from NIH (EBR01007351) is greatly acknowledged.

Reference: 1) Banerjee SS, Nanotechnology. 2008; 19; 505104- 505111.
2) Banerjee, Chem. Mater. 2007; 19; 6345-6349.