Calcium Phosphate System in Sensing and Drug Delivery

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Introduction: Calcium phosphates (CaPs) are widely used in bone tissue engineering applications due to their excellent bioactivity and non-immunogenic response to them by the body. CaPs have relatively low solubility in physiological pH, but have higher solubility in acidic environments of endolysomes or around solid tumors. This pH dependent solubility makes CaPs suitable for intracellular delivery of therapeutics encapsulated in CaPs. Alendronate (AD), a bisphosphonate drug, is widely used for the treatment of various metabolic bone diseases because of its potent inhibitory effect on osteoclastic bone resorption [1]. Glutamic acid, an amino acid, in addition to being a neuro-transmitter can also regulate bone cell activities by glutamate signaling [2]. **Objective** of this research is to fabricate novel multifunctional CaP based system that will deliver the drug at its target site and simultaneously facilitate sensing. Our **hypothesis** is that chemistry of CaP and the therapeutic agent play a vital role in controlling the delivery in the physiological pH. The **rationale** is that once we determine the adsorption and release kinetics depending on CaP and therapeutic agent's chemistry, and solution pH, we should be able to design better bone grafts for simultaneous sensing and controlled drug delivery.

Methods: Calcium phosphate–alendronate nanocomposite (CaP/AD) was synthesized using alendronate sodium trihydrate and calcium nitrate. Synthesis of rhodamine B (RDB)-CaP/AD composite was performed by surface modification of CaP/AD nanocomposite with rhodamine B (RDB), as shown in **Fig. 1**. Glutamic acid (GA) adsorption was carried out on hydroxyapatite(HA) and β-tricalcium phosphate (β-TCP) 1:1 composite. Particle size of the synthesized nanopowder was measured by transmission electron microscope (TEM). Phase analysis was performed by XRD. GA adsorption was done in 5, 7.5, and 10 mg/ml GA for 6 and 12 h, respectively. Release study was carried out at 37 ^{o}C in PBS of pH 5.0 and 7.4. Release medium was withdrawn at predetermined time intervals and the drug concentration was analyzed using a Spectronic UV-visible spectrophotometer. A BIOTEK synergy HT microplate reader was used to measure the fluorescence.

Results: TEM analysis reveals that average dimension of the CaP/AD nanocomposite is 20 × 44 nm. The dimension of the synthesized CaP nanoparticle is found to be 55 x 136 nm. A reduction in the crystal dimension of the composite is caused by AD addition and hence the dimension of CaP/AD composite is smaller than CaP. The smaller dimension of CaP/AD nanocomposite clearly shows that the product is a composite and not a mixture of CaP and AD. XRD analysis confirms that a calcium deficient hydroxyapatite as the major phase (CDHA) is obtained in CaP and CaP/AD nanocomposite. Release study of AD in slightly acidic environment (pH 5.0) was performed to understand the AD release in endolysosomal microenvironments inside the cell. Around 81% AD release is observed in 48 h in pH 5.0 from the CaP/AD nanocomposite system and no further release is observed beyond this. AD release from CaP/AD system is decreased to ~19% with an increase in pH of the release medium from 5.0 to 7.4. This difference in release rate can directly be attributed to the difference in solubility of CaP at different pH. Higher dissolution of CaP/AD is caused by high release of AD at low pH. This indicates the good storage capacity of CaP/AD in physiological pH of 7.4 and pH dependence of the release.

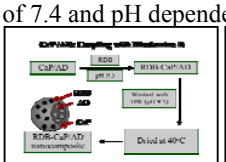

Figure 1. Synthesis of CaP/AD-RDB

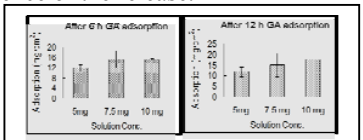

Figure 2. Adsorption of GA on HA and β-TCP (1:1) composite after 6h and 12 h.

The coupling process of RDB with CaP/AD is based on the electrostatic interaction between the positively charged amide group of RDB and the negatively charged CaP/AD surface. The fluorescence property of RDB-CaP/AD is observed at an excitation wavelength of 530 nm. There was not enough increase in adsorption of GA after 12h as compared to 6h (**Fig. 2**). A maximum of 15 mg/cm^2 GA is adsorbed after 6 h from 7.5 and 10 mg/ml GA solution. FTIR spectra shows the evidence of GA adsorption on HA/TCP composite (**Fig. 3**). Slow release of GA from HA/TCP composite (**Fig. 4**) may be due to strong chemical interaction between GA and Ca^{2+} cation of TCP. GA is a metal chelating agent which makes it capable of strong chemical interactions with metal ions [2].

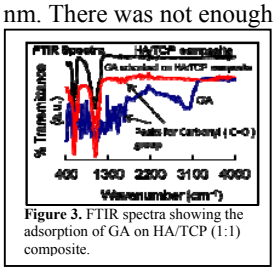

Figure 3. FTIR spectra showing the adsorption of GA on HA/TCP (1:1) composite.

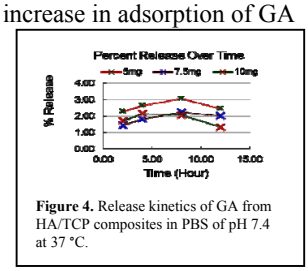

Figure 4. Release kinetics of GA from HA/TCP composites in PBS of pH 7.4 at 37 °C.

Conclusions: Rhodamine B (RDB) is introduced on the CaP/AD nanocomposite surface for sensing of drug release. Our results suggest that this pH tunable CaP nanocarrier system can potentially be used for both controlled drug delivery and sensing. GA adsorbed HA/TCP based CaPs could also potentially be used for local application to enhance osteogenesis or control bone resorption. The authors would like to acknowledge financial support from the National Institutes of Health.

References: 1. Henneman JZ. J. Biomed. Mater. Res. 2008, 85A, 993-1000; **2**. Seidlitz EP. Can. J. Physiol. Pharmacol. 2010;88: 929–936.