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Short communication

Controlled release of alendronate from nitrogen-doped mesoporous carbon

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ABSTRACT

We have synthesized a nitrogen doped mesoporous carbon with the BET surface area of 1066 m^2/g, total pore volume 0.6 cm^3/g and nitrogen content of 0.5%. Total alendronate adsorption in this carbon was ~5%. The release experiments were designed in four different media with sequential pH values of 1.2, 4.5, 6.8 and 7.4 for 3, 1, 3 and 5 h, respectively and at 37 °C to imitate the physiological conditions of stomach, duodenum, small intestine and colon, respectively. Release of the drug demonstrated a controlled fashion; only 20% of the drug was released in the media with pH = 1.2, whereas 64% of the drug was released in pH = 7.4. This is in contrary to pure alendronate that was completely dissolved within 30 min in the first release media (pH = 1.2) only. The relatively larger uptake of alendronate in this carbon and its sustained fashion of release can be attributed to the hydrogen bonding between the drug and the nitrogen functionalities on carbon surface. Based on this result, it can be inferred that this formulation may lower the side effects of oral delivery of alendronate.

1. Introduction

Bisphosphonates are one type of synthetically produced drugs that are structurally similar to that of naturally occurring pyrophosphates. Alendronate and Zoledronate are two typical bisphosphonates, in which alendronate has the most common application in bone related problems. Alendronate is an aminobisphosphonate (Fig. 1) and it can suppress the activity of osteoclast cells thereby lowering the osteoclast-mediated bone resorption [1–5]. In addition to that, *in-vivo* and *in-vitro* studies proposed that bisphosphonates, like alendronate could possess osteo-stimulative properties [6,7] and mineralize nodules to help early osteoblastogenesis [8,9]. Recent studies demonstrated a possible relation between systematics usages of alendronate and avascular osteonecrosis of the jaw [10,11]. Alendronate has also shown a positive role in the treatment of intra-bony defects in patients with chronic periodontitis [12] or bone metastasis [13,14]. It is a well-prescribed medicine for osteoporosis, like post-menopausal osteoporosis, that causes bone weakening and easy fracturing of bones. Despite helpful effects in bone-related abnormalities, there are some severe side effects of this drug, especially for oral delivery. It causes severe burning in the esophagus followed by other gastrointestinal problems [15]. It has been suggested that the patient should follow a strict regulation of staying upright for at least 30 min after taking this medication to minimize such effect. Besides that, it has very low bio-availability (~1%) owing to poor absorption in the intestine [16,17] and rapid clearance from blood [18]. In order to enhance its total uptake, a higher dose is usually prescribed that cumulatively worsens the side effects.

Several passive formulations for alendronate delivery have been developed, but majority of them were focused in bio-ceramics for direct or local delivery towards rapid bone healing. Calcium phosphate or hydroxyapatite-based local delivery system for controlled release of alendronate has been reported successfully. The drug was directly loaded onto the bulk ceramic [19–21], functionalized with gelatin [22] or formulated with calcium phosphate and loaded into porous titanium [23]. Besides phosphates, silica based materials were quite popularly employed towards local delivery of alendronate. Ordered mesoporous silica [24,25] and aminopropyl [26] or phosphorous [27] doped silica were successfully developed for alendronate release. Different biocompatible polymeric materials were also developed for direct release of alendronate. Poly (lactic-co-glycolic acid) (PLGA) loaded hydroxyapatite microspheres were developed for control release of alendronate for bone repair [28];

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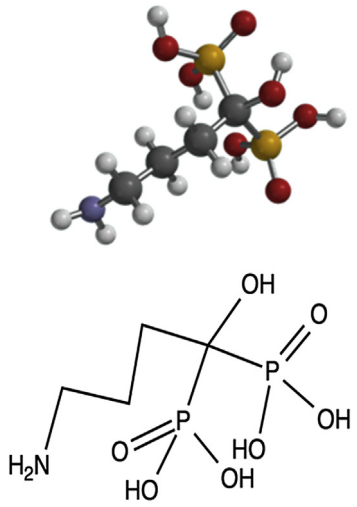

Fig. 1. Molecular structure of alendronate, stabilized ball and stick configuration by Spartan software [45], grey: carbon, white: hydrogen, blue: nitrogen, orange: phosphorous, red: oxygen (top), molecular formula (bottom). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pure PLGA/alendronate formulations were also employed for dental applications [29]. Polyethylene glycol (PEG)-dendrimer and H_2N-PEG-dendrimer-$(COOH)_4$ based polymeric systems were successfully reported as combined carriers of alendronate and paclitaxel [30]. Different types of bisphosphonate carriers are discussed in the review [31]. In order to enhance the oral bioavailability of alendronate, Han et al. [32] employed chitosan-coated liposomes with 200 nm size that enhanced the cellular uptake of the drug in Caco-2 cells. These drug carriers increased the oral bioavailability by 2.6 times compared to free drug in rat-based animal model study.

In today's perspective, carbon-based drug carriers have gained lot of interests in biomedical platforms. Traditionally, activated carbon material was employed as a remedy for drug overdose or poison arrester upon accidental indigestion. Different carbon materials that have already demonstrated their potential role in controlled drug delivery are carbon nanotube, carbon dots or mesoporous carbons. Especially, mesoporous carbons have demonstrated a successful role in both bulk and nano forms [33,34]. Different research groups, including ours reported the control release of multitude of drugs from mesoporous carbon-based materials, including captopril [35,36], furosemide [36], ranitidine hydrochloride [37], ibuprofen [37], antipyrine [38,39] indomethacin [39] and others. Our recent study confirmed the biocompatibility of mesoporous carbon materials in terms of cytotoxicity with HeLa cell, cell viability with fibroblast cells, hemolysis, and protein adsorption results [40]. Our recent study also confirmed the benign nature of mesoporous carbons compared to nano-carbons through the measurement of reactive oxygen generation, Adenosine Triphosphate (ATP) depletion and cellular uptake through TEM visualization [41]. Motivated by the potential role of mesoporous carbons in controlled drug delivery, we have employed nitrogen doped mesoporous carbon in the controlled release of alendronate towards oral delivery.

2. Experimental

2.1. Synthesis of nitrogen doped mesoporous carbon

Nitrogen doped mesoporous carbons were synthesized from the modified procedures reported by Wei et al. [42] Typically, 5 g resorcinol and 5 g Pluronic F127 were dissolved in 30 cm^3 solvent composed of 1:1 volumetric ratio of water and ethanol along with 0.6 cm^3 36% HCl. Upon mixing, 5 cm^3 37% formaldehyde solution was added as cross-linking agent and stirred for 3 h. After that, 5 g dicyandiamide was added to the mixture and stirred for 48 h. Then the mixture was separated from the solvent and cured at 200 °C for 2 h in air and then put to carbonization in a porcelain boat in a tube furnace. In the course of carbonization, it was heated to 400 °C in 1 °C/min followed by 1000 °C at 2 °C/min and then cooled down to ambient temperature. In order to further activate the carbonized material, it is mixed solid KOH in 1:3 ratio and heated in nitrogen atmosphere up to 1000 °C in 5 °C/min and then cooled down to ambient temperature. All the heating and cooling operations are performed in nitrogen atmosphere. The activated carbon material was washed with DI water several times and then filtered and dried before any characterization.

2.2. Materials characterization

The mesoporous carbon was characterized with pore size distribution by N_2 adsorption-desorption at liquid nitrogen temperature (77 K) and CO_2 adsorption at 273 K, electron microscopy by TEM and SEM-EDX and thermal stability by thermogravimetric analysis (TGA). The N_2 and CO_2 adsorption-desorption measurements were performed in Quantachrome's Autosorb–iQ instrument. To maintain 273 K for CO_2 adsorption, a Julabo® temperature controller was employed with 1:1 mixture of water and propylene glycol as chilling fluid. N_2 adsorption at 77 K was employed to calculate Brunauer-Emmett-Teller (BET) surface area, whereas both N_2 and CO_2 adsorption were used to calculate pore size distribution by non-local density function theory (NLDFT) in instrument's built-in software. Thermogravimetric Analysis (TGA) was performed in TA instruments' SDT Q600 instrument. TEM images were captured in Carl Zeiss Libra 120 TEM operating at 120 kV. The scanning electron microscopy (SEM) imaging was performed in a Carl Zeiss Merlin SEM equipped with EDX facility.

2.3. Alendronate loading and release

In order to load (adsorb) alendronate onto nitrogen doped mesoporous carbon, 0.1 g of pure alendronate is dissolved onto 10 cm^3 DI water adjusted with pH of 1.2 by HCl and then, 0.5 g of pure mesoporous carbon was added and stirred overnight. After that, the carbon is separated from the solution by filtration and quickly washed with DI water to remove alendronate that might have accumulated outside from the solution. The carbon was dried in oven at 100 °C overnight and used thereafter. The drug-loading amount was determined by comparing thermogravimetric analysis (TGA) data of pure drug, pure carbon and drug-loaded carbons. During TGA study, all the materials were heated upto 105 °C for 3 min to eliminate effect of pre-loaded moisture or HCl.

In order to investigate the controlled release of alendronate from mesoporous carbons, all the release experiments were conducted for 0.1 g of alendronate loaded carbon in 50 mL release media at 37 °C. The pH and exposure time of the release media were sequentially set to 1.2, 4.5, 6.8 and 7.4 for 3, 1, 3, and 5 h, respectively to mimic the physiological conditions of stomach, duodenum, small intestine and colon, respectively [39]. The medium with pH = 1.2 (simulated gastric fluid, SGF) was composed of HCl and 1% NaCl without any enzyme. Rest of the pH was set by HCl adjustment only. At the specific time intervals, 2.5 cm^3 of the release medium was withdrawn and replenished immediately with fresh release media. The collected sample was preserved for further analysis. After completing release medium of one pH, the carbon is separated, dried and inserted onto the