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Research paper

Montmorillonite as imaging and drug delivery agent for cancer therapy

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ABSTRACT

Montmorillonite (Mt) can be used as a simultaneous medical imaging and drug delivery agent for transcatheter arterial embolization. In this technique, the arterial vessels around tumors are blocked by drug loaded micro-sized Mt particles so oxygen and nutrition from the blood flow are blocked and drugs are released by the particles into the tumor site. Both devascularization (achieved by cutting off the oxygen and the nutrition) and drug release around tumor site can result in the progressive shrinkage of the tumor size. Mt is highly applicable and unique for transcatheter arterial embolization due to the size, adsorption capability, and biocompatibility of the particles. Mt has been considered previously as a targeted drug delivery agent to the gastrointestinal tract only for oral applications due to its slow and sustained drug releasing properties, but it has not yet been investigated as a targeted drug delivery agent for tumors at different or highly-specific areas. In this study, interactions of purified montmorillonite (PMt) with a cone beam computer tomography (CT) contrast material and an antitumor drug were investigated to prepare drug releasing arterial embolic clay mineral particles that enable medical imaging. *In-vitro* studies were carried out to show the biocompatibility of PMt The swelling and adsorption properties of PMt in the presence of the CT contrast material or antitumor drug were investigated to find a proper concentration for possible embolization. The results show that both the CT contrast material and antitumor drug could penetrate to the interlayer spaces and were adsorbed by the surfaces of the PMt particles. The size of the PMt increased due to both the adsorption and coagulation, which made the particles suitable for the arterial embolization procedure where a specific particle size is required to successfully obtain embolization. *In-vivo* tests of PMt as a CT contrast carrier and *in-vitro* drug activity on MCF-7 (human breast adenocarcinoma) cells were also satisfactory using the prepared embolic PMt particles.

1. Introduction

Clay and clay minerals have always played an important role in the field of health products as raw pharmaceutical materials. In ancient Egypt, China, and Rome, clay was used for therapeutic wound-healing purposes (Choy et al., 2007; Yang et al., 2016; Jayrajsinh et al., 2017). At present, clay and clay minerals are used as oral medication ingredients for antacids, gastrointestinal protectors, dermatological protectors, and antidiarrheatics. The drugs can easily be adsorbed by clay and clay minerals due to their high adsorption capacity, high specific surface area, ion exchange capacity, swelling property, and colloidal structure. Besides, clay and clay minerals show low toxicity for oral administration. The strong interactions between the clay and the drugs result in high adsorption rates and release of the drugs over an extended period of time. Due to the slow rate of drug release from clay and clay minerals and their modified forms, clay and clay minerals are considered as targeted drug delivery agents for oral applications to the stomach or colon; however, they have not yet been investigated as targeted drug delivery agents for tumors at different or specific areas (Bergaya et al., 2006; Aguzzi et al., 2007; Choy et al., 2007; Viseras et al., 2010; Yang et al., 2016; Jayrajsinh et al., 2017).

Transcatheter arterial embolization is a treatment procedure to shrink the tumor by depriving it of the oxygen-carrying blood and other substances that it needs to grow. Micro-size (at least 20 μm) particles are injected into an artery near the tumor or abnormal tissue using a catheter (thin, flexible tube) (Berenstein and Kricheff, 1981; Wright et al., 1982). These particles block the flow of the blood supply to the tumor tissue and the ischemia at the tumor site induces tumor size reduction or necrosis. If the embolization particles are loaded with chemotherapeutic drugs, drug delivery to tumor site can be achieved along with ischemia. Arterial embolization is currently performed clinically for the treatment of some cancer types such as liver, kidney, and neuroendocrine tumors.

Mt particles are highly suitable for developing drug-carrier

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embolization agents due to their high adsorption capacity, high specific surface area, swelling, and ability to achieve suitable particle sizes for embolization. The interactions between Mt and the drug as well as contrast material for imaging can lead to an increase in Mt particle sizes. The drug and contrast material can expand the interlayer space of Mt while changing the state of the surface charges of Mt. Both expansion and coagulation (due to the reduced surface charges) can increase the size of the Mt particles to larger particle sizes which is necessary for performing arterial embolization successfully. Once the arterial embolization has occurred, the drug may be released for an extended period of time unlike other embolic particles that are used such as iron and iron oxide particles (Moroz et al., 2002a, 2002b).

The aim of this study was to investigate the interactions of Mt with a CT contrast material and an antitumor drug to prepare drug releasing arterial embolic clay mineral particles that can also enable medical imaging. This study demonstrates purification of Mt with a purification process (Bergaya et al., 2006) including decomposition of carbonates, dissolution of hydroxides, oxidation of organic materials, dialysis, and sedimentation procedures. *In vitro* cytotoxicity tests were carried out to assess the biocompatibility of the purified montmorillonite (PMt) on normal cell lines. The PMt was dispersed in a CT contrast material in order to investigate the interaction of PMt and the contrast material used as an imaging agent and whether this interaction would result in appropriate particle sizes which is in between 20 and 200 μm (Berenstein and Kricheff, 1981; Wright et al., 1982) for embolization. Additionally, *in vivo* experiments were carried out to determine if CT contrast material adsorbed PMt would result in successful arterial embolization. The PMt was also subjected to an antitumor drug doxorubicin (DOX) adsorption and characterized with x-ray diffraction (XRD), zeta potential, particle size measurements, and Fourier Transform infrared (FTIR) spectroscopy analysis. *In vitro* drug release studies of DOX loaded PMt particles were carried out using phosphate buffered saline (PBS) media at pH 7.4 and pH 6.8 to simulate pH profiles of normal tissue and pathological tissues such as cancerous tissues (Ganta et al., 2008). Finally, besides determining the drug-releasing behavior of PMt, *in vitro* cytotoxicity tests were carried out on cancer cell lines to determine the toxicity of drug loaded PMt particles.

2. Experimental section

2.1. Materials

The raw Mt sample was collected from Edirne, Enez, Turkey, and later Mt was activated with 4 wt% Na_2CO_3 to obtain NaMt by the supplier Bensan Co. The NaMt was subjected to a purification process (Bergaya et al., 2006) including decomposition of carbonates, dissolution of hydroxides, oxidation of organic materials, dialysis, and sedimentation procedures.

The CT contrast material (U) contained 0.1 g sodium amidotrizoate and 0.66 g meglumine amidotrizoate in 1 mL and sodium calcium edetate as supporting material.

Physiological saline (PS) polifarma, 0.9% isotonic contained 0.90% w/v of NaCl in water.

Doxorubicin hydrochloride (DOX; (8 s-cis)-10-[(3-amino-2,3,6-trideoxy-alpha-l-lyxo hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxynaphthacene-5,12-dione hydrochloride) was purchased from EDQM (D2975000, Strasbourg, France).

2.2. Preparation of imaging agents

PMt (2% w/w) was dispersed in U by diluting with PS at four different concentrations: 25% w/w U + 75% w/w PS, 50% w/w U + 50% w/w PS, 75% w/w U + 25% w/w PS, and 100% w/w U named as PMt + 0.25 U, PMt + 0.5 U, PMt + 0.75 U, and PMt + 1 U respectively. The dispersions were ultrasonicated for 10 min and shaken overnight.

2.3. Preparation of drug delivery agents and drug release studies

PMt was loaded with different concentrations of a cancer drug, DOX, by adsorption method (Wise, 2000). Various concentrations of DOX (0.2, 0.4, 0.6, and 0.8 mg/mL) were dissolved in potassium buffered saline, PBS (Sigma-Aldrich, St Louis, MO) at pH 5 and were continuously shaken. Then PMt (2% w/w) was introduced into the DOX solution, and the dispersions were sonicated for 10 min and shaken with a rotator overnight inside light-protected tubes at room temperature. DOX was loaded to the particles at different concentrations of 0.2, 0.4, 0.6, and 0.8 mg/mL, and loaded particles were named PMt + 200DOX, PMt + 400DOX, PMt + 600DOX, and PMt + 800DOX respectively. After loading DOX to PMt, dispersions were centrifuged at 4500 rpm for 10 min and the supernatants were collected in order to determine loading efficiency. Unloaded DOX amount in the supernatant was determined by the absorption at 480 nm using a UV-spectrophotometer (BIO-RAD Benchmark Plus, Hercules, CA). Drug loading efficiency (LE %) of the particles was determined using the Eq. (1) below (Unsoy et al., 2014):

$$LE\% = \frac{(\text{total } \mu g \text{ of DOX} - \mu g \text{ of DOX in supernatant})}{(\text{total } \mu g \text{ of DOX})} \quad (1)$$

In vitro drug release studies of DOX loaded PMt particles were carried out using PBS at pH 7.4 and pH 6.8. DOX loaded PMts were washed with PBS two times before the release studies. 10 mg of PMt + 800DOX was introduced to 20 mL of PBS and then was shaken in a rotary shaker at 100 rpm in a 37 °C room for 20 days. At fixed time intervals, sample aliquots of 1 mL were withdrawn and 1 mL of fresh PBS was introduced into the release media. Dispersions were centrifuged to separate particles and drug concentrations were obtained from supernatants using an UV–Vis spectrophotometer (at 480 nm). Cumulative drug release percentage was calculated from the calibration curve equation of DOX. Each experiment was replicated at least 3 times.

2.4. Mammalian cell culture

All cell lines used in this study were cultured using the same mammalian tissue culture protocol. Cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) (HyClone), 1% penicillin/streptomycin (Sigma-Aldrich, St Louis, MO) and 1% L-Glutamine solution (Sigma- Aldrich, St Louis, MO). All incubations were performed in a humidified incubator containing 5% CO_2 at 37 °C (BINDER C150 E2, Tuttlingen, Germany). For experimental procedures, all cells were detached by trypsin 0.25% /EDTA 0.02% (PAN-Biotech P10–019100, Aidenbach, Germany) and resuspended in DMEM. Medium renewals of all cell cultures were performed 2 to 3 times per week.

2.5. Cytotoxicity assays

Cytotoxicity of PMt and DOX loaded PMTs was determined using human osteoblast (hFOB) and human breast adenocarcinoma (MCF-7) cell lines. Both cell lines were purchased from American Type Culture Collection (Manassas, VA). Particles were incubated with each cell line at different concentrations in order to assess their effect on cell viability. Cytotoxicity to the cells was determined using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan), which contains tetrazolium salt (WST-8, [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt]). Produced formazan dye by WST-8 was quantified from absorbance at 450 nm using an ELISA multiwell spectrophotometer (BIO-RAD Benchmark Plus, Hercules, CA). The relative cell viability (%) was calculated by Eq. (2) where OD is optical density: