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In Vitro–In Vivo Evaluation of an Oral Ghost Drug Delivery Device for the Delivery of Salmon Calcitonin

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ABSTRACT

An orally administered site-specific Oral Ghost Drug Delivery (OGDD) device was developed and evaluated for the administration of salmon calcitonin. *In vitro* drug release studies have been undertaken using biorelevant media and aspirated gastrointestinal fluid from a large white pig in addition to characterization of a formulated trimethyl chitosan blend formulated and prepared into a loaded mini-pellet system. *In vivo* drug release analysis in a large white pig model has further been undertaken on the OGDD device and a commercial intramuscular injection to ascertain the release properties of the OGDD device in an animal model in comparison with the currently used treatment option for the administration of salmon calcitonin. Results of this study have detailed the success of the prepared system during both *in vitro* and *in vivo* analyses with the OGDD providing a greater control of release of salmon calcitonin when compared to the commercial product.

Introduction

Proteins and peptides are examples of macromolecule compounds that contain unique combinations of amino acid monomers that function in the human body in a variety of physiological processes. These peptides have been effectively utilized for the treatment of chronic conditions such as type I diabetes and osteoporosis.[1-4] The chronic administration of peptide therapeutic agents, such as calcitonin and insulin, is however usually delivered as subcutaneous or intramuscular injections at a high dosing frequency. Some of these macromolecules often require daily dosing which is painful and inconvenient for the patient. Oral delivery systems comprising peptide molecules have been seen as a potential option for the peptide administration due to its noninvasive nature, cheaper production costs, and increased patient compliance.[5] The major concern for the oral delivery of peptide molecules is the harsh gastric environment that denatures protein molecules rendering them ineffective for absorption and exerting a therapeutic effect.[6]

An Oral Ghost Drug Delivery (OGDD) device has been designed and developed for advanced site-specific oral peptide delivery to enhance patient compliance and efficacy and to reduce toxicity. The device has been proposed as a significant step in administering oral peptides as an alternative to parenteral administration that may benefit the therapeutic outcome of chronic conditions such as osteoporosis or acute conditions such as the administration of vaccines. The OGDD device has a high customizable potential that could be augmented to achieve the successful oral administration of many biopharmaceuticals within targeted locations in the gastrointestinal tract which will become increasingly important as greater quantities and variants of biopharmaceuticals become clinically certified.

The OGDD device comprises a nonresponsive, nondegradable shell component produced from a U.S. Food and Drug Administration

Abbreviations used: OGDD, Oral Ghost Drug Delivery; UHMW-PE, ultra–high-molecular-weight polyethylene; AAm, polyethylene: acrylamide monomers; MAA, methyacrylic acid; BIS, N-N'-Methylenebisacrylamide; TEMED, tetramethylethylenediamine; TMC:Cl, trimethyl chitosan chloride; EDTA, ethylenediaminetetraacetic acid; ATR-FTIR, attenuated total reflectance-Fourier transform; SEM, scanning electron microscope; FaSSGF, fasted state simulated gastric fluid; FaSSIFV2, fasted state simulated intestinal fluid version 2; FaSSGFc, fasted state simulated gastric fluid canine; AUC, area under the curve; AUMC, area under the first moment curve; K_{10}, elimination rate constant; K_{01}, rate constant of absorption; C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; MDT, mean dissolution time; MRT, mean residence time; VSS, volume of distribution at steady state.

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approved ultra−high-molecular-weight polyethylene plastic, Polystone® M. This rigid plastic displays a high mechanical resistance, low water retention abilities, resistance to corrosive chemicals, and a high abrasion resistance and is utilized within surgical implants during knee and hip operations.[7] The nondegradable shell has been used for structural support of the OGDD device due to its robust nature and resistance to corrosive gastric media and is excreted via the gastrointestinal tract.[7] The rate of excretion of the nondegradable shell is dependent on the gastrointestinal transit time of the dosed subject (50 h for a pig).[8] The gastrointestinal transit time for rigid nonerodible oral dosage forms, however, may be increased up to 33 days dependent on the density and size of the system.[8]

A dynamically equilibrating stimuli responsive hydrogel which has been developed previously was placed within the base of the shell component which displaces a peptide-loaded trimethyl chitosan chloride mini-pellet (TMC:Cl MiPe) and a ethylenediaminetetraacetic acid (EDTA) *in situ* hot melt dispersion mini-pellet, also developed previously, from the OGDD device.[9,10] A Eudragit® cap was added at the end of the OGDD to facilitate both the release of the mini-pellets at the target location and the generation of a positive pressure within the OGDD device. A positive pressure was used to allow for release of the mini-pellets within the small intestine. Salmon calcitonin has been utilized as the model drug for systemic peptide administration; due to its poor intestinal permeability and sensitivity to pancreatic serine proteases, salmon calcitonin is useful in evaluating delivery technologies.[11,12]

In vitro drug release analysis in biorelevant media has been undertaken to ascertain the release properties of the OGDD device. Additionally, a porcine model was employed to evaluate the OGDD device *in vivo* due to the ability of the porcine model to achieve a body mass similar to an adult human (≥50 kg) and displays tolerance to frequent blood sampling.[13] The porcine model has been implemented for a multitude of multisampling studies that investigated drug transport and metabolism due to the close similarities of these processes with respect to humans.[14-17] In addition, the gastrointestinal tract of the pig model is very similar to that of the human with respect to the gastrointestinal pH profile, dietary similarities, and transition times within the gastrointestinal tract.[18] A further similarity can be found in the coronary artery distribution of the porcine model, which is very similar to that of the human model.[19] This study therefore provides for the *in vitro* and *in vivo* evaluation of the OGDD device in a large white pig model.

Materials and Methods

Materials

Chitosan (medium molecular weight; degree of deacetylation of 77.0%) and Methacrylic acid (99% pure, 86.09 g/mol) was purchased from Aldrich (Schnelldorf, Germany). Salmon calcitonin (good manufacturing practice grade) and the salmon calcitonin immunoassay kit (S-1166) were purchased from Bachem (Bachem AG, Bubendorf, Switzerland). Acrylamide (≥98 pure, MW 71.08 g/mol) was purchased from Fluka (Sigma-Aldrich, Buchs, Switzerland). Methoxypolyethylene glycol 2000 (≥99%), sodium iodide (99.999%), methyl iodide (≥99.0%), sodium deoxycholate (≥98.0%), sodium taurocholate (≥90.0%), sodium taurodeoxycholate (≥97.0%), tetramethylethylenediamine (99%, 116.20 g/mol), ammonium persulfate (≥98.0%, 228.20 g/mol), Pluronic F-127, N,N'-methylenebis(acrylamide) (99%, 154.17 g/mol), maleic acid (99%), and EDTA (≥99.995%) were purchased from Sigma-Aldrich (St. Louis, MO). Ac-Di-Sol® and Avicel® RC/CL type RC-591 (co-dried blend of microcrystalline cellulose and sodium carboxymethylcellulose) was purchased from FMC BioPolymer (Philadelphia, PA). Egg phosphatidylcholine (97.5%) was purchased from Lipoid GmbH (Ludwigshafen, Germany). All other chemicals were of analytical grade and used as received.

Preparation of the OGDD Device

Manufacture of Nonresponsive Nondegradable Shell Component

The nonresponsive nondegradable shell component was manufactured utilizing Polystone® M. The plastic was composed of ultra−high-molecular-weight polyethylene (UHMW-PE). Rods of UHMW-PE were lathed to an accurate diameter of 6 mm. The lathed rods of UHMW-PE were cut into 10-mm segments. A 750 CNC Bed milling machine (Ajax Machine Tools, Hampshire, UK) was utilized to hollow out the rod segments to a depth of 9 mm and a diameter of 5.5 mm. A rod segment was then placed in a table top vice grip and 8 1-mm diameter orifices were drilled along the base (part of the rod segment which was not hollowed out), and an additional 4 1-mm orifices were drilled into the base of the UHMW-PE segment. This formed the nonresponsive nondegradable shell component.

Synthesis of the Stimuli Responsive Hydrogel as the Trigger Mechanism of the OGDD Device

As detailed by Hibbins and co-workers,[10] to an aqueous solution containing 50% w/v of acrylamide and methyacrylic acid, respectively, N-N'-Methylenebisacrylamide (2.5% w/v), deionized water (30 mL), and Pluronic F-127 (10% w/v) were sequentially added. The formulation was allowed to react under constant magnetic stirring (3500 rpm); thereafter, ammonium persulfate (10% w/v) and tetramethylethylenediamine (20% w/v) were added at room temperature. Sodium bicarbonate (100 mg) was subsequently added to induce the formation of a porous gel. Molds were produced that would allow the *in situ* curing process to take place within a cylindrical shape (inner diameter of 4.77 mm). The molds were then placed in a glass beaker and incubated in a water bath at 37 ± 0.5°C for 12 h. Once the curing process was completed, the *in situ* cured rods were then removed from the cylindrical molds and cut transversely into 0.5 mm pieces and then longitudinally into 0.3 mm pieces.

Manufacture of the Erodible Methylcellulose Disc

A methylcellulose disc was placed between the stimuli responsive hydrogel and the drug carriers to restrict contact with water within the drug delivery system. Additionally, the ability of the stimuli responsive hydrogel to act as the trigger mechanism within the drug delivery system was enhanced by the presence of the methyl cellulose disc. For the preparation of the methylcellulose disc, methylcellulose was accurately weighed out (20 mg) and placed within a punch and die set to produce mini-pellets with a diameter of 4 mm and a width of 1 mm.

Manufacture of the Avicel®-EDTA-mPEG In Situ *Hot Melt Dispersion Mini-Pellet*

The Avicel®-EDTA-mPEG *in situ* hot melt dispersion mini-pellet was manufactured as detailed in Hibbins et al.[9] Briefly, melt dispersions of EDTA-mPEG were synthesized at the respective ratio of 33.5% w/w mPEG, 33.5% w/w EDTA, and 33% w/w Avicel® RC/CL type R-591. The mPEG was first melted to 60°C on a watch glass heated by a calibrated hot plate magnetic stirrer. Once the solid mass of mPEG was melted to a liquid phase, EDTA was added to the molten mPEG. The EDTA was homogenously distributed within the molten mPEG with a wooden spatula for 1 min. Once a uniform distribution was obtained, the EDTA-mPEG hot melt dispersion was removed from the hot plate magnetic stirrer and allowed to cool under constant stirring. Once the EDTA-mPEG hot melt dispersion had become a cool solid, it was passed through a metal sieve with a