



Full length article

Thermoresponsive nanospheres with independent dual drug release profiles for the treatment of osteoarthritis



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ABSTRACT

Dual drug delivery of drugs with different therapeutic effects in a single system is an effective way to treat a disease. One of the main challenges in dual drug delivery is to control the release behavior of each drug independently. In this study, we devised thermo-responsive polymeric nanospheres that can provide simultaneous and independent dual drug delivery in the response to temperature change. The nanospheres based on chitosan oligosaccharide conjugated pluronic F127 grafting carboxyl group were synthesized to deliver kartogenin (KGN) and diclofenac (DCF) in a single system. To achieve the dual drug release, KGN was covalently cross-linked to the outer part of the nanosphere, and DCF was loaded into the inner core of the nanosphere. The nanospheres demonstrated immediate release of DCF and sustained release of KGN, which were independently controlled by temperature change. The nanospheres treated with cold temperature effectively suppressed lipopolysaccharide-induced inflammation in chondrocytes and macrophage-like cells. The nanospheres also induced chondrogenic differentiation of mesenchymal stem cells, which was further enhanced by cold shock treatment. Bioluminescence of the fluorescence-labeled nanospheres was significantly increased after cold treatment *in vivo*. The nanospheres suppressed the progression of osteoarthritis in treated rats, which was further enhanced by cold treatment. The nanospheres also reduced cyclooxygenase-2 expression in the serum and synovial membrane of treated rats, which were further decreased with cold treatment. These results suggest that the thermo-responsive nanospheres provide dual-function therapeutics possessing anti-inflammatory and chondroprotective effects which can be enhanced by cold treatment.

Statement of Significance

We developed thermo-responsive nanospheres that can provide a useful dual-function of suppressing the inflammation and promoting chondrogenesis in the treatment of osteoarthritis. For a dual delivery system to be effective, the release behavior of each drug should be independently controlled to optimize their desired therapeutic effects. We employed rapid release of diclofenac for acute anti-inflammatory effects, and sustained release of kartogenin, a newly found molecule, for chondrogenic effects in this polymeric nanospheres. This nanosphere demonstrated immediate release of diclofenac and sustained release of kartogenin, which were independently controlled by temperature change. The effectiveness of this system to subside inflammation and regenerate cartilage in osteoarthritis was successful demonstrated through *in vitro* and *in vivo* experiments in this study. We think that this study will add a new concept to current body of knowledge in the field of drug delivery and treatment of osteoarthritis.

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Abbreviations: EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; DMAP, 4-dimethylaminopyridine; ACLT, anterior cruciate ligament transection; BSA, bovine serum albumin; COS, chitosan oligosaccharide; COL2A1, collagen type II; CMC, critical micelle concentration; COX-2, cyclooxygenase-2; DI, deionized; DMM, destabilization of the medial meniscus; D₂O, deuterated water; F127-COOH, dicarboxylate pluronic F127; DCF, diclofenac; dDMSO, dimethyl sulfoxide; DMEM/F-12, Dulbecco's modified Eagle's medium/F-12; DLS, dynamic light scattering spectrophotometer; ELISA, enzyme-linked immunosorbent assay; FE-SEM, field-emission scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAG, glycosaminoglycan; HPLC, high-performance liquid chromatography; hBMSCs, human bone marrow stromal cells; ITS, insulin/transferrin/selenium; IL-6, interleukin-6; IA, intra-articular; KGN, kartogenin; LPS, lipopolysaccharide; MSCs, mesenchymal stem cells; MWs, molecular weights; Sulfo-NHS, N-hydroxysulfosuccinimide; OA, osteoarthritis; OARS, Osteoarthritis Research Society International; PBS, phosphate buffered saline; PEG, poly(ethylene glycol); PEI, polyethylenimine; PEOx-PPOy-PEOz, poly(oxyethylene)-block-poly(oxypropylene)-block-poly(oxyethylene); ¹H NMR, proton nuclear magnetic resonance; RT-qPCR, real-time quantitative polymerase chain reaction; ROI, region of interest; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; TEM, transmission electron microscopy.

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1. Introduction

In a drug delivery system which contains two different drugs, the respective optimal dose and release characteristics of each drug should be retained to achieve the desired synergistic effect. Thus, a system that can control the release behavior of each drug independently is required to optimize the therapeutic effects of both drugs. Several approaches have been taken to co-encapsulate multiple therapeutic agents into a single carrier, such as physical loading into the particle core [1–4], incorporation of an additional media compartment to the particle surface [5–8], covalent conjugation of multiple drugs to the polymer backbone [9–11], and combinations of direct encapsulation into the polymeric core and covalent linkage between the polymer and the other drug [12]. However, selective loading of multiple functional drugs with independent release profiles is still a challenging issue.

Thermo-responsive pluronics are a family of ABA-type triblock copolymers with the composition of poly(oxyethylene)-block-poly(oxypropylene)-block-poly(oxyethylene) (PEOx-PPOy-PEOz). Due to their amphiphilic nature, pluronics form a micellar structure in an oil-in-water emulsion. When pluronics are conjugated with other polymers, such as poly(ethylene glycol) (PEG) [13], polyethylenimine (PEI) [14,15] or chitosan [16], thermo-responsive nanocapsules can be produced. Hydrophobic drugs

are solubilized by being located in the hydrophobic core of the micelle. In general, pluronics with longer PPO blocks and higher molecular weights (MWs) more strongly solubilize hydrophobic drugs [17].

Chitosan, derived from chitin, is one of the natural polymers that have been frequently used in the development of drug delivery systems. It is distinguished from other biopolymers by its amine group that provides useful binding properties to anionic surfaces of cellular membranes [18], and anionic DNA or RNA [19]. Moreover, the amine groups allow for chemical modification with many other molecules [20,21]. This characteristic is particularly important because amide coupling of amines and carboxylic acid groups is a very useful conjugation method.

The concept of polymer-drug conjugates for the delivery of hydrophobic small molecular drugs was first proposed by Ringsdorf in 1975 [22]. Drug conjugation to a hydrophilic polymer offers several significant advantages, such as enhancement of the aqueous solubility of a drug, the potential for a drug to be delivered in a controlled manner, and an opportunity to alter drug pharmacokinetics and biodistribution [23]. Kartogenin (KGN), a hydrophobic small molecule drug (MW = 317.34 Da), is a recently characterized compound that promotes chondrogenic differentiation of mesenchymal stem cells (MSCs) and induces regeneration of the cartilage in osteoarthritis (OA) [24]. Because of its carboxyl

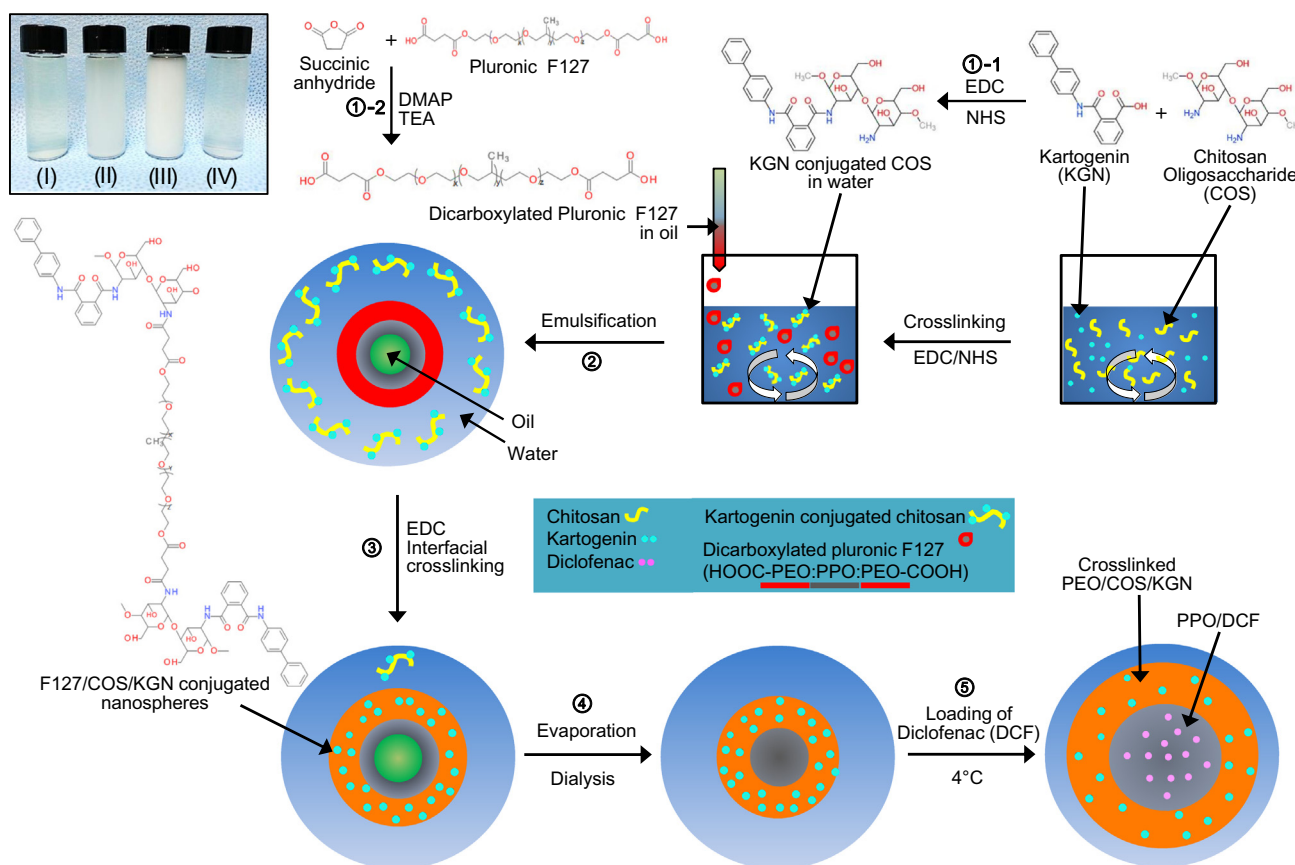


Fig. 1. Illustration of the procedure and chemistry to synthesize the F127/COS/KGN_{DCF} nanospheres. The carboxyl group of KGN and the amine group of COS were covalently conjugated by EDC/NHS catalysis before nanosphere synthesis. Each hydroxyl end of pluronic F127 was grafted with a carboxyl group derived from succinic anhydride. Then, the remaining amine groups in the KGN-conjugated COS were covalently cross-linked with carboxyl groups of dicarboxylated pluronic F127 using an EDC catalyst during oil-in-water emulsification. DCF was loaded into the cross-linked F127/COS/KGN nanospheres by increased wall permeability due to the expansion after cold shock treatment. The pictures on upper left show the typical appearance of the KGN-conjugated COS (I), the oil-in-water emulsion of dicarboxylated pluronic F127 and KGN-conjugated COS before (II) and after (III) sonication, and after rotary evaporation to remove the organic phase (IV) [EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, N-hydroxysuccinimide; DMAP, 4-dimethylaminopyridine; TEA, triethylamine; COS, chitosan oligosaccharide; KGN, kartogenin; DCF, diclofenac; PEO, polyethylene oxide; PPO, polypropylene oxide].

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