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Magnetic hyaluronic acid nanospheres via aqueous Diels–Alder chemistry to deliver dexamethasone for adipose tissue engineering

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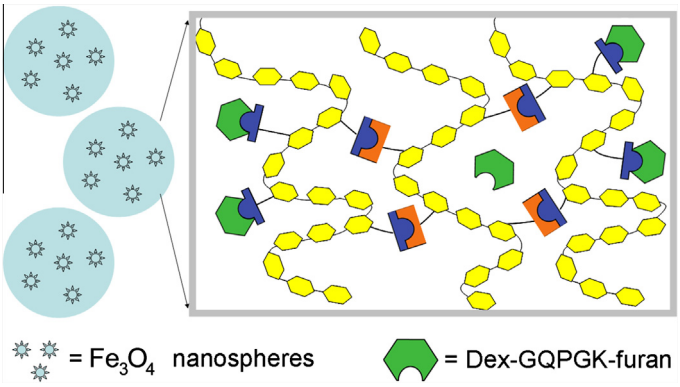
GRAPHICAL ABSTRACT



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ABSTRACT

Biopolymer-based nanospheres have great potential in the field of drug delivery and tissue regenerative medicine. In this work, we present a flexible way to conjugate a magnetic hyaluronic acid (HA) nanosphere system that are capable of vectoring delivery of adipogenic factor, e.g. dexamethasone, for adipose tissue engineering. Conjugation of nanospheres was established by aqueous Diels–Alder chemistry between furan and maleimide of HA derivatives. Simultaneously, a furan functionalized dexamethasone peptide, GQPGK, was synthesized and covalently immobilized into the nanospheres. The magnetic HA nanospheres were fabricated by encapsulating super-paramagnetic iron oxide nanoparticles, which exhibited quick magnetic sensitivity. The aqueous Diels–Alder chemistry made nanospheres high binding efficiency of dexamethasone, and the vectoring delivery of dexamethasone could be easily controlled by a external magnetic field. The potential application of the magnetic HA nanospheres on vectoring delivery of adipogenic factor was confirmed by co-culture of human adipose-derived stem cells (ASCs). *In vitro* cytotoxicity tests demonstrated that incorporation of dexamethasone into magnetic HA nanospheres showed high efficiency to promote ASCs viabilities, in particular under a magnetic field, which suggested a promising future for adipose regeneration applications.

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1. Introduction

Biodegradable and biocompatible materials have served as highly functional cell scaffolds for drug delivery which increases the effectiveness in regulation of tissue regeneration [1–5]. The convergence of biomaterials and nanotechnology has enabled the development of nano-biomaterials including nanospheres and nanogels as intelligent drug delivery systems [6–8]. In tissue engineering, biopolymer-based nanospheres have been fabricated as microscale building blocks to create tissue constructs using bottom-up tissue engineering approaches [9–12].

For practical soft tissue applications, dexamethasone (Dex) is one of key adipogenic factors to induce adipogenesis for adipose regeneration [13–15]. The establishment of vectoring delivery systems is necessary for ensuring Dex target the defect sites in the use of nanospheres. Although some microspheres and nanospheres have been served as delivery systems, conventional delivery methods are limited by the intrinsic inability to translate Dex into soft tissue engineering due to the un-controlled release [16–18]. Dex that is covalently immobilized into carriers can be more efficiently transported to a localized site and be released in a sustained-dosage form.

We hypothesize that magnetic biopolymer-based nanospheres have potential to broad pharmaceutical applications under controlled magnetic fields, such as vectoring delivery of Dex to target defect sites of adipose tissue. Super-paramagnetic iron oxide nanoparticles have been widely used in biomedical applications, such as clinical imaging, cell patterning, blood purification and vectoring delivery agents in the treatment of tumors by hyperthermia techniques [19–24]. Due to their innate magnetic characteristics and biocompatibility, iron oxide nanoparticles have been encapsulated in biomaterials to fabricate novel cell scaffolds [25,26].

Technically, microspheres and nanoparticles should be crosslinked by physical interactions or chemical agents such as glutaraldehyde, carbodiimide (EDAC) and genipin. These chemical agents are the major obstacles in the use in cell scaffolds due to their toxicity to cells and tissues, thus limiting their clinical applications on tissue engineering [27,28]. Bioconjugation has the ability to overcome these obstacles through biocompatible crosslinking, increasing bioactive efficacy and decreasing adverse effects [29,30]. In the past few years, the use of aqueous Diels–Alder chemistry has been known in conjugation strategy in preparation of biomaterials [31,32]. For example, conjugation of biopolymers through aqueous Diels–Alder chemistry has been employed for preparation of biodegradable hydrogels for cell culture and drug delivery [33–35]. These successful results clearly illustrate that aqueous Diels–Alder chemistry promise as conjugation strategy for cell scaffolding materials.

In this study, we put forward this method to broaden biopolymer-based nanosphere system. We described a combined approach of emulsification–precipitation polymerization to prepare magnetic hyaluronic acid (HA) nanospheres via aqueous Diels–Alder chemistry. HA is a linear high-molecular-weight polysaccharide, which is the main glycosaminoglycan and the backbone of proteoglycans in extracellular matrix (ECM). Due to its excellent properties, HA has been extensively applied in cell scaffolds for tissue regeneration [36–38]. This study describes the synthesis, properties and cytotoxicity of HA nanospheres with super-paramagnetic characteristics, as a Dex delivery system for soft tissue engineering.

2. Materials and methods

2.1. Materials

Hyaluronic acid sodium (MW $\sim 1.6 \times 10^6$), dexamethasone (Dex), furfurylamine, hyaluronidase, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) were purchased from Sigma–Aldrich. 4-(4-*N*-Maleimidophenyl) butyric acid hydrazide hydrochloride (MPBH) and *N*-hydroxysulfosuccinimide (Sulfo-NHS) were obtained from Thermo Fisher Scientific Inc. (Rockford, IL, USA). All other chemicals were used as received without purification.

2.2. Synthesis of maleimide-functionalized HA

0.3 g HA sodium was dissolved in 100 mL nanopure H_2O and activated by EDAC/Sulfo-NHS. 0.3 g MPBH was dissolved in DMSO and then slowly added to the activated HA sodium solution under stirring. The maleimide-functionalized HA (HA-maleimide) derivative was collected as a white foam by freeze-drying after exhaustive dialysis against nanopure for 3 days (MWCO 10,000, Spectra/Por membrane, Rancho Dominguez, CA, USA). ^{1}H NMR spectra of product was measured (300 MHz, Bruker Avance) at ambient temperature using D_2O as a solvent and the percentage of maleimide substitution in the derivative was quantified as 44.2%.

2.3. Synthesis of furan-functionalized HA

0.4 g HA sodium was dissolved in 40 mL of MES buffer (100 mM, pH 5.5) to which 0.56 g DMTMM was added and stirred for 10 min. 95 μL Furfurylamine was then added dropwise. The reaction was conducted at room temperature for 24 h and then dialyzed against nanopure H_2O for 3 days (MWCO 10,000 Da). Water was removed by lyophilization to obtain HA-furan as a white foam. The degree of substitution was determined as 48.7% from ^{1}H NMR spectra by comparing the ratio of the areas under the proton peaks at 6.26, 6.46, and 7.65 ppm (furan protons) to the peak at 1.9 ppm (*N*-acetyl glucosamine protons).

2.4. Synthesis of furan-functionalized Dex peptide

To achieve Dex immobilization, a peptide with the sequence GQPGK-furan was synthesized using standard Fmoc-mediated solid phase peptide synthesis methods as described previously [39,40]. The 3-furoic functionality was directly incorporated during the solid phase synthesis using a synthetic Fmoc protected amino acid analogue. Dex-NHS ester was then introduced to the resin-bound peptide sequence and allowed to react with the *N*-terminal primary amine overnight. After synthesis and final modification, the Dex-GQPGK-furan peptide was cleaved from the solid support using a mixture of trifluoroacetic acid, triisopropyl silane and water (95/2.5/2.5 v/v), and precipitated in cold diethyl ether. The crude peptide was purified by means of high performance liquid chromatography (HPLC).

2.5. Conjugation of nanospheres

The preparation of Fe_3O_4 nanospheres was followed by a chemical co-precipitation of Fe^{2+} and Fe^{3+} ions described previously [22,23]. With some modifications, 28 mmol $FeCl_2$ and 16 mmol $FeCl_3$ were prepared in 50 mL nanopure H_2O in two beakers, and then transferred to a 250 mL three-necked flask together, stirred under nitrogen. When the solution was heated to 60 °C, $NH_3{\cdot}H_2O$ (25 wt%) was added drop wise until pH 10. After base was added, the solution immediately became dark brown, which indicates iron oxide has been formed in the system. The solution was heated at 80 °C for 1 h. The precipitates were isolated from the solvent by magnetic decantation and repeatedly washed with nanopure H_2O until neutral, then were dried at room temperature under vacuum for 24 h.