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Preparation and Characterization of DHAD/HRP Co-loaded Multivesicular Liposomes

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Abstract

The structure of carriers play an important role in the space distribution of different drugs, therefore this paper studies multivesicular liposomes (MVLs), which have a unique nano-in-micro structure as possible carriers in combination therapy. Horseradish peroxidase (HRP) and mitoxantrone hydrochloride (DHAD) were selected as model drugs for protein and small molecule drug respectively. The results show that the MVLs are spherical and that the internal space is divided up into numerous compartments. The particle size ranged from 15 to 20 μm and the system was stable according to the zeta potentials. In vitro release studies display that the DHAD/HRP co-loaded MVLs has better sustained release profiles than one drug alone, and the MVLs exhibited an orderly release behavior which suggested that MVLs might be used as drug carriers in combination therapy applications.

Keywords: Multivesicular Liposomes (MVLs); Combination Therapy; Protein; Small Molecule Drug

1 Introduction

At present, drug therapy is still one of the most important therapeutic regimens in human health and cure of diseases [1-3]. While in most cases, especially in cancer therapy, single-drug therapy is generally ineffective to completely treat the diseases. The one-dimensional action mechanism often activates and strengthens the alternative pathways, prompting the emergence of chemoresistance mutations and tumor relapse [4, 5]. In order to increase treatment efficacy, drug combination therapy provides an alternative strategy by taking synergetic actions against some diseases. This combination strategy now has been widely studied in many fields such as tissue engineering [6], hypertension [7], diabetes [8], urinary tract symptoms [9], papulosis [10], hepatitis [11] and cancers [12-14]. Woodcock et al. [15] also highlighted the novel combination therapies in a perspective form. Furthermore, some researchers studied both combination therapy and drug delivery systems (DDS) to coordinate the release behavior and extend the release period of different drugs

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for better cure efficacy. For example, Jäger et al. [16] prepared core-shell polymeric nanoparticles simultaneously loaded with docetaxel and doxorubicin. The results indicate that the combination provides a more efficient suppression of tumor-cell growth in mice bearing EL-4 T cell lymphoma when compared to the effect of nanoparticles loaded with either docetaxel or doxorubicin separately. Greco et al. [17] also highlighted that combination therapy supplies opportunities and challenges for polymer-drug conjugates such as nanomedicines.

In order to improve the sustained-release properties and control the burst-release effects of carriers, some researchers show interests in designing carriers with a special structure that often plays an important role in the space distribution of different drugs. In our previous work, we prepared nanoparticles embedded microcapsules (NEMs) that had a unique structure for drug allocation in different space, thus led to excellent performances such as sequential release and longer sustained release period without initial burst release effect [18-20]. Therefore, we have particular interests in finding DDS with unique structure. Multivesicular liposomes (MVLs) are unique lipid-based systems containing numerous discontinuous internal aqueous chambers bounded by continuous, non-concentric network [21, 22]. Having considered the structure similarity of NEMs and MVLs, we are curious to whether MVLs can be used in combination therapy. In this paper, MVLs were prepared and characterized using horseradish peroxidase (HRP) and mitoxantrone hydrochloride (DHAD) as model drugs for protein and small molecule drug respectively. We anticipated that the MVLs would show promising use in the future drug combination therapy.

2 Experimental Section

2.1 Materials

Glycerol trioleate (CP), cholesterol (Chol) and oleic acid were all purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd (China). Soybean lecithin (≥96%) was supplied by Lipoid GmbH (Germany). L-lysine, glucose, dichloromethane and Triton X-100 (CP) were purchased from National Pharmaceutical Group Chemical Reagent Co., Ltd (China). Sucrose was obtained by Shantou Dahao Fine Chemicals Co. (Shantou, China). Mitoxantrone hydrochloride (DHAD) (≥98%) was purchased from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). Horseradish peroxidase (HRP, Rz≥3, 250 U/mg) was purchased from Beijing Biodee Biotechnology Co., Ltd (Beijing, China). Millipore filters (0.22 μm filters, Mixed Cellulose Ester membranes) were purchased from Shanghai Xinya Purification Device Factory (Shanghai, China). All other reagents and chemicals were of analytical grade.

2.2 Preparation of blank or DHAD/HRP co-loaded MVLs

The blank or DHAD/HRP co-loaded MVLs were prepared by the conventional water-in-oil-in-water (w/o/w) double emulsification process [23, 24] with a little modification. The optimized preparation condition in this research are as follows: a lipid combination of 21.25 mM soybean lecithin, 31.04 mM cholesterol, 13.55 mM glycerol trioleate and 3.54 mM oleic acid were dissolved in dichloromethane to form the oil phase, which was emulsified with an equal volume of aqueous solution (the internal aqueous solution) blank or containing drugs (0.5 mg/ml DHAD, 0.5 mg/ml HRP, the DHAD:HRP ratios were varied as follows: 1:1, 1:3 and 3:1 (w/w)) in 5% sucrose, using a high-speed homogenizer (T25, IKA, Germany) at 20 000×g for 10 min, to produce a