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Lipogels responsive to near-infrared light for the triggered release of therapeutic agents

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ABSTRACT

Here we report a composite system based on fibrin hydrogels that incorporate in their structure near-infrared (NIR) responsive nanomaterials and thermosensitive liposomes (TSL). Polymerized fibrin networks entrap simultaneously gold-based nanoparticles (NPs) capable of transducing NIR photon energy into heat, and lysolipid-incorporated TSL (LTSL) loaded with doxorubicin hydrochloride (DOX). NIR irradiation of the resulting hydrogels (referred to as "lipogels") with 808 nm laser light increased the temperature of the illuminated areas, leading to the release of the liposomal cargo. Levels of DOX that release from the "smart" composites were dependent on the concentration of NIR nanotransducers loaded in the lipogel, the intensity of the electromagnetic energy deposited and the irradiation regime. Released DOX retained its bioactivity, as shown in cultures of epithelial carcinoma cells. Finally, the developed drug delivery platform was refined by using NIR-photoabsorbers based on copper sulfide NPs to generate completely biodegradable composites as well as through the incorporation of cholesterol (Ch) in LTSL formulation, which lessens leakiness of the liposomal cargo at physiological temperature. This remotely controlled system may suit well for those therapies that require precise control over the dose of delivered drug in a defined spatiotemporal framework.

Statement of Significance

Hydrogels composed of fibrin embedding nanoparticles responsive to near infrared (NIR) energy and thermosensitive liposomes loaded with doxorubicin hydrochloride (DOX), were prepared by *in situ* polymerization. NIR-light irradiation of these constructs, referred to as "NIR responsive lipogels", results in the controlled release of DOX to the surrounding medium. This technology may use fully degradable components and can preserve the bioactivity of liposomal cargo after remote triggering to finely regulate the dose and bioavailability of delivered payloads. NIR responsive lipogels technology overcomes the limitations of drug release systems based on the combination of liposomes and degradable polymeric materials, which in many cases lead to insufficient release at therapy onset or to overdose during high degradation period.

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1. Introduction

Hydrogels and other polymer-based carriers have emerged as promising systems to provide reservoirs for pharmaceuticals acting in a variety of anatomical regions. Highly biodegradable and biocompatible fibrin gels, obtained by thrombin-catalyzed polymerization of fibrinogen, have been used since last century with remarkable success as post-surgery sealants and stand out for their potential as controlled delivery systems of various pharmaceutical agents [1]. After addition of thrombin to a fibrinogen solution, fibrin networks can be prepared in the form of macroscopic gels, films, threads, microbeads or nanoparticles. The final properties of the gels, including porosity and degradability, can be tailored by the process and formulation parameters [2]. The release of drugs from fibrin gels occurs via diffusion once the pores in the network are bigger than the hydrodynamic size of the entrapped therapeutics [3]. Moreover it has been shown that the release can be modulated by entrapment of protease inhibitors in the protein matrix that slow down the *in vivo* degradation, by crosslinking with bifunctional reagents that enhance the retention of the therapeutic agents in the protein network or by covalent linking of the payloads to the matrix [4–6]. Entrapment of drug-loaded nanoparticles, e.g. liposomes, can remarkably lower the release rates of water-soluble therapeutics that readily diffuse out from fibrin gels [7,8].

Liposomes are sphere-shaped, unilamellar or multilamellar vesicles typically made from phospholipids. Hydrophobic drugs can be solubilized in the lipid bilayer while more hydrophilic drugs can be loaded in the aqueous core of liposomes. Pharmaceutical agents retained by liposomes become bioavailable only after being released. Most attempts to use liposomes as drug delivery vehicles are based on considering liposomes as entities that circulate in the blood, being taken up by certain cells or tissues to slowly release their therapeutic cargo as they degrade [9–11]. Optimization of the release rate of entrapped drug is essential to control local drug concentration and increase the therapeutic outcome of liposomes [12,13]. Several drug delivery systems aiming sustained release of therapeutic peptides, proteins or drugs from liposomes have been proposed, including their encapsulation in fibrin networks [7,8,10,11]. Also, triggering modalities for site-specific release of therapeutics have been developed to improve the therapeutic efficacy of liposomal formulations. These strategies include local triggers which are specific to target sites (e.g., pH and enzymes) and remote triggers (e.g., temperature, ultrasound, magnetic field, radiofrequency and light) through the use of specific lipid compositions and coatings [14–17]. Among the latter approaches, heat-based delivery appears to be the most promising to date [18]. TSL are special formulations capable of releasing encapsulated cargos in response to an increase of temperature, whilst preventing premature leakage at physiological temperature [19]. Incorporation of lysolipids, phospholipids with one hydrocarbon chain, in the formulation of TSL can lower the phase transition temperature of the lipid bilayer to the mild hyperthermia range [20–22]. Upon heating, lysolipids create and stabilize water-filled pores along liquid-solid boundaries formed in the lipid bilayer of liposomes, enhancing its permeability [18]. An example of LTSL is ThermoDox®, a heat-activated liposomal formulation of doxorubicin that upon intravenous administration releases the entrapped drug at temperatures above 40 °C. This formulation, the first and only thermosensitive liposome formulation to reach clinical development to date [23], is currently being evaluated in combination with radiofrequency thermal ablation for the treatment of hepatocellular carcinoma in the OPTIMA trial [24], a clinical phase III trial based on the promising findings obtained from *post-hoc* analysis of the HEAT trial [25]. A major drawback of systemically administered liposomes, as Thermodox®, is their poor and inhomogeneous distribution at distances away from the tumor vasculature which can compromise clinical outcomes. Furthermore, radiofrequency energy is difficult to focus on a discrete target site. These limitations may be circumvented by technologies that allow direct disposal of thermosensitive liposomes in the tumor region and that employ focusable sources of heat to trigger drug release.

In the present work, we exploit the ability of fibrin hydrogels to incorporate in their structure inorganic photoabsorbers such as hollow gold nanoparticles (HGNP) [26] or copper sulfide nanoparticles (CuSNP) [27] during gelation [28]. Both NPs exhibit strong light absorption at near-infrared (NIR) wavelengths (650–1050 nm) and can convert light energy into thermal energy through localized surface plasmon resonance, and in the case of CuSNP, also through the excitation of direct (band-to-band) or indirect electronic transitions due to their semiconductor character [29]. We hypothesized that incorporating NIR nanotransducers (NIR-NT) in fibrin matrices that include DOX-loaded LTSL (LTSL-DOX), referred to as "lipogels" [30,31], may result in an advanced drug delivery platform exogenously regulated by NIR electromagnetic energy, which has its maximum depth of penetration in biological tissue (Fig. 1).

2. Materials and methods

2.1. Chemical reagents

The phospholipids 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-N-poly(ethylene glycol)-2000 (DSPE-PEG2000) were provided by Lipoid (Ludwigshafen, Germany). Monostearoylphosphatidylcholine (MSPC) was purchased from Avanti Polar Inc (Alabaster, AL, USA). Other chemical reagents were purchased from Sigma Aldrich unless otherwise specified.

2.2. DOX-loaded liposomes

LTSL or cholesterol-containing LTSL (LTSL-Ch) were prepared as follows. DPPC:MSPC:DSPE-PEG2000 or DPPC:MSPC:DSPE-PEG2000:Ch were dissolved in chloroform in a molar ratio of 86:10:4 or 81.7:9.5:3.8:5, respectively. A lipid film was formed in a rotavapor under vacuum at 40 °C. Residual solvent was removed by flushing the film with nitrogen gas for 1 h. Liposomes were prepared by hydrating the film with 240 mM ammonium sulfate buffer (pH 5.5) at 60 °C, aiming to a final lipid concentration of 25 mg mL^{-1}. Polycarbonate membrane filters (650, 200 and 100 nm) were employed to extrude the resulting liposomes at 60 °C. Loading of DOX into liposomes was performed using the pH gradient loading method as previously described [32,33], with initial DOX-to-lipid ratio of 1:20 (mol:mol). Briefly, extravesicular buffer was exchanged using 10 kDa cut-off membranes with dialyzing medium HBS pH 7.4 (20 mM HEPES, 150 mM NaCl) at 500 volumes for 1 volume of liposomal dispersion in three repeated cycles, establishing the gradient. Liposomes were loaded with DOX by incubation at 37 °C for 1 h. Unencapsulated DOX molecules were removed using Sephadex G-25 gel filtration resins. LTSL-DOX or LTSL-Ch-DOX were suspended in HBS pH 7.4 and stored at 4 °C. Liposome size was measured by dynamic light scattering (DLS) using a Malvern 4700 compact goniometer (Malvern GmBH, Germany). Calorimetric studies were carried out in a Mettler-Toledo differential scanning calorimeter with a high-sensitivity sensor. Scans were performed in a range of 20–50 °C and recorded