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In vitro investigation of methylene blue-bearing, electrostatically assembled aptamer–silica nanocomposites as potential photodynamic therapeutics

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ABSTRACT

Photodynamic therapy, that is, excitation of a photosensitizer with light to generate reactive oxygen species such as singlet oxygen, has emerged as a noninvasive technique for cancer theranostics. However, the clinical use of many photosensitizers is impeded by their hydrophobicity, the nonspecific damage they cause to normal tissues, and their susceptibility to environmental degradation. In this study, we developed a simple electrostatic adsorption strategy to fabricate aptamer–silica nanocomposites by sequentially functionalizing nanocomposites with the cell surface-associated mucin 1 aptamer for tumor targeting and a hydrophilic photosensitizer, methylene blue, for photodynamic therapy applications. We investigated the relationship between the biophysical properties and cellular uptake of such nanocomposites to improve their formulation. Effective generation of singlet oxygen was achieved with a low photosensitizer dosage (0.5 μM) and a short, low-power irradiation (1 min, 10 mW/cm^2). With the current strategy, the efficiency of photodynamic therapy was determined by the cellular uptake of nanocomposites and the targeting molecules used.

1. Introduction

Photodynamic therapy (PDT) has emerged as a popular adjuvant treatment for cancer [1,2]. PDT is a noninvasive method that involves the delivery of light-sensitive molecules called photosensitizers (PSs) to target sites [3]. Upon irradiation with light of the appropriate wavelength, PSs absorb energy and transfer it to the surroundings, generating reactive oxygen species (ROS) such as singlet oxygen or free radicals and inducing cell death via apoptosis, necrosis, or both [4]. However, the use of most existing PSs is hampered by disadvantages such as nonspecific damage to normal tissues, hydrophobicity, and poor stability [5,6].

To overcome this barrier to PDT, various PS-bearing nanoscale vehicles that enable tumor site targeting and offer improved therapeutic efficacy and resistance to environmental degradation have been developed [7–11]. Silica-based nanomaterials have been extensively employed in PDT because of their great biocompatibility (Table S1). A few studies [12,13] have used specific tumor-targeting molecules to functionalize the nanocarrier surface, thereby improving PDT selectivity. Targeting agents frequently applied to improve specificity include antibodies, aptamers, short peptides, and other small molecules [14]. Aptamers are single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules that can fold into three-dimensional structures to enable specific recognition of target molecules with high affinity [15]. Compared with other targeting agents, aptamers possess several advantages: they are simple to synthesize, have low immunogenicity, and are readily applicable to small ligands, and their small size facilitates target recognition. Combinations of aptamers with inorganic colloids, particularly silica nanomaterials, have been extensively studied [16,17] and successfully utilized in biosensing [18,19] or to enhance the efficiency of drug delivery [20]. The aptamer of mucin 1 (MUC1), a large transmembrane glycoprotein with ≥10-fold increased expression in most malignant breast, lung, and colon adenocarcinomas [21], is an ideal targeting agent for silica-based anticancer drug delivery systems [14,22].

Additionally, clinically useful PSs must be nontoxic and should be retained in malignant tissue. PSs that respond to longer wavelengths (>600 nm) are relatively efficient photochemically and are preferred over those that are excited by wavelengths in the conventional UV–vis range, as absorption of light in this region is rather weak in tissues, and low background signals in the longer

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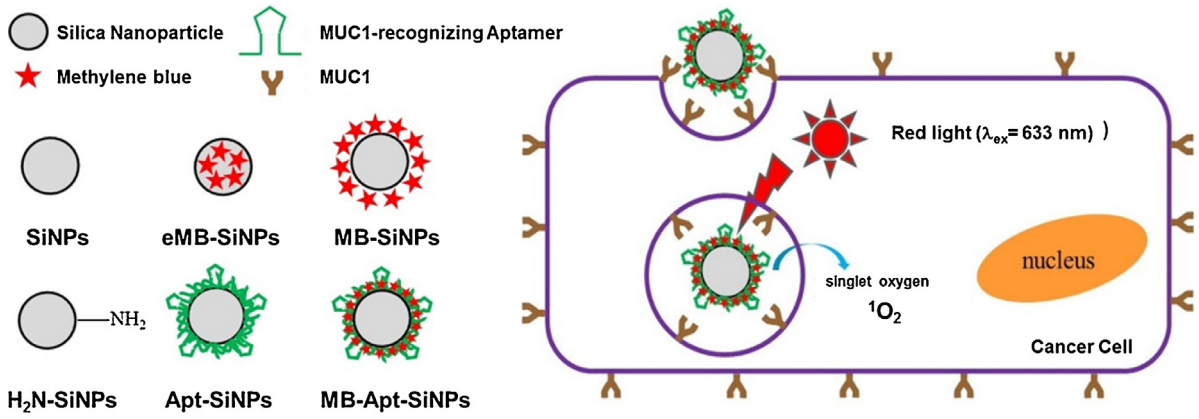

Fig. 1. Schematic illustration of methylene blue (MB) delivery to cancer cells by silica nanocomposites (Si NPs). Singlet oxygen was generated upon irradiation of MB at 633 nm to achieve photodynamic therapy.

wavelength region enable penetration to deeper tissue sections [23]. Methylene blue (MB) is an attractive candidate PS for PDT because of its hydrophilicity, cost effectiveness, strong absorption at 550–700 nm, and high singlet oxygen quantum yield ($\Phi_D \approx 0.5$) [24,25]. Furthermore, MB has been approved by the United States Food and Drug Administration for the treatment of methemoglobinemia [26].

Despite success in treating cancer with PDT, several limitations remain, including complicated vehicle preparation, high PS dosages, extended nanocarrier incubation and irradiation times, and poor selectivity. Besides, the MB absorption spectrum varies during isomerization and polymerization (Table S2), which may influence singlet oxygen production and result in low PDT efficiency. However, no studies have evaluated and compared nanocarriers with different formulations. Herein, a silica nanocomposite was fabricated by simple electrostatic adsorption; the nanocomposite was functionalized with the MUC1 aptamer [27,28] for tumor targeting, and MB was used as the singlet oxygen generator for PDT. Various alternative nanocomposites were evaluated to improve the efficiency of PDT and achieve <50% viability in high MUC1-expressing cancer cell lines MCF-7, in comparison with low MUC1-expressing HCT-116 and normal cells (Fig. 1).

2. Material and methods

2.1. Chemicals

Tetraethyl orthosilicate (TEOS), (3-aminopropyl) trimethoxysilane (APTMS), aqueous ammonia (NH_4OH) solution (70% (w/w) water, 30% (w/w) ammonia), fluorescein isothiocyanate (FITC), and ethanol (99.9% purity) were purchased from Sigma–Aldrich (St. Louis, MO, USA). *n*-Hexanol and methylcyclohexane were purchased from Alfa Aesar (Ward Hill, MA, USA). Triton X-100 was from AMRESCO (Solon, OH, USA), and MB was provided by Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Tris base was purchased from J.T. Barker (Center Valley, PA, USA). The MUC1 aptamer (Apt), 5′-T6 (spacer)-GCAGTTGATCCTTTGGATACCCTGG, was synthesized by MDBio, Inc. (New Taipei City, Taiwan). Fetal bovine serum (FBS), penicillin/streptomycin, AlamarBlue® Cell Viability Reagent, and the singlet oxygen fluorescence probe, Singlet Oxygen Sensor Green (SOSG), were obtained from Molecular Probes (Thermo Fisher Scientific Inc., Waltham, MA, USA). Dulbecco's phosphate-buffered saline (PBS) was purchased from BioSource (Camarillo, CA, USA). For cell experiments, all reagents, buffers, and culture media were sterilized by autoclaving (121 °C, 15 min) or filtration (pore size, 0.22 μm; Millipore, Bedford, MA, USA) and stored under sterile conditions. The 100-kDa MW cutoff spin filters were purchased from Pall Corporation (Port Washington, NY, USA).

2.2. Synthesis of silica nanoparticles (Si NPs) and MB-encapsulated Si NPs (eMB-Si NPs)

Si NPs and eMB-Si NPs were prepared using the reverse microemulsion method. Briefly, a water-in-oil microemulsion was prepared by mixing 1.8 mL of Triton X-100, 7.5 mL of methylcyclohexane, 1.6 mL of *n*-hexanol, and 400 μL of deionized (DI) water (to synthesize eMB-Si NPs, 480 μL of 2.5 mM MB solution was also included in the mixture); this was followed by 30 min of stirring and subsequent addition of 200 μL of TEOS and 100 μL of NH_4OH. The solution was stirred at room temperature for 24 h. Ethanol was added to disrupt the microemulsion, and particles were recovered, washed three times with ethanol, and washed once with DI water [29]. The nanocomposites were stored at 4 °C in ethanol.

2.3. Synthesis of MB-coated Si NPs (MB-Si NPs)

MB was loaded on the surface of Si NPs by simple mixing. Briefly, Si NPs (6 mg) were suspended in water, and MB solution was added to a final concentration of 100 μM in a total volume of 6 mL. The mixture was stirred for 2 h, and unabsorbed free MB was removed by using a 100-kDa MW cutoff spin filter. The MB-Si NPs were stored at 4 °C in DI water.

2.4. Synthesis of NH_2-functionalized Si NPs (H_2N-Si NPs)

Amine-functionalized Si NPs were synthesized by suspending Si NPs (10 mg) in 4 mL of ethanol and adding 200 μL of NH_4OH, 375 μL of DI water, and 175 μL of APTMS. The mixture was vigorously stirred for 2 h and ultrasonicated for 1 h at room temperature. The suspension was kept in a 60 °C water bath for 6 h [30], and then the recovered particles were washed three times with ethanol and once with DI water. The H_2N-Si NPs were stored at 4 °C in ethanol.

2.5. Synthesis of aptamer-functionalized Si NPs (Apt-Si NPs)

Modification of H_2N-Si NPs with the MUC1 aptamer was accomplished by electrostatic adsorption. Briefly, 1 nmol of the aptamer was dissolved in 100 μL of PBS, heated at 95 °C for 5 min, and gradually cooled to 25 °C. A previously prepared H_2N-Si NP suspension (1 mg in 300 μL PBS) was reacted with the aptamer solution at 4 °C for 24 h. The Apt-Si NPs were recovered, washed three times with PBS, and stored at 4 °C in PBS.