Nanotechnology for medical and environmental applications.

Gu F. Rosen J. and Carty AJ.
University of Waterloo, Waterloo, Canada (frank.gu@uwaterloo.ca)

INTRODUCTION AND OBJECTIVES

Over the past decade, research interest in the application of biomedical nanotechnology to the diagnosis and treatment of disease has grown significantly. Continual advancements in nanofabrication have led to the production of increasingly elaborate nanoparticle devices employing stimuli-responsive controlled drug release, novel radiation-coupled therapies, or finely-tuned physiological interactions, and a wide variety of such innovative designs are reported weekly in the scientific literature. The goal of this study is to demonstrate a proof-of-concept for one proposed method of facilitating particle aggregation via an external stimulus. For this project, we chose to use the presence of a particular wavelength of light as the stimulus to induce aggregation. Light has a number of features making it ideal for this purpose; its high spatial resolution allows for precise locational control of aggregation behavior. For example, one could cause aggregation in only a certain area of a fluid, or in a particular well of a microplate. Furthermore, one can exert precise temporal control over light, which cannot be achieved to the same degree with other stimuli such as pH or ionic strength. Light is also a true external stimulus; in contrast to alternatives such as a change in pH, ionic strength, or the addition of an antigen, light requires no additives be placed into the solution itself. Since parameters such as pH, ionic strength, and even temperature can fluctuate in different biological compartments or disease states, there is a greater potential for interference in the external control of aggregation state. In order to create particles capable of changing their aggregation state in response to a light stimulus, a molecule must first be chosen that changes its intermolecular interactions in response to incident light of particular wavelengths. We examined various light responsive materials including azobenzene, IR dyes, and gold nanomaterials. Azobenzene can undergo a reversible transformation (from cis to trans conformation) upon light irradiation, however this is a physical /mechanical change as opposed to a chemical one and we could not readily determine how to translate this into an aggregation behavior in a simple way. IR dyes and gold nanomaterials, while responsive to light, generally exert their effects through a temperature change caused by the light exposure. We sought a method that relied purely on the irradiated light as the stimulus, as we did not want interference from environmental temperature fluctuations. Thus we based our nanoparticle system on the well-known behavior of coumarin and its derivatives. Coumarins are a broad family of chemicals typically derived from plants which all share the same basic fused ring structure seen in Fig. 1. They have been used extensively in biological research as dyes and fluorescent tags owing to their well-characterized UV absorbance and fluorescence properties. Coumarin is known to undergo a reversible photo-dimerization reaction when stimulated with UV light of a specific wavelength (Fig. 1). By irradiating with two different wavelengths of light, it is possible to both form and break the dimers. Coumarin has been used to facilitate photo-polymerization and cross-linking, to control the release of drug molecules, and it has recently been functionalized onto a nanoparticle surface. It is our intention to use this mechanism to facilitate the aggregation of nanoparticles in response to light.

MATERIALS AND METHODS

Synthesis of amine functionalized silica nanoparticles Ethanol and acetic acid were mixed at a 1:1 volume ratio in a flask and 19 ml of LUDOX AS-40 was added with a magnetic stir bar. The solution was placed in a 50 °C oil bath and 3 ml of APTS was added to the mixture. The solution was placed in a sealed vial for storage and a portion was dried to determine the concentration. 50 mL of PBS (0.1 M, pH 7.4), 200 µL of acetic acid, and 155 mg of dextran (Mw = 6 K) were added to a flask, pH was approximately 4. 400 mg of the amine-functionalized particles were added to the flask, which was covered and allowed to stir for 24 hours in order to allow the dextran to absorb to the surface of the nanoparticles (if this step is not performed, the particles will crash out of solution).

Periodate oxidation of dextran-functionalized particles All of the dextran-functionalized particles from the previous step were placed into a flask covered with foil to protect it from light. 95 mg of NaIO4 was added to the solution under stirring and the flask was covered.37 After 3 hours, 0.5 ml of glycerol was added and allowed to react for 30 minutes to quench the remaining NaIO4. The particles were re-suspended in 3 ml of DI H2O, removed from the Amicon tube and sonicated for 30 seconds at 30 % amplitude. A 70:30 mixture of DMSO to acetic acid (50 ml total volume) was placed in a flask. 315 mg of AMC and the oxidized particles (in a minimal volume of DI H2O) were placed in the flask, which was covered with foil to protect from light. 40 mg of NaCNBH3 was added and the reaction was allowed to proceed for 18 to 24 hours at room temperature. The solution was placed in a 12 K dialysis membrane and

dialyzed against 800 ml of DMSO for at least 10 hours.

RESULTS AND DISCUSSIONS

The response of AMC functionalized particles to 350 nm UV light was assessed using TEM, DLS, and UV-VIS. Fig. 1 shows how the hydrodynamic size of the particles change over time when they are exposed to 350 nm light. As they were exposed to UV light, the particles hydrodynamic size increased, as measured by DLS, and the solution began to appear noticeably cloudier. After the entire time-course of the experiment the particle solution, which was originally only faintly cloudy, had turned quite cloudy and opaque. The exposure to UV light thus caused a clear increase in average particle size to occur. Up to an exposure time of 40 minutes, the particle size appears to increase in a relatively controlled manner, however around the 40-minute mark there is a rapid increase in particle size. We theorize that this rapid size increase could be due to spontaneous particle aggregation, which may begin to occur only after the UV-light induced aggregates reach a critical size. These light-induced aggregates could then serve as seeds for spontaneous aggregation processes, leading to the rapid and uncontrollable increase in particle size seen in Fig. 1.

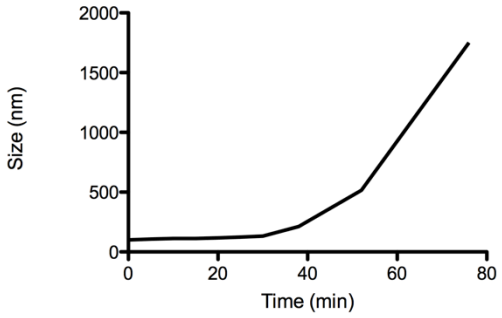

Fig. 1. Changes in hydrodynamic size of AMC functionalized particles over time during exposure to 350 nm UV light.

The particles were observed using TEM both before and after exposure to 350 nm light as shown in Fig. 2. Prior to exposure (Fig. 2a) the particle are seen to be fairly well dispersed; individual silica cores (D ~ 31.4 nm) can be readily observed on the image, and the clusters that are observed are most likely due to aggregation caused by the drying and sample preparation process. The diffuse material that can be seen around and between silica cores is most likely the dextran polymer coating functionalized with the AMC moieties. Fig. 2b shows the particles after exposure to 350 nm UV light; large aggregates of particles (D ~ 2.3 μm) with many overlapping silica cores are readily visible. From these images, the aggregation caused by irradiation of 350 nm UV light can be directly observed, further supporting the data in Fig. 1.

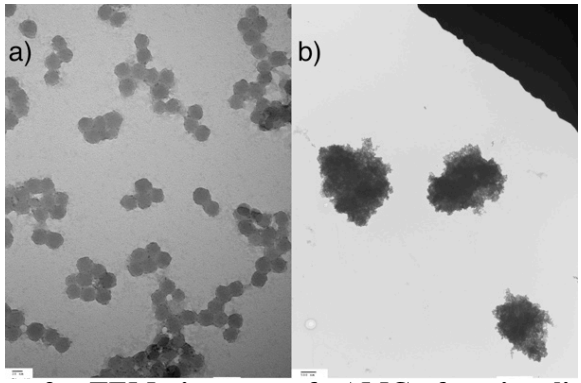

Fig. 2. TEM images of AMC functionalized particles before (a) and after (b) exposure to 350 nm light. Scale bar in (a) is 20 nm, scale bar in (b) is 500 nm.

CONCLUSION

Here we demonstrated proof-of-concept that the dimerization of coumarin molecules can serve as a mechanism for stimulus-induced aggregation of nanoparticles. Particles with this type of stimuli-responsive aggregation could have numerous applications in both medical therapeutics and diagnostics. Aggregation control can be used to immobilize particles in a particular biological compartment, modify drug release kinetics, or increase the signal strength of diagnostic nanoparticles. Due to its great potential applications, and highly transferrable chemistry, we believe that the methods examined in this study could be applied to a wide array of nanoparticles for various biomedical applications.

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