OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 1, No. 11, November 2007, p. 644 - 647

Remote radio-control of siRNA release from magnetite-hydrogel composite

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Magnetically responsive gel network formed by copolymerization of N-isopropylacrylamide and acrylamide, with a critical solution temperature tuned slightly above the body temperature, containing embedded magnetic nanoparticles was prepared. Magnetic nanoparticles were used for the gel heating when exposed to radiofrequency field with frequency 760 KHz. When the temperature of the copolymer exceeded the critical solution temperature, the material collapsed and a fast release of small interefering RNA co-embedded inside the gel network was observed, which may have far reaching therapeutic implications.

(Received September 4, 2007; accepted October 31, 2007)

Keywords: Superparamagnetic hydrogel, Radiofrequency field, Gene therapy, siRNA controlled release

1. Introduction

Various methods of accomplishing modulated in vivo drug delivery have been described in the literature and are currently in use or undergoing investigation. Mechanical pumps are one type of device that is commonly employed. Another method that has been examined is the use of ultrasound for "blasting off" a layer of material from a drug-containing polymer matrix to alter drug release. That method requires use of rigid, hydrophobic polymers that are generally incompatible with proteins and other hydrophilic macromolecular drugs. Other potential problems with the routine implementation of such ultrasound techniques may exist, as suggested by the widespread concern about the long term safety of repetitive exposure of body tissues to ultrasonic energy. The formulation and application of polymer particles and hybrid particles composed of polymeric and magnetic material are of great interest for applications in biotechnology [1,2]. Recently a new kind of magnetically responsive materials were prepared, namely the magnetosensitive gels [3-6]. These materials are usually polymer networks sustaining solution containing magnetic particles. Their possible applications include controlled material release, separation systems and artificial muscles. Due to their superparamagnetic properties they are a potential candidates as a agents for electromagnetic hyperthermia [7]. These subdomain superparamagnetic particles produces substantially more heat per unit mass than the 1000 times larger multidomain ferrite particles of similar composition, when exposed to radiofrequency field [8-10]. The mechanism of heating is based on Brownian relaxation (rotation of the particle as a whole according to external magnetic field) and Neél effect (reorientation of the magnetization vector inside the magnetic core against an energy barrier).

Our aim in this paper is to evaluate the properties of a gel network formed by copolymerization of N-isopropylacrylamide (NIPAAm) and acrylamide (AAm), which exhibits a critical solution temperature (LCST) slightly above the body temperature. When the temperature of the copolymer exceeds the LCST, the material collapses causing a fast release of material (siRNA) embedded inside the gel network. Embedded magnetic particles are used for the gel heating when exposed to radiofrequency field.

The 2006 year Nobel prize winning phenomena of RNA interference (RNAi) were first reported in 1998 by Firo et al. [11] who demonstrated that double-strand RNA induced sequence-specific silencing of gene expression in nematode cells. Elbashir et al. [12] demonstrated that RNAi can be achieved in the mammalian cells by using oligoribonucleotide duplex 21 or 22 bases in length (small interfering RNA; siRNA). More recently, several studies applied RNAi to treatment of various disorders in animal models [13] and showed that RNAi may provide promising strategies to treat diseases by suppressing disease responsible genes. However, the efficacy of RNAi depends upon efficient delivery of siRNA, because siRNAs are rapidly cleared from plasma so effective treatment of chronic diseases may rely on the repeated administration of these agents to maintain therapeutic concentrations. An alternative delivery approach for local siRNA administration may be to encapsulate the agents in polymeric compositions that can protect the nucleotide chain an allow controlled release in response to radiofrequency radiation.

2. Experimental procedure

2.1. Preparation of magnetic nanoparticle containing hydrogel [14, 15]

NIPAAm was mixed with acrylamide (to set up LCST to ~ 40 ^{o}C) and bis-acrylamide as a crosslinker. A free radical initiator was added, and the liquid was converted to a hydrogel. NIPAAm was obtained from Aldrich (Milwaukee, USA) and recrystallized in n-hexane. AAm, N,N'-methylenebisacrylamide (MBAAm), ammonium persulfate (APS), N,N,N',N'-tetramethylethylenediamine (TEMED) were all obtained from Sigma (St. Louis, USA). In this study, hydrogels were constructed of two layers of 1.75 M poly(NIPAAm-co-AAm). The primary monomer solution was formed by placing a total of 15 ml of NIPAAm and AAm in a round-bottomed flask in a 95/5 molar ratio (NIPAAm-co-AAm). MBAAM was added as a crosslinker at a molar ratio of 1/750 (crosslinker/monomer). The flask was evacuated, and 50 µl of 1% APS solution (w/w) and 10 µl TEMED (6.6 µM) were added to initiate the redox reaction that forms the hydrogel. The hydrogel precursor solution was then poured into molds consisting of two glass slides separated by 1.5 mm Teflon spacers. After curing at 30 ^{o}C for 2 hours, the faceplate of the mold was removed, and the walls of the mold were extended by 1 mm. The faceplate was then replaced on the mold. An additional 10 ml of the monomer solution was prepared as described above, with the addition of 2 wt. % of the PEGdiphosphate stabilized magnetic nanoparticles with diameter of 80 nm (Chemicell GmbH, Berlin, Germany) at the same time as the APS and TEMED. This secondary copolymer solution was then poured into the mold, over the initial hydrogel, and allowed to cure for 2 hours at 22 ^{o}C. The resulting bilayer hydrogel was removed from the mold and allowed to swell in deionized water for 24 hours, after which it was cut into 1 cm diameter disks with a cork borer and dried overnight in a vacuum oven. Control bilayer hydrogels, lacking the magnetic nanoparticles, were formed in the same manner but without the addition of the magnetite suspension to the second monomer solution.

2.2. siRNA loading and release quantification

Dry nanoshell-composite hydrogels were placed in a 5 nM siRNA solution (synthetic siRNA targeting β-galactosidase gene sequence CTACACAAATCAGCGATTT provided by Dharmacon (Chicago, USA) was used) and allowed to swell for 3 days at 10 ^{o}C. After loading with siRNA the 1 cm diameter hydrogel disks were removed from the dye solution, quickly rinsed in fresh Tris buffer, and placed in a glass vial containing 1.8 ml of Tris buffer. The vial was then irradiated in radiofrequency generator. Samples of the Tris buffer were removed from the vial at set intervals, and the absorbance at 260 nm was measured to determine the concentration of siRNA in the release buffer.

2.3. Experimental setup for radiofrequency irradiation

For this study we have modified experimental setup (Fig. 1) used previously in the study of influence of electromagnetic field on the liposomes [16-19]. The magnetic field with the amplitude 9.6 kA/m and frequency 760 kHz was achieved inside the water-cooled cooper induction coil with radius r=12 cm (n=10 turns with turn to turn distance z=0.7 cm).

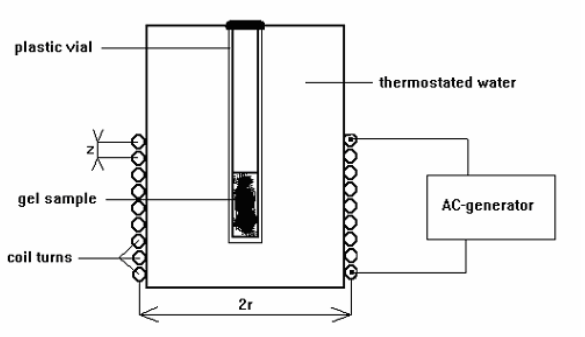

Fig. 1. Experimental setup for the heating of a magnetic hydrogel in a radiofrequency field and controlled release of siRNA.

3. Results and discussion

The effect of radiofrequency irradiation on the release of siRNA compared with non- irradiated sample is shown in Fig. 2.

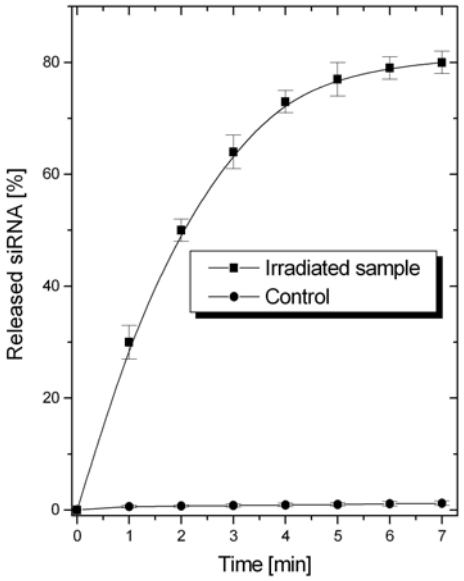

Fig. 2. Release of siRNA from non-irradiated magnetite-hydrogel composite compared with the effect of radiofrequency irradiation. Data are mean ± SD from 5 independent measurements.

If all siRNA is not released during the initial irradiation sequence, additional bursts of release of the drug can be elicited by subsequent irradiation, as shown in Fig. 3. Once the radiofrequency irradiation is stopped, the driving force for the convective transport of material out of the hydrogel matrix is removed. During this time, the drug release is driven by diffusion, and the amount released is much less than that generated by irradiation. The hydrogel will begin to swell as soon as the radiofrequency radiation is turned off, returning to its equilibrium state. A second irradiation sequence delivered at this time will cause the hydrogel to collapse again, resulting in another burst of release of the drug molecule.

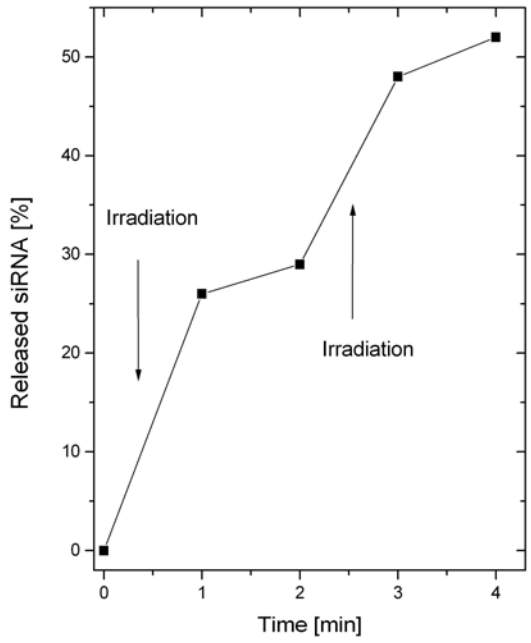

Fig. 3. Repeated release of siRNA from magnetite-hydrogel composite using 1 min periods of radiofrequency irradiation.

An important property of hydrogels is the ability to undergo abrupt changes in volume without dissolving in the immersed medium. "Smart gels" are able to swell or shrink up to 1000 times in response to small changes in temperature, pH level, electric fields or solvent and ionic composition. The temperature-sensitive NIPAAm-co-AAm hydrogels were found to exhibit volume changes of over 800 %. The collapse of hydrogel at LCST temperature provides a convective force for the transport of the siRNA outside the hydrogel. Without the irradiation small amount of siRNA is released via diffusion through the porous hydrogel.

These our first results therefore represents magnetically responsive hydrogels as a promising material suitable for remote radio-controlled release of siRNA.

One important possible applications include treatment of a residual neoplastic cells which are almost always present after tumor excision from the brain, by filling the empty place in the brain by the biocompatible magnetic gel, and then release suitable siRNA using electromagnetic hyperthermia. As has been recently found hydrogel polymer matrices formed from poly-N-(2-hydroxypropyl)-methacrylamide are suitable also as a substrate for culturing rat neuronal cells [20] therefore suitable magnetic gels may have multifold applications for the treatment of brain cancer and restoring the impaired brain tissue.

The siRNA can also be encapsulated to magnetically responsive thermo-sensitive NIPAAm-co-AAm microparticles, and then high-gradient magnetic targeting [21-23] can be used for delivery of siRNA to desired sites with subsequent radiofrequency mediated release.

Acknowledgements

We acknowledge support from European Union grant No. LSHB-CT-2004-005213 (STREP project Improved Precision) and VEGA grant 1/2012/05.

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