<Review>

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Review on PEGylation (Surface Coating with Polyethylene Glycol) and Potential Use of PEGylated Silica-based Materials for Biomedical Applications

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Abstract

In clinical treatment of diseases, diagnostics and drug delivery are the main issue to obtain successful therapeutic effect. Various kinds of probes and/or carriers are used as a tools for diagnostics and delivery of the drugs to the target areas. Biocompatibility of the devices/tools is the major concern for using them in biological systems. Surface modification by polyethylene glycol (PEG) polymer (PEGylation) of devices/tools is widely used to make them biocompatible. PEGylated silica-based nanocomposites earn much attention in using them as a tools for diagnostics and drug delivery systems because of their enhanced biocompatibility and versatility. This review demonstrates potential using of PEGylated silica-based nanocomposites in diagnostics and drug delivery systems.

1. Introduction

Biomaterials when reduced to the size scale of nano (nanobiomaterials), properties like surface-area, quantum, and optical effects, electrical and magnetic behaviors are unassailable for diagnosis and treatment of diseases. Among many structurally stable non-metallic nanobiomaterials that have been investigated for biomedical applications, silica materials with defined structures and surface properties occupy a prominent position because of their easy preparation and suitable surface properties. Monodisperse spherical silica nanoparticles can be easily synthesized by the hydroly-

sis of tetraethyl orthosilicate (TEOS) in alcohol media under catalysis by ammonia¹⁾. Different-sized silica spheres in the whole colloidal size range can be obtained simply by changing the initial concentrations of the reagents.

Scheme 1 Synthesis of silica nanoparticles

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Lee et. al.²⁾ proposed the reaction mechanism of hydrolysis of TEOS are as follows

Scheme 2 Possible chemical reactions encountered in the synthesis of silica nanoparticles by the Stöber method

In the last decade, with the increasing interest toward nanotechnology and nanomedicine, silica nanoparticles have experienced a new use and several applications in the field of biotechnology and medicine have been proposed, such as diagnostics^{3~6)}, drug delivery^{7~10)}, gene transfection^{11,12)}, and multimodal imaging^{13~16)}. As compared to other nanomaterials, silica nanoparticles do not acquire any peculiar properties except for the corresponding increase in surface area¹⁷⁾. As a matter of fact, they can be simply considered as extremely small glass spheres. Hence, silica nanoparticles are so appealing mainly because of their accessibility and versatility, which are probably unmatched by any other nanomaterial. They are easily prepared from inexpensive precursors and with the desired size by straightforward and mild procedures. Silica is rigid, relatively chemically inert and does not swell with solvent or pH variation.

As a drawback, the use of silica nanoparticles in medicine presents problems of biocompatibility. Colloidal stability is granted by the electrostatic repulsion between the negative charges of the deprotonated silanol groups present on the particle surface. Thus, disper-

sion in high ionic strength media, such as biological fluids, causes particles aggregation and precipitation^{17, 18)}.

Silica is also very well suited to adsorb proteins on its surface exploiting both hydrogen bonding and electrostatic interaction¹⁷⁾. This is why the most important industrial application of silica colloids is beverage clarification. Unfortunately, this property implies that once the silica nanoparticles are injected in the blood stream, they are rapidly coated by serum proteins (opsonins) that activate the capture by reticuloendothelial system (RES) and cause the consequent rapid removal by circulation^{9, 19~21)}. The most popular strategy to avoid RES capture of nanoparticles is surface passivation with hydrophilic and neutral species such as PEG, which are able to prevent opsonization and consequently to increase circulation lifetime²²⁾.

2. PEGylation

PEGylation is the process of covalent attachment of PEG polymer chains to another molecule, normally a drug or therapeutic protein. PEG polymers contain numerous inherent favorable biological characteristics including high water solubility and a lack of toxicity and immunogenicity. Thus, the chemical modification of biologically active compounds, such as peptides, antibody fragments, enzymes, or small molecules with PEG chains often leads to improved pharmacokinetics and biological function in many applications. PEGylation can be used as a tool to facilitate drug delivery of nanocarrier systems, nanoparticles, and microparticles. Also, in contrast to increasing water

Fig. 1 Illustration of coagulation of silica nanoparticles in high ionic strength environment.

Fig. 2 Polyethylene glycol (PEG)

solubility of certain compounds and materials, PEG-enzyme complexes have been shown to increase the solubility, stability, and activity of the enzymes in hydrophobic organic solvents.

The addition of PEG to the nanoparticle surface (PEGylation) can help to overcome disadvantages associated with some biopharmaceuticals. PEG is a coiled polymer of repeating ethylene ether units with dynamic conformations (Fig. 2). In both drug-delivery and imaging applications, the addition of PEG to nanoparticles reduces RES uptake and increases circulation time versus uncoated counterparts²³. Aggregation decreases owing to passivated surfaces, and association with nontargeted serum and tissue proteins is diminished, resulting in so-called 'stealth' behavior. The PEG chains reduce the charge-based contact typical of proteins and small-molecule interactions. Solubility in buffer and serum increases due to the hydrophilic ethylene glycol repeats and the Enhanced permeability and retention (EPR) effect is modulated due to nanoparticle size changes via addition of a PEG coat^{24, 25}. Due to these attributes, PEGylated nanoparticles generally accumulate in the liver a half to a third of the amount of non-PEGylated nanoparticles and demonstrate higher tumor accumulation versus background²⁶. PEG is inexpensive, versatile, and Food and Drug Administration (FDA) approved for many applications²⁷.

3. PEGylated silica nanoparticles

The problem one encounters with silica nanoparticles as carriers for biomedical applications is that the particles as such are not easily biodegraded, toxic, or easily taken up by the body's RES and are deposited in the body, which may cause other side effects in the future. To overcome these difficulties, the PEGylation of silica nanoparticles is become the center of one's interest in which the silicon atom has been organically modified by attaching through suitable chemical conjugation. PEG has been bound to silica through direct surface esterification of its surface hydroxyl groups and the silica surface silanols or through a urethane linkage in

order to stabilize colloidal silica in water^{28, 29}. In some cases, PEG is attached to the external surface of the silica particles by means of hydrogen bonding, in other cases the synthesis of the silica particles takes place directly in the presence of PEG rendering ester linkages (Si-O-C).

Yagüe et.al. reported the synthesis of PEGylated silica micro- and nanoparticles, and characterized the prepared particles in order to study their potential as drug-delivery vectors³⁰. They obtained that, while hydrogen bonding between PEG and silanol groups occurs, the stability of the bonding is poor both in a non-ionic detergent (Tween-20) and in a medium with high ionic force. In addition, this route seems less desirable, since particles with covalently bound PEG chains achieve longer blood circulation half-lives than similar particles with only surface adsorbed PEG³¹. A standard protein adsorption experiments (immobilization of bovine serum albumin, BSA) were carried out in order to analyze the stability and the potential avoidance of the RES achieved after PEGylation. From the results, it was clearly observed that the presence of PEG on the surface of the silica nanoparticles significantly reduces BSA adsorption at both acidic and alkaline pH values. This could be attributed to the increased water-retaining capacity, charge neutrality and steric repulsion effects caused by PEG³². An electrostatic self-assembly of a graft copolymer of polyethyleneimine (PEI) with PEG (PEI-PEG) onto porous silica nanoparticles has been reported by Thierry et.al., where the PEGylated silica nanoparticles showed excellent colloidal stability³³. *In vitro* protein adsorption tests using 5 % serum demonstrated the ability of PEI-PEG copolymer layers to resist biologically nonspecific fouling and to prevent aggregation of the nanoparticles in physiological environments. Several other studies have also discussed about the stability and nonspecific protein binding of PEGylated silica nanoparticles in physiological condition^{34~36}. However, along with stability and reduced nonspecific protein binding, some additional properties such as stimuli sensitivity and/or inflammation reduction ability will be highly desirable in the biomedical application including drug delivery. Recently, we reported a simple method for the synthesis of PEGylated silica nanoparticles by matrix-catalyzed hydrolysis of TEOS in the presence of PEGylated polyamine nanogel³⁷. Hayashi et.al.

previously reported that the PEGylated nanogel is composed of a cross-linked poly[2-(N,N-diethylamino)ethyl methacrylate] (PEAMA) core and a tethered PEG shell³⁸⁾. At the end of the tethered PEG chain, an acetal functional group is present where we can easily install biomolecules or drugs in order to deliver them for therapeutic treatment. The PEGylated nanogel shows high stability against extremely dilute and high salt conditions, excellent biocompatibility, negligible toxicity, and prolonged blood circulation³⁹⁾. The nanogel shows significant volume phase transitions (swelling/deswelling) in response to pH changes^{38, 40)}. Silica nanoparticles were obtained in the nanogel core as shown in **Fig. 3**. The prepared PEGylated nanogel containing silica nanoparticles (SiNG) showed excellent stability under physiological conditions. The prepared SiNG also show reversible volume phase transitions in

Fig. 3 TEM photograph of SiNG. The photograph is reproduced from ref. 37 with the permission of © 2012 Elsevier B.V.

Fig. 4 pH dependency of the diameters of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles measured by DLS in water containing 0.15 M NaCl at 37°C; detector position at 173° with incident laser beam.

response to pH changes similar to those of the original nanogel as shown in **Fig. 4**. Considering the high stability in physiological condition, functionality, and pH sensitivity of the prepared SiNG, it is expected that this promising material can be used in several biomedical applications, such as bioimaging and drug delivery system. SiNG can be a suitable vehicle for bioimaging by doping metals or fluorescent dye on the silica surfaces present in the core of the particles. An attempt was taken to synthesize core-shell silica-gold hybrid nanoparticles in the nanogel core which will be discussed in the later section.

More recently, we designed and synthesized a PEGylated silica nanocomposite consisting of silica nanoparticles in the core of an amphiphilic block copolymer, poly(ethylene glycol)-b-poly(4-[2,2,6,6-tetramethylpiperidine-1-oxyl]amino- methylstyrene) (PEG-b-PMNT), possessing stable nitroxide radicals in the hydrophobic segment as a side chain via an amine linkage⁴¹⁾. Yoshitomi et. al. reported previously that PEG-b-PMNT forms core-shell type micelles in aqueous medium termed redox nanoparticle (RNP), and shows reactive oxygen species (ROS) scavenging ability⁴²⁾. The nanocomposites of silica and RNP (siRNP) synthesized in this study showed extremely high stability over very low pH conditions such as pH 1.2, and high drug (Rebamipide, an anti-ulcer drug) loading capacity. Accordingly, the siRNP is very promising as an oral drug carrier. We evaluated the ability of siRNP as oral rebamipide carrier, however, will discuss detail in the following Section 5.

4. PEGylated silica nanoparticles for bioimaging

Silica nanoparticle is an excellent building block for contrast agent in bioimaging. Doping of silica particles with rare earth metals is one of the common methods to prepare contrast agent for bioimaging. Several studies have been reported about rare earth doping of silica for the predation of contrast agents for bioimaging so far^{43~46)}. For example, Bhakta et.al. reported the method of preparation of gadolinium oxide-doped silica nanoparticles whose surface has been functionalized to anchor DNA onto it. The gadolinium oxide-doped silica nanoparticles are paramagnetic as observed from the nuclear magnetic resonance (NMR) line-broadening effect on proton spectrum of the surrounding water. Gadolinium oxide encapsulated silica

Fig. 5 Schematic representation of the synthesis of PEGylated nanogel containing SiO₂/Au hybrid nanoparticles.

nanoparticles conjugated with DNA showed about 75% and 77% *in vitro* transfection efficiencies in comparison with the Polyfect in COS-7 and 293T cells, respectively. From these investigations it is evident that the surface-modified silica nanoparticles were able to facilitate the transfer of plasmid DNA into mammalian cells *in vitro*. Considering the chemical inertness and biocompatibility of silica, this technology may provide a new platform for nonviral gene delivery as well as a contrasting agent in magnetic imaging.

The silica-based nanoparticles can be easily conjugated with any fluorophore type, leading to the generation of robust, fluorescent nanoparticles^{47,48}. In recent years, organically modified silicate (ORMOSIL) nanoparticles doped with organic dyes have been reported as promising probes for safe *in vivo* bioimaging⁴⁹. These nanoparticles facilitate optical bioimaging in the near-infrared (NIR) window, with maximum tissue penetration of light and minimum background signal^{50,51}.

Another promising silica based diagnostic tool gold nanoshell was reported in 1998 by Oldenburg et al⁵². The nanoshell is a composite spherical nanoparticle consisting of a dielectric silica core covered by a thin gold shell. It possesses highly favorable optical and chemical properties for biomedical imaging and therapeutic applications. By varying the thickness ratio of the core and the shell, the optical resonance of these nanoparticles can be tuned over a broad region ranging from the near-UV to the mid-infrared. This range includes the NIR wavelength region where tissue transmissivity peaks. In addition to spectral tunability, nanoshells offer other advantages over conventional organic dyes including improved optical properties and reduced susceptibility to chemical/thermal denaturation. We recently reported a simple method for the synthesis of SiO₂/Au hybrid nanoparticles by

Fig. 6 UV-vis absorption spectra of the pH-responsive PEGylated nanogel containing SiO₂/Au hybrid nanoparticles prepared at various pH conditions. The figure is reproduced from ref. 37 with the permission of © 2012 Elsevier B.V.

matrix-catalyzed hydrolysis of TEOS, followed by the reduction of HAuCl₄ in presence of PEGylated polyamine nanogel³⁷. A schematic diagram of the synthesis of the SiO₂/Au hybrid nanoparticles inside the pH-responsive PEGylated nanogel is shown in Fig. 5. In the first step, SiNG was prepared as described in the previous section (Fig. 3). Then, HAuCl₄ was added into the SiNG solution at pH 9.0 condition. As a result of the reduction of HAuCl₄ by the deprotonated tertiary amino groups at pH 9.0 condition surrounding the silica particles, SiO₂/Au hybrid nanoparticles were formed in the nanogel core. The obtained PEGylated nanogel containing SiO₂/Au hybrid nanoparticles show optical resonance at longer wavelength (618 nm) as compared to the optical resonance of typical gold nanoparticles (520 nm), as shown in Fig. 6. However, the absorption at 618 nm is not as longer wavelength as typical nanoshell shows at the NIR region. This might be due to the thicker gold layer (~40 nm) grown on smaller silica particles (~8 nm). Further studies are needed to control the thickness of gold layer on the controlled silica surface in order to obtain the absorption at the NIR region. However, our prepared

Fig. 7 a) Size distribution of the pH-responsive PEGylated nanogel containing SiO_2/Au hybrid nanoparticles at different pH conditions, and b) pH dependency of the diameters of the pH-responsive PEGylated nanogel containing SiO_2/Au hybrid nanoparticles, measured by DLS in water containing 0.15 M NaCl at 37°C ; detector position at 173° with incident laser beam. Both figures are reproduced from ref. 37 with the permission of © 2012 Elsevier B.V.

PEGylated nanogel containing SiO_2/Au hybrid nanoparticles show extremely high stability under physiological conditions, and show reversible volume phase transition (swelling) in response to changes in pH as shown in Fig. 7 a) and b) respectively.

5. PEGylated silica nanoparticles for drug delivery

From last few decades, drug delivery systems have been moving from sustained, but uncontrolled delivery to controlled-release systems. Enhance therapeutic efficacy and patient compliances by using controlled-release systems are driving the demand for versatile, high-performance controlled-release systems. Controlled delivery systems ensure both the control of release rate of a drug, and the site specific delivery. The selective augmentation of drug concentrations within the target sites is a major challenge in reducing the severity of side-effects as well as in improving therapeutic efficacy^{53~55}. Several nano-sized drug carriers, which include water-soluble polymer-drug conjugates^{56~57}, long-circulating liposomes^{58~60}, and polymeric micelles^{61, 62}, have been developed to deliver precisely and safely the appropriate concentrations of drugs to the target sites. Some of these carriers have been approved for clinical use⁶³ or are currently in clinical trials^{64, 65}, since they show extended bioavailability with reduced nonspecific accumulation in normal tissues and preferential accumulation in target sites due to the EPR effect⁶⁶. However, even though these carriers reach the target sites, the concentrations of the active anticancer

drugs within the target cells are often insufficient due to inefficient release of the drugs from the carriers into the cytoplasm, which leads to a requirement for higher dosages of the anticancer drugs⁶⁷. In addition, the carrier matrixes often cause inflammation by generating ROS⁶⁸, which reduces the therapeutic efficacy. Recently, silica nanoparticles became very good candidates for drug delivery systems^{10, 11}, because, they show the desirable features of hydrophilicity, biocompatibility and low toxicity unlike well-known delivery platforms such as micelles, liposomes, and polymers⁶⁹. Several research groups reported the potential of surface modified silica nanoparticles in drug delivery systems^{70~74}. One of our recent works is reported about the design and synthesis of siRNP for oral drug delivery system using an amphiphilic block copolymer micelle⁴¹, as mentioned in section 3. Yoshitomi et.al. reported previously that the block copolymer, PEG-b-PMNT, possesses stable nitroxide radicals in its hydrophobic segment as a side chain via an amine linkage, and forms core-shell type micelles in aqueous medium termed RNP⁴². Nitroxide radicals are confined in the core of the micelle which can catalytically scavenge ROS^{75, 76}. The *in vivo* studies have been confirmed that the orally administered RNP shows protective effect on non-steroidal anti-inflammatory drugs (NSAID)-induced small intestinal inflammation⁷⁷, and reduces dextran sodium sulfate (DSS)-induced ulcerative colitis⁷⁸ as a result of scavenging ROS in the inflammation area. However, the nanocomposite, siRNP, is anticipated to 1) have ROS scavenging ability due to

presence of nitroxide radical in the nanocomposite core; and 2) have an increased drug loading capacity through adsorption on the silica surfaces. It has been confirmed that the prepared siRNP shows extremely high stabilities in very low pH conditions such as pH 1.2, and high rebamipide (an anti-ulcer drug) loading capacities. When the rebamipide-loaded siRNP (rebamipide@siRNP) was administered orally, a significant increase in the blood uptake of rebamipide was observed when compared to orally administered free rebamipide. Because siRNP possesses nitroxide radicals, which catalytically scavenge ROS, rebamipide@siRNP did not cause any inflammation in gastrointestinal (GI) tract. Rather, it is confirmed to suppress inflammation of DSS-induced colitis model mice effectively. As it is mentioned that the siRNP is designed for oral drug delivery system, however, it is very important to ensure the stability of the nanocomposite in gastric environment because successfully delivering drugs via oral administration highly depends on the stability of the drug-loaded carrier particles in the stomach, a low pH environment. In order to check the stability of the particles under stomach conditions, the size distributions of rebamipide@siRNP was measured by DLS in a pH 1.2 hydrochloric acid buffer solution, with a NaCl concentration of 150 mM, at 37°C. After 4 h of incubation, rebamipide@siRNP showed uniform size distributions with a low polydispersity index of approximately 0.147, suggesting the high stability of the particles under conditions of very low pH, as shown in Fig. 8. Another point in oral drug delivery system is the release behavior of drugs by the carrier matrixes. Avoid releasing in stomach (pH 1.2), and enhance releasing in intestinal track (pH 7~9) are desirable to improve the oral bioavailability of the drugs through the absorption by intestinal membranes.

The drug-release behavior of rebamipide@siRNP particles was measured *in vitro* and *in vivo* to study their potential uses in drug delivery systems. In order to better mimic the *in vitro* release of rebamipide from the as-prepared siRNP nanocomposites, simulated intestinal fluid (PBS, pH 7.4) and simulated gastric fluid (HCl buffer solution, pH 1.2) were chosen as the release fluids. The release percentages of rebamipide from the nanocomposite particles as a function of time are shown in Fig. 9. At the physiological pH (around 7.4), the silanol groups (Si-OH) in the positively-charged

Fig. 8 Size distributions of 5 % rebamipide@siRNP after 4 h of incubation at pH 1.2, as measured by DLS in a 0.15 M NaCl solution at 37°C; detector position at 173° with incident laser beam. The figure is reproduced from ref. 41 with the permission of © 2014 Informa Plc.

Fig. 9 The release of rebamipide from siRNP nanocomposites at pH 7.4 (●) and 1.2 (■), as a function of time, at 37°C. The figure is reproduced from ref. 41 with the permission of © 2014 Informa Plc.

nanocomposite core should become deprotonated, and a strong electrostatic repulsion between the negative charges of SiO⁻ groups and the negative charge of rebamipide molecule should be generated. Consequently, the pH value of 7.4 in the physiological buffer promoted the release of the anionic molecules. Under the low pH conditions of the simulated gastric fluid (pH 1.2), the Si-OH groups in the positively-charged nanocomposite core were fully protonated, so that it was highly positive. Accordingly, rebamipide molecules have a tendency to be adsorbed strongly on the silica surface via electrostatic interaction. The *in vivo* studies showed that higher accumulation of rebamipide was obtained in the intestinal area as shown in Fig. 10. This fact agrees with the *in vitro* release data where an accelerated release was observed under pH 7.4 conditions. Considering the results just discussed, rebamipide-installed siRNP would be successful as an oral drug carrier. This promising siRNP would also be

Fig. 10 The amount of rebamipide accumulated in different parts of the small intestine after oral administration for rebamipide@siRNP (■) and free rebamipide (●) at predetermined time intervals and for different administered doses, a) 5 mg/kg, duodenum; b) 5 mg/kg, jejunum; c) 5 mg/kg, ileum; d) 10 mg/kg, duodenum; e) 10 mg/kg, jejunum; f) 10 mg/kg, ileum; g) 20 mg/kg, duodenum; h) 20 mg/kg, jejunum; i) 20 mg/kg, ileum. Each data point is expressed as a mean of the data obtained from 5 mice. The error bar represents the standard error of the mean (SEM), * = P value < 0.05 , ** = P value < 0.01 , and *** = P value < 0.001 vs. free rebamipide groups. The figure is reproduced from ref. 41 with the permission of © 2014 Informa Plc.

used for oral delivery of other drugs such as insulin. The oral anti-cancer therapy by delivering anti-cancer drugs such as doxorubicin using siRNP would be effective because siRNP could reduce the severe side-effects of doxorubicin by scavenging ROS.

6. Conclusion

The development of nano-biomaterials for biomedical applications has been driven by the demand for highly efficient systems for bioapplications, such as clinical diagnostics and drug delivery. Among the various developed systems, PEGylated silica-based nanocomposites have shown their notable advantages

including biocompatibility and versatility. A variety of *in vitro* and *in vivo* studies have demonstrated the advantages of using PEGylated silica-based nanocomposites in biomedical applications. As oral drug delivery became the most popular and successful route for drug administration because of patient convenience and compliance, however, many drugs are currently used as parenteral formulations because of their poor oral bioavailability. Using PEGylated silica-based materials as a carrier or device could open a new door for improving the bioavailability of orally administered drugs. As oral anti-cancer therapy is still hardly studied and reported, it would be a new area of

research where PEGylated silica-based materials could show their potential as a carrier or device for orally administered anti-cancer drugs. Our prepared siRNP would be expected to be a promising carrier for orally administered anti-cancer drugs. siRNP could improve the delivery of anti-cancer drugs in the intestinal area, and could reduce the toxicity of drugs to the GI track by scavenging ROS; which might enhance the therapeutic effect of anti-cancer drugs. However, the toxicity of anti-cancer drugs after absorption to the blood-stream, and their accumulation are still needed to be taken

into account in order to achieve successful oral anti-cancer therapy.

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Abbreviations and Terminologies

Abbreviations/Terms	Elaborations/meanings
293T cells	Human Embryonic Kidney 293 cells
BSA	Bovine serum albumin
COS-7 cells	The COS-7 cell line was derived from the kidney of the African Green Monkey, <i>Cercopithecus aethiops</i> .
EPR effect	Enhanced permeability and retention effect
GI track	Gastrointestinal tract
<i>In vitro</i>	In an artificial environment outside a living organism.
<i>In vivo</i>	An experiment that is done inside a living organism.
NIR	Near-infrared
P value	P-value is a statistical term by which the significant difference of two data sets can be evaluated.
PEG	Polyethylene glycol
PEGylation	Surface coating with polyethylene glycol
PEI	Polyethyleneimine
Polyfect	PolyFect is a Transfection Reagent that has been specifically developed and designed for transfection of commonly used cell lines such as COS-7, NIH/3T3, HeLa, 293, and CHO cells.
RES	Reticuloendothelial system
RNP	Redox nanoparticle
ROS	Reactive oxygen species
Rebamipide@siRNP	Rebamipide-loaded siRNP
SiNG	PEGylated nanogel containing silica nanoparticles
siRNP	The nanocomposites of silica and RNP
TEOS	Tetraethyl orthosilicate

References

- 1) W. Stöber, A. Fink and E. Bohn, *J. Colloid Interface Sci.*, **26**, 62 (1968).
- 2) K. Lee, J.-L. Look, M. T. Harris and A. V. McCormick, *J. of Colloid and Interface Sci.*, **194**, 78 (1997).
- 3) I. I. Slowing, B. G. Trewyn, S. Giri and V. S. Y. Lin, *Adv. Funct. Mater.*, **17**, 1225 (2007).
- 4) C. W. Lu, Y. Hung, J. K. Hsiao, M. Yao, T. H. Chung, Y. S. Lin, S. H. Wu, S. C. Hsu, H. M. Liu, C. Y. Mou, C. S. Yang, D. M. Huang and Y. C. Chen, *Nano Lett.*, **7**, 149 (2007).
- 5) Y. S. Lin, C. P. Tsai, H. Y. Huang, C. T. Kuo, Y. Hung, D. M. Huang, Y. C. Chen and C. Y. Mou, *Chem. Mater.*, **17**, 4570 (2005).
- 6) L. Wang, C. Y. Yang and W. H. Tan, *Nano Lett.*, **5**, 37 (2005).
- 7) M. Vallet-Regí, F. Balas and D. Arcos, *Angew. Chem., Int. Ed.*, **46**, 7548 (2007).
- 8) J. Lu, M. Liong, J. I. Zink and F. Tamanoi, *Small*, **3**, 1341 (2007).
- 9) C. Barbe, J. Bartlett, L. G. Kong, K. Finnie, H. Q. Lin, M. Larkin, S. Calleja, A. Bush and G. Calleja, *Adv. Mater.*, **16**, 1959 (2004).
- 10) I. Roy, T. Y. Ohulchanskyy, H. E. Pudavar, E. J. Bergey, A. R. Oseroff, J. Morgan, T. J. Dougherty and P. N. Prasad, *J. Am. Chem. Soc.*, **125**, 7860 (2003).
- 11) I. Roy, T. Y. Ohulchanskyy, D. J. Bharali, H. E. Pudavar, R. A. Mistretta, N. Kaur and P. N. Prasad, *Proc. Natl. Acad. Sci., USA*, **102**, 279 (2005).
- 12) E. H. Chowdhury and T. Akaike, *Curr. Gene Ther.*, **5**, 669 (2005).
- 13) K. M. L. Taylor, J. S. Kim, W. J. Rieter, H. An, W. Lin and W. Lin, *J. Am. Chem. Soc.*, **130**, 2154 (2008).
- 14) D. Gerion, J. Herberg, R. Bok, E. Gjersing, E. Ramon, R. Maxwell, J. Kurhanewicz, T. F. Budinger, J. W. Gray, M. A. Shuman and F. F. Chen, *J. Phys. Chem. C*, **111**, 12542 (2007).
- 15) W. J. Rieter, J. S. Kim, K. M. L. Taylor, H. Y. An, W. L. Lin, T. Tarrant and W. B. Lin, *Angew. Chem., Int. Ed.*, **46**, 3680 (2007).
- 16) S. T. Selvan, P. K. Patra, C. Y. Ang and J. Y. Ying, *Angew. Chem., Int. Ed.*, **46**, 2448 (2007).
- 17) Colloidal Silica, Fundamentals and Applications (ed.: H. E. Bergna and W. O. Roberts), *CRC Press, Boca Raton, USA* (2006).
- 18) A. Vanblaaderen, J. Vangeest and A. Vrij, *J. Colloid Interface Sci.*, **154**, 481 (1992).
- 19) X. He, H. Nie, K. Wang, W. Tan, X. Wu and P. Zhang, *Anal. Chem.*, **80**, 9597 (2008).
- 20) M. M. van Schooneveld, E. Vucic, R. Koole, Y. Zhou, J. Stocks, D. P. Cormode, C. Y. Tang, R. E. Gordon, K. Nicolay, A. Meijerink, Z. A. Fayad and W. J. M. Mulder, *Nano Lett.*, **8**, 2517 (2008).
- 21) S. M. Moghimi, A. C. Hunter and J. C. Murray, *Pharmacol. Rev.*, **53**, 283 (2001).
- 22) Stealth Liposomes (Eds: D. Lasic and F. Martin), *CRC Press, Boca Raton, USA* (1995).
- 23) L. E. van Vlerken, T. K. Vyas and M. M. Amiji, *Pharm. Res.*, **24**, 1405 (2007).
- 24) A. G. Kanaras, F. S. Kamounah, K. Schaumburg, C. J. Kiely and M. Brust, *Chem. Commun.*, **20**, 2294 (2002).
- 25) G. S. Kwon, *Crit. Rev. Ther. Drug Carrier Syst.*, **20**, 357 (2003).
- 26) R. Gref, Y. Minamitake and M. T. Peracchia, *Science*, **263**, 1600 (1994).
- 27) K. Knop, R. Hoogenboom, D. Fischer and U. S. Schubert, *Angew. Chem. Int. Ed. Engl.*, **49**, 6288 (2006).
- 28) G. J. Soler-Illia, Clement Sánchez, B. Lebeau and J. Patarin, *Chem. Rev.*, **102**, 4093 (2002).
- 29) R. D. Badley, W. T. Ford, F. J. Mcenroe and R. A. Assink, *Langmuir*, **6**, 792 (1990).
- 30) C. Yagüe, M. Moros, V. Grazú, M. Arruebo and J. Santamaria, *Chemical Engineering Journal*, **137**, 45 (2008).
- 31) D. E. Owens and N. A. Peppas, *Int. J. Pharm.*, **307**, 93 (2006).
- 32) J. Andrade, V. Hlady, A. P. Wei, C. H. Ho, A. Laea, S. Y. Jeon and E. Dtraup, *J. Clin. Mater.*, **11**, 67 (1992).
- 33) B. Thierry, L. Zimmer, S. McNiven, K. Finnie, C. Barbé and H. J. Griesser, *Langmuir*, **24**, 8143 (2008).
- 34) I. M. Rio-Echevarria, F. Selvestrel, D. Segat, G. Guarino, R. Tavano, V. Causin, E. Reddi, E. Papini and F. Mancin, *J. Mater. Chem.*, **20**, 2780 (2010).
- 35) F. Branda, B. Silvestri, G. Luciani, A. Costantini and F. Tescione, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **367**, 12 (2010).
- 36) P. K. Sudeep, Z. Page and T. Emrick, *Chem. Commun.*, **46**, 6126 (2008).
- 37) M. A. Hossain, Y. Ikeda, T. Hara and Y. Nagasaki, *Colloids and Surfaces B: Biointerfaces*, **102**, 778 (2013).
- 38) H. Hayashi, M. Iijima, K. Kataoka and Y. Nagasaki, *Macromolecules*, **37**, 5389 (2004).
- 39) M. Tamura, S. Ichinohe, A. Tamura, Y. Ikeda and Y. Nagasaki, *Acta Biomater.*, **7**, 3354 (2011).
- 40) M. Oishi and Y. Nagasaki, *Nanomedicine*, **5**, 451 (2010).
- 41) M. A. Hossain, M. Yamashita, L. B. Vong, Y. Ikeda and Y. Nagasaki, *Journal of Drug Targeting*, **22**, 638 (2014).
- 42) T. Yoshitomi, A. Hirayama and Y. Nagasaki, *Biomaterials*, **32**, 8021 (2011).
- 43) G. Bhakta, R. K. Sharma, N. Gupta, S. Cool, V. Nurcombe and A. Maitra, *Nanomedicine: Nanotechnology, Biology, and Medicine*, **7**, 472 (2011).
- 44) F. Enrichi, R. Ricco, P. Scopece, A. Parma, A. R. Mazaheri, P. Riello and A. Benedetti, *J Nanopart Res*, **12**, 1925 (2010).
- 45) X. H. Chuai, H. J. Zhang, F. Sh. Li, S. B. Wang and G. Z. Zhou, *Materials Letters*, **46**, 244 (2000).
- 46) B. Yan and J. Y. You, *Inorganic Materials*, **42**, 1046 (2006).
- 47) H. Ow, D. R. Larson, M. Srivastava, B. A. Baird, W. W. Webb and U. Wiesner, *Nano Lett.*, **5**, 113 (2005).
- 48) X. Zhao, L. R. Hilliard, S. J. Mechery, Y. Wang, R. P. Bagwe, S. Jin and W. Tan, *Proc. Natl Acad. Sci. USA*, **101**, 15027 (2004).
- 49) R. Kumar, I. Roy, T. Y. Ohulchanskyy, L. A. Vathy, E. J. Bergey, M. Sajjad and P. N. Prasad, *ACS Nano*, **4**, 699 (2010).
- 50) R. Weissleder, *Nature Biotechnol.*, **19**, 316 (2001).
- 51) W. C. Chan, D. Maxwell, X. Gao, R. Bailey, M. Han and S. Nie, *Curr. Opin. Biotechnol.*, **13**, 40 (2002).
- 52) S. J. Oldenburg, R. D. Averitt, S. L. Westcott and N. J. Halas, *Chemical Physics Letters*, **288**, 243 (1998).
- 53) R. Duncan, *Nat. Rev. Drug Discovery*, **2**, 347 (2003).
- 54) H. Maeda, *Adv. Drug Delivery Rev.*, **46**, 149 (2001).
- 55) R. K. Jain, *Eur. J. Pharm. Sci.*, **20**, 1 (2003).

-
- 56) D. Avichechter, B. Schechter and R. Arnon, *React. Funct. Polym.*, **36**, 59 (1998).
- 57) A. Jr. Bogdanov, S. C. Wright, E. M. Marecos, A. Bogdabova, C. Martin, P. Petherick and R. Weissleder, *J. Drug Targeting*, **4**, 321 (1997).
- 58) C. M. Lee, T. Tanaka, T. Murai, M. Kondo, J. Kimura, W. Su, T. Kitagawa, T. Ito, H. Matsuda and M. Miyasaka, *Cancer Res.*, **62**, 4282 (2002).
- 59) A. N. Gordon, J. T. Fleagle, D. Guthrie, D. E. Parkin, M. E. Gore and A. J. Lacave, *J. Clin. Oncol.*, **19**, 3312 (2001).
- 60) M. S. Newman, G. T. Colbern, P. K. Working, C. Engbers and M. A. Amantea, *Cancer Chemother. Pharmacol.*, **43**, 1 (1999).
- 61) T. Nakanishi, S. Fukushima, K. Okano, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai and K. Kataoka, *J. Controlled Release*, **74**, 295 (2001).
- 62) M. Yokoyama, T. Okano, Y. Sakurai, S. Suwa and K. Kataoka, *J. Controlled Release*, **39**, 351 (1996).
- 63) H. Maeda, T. Sawa and T. Konno, *J. Controlled Release*, **74**, 47 (2001).
- 64) T. Lian and R. J. Y. Ho, *J. Pharm. Sci.*, **90**, 667 (2001).
- 65) P.A.Vasey, S.B.Kaye, R.Morrison, C.Twelves, P.Wilson, R. Duncan, A. H. Thoson, L. S. Murray, T. E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio and J. Cassidy, *Clin. Cancer Res.*, **5**, 83 (1999).
- 66) Y. Matsumura and H. Maeda, *Cancer Res.*, **46**, 6387 (1986).
- 67) Y. Tsukioka, Y. Matsumura, T. Hamaguchi, H. Koike, F. Moriyasu and T. Kakizone, *Jpn. J. Cancer Res.*, **93**, 1145 (2002).
- 68) Yang YW and Hsu PYJ, *Biomaterials*, **29**, 2516 (2008).
- 69) D. Luo and W. M. Saltzman, *Gene Ther.*, **13**, 585 (2005).
- 70) Li Tang, Timothy M. Fan, Luke B. Borst, and Jianjun Cheng, *ACS Nano*, **6**, 3954 (2012).
- 71) W. Qu, Y. Li, L. Hovgaard, S. Li, W. Dai, J. Wang, X. Zhang and Q. Zhang, *International Journal of Nanomedicine*, **7**, 4983 (2012).
- 72) M. Liu, L. Gan, L. Chen, Z. Xu, D. Zhu, Z. Hao, and L. Chen, *Langmuir*, **28**, 10725 (2012).
- 73) S. Radin, S. Falaize, M. H. Lee and P. Ducheyne, *Biomaterials*, **23**, 3113 (2002).
- 74) P. Kortessuo, M. Ahola, S. Karlsson, I. Kangasniemi, A. Yli-Urpo and J. Kiesvaara, *Biomaterials*, **21**, 193 (2000).
- 75) B. P. Soule, F. Hyodo, K. Matsumoto, N. L. Simone, J. A. Cook, M. C. Krishna and J. B. Mitchell, *Free Radic. Biol. Med.*, **42**, 1632 (2007).
- 76) M. C. Krishna, A. Russo, J. B. Mitchell, S. Goldstein, H. Dafni and A. Samuni, *J. Biol. Chem.*, **271**, 26026 (1996).
- 77) S. Sha, L. B. Vong, P. Chonpathompikunlert, T. Yoshitomi, H. Matsui and Y. Nagasaki, *Biomaterials*, **34**, 8393 (2013).
- 78) L. B. Vong, T. Tomita, T. Yoshitomi, H. Matsui and Y. Nagasaki, *Gastroenterology*, **143**, 1027 (2012).
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