TEMPERATURE-SENSITIVE POLYMER-GOLD NANOCOMPOSITES AS INTELLIGENT THERAPEUTIC SYSTEMS

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Introduction

Research in the field of biomaterials and drug delivery is moving toward individually tailored intelligent therapeutic systems which are capable of responding to and correcting undesirable conditions, on a molecular level, within the body (1, 2). These systems will mimic natural biosystems in their size, structure, and function, and will therefore need to be synthesized using advanced nanofabrication techniques. The development of these and other nanoscale polymer systems is of interest in modern research as well, due to the possibility of combining the advantages of traditional macroscale polymer properties, such as swelling, with nanoscale properties, such as faster drug release rates due to shorter characteristic lengths and spatially localized heating effects. Also, due to the size limitations imposed by the body's natural defense mechanisms, such as the reticuloendothelial system, polymer systems 0.3 μm in diameter and smaller will be required for the development of effective *in vivo* intelligent therapeutic systems (3-5).

Over the last few years a variety of parenteral synthetic polymer systems (i.e. biodegradable, osmotic, pH responsive, and others) and medical devices (i.e. insulin pumps) have been developed in the hopes of achieving intelligent therapeutic function (1, 6-8). However, to date none of these systems has been able to successfully combine the aspects of molecular recognition, biocompatibility, intelligent response, and non-invasive external therapeutic control. Therefore, there is a need to design intelligent drug delivery modalities that can synergistically incorporate all of these capabilities into one complete system. Intelligent therapeutic systems based on thermally responsive metal-polymer nanocomposites have the ability to achieve these goals and thereby revolutionize drug delivery and disease treatment.

The intelligent therapeutic nanocomposite systems presented here are composed of a metal nanoparticle core, surrounded by a temperature-responsive interpenetrating polymer network (IPN) layer, which encapsulates desired molecules or compounds, and exhibits a sharp positive swelling transition, which is ideal for drug delivery applications. The metal nanoparticle core of these systems can be tuned to absorb visible to near infrared (530 – 1200 nm) wavelengths of light depending on either the core to shell thickness in metal nanoshells or the diameter in solid metal nanoparticles. This absorbed light energy is then converted to heat and transmitted locally to the surrounding IPN layer, which triggers the swelling of this temperature-response polymer layer and hence the triggered release of any encapsulated agents.

Experimental Part

Acrylamide (AAm), acrylic acid (AA), N,N'-methylenebisacrylamide (MBAAm), and polyethylene glycol laurylether (Brij 30) were obtained from Aldrich, ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were obtained from FisherBiotech, and sodium bis(2-

ethylhexyl) sulfosuccinate (AOT) was obtained from Fluka. All were used as received for the preparation of the PAAm/PAA interpenetrating polymer network layer.

Tetrakis(hydroxymethyl)phosphonium chloride (THPC), 3-aminopropyltriethoxysilane (APTES), and sodium citrate tribasic dehydrate were obtained from Aldrich, potassium carbonate and formaldehyde were obtained from Fisher Scientific, chloroauric acid was obtained from Acros Organics, methoxy poly(ethylene glycol) (MW = 5,000) thiol was obtained from Nektar and 0.33 μm monodisperse silica microspheres were obtained from Bangs Laboratories. All were used as received for the preparation of solid gold nanospheres and gold-silica nanoshells.

Solid gold nanospheres (~50 nm diameter) were prepared via the common technique of citrate reduction, which has been previously described in detail (9). Briefly, a 50 ml solution of 0.25 mM chloroauric acid was prepared in a round bottom flask and dark aged overnight. The chloroauric acid solution was then brought to a boil under reflux and 0.5 ml of 40 mM sodium citrate was injected into the round bottom flask under vigorous stirring. This solution was allowed to react for 1 hour at room temperature and then capped with the addition of enough mPEG-SH to achieve a final concentration of 1 μM.

Gold nanoshells (~600 nm diameter) were also prepared as previously described (10). Briefly, 330 nm diameter silica particles were surface terminated with amine groups by reaction with APTES in ethanol. These particles were then seeded with small gold clusters, which associate with the amine group of the APTES on the surface of the silica particles. Finally, a complete gold nanoshell was then formed around the silica particles by the growth and eventual coalescence of the surface gold seeds via the reduction of aqueous gold from solution onto the seed particles.

Polymer-gold nanocomposites were then formed using a two step sequential IPN synthesis method. First, gold nanoparticles were encapsulated inside of polyacrylamide nanoparticles by *in situ* inverse emulsion polymerization with the gold nanoparticles located in the aqueous monomer droplet phase. This inverse emulsion solution consisted of an 81% hexane continuous phase, with a 13% surfactant phase (AOT and Brij 30 in a 2:1 ratio), and a 6% aqueous phase. In a typical experiment, 1 ml of previously prepared gold nanoparticles suspended in aqueous solution were added directly to a 3-neck round bottom flask containing the entire hexane continuous phase, and equipped with a condenser, nitrogen purge line, and overhead mechanical stirrer. Emulsifiers were then added to the mixture and dissolved under vigorous stirring. The remaining portion of the aqueous phase was then split into two separate premixes containing each containing 11.7% monomer, 4% crosslinker (MBAAm), 5.3% initiator (APS), and 79% deionized distilled water by weight. The only difference in these two premixes was that one contained only acrylamide monomer and the other only acrylic acid. In the first step of the sequential IPN synthesis method the acrylamide only aqueous premix was added to the same 3-neck round bottom flask already containing the hexane, gold particles, and emulsifiers. This mixture was then purged with nitrogen gas for 30 minutes to remove oxygen, after which the polymerization was initiated with the injection of TEMED (0.3 ml) and allowed to react for two hours at room temperature. After two hours the reaction was cooled to 0˚C and opened to the atmosphere to terminate the reaction. The second step of the IPN synthesis was then carried out by the addition of the acrylic acid only premix to the reaction vessel followed again by purging and the addition of TEMED. The final IPN polymer-gold nanocomposites were then purified and collected using reduced pressure to remove the hexane solvent phase and centrifugation in ethanol to precipitate out the particles. Finally these polymers were then dried and re-suspended in deionized distilled water.

Results

The size and relative shape of solid gold nanospheres and gold nanoshells were examined using a JOEL 2010F transmission electron microscope (TEM) operating at 200kV. The composition of these particles was also confirmed using an Oxford INCA energy dispersive spectroscopy (EDS) detector with a probe spot size of 0.5 nm attached to the JOEL 2010F TEM. Images (a) and (c) in Figure 1 illustrate

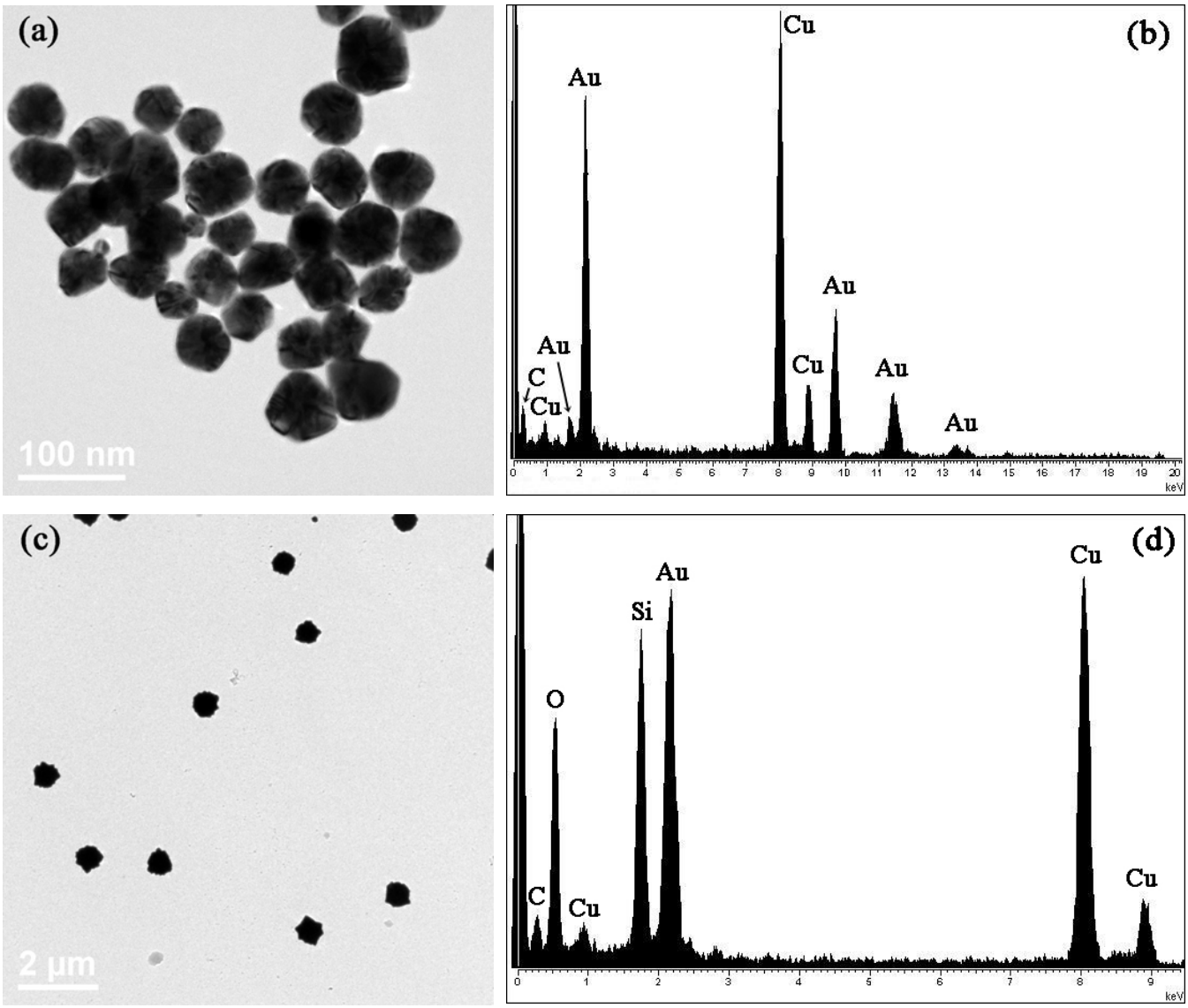

Figure 1. TEM image and individual particle EDS spectrum of both solid gold nanospheres (d ~50 nm) (a and b), and gold nanoshells (d ~600 nm) (c and d).

that the synthesized gold nanoparticles were roughly spherical in shape with an average diameter of ~50 nm for the solid gold spheres and ~600 nm for the gold nanoshells. The EDS spectrums, shown in Figure 1 (b) and (d), confirm that the particles in (a) are solid gold particles and the particles in (c) are gold-silica nanoshells. The copper and carbon peaks present in both the EDS spectrums are due to the carbon coated 300 mesh copper TEM support grids on which the samples were imaged.

The swelling behavior of the IPN nanoparticles was examined using a Brookhaven ZetaPlus dynamic light scattering (DLS) instrument operating at a 90˚ scattering angle with a 635 nm diode laser source. The IPN nanoparticles exhibited a positive sigmoidal swelling response with temperature at around 40 ± 5 ˚C with a linear swelling ratio increase of greater than four times the collapsed particle diameter (~ 300 nm) as shown in Figure 2.

The size, morphology, and composition of the final IPN polymer-gold nanocomposites were examined using a Philips EM 208 TEM operating at 100kV and a LEO 1530 field emission scanning electron microscope (FE-SEM) operating at 10kv with attached Oxford

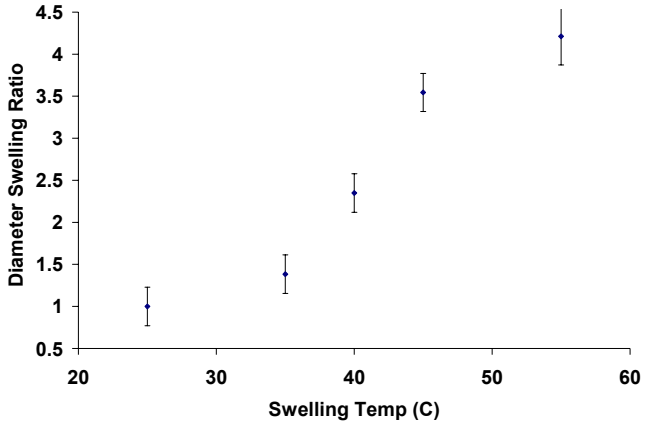

Figure 2. The change in hydrodynamic diameter of IPN nanospheres suspended in aqueous buffer as a function of temperature. Error bars represent one standard deviation, n=3.

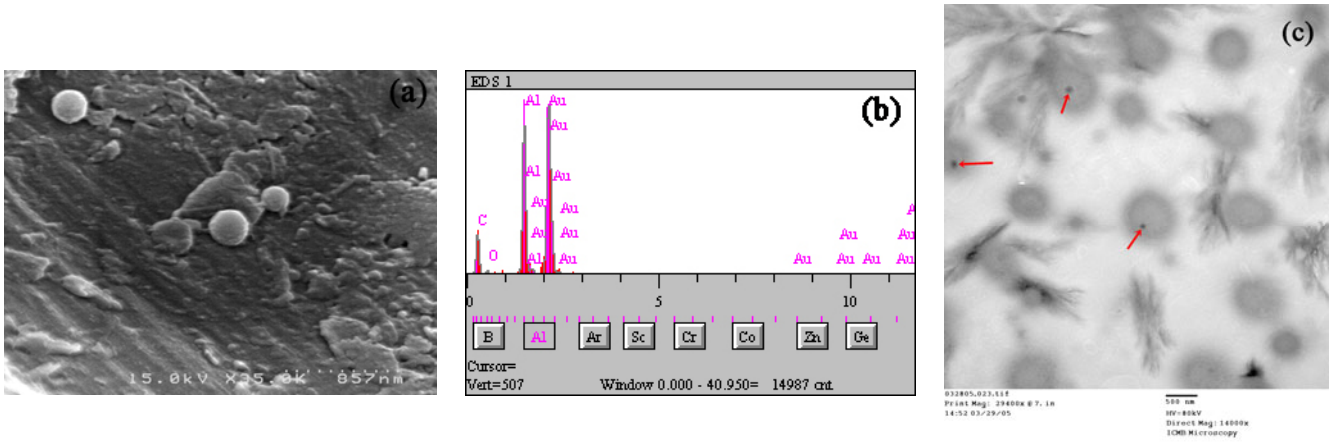

Figure 3. SEM (a), EDS (b), and TEM (c) images of polymer-gold nanocomposites. The red arrows in image (c) indicate the presence of gold spheres entrapped inside of the polymer particles.

INCA EDS detector. Figure 3a clearly illustrates that the final morphology of the polymer-gold nanocomposites is spherical with an overall particle diameter of roughly 300 nm. The EDS and TEM images in Figure 3b and 3c also confirm the presence of solid gold, ~50 nm diameter, nanoparticles inside of some of the nanocomposites as indicated by red arrows in Figure 3c and the presence of gold, carbon, and oxygen peaks in the EDS spectrum. The aluminum peak in the EDS is due to aluminum SEM stub of which the sample was mounted.

Discussion

This report illustrates the successful synthesis of a thermally responsive IPN polymer-gold nanocomposite system. TEM and EDS analysis confirm the successful synthesis and incorporation of solid gold nanoparticles inside of IPN nanospheres. DLS analysis also confirms that these IPN nanospheres exhibit a positive and sharp sigmoidal swelling response with increases in temperature. Therefore the swelling and hence therapeutic release of these systems can be controlled externally using

non-invasive laser heating of the gold nanoparticle cores of these systems making them an externally triggered intelligent therapeutic system. DLS studies of the laser induced swelling and therapeutic release properties of these novel thermally responsive polymer-gold nanocomposites will be the focus of future publications.

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