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Research review paper

Polymeric nanoparticles for optical sensing

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ABSTRACT

Nanotechnology is a powerful tool for use in diagnostic applications. For these purposes a variety of functional nanoparticles containing fluorescent labels, gold and quantum dots at their cores have been produced, with the aim of enhanced sensitivity and multiplexing capabilities. This work will review progress in the application of *polymeric* nanoparticles in optical diagnostics, both for *in vitro* and *in vivo* detection, together with a discussion of their biodistribution and biocompatibility.

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1. Introduction — nanotechnology and optical diagnostics

In the past decade, progress in nanotechnology has produced a wide variety of nanoparticles (NPs) useful for diagnostic applications. The use of nanotechnology in diagnostics is attractive because only a small volume of sample is required, allowing a low limit of detection (LOD) to be achieved. Often the use of NPs in diagnostic tests makes them faster to perform and more sensitive than comparable assays which use biomolecules (Jain, 2005). Furthermore, NPs possess unique optical and magnetic properties depending on their composition. Moreover, according to their intended application, nanoparticles can be engineered to impart the required properties. The use of NPs in molecular diagnostics can be termed "nanodiagnostics" and has been successfully employed both *in vivo* and *in vitro* assays (Jain, 2007). In recent years, the global market for *in vitro* diagnostics (IVD) has increased at an incredible rate; from US$44 billion in 2010 to a predicted US$60 billion by the end of 2014 (Renub-Research, 2010). The "point-of-care" sector represents the largest part of the IVD global market, followed by immunochemistry and molecular diagnostics (RNCOS, 2011).

Currently the most important application areas for polymeric NPs in optical diagnostics are biomarker analysis, cancer diagnosis, diagnostic imaging, and immunoassays. The binding of the target biomolecule to NPs is the pivotal step in most nanoparticle-based assays. In order to detect the target analyte, this binding should result in a measurable signal that can be quantified. For this purpose, the most commonly

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used labels are enzymes, which can catalyze the formation of colored products that can be detected by a change in color of a solution, or molecules/materials capable of emitting a fluorescent signal. Dyes have been popular tools employed in optical diagnostics, allowing the detection of analytes with good sensitivity, either through color changes or by emission of fluorescence. Unfortunately, dyes suffer from photobleaching and often have an asymmetric emission spectrum. Photostability is important in the case of prolonged observation, where photobleaching severely impacts on their capability to detect single molecules. Furthermore some dyes, such as fluoresceins and rhodamines, suffer from quenching phenomena when present in solution at high concentration (Demchenko, 2010). Despite the aforementioned drawbacks, organic dyes are widely employed due to their low cost and ease of use. When the dye is embedded in a polymeric matrix however, increased photostability is often observed due to the "protective" effect of the polymer. Furthermore, covalently coupling the dye to the polymer will also reduce the chance of leakage, which is possible with entrapped dyes, which can still diffuse out of the polymer. NPs can also contain hundreds of dye molecules which will increase the intensity of color or the brightness of emission. Furthermore, the hydrophobic microenvironment created within polymeric NPs can enhance the quantum yield of certain fluorescent reporters (Cui et al., 2013; Hu and Liu, 2010; Shiraishi et al., 2010; Wang et al., 2009). In addition, the presence of reactive functional groups along the polymer backbone allows specific labeling with other molecules as well as allowing the particle surface to be modified for specific applications.

Reporters for optical sensing can be divided into two main categories. The first includes reporters used as *molecular sensors* in systems capable of detecting changes in their surroundings and respond to the presence of specific molecules. The second involves reporters used as *labels*, the aim of which is to give rise to a fluorescent signal which depends only on the presence of the reporter in a particular area of a diagnostic device, without any variation in the fluorescence properties (Demchenko, 2010). The most important considerations when using labels are how to obtain high brightness and provide protection from the surrounding environment. The ideal molecular sensor should possess high molar absorption coefficient and high quantum yield, it should be photochemically and thermally stable, and give an optical response proportional to the concentration of the target analyte without any interactions with other analytes in the sample. A large Stokes shift is highly desirable for both applications because it allows the fluorescent signal to be detected without problems associated with the overlapping of excitation and emission spectra of the dye (Demchenko, 2005). For *in vivo* use, reporters must be non-toxic and their biodistribution should be well-known. Herein, we describe recent advances in the synthesis and applications of polymeric NPs in optical *in vitro* and *in vivo* diagnostics.

2. Synthesis of polymeric nanoparticles

Polymeric NPs can be made from organic polymers or inorganic materials such as silica. Generally, organic NPs are prepared either by polymerization of monomers or by processing of preformed polymers. In the latter case, the techniques most frequently employed are: solvent evaporation, salting-out, nanoprecipitation, dialysis and supercritical fluid technology (Rao and Geckeler, 2011). Otherwise, polymeric NPs can be produced from monomers by several methods, such as: dispersion, precipitation and interfacial polymerizations. For diagnostic applications, the most commonly employed techniques are emulsion and living free radical polymerization. The former is carried out in water as the dispersion medium, with or without the addition of surfactant, and allows excellent control to be exerted over the size distribution of the NPs produced, including the production of monodisperse emulsions. Similarly, the living free radical polymerization methods permit excellent control over the molecular weight, polydispersity and composition of NPs (Zetterlund et al., 2008). These latter procedures are based on the establishment of dynamic equilibria between a small number of growing radicals and a large majority of the dormant species. Even though this method can have some issues related to the colloidal stability of NPs, living polymerization is an excellent technique for the production of functionalized NPs. Moreover, in contrast to conventional free radical polymerizations, living polymerizations do not undergo exothermic autoacceleration, allowing superior control of parameters such as the polymer chain length and particle size.

Silica NPs are generally synthesized either in an emulsion-based technique performed in organic solvents or by the Stöber method. The first process requires large amounts of surfactant, whereas the Stöber method is performed in a mixture of water and ethanol in the absence of surfactants and allows spherical NPs to be obtained in a narrow size distribution. It was recently shown however that the silica layer present on NPs produced by the Stöber method can be inhomogeneous (Wong et al., 2011). In both approaches for the synthesis of NPs, dye can be added to the monomer mixture prior to initiating the polymerization, with the results that it becomes entrapped within the polymeric matrix. Copolymerization with a polymerizable form of the dye can eliminate the leaching of dye from the particle, but generally has a requirement

Table 1
Comparison of the most frequently employed methods for the synthesis of NP for biomedical application.

Organic nanoparticles			
Synthetic method	Particle size/initiator	Advantages	Drawbacks/issues
Mini- and micro-emulsion polymerization	10–200 nm Initiator: persulfate; azoisobutyronitrile	Performed both in water and organics. Good monodispersity	Size control. Surfactant needed to obtain smaller particles
Living radical polymerization	30–300 nm Initiator: alkoxyamines, thioesters, alkyl halides, iniferters	Performed both in water and organics. Ideal for synthesis of functionalized nanoparticles	Residues of initiator and surfactant (if employed). Colloidal stability
Precipitation and dispersion polymerization	200 nm–10 μm Initiator: persulfate; azoisobutyronitrile	With/without surfactant. Moderate-good monodispersity	Size control. Possible irregular shape. Difficult to obtain particles < 200 nm
Inorganic nanoparticles			
Synthetic method	Particle size/catalysis	Advantages	Drawbacks/issues
Microemulsion polymerization	10–70 nm Basic catalysis	Good monodispersity	Presence of surfactant. Performed in organics
Sol–gel method	50 nm–2 μm Acid/basic catalysis	Good monodispersity	Presence of surfactant
Stöber method	15 nm–2 μm Basic catalysis	Surfactant-free excellent monodispersity	Difficult to obtain particles <50 nm

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