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Review article

Fate of micelles and quantum dots in cells

Dusica Maysinger [a,*], Jasmina Lovrić [a,b], Adi Eisenberg [c], Radoslav Savić [a]

[a] Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada
[b] Department of Pharmaceutical Technology, University of Zagreb, Zagreb, Croatia
[c] Chemistry Department, McGill University, Montreal, Canada

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Abstract

Micelles and quantum dots have been used as experimental drug delivery systems and imaging tools both in vitro and in vivo. Investigations of their fate at the subcellular level require different surface-core modifications. Among the most common modifications are those with fluorescent probes, dense-core metals or radionucleids. Cellular fate of several fluorescent probes incorporated into poly(caprolactone)-b-copolymer micelles (PCL-b-PEO) was followed by confocal microscopy, and colloidal gold incorporated in poly 4-vinyl pyridine-PEO micelles were developed to explore micelle fate by electron microscopy. More recently, we have examined quantum dots (QDs) as the next-generation-labels for cells and nanoparticulate drug carriers amenable both to confocal and electron microscopic analyses. Effects of QDs at the cellular and subcellular levels and their integrity were studied. Results from different studies suggest that size, charge and surface manipulations of QDs may play a role in their subcellular distribution. Examples of pharmacological agents incorporated into block copolymer micelles, administered or attached to QD surfaces show how the final biological outcome (e.g. cell death, proliferation or differentiation) depends on physical properties of these nanoparticles.

Keywords: Block copolymer micelles; Quantum dots; Internalization; Stability; Confocal microscopy; Multiple labeling; Fluorescent dyes; Organelles

1. Block copolymer micelles

1.1. Definition and properties

Block copolymer micelles are nanosized (10–100 nm) particles dispersed in water. They are prepared from synthetic polymers that contain hydrophilic and hydrophobic parts. The hydrophilic part is commonly poly(ethylene oxide), PEO, and the hydrophobic part is varied from amino acids, polyesters and poloxamers [1]. PEO is usually used to decrease contact between macromolecules in the surrounding environment and surfaces coated with PEO. The molecular mass of PEO blocks [2] and surface density [3] must be tailored to achieve the protein repelling effect. Presence of PEO in the outer shell (exposed to the surrounding environment) of block copolymer micelles contributes to maintaining the micelles dispersed in solution and hinders their contact with cells [4]. The most commonly investigated micelles are of spherical shape. However, various morphologies are attainable, and transition between micelles and vesicles [5] by varying the solution properties is well documented [6,7]. To create spherical micelles the hydrophilic part of the polymer should not outweigh the hydrophobic portion [8]. The characteristic feature of block copolymer micelles is their core-shell structure (Fig. 1).

The core is comprised of hydrophobic parts of the polymer, and its physical state can vary from swollen to frozen depending on the glass transition temperature (T_g) of the polymer. The defining properties of micelles include critical micellar concentration (CMC), aggregation number, size and shape [9]. The size of micelles allows their selective accumulation in sites with leaky vasculature, through enhanced permeability and retention effect [10,11].

* Corresponding author. Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir William Osler, Montreal, Que., Canada H3G 1Y6. Tel.: +1 514 3981264; fax: +1 514 3986690.
E-mail address: dusica.maysinger@mcgill.ca (D. Maysinger).

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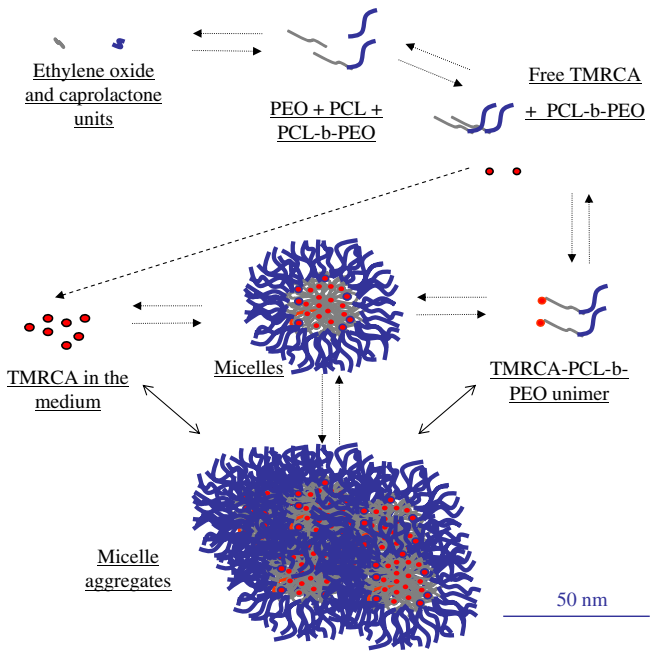

Fig. 1. Micelle dynamics and stability. PCL-b-PEO micelles are dynamic systems which accommodate lipophilic agents (e.g. drugs and fluorescent dyes such as TMRCA) in their hydrophobic PCL cores. The hydrophilic PEO parts form corona that separate core from aquatic environment. Multiple equilibria exist between micelles and their components, i.e. entrapped agents (TMRCA) and PCL-b-PEO unimers. Under physiological conditions micelles can form large aggregates.

1.2. Micelles as drug delivery vehicles

The purpose of using block copolymer micelles in medical sciences is to minimize the hydrophobic drugs of solubility problems, toxicity and inadequate pharmacokinetics. The most significant advances were made with anticancer agents and these delivery systems have recently reached clinical trials [12–14]. Steroid hormones can be also very effectively incorporated into block-copolymer micelles [15,16]. Such micelles could provide drug-loaded depot systems with controllable release that could find use in hormone replacement therapy. Immunosuppressant agent FK506, incorporated into PCL-b-PEO micelles administered at the site of sciatic nerve lesion, can facilitate repair of the peripheral nerves [17]. Micelles seem to be suitable nanocarriers for a number of anti-inflammatory agents, and the local delivery of anti-inflammatory drugs through accumulation of micelles effectively reduces vascular leakage at inflamed sites [18–20]. Block copolymer micelles made of charged block copolymers have been investigated for non-viral delivery of genetic material [21,22] and other charged molecules [23]. The self-assembly of polyion complex micelles (PICM) proceeds through the neutralization and segregation of oppositely charged polyions in a way that combines features of amphiphilic micelles and interpolyelectrolyte complexes. Polymers having protonated amines at physiological pH may be considered good candidates for the preparation of PICM incorporating polyanionic molecules such as plasmid DNA [24], oligonucleotides [25] and enzymes [26]. Examples of such polymers are poly(ethyleneimine) (PEI), PLys, polyamidoamide and poly(2-(*N*,*N*-dimethylamino)ethyl methacrylate) [27]. However, some of these polymers are cytotoxic (e.g. PEI). In order to form PICM with polycationic drugs, polymers exhibiting negatively charged units, including poly(methacrylic acid) and PAsp, are required [28]. Properties and application of ionomers forming micelles have been recently reviewed [23].

1.3. Selected approaches to labeling the micelles

The cellular fate of block copolymer micelles is relevant for evaluating and optimizing the delivery system. The distribution of micelles inside living cells can be followed by fluorescent labeling of the constitutive block copolymers. For example, red fluorescent dyes [29], green fluorescent dyes [30] and drugs [31] have been covalently coupled to the polymer and fate of the micelles investigated by confocal microscopy. Combined with the use of organelle selective dyes (Table 1) the details of the cellular distribution of micelles in live cells were assessed [32] (Fig. 2). Alternatively block copolymers can be labeled with radioactive probes [16,17,33–35], and heavy atoms such as gold [36,37], so that they can be revealed by electron microscopy. Gold, because of its high electron density and stability, can be incorporated into micelles to allow visualization of individual micelles by TEM. This technique provides information on intracellular location of gold-associated particles with much better resolution than is achievable by optical microscopy. To date, however, there are no TEM reports showing the presence of individual micelles in subcellular compartments, although many groups have investigated the association between heavy metals and polymers for various applications [38–43]. We have prepared and characterized gold-incorporated micelles [36]. Such micelles can enter the cells when incubated for at least one hour and can be seen as agglomerates by TEM (Fig. 2). Similar to the findings with fluorescent-labeled micelles, the data suggest that the micelles do not enter the nucleus but they can be found in endosomes/lysosomes. Although this approach cannot be used to study the fate of micelles in real time [44], it has the advantage of providing more refined information on subcellular distribution within cell organelles (Fig. 2).

In summary, using both fluorescently labeled agents and fluorescent polymers we were able to distinguish between micelle-incorporated agents and unlabeled micelles and to provide the evidence for their intracellular location. The most compelling evidence for internalized micelles containing fluorescent agents was obtained by fluorescence lifetime imaging (FLIM) [45–47]. The principle of the FLIM experiments consists of the following steps: (i) the life-time of the donor fluorescence is measured in the absence of an acceptor fluorophore (control); (ii) another sample is prepared where both donor and acceptor fluorophores are present and both are imaged by confocal microscope; (iii) FLIM instrument measures the lifetime of the donor fluo-