Polyethylene glycol – Indocyanine green Nanoparticles for Photodynamic Therapy Technique

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Abstract
Nanoparticles formulated from biodegradable polymers such as polyethylene glycol (PEG) with molecular weight 6000 and 15,000 respectively are being extensively investigated as drug (Indocyanine green, ICG) delivery system due to their controlled release characteristics and biocompatibility. PEG nanoparticles for ICG delivery are mainly formulated by a routine technique using PVA as a stabilizer generating negatively charged particles and heterogeneous size distribution. The objective of the present study is to formulate cationic PEG nanoparticles with defined size and shape that can efficiently bind ICG. This technique to make cationic nanoparticles with very low size composed of biodegradable and biocompatible. PVA-chitosan blend was used to stabilize the PEG nanoparticles.

Keywords: Nanoparticles; Polyethylene glycol; Indocyanine green

Introduction
Nanoparticles (NPs) are synthetic materials with dimensions from one to hundreds of nanometers, and remarkable applications in biomedicine due to the unique way in which they interact with matter [1,2]. There are currently more than 35 US FDA-approved NPs often incorporating polyethylene glycol (PEG), with a larger number in preclinical studies for both imaging and therapy (Figure 1A) [1,3-9]. NPs have large payloads, stability, avidity, signal enhancement and the capacity for multiple, simultaneous applications owing to their unique size and high surface area: volume ratio [10]. While they are bigger than molecules and many proteins, yet smaller than cells, they behave differently to other therapies and imaging agents, affecting their in vivo applications. For example, in cancer tissue, NPs not only extravasate from the leaky tumor vasculature, to a higher degree than healthy tissue, but also remain in the area by the enhanced permeability and retention (EPR) effect [11]. NPs lodged in the tumor can then perform signaling and/or therapy [10]. Despite these advantages, some fundamental challenges hamper NP deployment to the clinic. These include uptake by the reticuloendothelial system (RES), in which NPs are rapidly shuttled out of circulation to the liver, spleen or bone marrow, and nonspecific binding of NPs to nontargeted or nondiseased areas. Concerns about NP toxicity often arise because of this RES accumulation. Aggregation can lead to NP entrapment in the liver, lungs or elsewhere due to capillary occlusion [12]. The addition of PEG to the NP surface (PEGylation) can reduce many of these challenges (Figure 1B). PEG is a coiled polymer of repeating ethylene ether units with dynamic conformations (Figure 1C). In both drug-delivery and imaging applications, the addition of PEG to NPs reduces RES uptake and increases circulation time versus uncoated counterparts [13]. 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 EPR effect is modulated due to NP size changes via addition of a PEG coat [14,15]. Due to these attributes, PEGylated NPs generally accumulate in the liver a half to a third of the amount of non-PEGylated NPs and demonstrate higher tumor accumulation versus background [16]. PEG is inexpensive, versatile and FDA approved for many applications [12]. All NPs contain at least two fundamental spatial components: the core and the corona that interact with the environment or solvent. While core/shell, core/multishell systems add further complexity, for example [18], all still possess an area in which NP interfaces with the solvent (Figure 1B). PEG chains modify this interface layer and increase circulation time. Circulation half-time ($t_{1/2}$) describes blood pool residence and is the period over which the concentration of circulating NPs remains above 50% of the injected dose, analogous to a drug’s half-life [19]. NP efficacy requires sufficient $t_{1/2}$ to not only reach the target, but also remain in the affected area (at concentrations sufficiently above background tissue) long enough for image capture or drug delivery. The RES system prevents site-specific accumulation because it removes the NPs from circulation, acting as a competitor to the intended target site [20]. In addition, the NPs must clear from the nontargeted area to produce imaging contrast or dosing efficiency. The ideal $t_{1/2}$ is dependent on application. In imaging, 2-6 h is optimal for injection, accumulation at targeted site, clearance from nontargeted areas and data collection. The ideal circulation time for therapeutic NPs is longer (days) to allow repeated exposure to affected area. Unfortunately, this can also expose healthy organ systems to the drug and is the motivation for targeted NPs, as such systems preferentially...
accumulate in the diseased area. Approaches to measuring t½ vary with NP type. When labeled with radionuclides, g counting of either specific organ systems or blood aliquots determines NP circulation time. One limitation is dissociation of radionuclide from NPs; however, radioactivity measurements may always be carried out noninvasively [21].

Measurement of t½ via fluorescence, Raman, inductively coupled plasma or chromatography/mass spectrometry is very specific to the NP, but requires sequential sampling of the blood pool. The RES is an immune system component, utilizing circulating macrophages and monocytes, liver Kupffer cells and spleen and other lymphatic vessels to remove foreign material, such as bacteria and viruses, from the body [20]. Figure 2 illustrates how opsonin proteins associate with foreign bodies and coat its surface [22]. As bacteria and viruses have the same negative surface charge as phagocytic cells, opsonins are critical to reducing the charge repulsion between the two systems [13]. Next, phagocytic cells engulf the material and transport it to the liver or spleen for degradation and excretion (Figure 2 A3–A4). Additional phagocytic macrophages are permanently located in the liver. Known as Kupffer cells, these cells serve as a major filter for many types of NPs and are a major interference with long t½ [23]. The PEG polymer on a NP surface increases t½ by reducing this opsonization process (Figure 2B2), thus preventing recognition by monocytes and macrophages, allowing the NPs to remain in the blood pool [13,22]. Hydrophobic particles are more vulnerable to the RES and hydrophilic PEG reduces these complications [22]. In addition to NP-RES interactions, poor t½ can also result from NP-NP interactions (i.e., aggregation). NPs aggregate primarily because the attraction between particles is stronger than the attraction for solvent [13,24]. NPs with a high surface energy have a greater tendency to aggregate as described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [25,26]. For spherical NPs, the interaction potential is related to the electrostatic repulsive potential and the van der Waals attraction potential [26]. PEG decreases the surface energy of NPs and minimizes van der Waals attraction [27-29]. Aggregation can be induced by solvents of high (>100mM) ionic strength (shielding of solvent from NP), highly concentrated solutions of NPs (less distance between the NPs), time from synthesis, or NP preparations with a very neutral (~±5 mV) zeta potential [30]. PEG decreases the amount of attraction between NPs by increasing the steric distance between them and increasing hydrophilicity via ether repeats forming hydrogen bonds with solvent. Other benefits to PEGylation include modifying the size of the particle. The reduced renal filtration of particles larger than 10 nm increases t½ however, at too large a size (>100 nm), liver uptake increases and EPR extravasation may decrease [31]. PEG modifies the NP flexibility and the NP can become ‘softer’ after PEGylation than the underlying material, influencing extravasation.

**PEG Applications**

Prior to NP applications, PEG was used as a nontoxic, water-soluble dispersant/stabilizer. Also known as Carbowax®, it is present in health and beauty aids, including laxatives, toothpastes and eye drops, and is an excipient in tablet formulations [32]. PEG stabilizes organ and blood donations. Early work with PEGylated NPs stemmed mostly from drug delivery [16,33-36]. One of the first reports on PEGylation was described by Davis and Abuchowski [37,38], where they covalently attached methoxy-PEGs (mPEGs) of 1900 and 5000 Da to bovine serum albumin and to liver catalase. Later, acrylic microspheres functionalized with PEG-modified human serum albumin increased t½ in vivo [39]. Li and colleagues found that 75-nm latex particles remained in rat circulation 40-times longer (half-life 20 min vs 13 h) when coated with PEG larger than 5000 kDa [33]. Klibanov and Huang found that incorporation of dioleoyl N-(monomethoxy polyethylene glycol succinyl) phosphatidylethanolamine (PEG-PE) into phosphatidylcholine: cholesterol liposomes increased t½ from 30 min to 5 h without increasing leakage of the liposome interior [35]. In the mid-1990s, Doxil® (liposomal delivery vehicle for doxorubicin) and oncospar (PEG-l-asparaginase) became the first FDA-approved NP therapeutics [40]. Doxil increases doxorubicin bioavailability nearly 90-fold at 1 week.
from injection of PEGylated liposomes versus free drug [41]. The use of PEG on the doxorubicin carrier yields a drug half-life of 72h with circulation half-life of 36h [42,43]. Later, Abraxane® was introduced as an albumin-functionalized NP for delivery of taxane without cremophor to enhance drug efficiency [44]. Thadakapally et al., developed a novel serum stable long circulating polymeric nanoparticles for curcumin with a modification to the well known and novel nanoparticle albumin bound technology. polyethylene glycol-albumin- curcumin nanoparticles were prepared using serum albumin and poly ethylene glycol using desolvation technique. Nanoparticles were characterized for encapsulation efficiency, particle size and surface morphology. Drug excipient compatibility was determined using fourier transform infrared spectroscopy. Physical state of the drug in the formulations was known by differential scanning colorimetry. In vitro release and solubility of the drug from nanoparticles were determined. In vivo Drug tissue uptake and kupffer cell uptake was determined with optimized nano formulation in rats after intravenous administration. Cell viability assay was determined using breast cancer cell line MD-MB-231. Entrapment efficiency for prepared nanoparticle was above 95%. A sustained release of drug from nanoparticles was observed for 35 days in both in vitro and in vivo studies with the optimized formulation. Polyethylene glycol-albumin-curcumin nanoparticles showed lesser liver and kupffer cell uptake as compared to that of curcumin-albumin nanoparticles suggesting the bestowment of stealthness to nanoparticles with pegylation. Also, the antiproliferative activity of polyethylene glycol-albumin- curcumin nanoparticle formulation was more as compared to native curcumin. Polyethylene glycol-albumin-curcumin nanoparticles thus developed can be conveniently used in breast cancer with improved efficacy compared to conventional therapies and as an alternate to nanoparticle albumin bound technology which is used in producing Abraxane, albumin based breast cancer targeting nanoparticles of paclitaxel [45]. Photothermal therapy (PTT) and photodynamic therapy (PDT) are emerging physical tumor treatments utilizing near infrared (NIR) light-absorbing agents which lead to thermal ablation of cancer cells or generate highly reactive oxygen species (ROS) via photosensitizer to ablate tumors [46,47]. PTT and PDT possess several advantages, such as minimal invasion, high therapeutic efficacy, limited side-effects, selective localized treatment and reproducible properties [48,49], and hence have received much attention in recent years [50,51]. Until now, a variety of materials has been explored as PTT or PDT agents due to their high absorption in the tissue-transparent NIR wavelength range, including organic fluorescent dyes [52], gold nanorods [53], CuS nanoparticles (NPs) [54], polymer NPs [55], carbon nanomaterials [56], etc. [57,58]. However, fluorescent dyes may be removed rapidly from the systemic circulation and lack specificity to a tumor, and inorganic photothermal agents have potential long-term toxicity due to the difficulty of degrading in the body [59]. Therefore, exploiting biocompatible and targeted therapeutic nanoagents with enhanced photothermal conversion capability and ROS generation ability to amplify PTT and PDT treatments remains challenging. Indocyanine green (ICG) is a clinical infrared imaging agent approved by the U.S. Food and Drug Administration (FDA), and has been applied in optical imaging of human vasculature, tissue and cells due to its biocompatibility and unique optical properties [60]. Due to strong absorption at 780 nm, ICG can effectively convert absorbed NIR optical energy into heat for PTT [61], and produce ROS for PDT [62], under NIR laser irradiation [63]. Nevertheless, the application of ICG in tumor phototherapy is limited by its tendency to aggregate, rapid degradation in aqueous solution [64], poor photo-stability and non-specific binding to proteins [65]. To overcome those limitations, various nanoparticle delivery systems have been developed to encapsulate ICG [66]. Lv et al., used a mesoporous silica (mSiO₂) matrix to load ICG molecules, and demonstrated that loaded ICG displayed a more enhanced photothermal effect than pure ICG [67]. ICG-loaded mesoporous silica NPs also could not only limit the degradation of ICG, but reach and stay at a tumor for a long period of time due to its enhanced permeability and retention (EPR) effect [68]. Hence, loading of ICG within targeting
nanocarriers with high efficiency is shown to be an effective way to promote the application of ICG in PTT and PDT treatment.

Conclusions

To improve the efficacy of PTT, polyethylene glycol (PEG) nanoparticles (NPs), indocyanine green (ICG) for NIR laser-induced PTT. ICG after administered intravenously will be readily bound with blood proteins and hence leads to mostly 2-4 min of plasmatic half-life. Among various pharmaceutical polymers, PEG is one of the best defined biomaterials with FDA approval for drug encapsulation due to its biocompatibility, biodegradability, and controllability for drug release.

References


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