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Multimodal tumor imaging by iron oxides and quantum dots formulated in poly (lactic acid)-D-alpha-tocopheryl polyethylene glycol 1000 succinate nanoparticles

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ABSTRACT

This work developed a multimodal imaging system by co-encapsulating superparamagnetic iron oxides (IOs) and quantum dots (QDs) in the nanoparticles of poly (lactic acid) - d-α-tocopheryl polyethylene glycol 1000 succinate (PLA-TPGS) for concurrent imaging of the magnetic resonance imaging (MRI) and the fluorescence imaging to combine their advantages and to overcome their disadvantages as well as to promote a sustained and controlled imaging with passive targeting effects to the diseased cells. The QDs and IOs-loaded PLA-TPGS NPs were prepared by a modified nanoprecipitation method, which were then characterized for their size and size distribution, zeta potential and the imaging agent encapsulation efficiency. The transmission electron microscopy (TEM) images showed direct evidence for the well-dispersed distribution of the QDs and IOs within the PLA-TPGS NPs. The cellular uptake and the cytotoxicity of the PLA-TPGS NPs formulation of QDs and IOs were investigated *in vitro* with MCF-7 breast cancer cells, which were conducted in close comparison with the free QDs and IOs at the same agent dose. The Xenograft model was also conducted for biodistribution of the QDs and IOs-loaded PLA-TPGS NPs among the various organs, which showed greatly enhanced tumor imaging due to the passively targeting effects of the NPs to the tumor. Images of tumors were acquired *in vivo* by a 7T MRI scanner. Further *ex vivo* images of the tumors were obtained by confocal laser scanning microscopy. Such a multimodal imaging system shows great advantages of both contrast agents making the resultant probe highly sensitive with good depth penetration, which confirms the diagnosis obtained from each individual imaging. With therapeutics co-encapsulation and ligand conjugation, such nanoparticles system can realize a multi-functional system for medical diagnosis and treatment.

1. Introduction

Early stage diagnosis plays a key role to determine prognosis for diseases, especially for fatal ailments such as cancer and cardiovascular diseases. Molecular imaging provides critical information to diagnose a disease in its earliest stage, which is an *in vivo* characterization and measurement of the disease process at the cellular and molecular level. Its objective is to investigate molecular basis and diagnose abnormalities of cellular functions as well as follow up molecular processes in living organisms in a non-invasive way. To image molecules *in vivo*, criteria such as the availability of high affinity probes, the ability of probes to overcome physiological barriers, the use of signal amplification strategies and the availability of sensitive, fast and high-resolution imaging techniques must be met [1].

Three medical imaging techniques, which are used most often in the current clinical practice, are the X-ray computed tomography (CT), positron emission tomography (PET) and magnetic resonance imagery (MRI). All these three imaging techniques involve using

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contrast agents. In CT scans, for example, radiocontrast agents are used. They are typically iodine compounds and adverse reactions have been a concern. The risk of adverse reaction is 4%–12% with ionic contrast materials and 1%–3% with non-ionic contrast materials [2]. Besides the potential risks from using the radiocontrast agents, CT scans also expose patients to harmful X-ray radiation. On the same note, PET scans also involve the use of radioactive tracer isotopes to promote imaging. These radiotracers are extremely unstable and ionize, resulting in radiation during imaging. In view of the radiation exposures of CT and PET scans, it is obvious that MRI is the preferred imagery technique, as it is non-invasive and will not cause radiation injury. However, one drawback of MRI is its natural insensitivity of imaging for label detection. This can be overcome by using targeted MRI contrast agents coupled with biologic amplification strategies. One example is the cellular internalization of superparamagnetic probes such as monocrystalline iron oxide nanoparticles [3,4]. A few superparamagnetic IO contrast agents were developed for MRI. These probes enable clearly defined anatomy imaging post contrast. Imaging molecular targets for early stage disease diagnosis requires probes with greater ability to amplify MRI signals [1,5]. Besides IOs, another probe used for amplification strategy is quantum dots (QDs) as luminescence probes in fluorescence imaging. A wide range of cells have been visualized by using QDs, which are also employed in DNA hybridization detection [6] and immunoassays [7]. *In vivo* longevity is one major advantage of QDs [8]. QDs can be utilized as an effective imaging probe for imaging tumor because of their strong and bright fluorescence, excellent photo stability and sensitivity [9]. Although necessary, amplification strategies are not enough to produce high quality images. Sufficient concentrations of probes must be gathered at the intended imaging area for an adequate period *in vivo*. Nevertheless, the agent dose is limited by the side effects of the agent itself and the rapid removal of probes from the blood system due to the body's mononuclear phagocyte system (MPS) interactions after opsonization [10,11]. Methods to cloak nanoparticles from MPS recognition and therefore increase their half-life in circulation involve surface modification of the probes [12–14]. In general, hydrophilic particles opsonize slower than hydrophobic particles [15–17] and neutrally charged particles opsonize slower than charged particles [18]. Thus, non-charged, hydrophilic groups can be grafted onto the probes to hinder opsonization. These groups are usually long hydrophilic polymers and non-ionic surfactants, which can shield hydrophobic and charged particles from opsonin proteins [11]. To date, the most popularly used shielding groups are polyethylene glycol (PEG) and PEG-containing copolymers.

Besides the problem of MPS recognition, certain probes may have good affinity with certain targets of imaging interest but pose to be toxic. An example is QDs, which are made up of elements that are toxic in individual elemental form. An appropriate modification and formulation of QDs could minimize their toxicity [19,20].Formulation of imaging probes such as IOs and QDs in nanoparticles of biodegradable polymers may thus provide an ideal solution as well as enhance cellular uptake, hence improving imaging effects [21]. Moreover, the imaging agent-loaded nanoparticles can be further conjugated with biological ligand to realize targeted delivery of the imaging agent to the diseased cells, which can be distinguished from healthy ones. The nanoparticles surface decorated with targeting ligand enables the selective delivery of imaging agent into diseased cells by the ligand-mediated approach, which achieves high specificity and sensitivity of cancer detections, allowing the diagnosis of cancer at its earliest stage.

IO and QD probes are effective probes for amplification in molecular imaging. However, individual imaging probes have their advantages and disadvantages. For instance, IO probes provide high spatial resolution and unlimited depth penetration [22] but their sensitivity in imaging fails in comparison to optical fluorescence imaging probes such as QDs. QDs, in turn; have excellent imaging effects and long half-life, but their ability for tissue penetration is limited due to the refraction and adsorption of light in the living organism. Multimodal imaging can be developed to make use of the advantages and overcome the limitations, which can be realized by co-encapsulation of QDs and IOs in ligand-conjugated nanoparticles of biodegradable polymers.

There have been some studies involving remodelling imaging probes suited for dual modality imaging capabilities. Jyun-Han Ke et al. decorated poly(acrylic acid) onto IOs resulting in a highly water-soluble superparamagnetic iron oxides which permit applications in MRI imaging. The free carboxylic groups exposed on the surface allow for covalent attachment of a fluorescent dye, Rhodamine 123 (Rh123), which permits applications in fluorescence imaging [23]. In another study by Zhou et al., the concept of upconversion luminescence (UCL) and MR dual-modality imaging *in vivo* of whole-body animals was explored. In the work, Tm^{3+}/Er^{3+}/Yb^{3+} co-doped NaGdF4 was synthesized with near-infrared to near-infrared upconversion luminescent and magnetic resonance properties [24]. Also, Choi et al. explored hetero-structured complexes formed by magnetic iron oxide nanoparticles and near-infrared (NIR) fluorescent single-walled carbon nano-tubes (SWNT) [25]. These complexes, when further conjugated with monoclonal antibodies to target specific receptor sites, could be used to provide molecular-level contrast and bio-sensoring. Most of the studies listed above, however, are related either to *ex vivo* or *in vitro* analysis. Furthermore, some of the studies lack clinical feasibility as they involve the use of probes for imagers, which are either not available or impractical in the current medical scene.

In this study, contrast agent IOs and fluorescent QDs are co-encapsulated in nanoparticles of poly(lactide)—tocopheryl polyethylene glycol succinate (PLA-TPGS), which was a new type of biodegradable copolymer recently synthesized in our laboratory [26]. PLA provides the needed mechanical strength and biodegradability, while TPGS component enhances the biocompatibility and provides stealth from RES as well as inhibits the multiple drug resistance (MDR) [27,28]. The IOs and QDs-loaded PLA-TPGS NPs were prepared by a modified nanoprecipitation technique, which were characterized for their various physiochemical properties and assessed for their *in vitro* cytotoxicity and cellular uptake. Such a multimodal probe was then tested *in vivo* and *ex vivo* on a xenograft tumor model for MRI and fluorescent imaging effects.

2. Materials and methods

2.1. Materials

Organic Quantum Dots (Qdot®655 ITK™; catalog number Q21721MP) and Carboxyl Quantum Dots (Qdot®655 ITK™; catalog number Q21321MP) were purchased from Invitrogen Corporation Singapore. Iron Oxide (IO) dispersed in THF were synthesized as explained earlier [29]. Tetrahydrofuran (THF), Penicillin-streptomycin solution and trypsin–EDTA solution were provided by Sigma–Aldrich (Sigma–Aldrich Pte Ltd, Singapore). Fetal bovine serum (FBS) was purchased from Gibco (Life Technologies AG, Switzerland). DMEM medium was from Invitrogen Corporation. All chemicals used in this study were HPLC grade. Millipore water was produced by the Milli-Q Plus System (Millipore Corporation, Bedford, USA). MCF-7 breast cancer cells were provided by American Type Culture Collection. PLA-TPGS copolymer was synthesized according to a method described in our previous work [26,29]. The PLA:TPGS component ratio for the PLA-TPGS copolymer used in this research is 90:10 w/w with number-averaged molecular weight (Mn) = 17,027.

2.2. Flocculation of QDs

The Organic QDs from Invitrogen were dispersed in n-decane. To prepare the QDs in THF, 1200 μL of alcohol mixture (75% methanol: 25% propanol) was added to 200 μL of organic QDs (equivalent of 0.23 mg Cd as determined by ICP-MS). The solution was then vortexed for 2 min and subjected to centrifuging for 15 min at