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Cellular uptake and biocompatibility of bismuth ferrite harmonic advanced nanoparticles

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Abstract

Bismuth Ferrite (BFO) nanoparticles (BFO-NP) display interesting optical (nonlinear response) and magnetic properties which make them amenable for bio-oriented diagnostic applications as intra- and extra membrane contrast agents. Due to the relatively recent availability of this material in well dispersed nanometric form, its biocompatibility was not known to date. In this study, we present a thorough assessment of the effects of *in vitro* exposure of human adenocarcinoma (A549), lung squamous carcinoma (NCI-H520), and acute monocytic leukemia (THP-1) cell lines to uncoated and poly(ethylene glycol)-coated BFO-NP in the form of cytotoxicity, haemolytic response and biocompatibility. Our results support the attractiveness of the functional-BFO towards biomedical applications focused on advanced diagnostic imaging.

From the Clinical Editor: Bismuth Ferrite nanoparticles (BFO-NP) have been recently successfully introduced as photodynamic tools and imaging probes. However, how these nanoparticles interact with various cells at the cellular level remains poorly understood. In this study, the authors performed in vitro experiments to assess the effects of uncoated and PEG-coated BFO-NP in the form of cytotoxicity, haemolytic response and biocompatibility.

Key words: Nanophotonic; Non-linear imaging; Bismuth ferrite; PEGylation; Biocompatibility

Most of nanophotonics approaches (quantum dots, plasmonic nanoparticles (NP), up-conversion NP) for health applications present static optical properties (absorption bands, surface plasmon resonances) often in the UV-visible spectral region and do not fully allow for exploiting the tuning capabilities of new laser sources and their latest extensions in the infrared. To circumvent these limitations, a few research groups in the last years have introduced a new nanotechnology approach based on inorganic nanocrystals with non-centrosymmetric structures. Such nanomaterials present a very efficient nonlinear response, and can be easily imaged by their second harmonic generation

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(SHG) in multi-photon imaging platforms.[1-6] Such harmonic NP (HNP) do not suffer from conventional optical limitations such as photobleaching and blinking allowing long-term monitoring of developing tissues.[5,7] Several HNP have been recently synthesized and tested for biological applications.[4-7] Particular care should be paid to assess the ability of these NP to reach intracellular targets without causing major interferences to the cell metabolism. In this context, this subcellular targeting becomes increasingly important as key parameters for the understating of complex events in living cells.[8,9] In fact, the possibility to freely change detection wavelength can be exploited for subtle co-localization studies with organelle-specific dyes, as the signal from NP can be freely shifted to avoid overlap with fluorophores emission bands. However, one aspect that is not fully understood and[10] remains uncertain is how nanomaterials interact with cellular interfaces such as cytoskeletal membranes since it is known that small alterations in their physicochemical properties can drastically influence the cells-NP interactions, especially the uptake mechanisms.[9,11] Therefore, lead-NP-candidate identification process based on high throughput screening as decision-making process is a prerequisite for the validation of new SHG NP for bio-imaging applications. Here we present a study based on $BiFeO_3$ (bismuth ferrite, abbreviated as BFO) NP (BFO-NP), which were recently successfully introduced as photodynamic tools and imaging probes.[12] Nonetheless, such is the technological novelty of this new group of materials that there is still a knowledge gap that requires the scientific community attention towards the investigation of the interaction at the cellular and subcellular levels. The opportunity of closing this gap is presented by providing the first thorough investigation on the effects of BFO-NP in cellular metabolism and uptake mechanisms. Toxicity and biocompatibility were assessed by automated high content screening, recording cytotoxicity, lysosomal mass and cell permeability, in line with previously published works.[13,14] Cellular uptake was investigated by co-localizing the NP with specific fluorophores for cell membranes and endosomes. Moreover, in this paper we present for the first time to our knowledge the most efficient protocol for the coating of these HNP with poly(ethylene glycol) (PEG) derivatives to promote colloidal stability and biocompatibility in biological media, and to allow post-functionalization with bioactive molecules.[15,16] In this context, the biocompatibility, cellular uptake and intracellular localization of free and PEG coated BFO-NP were compared.

Methods

Preparation of a polydisperse suspension of BFO

The starting BFO suspension (2 mL, 62.5 wt % in ZrO2 balls), provided by the company FEE (Germany) under a collaboration agreement, was diluted in EtOH (2 L) and ultra-sonicated for 12 h. After 10 days sedimentation, the upper portion of the polydisperse suspension (50 mL) was taken and mixed with oleic acid (4 mL). The volatiles were removed under vacuum and the residue was weighed and suspended in EtOH to obtain a stock solution at 3.6 mg/mL.

Coating of BFO-NP

BFO-NP (suspended in EtOH, 3.6 mg/mL, 583 μL) were diluted in EtOH:toluene:25% aqueous ammonia (0.50:0.50:0.32 mL) and ultra-sonicated for 30 min. PEG oligomers **1** and **2**[14] (1:1 ratio, 100 mg) were added and the suspension ultra-sonicated at 40 °C for 16 hr. The suspension was reduced to a small volume and distributed in plastic tubes for dilution with a mixture of dichloromethane (DCM):EtOH:water (1:1:1, 1 mL). After centrifugation (10 min, 13 000 rpm), the aqueous layer, containing the excess of unreacted polymers, was removed and a mixture of EtOH:water (1:1, 0.5 mL) was added to each plastic tube. The procedure was repeated 5 times to obtain a pure suspension of coated BFO-NP in DCM. After evaporation of DCM and addition of EtOH, the BFO-NP concentration was calculated by measuring the turbidity of the solution by spectrometry at 600 nm (Synergy HT) and by comparing the values with a standard-curve prepared using the stock solution at 3.6 mg/mL.

Characterization of uncoated and coated BFO-NP

Advanced physico-chemical characterization of uncoated and coated BFO-NP was recently performed and reported. In the present work, BFO-NP were characterized by dynamic light scattering (DLS) using a Zetasizer NanoZ (Malvern) for determination of mean hydrodynamic volume and zeta potential. Suspension of uncoated or coated BFO-NP (20 μL) was diluted in 1 mL of distilled water. Acetic acid (100 μL) was added and the resulting suspensions were ultra-sonicated for 30 min and analysed by DLS.

Nanoparticles characterization in biological media

The physico-chemical characterization of the NP was carried out by nanoparticle tracking analysis (NTA, Nanosight NS500). BFO-NP at 25 μg/mL were vortexed for 5 s to disperse the particles and then diluted at 1 μg/mL in different solutions (0.22 μm filtered): diethylpyrocarbonate (DEPC) water, Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Memorial Institute (RPMI) and Ham's F-12 K (Kaighn's) Medium (F12K) culture media, and their supplemented form with 10% fetal bovine serum (FBS). Three culture media were chosen since commonly utilised for the in vitro culture cell models selected. The different dispersions were then analyzed via NTA for the physico-chemical characterization measurement of hydrodynamic radius and polydispersity index (PDI) at room temperature of BFO-NP. All measurements were carried out three times at physiologically relevant pH (pH = 7.4) and means and standard deviations (SD) were calculated. Quality assurance over the measurements carried out was guaranteed by the adoption of Quality Nano (QNano, FP7 project) standard operating procedures (SOPs), which have been developed as part of large inter laboratory comparative study focused on nanoparticle physico-chemical characterization.[17]

Cell model and culturing conditions

Human lung-derived A549 and NCI-H520 cancer cell lines and human monocytic THP-1 cell line are available from ATCC