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Surface Engineering of Bismuth Nanocrystals to Counter Dissolution†

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Abstract

Rapid dissolution of Bi Nanocrystals (NCs) in lysosomal conditions results in poor biocompatibility. We report that an *in situ* surface coating of Bi nanocrystals with Ganex[®] V216, a cosmetic dispersant, limits its dissolution under physiological conditions. These Bi Ganex (BiG) NCs are readily encapsulated in FDA approved polymer Poly(DL-Lactic-co-Glycolic Acid) (PLGA) by an oil-in-water emulsion technique and also undergo facile SiO₂ coating. BiG NCs in BiG@PLGA and BiG@SiO₂ nanoparticles dissolve slowly under physiological conditions and exhibit excellent biocompatibility, as opposed to uncoated Bi NCs. Finally, these Bi nanoconstructs are shown to be strong CT CAs, even at relatively low Bi concentrations.

Recent advancements in nanomaterial design can potentially transform Computed Tomography (CT) into a robust molecular imaging platform.¹ Over the past decade, there has been considerable research to establish various formulations of Gold NanoParticles (AuNPs) as Contrast Agents (CAs) for CT.² The higher atomic number of Gold ($Z = 79$), as compared to iodine ($Z = 53$), together with the synthetic ease and high biocompatibility of AuNPs make them a viable alternative to the iodine based CAs approved for clinical use, particularly at high kVp.³ Although bulk gold has a long history of medical use and is rendered non-toxic for humans, there are multiple reports that challenge the safety profile of AuNPs.⁴ Concerns range from nephrotoxicity due to accumulation of AuNPs in kidneys to inflammatory and apoptotic response in liver tissues due to protracted elimination from the liver.^{5,6} *In vitro* studies also correlate the increase in cellular apoptosis and alterations in cellular morphology to elevated AuNP concentration.^{7,8} High market cost of gold is also a major deterrent towards its routine use in clinical CT. The search for alternatives to AuNPs as CT CAs is an active area of research and Bi ($Z = 83$), a close neighbour to gold in the periodic table, is an exciting prospect.

In recent years, Bi NPs have generated tremendous interest for applications in biomedicine, particularly in the design of novel CAs for CT.⁹ Bi has a higher X-ray attenuation coefficient in comparison to Au (Bi 5.74 vs. Au 5.16 cm² kg⁻¹ at 100 keV) and is 1000-fold cheaper. The most widely studied examples of Bi NPs as CT CAs are Bi₂S₃ NPs and various

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nanocolloid formulations of Bi (e.g. *NanoK*, bismuth-n-decanoate complexes encapsulated in a phospholipid mono-layer)^{10,11}. These NPs have been shown to produce effective *in vivo* CT contrast. However, the compound form of Bi in these examples, which includes atoms other than Bi, limits the theoretical maximum Bi payload in the final NP construct and this diluted Bi content requires higher NP concentrations to produce effective contrast. Efficient packing of radiopaque material in a confined volume is of utmost importance so as to facilitate molecular imaging by CT. To achieve this goal, our group has been working to utilize elemental Bi NanoCrystals (NCs) for the development of novel, biocompatible CAs for CT. Taking into account density and atomic mass, there is nearly two-fold increase in Bi concentration in identically sized elemental Bi NCs versus Bi₂S₃ NPs. However, elemental Bi NCs rapidly dissolve within lysosomes in cells, resulting in poor biocompatibility. Indeed, free Bi ions have LD₅₀ = 8 mmol/L as compared to Bi in stable Bi₂S₃ NPs with LD₅₀ = 100 mmol/L.¹² Surface engineering to reduce their rapid dissolution is key towards continued development of Bi NCs for biological use.

We herein report the *in situ* surface coating of Bi NCs using a commercially available PHD-co-PVP copolymer [poly(1-hexadecene-co-1-vinylpyrrolidinone), Trade name Ganex[®] V216, or simply Ganex]. The resulting BiG (Bismuth-Ganex) NCs were highly monodisperse and compared to elemental Bi NCs with no Ganex coating on them, had significantly less dissolution under physiological conditions *in vitro*. Ganex is widely used in the cosmetic industry as a pigment dispersant or suspending agent. It is also an excellent film former, which lead us to envision that BiG NCs with a Ganex dressing can impart stability against dissolution. To enhance their stability, water solubility and biocompatibility, these unique NCs were encapsulated in the FDA approved Poly(DL-Lactic-co-Glycolic Acid) (PLGA) polymer. This research builds on our earlier work with fluorescent PLGA-encapsulated Bi NPs for dual CT/fluorescence applications.¹³ We also report an efficient protocol for the SiO₂ coating of BiG NCs. Similar to the oleic acid coating of Iron oxide NCs prior to NP formulation, a pre Ganex coating facilitated BiG NC encapsulation in PLGA polymer and SiO₂.¹⁴ *In vitro* cell viability and cell internalization experiments, together with preliminary μ CT studies reveal the potential of these BiG NPs as CAs for CT.

Buhro *et. al.* have pioneered the synthesis of near monodisperse Bi NCs in a diameter range of 3–30 nm.^{15,16} Their method (Method A) involves a Ganex stabilized, high temperature thermolysis reaction between BiCl₃ and Na[N{Si(CH₃)₃}₂] to form Bi NCs (Fig. 1a, synthetic details in Electronic Supplementary Information or ESI[†]). Employing an exact protocol as reported, we used a 25 wt% solution of Ganex in 1-Octadecene to generate an oily residue (Scheme S1, ESI[†]) that on Transmission Electron Microscopy (TEM) confirmed NC formation (BiG-25 NCs, Fig. 1b; Fig. S1, S2 in ESI[†]). Energy Dispersive Spectroscopy (EDS) of this residue affirmed the presence of Bi in the NCs, while Fourier Transform Infra-Red (FTIR) and Thermo Gravimetric Analysis (TGA) revealed a thick Ganex coat with less than 20% overall Bi content (Fig. S3 in ESI[†]). Multiple attempts to isolate NCs from this thick oil by centrifugation with various solvents were unsuccessful. Dry NCs are a precondition for their facile encapsulation in PLGA or SiO₂. To streamline the isolation of

[†]Electronic Supplementary Information (ESI) available: [Detailed procedures, characterization, cellular, μ CT data and ESI figures]. See DOI: 10.1039/x0xx00000x

BiG NCs and to enhance the overall Bi content, we modified the protocol by altering the amount of Ganex used in the reaction precursor.

The high viscosity and film forming ability of Ganex prompted us to decrease its precursor load from 25 wt% to as low as 5 wt% in a solution of 1-Octadecene. The resulting BiG NCs (BiG-5) were highly monodisperse ($12 \text{ nm} \pm 1.2$; average size $\pm$ S.D.) as is evident from the TEM images (Fig. 1c; Fig. S4 in ESI[†]). FTIR analysis revealed a surface coating of Ganex on the NCs, EDS confirmed the presence of Bi while TGA conferred the overall Bi content to 75.7 % (Fig. S5 in ESI[†]). Most importantly, the isolation of the NCs was straightforward (detailed in ESI[†]). Further decrease in precursor Ganex content to 2.5 wt% led to less uniform size distribution for the isolated BiG NCs ($14 \text{ nm} \pm 4.1$, Fig. 1d; Fig. S6 in ESI[†]), while an increase to 10 wt% Ganex lead to incomplete NC formation. EDS and FTIR spectra of the BiG NCs formed using 2.5 wt% Ganex (BiG-2.5 NCs) confirmed the presence of Bi and a Ganex surface coating respectively, while TGA revealed an overall Bi content of 80.9 % (Fig. S7 in ESI[†]).

In order to compare the influence of Ganex coating on their degradation in lysosomal media, Bi NCs were also made employing a method proposed by Son *et. al.* (Method B) that does not involve the use of Ganex (Scheme S2 in ESI[†]).¹⁷ This method is based on the Tri-n-octylphosphine assisted reduction of a bismuth dodecanethiolate complex, formed *in situ* by the reaction of bismuth neodecanoate and dodecanethiol. Adapting method B, bismuth NCs (Bi NCs) in a diameter range of $48 \text{ nm} \pm 8$ were formed as observed by TEM (Fig. S8 in ESI[†]). FTIR analysis revealed a coating of dodecanethiol on the NCs; EDS confirmed the presence of Bi while TGA showed a 98.2 % overall Bi content in the NCs (Fig. S9 in ESI[†]). Table 1 lists various Bi NCs obtained using different synthetic methods and their characterization details.

For the *in vitro* release study, dissolution of Bi from various NCs was studied in phosphate buffer saline (PBS, pH 7.4) and sodium citrate (NaCit, pH 5.5) at 37 °C for a period of 4 weeks. Incubation in PBS mimics the cytosolic and extracellular pH; while NaCit, pH 5.5 mimics the post endocytosis environment of the lysosome. A low pH and the presence of citrate are conditions that endocytosed NPs will experience as they follow an intracellular transport pathway through endosomes to lysosomes. The procedural details for this experiment carried out with BiG-5, BiG-2.5 and Bi NCs, suspended in 1 mL each of PBS and NaCit at 37 °C are described in Section 3 of the ESI[†]). Briefly, aliquots from a suspension of the NCs in PBS and NaCit were withdrawn at regular time points for the entire period of the study and analysed for Bi content using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and then normalized to obtain cumulative Bi release. The data obtained is plotted in Fig. 2 and shows a significant decrease in the rate of Bi dissolution in NaCit for BiG NCs as compared to non-ganex coated Bi NCs (Fig. 2b). This reduction in NC dissolution rate is most prominent within the first 24 hours, wherein < 20 % of total Bi is lost from the BiG-5 NCs, as compared to a loss of > 85 % from the uncoated Bi NCs. For the BiG-2.5 NCs, the cumulative Bi dissolution was > 71 % which is markedly higher than that observed for BiG-5 NCs; clearly signifying the importance of an optimal Ganex coating for controlled NC dissolution. After this initial burst there were small increments in Bi dissolution, and over the course of 4 weeks, the dissolution values

plateaued for all the NCs; 85.8 % for uncoated Bi NCs, 73.6 % for BiG-2.5 NCs and 34.6 % for BiG-5 NCs. This demonstrates that the Ganex coating also limits the long-term dissolution of BiG NCs. In stark contrast, different BiG and Bi NCs were found to be almost completely resistant to the dissolution process in PBS, with < 2.3 % loss over 4 weeks (Fig. 2a).

Method A thus provides us with an easy protocol for the *in situ* surface engineering of Bi NCs to limit their dissolution. The most exciting aspect of this method lies in its ability to tune the extent of dissolution based on the amount of precursor Ganex used in the reaction. Over the past decade, a variety of inorganic nanomaterials based on different formulations of iron, manganese, gold, silver, bismuth and gadolinium etc. have been proposed for a range of theranostic applications.^{18–24} During synthesis, most inorganic NCs are isolated with a surface coating of short ligand molecules such as oleic acid, mercapto succinic acid, citric acid and others that are used *in situ* to initiate NC growth and stabilize the as synthesized NCs. To our knowledge, there have been no reports on the ability of any of these surface ligands to limit NC dissolution. Although the use of Ganex as a stabilizer for the synthesis of Bi NCs was originally proposed by Buhro and co-workers, the unique ability of Ganex to restrict NC dissolution in lysosomal media was not mentioned or has never appeared in subsequent reports. We for the first time have tweaked this protocol to generate BiG NCs with a handle on the extent of Ganex coating. Again, PLGA encapsulation of bare Bi NCs with no Ganex coating on them did not restrict their dissolution in lysosomal media.¹³ In a marked improvement, the unique BiG NCs reported in this work limits such dissolution significantly. Although silica and other polymeric coating of inorganic NCs have been reported to limit dissolution, such surface engineering is mostly carried out after NC formation in a separate synthetic step, unlike the Ganex surface coating obtained *in situ* during regular synthesis of Bi NCs in the present work.^{25,26} We believe that Ganex can be easily incorporated within the synthetic protocol of other types of inorganic NCs to display a similar ability to limit NC dissolution.

Our next step involved the encapsulation of BiG NCs in PLGA and SiO₂. The general fabrication technique for making the BiG@PLGA NPs (Method C; Scheme S3 and S4 in ESI[†]) is based on using tip sonication to form an oil-in-water emulsion, comprising the BiG NCs and PLGA polymer in the oil (dichloromethane, DCM) layer and a water soluble emulsifier Poly(vinyl alcohol) (PVA) in the aqueous layer, followed by removal of DCM over time to form hardened BiG@PLGA NPs.

Using this general protocol, BiG-5@PLGA and BiG-2.5@PLGA NPs were made employing BiG-5 and BiG-2.5 NCs, respectively. In order to track the cellular uptake of these BiG@PLGA NPs, we decided to incorporate a fluorescent Coumarin-6 (C6) tag for both the NC variants. Employing the current protocol, this was straightforward and was carried out by simply adding C6 to a mixture of BiG NCs and PLGA in the DCM layer prior to their addition to aqueous PVA and subsequent tip sonication to form the desired fluorescent NPs (Fig. 3a, Method C).

To further test the functionality of BiG NCs and counter their inherent hydrophobicity, we attempted surface coating using SiO₂ to form core-shell NPs. Using a protocol proposed by

Yi *et. al.*, BiG-5@SiO₂ NPs were easily synthesized by employing IGEPAL[®]-CO-520, a non-ionic surfactant with 50 mol % hydrophilic groups and Tetraethyl orthosilicate (TEOS) as the SiO₂ source (Method D; Fig. 3b; Scheme S4 in ESI[†]).²⁷ Using this approach, a uniform SiO₂ shell (~10 nm thickness) around the BiG-5 NCs could be routinely prepared.

All the resulting NP types were characterized for size and surface charge using Dynamic Light Scattering (DLS). Table 2 lists the size, zeta potential and PDI for all the five NPs (BiG-5@PLGA., BiG-2.5@PLGA, both with and without C6 and BiG-5@SiO₂). C6 incorporation in NPs lead to a decrease in size and an increase in zeta potential of the resulting NPs. Bi and Si content in NPs was confirmed using EDS and TGA; FTIR of dry NPs revealed the presence of PLGA, Ganex and C6 in the respective NPs and the morphology of the NPs was determined using TEM and Scanning Electron Microscopy (SEM) that showed spherical particles across all the NP types (Fig. 3c–h; Fig. S10–S22 in ESI[†]).

Next, Bi dissolution from various BiG NPs was evaluated in PBS (pH 7.4) and NaCit (pH 5.5). Fig. 4 shows the cumulative Bi dissolution as obtained from the corresponding ICP-OES study and confirms the utility of a Ganex coating on limiting the NP dissolution in NaCit; 73.4 % for BiG-2.5@PLGA NPs, 16% for BiG-5@PLGA NPs and 22.1 % for BiG-5@SiO₂ NPs within the first 24 hours. Over the entire 4 week experiment, the dissolution of Bi from BiG-5@PLGA and BiG-5@SiO₂ NPs was limited to 27.9 % and 27.5 % respectively, while BiG-2.5@PLGA NPs suffered 88.6 % Bi dissolution. This can be attributed to an inefficient PLGA encapsulation as well as a faster dissolution rate of BiG-2.5 NCs that compromises NP integrity leading to faster dissolution. These results also indicate that an optimal surface coating using Ganex can withstand and facilitate encapsulation in PLGA and/or SiO₂ and continues to limit the dissolution of Bi from the NPs.

To ascertain the ability of BiG-5 and BiG-2.5@PLGA NPs to label macrophages, cells were incubated with C6 loaded NPs (0.1 mg/mL) for 3 h and visualized via confocal microscopy (Fig. 5a–d; Fig. S23 in ESI[†]). For both variants, high uptake by macrophages was observed, irrespective of the NC type. Further, the biocompatibility of BiG-2.5, BiG-5@PLGA and BiG-5@SiO₂ NPs was ascertained by MTT assay using Raw 264.7 macrophage cells following 24, 48 and 72 h incubation with varied NP concentrations (Fig. 5e; Fig. S24 in ESI[†]). Optimal Ganex coating thus resulted in a higher biocompatibility (up to 0.1 mg/mL) for the BiG-5@PLGA and BiG-5@SiO₂ NPs as compared to BiG-2.5@PLGA NPs.

Finally to test the CT viability of the BiG NPs, various concentrations of the different NP types were dispersed in a 0.5% agarose gel (0 to 80 mM Bi) and placed into 0.5 mL centrifuge tubes for phantom CT imaging using a GE eXplore Locus μ CT scanner operating at a tube voltage of 80 kVp and 450 μ A. For comparison, clinically used CT agent ISOVUE-300 at various concentrations (0 to 80 mM I) was also scanned. High CT contrast for the BiG-5@PLGA NPs was observed as compared to ISOVUE-300 even at a relatively low concentration of 20 mM (Fig. 5f). A similar, three-fold contrast enhancement was also observed for the corresponding BiG-5@SiO₂ NPs (Fig. S25A in ESI[†]) that signifies the potential of these novel BiG NPs as future CT CAs. For both BiG-5@PLGA and

BiG-5@SiO₂ NPs with a stark difference in size and Bi content, elevated values for the X-ray attenuation coefficients (15.6 and 15.1 HU per mM Bi respectively, Fig. S25B in ESI[†]) were observed. Such non-variance in X-ray attenuation over NP size has already been reported for AuNPs.²⁸ In comparison, PVP-coated Bi₂S₃ NPs have been reported to have a CT value of 9.3 HU per mM in one article²⁹ and 6.7 HU per mM in another paper.³⁰ Similarly, PEGylated AuNPs and TaO_x NPs have been reported with CT values of 5.3 HU per mM³¹ and 6.0 HU per mM respectively.³² Our unique BiG NP system clearly has higher CT value on the molarity basis. Huang and co-workers have recently reported BiOI NPs with a CT value of ~20 HU per mM, but that can be attributed to a combination of both Bi and Iodine within the NP system.³³ The high X-ray attenuation for Bi, together with efficient packing of BiG NCs in the BiG-5@PLGA NPs and BiG-%@SiO₂ NPs leads to such high CT contrast as compared to values reported for other NP based CT CAs.^{34,35}

Conclusions

In summary, we have reported the synthesis and characterization of PLGA and SiO₂ encapsulated Bi NPs surface engineered to display restricted dissolution in lysosomal media. The Bi-based NP CT CAs reported till date suffer from low contrast and are toxic due to a rapid dissolution profile. Our unique design of the Ganex coated Bi NCs successfully limits their dissolution and a highly efficient packing in SiO₂ and PLGA envelope generates a X-ray dense CT CA with an enhanced contrast at relatively low Bi concentrations. We believe that these unique NP constructs will act as a platform to trigger further investigations into Bi based NP CT CAs. Future attempts at the Ganex coating of other inorganic NCs and their subsequent encapsulation within a host of biocompatible polymers such as cellulose, chitin and PEG can lead to the design of a new generation of nanomaterials with various theranostic and material science applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notes and references

1. Liu Y, Ai K, Lu L. *Acc Chem Res.* 2012; 45:1817–1827. [PubMed: 22950890]
2. Xi D, Dong S, Meng X, Lu Q, Meng L, Ye J. *RSC Adv.* 2012; 2:12515.
3. Hainfeld JF, Slatkin DN, Focella TM, Smilowitz HM. *Br J Radiol.* 2006; 79:248–253. [PubMed: 16498039]
4. Dorsey JF, Sun L, Joh DY, Witztum A, Al Zaki A, Kao GD, Alonso-Basanta M, Avery S, Tsourkas A, Hahn SM. *Transl Cancer Res.* 2013; 2:280–291. [PubMed: 25429358]
5. Khlebtsov N, Dykman L. *Chem Soc Rev.* 2011; 40:1647–1671. [PubMed: 21082078]
6. Cho WS, Cho M, Jeong J, Choi M, Cho HY, Han BS, Kim SH, Kim HO, Lim YT, Chung BH, Jeong J. *Toxicol Appl Pharmacol.* 2009; 236:16–24. [PubMed: 19162059]

7. Mironava T, Hadjiargyrou M, Simon M, Jurukovski V, Rafailovich MH. *Nanotoxicology*. 2010; 4:120–137. [PubMed: 20795906]
8. Pernodet N, Fang X, Sun Y, Bakhtina A, Ramakrishnan A, Sokolov J, Ulman A, Rafailovich M. *Small*. 2006; 2:766–73. [PubMed: 17193121]
9. Pan, D., Misra, S., Kim, S. *Nanomedicine*. CRC Press; 2014. p. 131-150.
10. De La Vega JC, Häfeli UO. *Contrast Media Mol Imaging*. 2015; 10:81–95. [PubMed: 25044541]
11. Pan D, Roessl E, Schlomka JP, Caruthers SD, Senpan A, Scott MJ, Allen JS, Zhang H, Hu G, Gaffney PJ, Choi ET, Rasche V, Wickline SA, Proksa R, Lanza GM. *Angew Chem Int Ed Engl*. 2010; 49:9635–9. [PubMed: 21077082]
12. He W, Ai K, Lu L. *Sci China*. 2015; 58:753–760.
13. Swy ER, Schwartz-Duval AS, Shuboni DD, Latourette MT, Mallet CL, Parys M, Cormode DP, Shapiro EM. *Nanoscale*. 2014; 6:13104–13112. [PubMed: 25248645]
14. Xu S, Yang F, Zhou X, Zhuang Y, Liu B, Mu Y, Wang X, Shen H, Zhi G, Wu D. *ACS Appl Mater Interfaces*. 2015; 7:20460–20468. [PubMed: 26327472]
15. Wang F, Tang R, Yu H, Gibbons PC, Buhro WE. *Chem Mater*. 2008; 20:3656–3662.
16. Wang F, Buhro WE. *Small*. 2010; 6:573–81. [PubMed: 20108241]
17. Son JS, Park K, Han MK, Kang C, Park SG, Kim JH, Kim W, Kim SJ, Hyeon T. *Angew Chem Int Ed Engl*. 2011; 50:1363–6. [PubMed: 21290513]
18. Lee N, Yoo D, Ling D, Cho MH, Hyeon T, Cheon J. *Chem Rev*. 2015; 115:10637–10689. [PubMed: 26250431]
19. Kulkarni VM, Bodas D, Paknikar KM. *RSC Adv*. 2015; 5:60254–60263.
20. Yang X, Yang M, Pang B, Vara M, Xia Y. *Chem Rev*. 2015; 115:10410–10488. [PubMed: 26293344]
21. Dreaden EC, El-Sayed MA. *Acc Chem Res*. 2012; 45:1854–1865. [PubMed: 22546051]
22. Li Z, Hu Y, Howard KA, Jiang T, Fan X, Miao Z, Sun Y, Besenbacher F, Yu M. *ACS Nano*. 2016; 10:984–997. [PubMed: 26655250]
23. Lux F, Sancey L, Bianchi A, Crémillieux Y, Roux S, Tillement O. *Nanomedicine*. 2015; 10:1801–1815. [PubMed: 25715316]
24. Cormode DP, Sanchez-Gaytan BL, Mieszawska AJ, Fayad ZA, Mulder WJM. *NMR Biomed*. 2013; 26:766–780. [PubMed: 23303729]
25. Lee YC, Chen DY, Dodd SJ, Bouraoud N, Koretsky AP, Krishnan KM. *Biomaterials*. 2012; 33:3560–7. [PubMed: 22341582]
26. Fang J, Chandrasekharan P, Liu XL, Yang Y, Lv YB, Yang CT, Ding J. *Biomaterials*. 2014; 35:1636–42. [PubMed: 24290697]
27. Yi DK, Selvan ST, Lee SS, Papaefthymiou GC, Kundaliya D, Ying JY. *J Am Chem Soc*. 2005; 127:4990–4991. [PubMed: 15810812]
28. Ross RD, Cole LE, Tilley JMR, Roeder RK. *Chem Mater*. 2014; 26:1187–1194.
29. Rabin O, Manuel Perez J, Grimm J, Wojtkiewicz G, Weissleder R. *Nat Mater*. 2006; 5:118–122. [PubMed: 16444262]
30. Ai KL, Liu YL, Liu JH, Yuan QH, He YY, Lu LH. *Adv Mater*. 2011; 23:4886. [PubMed: 21956662]
31. Kim D, Park S, Lee JH, Jeong YY, Jon S. *J Am Chem Soc*. 2007; 129:7661–7665. [PubMed: 17530850]
32. Oh MH, Lee N, Kim H, Park SP, Piao Y, Lee J, Jun SW, Moon WK, Choi SH, Hyeon T. *J Am Chem Soc*. 2011; 133:5508–5515. [PubMed: 21428437]
33. Kandanapitiye MS, Gao M, Molter J, Flask CA, Huang SD. *Inorg Chem*. 2014; 53:10189–10194. [PubMed: 25283335]
34. Jakhmola A, Anton N, Vandamme TF. *Adv Healthc Mater*. 2012; 1:413–431. [PubMed: 23184772]
35. Lee N, Choi SH, Hyeon T. *Adv Mater*. 2013; 25:2641–2660. [PubMed: 23553799]

Fig. 1.

a) Schematic representation for synthesis of BiG nanocrystals employing Method A; representative TEM images for b) BiG-25 NCs; c) BiG-5 NCs; and d) BiG-2.5 NCs.

Fig. 2. Dissolution of BiG and Bi NCs in a) PBS (pH 7.4) and b) Sodium Citrate (NaCit; pH 5.5) over 4 weeks, obtained using ICP-OES ($n = 4$, S.D. < 0.5).

Fig. 3. Schematic representation for synthesis of a) BiG@PLGA nanoparticles by Method C; and b) BiG-5@SiO₂ nanoparticles by Method D. Characterization of BiG-2.5@PLGA NPs by TEM c) and SEM e); BiG-5@PLGA NPs by TEM f) and SEM h) and BiG-5@SiO₂ NPs by TEM i) and SEM k).

Fig. 4. Bi dissolution from BiG-5 and BiG-2.5@PLGA NPs and BiG-5@SiO₂ NPs in a) PBS (pH 7.4); and b) Sodium Citrate (NaCit; pH 5.5) over 4 weeks using ICP-OES (n = 4, S.D. < 0.5).

Fig. 5. Cellular uptake of BiG-5@PLGA-C6 NPs at concentration of 0.1 mg per mL after 3 h incubation with Raw 264.7 macrophage cells; a) Nuclei stained by DAPI; b) Cytoskeleton dyed by phalloidin; c) NPs loaded with Coumarin-6 (C6) and d) all the channels merged; e) Cell viability (%) over 24 h for BiG NPs in Raw 264.7 macrophage cells; f) μCT phantom images of BiG-5@PLGA NPs and ISOVUE-300; the scale bar presents CT value in HU.

Table 1

Synthetic details, nanocrystal content and size of various Bi NCs.

NC type	Synthetic Method	Ganex in 1-ODE ^a (wt%)	NC Content ^b (%)	Size ^c (nm)
BiG-25	A	25	80.9	14 ± 4.1
BiG-5	A	5	75.7	12 ± 1.2
BiG-2.5	A	2.5	18.1	9.0 ± 1.1
Bi NCs	B	NA	98.2	48 ± 8.0

^a1-ODE is 1-Octadecene;

^bNC content obtained using TGA;

^csize reported from TEM images analysed using Image J software.

Synthetic details, nanoparticle formulations employed, final NC content (by TGA), and the hydrodynamic radii (D_h , nm), Polydispersity index (**PDI**) and zeta potential (ζ , mV) of different nanoparticles obtained using Dynamic Light Scattering (DLS).

Table 2

NP type	Synthetic Method	Polymer Type	NC Type	Polymer:NC:C6 (w:w:w)	Bi NC Content (%)	D_h (nm)	PDI	ζ (mV)
BiG-2.5@PLGA	C	PLGA	BiG-2.5	1:2:0	40.8	294.0 $\pm$ 5.1	0.31 $\pm$ 0.01	-8.06 $\pm$ 0.43
BiG-2.5@PLGA-C6	C	PLGA	BiG-2.5	5:10:1	39.1	248.7 $\pm$ 1.7	0.24 $\pm$ 0.02	-9.21 $\pm$ 0.39
BiG-5@PLGA	C	PLGA	BiG-5	1:2:0	71.6	138.6 $\pm$ 5.1	0.22 $\pm$ 0.03	-12.07 $\pm$ 0.38
Bi-5@PLGA-C6	C	PLGA	BiG-5	5:10:1	63.0	117.6 $\pm$ 4.1	0.18 $\pm$ 0.03	-13.83 $\pm$ 0.55
BiG-5@SiO2	D	Silica	BiG-5	7:1:0	80.1	115.3 $\pm$ 3.5	0.14 $\pm$ 0.03	-13.27 $\pm$ 0.40