Boron clusters-based systems for molecular imaging and BNCT

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Icosahedral boron clusters or borohydride clusters, carboranes and metallacarboranes, do not exist in nature and are purely abiotic, man-made compounds. The structure of boron cluster compounds differs from that of organic ones, as they have a polyhedral cage-like structure, which consists of different numbers of vertices occupied by boron atoms or heteroatoms to usually form an icosahedral. They are highly thermally and chemically stable. This stability results from the 3-dimensional aromaticity of boron cluster compounds, in which σ bonds are delocalized, as opposed to delocalized π bonds of the 2-dimensional aromaticity of organic compounds [1]. Their unique properties as rigidity, chemical stability, 3D aromaticity, low toxicity, as well as their hydrophilicity, hydrophobicity, or amphiphilicity (depending on the structure), and the ability to form dihydrogen and σ-hole bonding, give them the ability to interact with biological molecules in a different way than organic compounds and make them useful in biomedical applications as pharmacophores, especially in the design of boron carriers for Boron Neutron Cancer Therapy (BNCT).[2]

Furthermore, due to their unique structural and electronic properties, boron clusters are excellent entities when applying them to tailor the fluorescence emission of any fluorophore group.[3],[4] Then, more recently, the interest has been focused on developing boron rich photoluminescence systems by linking different light emitting fluorophores to neutral and anionic boron clusters. The main goal has been to design new boron-based molecules and materials as theranostic agents for diagnosis (bioimaging) and boron carriers for BNCT. In the last decade, our group have developed boron cluster-based molecules as fluorescent probes for in vitro fluorescent bioimaging, by linking different neutral or anionic boron clusters: $C_2B_{10}H_{12}$, $[B_{12}H_{12}]^{2-}$, $[3,3'\text{-}M(C_2B_9H_{11})_2]^-$ (M = Co, Fe) to diverse fluorophores. We have used well-known fluorophores as BODIPY, *aza*BODIPY, anthracene, or fluorescent organotin complexes. All of them have shown exceptional cellular uptake and intracellular boron release that together with their fluorescent properties and biocompatibility make these compounds good candidates for cell tracking and boron delivery systems.

BODIPY-anionic boron cluster conjugates bearing $[B_{12}H_{12}]^{2-}$ and $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$ (COSAN) and $[3,3'\text{-}Fe(1,2\text{-}C_2B_9H_{11})_2]^-$ did not show cytotoxicity at low concentration (5 µg B/ml). All these compounds were well internalised by Hela cells, being the BODIPY-COSAN derivative the one that showed an exceptional cellular uptake and intracellular boron release. These properties together with its fluorescence, biocompatibility and high boron content make this compound potential candidate for cell labelling agents towards medical diagnosis and boron carrier for BNCT(Figure 1).[5]

Apart from previous BODIPY derivatives bearing anionic boron clusters, our group has also developed neutral BODIPY- and aza-BODIPY-carborane conjugates bearing C-substitued *ortho*- and *meta*-carborane clusters.[6] HeLa cells were incubated with our set of BODIPYs that exhibited different behaviour regarding cellular uptake and subcellular distribution. The differences seem to be originated in their unlike static dipole moments and partition coefficients, which depend on the type of cluster isomer (o- or m-) linked to the BODIPY and that modulate the ability of these molecules to interact with the lipophilic microenvironments in cells. It can be highlighted that the *m*-carborane derivatives with a higher lipophilicity were much better internalized by cells than their *ortho*-analogues. These behaviour was also observed in anthracenyl-containing iodo-*m*-carborane derivatives, that were internalised by HeLa cells by endocytosis and accumulated in the cytoplasm, which allowed their cellular imaging by confocal microscopy.[7]

Organotin compounds are based on 4-hydroxy-N'-((2-hydroxynaphthalen-1-yl)methylene)benzohydrazidato that was derivatized with $[B_{12}H_{12}]^{2-}$ and COSAN.[8] These compounds showed luminescence properties in solution with Φ_F values in the range from 24% to 49%. Mouse melanoma B16F10 cells were incubated with 10 µg/mL of the different compounds for 2 h and then analysed by confocal laser microscopy. Noticeable different staining effect was observed depending on the type of boron cluster; compounds bearing the COSAN anion showed an important fluorescence in the cytoplasm, whereas those bearing $[B_{12}H_{12}]^{2-}$ produced extraordinary nucleoli and cytoplasmic staining. The remarkable fluorescence staining properties of these organotin compounds in B16F10 cells make them excellent candidates for in vitro fluorescent bioimaging.

Besides previous luminescent materials, our group has also prepared carbon-based nanomaterials which consists of graphene oxide functionalized in the surface by monoiodinated

cobaltabis(dicarbollide).[9] We have efficiently developed a new nanomaterial (GO-I-COSAN, Fig. 2) based on graphene oxide (GO) functionalized with radiolabeled COSAN (I-COSAN) and demonstrated that our nanomaterial can potentially act as a theranostic agent for diagnosis (radioimaging) and therapy (anticancer agent for BNCT). TEM analyses confirm the internalisation of the nanomaterial by cells and its accumulation in the cytoplasm, without causing changes neither in the size nor in the morphology of cells. The GO-I-COSAN does not show cytotoxicity in vitro for HeLa cells, with a cell viability greater than 90 %. Furthermore, GO-I-COSAN is ingested by *C. elegans* resulting in a survival rate of around 100 %, revealing the absence of toxicity in vivo for the worms and supporting the results observed in the in-vitro studies. Radiolabeling of the material with the positron emitter ^{124}I was achieved via isotopic exchange of the I-COSAN to obtain [^{124}I]I-COSAN followed by its grafting onto GO. Biodistribution studies by Positron Emission Tomography (PET) indicate accumulation of the nanomaterial in the liver at early time, as well as accumulations in lungs and kindness. The nanomaterial shows radiochemical stability in vivo and relative long circulation time. Taking into account that long-circulating nanomaterials tend to accumulate in tumors to a certain extent due to enhanced permeability and retention (EPR) effect, it is reasonable to assume that our nanomaterial would show important tumor uptake, resulting in a high concentration of boron atoms in the tumor tissue, which is a requirement for BNCT. Notably, a favorable biodistribution profile suggests the potential use of this nanomaterial as a theranostic agent for in vivo bioimaging and boron carriers for BNCT.

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Figures

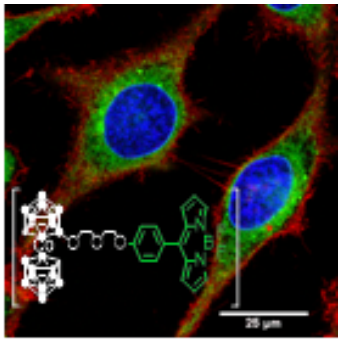

Figure 1. Confocal image of HeLa Cells incubated with Bodipy-Cosane conjugates after incubation for 2 hours.

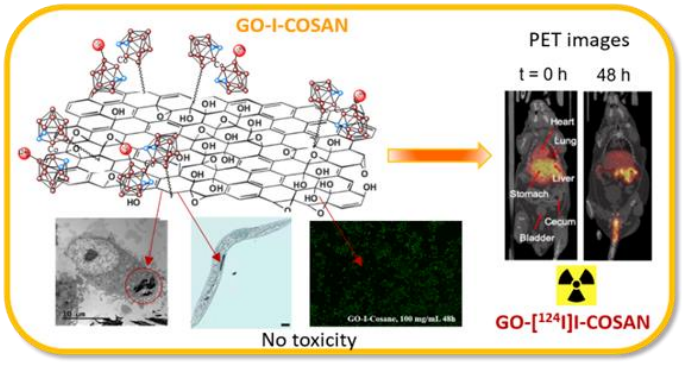

Figure 2. Representation of GO functionalised with radiolabelled GO-[124I]I-COSAN including TEM and PET images.