Boron-rich, cytocompatible block copolymer nanoparticles by polymerization-induced self-assembly†

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Core–shell nanoparticles (NPs) with a boron-rich core were synthesized by RAFT-mediated polymerization-induced self-assembly using a new methacrylic boronate ester monomer. Under specific conditions, sub-100 nm spherical NPs could be obtained at high conversions by either emulsion or dispersion RAFT polymerization using poly(oligo(ethylene glycol) methacrylate) (POEGMA) dithiobenzoate-based chain transfer agents. Phenylboronic acid surface-functionalized NPs were obtained using a telechelic POEGMA. Primary data on biocompatibility is provided and suggests suitability as boron delivery agent for boron neutron capture therapy.

Among the various potential techniques to fight cancer and eliminate tumor cells, boron neutron capture therapy (BNCT) is an attractive binary treatment based on (i) delivery of non-toxic boron drugs into tumor cells and (ii) irradiation with a thermal neutron beam to trigger nuclear fission of boron-10 and subsequent production of high-energy alpha particles.†

The key challenge for successful BNCT treatment is the specific accumulation of boron – in any form – in tumor cells. Boronophenylalanine (BPA) has been one of the two major compounds used in BNCT clinical trials for decades, yet requires rather large injected amounts to reach sufficient boron concentrations.‡ The low tumor uptake and selectivity, and short retention of BPA are due to its non-selective tumor-targeting functionality, negative charge, and low molecular weight.† Similar issues are encountered with the other major compound used in BNCT, namely sodium borocaptate. To improve the longer circulation of boron agents and therefore facilitate specific tumor accumulation, several types of nanoparticles (NPs) such as liposome, dendrimer, and block copolymer (BCP) NPs have been evaluated as potential delivery systems. For instance, boronic acid-containing BCP NPs have been designed and synthesized for BNCT. In these examples, boron compounds were either encapsulated in or covalently linked to nanoparticles supposed to accumulate in neoplastic regions via the enhanced permeability and retention (EPR) effect.11,12 While they may improve specific delivery, macromolecular approaches to boron delivery for BNCT still exhibit shortcomings such as low boron content per unit weight, and/or complicated and time-consuming particle synthesis. In the present contribution, we sought to harness polymerization-induced self-assembly (PISA) to prepare boron-rich nanoparticles because this method precisely addresses such shortcomings.

PISA has become a very popular method over the last decade to design core–shell polymeric NPs with specific shapes and controlled diameters.13–17 Indeed, PISA not only yields colloidal suspensions with homogeneous morphology directly at high concentrations, but also simultaneously gives access to precise macromolecular architecture with high control over functionality,18,19 with excellent potential for a range of applications.20–27 We thus anticipated that the PISA-based synthesis of nanoparticles with a boron-rich core and a biocompatible shell in a time-efficient manner could be possible. Note that, while boron-containing polymers have recently...
been in vogue,\textsuperscript{28–30} no example of boron-containing PISA-
made nanomaterials can be found. To this end, we designed a
new boronate ester-functionalized methacrylate based on a
benzyl methacrylate (BzMA) scaffold – a monomer well-known
in the PISA realm – and polymerized it in polar media under
radical initiation in the presence of a poly(oligo(ethylene
glycol) methacrylate) (POEGMA) macromolecular chain trans-
fer agent (macroCTA). POEGMA was selected for its high solu-
ability in a wide range of solvents, as well as its biocompatibility
and propensity to provide a stealth character to coated nano-
particles, e.g. particles, thereby enabling application of our NPs as nanome-
dicines, hence in the BNCT field.\textsuperscript{31–33} Moreover, since the
POEGMA macroCTA is synthesized by reversible addition–frag-
mentation transfer (RAFT) polymerization, \( \alpha \)-functionalized
POEGMA macroCTAs can be readily obtained, which allows a
straightforward post-functionalization on the surface of nano-
particles, \textit{e.g.}, for targeted delivery. Here, phenylboronic acid
was introduced as \( \alpha \) end group for specific targeting of sialy-
lated epoites on the membrane of solid tumors.\textsuperscript{34} The overall
design of the present study is displayed in Scheme 1.

As mentioned above, we designed the boronate ester-con-
taining methacrylate based on BzMA because the latter was
successfully employed in multiple RAFT polymerization-
induced self-assembly (RAFTPISA) studies.\textsuperscript{35–39} We chose the
ester form of boronic acid to prevent potential issues related to
self-dimerization and subsequent uncontrolled crosslinking
during synthesis,\textsuperscript{40,41} as well as to tune solubility with regards
to the PISA process.\textsuperscript{16} 4-Pinacolboronylbenzyl methacrylate
monomer \textbf{PBBMA} was synthesized in two steps: (i) esterifica-
tion of commercially available 4-(hydroxymethyl) phenyl-
boronic acid with pinacol and (ii) Steglich esterification with
methacrylic acid (see ESI†). Combination of \( ^1 \text{H}, ^{13} \text{C}, \) and \( ^{11} \text{B} \)
NMR spectroscopy and high-resolution mass spectrometry
characterizations confirmed structure and purity of \textbf{PBBMA},
obtained as an oil (Fig. S1–S4†). Before implementing it for
the synthesis of amphiphilic block copolymers in dispersed
media, the propensity of this monomer to be polymerized by
RAFT solution polymerization with a low-molar-mass CTA,
many \( 2\text{-cyano-2-propyl benzodithioate (CPBD),} \) was first
assessed. The polymerization was conducted in \( N,N \)-dimethyl-
acetamide (DMAc) at various \([\textbf{PBBMA}][\text{CPBD}] \) ratios (100, 200,
and 400) with a constant [AIBN]/[CPBD] ratio equal to 0.2, at
either 0.3 M or 0.6 M of \textbf{PBBMA} (9 and 18 wt\%, respectively).
As expected, the higher concentration of monomer led to a
faster polymerization (Fig. 1A). In most cases, the polymeri-
ization fulfilled elementary criteria for a controlled/living
polymerization, \textit{i.e.}, linear increase in pseudo-first order plot –
at least in the first 8 hours of polymerization, linear increase
of number-average molar mass with conversion, and clear
shift of size-exclusion chromatography (SEC) traces (Fig. 1 and
S5†). However, it was possible to achieve high conversions in a
reasonable time frame only at the lowest targeted degree of
polymerization (\( D_{\text{Pth}} = 100 \)), \textit{i.e.}, at the highest CPBD and
AIBN concentrations. Higher \( D_{\text{Pth}} \) systematically involved
higher \( D \) values. Nevertheless, \textbf{PBBMA} showed a sufficient
amenability to polymerize \textit{via} the RAFT process and was sub-
sequently used in emulsion and dispersion polymerizations,
without surfactant, in the presence of POEGMA macroCTAs.

A set of macroCTAs was prepared by RAFT solution
polymerization of two distinct OEGMA oligomonomers (\( M_2 \)
= 300 or 500 g mol\(^{-1}\)) with either CPBD or an
\( N \)-hydroxysuccinimide ester derivative of CPBD, to introduce \( \alpha \)
functionality, which should then eventually be presented at
the particle surface after RAFTPISA (see Scheme 1). All
macroCTAs were obtained with low dispersity (\( D < 1.2 \))
(Table S1†).

First, we examined the emulsion polymerization of \textbf{PBBMA}
in water/EtOH (3 : 1 vol/vol). All sets of conditions can be
found in Table S2.† For this series, \textbf{POEGMA}_{500} was chosen for
its high water solubility (even at high temperatures), which is a
vital parameter in PISA. For instance, emulsion RAFTPISA at
70 °C with \([\textbf{PBBMA}][\text{POEGMA}_{500}]/[\text{ACVA}] = 100/1/0.2 \) at 10 wt\%
solids content (Table S2,† entry 1) led to about 60% conversion
after 2 h with a reasonably narrow molar mass distribution
(MMD), yet did not significantly proceed further (Fig. S8†).
The resulting NPs were stable monodisperse spheres of about
50 nm, as determined by dynamic light scattering (DLS)
(Fig. S9†), accompanied by a slight coagulum. Increase in total
solids content to 20 wt\% allowed higher \textbf{PBBMA} conversions
to be reached (94% in 2 h; Table S2,† entry 4), yet with a broad-

\[ \begin{align*}
\text{POEGMA} & \quad \text{PBBMA} \\
\text{ACVA} & \quad \text{H}_2\text{O/EtOH} \\
70 °C & \quad \text{RAFTPISA in} \\
\text{emulsion} & \quad \text{or} \\
& \quad \text{dispersion} \\
(\text{ACVA}/\text{H}_2\text{O/EtOH}) & \quad \text{(AIBN/MeOH)}
\end{align*} \]

\textbf{Scheme 1}. Synthetic route and compounds employed in the current study for boron-rich nanoparticle formation by RAFT polymerization-induced self-assembly (RAFTPISA). ACVA = 4,4’-azobis(4-cyanopentanoic acid). AIBN = azobisisobutyronitrile.
ening of the MMD (Fig. 2A). Although the final dispersion contained a homogeneous population of spheres of around 50 nm, as observed by transmission electron microscopy (TEM) (Fig. 2B) and DLS (Fig. S10†), a small amount of coagulum was again present. Increasing \([PBBMA]/[POEGMA_{500}]\) to 200 or 400 at solids contents of 10 or 20 wt% systematically led to early destabilization and low conversions (entries 2, 3, 5, and 6, Table S2†).

In order to produce a colloidally stable PISA system and achieve easy removal of potentially unreacted monomer, we turned to a dispersion process. Methanol was chosen as polymerization medium because (i) \(PBBMA\) was found to be highly soluble in it as opposed to its polymer – which is a requirement for PISA – and (ii) because it is considered a green solvent.⁴² As the thermoresponsive character of POEGMAs with shorter oligoethylene glycol side chains becomes irrelevant in methanol, we chose to start our investigation on dispersion RAFTPISA with \(POEGMA_{300}\), because this is also a more practical polymer in terms of purification (i.e., separation of residual monomer). A series of experiments was performed by varying \(DP_{th}\) (100, 200, and 400) and the total solids content (10, 15, and 30 wt%). With the previously employed \([\text{initiator}]/[\text{CTA}]\) ratio of 0.2, far-from-complete \(PBBMA\) conversions were obtained after 24 h (Table S3, entries 1–6, as well as Fig. S11†). Increasing \([\text{initiator}]/[\text{CTA}]\) to 0.5 allowed a nearly full \(PBBMA\) conversion for \(DP_{th} = 100\) at 15 and 30 wt%. In those cases, as well as for \(DP_{th} = 200\) at 30 wt%, more than 90% \(PBBMA\) was polymerized within 6–8 h and nearly full conversion was eventually reached (Fig. 3A). Molar masses increased with time and conversion, with however a significant apparent amount of remaining macroCTA, and therefore increasing \(\mathcal{D}\) (Fig. 3B). Nevertheless, particle size increased with conversion and retained a homogeneous character (Table S4†). As observed by TEM, pure spherical NPs were obtained with \(DP_{th} = 100\) at 15 wt%
amount of free pinacol (13 mol%) was instantaneously formed (Fig. S17†). For DPth = 100 at 15 wt%, a colloidally stable dispersion was obtained, however with conversion-dependent evolution of the dispersion characteristics, following classic observations made in PISA formulations leading to higher-order morphologies: first, fluid dispersions of small nanoparticles; then, gel with large heterogeneous particle size distribution (PSD); finally, viscous dispersions with homogeneous PSD (Fig. S15 and Table S6†). As of now, we have not investigated all these samples departing from classic core–shell spheres in depth, since BNCT has so far typically investigated all these samples departing from classic core–shell spheres in depth, since BNCT has so far typically assessed the biocompatibility of the RAFTPISA–POEGMA500NHS NPs. For imaging purposes, fluorescent NPs were prepared by adding a small amount of fluorescein methacrylate (FMA) to a PISA recipe.46 Briefly, nanoparticles NP1 and NP2, without and with surface functional groups, respectively, were obtained from POEGMA300 and POEGMA500NHS, respectively (see ESI†). NHS ester-surface-functionalized NP2 were subsequently reacted with 3-aminophenylboronic acid to produce NP3 with specific targeting agent for sialylated epitopes (Fig. 4A), as mentioned earlier. Successful surface modification to obtain NP3 was confirmed by the appearance of aromatic signals in deuterated polar media, in which the core is invisible (Fig. S20†). The NP dispersions were dialyzed against MeOH, then water, to remove unreacted monomer and switch the dispersant, respectively. Their colloidal characteristics in cell culture media are collated in Table S7.† The fluorescent nature of the NPs was confirmed by fluorescence spectroscopy with \( \lambda_{em,max} = 515 \) nm (Fig. S20†). Next, we analyzed interactions of the NPs with scavenger cells. Indeed, clearance by the reticuloendothelial system (RES) typically accounts for the poor delivery of nanomedicines to target tissues.47 Therefore, uptake and cytocompatibility of NP1 and NP3 were tested in RAW 264.7 and HUVEC cells,48

(Fig. 3C). Importantly, the hydrodynamic diameters of the NPs remained essentially unchanged when transferred to water with no sign of aggregation (Table S4†). At higher target DPth and/or solids content, PISA syntheses gave more condensed jelly-like solution or gel sedimentation indicating possible formation of worm-like filomicelles/nanofibers (entries 5, 6, and 9, Table S3†). At higher DPth and solids content, i.e., 200–400 and 30 wt%, control was lost both in terms of MMDs (\( \bar{D}_n > 2 \)) and colloidal stability (precipitation). When POEGMA300 was replaced with POEGMA300 (Table S5†), kinetics remained similar (Fig. S14†). For DPth = 100 at 15 wt%, a colloidal stable dispersion was obtained, however with conversion-dependent evolution of the dispersion characteristics, following classic observations made in PISA formulations leading to higher-order morphologies: first, fluid dispersions of small nanoparticles; then, gel with large heterogeneous particle size distribution (PSD); finally, viscous dispersions with homogeneous PSD (Fig. S15 and Table S6†). As of now, we have not investigated all these samples departing from classic core–shell spheres in depth, since BNCT has so far typically assessed the biocompatibility of the RAFTPISA–POEGMA500NHS NPs. For imaging purposes, fluorescent NPs were prepared by adding a small amount of fluorescein methacrylate (FMA) to a PISA recipe.46 Briefly, nanoparticles NP1 and NP2, without and with surface functional groups, respectively, were obtained from POEGMA300 and POEGMA500NHS, respectively (see ESI†). NHS ester-surface-functionalized NP2 were subsequently reacted with 3-aminophenylboronic acid to produce NP3 with specific targeting agent for sialylated epitopes (Fig. 4A), as mentioned earlier. Successful surface modification to obtain NP3 was confirmed by the appearance of aromatic signals in deuterated polar media, in which the core is invisible (Fig. S20†). The NP dispersions were dialyzed against MeOH, then water, to remove unreacted monomer and switch the dispersant, respectively. Their colloidal characteristics in cell culture media are collated in Table S7.† The fluorescent nature of the NPs was confirmed by fluorescence spectroscopy with \( \lambda_{em,max} = 515 \) nm (Fig. S20†). Next, we analyzed interactions of the NPs with scavenger cells. Indeed, clearance by the reticuloendothelial system (RES) typically accounts for the poor delivery of nanomedicines to target tissues.47 Therefore, uptake and cytocompatibility of NP1 and NP3 were tested in RAW 264.7 and HUVEC cells,48

Fig. 3 (A) Conversion vs. time plots for PBBMA dispersion RAFTPISA with POEGMA300 at [AIBN]/[POEGMA300] = 0.5 at various solids contents and DPth. (B) Size-exclusion chromatograms of polymers obtained by PBBMA dispersion RAFTPISA in methanol at 70 °C with POEGMA300 at [AIBN]/ [POEGMA300] = 0.5, DPth = 100, and 15 wt% solids content (entry 7, Table S3†). (C) Transmission electron micrograph of nanoparticles obtained after 25 h under conditions corresponding to B (entry 7, Table S3†).
which are widely used as model systems in nanotoxicology and represent macrophages and endothelial cells, respectively, belonging to the RES.\textsuperscript{45} Fluorescein-labeled, carboxylated poly(styrene) (PS-COOH) NPs were included as a reference polymeric nanomaterial.\textsuperscript{49} As seen in Fig. 4B, nanoparticles NP1 and NP3 were non-toxic up to the concentration of 100 $\mu$g mL\textsuperscript{-1} over 24 h of exposure. Note that NP1 could not be assessed with HUVECs because large agglomerates were formed in the corresponding M200/LSGS cell medium (Table S7\textsuperscript{†}) and interfered with image analysis. Compared to PS-COOH NPs, non-functionalized NP1 were taken up at very low amounts in macrophages and adhered to endothelial cells (presumably due to agglomeration). However, 3-aminophenylboronic acid-functionalized NP3, intended for targeting selected cells of interest, did not accumulate in macrophages and endothelial cells (Fig. S21−S23\textsuperscript{†}). Hence, future investigations on clearance of these NPs \textit{in vivo} as well as their potential to target specific cell types are warranted. The difference in colloidal stability between NP1 and NP3 in these conditions remains to be elucidated. Nevertheless, functional nanoparticles NP3 appear to be promising for BNCT.

While their stealth core–shell architecture and their ease of functionalization with targeting moieties or labels confer decisive advantages over boronophenylalanine (BPA) – the classic BNCT agent – it is particularly interesting to put into perspective the “solubilizing effect” of the present NPs with the maximum solubility of BPA. The latter is indeed soluble at physiological pH only up to about 6 mg mL\textsuperscript{-1}, that is, 0.031 wt\% of boron.\textsuperscript{50} Considering the best working systems here (entries 4 and 7 of Tables S2 and S3,\textsuperscript{†} respectively), boron mass concentrations with one order of magnitude higher are achieved (0.42 and 0.33 wt\%, respectively), within non-optimized systems (see calculations in ESI\textsuperscript{†}). At the same time, the boron concentration per mass unit of compound remains in the same range: 5.2 wt\% for BPA vs. 2.8 and 2.2 wt\%, respectively.

**Conclusions**

In this communication, we described for the first time the synthesis of amphiphilic diblock copolymer nanoparticles with a boron-rich core by polymerization-induced self-assembly (PISA). A new methacrylic monomer with a pinacol boronate ester was synthesized with good yields from commercially available and cheap reagents in two steps. Its efficient RAFT polymerization was demonstrated for limited degrees of polymerization, both in homogeneous and heterogeneous systems. Using hydrophilic macromolecular transfer agents, PISA could be carried out to obtain sub-100 nm spherical nanoparticles using both emulsion and dispersion polymerization in (hydro)alcoholic media. While they may not be useful for the envisioned application, \textit{i.e.}, boron-neutron capture therapy (BCNT), typical PISA higher-order morphologies such as nanorods/fibers and nano/microvesicles were not investigated, yet could be part of further investigations. Future work should also be directed to refining the optimal block copolymer composition (DP of 1\textsuperscript{st} block vs. DP of 2\textsuperscript{nd} block) to increase the relative boron concentration within the nanoparticles. Finally, encouraged by the preliminary results on biocompatibility reported here, implementation of the new nanoparticles in BNCT, first \textit{in vitro}, then possibly \textit{in vivo}, should be performed.
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Conflicts of interest

There are no conflicts to declare.

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