Polymersome nanoparticles for delivery of Wnt-activating small molecules

Edoardo Scarpa, PhD\textsuperscript{a,b}, Agnieszka A Janeczek, PhD\textsuperscript{a}, Alethia Hailes, BSc(Hons)\textsuperscript{a,b}, Maria C de Andrés, PhD\textsuperscript{a}, Antonio De Grazia, MSc(Hons)\textsuperscript{a}, Richard OC Oreffo, DSc\textsuperscript{a,b}, Tracey A Newman, PhD\textsuperscript{b,d,*}, Nicholas D Evans, PhD\textsuperscript{a,b,c,*}

\textsuperscript{a}Centre for Human Development, Stem Cells and Regeneration, Bone and Joint Research Group, University of Southampton Faculty of Medicine, Southampton, United Kingdom
\textsuperscript{b}Institute for Life Sciences, Centre for Biological Sciences, B85, University Road, University of Southampton, Southampton, United Kingdom
\textsuperscript{c}Bioengineering Sciences Group, Faculty of Engineering and the Environment, University of Southampton, Highfield, Southampton, United Kingdom
\textsuperscript{d}Clinical and Experimental Sciences, Medicine, University of Southampton, Southampton, United Kingdom

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Abstract

Spatiotemporal control of drug delivery is important for a number of medical applications and may be achieved using polymersome nanoparticles (PMs). Wnt signalling is a molecular pathway activated in various physiological processes, including bone repair, that requires precise control of activation. Here, we hypothesise that PMs can be stably loaded with a small molecule Wnt agonist, 6-bromoindirubin-3′-oxime (BIO), and activate Wnt signalling promoting the osteogenic differentiation in human primary bone marrow stromal cells (BMSCs). We showed that BIO-PMs induced a 40% increase in Wnt signaling activation in reporter cell lines without cytotoxicity induced by free BIO. BMSCs incubated with BIO-PMs showed a significant up-regulation of the Wnt target gene \textit{AXIN2} (14 ± 4 fold increase, \textit{P} \textless 0.001) and a prolonged activation of the osteogenic gene \textit{RUNX2}. We conclude that BIO-PMs could represent an innovative approach for the controlled activation of Wnt signaling for promoting bone regeneration after fracture.

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Key words: Polymersomes; Wnt; Drug delivery

Controlled, spatiotemporal delivery of therapeutic molecules is a major goal in medicine that may be achieved by the use of nanosized carriers. \textsuperscript{1} Polymersomes (PMs) are an attractive candidate delivery agent. They are vesicles formed by the self-assembly of amphiphilic copolymers in aqueous solutions. \textsuperscript{2} PMs possess a hydrophilic core and an amphiphilic shell which can accommodate a variety of hydrophilic and hydrophobic molecules and drugs at high concentration. PMs can be synthesised from a wide range of biodegradable polymers, and may be engineered to release payloads in response to environmental stimuli. \textsuperscript{3–5} Furthermore, the outer surface of PMs can be composed entirely of polyethylene glycol (PEG) in order to prolong their \textit{in vivo} circulation time, \textsuperscript{6} and/or can be coupled with cell or tissue targeting motifs. \textsuperscript{7,8} To date, the majority of research on PMs as drug delivery vehicles has focused on cancer chemotherapy, where there is a need to deliver high concentrations of toxic and often highly water-insoluble drugs selectively to tumours. \textsuperscript{9} Various studies have shown that PMs can incorporate a wide variety of chemotherapeutic small molecules. \textsuperscript{10,11} Very recently PMs have been shown to increase...
the therapeutic index of paclitaxel in a model of gastric cancer in comparison to the albumin-bound formulation of paclitaxel currently commercialized as Abraxane\textsuperscript{6,12}. Building on these encouraging results, there is emerging data supporting the utility of PMs as drug delivery vehicles beyond oncology.\textsuperscript{33,14} These studies open up the possibility of applications of PMs in disease conditions where control over intracellular delivery, pharmacokinetic profile, and spatiotemporal delivery are fundamentally important.

We are investigating the notion that PMs might be exploited for the spatiotemporally-controlled delivery of therapeutic molecules to bone injury sites to promote fracture repair. Bone fracture is a highly regulated, reparative process that involves a sequence of distinct but overlapping physiological processes.\textsuperscript{15} Uncomplicated fractures generally heal well, but in some circumstances - for example in high-energy fractures, or when there is significant underlying pathology - fracture healing is delayed or may not occur. Delayed or non-union fractures are a significant clinical burden\textsuperscript{24,25} and new therapies are urgently required, but there are currently no clinically-approved drugs available for systemic administration that promote fracture healing.\textsuperscript{17}

The canonical (β-catenin-dependent) Wnt signalling pathway is a highly conserved molecular pathway that in bone tissue plays a fundamental role controlling bone homeostasis and repair,\textsuperscript{15} and it is an attractive drug target in this context. Several Wnt-targeting drugs have shown exceptional promise in increasing bone mass in patients with osteoporosis.\textsuperscript{18} However, there is a paucity of evidence to suggest that these therapeutics could be successful in fracture healing in humans. This may stem from the pleiotropy of Wnt signalling - elevated Wnt signalling can exert strongly stimulatory effects on bone fracture, but can also inhibit bone healing if activation is not timed appropriately.\textsuperscript{19,20} Furthermore, we recently demonstrated that Wnt activation in bone stem cells can both promote and inhibit osteogenic differentiation in a similar manner.\textsuperscript{21} For this reason, it is likely that if Wnt signalling is to be exploited in fracture healing, its activation must be spatially and temporally regulated.

Nanodelivery provides a potentially attractive answer to this challenge. Previous work has indicated that Wnt3A protein can be stably associated with nanosized liposomes and improve bone healing,\textsuperscript{20} osseointegration\textsuperscript{22} and stem cell activation.\textsuperscript{23} The use of recombinant proteins as therapeutic agents however has several disadvantages, including expense of production and poor stability during storage or following administration.\textsuperscript{24} An alternative approach is to exploit stable, inexpensive, synthetic small molecules that activate Wnt signalling. Examples are AZD 2858 and AR-28, small molecules that have been shown to enhance bone mass\textsuperscript{25,26} and augment fracture repair\textsuperscript{27} in rats and mice, or 6-bromoindirubin-3'-oxime (BIO), the administration of which resulted in a significant increase in bone trabecular number and thickness,\textsuperscript{28} as well as increased dentin mineralization following injury in healthy mice.\textsuperscript{29} Nonetheless, the clinical application of many of these molecules including BIO is limited by poor solubility, bioavailability and cytotoxicity at high concentrations.\textsuperscript{30}

In this study, we hypothesized that encapsulation of BIO in PMs would allow the activation of the Wnt pathway in a controlled manner while reducing the cytotoxicity associated with the delivery of high concentrations of BIO. We produced PMs using polyethylene glycol- polyacaprolactone (PEG-PCL) block copolymer. Both PEG and PCL polymers are approved for the use in clinic, therefore enabling rapid translational of research findings. We provide evidence of encapsulation and retention of a BIO in PMs and for a cell-protective function promoted by drug encapsulation within PMs. Finally, we demonstrate BIO loaded-PMs promote osteogenic differentiation in human primary cells, findings that highlight the potential of this system in regenerative medicine.

### Methods

Extended materials and methods can be found in the Supplementary Data.

### Results

**6-Bromoindirubin 3’-oxime (BIO) can be stably loaded in PCL-PEG polymersomes**

We first loaded PCL-PEG PMs with 6-bromoindirubin-3’-oxime (BIO), an ATP-competitive inhibitor of glycogen-synthase-kinase-3β (GSK-3β) by dissolving BIO either in the organic solvent fraction or in the aqueous PBS (Figure 1).

To differentiate these techniques, we termed these resulting PM preparations ‘DMF-dissolved’ or ‘PBS-dissolved’ respectively.

PBS- and DMF-dissolved PMs (Figure 2, A) displayed uniform size distributions of ~65 nm ± 18 nm and similar final concentrations of 1.5 × 10\textsuperscript{13} PMs ml\textsuperscript{-1} as measured by nanoparticle tracking analysis (NTA) (Figure 2, B). These sizes were in good agreement with the hydrodynamic diameter measured by dynamic light scattering (DLS) (~62 nm ± 22 nm) (Figure 2, C). Unloaded PMs were slightly smaller than BIO-loaded PMs (~57 nm vs. ~62 nm, Figure 2, C and Table 1), but this difference was not significant (\( P = 0.70 \)). Neither were there any significant differences in the charge of PMs, as measured by ζ-potential (Table 1).

On storage of PMs for up to 14 days, minor (<10%) changes in size were observed for empty or DMF-dissolved PMs (Figure 2, D). In contrast, core-loaded PMs were unstable, with an increase in size from 65 nm to 210 nm between 7 and 14 days and concurrent increase in the polydispersity index (Pdi) from 0.19 to 0.31, (Figure 2, D).

Next, we quantified the amount of BIO loaded in the two preparations following synthesis and purification using absorption spectroscopy. BIO has absorbance peaks at 535 and 580 nm where there is little interference from Rayleigh scattering from PMs (Supplementary Figure 1, A). We used absorbance of BIO at 535 nm to determine a standard curve for BIO (Supplementary Figure 1, B). Following purification, comparable concentrations of BIO were measured in bulk suspensions (15 × 10\textsuperscript{12} PMs ml\textsuperscript{-1}) of core-loaded PMs (187 ± 36 μM) and DMF-dissolved PMs (198 ± 32 μM). Based on these data we estimated an encapsulation efficiency of ~90% for both formulations. Finally, we measured the stability of the
incorporation of BIO in the stable, DMF-dissolved PMs under continuous dialysis sink conditions (Figure 2, E). BIO association with PMs was stable for 1–2 days with negligible release, after which release occurred steadily with ~10 ± 1% lost at day 3, and 56.68 ± 2.5% of BIO lost between day 3 and 5. After this period, the residual BIO was slowly but constantly released from the PMs, as demonstrated by the presence of 22.3 ± 4.2% and 12.3 ± 6.7% of the original concentration loaded still present after 10 and 14 days, respectively (Figure 2, E). In summary, these data illustrate that the Wnt pathway agonist BIO can be loaded in PCL-PEG PMs at high concentrations for time periods of up to 48 hours.

BIO-loaded PMs are taken up by Wnt reporter cells and bone marrow stromal cells (BMSCs)

Then we investigated the internalization of PMs in Wnt-responsive and functionally relevant cells.31 This relies on the incorporation within PMs of self-quenched high concentration of fluorescein, which then becomes detectable following release from nanoparticles (Figure 3, A).

After 24 hours of incubation with PMs, both 3T3 Leading Light® Wnt reporter cells and bone marrow stromal cells (BMSCs) were positive for fluorescein as measured by microscopy, indicating internalization of PMs and release of payload (Figure 3, B). To investigate the kinetics of PMs uptake by these two cell types we used flow cytometry. Figure 3, C and D show that within 1 hour of incubation 100% of 3T3 cells are positive for fluorescein, compared to 91% ± 11% of BMSCs. 100% positivity of BMSCs for fluorescein was not reached until 3 hours (Figure 3, C and D). We observed differences in the relative mean fluorescence intensity of each cell line, indicating differential PM uptake between cell types (Figure 3, E). There was also a difference in the kinetics of uptake between cells. The time at which half-maximal fluorescence intensity (τ1/2) was reached was ~1 hour for BMSCs compared to ~2.5 hours for 3 T3 cells. Inclusion of BIO in fluorescein-containing PMs did not affect the total number of cells that became positive for fluorescein, but resulted in a decrease in the mean cellular fluorescence of 10 ± 3% and 21 ± 2% for reporter cells and BMSCs, respectively (Supplementary Figure 2).

Together this data indicate that PMs are rapidly taken up by 3T3 Wnt reporter cells and human BMSCs, that the payload is released intracellularly, and that inclusion of BIO has a minor effect on the degree of uptake.

BIO-PMs induce Wnt signalling in a dose-dependent manner

To determine whether PM-delivered BIO was able to induce Wnt signalling we incubated reporter cells with PBS-, DMF-dissolved PMs or free BIO for 24 hours and measured the level of Wnt activation by luciferase production (Figure 4, A). Both PM preparations induced Wnt signalling activation. Compared to free BIO, which has a maximum activation at a concentration of ~5 μM, DMF-dissolved PMs induced a maximum response at a factor of 0.46 ± 0.03 of the readout measured using 5 μM free BIO, while PBS-dissolved PMs induced a maximal response at a factor of 0.15 ± 0.03 of free BIO. This was despite PBS- and DMF-dissolved PMs contained comparable concentration of BIO (Figure 4, B).
We also confirmed the stability of the association between BIO and PMs. We ultracentrifuged DMF-dissolved PM suspensions to separate the PMs from supernatant and demonstrated that the activity of the preparation (i.e. the ability to induce the activation of the Wnt pathway in reporter cells) was retained in the pellet fraction consisting of PMs (~90% of the total activity measured) (Figure 4, C). As suggested by data in Figure 2, E, we confirmed that the association of BIO with PMs was stable for up to 36 hours. Continuous dialysis under sink conditions did not diminish the activity of the PM-BIO pellet, with no activity lost to the supernatant or through the dialysis tubing (Figure 4, D).

PM encapsulation protects cells from BIO-induced toxicity but retains Wnt signalling activity

Although being an established inhibitor of GSK-3\( \beta \), BIO is relatively membrane impermeable, and its IC50 is ~5 nM for GSK-3\( \beta \). However, extracellular concentrations of 500–1000×
greater (~2 μM) are required to induce half maximal response in the Wnt-signalling pathway (Figure 5, A). In parallel, BIO is known to cause cytotoxicity by unknown mechanisms, probably through binding to other intracellular kinases such as CDKs, JAK–STAT. We hypothesized that delivery of BIO using PMs would reduce these non-specific effects through a more controlled delivery.

To investigate this, we incubated 3T3 reporter cells with increasing concentrations of either DMF-dissolved PMs (hereafter referred to as BIO-PMs) or free BIO and compared their effects on activity and cell-induced toxicity. Free BIO promoted Wnt signalling activity in a dose-dependent manner with a maximal response at a concentration of 5 μM, and a progressive decline at higher concentrations. The maximal activation of Wnt signalling using BIO-PMs was reached at a concentration of \(15 \times 10^{11}\) PMs ml\(^{-1}\), corresponding to 46 ± 3% of the maximal activity measured using 5 μM BIO. Similar to free BIO, at concentrations higher than \(15 \times 10^{11}\) PMs ml\(^{-1}\), BIO-PMs also resulted in a decrease in reporter cell activity (Figure 5, A), albeit less markedly than with free BIO. We found a dose-dependent decrease in cell viability in response to free BIO (57 ± 10% of cell survival at an extracellular concentration of 5 μM), but no decrease in cell viability in response to BIO-PMs, even at concentrations that induced a decline in pathway activation (Figure 5, B and C). This suggests that in contrast to free BIO, BIO-PMs at any extracellular concentration tested do not have cytotoxic effect on cells. The decreased pathway activation at high PM concentrations must therefore occur by non-cytotoxic means.

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<th>Polymersome (PM) preparations over time.</th>
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Summary of size, polydispersity index (PI) and ζ-potential of PBS- and DMF-dissolved PMs and empty-PMs over 21 days. Data presented as mean ± SD (n = 3). PDI: polydispersity index; PM: polymersome.
mechanisms. Notably, we did not find any effect of empty PMs on free-BIO induced Wnt pathway activation, nor of increases in PBS buffer solution (Supplementary Figure 3). It is likely that the protection of cytotoxicity conferred by encapsulation of BIO may result from an uptake route that provides more specific delivery to its intended target. To investigate this, we measured the temporal activation of Wnt signalling in the reporter cell line by performing a pulse chase experiment, where cells were exposed to either PMs or free BIO for fixed periods of times, before wash-out and subsequent assessment of pathway activation at 24 hours (Figure 5, E). In order to compare free BIO and BIO-PMs, extracellular concentrations were selected (i.e. final concentrations in the cellular media) that elicit an equivalent activation of the Wnt signalling pathway in a 24 hour activation experiment. These concentrations were calculated from the experiments reported in Figure 5, A and are 2 μM and $15 \times 10^{11}$ mL$^{-1}$ for free BIO and BIO-PMs, respectively. As shown in Figure 5, F, the activity measured was greater for free BIO than for BIO-PMs for short exposure times (1–6 hours), but was similar at time periods of <12 hours. Supporting this, at 48 hours post incubation, BIO-PMs induced a significantly higher level of Wnt signalling activation than 2 μM free BIO (Supplementary Figure 4, $p < 0.05$). These results suggest that free BIO enters cells more quickly than BIO-PM and that BIO delivered by PMs requires a longer period of time to accumulate and activate signalling. Together these data indicate that BIO-PMs activate Wnt signalling, are not cytotoxic, and allow for a time-controlled delivery of high concentrations of payload, which would be cytotoxic if administered in a ‘free’ form.

**Application of BIO-PMs to achieve osteogenic priming of human multipotent stromal cells**

Activation of Wnt signalling is crucial in many repair processes, including bone regeneration following injury. Multipotent BMSCs contribute to bone repair and their differentiation to osteoblasts is known to be modulated by canonical Wnt signalling$^{21,23,39}$, therefore, they represent a good target for assessing the biological activity of PM-mediated Wnt activation (Figure 6, A).

Incubation of BMSCs with BIO-PMs resulted in a significant increase in the expression of the Wnt target gene $AXIN2$ at 24 hours ($12 \pm 6$ fold increase, $p < 0.001$, for $6 \times 10^{11}$ BIO-PMs mL$^{-1}$ and $9 \pm 3$ fold increase, $p < 0.05$, $15 \times 10^{11}$ BIO-PMs mL$^{-1}$). Similarly, 2 μM free BIO also
promoted AXIN2 expression at 24 hours (14 ± 4 fold increase, \( p < 0.001 \)). However, BIO-PMs promoted a sustained activation of Wnt signalling compared to free BIO (\( p < 0.0001 \)), before declining to baseline levels or lower after 4 days (Figure 6, B). These data indicate that BIO-PMs are internalized by BMSCs and that BIO-PMS promote a prolonged activation of the Wnt signalling pathway.

Next, we investigated whether the activation of Wnt induced by BIO-PMs would also promote osteogenic differentiation of BMSCs. As shown in Figure 6, C, the osteogenic ‘master gene’ RUNX2, was markedly up-regulated following 24 hour treatment with BIO-PMs (more than 1.5 fold increase compared to control), and significantly increased after incubation with 2 \( \mu \)M BIO (2.5 ± 0.5 fold increase compared to control, \( p < 0.05 \)). Importantly, incubation of BMSCs
with BIO-PMs at a concentration of $15 \times 10^{11}$ PMs ml$^{-1}$ resulted in a prolonged up-regulation of RUNX2, as evidenced by the gene expression 4 days post exposure ($2.3 \pm 0.96$ fold increase compared to control, $p < 0.05$) (Figure 6, C). Similar to free BIO incubation of BMSCs with BIO-PMs also promoted the up-regulation of SP7 (also known as Osterix), which is another key gene involved in the osteogenic commitment (Figure 6, D). Together this data indicates that BIO-PMs activate Wnt signalling in human BMSCs and promote their initial osteogenic commitment.

Discussion

Polymeric nanoparticles have potential for drug delivery where spatiotemporal delivery of a drug is key. Canonical Wnt signalling in bone repair is one such example. In this study, we have demonstrated that: (i) PMs can carry high concentrations of a relatively water-insoluble activator of Wnt signalling, (ii) PMs are taken up by cells important in the bone reparative process, and (iii) that activation of canonical Wnt signalling and osteogenic differentiation can be achieved while avoiding the toxicity that BIO causes when delivered with no carrier.

In cases of severe fractures, a pharmacological treatment aimed to promote bone repair could be beneficial in order to limit the incidence non-healing fractures. It is estimated that 10% to 50% of all the fractures diagnosed, depending on their anatomical localization, will not heal adequately, resulting in what is defined as ‘non-union’. Currently, bone morphogenetic proteins (BMPs) are the only growth factors approved for aiding fracture healing. However, there are several limitations when using BMPs in clinic, including osteolysis, soft tissue swelling, ectopic bone formation due to the use of supra-physiological doses, and the high cost of the drug. Wnt could therefore be a more robust and reliable molecular target. Encapsulation of Wnt agonist in a drug delivery system could limit the off-target accumulation and dramatically reduce the doses administered.

Liposomes loaded with Wnt proteins have been shown to be effective in promoting bone repair following local administration, and in inducing the osteogenic differentiation of human skeletal stem cell populations. However, liposomes are limited by short half-life in vivo and have a limited ability to retain payloads. Conversely, PMs are able to protect the payload from degradation in addition to high circulation half-life and low off-site accumulation in vivo. PMs core-shell structure allows for accommodation of hydrophilic and hydrophobic payloads simultaneously, including molecules soluble in both phases. BIO is soluble in organic solvents (i.e. DMF) and in
water (up to a concentration of 14 mM). Here, PMs were loaded with BIO by incorporating it either in the polymer/DMF solution or in the PBS bath. In the PBS-dissolved preparation the inclusion of BIO led to PM instability with changes in size 14 days post-production. An explanation for this observation is that BIO precipitates out of solution within the PM core causing perturbations to the PM structure and its premature collapse. The final concentration of BIO encapsulated in the two preparations was investigated using absorbance spectroscopy. This technique has inherent limitations including poor quantitative sensitivity and may underestimate the final concentration of BIO if any of it is in the solid state. Future studies using high performance liquid chromatography will be needed to better characterize BIO encapsulation.

We demonstrated that PMs are internalized by Wnt reporter cells and that the presence of BIO does not affect cellular uptake. Using a luciferase assay we have shown that DMF-dissolved PMs were significantly more active than PBS-dissolved PMs. This may be due to the fact that the 6 carbon atoms present in PCL backbone structure create strong supramolecular hydrophobic interactions between the molecule and the polymer itself when the two are dissolved in the same phase. Previous work has demonstrated that the solubility of hydrophobic drugs within a PM preparation is determined by the number of hydrogen bonds between payload and polymer, and given a constant concentration of drug, these bonds are directly proportional to the number of PCL monomers present. This would also explain the high level of association between BIO molecules and PMs demonstrated by dialysis and ultracentrifugation.

Regardless of the mechanism by which BIO was incorporated in PMs, drug release occurs rapidly following uptake, as determined by Wnt reporter cell assays. It can be hypothesized that hydrolytic processes taking place at the interface between PEG and PCL would promote the progressive exposure of BIO molecules to the extra-vesicular environment, which may result in an increased gradient pressure with subsequent release through diffusion.

A concentration of $15 \times 10^{11}$ BIO-PMs ml$^{-1}$ and an extracellular concentration of 2 $\mu$M BIO induced similar levels of Wnt signalling activation in reporter cells, which was approximately $\sim 40\%$ of the maximal activation measured (following incubation with an extracellular concentration of 5 $\mu$M BIO). The relationship between concentration of BIO-PMs used and intensity of Wnt signalling measured was biphasic, similar to that observed with free BIO. However, the two conditions had the opposite effect on cellular viability. High concentrations of free BIO were cytotoxic for the reporter cells, in line with previous observations on several cancer cells. In contrast, incubation with BIO-PMs did not induce cellular toxicity indicating that intracellular delivery using PMs avoids the activation of molecular pathways that are switched on when BIO is delivered in its free form, or PMs may mediate a protective action that inhibits BIO cytotoxicity. This may be mediated by the slow release of the payload into the cytoplasm. Pulse-chase experiments suggested that maximal activation of the Wnt pathway following incubation with BIO-PMs is delayed compared to free BIO. It has been demonstrated that intact PEG-PCL nanoparticles are still present intracellularly 24 hours after their initial cellular internalisation. These data suggest that PMs could function as intracellular depot of payloads and may explain the protective mechanism mediated by PMs. If it is assumed that BIO cytotoxicity is induced by the total inhibition of one of the CDKs, it is possible that the high intracellular concentration of free BIO is inhibiting the kinase at any given time, while the slow release from PMs is sufficient to transiently inhibit the kinase, but not enough to prevent its reactivation over time resulting in the absence of cytotoxicity. Furthermore, the depot effect mediated by PMs is induces a sustained activation of the Wnt signalling pathway over the course of 48 hours. A limitation of these findings is that the level of activity recorded using the reporter cells is a reflection of the amount of functional luciferase enzyme produced, and the luciferase readout can be significantly affected by a number of factors (i.e. the number of empty-PMs present in solution). In order to overcome these limitations, it would be important to quantify the intracellular concentration of BIO at different time points following incubation with either free BIO or BIO-PMs. For example, in future experiments it would be useful to create isotope-labeled variants of both BIO and PEG-PCL copolymer in order to be able to accurately quantify their concentration within the cells at uptake.

Finally, we investigated the application of BIO-PMs as possible source of controlled Wnt signalling activation for therapeutic purposes. First, we demonstrated that BIO-PMs are internalized by BMSCs inducing increased expression of AXIN2, which demonstrates the activation of the Wnt pathway. The results obtained with BMSCs corroborated what observed in the reporter cells, whereby BIO-PMs induce the maximal activation of the Wnt pathway after a 48 hour incubation, reinforcing the hypothesis that PMs function as intracellular depot for BIO. Secondly, we demonstrated the increased relative expression of both RUNX2 and SP7 following incubation with BIO-PMs. These genes are involved in the early osteogenic differentiation of BMSCs. This is in agreement with previous studies. Very recently, Low and colleagues demonstrated that micelles loaded with BIO and tethered with a bone-targeting peptide were able to increase both bone mineral density and bone volume in mice models of bone fracture. Future studies will address the functionality of BIO-loaded PMs in augmenting bone repair following fracture. Nevertheless, it was demonstrated that PMs could be loaded with BIO and induce significant activation of the Wnt signalling pathway following incubation with either reporter cells or BMSCs. There has not been previous research demonstrating the encapsulation of a GSK-3b inhibitor in PMs, making the results here especially novel.

In summary, the results presented demonstrate that PMs loaded with BIO can induce the activation of the Wnt signalling pathway. This encapsulation of a small molecule into PMs results in reduced cytotoxicity and in sustained Wnt signalling activation that promotes the early osteogenic differentiation of human stem cells. In view of the continuous advances in polymer chemistry and nanoparticle formulations, we demonstrated that “basic” PEG-PCL PMs incorporating a commercially available small molecule, may be a simple good candidate for a therapeutic approach focused on promoting bone regeneration following injury.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nano.2018.02.014.

References


